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# Multilocus phylogenetic inference in subfamily Chlorogaloideae and related genera of Agavaceae – Informing questions in taxonomy at multiple ranks

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ABSTRACT

A series of taxonomic questions at the subfamilial, generic, and intrageneric levels have remained within subfamily Chlorogaloideae s.s. (comprising *Camassia*, *Chlorogalum*, *Hastingsia*, and *Schoenolirion*) and relatives in Agavaceae. We present the first phylogenetic hypotheses focused on Chlorogaloideae that are based on multiple independent loci and include a wide sampling of outgroups across Agavaceae. In addition to chloroplast regions *ndhF* and *trnL–trnF*, we used nrDNA ITS for phylogenetic inference. Incomplete concerted evolution of the latter is indicated by intra-individual site polymorphisms for nearly half of the individuals. Comparisons of four coding and analysis methods for these characters indicate that the region remains phylogenetically informative. Our results confirm that Chlorogaloideae s.s. is not monophyletic, due to the close relationship of *Schoenolirion* with *Hesperaloe* and *Hesperoyucca*, as well as the likely sister relationship between *Hesperocallis* and core Chlorogaloideae (*Camassia*, *Chlorogalum*, and *Hastingsia*). *Chlorogalum* is also not monophyletic, being divided with strong support into vespertine and diurnal clades. This study produced the first phylogenetic hypotheses across *Hesperaloe*, allowing initial tests of several taxonomic disagreements within this genus. Our results reveal the lack of cohesion of *H. funifera*, indicating that *H. funifera* ssp. *funifera* may be more closely related to *H. campanulata* than to *H. funifera* ssp. *chiangii* (=*H. chiangii*). With potential gene flow between many members of *Hesperaloe* and a possible hybrid origin for *H. campanulata*, the genetic relationships within this genus appear complex. Further population-level investigation of many of the taxa in Chlorogaloideae s.l. would benefit our understanding of the evolution and taxonomy of these groups; *Camassia* and *Hastingsia* are the current focus of ongoing study.

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## 1. Introduction

Although there are groups of plants whose taxonomic limits have been clearly and robustly inferred since the time of Linnaeus (e.g., Asteraceae; Funk et al., 2009), lilies and their relatives are certainly not among them. Massive family-level rearrangements of Liliaceae s.l. into potentially over 20 families have resulted from efforts to discern their evolutionary relationships (e.g., APG, 2003, 2009; Cronquist, 1981). One challenging family within this complex group of plants is Agavaceae, also known as Agavoideae in Asparagaceae (APG, 2009; Chase et al., 2009). Some members of this family have received significant attention, such as the classic study system of *Yucca* – yucca moth mutualisms (Pellmyr, 2003) and economically-relevant taxa (e.g., FNA, 1993+; Zizumbo-Villarreal et al., 2013). Within Agavaceae, the less well-studied Chlorogaloideae s.l. (Halpin and Fishbein, 2013) provides many potential avenues for evolutionary studies. This group comprises a diverse assemblage of species that inhabit deserts to wetlands (FNA, 1993+); the distributions of some species cover broad regions of the United States while others are narrowly endemic on serpentine soils (Halpin and Fishbein, 2013). These rosette-forming plants vary greatly in morphology. For example, they range in height from 18 to 400 cm (Hochstätter, 2009; Sherman, 1969; Starr, 1997) and vary in floral shape among tubular, campanulate, and rotate types (zygomorphic and actinomorphic;

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FNA, 1993+; Starr, 1997). As in Agavaceae as a whole, Chlorogaloideae includes many plants utilized by humans, such as those used in horticulture (e.g., McGary, 2001; Starr, 1997), for pulp and paper applications (Sanchez et al., 2011), and as historically important food sources (Mehalchick et al., 2004; Moerman, 1986).

Chlorogaloideae s.s. (Speta, 1998) has traditionally included four genera with up to 18 species and 29 total infrageneric taxa: *Camassia* (camas), *Chlorogalum* (soap plants), *Hastingsia* (rush lilies), and *Schoenolirion* (rush lilies; Fig. 1 and Table 1). The latter three genera were first recognized as subtribe Chlorogaleae (Watson, 1879). This grouping was maintained for nearly 100 years, although Engler (1887) added *Hemiphylacus* and included *Hastingsia* in *Schoenolirion* within his Chlorogalinae. A link between *Camassia* and the original three genera of subtribe Chlorogalinae was supported by the cytological and morphological study of *Schoenolirion* by Sherman (1969), who also suggested that *Hemiphylacus* be excluded from the subtribe. Later, Speta (1998) officially proposed Chlorogaloideae as one of five subfamilies in Hyacinthaceae.

Considerable taxonomic fluidity characterizes Chlorogaloideae, including at the family level. Some genera have been placed in Liliaceae (Baker, 1873; Engler, 1887; Watson, 1879), Anthericaceae (Schulze, 1982), Hyacinthaceae (Batsch, 1786; Dahlgren et al., 1985; Speta, 1998), Camassiaceae (Cupov, 1994), Chlorogalaceae (Hoogland and Reveal, 2005), and Agavaceae. The inclusion of Chlorogaloideae in Agavaceae is well supported by phylogenetic evidence and by their shared bimodal karyotype, i.e., with chromosomes of two distinct size classes (Cave, 1970; McKain et al., 2012; Sato, 1935). A potential relationship of Chlorogaloideae with *Hesperaloe*, *Hesperocallis*, and *Hesperoyucca* (Fig. 1 and Table 1) is also supported by recent phylogenetic analyses (Halpin, 2011; Halpin and Fishbein, 2013). For simplicity, we will refer to these three genera plus the four genera of Chlorogaloideae s.s. as Chlorogaloideae s.l. (following Halpin and Fishbein, 2013, who summarized key aspects of their taxonomic history).

Preliminary phylogenetic insights for some members of Chlorogaloideae s.l. have been provided by several studies. Based on sampling 1–3 species per genus, both a *Camassia* – *Chlorogalum* clade and a *Hesperaloe* – *Hesperoyucca* clade were supported as members of Agavaceae s.l. by Bogler et al. (2006; using ITS, *ndhF*, and *rbcL*) and Smith et al. (2008; using *trnL–trnF*), but relationships with other members of Agavaceae have not been well supported. *Hesperocallis* was also sampled by Bogler et al. (2006) and placed in Agavaceae. Other phylogenetic studies of Agavaceae, Hyacinthaceae, and Asparagales similarly resolved sampled genera of Chlorogaloideae s.l. within Agavaceae (Good-Avila et al., 2006; Pfosser and Speta, 1999; Seberg et al., 2012). Although helpful in clarifying the family-level placement of Chlorogaloideae s.l., the broad focus of each of these studies led to very low sampling within the subfamily.

Halpin and Fishbein (2013) recently provided the first phylogenetic hypotheses focused on Chlorogaloideae s.s., using four cpDNA regions (*rpl16*, *trnD–trnT*, *psbJ–petA*, and *trnS–trnfM*). They sampled at least one population from each species in Chlorogaloideae s.s. and *Hesperocallis undulata*, one species from *Hesperaloe* and *Hesperoyucca*, and seven outgroups; this was the first inclusion of *Schoenolirion* in a phylogenetic study. Their tree resolved a clade that they designated "Core Chlorogaloideae" (*Camassia*, *Chlorogalum*, and *Hastingsia*), with *Hesperocallis* weakly supported as sister, followed by their "SHH" clade comprising *Schoenolirion*, *Hesperaloe*, and *Hesperoyucca*. Concordantly, recent analyses of whole chloroplast genomes strongly place *Camassia*, *Chlorogalum*, and *Hesperocallis* in a clade sister to a *Schoenolirion* – *Hesperaloe* – *Hesperoyucca* clade; in that case only one species was sampled for each genus except *Hesperaloe* (2 spp.; Michael McKain, pers. comm.). Halpin and Fishbein (2013) highlighted the need for more data, such as independent loci, and posed the possibility of dividing Chlorogaloideae s.s. or expanding the subfamily to encompass core Chlorogaloideae, *Hesperocallis*, and the SHH clade.

The present study focuses on providing some essential missing links in our understanding of phylogenetic relationships in these genera. The monophyly of four of the seven genera in Chlorogaloideae s.l. was supported by the phylogeny of Halpin and Fishbein (2013), with three genera remaining unsupported: *Hesperaloe* (not tested), *Hesperoyucca* (not tested), and *Chlorogalum* (paraphyletic). At the intrageneric level, only the phylogenetic analysis of Halpin and Fishbein (2013) evaluated *Chlorogalum* and *Schoenolirion*, although separate chloroplast phylogenies are available for *Camassia* (Fishbein et al., 2010) and *Hastingsia* (Halpin, 2011); each tree provided an initial outline of relationships, but with many unanswered questions. A multilocus phylogenetic hypothesis for both genera is being developed as part of a separate integrative taxonomic study (S. Kephart, J. Archibald, T. Culley, K. Theiss, unpub.). *Hesperocallis* is monotypic, but the phylogenetic relationships within *Hesperaloe* and *Hesperoyucca* are not well known. Only one or two species in these two genera had been sampled for broad phylogenetic studies, limiting assessment of relationships across all Chlorogaloideae s.l. prior to our work.

No previous phylogenetic analyses have focused on relationships within *Hesperaloe*, although the ITS phylogeny of Clary (2001) included three of the 6–9 taxa in the genus and strongly supported its monophyly. *Hesperaloe* has been consistently placed in Agavaceae (see FNA, 1993+), with some species formerly recognized as members of *Yucca* or *Aloe*. The exact number and ranks of taxa within *Hesperaloe* vary depending on the taxonomic treatment (Hochstätter, 2009; Hochstätter and Martínez-Ávalos, 2010; Starr, 1997).

*Hesperoyucca* currently encompasses three species (Clary, 2001), with *Hesperoyucca whipplei* being divided into 0–5 infraspecific taxa recognized at the variety or subspecies level (e.g., Haines, 1941; McKinney and Hickman, 1993). Formerly part of *Yucca* (e.g., Bogler et al., 2006; McKelvey, 1938, 1947), the genus is now recognized as distinct based on morphological, phenological, pollination, and phylogenetic criteria (Clary, 2001). Bogler et al. (2006) placed *Hesperoyucca* in Agavaceae s.s. as a close relative of *Hesperaloe*. The first phylogeny focused on this genus was the ITS tree of Clary (2001), with two populations of *H. whipplei* and one of each of the other two species. The genus as a whole and *H. whipplei* were each well supported as monophyletic.

*Hesperocallis* is a monotypic genus whose relationship to other monocots has been a long-standing puzzle. Treated alternatively in Liliaceae, Hemerocallidaceae, Funkiaceae (=Hostaceae), and Hesperocallaceae (putatively related to Alliaceae), it is placed currently in Agavaceae (Pires et al., 2004), consistent with its bimodal karyotype. Pires et al. (2004) supported this placement with the first phylogeny that included *Hesperocallis*. With greater sampling in the family, Bogler et al. (2006) also strongly supported *Hesperocallis* in an Agavaceae s.l. clade sister to an "extended Agavaceae" clade. We follow Chase et al. (2009) in considering Agavaceae to encompass both clades. Analyses by Halpin and Fishbein (2013) intriguingly placed *Hesperocallis* as sister to core Chlorogaloideae, but lacked strong support. To our knowledge, no prior phylogenies have strongly-supported resolution of the close relatives of *Hesperocallis*. Few morphological studies have explicitly linked this genus with the genera of Chlorogaloideae s.s. However, in a monograph of *Chlorogalum*, Hoover (1940) considered *Camassia*, *Hastingsia*, and *Schoenolirion* as most closely related to *Chlorogalum*, followed by *Hesperocallis* and *Odontostomum* Torr. The latter is now known to be well separated from Agavaceae (Stevens, 2001+). Gould (1942) similarly noted an affinity among *Camassia*, *Chlorogalum*, *Hesperocallis*, and *Schoenolirion*. Regardless, the exact placement of *Hesperocallis* within Agavaceae remains uncertain.

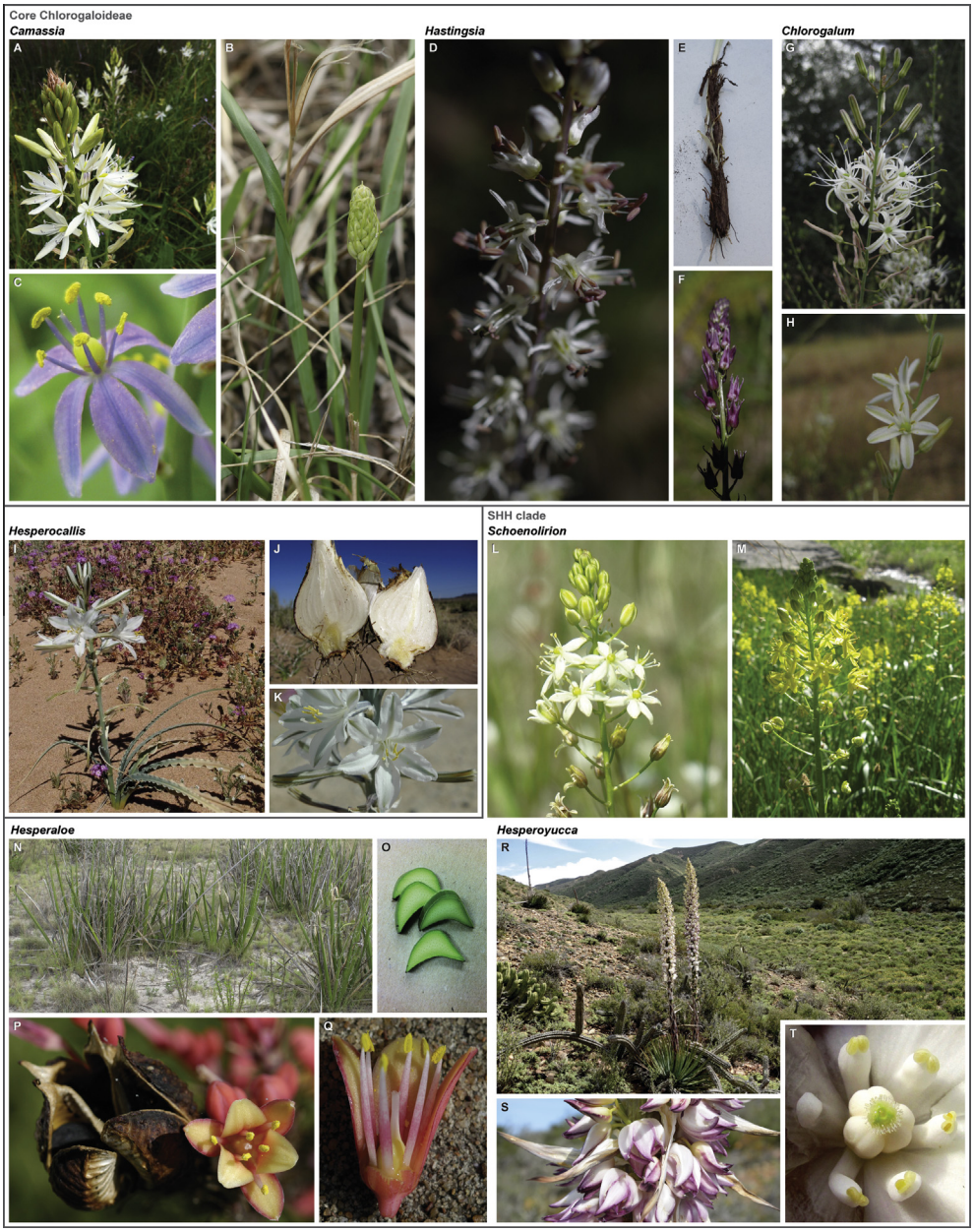


**Fig. 1.** Photographs across Chlorogaloideae s.l., representing a subset of the morphological diversity. (A) *Camassia leichtlinii* ssp. *leichtlinii*, inflorescences. (B and C) *C. angusta*, plant in bud (note keeled leaves), flower (note 3-lobed stigma). (D and E) *Hastingsia alba*, inflorescence, bulbs. (F) *H. atropurpurea*, inflorescence. (G) *Chlorogalum pomeridianum* var. *pomeridianum*, inflorescence. (H) *Ch. angustifolium*, inflorescence. (I–K) *Hesperocallis undulata*, plant, bulb, flowers. (L) *Schoenolirion wrightii*, inflorescence. (M) *S. croceum*, inflorescences. (N) *Hesperaloe funifera* spp. *chiangii*, vegetative plants. (O) *H. funifera* ssp. *funifera*, leaf cross sections. (P and Q) *H. engelmannii*, flower and dehisced capsule, flower with three tepals removed (note stout style). (R and S). *Hesperoyucca peninsularis*, plants and habitat, flowers. (T) *H. newberryi*, carpel and stamens (note apparently capitate stigma with trichomes). Photo credits: (A, B, and O) JKA; (C) Jim Kephart; (D–F) Linnea Hardlund; (G and H) Julie Kiersted Nelson; (I) Sue Carnahan via SEINet.org; (J and T) Wendy Hodgson; (K) Linda Prince; (L) Louisiana Natural Heritage Program; (M) Mason Brock; (N) Greg Starr; (P and Q) James Henrickson; (R and S) James Riley.

In all, previous studies have contributed new insights yet also challenging questions for understanding the evolutionary relationships of the taxonomically complex 'lilies' known as Chlorogaloideae. Here we provide a nuclear and chloroplast phylogeny of Chlorogaloideae s.l., with nearly complete taxon sampling of all putative species and infraspecific taxa. By sampling three genetic regions previously used for study within Agavaceae (ITS, *ndhF*, *trnL–trnF*), we were able to incorporate a wide array of outgroups across Agavaceae. Our goals were to: (1) Provide the first phylogenetic test based on multiple independent loci of the monophyly of Chlorogaloideae s.s. and of each of the seven study genera. (2) Infer the placement of each genus of Chlorogaloideae s.l. relative to the major clades of Agavaceae, with particular focus on the enigmatic *Hesperocallis*. (3) Produce the first phylogenetic tree across *Hesperaloe*, allowing preliminary phylogenetic tests of the differing taxonomic schemes proposed for this genus. (4) Develop a broad phylogenetic framework for subsequent species-delimitation and speciation studies within *Camassia* and *Hastingsia*.

## 2. Materials and methods

### 2.1. Taxon collection and DNA extraction

Leaf material (ca. 20 mg or more per individual) was collected on silica gel from each population. DNA was extracted from leaf samples using standard CTAB methods (Doyle and Doyle, 1987)

or a DNeasy® Plant Mini Kit (Qiagen, Valencia, California, USA). Initial analyses included 119 accessions, which were pruned to 92, 87, and 95 accessions for the final concatenated (ITS, *ndhF*, and *trnL–F*), ITS, and cpDNA (*ndhF* and *trnL–F*) analyses, respectively (Table 2 and Fig. 2). The differences in sampling among loci are due to different availability of outgroup (OG) sequences from GenBank (accessions designated "GB"). Outgroups were included in the final concatenated analysis if sequences from at least two of the three DNA regions were available. Other accessions were removed for the final analyses if there was evidence of hybridization, cultivation, unconfirmable taxonomic identification, or very long phylogenetic branches (see below). Final sampling of 87–95 accessions included 41 putative taxa of Chlorogaloideae s.l. and up to 14 OG taxa. At least one, and usually two or more, populations were sampled from all taxa within Chlorogaloideae s.l. (Tables 1 and 2), except the putative infraspecific taxa of *Hesperoyucca whipplei* and the rare Mexican *Hesperaloe malacophylla* (Hochstätter and Martínez-Ávalos, 2010). Outgroups represent each of the major clades of Agavaceae supported in Bogler et al. (2006); we based the choice of individuals for rooting on consistent relationships in previous phylogenies focused at larger taxonomic scales (Bogler et al., 2006; Chen et al., 2013; Seberg et al., 2012).

In addition to the outgroup targeting noted above, three other taxa were included from GenBank because prior taxonomic hypotheses suggested a relationship to the ingroup (i.e., Chlorogaloideae s.l., Table 1). These included *Hemiphylacus* S. Watson, formerly a member of subtribe Chlorogalinae (Engler, 1887; Krause, 1930), and *Oziroe biflora* (Ruiz & Pav.) Speta, formerly placed in *Camassia* (Tropicos.org). Previous analyses (e.g., Bogler et al., 2006) resolved a more distant relationship for *Hemiphylacus* and *Oziroe* with Agavaceae. Currently, *Hemiphylacus* is in Asparagaceae/Asparagoideae, and *Oziroe* is in Hyacinthaceae (or Scilloideae of Asparagaceae; Chase et al., 2009). Our initial analyses confirmed this distant relationship, placing both accessions on very long branches. We excluded them from final analyses to avoid long-branch attraction or repulsion (Felsenstein, 1978; Siddall, 1998). Finally, *Yucca queretaroensis* was included. Pellmyr et al. (2007) resolved this species as sister to the rest of *Yucca* using AFLP markers. They recommended further phylogenetic tests with greater sampling of *Hesperaloe* and *Hesperoyucca* to verify the generic placement of this species. Smith et al. (2008) sequenced *trnL–trnF* across Agavaceae, focusing on *Yucca*. They sequenced an accession of *Y. queretaroensis* that may be influenced by introgression (designated GB2 in this study) and included data from an accession sampled by Good-Avilla et al. (2006; GB3). The latter was sister to the remainder of *Yucca*, but with weak support and alternate placements depending on details of analysis (including a possible placement as sister to *Hesperoyucca*). The other accession was nested within *Yucca*. We included each sequence in separate analyses as well as an *ndhF* sequence from another population (*Y. queretaroensis* GB1). Our analyses showed no major differences in the inferred relationships regardless of the accession(s) used of *Y. queretaroensis*, likely due to much smaller sampling within *Yucca* (3 species) compared to Pellmyr et al. (2007) and Smith et al. (2008).

GenBank sequences for different gene regions from the same species were included in initial analyses as different putative individuals (numbered GB1, GB2, etc.), unless voucher citations from source papers verified origin from the same individual. Initial analyses confirmed consistent phylogenetic placement for each species, and their sequences were concatenated for subsequent combined analyses. This is indicated in accession labels; GB12 indicates concatenation of sequences GB1 and GB2 for a species.

### 2.2. DNA sequencing

We amplified one nuclear and two chloroplast regions: the nrDNA internal transcribed spacer regions (ITS-1 – 5.8S – ITS-2; Baldwin et al., 1995; Soltis and Soltis, 1998), as well as *ndhF* (Terry et al., 1997) and the *trnL* intron, *trnL* exon, and *trnL–trnF* spacer region (referred to here as the *trnL–F* region) from the

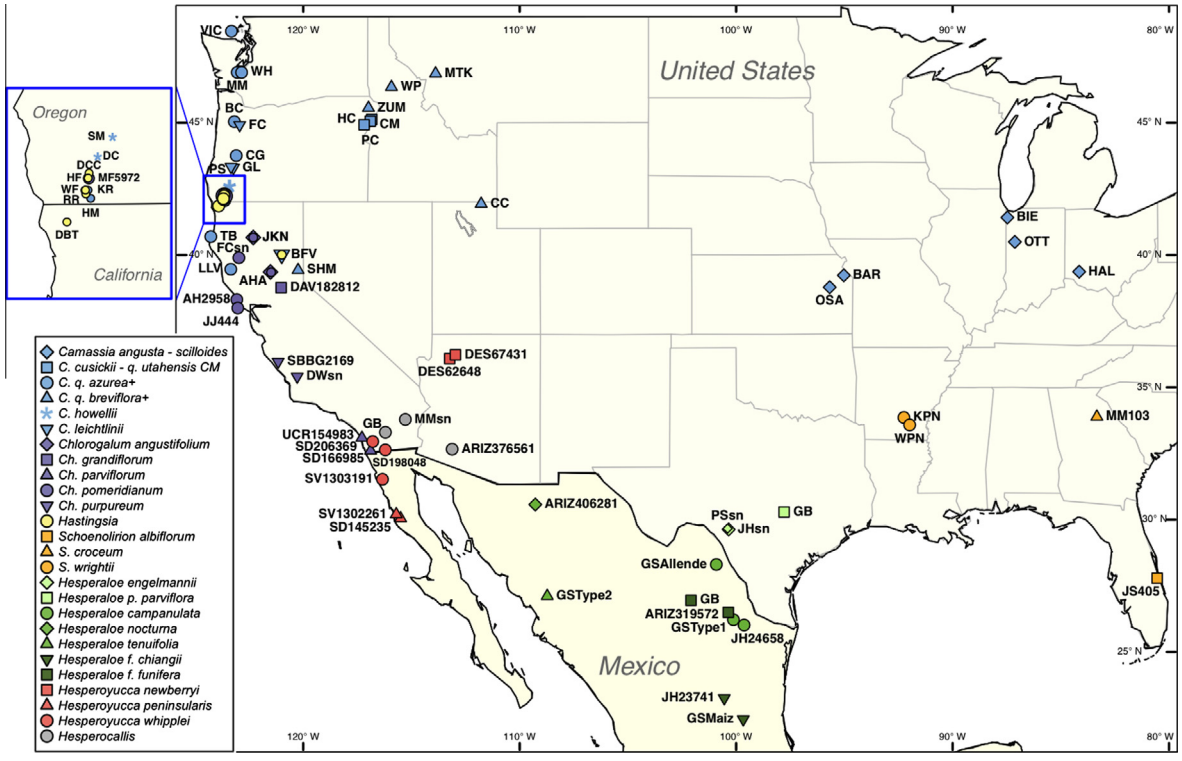


**Fig. 2.** Map of the United States and Mexico, showing all known locations for the sampled populations of Chlorogaloideae s.l.; the original sites for *Hesperaloe campanulata* MMsn and *H. parviflora* ssp. *bechtoldii* JH24815 are unknown and not mapped. Site code is indicated next to the symbol. Taxon or clade membership is indicated by symbol shape and color (see Fig. 3). Some symbols differ in size to facilitate visibility.

**Table 1**
Previously-hypothesized groups that include some genera of Chlorogaloideae s.l. and purported relatives. Genera that are not treated in a given grouping are indicated by an "–".

| No. intrageneric taxa | Chlorogaloideae s.l. | | Subfamily Chlorogaloideae s.s. | *Schoenolirion* and relatives | *Chlorogalum* and relatives | Subtribe Chlorogalinae | Subtribe Chlorogaleae |
|---|---|---|---|---|---|---|---|
| FNA (1993+) and others[a] | Halpin and Fishbein (2013) | | Speta (1998) | Sherman (1960) | Hoover (1940) | Engler (1887) | Watson (1879) |
| 6 spp., 10 sspp. | *Camassia* | Core Chlorogaloideae | *Camassia* | *Camassia* | *Camassia* | - | - |
| 5 spp., 5 vars. | *Chlorogalum* | | *Chlorogalum* | *Chlorogalum* | *Chlorogalum* | *Chlorogalum* | *Chlorogalum* |
| 2–4 spp., 0–2 vars. | *Hastingsia* | | *Hastingsia* | *Hastingsia* | *Hastingsia* | *Hastingsia*[b] | *Hastingsia* |
| 3 spp. | *Schoenolirion* | SHH clade | *Schoenolirion* | *Schoenolirion* | *Schoenolirion* | *Schoenolirion* | *Schoenolirion* |
| 5–9 spp., 0–2 sspp. | *Hesperaloe* | | - | - | - | - | - |
| 3 spp., 0–5 sspp., 0–4 vars. | *Hesperoyucca* | | - | - | - | - | - |
| 1 sp. | *Hesperocallis* | | - | - | *Hesperocallis* | - | - |
| - | - | | - | - | - | *Hemiphylacus* | - |
| - | - | | - | - | *Odontostomum* | - | - |

[a] See text: Becking (1986), Hochstätter (2009), Hochstätter and Martínez-Ávalos (2010), Lang and Zika (1997), Starr (1997).
[b] Merged within *Schoenolirion*.

chloroplast genome (Soltis and Soltis, 1998). Standard primers were employed for ITS (Table 3; Wen and Zimmer, 1996). The sequencing of *trnL–F* was complicated by several polyT regions, including one near the beginning of the region that was disruptive enough to require design of a new forward primer ("c2," Table 3). Amplifications also used the standard reverse primer "f" and internal primers "d" and "e" when necessary (Taberlet et al., 1991). Other polyT regions, usually near the center of *trnL–F*, disrupted sequencing in at least 64% of the individuals, but it was possible to compensate via additional sequencing. Primers for *ndhF* were either standard or slightly modified versions of those from Terry et al. (1997; Table 3), being used to amplify the region in two or three fragments. High quality DNAs were amplified in two fragments, using primer combinations 032F – 1318bR and 1101bF – 2110R.

PCR reactions (25–50 μl) consisted of 0.5–1 μl DNA, 0.4 μM of each primer, and 1× Premium Bullseye Taq DNA Polymerase Mix (75 mM Tris–HCl pH 8.5, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.1% Tween 20, 0.2 mM dNTPs, 0.025 units/μl Bullseye Taq polymerase, inert red dye and stabilizer; Midsci, Saint Louis, Missouri, USA). 10% DMSO was added to ITS reactions and 5% DMSO to *trnL–F* reactions. A Labnet MultiGene thermocycler (Labnet International Inc., Edison, New Jersey, USA) was used with the following settings: 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 50 °C (for ITS) or 48 °C (for *trnL–F* and *ndhF*), and 2 min at 72 °C; 10 min at 72 °C. Each thermocycler run included a negative control reaction with all reagents except for the DNA. PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, California, USA) and sequenced by Macrogen USA (Rockville, Maryland, USA). Sequences have been deposited in GenBank (KP008151–KP008366; Table 2).

### 2.3. Phylogenetic analyses and hypothesis testing

Individual contigs were edited, assembled, and aligned in Geneious v. 6.1.7 (Biomatters, Auckland, New Zealand). Alignments used MUSCLE settings and were adjusted by eye. Gaps were coded as characters using a modified version of the complex indel coding method of Simmons and Ochoterena (2000), as implemented by SeqState v. 1.4.1 (IndelCoder: modified complex coding option; Müller, 2005). Final analyses were run on concatenated, ITS, and cpDNA datasets using maximum parsimony (MP) and Bayesian inference (BI). Maximum likelihood (ML) analyses were run in Garli v. 2.01 (Zwickl, 2006) for comparison; the topologies of the favored trees were very similar to those from BI and will largely not be discussed. Parsimony analyses were conducted in PAUP* v. 4.0b10-x86 (Swofford, 2003) with the heuristic search option, MulTrees, no MaxTrees limit, swapping on all trees, and parsimony options set to collapse branches if minimum length is zero ("amb-"). Uninformative characters were excluded before all analyses and all remaining characters were equally weighted and unordered. One thousand quick searches with random taxon addition and NNI branch swapping were used to locate multiple islands of minimum-length trees, forming a starting pool of trees for more thorough searches employing TBR (Maddison, 1991). Separate TBR searches were run in different putative islands, but always inferred the same final relationships. Relative support for clades was assessed via jackknife (JK) analyses (5000 replicates). PAUP* was set to emulate Jac resampling with character deletion at 37%. Initial analyses were run using both nucleotide and indel characters, indel characters alone, and a subset of the indel characters that excluded potentially problematic characters. Results were consistent with or without indel characters; final MP analyses included all indels except three formed from variable single nucleotide repeats in the cpDNA dataset. Maximum likelihood and BI analyses were conducted on the nucleotide datasets only. Model parameters were estimated using the Akaike Information Criterion (AIC) of jModelTest v. 2.1.3 (Posada, 2008). Bayesian analyses were conducted in MrBayes v. 3.2.1 (Ronquist et al., 2012) and were partitioned by gene region, allowing independent evolutionary models for each. Two runs of metropolis-coupled Markov chain Monte Carlo simulations each used four linked chains (three heated and one cold) and default priors for all model parameters. The analyses were halted when the average standard deviation of split frequencies was below 0.0008, except for the cpDNA analyses, for which a cut-off of 0.008 was sufficient (Table 4). Parameter values were sampled every 100 generations. Convergence was assessed using Tracer v. 1.6 (Rambaut and Drummond, 2007) and AWTY (Nylander et al., 2008; Wilgenbusch et al., 2004; "compare" and "cumulative" utilities).

Topological incongruence was assessed by visually comparing the well-supported nodes from each analysis, considering nodes with at least 70% bootstrap/jackknife support (see Kellogg et al., 1996; Mason-Gamer and Kellogg, 1996) or 0.90 posterior probabil-

**Table 2**
Information for individuals sampled for phylogenetic analyses. Individual codes include site code and individual number (with collection year for *Camassia* and *Hastingsia*), or they are designated "GB" if those sequences were downloaded from GenBank. Voucher information includes collector(s), collection number, herbarium, and herbarium accession number if available. GenBank accession numbers are given for ITS, *trnL–F*, and *ndhF*. "MonAToL" indicates sequences pulled from genomic data gathered by M. McKain et al. for the Monocot Tree of Life project (http://www.botany.wisc.edu/givnish/monocotatol.htm). Inapplicable fields for a given individual are indicated by a "–".

| Taxon | Individual | Locality | Voucher | ITS | *trnL–F* | *ndhF* |
|---|---|---|---|---|---|---|
| *Agave dasylirioides* Jacobi & C.D. Bouché | GB | | | U23999, U24019 | – | DQ071892 |
| *Agave lechuguilla* Torr. | GB1 | | | U24000, U24020 | – | DQ071893 |
| *Agave lechuguilla* Torr. | GB2 | | | – | EU092542 | – |
| *Agave striata* Zucc. | GB | | | U24001, U24021 | – | DQ071896 |
| *Anthericum liliago* L. | GB | | | – | AF508513 | AF508402 |
| *Behnia reticulata* (Thunb.) Didr. | GB1 | | | – | – | AY191168 |
| *Behnia reticulata* (Thunb.) Didr. | GB2 | | | – | AF117007, AF117035 | – |
| *Beschorneria yuccoides* K. Koch | GB | | | U24008, U24028 | – | – |
| *Camassia angusta* (Engelm. & A. Gray) Blank. | OSA_1 (2012) | United States, Kansas, Osage Haymeadow, 38.7822, −95.67857 | Archibald, King, and Sharkan 2012-4 (KANU 393280) | KP008295 | KP008151 | KP008223 |
| *Camassia angusta* (Engelm. & A. Gray) Blank. | OTT_1M (2011) | United States, Indiana, Otterbein Prairie, 40.494493, −87.124091 | Homoya and Dana 90-06-14-66 (BUT 155014) | KP008296 | KP008152 | KP008224 |
| *Camassia cusickii* S. Watson | HC_2 (2006) | United States, Oregon, Hell's Canyon, 45.126083, −116.836117 | Kephart 600 (WILLU 50056) | KP008297 | KP008153 | KP008225 |
| *Camassia cusickii* S. Watson | PC_3 (2006) | United States, Oregon, Pine Creek, 45.0578, −116.9035 | Kephart 805 (WILLU 50117) | KP008298 | KP008154 | KP008226 |
| *Camassia howellii* S. Watson | DC_1 (2012) | United States, Oregon, Dutcher Creek, 42.4233, −123.54135 | Kephart, Bell, and Johnson 625 (WILLU 50091) | KP008299 | KP008155 | KP008227 |
| *Camassia howellii* S. Watson | SM_1 (2006) | United States, Oregon, Sexton Mountain, 42.594583, −123.37113 | Kephart 593 (WILLU 50057) | KP008300 | KP008156 | KP008228 |
| *Camassia leichtlinii* (Baker) S. Watson ssp. *leichtlinii* | GL_4M (2007) | United States, Oregon, Glide, 43.301217, −123.225967 | Kephart s.n. (WILLU 50067) | KP008301 | KP008157 | KP008229 |
| *Camassia leichtlinii* (Baker) S. Watson ssp. *leichtlinii* | PS_23 (2007) | United States, Oregon, Popcorn Swale, 43.301317, −123.225967 | Kotaich 105 (WILLU 50076) | KP008302 | KP008158 | KP008230 |
| *Camassia leichtlinii* (Baker) S. Watson ssp. *suksdorfii* (Greenm.) Gould | BFV_L24 (2006) | United States, California, Butterfly Valley, 40.012283, −120.994119 | Kephart and Theiss 604 (WILLU 50058) | KP008303 | KP008159 | KP008231 |
| *Camassia leichtlinii* (Baker) S. Watson ssp. *suksdorfii* (Greenm.) Gould | FC_1 (2006) | United States, Oregon, Fruitland Creek, 44.914334, −122.938023 | LaDouceur and Dick s.n. (WILLU 50087) | KP008304 | KP008160 | KP008232 |
| *Camassia quamash* (Pursh) Greene ssp. *azurea* (A. Heller) Gould | MM_1 (2007) | United States, Washington, Mima Mounds, 46.904633, −123.049567 | Swift s.n. (WILLU 50059) | KP008305 | KP008161 | KP008233 |
| *Camassia quamash* (Pursh) Greene ssp. *azurea* (A. Heller) Gould | WH_1 (2008) | United States, Washington, Wolf Haven, 46.90335, −122.848348 | Kotaich 100 (WILLU 50085) | KP008306 | KP008162 | KP008234 |
| *Camassia quamash* (Pursh) Greene ssp. *breviflora* Gould | SHM_1 (2006) | United States, California, Sagehen Meadow, 39.430217, −120.239651 | Kephart 525 (WILLU 50055) | KP008307 | KP008163 | KP008235 |
| *Camassia quamash* (Pursh) Greene ssp. *breviflora* Gould | ZUM_20 (2008) | United States, Oregon, Zumwalt Prairie, 45.55966, −116.986354 | Kephart 612 (WILLU 50079) | KP008308 | KP008164 | KP008236 |
| *Camassia quamash* (Pursh) Greene ssp. *intermedia* Gould | CG_11 (2008) | United States, Oregon, Cottage Grove, 43.758, −123.095 | Kotaich 104 (WILLU 50080) | KP008309 | KP008165 | KP008237 |
| *Camassia quamash* (Pursh) Greene ssp. *intermedia* Gould | PS_1 (2007) | United States, Oregon, Popcorn Swale, 43.301317, −123.225967 | Dennis 102 (WILLU 50071) | KP008310 | KP008166 | KP008238 |
| *Camassia quamash* (Pursh) Greene ssp. *linearis* Gould | LLV_1M (2012) | United States, California, Little Lake Valley, 39.46086, −123.346616 | Theiss and Hardlund s.n. (WILLU 50098) | KP008311 | KP008167 | KP008239 |
| *Camassia quamash* (Pursh) Greene ssp. *linearis* Gould | TB_1M (2012) | United States, California, Table Bluff, 40.6953, −124.2706 | Mesler 758 (WILLU 50073) | KP008312 | KP008168 | KP008240 |
| *Camassia quamash* (Pursh) Greene ssp. *maxima* Gould | BC_WS17 (2006) | United States, Oregon, Bethel Church, 45.041017, −123.183417 | Kephart 576 (WILLU 50060) | KP008313 | KP008169 | KP008241 |
| *Camassia quamash* (Pursh) Greene ssp. *maxima* Gould | VIC_Q1 (2004) | Canada, British Columbia, University of Victoria, 48.460967, −123.3189 | Allen 1310 (WILLU 50050) | KP008314 | KP008170 | KP008242 |
| *Camassia quamash* (Pursh) Greene ssp. *quamash* | MTK_7 (2008) | United States, Montana, Pattee Canyon, 46.860283, −113.88695 | Snustad-Clark s.n. (WILLU 50081) | KP008315 | KP008171 | KP008243 |
| *Camassia quamash* (Pursh) Greene ssp. *quamash* | WP_1 (2008) | United States, Idaho, Weippe Prairie, 46.349633, −115.9252 | Kotaich 111 (WILLU 50082) | KP008316 | KP008172 | KP008244 |
| *Camassia quamash* (Pursh) Greene ssp. *utahensis* Gould | CC_5 (2011) | United States, Utah, Cherry Creek, 41.931861, −111.77944 | Wolf 924 (WILLU 50097) | KP008317 | KP008173 | KP008245 |

(*continued on next page*)

**Table 2** (*continued*)

| Taxon | Individual | Locality | Voucher | ITS | *trnL–F* | *ndhF* |
|---|---|---|---|---|---|---|
| *Camassia quamash* (Pursh) Greene ssp. *utahensis* Gould | CM_1 (2008) | United States, Oregon, Carson Meadow, 44.922265, −117.190783 | Kotaich 123 (WILLU 50083) | KP008318 | KP008174 | KP008246 |
| *Camassia quamash* (Pursh) Greene ssp. *walpolei* (Piper) Gould | HM_1M (2013) | United States, Oregon, Hogue Meadow, 42.0568, −123.62955 | Archibald, Hardlund, and Kephart s.n. (WILLU 50103) | KP008319 | KP008175 | KP008247 |
| *Camassia quamash* (Pursh) Greene ssp. *walpolei* (Piper) Gould | KR_1 (2007) | United States, Oregon, Ken Rose, 42.1286, −123.6616 | Dennis 113 (WILLU 50074) | KP008320 | KP008176 | KP008248 |
| *Camassia scilloides* (Raf.) Cory | BAR_1 (2012) | United States, Kansas, Barnhardt Prairie, 39.23107, −95.02493 | Archibald 2011-4 (KANU 393269) | KP008321 | KP008177 | KP008249 |
| *Camassia scilloides* (Raf.) Cory | BIE_5 (2011) | United States, Indiana, Biesecker Prairie, 41.4178, −87.468283 | Schnabel s.n. (WILLU 50075) | KP008322 | KP008178 | KP008250 |
| *Camassia scilloides* (Raf.) Cory | HAL_10 (2011) | United States, Ohio, Hall's Creek, 39.36731861, −84.15142363 | Cartieri s.n. (CINC TMC13-1001) | KP008323 | KP008179 | KP008251 |
| *Chlorogalum angustifolium* Kellogg | AHA_1GH | United States, California, Ahart Ranch, 39.35775, −121.5136667 | Ahart 1397 (UCD 138418) | KP008324 | KP008180 | KP008252 |
| *Chlorogalum angustifolium* Kellogg | JKN_4 | United States, California, Redding, 40.661488, −122.3084 | Nelson 2013-008 (WILLU 50108) | KP008325 | KP008181 | KP008253 |
| *Chlorogalum grandiflorum* Hoover | DAV182812_a | United States, California, BLM Pine Hills Reserve, 38.76492237, −121.0214849 | CNPS SN Foothill Team SNFN0219 (UCD 80426) | KP008326 | KP008182 | KP008254 |
| *Chlorogalum parviflorum* S. Watson | UCR154983_a | United States, California, Near Encinatas, 33.092, −117.288 | Sanders 30293 (UCR 154983) | KP008327 | KP008183 | KP008255 |
| *Chlorogalum parviflorum* S. Watson | SD166985_a | United States, California, Otay Mountain Ecological Reserve, 32.60667, −116.88778 | Rebman, Gregory, and Rocks 10507 (SD 166985) | KP008328 | KP008184 | KP008256 |
| *Chlorogalum pomeridianum* (DC.) Kunth var. *divaricatum* (Lindl.) Hoover | AH2958_1 | United States, California, UC Davis Bodega Marine Reserve, 38.3138055, −123.0681944 | Howald and Sones 2958 (KANU 392053) | KP008329 | KP008185 | KP008257 |
| *Chlorogalum pomeridianum* (DC.) Kunth var. *divaricatum* (Lindl.) Hoover | JJ444_1 | United States, California, Point Reyes National Seashore, 37.995, −123.023 | Jernstedt 369 (UCD 113455) | KP008330 | KP008186 | KP008258 |
| *Chlorogalum pomeridianum* (DC.) Kunth var. *minus* Hoover | FCsn_a | United States, California, Near Sunnyside, 39.89739, −122.97322 | Callahan s.n. (–) | KP008331 | KP008187 | KP008259 |
| *Chlorogalum pomeridianum* (DC.) Kunth var. *pomeridianum* | AHA_1GH | United States, California, Ahart Ranch, 39.346833, −121.487055 | Ahart 19670 (WILLU 50112) | KP008332 | KP008188 | KP008260 |
| *Chlorogalum pomeridianum* (DC.) Kunth var. *pomeridianum* | MF5972_1 | United States, Oregon, 8 Dollar Mountain, 42.2315, −123.651 | Fishbein 5972 (HPSU) | KP008333 | KP008189 | KP008261 |
| *Chlorogalum pomeridianum* (DC.) Kunth var. *pomeridianum* | JKN_3 | United States, California, Redding, 40.661488, −122.3084 | Nelson 2013-009 (WILLU 50099) | KP008334 | KP008190 | KP008262 |
| *Chlorogalum purpureum* Brandegee var. *purpureum* | SBBG2169_a | United States, California, Near Jolon, 35.965, −121.161 | Wilken 15701 (SBBG 2169) | KP008335 | KP008191 | KP008263 |
| *Chlorogalum purpureum* Brandegee var. *reductum* Hoover | DWsn_1 | United States, California, Red Hill Road, 35.40293, −120.27959 | Hannon 898 (SBBG 114288) | KP008336 | KP008192 | KP008264 |
| *Chlorophytum alismifolium* Baker | GB | | | – | – | AY191163 |
| *Furcraea cahum* Trel. | GB | | | – | – | DQ071898 |
| *Hastingsia alba* (Durand) S. Watson | BFV_1M (2012) | United States, California, Butterfly Valley, 40.01185, −120.99238 | Kephart 661 (WILLU 50100) | KP008337 | KP008193 | KP008265 |
| *Hastingsia alba* (Durand) S. Watson | DBT_17M (2012) | United States, California, Darlingtonia Botanical Trail, 41.850778, −123.907972 | Barosh and Theiss s.n. (WILLU 50101) | KP008338 | KP008194 | KP008266 |
| *Hastingsia atropurpurea* Becking | WF_1 (2010) | United States, Oregon, Woodcock Fen, 42.12755, −123.697 | Kephart 640 (WILLU 50092) | KP008339 | KP008195 | KP008267 |
| *Hastingsia bracteosa* S. Watson | DCC_19M (2012) | United States, Oregon, Deer Creek Center, 42.277183, −123.648136 | Morse I181b (–) | KP008340 | KP008196 | KP008268 |
| *Hastingsia bracteosa* S. Watson | HF_1 (2010) | United States, Oregon, Howell's Fen, 42.233263, −123.659023 | Kephart 635 (WILLU 50093) | KP008341 | KP008197 | KP008269 |
| *Hastingsia serpentinicola* Becking | RR_3 (2010) | United States, Oregon, Rough & Ready, 42.095183, −123.686733 | Kephart 629 (WILLU 50095) | KP008342 | KP008198 | KP008270 |
| *Hastingsia serpentinicola* Becking | WF_17M (2012) | United States, Oregon, Woodcock Fen, 42.12922, −123.69105 | Theiss s.n. (WILLU 50116) | KP008343 | KP008199 | KP008271 |
| *Herreria salsaparilha* Mart. | GB | | | – | – | AY191178 |
| *Hesperaloe campanulata* G.D. Starr | GSAllende_1 | Mexico, Coahuila de Zaragoza, Near Allende, 28.30, −100.91 | | KP008344 | KP008200 | KP008272 |
| *Hesperaloe campanulata* G.D. Starr | GSType1_1 | Mexico, Nuevo León, Mamulique Microondas, 26.5167, −100.125 | Starr 93-001 (ARIZ 319675) | KP008345 | KP008201 | KP008273 |

**Table 2** (*continued*)

| Taxon | Individual | Locality | Voucher | ITS | *trnL–F* | *ndhF* |
|---|---|---|---|---|---|---|
| *Hesperaloe campanulata* G.D. Starr | JH24658_1 | Mexico, Nuevo León, Near Cerralvo, 26.0166, −99.6333 | Henrickson and Patterson 24658 (TEX) | KP008346 | KP008202 | KP008274 |
| *Hesperaloe campanulata* G.D. Starr | MMsn | Plant grown at New York Botanical Garden | | MonAToL | MonAToL | MonAToL |
| *Hesperaloe engelmannii* Krauskopf | JHsn_1 | United States, Texas, Plant grown in Federicksburg, Texas; originally from W. branch of Nueces river, 29, −100 | | KP008347 | KP008203 | KP008275 |
| *Hesperaloe engelmannii* Krauskopf | PSsn_3 | United States, Texas, Dobbs Run Ranch, 29.66672, −100.39512 | Smith s.n. (KANU 392050) | KP008348 | KP008204 | KP008276 |
| *Hesperaloe funifera* (K. Koch) Trel. ssp. *chiangii* G.D. Starr | GSMaiz_1 | Mexico, San Luis Potos, Near Ciudad de Maíz, 22.451597, −99.671136 | | KP008349 | KP008205 | KP008277 |
| *Hesperaloe funifera* (K. Koch) Trel. ssp. *chiangii* G.D. Starr | JH23741_1 | Mexico, San Luis Potos, Near Pozos Santa Clara, 23.25, −100.55 | Henrickson 23741 (TEX) | KP008350 | KP008206 | KP008278 |
| *Hesperaloe funifera* (K. Koch) Trel. ssp. *funifera* | GB | | | U23978, U24038 | – | DQ071899 |
| *Hesperaloe funifera* (K. Koch) Trel. ssp. *funifera* | ARIZ319572_a | Mexico, Nuevo León, Sabinas Hidalgo, 26.319, −100.379 | Starr 91-2 (ARIZ 319572) | KP008351 | KP008207 | KP008279 |
| *Hesperaloe nocturna* Gentry | ARIZ406281_a | Mexico, Sonora, Cañada el Tejano, 30.57194, −109.27916 | Reina-G., Van Devender, and Wolf 2010-217 (ARIZ 406281) | KP008354 | KP008210 | KP008282 |
| *Hesperaloe parviflora* (Torr.) J.M. Coult. ssp. *bechtoldii* Hochstätter | JH24815_1 | Plant grown in Sonora, Texas; original location unknown | Henrickson 24815 (TEX) | KP008355 | KP008211 | KP008283 |
| *Hesperaloe parviflora* (Torr.) J.M. Coult. ssp. *parviflora* | GB | | | U23979, U24039 | – | – |
| *Hesperaloe tenuifolia* G.D. Starr | GSType2_1 | Mexico, Sonora, Cerro Agujudo, 27.11389, −108.72861 | Meyer and Jenkins 90-63 (ARIZ 292741) | KP008358 | KP008214 | KP008286 |
| *Hesperocallis undulata* A. Gray | GB | | | – | AY561253 | AY225050 |
| *Hesperocallis undulata* A. Gray | ARIZ376561_a | United States, Arizona, Barry M. Goldwater Range, 32.665, −113.1218333 | McLaughlin and Bowers 10283 (ARIZ 376561) | KP008359 | KP008215 | KP008287 |
| *Hesperocallis undulata* A. Gray | MMsn | United States, California, Desert Lily Reserve, 33.789, −115.283 | Prince, Koontz, Pilapil, and Asanidze 674 (RSA 788238) | – | MonAToL | MonAToL |
| *Hesperoyucca newberryi* (McKelvey) Clary | DES62648_a | United States, Arizona, 193 Mile Canyon, 36.00136194, −133.0038972 | Hodgson 21151 (DES 62648) | KP008352 | KP008208 | KP008280 |
| *Hesperoyucca newberryi* (McKelvey) Clary | DES67431_a | United States, Arizona, Mohawk Canyon, 36.00379639, −112.0161333 | Hodgson, Makarick, Prince, Hahn, and Watters 21994 (DES 67431) | KP008353 | KP008209 | KP008281 |
| *Hesperoyucca peninsularis* (McKelvey) Clary | SD145235_a | Mexico, Baja California, Near Catavina, 30.04916667, −115.5013889 | Hodgson 9602 (SD 145235) | KP008356 | KP008212 | KP008284 |
| *Hesperoyucca peninsularis* (McKelvey) Clary | SV1302261_1 | Mexico, Baja California, Near Valle Tranquilo, 30.201833, −115.703833 | Vanderplank, Riley, and Simancas 130226-1 (SD) | KP008357 | KP008213 | KP008285 |
| *Hesperoyucca whipplei* (Torr.) Trel. | SD198048_a | United States, California, Jacumba, 32.63965, −116.20744 | Hendrickson 3697 (SD 198048) | KP008360 | KP008216 | KP008288 |
| *Hesperoyucca whipplei* (Torr.) Trel. | SD206369_a | United States, California, Cleveland National Forest, 32.95362, −116.7906 | Rebman 19027 (SD 206369) | KP008361 | KP008217 | KP008289 |
| *Hesperoyucca whipplei* (Torr.) Trel. | SV1303191_1 | Mexico, Baja California, Rancho los aquajitos, 31.526192, −116.339312 | Vanderplank and Arauz 1303191 (SD) | KP008362 | KP008218 | KP008290 |
| *Hosta ventricosa* (Salisb.) Stearn | GB | | | U23980 | AF508512 | AF508401 |
| *Hosta ventricosa* (Salisb.) Stearn | MM106 | Plant grown in cultivation; original location unknown | | – | MonAToL | MonAToL |
| *Leucocrinum montanum* Nutt. ex A. Gray | GB1 | | | – | – | AY225052 |
| *Leucocrinum montanum* Nutt. ex A. Gray | GB2 | | | – | AF117003, AF117031 | – |
| *Leucocrinum montanum* Nutt. ex A. Gray | LF_a | United States, Oregon, Lost Forest, 43.36411, −120.3293 | Ruedas s.n. (OKLA) | KP008363 | KP008219 | KP008291 |
| *Manfreda virginica* (L.) Salisb. ex Rose | GB | | | U23984, U24043 | – | DQ071901 |
| *Polianthes geminiflora* (Lex.) Rose | GB1 | | | U23989, U24047 | – | AY225048 |
| *Polianthes geminiflora* (Lex.) Rose | GB2 | | | – | DQ500903, DQ500937 | – |
| *Polianthes pringlei* Rose | GB1 | | | U23990, U24048 | – | DQ071902 |
| *Polianthes pringlei* Rose | GB2 | | | – | DQ500904, DQ500938 | – |
| *Prochnyanthes mexicana* (Zucc.) Rose | GB1 | | | U23991, U24049 | – | DQ071903 |
| *Prochnyanthes mexicana* (Zucc.) Rose | GB2 | | | – | DQ500917, DQ500952 | – |
| *Schoenolirion albiflorum* (Raf.) R.R. Gates | JS405_a | United States, Florida, Near Fellsmere, 27.7863611, −80.550556 | Scanlon 405 (FLAS 208612) | KP008364 | KP008220 | KP008292 |

(*continued on next page*)

**Table 2** (*continued*)

| Taxon | Individual | Locality | Voucher | ITS | *trnL–F* | *ndhF* |
|---|---|---|---|---|---|---|
| *Schoenolirion croceum* (Michx.) Alph. Wood | MM103 | United States, Georgia, Rock & Shoals Outcrop, 33.887633, −83.334665 | McKain 103 (WILLU 50104) | MonAToL | MonAToL | MonAToL |
| *Schoenolirion wrightii* Sherman | KPN_2.1GH | United States, Arkansas, Kingsland Prairie Natural Area, 33.86133, −92.24991 | Zollner s.n. (WILLU 50106) | KP008365 | KP008221 | KP008293 |
| *Schoenolirion wrightii* Sherman | WPN_1.4GH | United States, Arkansas, Warren Prairie Natural Area, 33.58208, −91.98305 | Witsell 01-0109 (ANHC 3379) | KP008366 | KP008222 | KP008294 |
| *Yucca brevifolia* Engelm. | MMsn | | Smith s.n. (–) | MonAToL | MonAToL | MonAToL |
| *Yucca queretaroensis* Pina | GB1 | | | – | – | JX903320 |
| *Yucca queretaroensis* Pina | GB2 | | | – | EU092616 | – |
| *Yucca treculeana* Carrière | GB1 | | | U23995, U24053 | – | DQ071904 |
| *Yucca treculeana* Carrière | GB2 | | | – | EU092632 | – |

**Table 3**
Primers used for amplification and sequencing. Those modified for this study are in bold.

| DNA region | Primer name | Sequence | Source |
|---|---|---|---|
| ITS | N-nc18S10 | AGGAGAAGTCGTAACAAG | Wen and Zimmer (1996) |
| ITS | C26A | GTTTCTTTTCCTCCGCT | Wen and Zimmer (1996) |
| ***trnL–F*** | **c2** | **CTCAATGGAAGCTGTTCTAA** | **Modified from** Taberlet et al. (1991) |
| *trnL–F* | f | ATTTGAACTGGTGACACGAG | Taberlet et al. (1991) |
| *trnL–F* | d | GGGGATAGAGGGACTTGAAC | Taberlet et al. (1991) |
| *trnL–F* | e | GGTTCAAGTCCCTCTATCCC | Taberlet et al. (1991) |
| *ndhF* | 032F | TACCTTTTCTTCCACTTCCAGTT | Terry et al. (1997) |
| ***ndhF*** | **451bF** | **TGGGAACTTGTAGGAATGT** | **Modified from** Terry et al. (1997) |
| ***ndhF*** | **451bR** | **ACATTCCTACAAGTTCCCA** | **Modified from** Terry et al. (1997) |
| *ndhF* | 745F | TGGTTACCTGATGCTATGGAAGG | Terry et al. (1997) |
| *ndhF* | 745R | CCTTCCATAGCATCAGGTAACCA | Terry et al. (1997) |
| ***ndhF*** | **1101bF** | **GGAACCTATTGTTGGRTATTCGCC** | **Modified from** Terry et al. (1997) |
| ***ndhF*** | **1101bR** | **GGCGAATAYCCAACAATAGGTTCC** | **Modified from** Terry et al. (1997) |
| ***ndhF*** | **1318bF** | **GGATTAACTGCATTTTATATGTTT** | **Modified from** Terry et al. (1997) |
| ***ndhF*** | **1318bR** | **AAACATATAAAATGCAGTTAATCC** | **Modified from** Terry et al. (1997) |
| ***ndhF*** | **1600bF** | **CCTCACGAGTCGGACAATACTATG** | **Modified from** Terry et al. (1997) |
| ***ndhF*** | **1600bR** | **CATAGTATTGTCCGACTCGTGAGG** | **Modified from** Terry et al. (1997) |
| *ndhF* | 2110R | CCCCCTATATATTTGATACCTTCTCC | Terry et al. (1997) |

ity (Simmons et al., 2004). Alternate phylogenetic hypotheses were tested using approximately unbiased (AU) tests in Consel (Shimodaira, 2002), using the concatenated dataset. Candidate trees for AU tests were produced in Garli via unconstrained ML and bootstrap (BSt) analyses (500 replicates) as well as those constrained to include or exclude clades of interest (100–500 replicates). Conclusions were consistent across multiple runs of Consel with differing candidate tree sets. Final AU *p*-values are from runs using all candidate trees from unconstrained and constrained ML and bootstrap analyses.

Nearly half of the ITS sequences displayed at least one strongly-supported double peak, suggesting incomplete concerted evolution for this region in Chlorogaloideae s.l. (Alvarez and Wendel, 2003). This may leave multiple ITS types within a given individual, while others may only have one type. Potts et al. (2014) provide the abbreviation 2ISP (pronounced "twisp") for such intra-individual site polymorphisms. While complicating the interpretation of ITS trees (Alvarez and Wendel, 2003), this does not eliminate their potential utility (Potts et al., 2014). Thorough cloning of ITS types and fluorescent or genomic in situ hybridization (FISH or GISH) would provide the best estimate of all ITS types in each individual and the genomic placement of those copies. However, such work is not feasible for many phylogenetic projects (Alvarez and Wendel, 2003), including the current study; and even those time and resource intensive methods do not guarantee complete sampling of all ITS types. Regardless, the impact of this intra-individual variation on phylogenetic analyses for Chlorogaloideae s.l. may be minimal.

Several types of analyses were run on the ITS data to account for these 2ISPs in alternate ways and assess their influence on phylogenetic inference with this data set. Indel characters were treated as missing for the following initial comparisons: (1) *All nucleotide characters with a 2ISP in any individual were removed.* As with any analysis based on regions within DNA arrays (such as ITS), this does not completely remove the potential for complex genetic processes to confound interpretation of phylogenies (Alvarez and Wendel, 2003). However, that would be true with or without obvious 2ISPs. (2) *2ISPs were coded as ambiguities and run using the typical settings described above.* (3) *Haplotypes were estimated for all 2ISPs using PHASE and kept as separate units in the phylogenetic analyses.* PHASE assumes that no more than two alleles are present per individual, which is not necessarily true for ITS. However, it allows for some separation of haplotypes. GenBank sequences were excluded from phasing, as were populations *Chlorogalum pomeridianum* var. *divaricatum* AH and *Hesperoyucca peninsularis* SV1302261. The latter two were excluded due to excessive missing data in the center of their sequences. With those exclusions, there were no missing data positions. SeqPHASE (Flot, 2010) transformed file formats (Stephens and Donnelly, 2003; Stephens et al., 2001). PHASE 2.1.1 was run five times with different random seeds in order to check for consistency, and the final run included five internal replicates. (4) *2ISPs were coded as ambiguities but run using the step-matrix method of* Potts et al. (2014). This assigns costs to changes between nucleotide codes, assuming that an ambiguity code states that *all* rather than *any* of the enclosed nucleotides are present at that site (i.e., R = A and G, not

**Table 4**
Characteristics of final DNA sequence datasets and resulting maximum parsimony (MP) and Bayesian inference (BI) analyses of Chlorogaloideae s.l. (i.e., the ingroup, IG) and outgroups (OG).

| | Concatenated[a] | ITS-unphased | ITS-phased | cpDNA[b] |
|---|---|---|---|---|
| *Dataset* | | | | |
| No. individuals | 92 (76 IG, 16 OG) | 87 (75 IG, 12 OG) | 71 (35 with two types; 70 IG, 1 OG) | 95 (76 IG, 19 OG) |
| No. characters | 3715 (3682 nuc., 33 indel) | 781 (756 nuc., 25 indel) | 765 (749 nuc., 16 indel) | 2943 (2926 nuc., 17 indel) |
| No. potentially parsimony informative characters | 425 (286 among IG) | 153 (122 among IG) | 133 (132 among IG) | 273 (166 among IG) |
| *MP analyses* | | | | |
| Length of most parsimonious trees (MPTs) | 844 | 401 | 253 | 438 |
| No. of MPTs | 6 | 96 | 216 | 24 |
| CI | 0.681 | 0.633 | 0.644 | 0.731 |
| RI | 0.925 | 0.917 | 0.956 | 0.943 |
| RC | 0.63 | 0.581 | 0.616 | 0.689 |
| *BI analyses* | | | | |
| No. generations | 25,198,000 | 103,678,000 | 95,659,000 | 695,000 |
| Potential scale reduction factors | 1.000–1.001 | 1.000 | 1.000 | 1.00–1.006 |
| Total effective sample sizes for each parameter | >12,230 | >100,567 | >95,459 | >466 |

[a] Concatenated refers to the concatenated analyses of the unphased ITS dataset, *ndhF*, and *trnL–F*.
[b] cpDNA refers to the concatenated analyses of *ndhF* and *trnL–F*.

A or G). Table 5 represents the step matrix used, as inferred from Fig. 1 of Potts et al. (2014) when indel characters are not included.

## 3. Results

### 3.1. Differences due to optimality criterion or DNA region

Parsimony and BI analyses result in no strongly-supported differences based on the standard criteria defined in our methods (Fig. 3 and Tables 4 and 6). The best-fit models were GTR + G for ITS, TPM2uf + I + G for *trnL–F*, and TVM + I + G for *ndhF*. Preliminary analyses treated *ndhF* and *trnL–F* as separate loci. As expected given the lack of independence of DNA regions within the chloroplast genome, resulting topologies were entirely congruent; the regions were concatenated for subsequent analyses. Results from ITS and cpDNA analyses were generally congruent; most differences were not strongly supported and concatenation usually resulted in higher resolution and support for relationships. The few strongly-supported exceptions with apparent incongruence between loci are discussed below.

### 3.2. Influence of method of analysis for ITS data

It is unlikely that our ITS data set includes pseudogenes given the presence of conserved sections of DNA without any substitutions. Atypically long branches are absent in the ingroup, although the most distantly-related OG sampled for ITS, *Leucocrinum montanum*, is unsurprisingly divergent. The maximum number of 2ISP positions seen in any given individual is just nine (found in one individual, *Schoenolirion wrightii* KPN), whereas there are over 150 potentially parsimony informative characters in ITS (Table 4). We do not have information on double peaks for GenBank sequences; of the individuals sequenced by JKA's research group, 53% have no 2ISPs, 33% have 1–2 2ISPs, and only 14% have 3–9 2ISPs. Results from all four types of coding for 2ISPs in ITS analyses are similar. Analyses 1 (removing 2ISP characters), 2 (coding 2ISPs as ambiguities), and 4 (coding 2ISPs as ambiguities with a Potts step matrix) gave nearly identical results, with increasingly higher resolution and support in a few clades. Analysis 3 (2ISP characters phased into two haplotypes) unsurprisingly gave the most divergent results, but again relationships were generally consistent. All runs in PHASE gave similar results and so haplotypes were used from the final run. There were no uncertain phase calls or genotypes and confidence probabilities for all phase calls and all haplotypes were 1.00. Further analyses and discussion are focused on comparing analysis types 4 (Potts coding) and 3 (phased) for MP, and 2 (ambiguity coding) and 3 (phased) for BI; final concatenated analyses used the Potts method for MP and ambiguity coding for BI.

Phasing of 2ISPs into separate haplotypes produced only a few differences in topology (not shown) compared to unphased analyses (Fig. 3A and C), particularly in relationships with strong clade support. In most cases, multiple haplotypes from a given individual group together in the phased analyses either as a separate clade or as members of the same polytomy. When this is not the case, it does not result in major differences in interpretation of that individual's relationship to others. Universally strongly-supported cases of non-monophyly of individuals include intermixing of haplotypes from two populations of the same species (i.e., *C. howellii* DC and SM, and *S. wrightii* KPN and WPN) and two cases where one haplotype groups with other taxa. *Hesperoyucca peninsularis* SD145_haplotypeB forms a clade with individuals of *H. whipplei* (PP = 1, JK = 86), while haplotypeA is sister to that clade. Similarly,

**Table 5**
Step matrix assigning the number of steps required to transition between each potential nucleotide character coded using IUPAC codes, under the assumptions of the 2ISP coding method of Potts et al. (2014).

| | A | C | G | T | M | R | W | S | Y | K | V | H | D | B | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0 | 2 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 3 | 2 | 2 | 2 | 4 | 3 |
| C | 2 | 0 | 2 | 2 | 1 | 3 | 3 | 1 | 1 | 3 | 2 | 2 | 4 | 2 | 3 |
| G | 2 | 2 | 0 | 2 | 3 | 1 | 3 | 1 | 3 | 1 | 2 | 4 | 2 | 2 | 3 |
| T | 2 | 2 | 2 | 0 | 3 | 3 | 1 | 3 | 1 | 1 | 4 | 2 | 2 | 2 | 3 |
| M | 1 | 1 | 3 | 3 | 0 | 2 | 2 | 2 | 2 | 4 | 1 | 1 | 3 | 3 | 2 |
| R | 1 | 3 | 1 | 3 | 2 | 0 | 2 | 2 | 4 | 2 | 3 | 3 | 3 | 3 | 4 |
| W | 1 | 3 | 3 | 1 | 2 | 2 | 0 | 3 | 2 | 2 | 3 | 1 | 1 | 3 | 2 |
| S | 3 | 1 | 1 | 3 | 2 | 2 | 3 | 0 | 2 | 2 | 1 | 3 | 3 | 1 | 2 |
| Y | 3 | 1 | 3 | 1 | 2 | 4 | 2 | 2 | 0 | 2 | 3 | 3 | 3 | 3 | 4 |
| K | 3 | 3 | 1 | 1 | 4 | 2 | 2 | 2 | 2 | 0 | 3 | 3 | 1 | 1 | 2 |
| V | 2 | 2 | 2 | 4 | 1 | 3 | 3 | 1 | 3 | 3 | 0 | 2 | 2 | 2 | 1 |
| H | 2 | 2 | 4 | 2 | 1 | 3 | 1 | 3 | 3 | 3 | 2 | 0 | 2 | 2 | 1 |
| D | 2 | 4 | 2 | 2 | 3 | 3 | 1 | 3 | 3 | 1 | 2 | 2 | 0 | 2 | 1 |
| B | 4 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 3 | 1 | 2 | 2 | 2 | 0 | 1 |
| N | 3 | 3 | 3 | 3 | 2 | 4 | 2 | 2 | 4 | 2 | 1 | 1 | 1 | 1 | 0 |

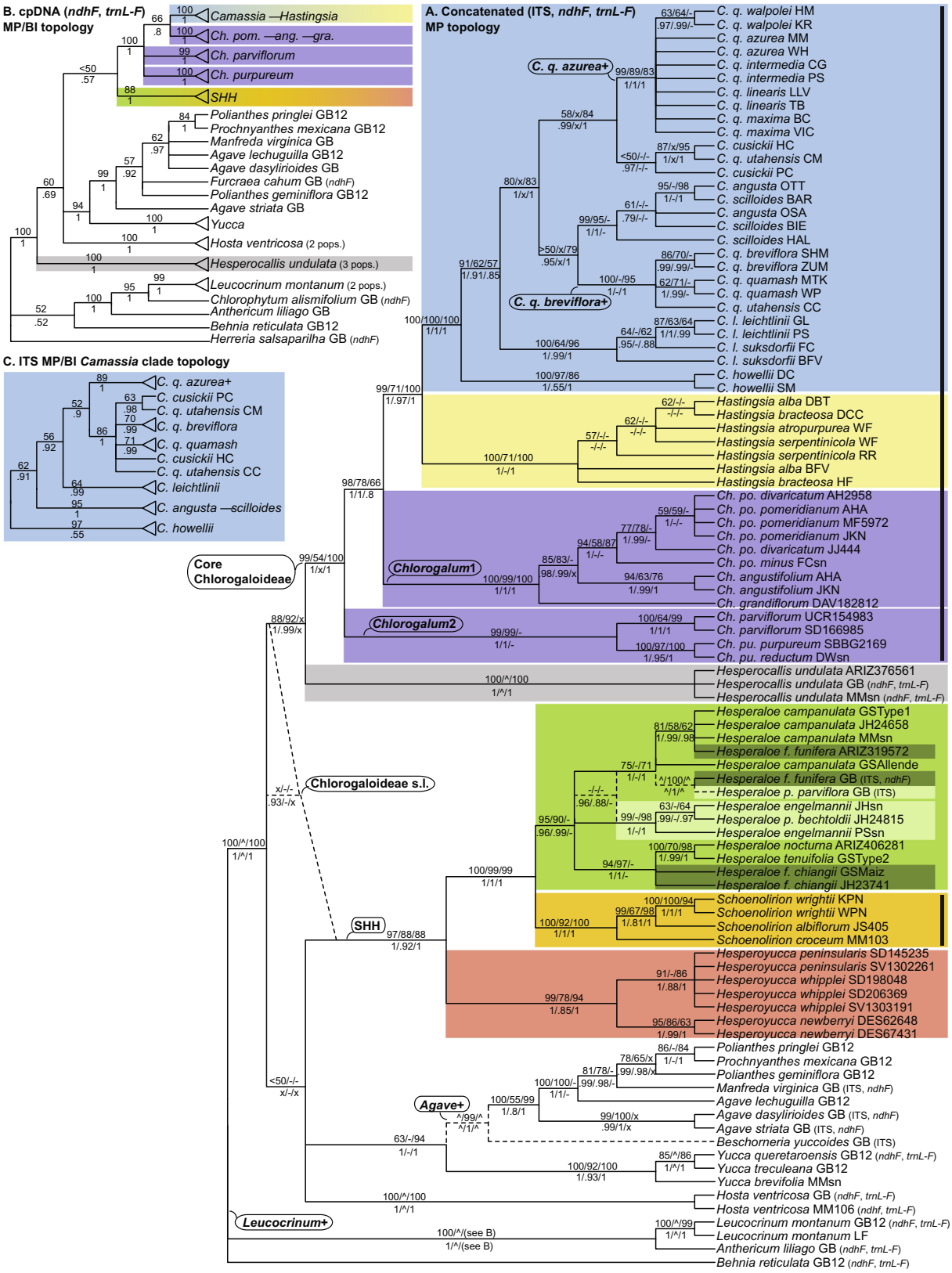


**Fig. 3.** Inferred phylogenies from analyses of ITS and two cpDNA regions (*ndhF* and *trnL–F*). (A) Strict consensus topology of most parsimonious trees for concatenated analyses. Membership in Chlorogaloideae s.s. is indicated by black bars to the right of taxon names. Support values are given for concatenated/ITS/cpDNA analyses, MP jackknife values greater than 50 are shown above the branches and BI posterior probabilities are shown below. "–" indicates a clade that is not resolved in that analysis; "x" indicates a clade that is contradicted by that analysis (see B and C for alternate resolutions); "^" indicates a clade that is not relevant to that analysis due to unsampled accessions for some loci. Accessions that are missing DNA regions have the sequenced regions noted after the accession name; if two DNA regions were missing, the accession was not included in concatenated analyses. Dotted lines indicate relationships inferred by BI and/or separate locus analyses that differ from the concatenated strict consensus. More complex alternate resolutions of relationships are given in two separate topologies. (B) cpDNA MP/BI topology with focus on outgroup relationships. (C) ITS MP/BI topology for the *Camassia* clade. Genera of Chlorogaloideae s.l. are color coded, as are sub-groups in *Hesperaloe*. Site codes are listed after taxon names.

*Camassia quamash* ssp. *intermedia* PS_haplotypeA joins the remainder of the *C. q. azurea*+ clade (PP = 1, JK = 83), excepting haplotypeB, which is sister to that clade. This may be due to deep coalescence, simply underscoring the close relationship among those subspecies given the low resolution within the *C. q. azurea*+ clade (e.g., Fig. 3). However, *C. q. intermedia* PS is sympatric with *C. leichtlinii* spp. *leichtlinii* PS, and the presence of individuals with intermediate traits such as floral color suggests gene flow. The DNA sequence of the partially "misplaced" *C. q. intermedia* PS_haplotypeB may have been influenced by introgression, although it remains close to the *C. q. azurea+* clade in the phylogeny. Further discussion of ITS results will focus on the unphased analyses, unless noted otherwise.

### 3.3. Phylogenetic hypotheses

Topologies are generally well resolved (Fig. 3), although some relationships require more data. Most inferences from ITS and cpDNA data are consistent. More OG sequences were available for cpDNA; we sampled five genera of the "Extended Agavaceae" clade of Bogler et al. (2006). As in that and other Family/Order-focused studies, our results strongly support this clade in the cpDNA trees (PP = 1, JK = 100; designated the "*Leucocrinum*+ clade"; Fig. 3B). Concatenated analyses also resolve this clade (PP = 1, JK = 100; Fig. 3A), with lower taxon sampling, while ITS analyses only included *Leucocrinum montanum*. Strongly inferred subclades within this group are completely consistent with Bogler et al. (2006).

The other OGs are divided among three clades: the *Hosta* clade, the *Yucca* clade, and the *Agave*+ clade (Fig. 3). The latter includes up to six of the sampled genera and is sister to the *Yucca* clade in concatenated (PP = 1, JK = 63) and cpDNA analyses (PP = 1, JK = 94), while those two clades are in a polytomy in the ITS BI tree, and *Yucca* is weakly placed as sister to a Chlorogaloideae s.l. – *Agave*+ clade (JK = 64) in the ITS MP tree. High-quality ITS sequences were not available for *Hosta*; cpDNA and concatenated analyses do not strongly resolve the placement of this genus.

Within the *Agave*+ clade, concatenated relationships are consistent with Bogler et al. (2006), although they are better supported or resolved in some cases. Relationships from ITS and cpDNA analyses are largely consistent, and the only two exceptions lack strong support in some analyses. Concatenated and ITS data resolve a *Polianthes* – *Prochnyanthes* clade (concat.: PP = 0.99, JK = 78; ITS: PP = 0.98, JK = 65; Fig. 3A), while *Polianthes geminiflora* is excluded from this clade in cpDNA analyses (PP = 0.97, JK = 62; Fig. 3B). Also, in concatenated and ITS analyses, the sampled species of *Agave* fall in a well-supported grade near the base of the *Agave*+ clade. In the cpDNA topologies, two of the *Agave* species are nested more deeply (PP = 0.97, JK = 62).

Chlorogaloideae s.s. is not inferred as monophyletic by any analysis nor supported by AU tests (Table 6). A separate SHH clade sensu Halpin and Fishbein (2013) is strongly supported (concat.: PP = 1, JK = 97; ITS: PP = 0.92, JK = 88; cpDNA: PP = 1, JK = 88), although almost 11% of the trees constrained to lack the SHH clade are accepted by AU tests. Core Chlorogaloideae has strong support (concat.: PP = 1, JK = 99; cpDNA: PP = 1, JK = 100), but *Hesperocallis* is nested within the clade by some ITS analyses (Core – *Hesperocallis* clade: PP = 0.99, JK = 92). The AU tests do not distinguish between trees in which the Core clade is monophyletic vs. has *Hesperocallis* nested, but regardless, AU tests accept fewer than 13% of trees lacking a Core – *Hesperocallis* clade. Although ITS sequences were only available for one population of *Hesperocallis undulata*, three populations were included in our final analyses of concatenated and cpDNA data and are strongly supported as monophyletic by all analyses, including AU tests. Separate analyses of the cpDNA data do not strongly resolve the relationship of *Hesperocallis* relative to Chlorogaloideae; it is placed in a weak grade/polytomy with all other major clades. Analyses of ITS strongly resolve *H. undulata* with the Core Chlorogaloideae (Core – *H. undulata*: PP = 0.99, JK = 92). *Hesperocallis* is placed sister to the *Ch. parviflorum* – *Ch. purpureum* clade by BI analyses (PP = 0.70) and sister to all of Core Chlorogaloideae by MP analyses (JK = 54). Concatenated analyses strongly resolve it as sister to the Core Chlorogaloideae clade (Core – *H. undulata*: PP = 1.0, JK = 88; Core: PP = 1.0, JK = 99). The AU tests do not significantly support or reject the monophyly of Chlorogaloideae s.l.; this group forms a polytomy or is weakly resolved either as monophyletic or not by phylogenetic analyses.

The monophyly of all tested genera in Chlorogaloideae s.l. is well supported by all phylogenies, with the exceptions of *Hastingsia*, *Hesperaloe*, and *Chlorogalum*. In addition, although the *Hesperoyucca* clade has moderate to strong support in the phylogenies, it is not completely supported by AU tests. However, only 6% of trees constrained to lack this clade were accepted by the tests. The monophyly of *Hastingsia* and *Hesperaloe* is strongly supported by concatenated analyses, but *Hastingsia* forms a polytomy in ITS BI analyses (vs. ITS MP: JK = 71; cpDNA: PP = 1, JK = 100) and *Hesperaloe* is a polytomy in cpDNA analyses (vs. ITS: PP = 0.99, JK = 90). Our AU tests rejected all but 6% of trees lacking a *Hastingsia* clade and did not significantly support the monophyly of *Hesperaloe*. *Chlorogalum* forms two (to three) very strongly-supported clades. These form a grade at the base of the *Camassia* – *Hastingsia* clade, with the branch dividing *Chlorogalum* being strongly inferred by concatenated (PP = 1, JK = 98, AU $p < 0.001$) and ITS (PP = 1, JK = 78) analyses, and more weakly by cpDNA (PP = 0.8, JK = 66).

Within the SHH clade, strong support exists for a sister relationship between *Hesperaloe* and *Schoenolirion* (concat.: PP = 1, JK = 100; ITS: PP = 1, JK = 99; cpDNA: PP = 1, JK = 99). Relationships among populations of *Schoenolirion* are fully resolved and well supported, with the strongest signal from cpDNA data. In *Hesperoyucca*, two major clades are strongly supported from concatenated analyses and strongly to weakly inferred by individual DNA regions. Our preliminary analyses included three other accessions of *H. whipplei* and one of *H. newberryi* that were later excluded due to missing data, but inferences within *Hesperoyucca* were entirely consistent with the final results.

*Hesperaloe funifera* and its subspecies are each not monophyletic. Specifically, *H. funifera* ssp. *funifera* groups with *H. campanulata* (concat.: PP = 1, JK = 81; ITS: PP = 0.99, JK = 58; cpDNA: PP = 0.98, JK = 62; Fig. 3) while *H. f. chiangii* groups with *H. nocturna* and *H. tenuifolia* (concat.: PP = 1, JK = 94; ITS: PP = 1, JK = 97; cpDNA: polytomy). All analyses place population *H. f. funifera* ARIZ319572 in a subclade of *H. campanulata*. The remaining accession of *H. f. funifera* and *H. campanulata* are sister to this subclade according to cpDNA; they form a polytomy with this and other *Hesperaloe* populations in the ITS trees. *Hesperaloe parviflora* s.l. is monophyletic in concatenated (PP = 1, JK = 99) and cpDNA trees (PP = 1, JK = 98), but largely unresolved in ITS. The single population of *H. p. bechtoldii* nests within the two populations of *H. engelmannii* based on concatenated and cpDNA analyses (concat.: PP = 0.99, JK = 63; ITS: polytomy; cpDNA: PP = 0.97, JK = 64); this lack of monophyly for *H. engelmannii* was significant according to AU tests (Table 6). Only ITS sequence data were available for *H. p. parviflora*; this accession grouped with *H. f. funifera* GB (PP = 1, JK = 100).

*Camassia* and *Hastingsia* are strongly placed as sister taxa (concat.: PP = 1, JK = 99; ITS: PP = 0.97, JK = 71; cpDNA: PP = 1, JK = 100; Fig. 3). The great majority of trees without a *Camassia* – *Hastingsia* clade were rejected by AU tests, but 3% of trees with a short branch linking *Hastingsia* and *Chlorogalum* were marginally accepted. Relationships within *Hastingsia* are largely

unresolved, whereas some clades of *Camassia* have strong support. One contrast between the two genomes involves the *C. cusickii* – *C. quamash* ssp. *utahensis* CM clade, which groups with the *C. q. breviflora*+ clade according to ITS (PP = 1, JK = 86), but with the *C. q. azurea*+ clade according to cpDNA (PP = 1, JK = 84). AU tests show no significant difference between the altering placements. Another difference, but one that is not strongly supported by MP analyses, is the placement of the *C. angusta* – *scilloides* clade. ITS data place it sister to the rest of *Camassia*, excluding *C. howellii* (PP = 0.92, JK = 56), but cpDNA data resolve it in a polytomy with the *C. q. breviflora*+ clade (PP = 1, JK = 79). Concatenated data also group it with the *C. q. breviflora*+ clade, but AU tests do not reject the alternate resolution.

**Table 6**
Clade support values (MP Jackknife/BI Posterior Probability/ML Bootstrap) and maximum AU *p*-values for trees that contain ("Pos") or contradict ("Neg") a given clade. Trees are significantly less likely than best trees (i.e., rejected) if AU *p*-values are <0.05 (shown in bold). "–" indicates a clade that is not resolved in that analysis; "x" indicates a clade that is contradicted by that analysis; "^" indicates a clade that is not relevant to that analysis due to unsampled accessions for some loci.

| Clade | Concat. JK/PP/BSt | ITS JK/PP | cpDNA JK/PP | AU *p*-values |
|---|---|---|---|---|
| ***Intergeneric*** | | | | |
| Chlorogaloideae s.l.[a] | x (SHH – OGs:<50)/0.93/– | –/– | x/x (*Hesperocallis* – OGs: 60/0.69) | Neg: 0.699, Pos: 0.999 |
| Chlorogaloideae s.l. excluding *Hesperocallis* (vs. Core – *Hesperocallis*, see below) | x/x/x | x/x | <50/0.57 | Pos: 0.184 |
| Chlorogaloideae s.s.[b] | x/x/x | x/x | x/x | **Pos: 0.018** |
| Core Chlorogaloideae – *Hesperocallis* | 88/1.00/96 | 92/0.99 | x/x (*Hesperocallis* – OGs: 60/0.69) | Neg: 0.168 |
| Core Chlorogaloideae[c] | 99/1.00/99 | 54/x (*Hesperocallis* nested) | 100/1.00 | Neg: 0.662 |
| SHH[d] | 97/1.00/97 | 88/0.92 | 88/1.00 | Neg: 0.422 |
| *Camassia* – *Hastingsia* | 99/1.00/99 | 71/0.97 | 100/1.00 | Neg: 0.066 |
| ***Generic*** | | | | |
| *Camassia* | 100/1.00/100 | 100/1.00 | 100/1.00 | **Neg: 0.011** |
| *Chlorogalum* (vs. *Chlorogalum* grade) | x/x/x (vs. 98/1.00/96) | x/x (vs. 78/1.00) | x/x (vs. 66/0.80) | **Pos:<0.001** |
| *Hastingsia* | 100/1.00/100 | 71/– | 100/1.00 | Neg: 0.193 |
| *Hesperaloe* | 95/0.96/53 | 90/0.99 | –/– | Neg: 0.985 |
| *Hesperocallis* | 100/1.00/100 | ^/^ (one *Hesperocallis* sampled) | 100/1.00 | Neg: 0.047 |
| *Hesperoyucca* | 99/1.00/99 | 78/0.85 | 94/1.00 | Neg: 0.246 |
| *Schoenolirion* | 100/1.00/100 | 92/1.00 | 100/1.00 | **Neg: 0.003** |
| ***Intrageneric*** | | | | |
| *Camassia* excluding *C. angusta, C. scilloides*, and *C. howellii* (vs. excluding *C. leichtlinii* and *C. howellii*) | x/x/x (vs. 80/1.00/84) | 56/0.92 | x/x (vs. 83/1.00) | Pos: 0.911 |
| *C. cusickii* – *C. q. breviflora*+ (vs. *C. cusickii* – *C. q. azurea*+) | x/x/x (vs. 58/0.99/66) | 86/1.00 | x/x (vs. 84/1.00) | Pos: 0.995 |
| *Camassia cusickii* (vs. *C. cusickii* HC or PC – *C. q. utahensis* CM) | x/x/x (vs. 87/1.00/91) | x/x (vs. 63/0.98) | x/x (vs. 95/1.00) | **Pos:<0.001** |
| *Hesperaloe engelmannii* (vs. *H. engelmannii* JHsn – H. *p. bechtoldii* JH24815) | x/x/x (vs. 63/0.99/61) | –/– | x/x (vs. 64/0.97) | **Pos:<0.001** |
| *Hesperaloe funifera* (vs. *H. f. funifera* ARIZ319572 – *H. campanulata*) | x/x/x (vs. 81/1.00/94) | x/x (vs. 58/0.99) | x/x (vs. 62/0.98) | **Pos:<0.001** |
| *Schoenolirion croceum* – *S. wrightii* (vs. *S. albiflorum* – *S. wrightii*) | x/x/x (vs. 99/1.00/99) | x/x (vs. 67/0.81) | x/x (vs. 98/1.00) | **Pos:<0.001** |

[a] Chlorogaloideae s.l. = *Camassia*, *Chlorogalum*, *Hastingsia*, *Schoenolirion*, *Hesperaloe*, *Hesperoyucca*, *Hesperocallis*.
[b] Chlorogaloideae s.s. = *Camassia*, *Chlorogalum*, *Hastingsia*, *Schoenolirion*.
[c] Core Chlorogaloideae = *Camassia*, *Chlorogalum*, *Hastingsia*.
[d] SHH = *Schoenolirion*, *Hesperaloe*, *Hesperoyucca*.

**Table 7**
A subset of morphological traits discussed by Sherman (1969), as applied to Chlorogaloideae s.l. Sources are Sherman (1969) and/or FNA (1993+) unless noted otherwise.

| | Rootstock | Stigma shape | Leaf shape |
|---|---|---|---|
| *Camassia* | Bulb | 3-lobed | Keeled |
| *Chlorogalum* | Bulb | 3-lobed | Keeled |
| *Hastingsia* | Bulb | 3-lobed | Keeled |
| *Schoenolirion* | Rhizome with or without bulb | Unlobed to slightly 3-lobed | Flat or rounded, not prominently keeled |
| *Hesperaloe* | Rhizome | Unlobed[a] | Curved cross-section[b] |
| *Hesperoyucca* | With or without rhizomes[c] | Unlobed[d] or slightly 3-lobed[e] | Plano-convex, subtriquetrous, or keeled on both faces[f] |
| *Hesperocallis* | Bulb | Unlobed to slightly 3-lobed | Keeled |

[a] Watson and Eaton (1871).
[b] Hochstätter (2009), JKA, pers. obs.
[c] FNA (1993+), W. Hodgson, pers. comm.
[d] FNA (1993+), Clary (2001), Trelease (1902).
[e] Watson and Eaton (1871), Engelmann (1873).
[f] Trelease (1902).

## 4. Discussion

### 4.1. Overview and concordance among analyses

Phylogenetic analyses of independent nuclear and chloroplast loci resolved many relationships in the ecologically diverse Chlorogaloideae s.l. (Fig. 3), allowing insights into taxonomic questions at the familial to infraspecific levels. Results from MP, ML, and BI analyses were generally concordant, as were those from different gene regions. Certainly caution is important when using ITS for phylogenetic inference (Alvarez and Wendel, 2003; Edger et al., 2014), yet results in this case indicate consistent phylogenetic signal reflecting evolutionary relationships. Multiple coding methodologies produced similar phylogenetic hypotheses, and results from ITS in general are consistent with patterns evident from cpDNA regions. The coding method of Potts et al. (2014) did appear useful in retaining more information from 2ISPs (intra-individual site polymorphisms) in that the resulting topologies were slightly better resolved and supported. Even so, most clades and support values were nearly identical regardless of the coding method. Although polyploidy may also complicate patterns of evolution for molecular characters, there is no indication here that the ancient polyploidy in Agavaceae (McKain et al., 2012) nor the chromosomal changes within Chlorogaloideae s.l. (Halpin and Fishbein, 2013) confounded inference of phylogenetic relationships.

### 4.2. Relationships among outgroups and placement of Chlorogaloideae s.l. within Agavaceae

This study provides the most extensive sampling to date of taxa in Chlorogaloideae s.l., and we assess their placement within Agavaceae using a diverse array of outgroups across the major clades of the family. All strongly-supported results are consistent with previous studies focused at the family or order level (e.g., Bogler et al., 2006; Chen et al., 2013; Seberg et al., 2012). The single study with results partially inconsistent with those reported here focused on Ruscaceae/Nolinoideae (Kim et al., 2010), sampling just two species of Chlorogaloideae s.l. (in *Camassia* and *Hesperocallis*) and 16 total members of Agavaceae. *Camassia* was strongly supported as sister to the remainder of Agavaceae except *Anemarrhena*, a result not upheld here or by other phylogenetic studies (e.g., Halpin and Fishbein, 2013).

Our results confirm the phylogenetic affinity of *Yucca queretaroensis* with *Yucca* rather than with *Hesperoyucca* or *Hesperaloe*, which is consistent with its stable taxonomic placement within that genus. This provides the test suggested by Pellmyr et al. (2007), verifying its placement with greater phylogenetic sampling of these other genera.

### 4.3. Monophyly of Chlorogaloideae s.s. and s.l

Chlorogaloideae s.s. (Speta, 1998) is not inferred as monophyletic by any of our analyses (Fig. 3 and Table 6). Instead, *Schoenolirion* is separated from Core Chlorogaloideae (*Camassia*, *Chlorogalum*, *Hastingsia*) and placed in a strongly-supported "SHH" clade with *Hesperaloe* and *Hesperoyucca*. Halpin and Fishbein (2013) shared these results, supporting the monophyly of Core Chlorogaloideae based on SH tests (Shimodaira and Hasegawa, 1999) of four cpDNA regions. Core Chlorogaloideae is strongly affirmed by our concatenated and cpDNA analyses, and also by ITS with the inclusion of *Hesperocallis* weakly nested or sister to it (see below). The SHH clade is strongly supported by all phylogenetic analyses but without complete support from AU tests, similar to results of Halpin and Fishbein (2013). Previous phylogenetic taxon sampling in the SHH clade had been sparse, with *Schoenolirion* included only by Halpin and Fishbein (2013), and minimal sampling from *Hesperaloe* and *Hesperoyucca* even in that study. *Schoenolirion* is strongly inferred as sister to *Hesperaloe* and the recognition of *Hesperoyucca* as a separate genus from *Yucca* is upheld by new phylogenetic evidence (see also FNA, 1993+).

*Schoenolirion* has been classified with some genera of Chlorogaloideae since Watson (1879; Table 1), but its separation from Core Chlorogaloideae is not entirely unexpected based on morphology. Sherman (1969) noted only a "weak affinity" between *Schoenolirion* and its purported cohorts of the time (*Chlorogalum*, *Hastingsia*, and *Hemiphylacus*). He hypothesized a closer relationship with *Camassia* and compared the morphology of the genera in Chlorogaloideae s.s., suggesting that *Schoenolirion* was less closely allied than the other three but without mentioning *Hesperaloe* or *Hesperoyucca* as possible relatives. *Schoenolirion* is unique in Chlorogaloideae s.s. for its unusual, vertical rhizome (vs. bulbs), entire stigma (vs. clearly three-lobed), and leaf shape that is not prominently keeled (Table 7; FNA, 1993+; Halpin and Fishbein, 2013; Sherman, 1969). In contrast, the three genera of Core Chlorogaloideae and *Hesperocallis* have bulbs (Fig. 1), while *Hesperaloe*, *Hesperoyucca* (excepting *H. newberryi*, Wendy Hodgson, pers. comm.), and much of the rest of Agavaceae are rhizomatous (Halpin and Fishbein, 2013; Stevens, 2001+). Also, all three genera in the SHH clade have largely unlobed stigmas (Clary, 2001; Engelmann, 1873; FNA, 1993+; Sherman, 1969; Trelease, 1902; Watson and Eaton, 1871). Stigmas of *Hesperoyucca* seen by JKA (herbarium specimens) and W. Hodgson (pers. comm.) appear capitate with trichomes (Fig. 1), although minute lobing is possible and may be the source of some mixed reports (Table 7). *Hesperocallis* stigmas are capitate to slightly three-lobed (FNA, 1993+). Finally, keeled leaves characterize Core Chlorogaloideae and *Hesperocallis*, while leaves from the SHH clade tend to lack keels (FNA, 1993+; Hochstätter, 2009; Sherman, 1969; Trelease, 1902; JKA, pers. obs.). In all, these morphological characters are consistent with a Core Chlorogaloideae clade (possibly joined by *Hesperocallis*) and a SHH clade (Table 7).

Although strongly placed in Agavaceae (Bogler et al., 2006; Pires et al., 2004), the specific affinities of monotypic *Hesperocallis* remained poorly supported. Our results from concatenated analyses now strongly place *H. undulata* sister to Core Chlorogaloideae, but AU tests are not conclusive regarding this resolution (Table 6). The exact position of *Hesperocallis* varies somewhat with DNA region, but these differences are not strongly supported and may be due to differing OG sampling. Halpin and Fishbein (2013) resolved a similar pattern of relationships with their analyses of four cpDNA regions, but with lower support. While the monophyly of Chlorogaloideae s.l. is not clearly supported or rejected by our data, it is clear that Chlorogaloideae s.s. is not supported by molecular phylogenetic data. Delimitation of alternate taxonomic subfamilies or other such groups would benefit from a global analysis focused at the family level.

### 4.4. Monophyly of genera of Chlorogaloideae s.l., with focus on and within Chlorogalum

The monophyly of each genus in Chlorogaloideae s.l. is strongly affirmed by all phylogenetic trees and AU tests, with the following exceptions. Despite strong support from concatenated MP and BI analyses, AU tests do not significantly support the monophyly of *Hastingsia*, *Hesperaloe*, or *Hesperoyucca*. The former two genera also form polytomies in at least one of the separate analyses, although the monophyly of *Hastingsia* is supported by the cpDNA phylogenies of Halpin (2011) and Halpin and Fishbein (2013). The only genus consistently supported as non-monophyletic is *Chlorogalum*

(Fig. 3), whose two (or three) strongly-supported clades form a grade at the base of the *Camassia* – *Hastingsia* clade. The main clades are *Ch. pomeridianum* – *angustifolium* – *grandiflorum* and *Ch. parviflorum* – *purpureum*; the species of the latter clade form a polytomy in the cpDNA tree. Halpin and Fishbein (2013) similarly resolved three groups of *Chlorogalum* using cpDNA data, although SH tests suggested that a monophyletic *Chlorogalum* was not statistically less likely (Halpin and Fishbein, 2013), in contrast to our AU test results.

Molecular phylogenetic data thus back the division of *Chlorogalum* into at least two genera, an assertion that also has morphological and cytological support. Both *Ch. parviflorum* and *Ch. purpureum* differ from other species of *Chlorogalum* based on smaller diurnal (vs. vespertine) flowers and styles longer than the perianth (Hoover, 1940). However, *Ch. pomeridianum* and *Ch. grandiflorum* can develop exserted styles (FNA, 1993+). Regardless, Hoover (1940) argued that "These differences are so correlated with some difference in general aspect that one is led to suspect that the genus as accepted is composed of two separate lines of descent." He nevertheless retained all five species in one genus because they were "so much alike morphologically and so close geographically" (p. 140). Chromosome number also distinguishes the diurnal species ($n = 30$) from the vespertine *Ch. po. pomeridianum* ($n = 18$ or 15), *Ch. po. divaricatum* and *Ch. po. minus* ($n = 18$), and *Ch. angustifolium* ($n = 17$; Cave, 1970). Chromosomes of *Ch. grandiflorum* have not been counted to our knowledge. Cave (1970) states that the $n = 15$ karyotype within *Chlorogalum* is similar to that of *Camassia* ($n = 15$), consistent with a close phylogenetic relationship and similar floral characters (FNA, 1993+). Overall, while molecular, morphological, and chromosomal data support division of *Chlorogalum*, a closer look with detailed field or morphological study is recommended to determine the classification that best reflects evolutionary relationships.

At least four taxa in *Chlorogalum* s.l. are of conservation concern due to restricted distributions (*Ch. grandiflorum*, *Ch. po. minus*, *Ch. pu. purpureum*, and *Ch. pu. reductum*). Each tested species in the genus is strongly supported as monophyletic by concatenated analyses; only one accession was available for *Ch. grandiflorum*. Hoover (1940) also noted that each species is easily distinguished, possibly in part due to allopatry of species pairs except *Ch. pomeridianum* and *Ch. angustifolium*. He detected no hybridization between these two species, although Cave (1970) observed cytological disturbances (e.g., irregular meiosis) in individuals of both species at one of several sites of sympatry. Our analyses revealed a closer relationship between *Ch. pomeridianum* var. *pomeridianum* and *Ch. po. divaricatum* compared to *Ch. po. minus*. This is consistent with morphology; *Ch. po. minus* is distinctive in lacking numerous coarse bulb fibers and is relatively small, although its short stature may reflect growth on serpentine soils (Hoover, 1964). Sometimes confused with *Ch. grandiflorum* (FNA, 1993+), *Ch. po. minus* is supported as a member of *Ch. pomeridianum* by concatenated analyses.

### 4.5. Intrageneric relationships within the SHH clade, with focus on taxonomic questions in Hesperaloe

Of three recognized species in *Hesperoyucca*, the populations of *H. newberryi* are resolved as sister to a *H. whipplei* – *H. peninsularis* clade. This is consistent with geography: *H. newberryi* is disjunct in Arizona, whereas the distributions of *H. whipplei* (California and Baja California) and *H. peninsularis* (Baja California) overlap (Fig. 2). The ITS phylogeny of Clary (2001) separates *H. whipplei* (2 populations, BSt = 73) and *H. peninsularis* (1 pop.), whereas our five populations of these two species form a polytomy. Population genetic studies may further resolve species boundaries for this genus.

In *Schoenolirion*, Sherman (1969) recognized three species, suggesting that *S. croceum* is the progenitor of derivative *S. wrightii*. Instead, our analyses strongly support a *S. albiflorum* – *wrightii* clade. Halpin and Fishbein (2013) inferred a *S. croceum* clade sister to a clade containing one population of each of the three species. Still, all these results might be consistent with Sherman's (1969) hypothesis given that *S. albiflorum* is polyploid ($n = 24?$, $2n = 49$), compared to *S. wrightii* ($n = 12$) and *S. croceum* ($n = 15$ or rarely 16; Sherman, 1969). As such, a bifurcating tree may not completely describe its relationships. Sherman (1969) proposed that *S. albiflorum* was an allopolyploid between a hypothetical now-extinct species and *S. croceum* (or its ancestor), but that remains to be tested. The current distribution of *S. albiflorum* partially overlaps with *S. croceum* but not with *S. wrightii*. However, the only two morphological traits given by Sherman (1969) to distinguish *S. croceum* and *S. wrightii* link the latter with *S. albiflorum* (white vs. yellow flowers and leaves shorter than scape vs. longer). If instead *S. wrightii* is a diploid parent of *S. albiflorum*, it would explain their close relationship in our phylogenies. Despite the small size of this genus, chromosomal evolution appears complex.

Taxonomic revisions of *Hesperaloe* provide testable hypotheses (Hochstätter, 2009; Starr, 1997; Turner and Turner, 2002). Currently, the main areas of disagreement involve whether *H. funifera* ssp. *chiangii* represents a distinct species (*H. chiangii*), whether to segregate *H. engelmannii* or treat it as a synonym of *H. parviflora*, and potential recognition of new taxa, *H. parviflora* ssp. *bechtoldii* (Hochstätter, 2009) and *H. malacophylla* (Hochstätter and Martínez-Ávalos, 2010).

The monophyly of *H. funifera* and each of its subspecies is not supported by our data (Fig. 3 and Table 6). Populations of *H. f. funifera* are intermixed in a clade with *H. campanulata* (including the type population: *H. campanulata* GSType1). The distributions of the two taxa overlap in northern Mexico (Fig. 2), where Starr (1997, pers. comm.) noted an unusual, potentially hybridizing population of *H. f. funifera* characterized by intermediate floral coloration and plant sizes. Our *H. f. funifera* ARIZ319572 is less than 20 km from that site and has flowers of similar color (Greg Starr, pers. comm.). Starr also produced hybrids between *H. f. funifera* and *H. campanulata*, as well as $F_1$ and $F_2$ hybrids of *H. f. funifera* and *H. parviflora*. In morphology, the latter hybrids resembled *H. campanulata*, raising the hypothesis of a hybrid origin for that species (G. Starr, pers. comm.). Placing hybrids in phylogenetic reconstructions is not straightforward (McDade, 1992), and population genetic or morphological work is needed. Regardless, either hybridization or deep coalescence may explain the phylogenetic intermixing of *H. campanulata* and *H. f. funifera*, suggesting incomplete genetic segregation of these taxa.

Our results separate *H. chiangii* from *H. funifera*. The ITS and concatenated analyses strongly support a clade with two accessions of *H. f. chiangii* (including JH23741 from the type locality) and a *H. nocturna* – *H. tenuifolia* subclade. Since the *H. campanulata* – *H. f. funifera* clade is strongly inferred by cpDNA and concatenated analyses, all analyses divide *H. funifera*. However, given the complex relationships in this group, further study of genetic and morphological patterns in multiple populations is needed prior to making taxonomic decisions. The distribution of *H. f. chiangii* is disjunct from *H. f. funifera* but also from *H. nocturna* and *H. tenuifolia* (Hochstätter, 2009; Fig. 2).

*Hesperaloe parviflora* s.l. includes *H. p.* ssp. *parviflora*, *H. p.* ssp. *bechtoldii*, and putative segregate *H. engelmannii*. The population of *H. p. parviflora* (represented by a single available ITS sequence) groups strongly with population *H. f. funifera* GB. Our population of *H. p. bechtoldii* is nested within two populations of *H. engelmannii* based on concatenated and cpDNA analyses with moderate to high support (Fig. 3). While this raises questions about the potential recognition of *H. engelmannii* as a species, not all sister species

are reciprocally monophyletic (e.g., Luckow, 1995). In fact, all progenitor-derivative species pairs are expected to display a paraphyletic phylogenetic pattern, at least initially (Crawford, 2010; Rieseberg and Brouillet, 1994).

Turner and Turner (2002) noted that most taxonomists had not recognized *H. engelmannii*, although Trelease (1902) treated it as a variety of *H. parviflora*. Hochstätter (2009) followed Turner and Turner (2002) in accepting *H. engelmannii*, in contrast to G. Starr (pers. comm.) and James Henrickson (pers. comm.), who have also worked extensively with this genus (e.g., Starr, 1997). *Hesperaloe engelmannii* was originally distinguished from *H. parviflora* based on a shorter, thicker style and longer anthers (Fig. 1; Baker, 1880; Krauskopf, 1878; Starr, 1997). Turner and Turner (2002) confirmed floral differences, stating that *H. engelmannii* styles are "mostly 1–2(3) times as long as the ovary" (p. 41) in contrast to 3–5 times for *H. parviflora*. They further stated that individuals of *H. engelmannii* are larger, with longer and darker leaves, but also noted significant variation in both putative species. This is corroborated by a detailed study revealing carpel lengths that varied as much as 44% in one population of *H. parviflora* s.s. (Pellmyr and Augenstein, 1997; identified as *H. p. bechtoldii*, J. Henrickson, pers. comm.). James Henrickson (pers. comm.) also noted extensive variation in *H. parviflora* s.l. and discovered that individuals transplanted from a highly exposed population of *H. parviflora* s.s. to a shaded habitat eventually produced much longer leaves, in the range expected for *H. engelmannii*. Our work confirms the close relationship between *H. engelmannii* and *H. p. bechtoldii*, but further studies are needed. In particular, the separate ITS placement of *H. p. parviflora* s.s. should be tested with other populations and loci.

Natural or artificial hybrids have been observed between multiple species of *Hesperaloe*, including *H. nocturna* and *H. parviflora*, *H. parviflora* and *H. funifera*, *H. campanulata* and *H. funifera*, *H. campanulata* and *H. parviflora* x *H. funifera*, and *H. parviflora* and *H. engelmannii* (Hochstätter, 2009; Starr, 1997). One factor that may provide partial reproductive isolation among some species is flowering time. This genus includes day flowering species (*H. parviflora* s.l.), night flowering species (*H. funifera*, *H. nocturna*, and *H. tenuifolia*), as well as *H. campanulata*, whose flowers are visited by bats and hawkmoths at night but partially close into floral tubes during the day that are visited by hummingbirds (Starr, 1997; J. Henrickson, pers. comm; G. Starr, pers. comm.).

### 4.6. Phylogenetic framework for Camassia and Hastingsia

The sister relationship of *Camassia* and *Hastingsia* is strongly supported in all phylogenies. Species delimitation within *Hastingsia* has been contentious in prior and current taxonomies (Becking, 1986; FNA, 1993+; Lang and Zika, 1997; Theiss, in press). None of our analyses strongly support intrageneric resolution. This suggests a rapid radiation, also supported by lack of resolution and intermixing of species in a chloroplast (*rpl16*, *trnD–T*, *psbJ–petA*) phylogeny focused on *Hastingsia* (Halpin, 2011).

In contrast, major clades in *Camassia* are resolved. For a cpDNA phylogeny of *Camassia*, Fishbein et al. (2010) analyzed *rpl16* and *trnD–trnT*. Thirty-seven of our sampled populations overlap with theirs, and we additionally conducted a 5-region concatenated analysis with those accessions. Results (not shown) are entirely concordant with those in Fig. 3; differences are simply changes in support values, which almost always increased. In Fishbein et al. (2010), *C. leichtlinii* and *C. howellii* formed a very weakly supported clade (the "L clade"; PP = 0.51, BSt < 50) sister to the remainder of *Camassia* (the "Q clade"; PP = 1, BSt = 72). Both clades are contradicted in our ITS trees because *C. angusta* – *scilloides* is sister to the rest of the Q clade and *C. leichtlinii* (with weak to moderate support, Fig. 3C). Our concatenated and cpDNA results confirm a strongly-supported Q clade, but infer that *C. leichtlinii* and *C. howellii* form a strongly-supported grade rather than the L clade (Fig. 3A). Consistent in all of our analyses, *C. howellii* is sister to the rest of *Camassia*. Both *C. leichtlinii* and *C. howellii* share the rare presence of branches in their racemes, a trait otherwise absent in *Camassia*, but found in *Chlorogalum* and *Hastingsia* (Gould, 1942).

Subspecies of *C. quamash* occurring west and east of the Cascade Mountains fall in separate clades (i.e., the *C. q. azurea*+ and *C. q. breviflora*+ clades, respectively; Fig. 3), consistent with prior genetic and phylogenetic studies (Fishbein et al., 2010; Tomimatsu et al., 2009). *Camassia cusickii* has a very narrow range in northeastern Oregon and adjacent Idaho, overlapping with the eastern group of *C. quamash* (*C. q. breviflora*, *C. q. quamash*, and *C. q. utahensis*). The same two populations of *C. cusickii* as in Fishbein et al. (2010) were again not monophyletic, a result now supported by AU tests and both nuclear and chloroplast data, although the placement of *C. cusickii* differs in the two trees. ITS data strongly place *C. cusickii* with other taxa distributed east of the Cascades (Fig. 3C), whereas our and Fishbein et al. (2010)'s cpDNA analyses clearly group it with the western subspecies of *C. quamash*, along with geographically-proximate population(s) of *C. q. utahensis* that remain sister to a population of *C. cusickii* (Fig. 3A). Recent fieldwork and specimen vouchers imply that *C. q. utahensis* and *C. cusickii* may grow within dispersal range, with potential for intermixing.

Midwestern *C. angusta* and *C. scilloides* form a strongly supported clade in our concatenated and ITS trees, as in the cpDNA tree of Fishbein et al. (2010). Our cpDNA data also inferred a nested *C. angusta* OTT – *scilloides* BAR subclade, disrupting the monophyly of both species. Although hybridization could cause this pattern, it is unlikely for these populations, with one occurring in Indiana and the other in Kansas. Regardless, close genetic ties characterize these species.

Overall, our results are consistent with many aspects of prior taxonomic and phylogenetic studies of *Camassia* (Gould, 1942; Fishbein et al., 2010; FNA, 1993+; Kephart, in press), despite the complexity of some taxonomic boundaries. Many questions remain regarding diversification within this clade. Detailed phylogenetic study of *Camassia* and *Hastingsia* is underway and further discussion of intrageneric relationships are reserved for separate papers. Combining those results with morphological, ecological, and reproductive isolation studies will allow a more comprehensive understanding of the patterns and processes of speciation in the *Camassia* – *Hastingsia* clade.

## 5. Conclusion

Within Chlorogaloideae s.l., our results demonstrate lack of monophyly at the subfamily, genus, species, and infraspecific levels – while suggesting stability of the Core Chlorogaloideae and SHH clades as well as many of their genera. Complex patterns of evolution for many of these groups were revealed by these data. A detailed investigation of forces behind diversification in Chlorogaloideae s.l. is outside the scope of this study, but it appears that a variety of factors may have been involved, including allopatry, hybrid speciation, divergence in floral traits and pollinator interactions, and chromosomal changes. While it is difficult to say whether some of the differences emerged before or after speciation of the relevant taxa (Templeton, 1982), regardless they may be important in maintaining isolation and allowing further divergence.

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