Evaluating the Monophyly and Biogeography of *Cryptantha* (Boraginaceae)

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Abstract—*Cryptantha*, an herbaceous plant genus of the Boraginaceae, subtribe Amsinckiinae, has an American amphitropical disjunct distribution, found in western North America and western South America, but not in the intervening tropics. In a previous study, *Cryptantha* was found to be polyphyletic and was split into five genera, including a weakly supported, potentially non-monophyletic *Cryptantha* s. s. In this and subsequent studies of the Amsinckiinae, interrelationships within *Cryptantha* were generally not strongly supported and sample size was generally low. Here we analyze a greatly increased sampling of *Cryptantha* taxa using high-throughput, genome skimming data, in which we obtained the complete ribosomal cistron, the nearly complete chloroplast genome, and twenty-three mitochondrial genes. Our analyses have allowed for inference of clades within this complex with strong support. The occurrence of a non-monophyletic *Cryptantha* is confirmed, with three major clades obtained, termed here the *Johnstonella/Albidae* clade, the *Maritimae* clade, and a large *Cryptantha* core clade, each strongly supported as monophyletic. From these phylogenomic analyses, we assess the classification, character evolution, and phylogeographic history that elucidates the current amphitropical distribution of the group. Revealing the timing, direction, and number of times of dispersal between North and South America gives insight as to the origin of the great biodiversity of these regions.

Keywords—Amphitropical distribution, Amsinckiinae, *Johnstonella*.

The Boraginaceae, the forget-me-not family, has been the focus of many recent phylogenetic studies (Långström and Chase 2002; Hasenstab-Lehman and Simpson 2012; Nazaire and Hufford 2012; Weigend et al. 2013; Cohen 2014; Otero et al. 2014; Chacón et al. 2016). This family of herbs, shrubs, and trees has been subject to differing circumscriptions over the years, being classified as one large family (Boraginaceae s. l., in the broad sense, e.g. APGIV 2016), with up to five subfamilies (Mabberley 2008), or treated more narrowly (Boraginaceae s. s., in the strict sense), with the subfamilies largely elevated to family status (e.g. Weigend et al. 2013; Cohen 2014; Luebert et al. 2016). In this study, we elect to treat the Boraginaceae as the latter, strict sense (s. s.), and our use of the name Boraginaceae is with this circumscription for the remainder of this paper.

From these recent phylogenetic analyses (Hasenstab-Lehman and Simpson 2012; Nazaire and Hufford 2012; Weigend et al. 2013; Cohen 2014, 2015; Otero et al. 2014; Chacón et al. 2016), the genus *Cryptantha* Lehmann ex G.Don has been consistently recovered to be part of a strongly supported clade containing the genera *Adelinia, Amsinckia, Andersonglossum, Cryptantha, Dasynotus, Eremocarya, Greeneocharis, Harpagonella, Johnstonella, Oncaglossum, Oreocarya, Pectocarya,* and *Plagiobothrys*, although not all of these genera were recognized in all studies. The clade containing *Cryptantha* and close relatives is classified in subfamily Cynoglossoideae Weigend, tribe Cynoglosseae W.D.J. Koch, and subtribe Amsinckiinae Brand (sensu Chacón et al. 2016). Thus, subtribe Amsinckiinae, the first available name for this group, is used here to designate this clade.

Studies assessing interrelationships within *Cryptantha* have used only morphological characteristics and phenetic assessments, such the classification of 15 series in the North American *Cryptantha* (Johnston 1925). These series were circumscribed based on nutlet number per fruit (1–4), nutlet sculpturing (generally smooth or "rough," the latter "granular" or "tuberculate"), and, if more than one nutlet, whether the nutlets are similar (homomorphic) or different (heteromorphic) in size and/or sculpturing. Johnston (1924) had alluded to the fact that the species previously classified in the genus *Oreocarya*, all of which are perennials, should be recognized in *Cryptantha*. This was accepted by Payson (1927), who erected *Cryptantha* section *Oreocarya*. Johnston (1927) later

studied the South American Boraginaceae, including the genus *Cryptantha*. In this work, he proposed three sections of the genus *Cryptantha*: *C.* sect. *Eucryptantha* (with four series), *C.* sect. *Geocarya* (with five series), and *C.* sect. *Krynitzkia* (with five series). (See Table 1 for an updated list of Johnston's sections and series, including additions from Johnston 1937, 1939, and the Payson (1927) treatment of *Cryptantha* section *Oreocarya*). *Cryptantha* section *Krynitzkia* is distinguished in having only chasmogamous (also termed "chasmogamic") flowers, which open to expose the sexual organs of the plant, potentially allowing for cross pollination. This section comprises all North American (currently 59 species), and most (24 of 46) South American *Cryptantha* species. Two species, *Cryptantha albida* (Kunth) I.M.Johnst. and *C. maritima* (Greene) Greene, are found in both North and South America. Members of the other two sections, in addition to forming typical chasmogamous flowers in the upper parts of the plant, develop cleistogamous (also termed "cleistogamic") flowers, in which the perianth does not open and the flower is self-pollinated. Members of *Cryptantha* section *Eucryptantha*, comprising ten species restricted to South America, bear cleistogamous flowers in leaf axils of the middle part of the plant and in the extreme lower portion of the upper inflorescence units; these cleistogamous flowers form fruits similar in morphology to those of the extreme upper chasmogamous ones. In *Cryptantha* section *Geocarya*, consisting of 12 species also restricted to South America, cleistogamous flowers similar to those of *C.* sect. *Eucryptantha* are produced. However, all members of *C.* sect. *Geocarya* develop more specialized cleistogamous flowers at the extreme base of the plant, these termed "cleistogenes" (Grau 1983). The fruits of these cleistogenes in *C.* sect. *Geocarya* are different morphologically, being typically larger, reduced in number, and having a different sculpturing pattern from either the chasmogamous or cleistogamous flowers above (Johnston 1927; Grau 1983).

Brand (1931) provided a somewhat different classification of *Cryptantha*, dividing the genus into two subgenera: *Cryptantha* subgenus *Archaeocryptantha*, inclusive of both of Johnston's (1927) *C.* sections *Eucryptantha* and *Geocarya*, and *C.* subgenus *Krynitzkia*, equivalent to Johnston's (1925, 1927) *C.* section *Krynitzkia*. Brand further divided *C.* subgenus *Krynitzkia* into three sections: *C.* sect. *Cryptokrynitzkia*, *C.* sect. *Microkrynitzkia*,

TABLE 1. Johnston's (1925, 1927, 1937, 1939, 1961) classification of *Cryptantha*, supplemented by Payson (1927, for section *Oreocarya*), Grau (1981), and Simpson and Rebman (2013), showing sections and series. Reference indicated for those taxa not classified in Johnston 1925 or 1927. Current genus is placement sensu Hasenstab-Lehman and Simpson 2012. Bold = Taxa sequenced in this study. * = Type species for *Cryptantha*. For distribution (Distr.), NA = North America; SA = South America.

Classification	Species/Infraspecies	Distr.	Current genus
Cryptantha* section *Eucryptantha			
Unplaced to Series	**C. aspera (Philippi) Grau**	SA	*Cryptantha s. s.*
	C. latefissa R.L.Pérez-Mor.	SA	*Cryptantha s. s.*
Series *Capituliflorae*	**C. capituliflora (Clos) Reiche**	SA	*Cryptantha s. s.*
	C. longifolia (Philippi) Reiche	SA	*Cryptantha s. s.*
	C. spathulata (Philippi) Reiche	SA	*Cryptantha s. s.*
Series *Glomeratae*	**C. alfalfalis (Philippi) I.M.Johnst.**	SA	*Cryptantha s. s.*
	C. glomerata Lehmann ex G. Don*	SA	*Cryptantha s. s.*
Series *Glomeruliferae*	**C. glomerulifera (Philippi) I.M.Johnst.**	SA	*Cryptantha s. s.*
Series *Haplostachyae*	**C. calycotricha I.M.Johnst.**	SA	*Cryptantha s. s.*
	C. haplostachya (Philippi) I.M.Johnst.	SA	*Cryptantha s. s.*
Cryptantha* section *Geocarya			
Unplaced to Series	C. chispae Grau	SA	*Cryptantha s. s.*
	C. marticorenae Grau	SA	*Cryptantha s. s.*
Series *Alyssoides*	**C. alyssoides (A.DC.) Reiche**	SA	*Cryptantha s. s.*
Series *Dimorphae*	**C. cynoglossoides (Philippi) I.M.Johnst.**	SA	*Cryptantha s. s.*
	[= *Cryptantha* section *Eucryptantha*, sensu Grau (1981)]		
	C. dimorpha (Philippi) Greene	SA	*Cryptantha s. s.*
	[= *Cryptantha* section *Eucryptantha*, sensu Grau (1981)]		
	C. involucrata (Philippi) Reiche	SA	*Cryptantha s. s.*
	C. volckmannii (Philippi) I.M.Johnst.	SA	*Cryptantha s. s.*
Series *Dolichophyllae*	C. dolichophylla (Philippi) Reiche	SA	*Cryptantha s. s.*
	C. gayi I.M.Johnst.	SA	*Cryptantha s. s.*
Series *Lineares*	C. aprica (Philippi) Reiche	SA	*Cryptantha s. s.*
	C. linearis (Colla) Greene	SA	*Cryptantha s. s.*
Series *Virentes*	**C. kingii (Philippi) Reiche**	SA	*Cryptantha s. s.*
Cryptantha* section *Krynitzkia			
Unplaced to Series	C. papillosa R.L.Pérez-Mor.	SA	*Cryptantha s. s.*
Series *Affines*	**C. affinis (A. Gray) Greene**	NA	*Cryptantha s. s.*
	C. glomeriflora	NA	*Cryptantha s. s.*
Series *Albidae*	**C. albida (Kunth) I.M.Johnst.**	NA&SA	*Cryptantha s. s.*
	(only NA sample sequenced in this study)		
	C. mexicana I.M.Johnst.	NA	*Cryptantha s. s.*
Series *Ambiguae*	**C. ambigua (A.Gray) Greene**	NA	*Cryptantha s. s.*
	C. crinita Greene	NA	*Cryptantha s. s.*
	C. echinella Greene	NA	*Cryptantha s. s.*
	C. excavata Brandegee	NA	*Cryptantha s. s.*
	C. hendersonii (A.Nelson) J.C.Nelson	NA	*Cryptantha s. s.*
	[=C. intermedia var. h. (A.Nelson) Jepson & Hoover]		
	C. incana Greene	NA	*Cryptantha s. s.*
	[C. hendersonii in Johnston (1925)]		
	C. mariposae I.M.Johnst.	NA	*Cryptantha s. s.*
	C. simulans Greene	NA	*Cryptantha s. s.*
	C. torreyana (A.Gray) Greene	NA	*Cryptantha s. s.*
	C. traskiae I.M.Johnst.	NA	*Cryptantha s. s.*
Series *Angustifoliae*	C. angelica I.M.Johnst.	NA	*Johnstonella*
	C. angustifolia (Torrey) Greene	NA	*Johnstonella*
	C. costata Brandegee	NA	*Johnstonella*
	C. diplotricha (Philippi) Reiche	SA	*Johnstonella*
	C. fastigiata I.M.Johnst.	NA	*Johnstonella*
	C. grayi (Vasey & Rose) J.F.Macbride	NA	*Johnstonella*
	C. holoptera (A.Gray) J.F.Macbride	NA	*Johnstonella*
	C. inaequata I.M.Johnst.	NA	*Johnstonella*
	C. lepida (A.Gray) Greene	NA	*Eremocarya*
	C. micrantha (Torrey) I.M.Johnst.	NA	*Eremocarya*
	var. micrantha	NA	*Eremocarya*
	var. pseudolepida M.G.Simpson, et al.	NA	*Eremocarya*
	C. parviflora (Philippi) Reiche	SA	*Johnstonella*
	C. pusilla (Torrey & A.Gray) Greene	NA	*Johnstonella*
	C. racemosa (A.Gray) Greene	NA	*Johnstonella*
Series *Barbigerae*	C. argentea I.M.Johnst.	SA	*Cryptantha s. s.*
	C. barbigera	NA	*Cryptantha s. s.*
	var. barbigera	NA	*Cryptantha s. s.*
	var. fergusoniae J.F.Macbride	NA	*Cryptantha s. s.*
	C. calycina (Philippi) Reiche	SA	*Cryptantha s. s.*
	C. chaetocalyx (Philippi) I.M.Johnst.	SA	*Cryptantha s. s.*
	C. corollata (I.M. Johnston) I.M.Johnst.	NA	*Cryptantha s. s.*

(Continued)

TABLE 1. (CONTINUED).

Classification	Species/Infraspecies	Distr.	Current genus
	C. decipiens **(M. E. Jones) A.Heller**	NA	*Cryptantha s. s.*
	C. diffusa **(Philippi) I.M.Johnst.**	SA	*Cryptantha s. s.*
	[incl. *C. debilis* (Philippi) Reiche]		
	C. filaginea (Philippi) Reiche	SA	*Cryptantha s. s.*
	C. filiformis (Philippi) Reiche	SA	*Cryptantha s. s.*
	C. foliosa (Greene) Greene	NA	*Cryptantha s. s.*
	C. globulifera **(Clos) Reiche**	SA	*Cryptantha s. s.*
	C. granulosa (Ruiz & Pav.) I.M.Johnst.	SA	*Cryptantha s. s.*
	C. grandiflora Rydberg	NA	*Cryptantha s. s.*
	[*C. intermedia* var. *grandiflora* in Johnston 1925]		
	C. intermedia **(A. Gray) Greene**	NA	*Cryptantha s. s.*
	var. *hendersonii* (A.Nelson) Jepson & Hoover	NA	*Cryptantha s. s.*
	var. *intermedia*	NA	*Cryptantha s. s.*
	var. *johnstonii* J.F.Macbride	NA	*Cryptantha s. s.*
	C. juniperensis **R.B.Kelley & M.G.Simpson**	NA	*Cryptantha s. s.*
	[*C. nevadensis* var. *rigida* I.M.Johnst.]		
	C. limensis (A.DC.) I.M.Johnst.	SA	*Cryptantha s. s.*
	C. nevadensis **A.Nelson & P.B.Kennedy**	NA	*Cryptantha s. s.*
	C. patagonica (Speg.) I.M.Johnst.	SA	*Cryptantha s. s.*
	C. patula Greene	NA	*Cryptantha s. s.*
	C. peruviana **I.M.Johnst.**	SA	*Cryptantha s. s.*
	C. romanii I.M.Johnst.	SA	*Cryptantha s. s.*
	C. scoparia **A.Nelson**	NA	*Cryptantha s. s.*
	C. subamplexicaulis **(Philippi) Reiche**	SA	*Cryptantha s. s.*
	C. taltalensis I.M.Johnst.	SA	*Cryptantha s. s.*
	C. werdermanniana I.M.Johnst.	SA	*Cryptantha s. s.*
Series *Circumscissae*	*C. circumscissa* (Hooker & Arnott) Rydberg	NA&SA	*Greeneocharis*
	var. *circumscissa* (only NA specimen sequenced)	NA&SA	*Greeneocharis*
	var. *rosulata*	NA	*Greeneocharis*
	C. similis **K.Mathew & P.H.Raven**	NA	*Greeneocharis*
Series *Flaccidae*	*C. flaccida* **(Douglas ex Lehmann) Greene**	NA	*Cryptantha s. s.*
	C. rostellata (Greene) Greene	NA	*Cryptantha s. s.*
	C. sparsiflora **(Greene) Greene**	NA	*Cryptantha s. s.*
Series *Gnaphalioides*	*C. gnaphalioides* **(A.DC.) Reiche**	SA	*Cryptantha s. s.*
	C. marioricardiana Teillier	SA	*Cryptantha s. s.*
Series *Graciles*	*C. gracilis* **Osterhout**	NA	*Cryptantha s. s.*
Series *Leiocarpae*	*C. clevelandii* **Greene**	NA	*Cryptantha s. s.*
	[*C. abramsii, C. brandegei*]		
	C. ganderi **I.M.Johnst.** (Johnston 1939)	NA	*Cryptantha s. s.*
	C. hispidissima **Greene**	NA	*Cryptantha s. s.*
	[*C. clevelandii* Greene var. *florosa* I.M.Johnst.]		
	C. leiocarpa **(Fischer & C.A.Meyer) Greene**	NA	*Cryptantha s. s.*
	C. microstachys **(A.Gray) Greene**	NA	*Cryptantha s. s.*
	C. nemaclada **Greene**	NA	*Cryptantha s. s.*
	C. wigginsii **I.M.Johnst.**	NA	*Cryptantha s. s.*
Series *Maritimae*	*C. dumetorum* **(A.Gray) Greene**	NA	*Cryptantha s. s.*
	C. echinosepala J.F.Macbride	NA	*Johnstonella*
	C. maritima **(Greene) Greene**	NA&SA	*Cryptantha s. s.*
	var. *cedrosensis* (Greene) I.M.Johnst.	NA	*Cryptantha s. s.*
	var. *maritima*	NA	*Cryptantha s. s.*
	var. *pilosa* I.M.Johnst.	NA&SA	*Cryptantha s. s.*
	C. micromeres (A.Gray) Greene	NA	*Johnstonella*
	C. recurvata **Coville**	NA	*Cryptantha s. s.*
Series *Mohavenses*	*C. mohavensis* **(Greene) Greene**	NA	*Cryptantha s. s.*
	C. watsonii **(A.Gray) Greene**	NA	*Cryptantha s. s.*
Series *Muricatae*	*C. clokeyi* **I.M.Johnst.**	NA	*Cryptantha s. s.*
	C. muricata **(Hooker & Arnott) A.Nelson & J.F.Macbride**	NA	*Cryptantha s. s.*
	var. *denticulata* (Greene) I.M.Johnst.	NA	*Cryptantha s. s.*
	var. *jonesii* (A.Gray) I.M.Johnst.	NA	*Cryptantha s. s.*
	var. *muricata*	NA	*Cryptantha s. s.*
	C. martirensis **M.G.Simpson & Rebman**	NA	*Cryptantha s. s.*
Series *Phaceloides*	*C. dichita* (Philippi) I.M.Johnst.	SA	*Cryptantha s. s.*
	C. hispida **(Philippi) Reiche**	SA	*Cryptantha s. s.*
	C. phaceloides **(Clos) Reiche**	SA	*Cryptantha s. s.*
Series *Pterocaryae*	*C. oxygona* **(A.Gray) Greene**	NA	*Cryptantha s. s.*
	C. pterocarya **(Torrey) Greene**	NA	*Cryptantha s. s.*
	var. *pterocarya*, f. *pterocarya*	NA	*Cryptantha s. s.*
	var. *purpusii* Jepson	NA	*Cryptantha s. s.*
	var. *stenoloba* I.M.Johnst.	NA	*Cryptantha s. s.*
	C. utahensis **(A.Gray) Greene**	NA	*Cryptantha s. s.*

(Continued)

TABLE 1. (CONTINUED).

Classification	Species/Infraspecies	Distr.	Current genus
Series *Ramulosissimae*	**C. fendleri (A.Gray) Greene**	NA	*Cryptantha s. s.*
Series *Texanae*	**C. crassisepala (Torrey & A.Gray) Greene**	NA	*Cryptantha s. s.*
	C. kelseyana Greene	NA	*Cryptantha s. s.*
	C. mendocina I.M. Johnston	SA	*Cryptantha s. s.*
	C. minima Rydberg	NA	*Cryptantha s. s.*
	C. pattersonii Greene	NA	*Cryptantha s. s.*
	C. texana Greene	NA	*Cryptantha s. s.*
Cryptantha **section** *Oreocarya* (Payson 1927)			
	C. abata I.M.Johnst.	NA	*Oreocarya*
	C. aperta (Eastwood) Payson	NA	*Oreocarya*
	C. atwoodii L.C.Higgins	NA	*Oreocarya*
	C. bakeri (Greene) Payson	NA	*Oreocarya*
	C. barnebyi I.M.Johnst.	NA	*Oreocarya*
	C. breviflora (Osterhout) Payson	NA	*Oreocarya*
	C. caespitosa (A.Nelson) Payson	NA	*Oreocarya*
	C. cana (A.Nelson) Payson	NA	*Oreocarya*
	C. capitata (Eastwood) I.M.Johnst.	NA	*Oreocarya*
	C. celosioides (Eastwood) Payson	NA	*Oreocarya*
	C. cinerea (Greene) Cronquist	NA	*Oreocarya*
	var. abortiva (Greene) Cronquist	NA	*Oreocarya*
	var. arenicola L.C.Higgins & S.L.Welsh	NA	*Oreocarya*
	var. cinerea (Greene) Cronquist	NA	*Oreocarya*
	var. laxa (MacBride) L.C.Higgins	NA	*Oreocarya*
	var. pustulosa (Rydberg) L.C.Higgins	NA	*Oreocarya*
	C. compacta L.C.Higgins	NA	*Oreocarya*
	C. confertiflora (Greene) Payson	NA	*Oreocarya*
	C. crassipes I.M.Johnst.	NA	*Oreocarya*
	C. creutzfeldtii S.L.Welsh	NA	*Oreocarya*
	C. crymophila I.M.Johnst.	NA	*Oreocarya*
	C. elata (Eastwood) Payson	NA	*Oreocarya*
	C. flava (A.Nelson) Payson	NA	*Oreocarya*
	C. flavoculata (A.Nelson) Payson	NA	*Oreocarya*
	C. fulvocanescens (S.Wats.) Payson	NA	*Oreocarya*
	var. nitida (Greene) R.C.Sivinski	NA	*Oreocarya*
	C. grahamii I.M.Johnst.	NA	*Oreocarya*
	C. gypsophila Reveal & C.R.Broome	NA	*Oreocarya*
	C. hoffmannii I.M.Johnst.	NA	*Oreocarya*
	C. humilis (A.Gray) Payson	NA	*Oreocarya*
	var. nana (Eastwood) L.C.Higgins	NA	*Oreocarya*
	C. hypsophila I.M.Johnston	NA	*Oreocarya*
	C. insolita (J.F.Macbride) Payson	NA	*Oreocarya*
	C. interrupta (Greene) Payson	NA	*Oreocarya*
	C. johnstonii L.C. Higgins	NA	*Oreocarya*
	C. jonesiana (Payson) Payson	NA	*Oreocarya*
	C. leucophaea (Douglas) Payson	NA	*Oreocarya*
	C. longiflora (A.Nelson) Payson	NA	*Oreocarya*
	C. mensana (M.E.Jones) Payson	NA	*Oreocarya*
	C. nubigena (Greene) Payson	NA	*Oreocarya*
	C. oblata (M.E.Jones) Payson	NA	*Oreocarya*
	C. ochroleuca L.C.Higgins	NA	*Oreocarya*
	C. osterhoutii (Payson) Payson	NA	*Oreocarya*
	C. palmeri (A.Gray) Payson	NA	*Oreocarya*
	C. paradoxa (A.Nelson) Payson	NA	*Oreocarya*
	C. paysonii (J.F.Macbride) I.M.Johnst.	NA	*Oreocarya*
	C. propria (A.Nelson & J.F Macbride) Payson	NA	*Oreocarya*
	C. rollinsii I.M.Johnst.	NA	*Oreocarya*
	C. roosiorum Munz	NA	*Oreocarya*
	C. rugulosa (Payson) Payson	NA	*Oreocarya*
	C. salmonensis (A.Nelson & J.F.Macbride) Payson	NA	*Oreocarya*
	C. schoolcraftii Tiehm	NA	*Oreocarya*
	C. semiglabra Barneby	NA	*Oreocarya*
	C. sericea (A.Gray) Payson	NA	*Oreocarya*
	C. setosissima (A.Gray) Payson	NA	*Oreocarya*
	C. shackletteana L.C.Higgins	NA	*Oreocarya*
	C. sobolifera Payson	NA	*Oreocarya*
	C. spiculifera (Piper) Payson	NA	*Oreocarya*
	C. stricta (Osterh.) Payson	NA	*Oreocarya*
	C. subcapitata Dorn & Lichvar	NA	*Oreocarya*
	C. subretusa I.M.Johnst.	NA	*Oreocarya*
	C. tenuis (Eastwood) Payson	NA	*Oreocarya*

(Continued)

TABLE 1. (CONTINUED).

Classification	Species/Infraspecies	Distr.	Current genus
	C. thompsonii I.M.Johnst.	NA	*Oreocarya*
	C. thrysiflora (Greene) Payson	NA	*Oreocarya*
	C. tumulosa (Payson) Payson	NA	*Oreocarya*
	C. virgata (Porter) Payson	NA	*Oreocarya*
	C. virginensis (M.E.Jones) Payson	NA	*Oreocarya*
	C. weberi I.M.Johnst.	NA	*Oreocarya*
	C. welshii K.H.Thorne & L.C.Higgins	NA	*Oreocarya*
	C. wetherillii (Eastwood) Payson		

and *C.* sect. *Eukrynitzkia*, the latter further divided into four subsections: *C.* subsect. *Kraterokrynitzkia* (plants perennial), *C.* subsect. *Leiocarpum*, *C.* subsect. *Pterygium*, and *C.* subsect. *Trachycaryum*.

In a recent molecular phylogenetic study of this complex, Hasenstab-Lehman and Simpson (2012), using one chloroplast and one nuclear marker, recovered *Cryptantha* as polyphyletic and split it into five genera, the four resurrected genera *Eremocarya*, *Greeneocharis*, *Johnstonella*, and *Oreocarya*, plus a newly delimited and reduced *Cryptantha* s. s., a classification preliminarily accepted here. In the study of Hasenstab-Lehman and Simpson (2012), *Cryptantha* s. s. was split into two groups (*Cryptantha* s. s. 1 and *Cryptantha* s. s. 2), which were united as a single clade (but with weak support) in their parsimony analysis, but separated relative to other Amsinckiinae (again with weak support) in their maximum likelihood and Bayesian trees. Moreover, in all recent studies of the Amsinckiinae, interrelationships of species within both clades of *Cryptantha* have been generally poorly resolved (Hasenstab-Lehman and Simpson 2012; Weigend et al. 2013; Cohen 2014, 2015; Otero et al. 2014; Chacón et al. 2016).

The distribution of *Cryptantha* species, restricted to the nontropical regions of western North America and western South America, is mirrored in several other plant groups. The cause of this "amphitropical" (or "amphitropic") distribution has long been debated by researchers (Raven 1963; Moore et al. 2006; Wen and Ickert-Bond 2009; Simpson et al. 2017b); possible explanations include both vicariance and long-distance dispersal. The most recent accepted explanation for amphitropical distribution is via long-distance dispersal by migratory birds (Raven 1963; Moore et al. 2006). Hasenstab-Lehman and Simpson (2012) found that the distribution of the Amsinckiinae is best explained by several unidirectional dispersal events from North to South America. However, they had a limited sample size of South American taxa and recovered one incident of possible dispersal from South to North America in their *Cryptantha* s. s. 1 clade.

To better assess the phylogenetic history of *Cryptantha* species, a larger sample size and considerably more sequence data are necessary. High-throughput sequencing allows for the acquisition of millions of base pairs. Genome skimming, also called shallow sequencing, can be used for obtaining near complete sequences of high copy regions, such as the chloroplast (cpDNA), mitochondria (mtDNA), and the ribosomal cistron (nrDNA) (Straub et al. 2011, 2012). This method of sampling of the genome has been shown to increase the resolution and support for phylogenetic hypotheses in plant groups (Straub et al. 2012). Work on the genus *Oreocarya*, a close relative of *Cryptantha*, has also proven this technique to be successful in greatly improving resolution in phylogenetic analyses (Ripma et al. 2014).

The main goal of this study is to infer a strongly supported phylogeny for the genus *Cryptantha* and close relatives. This phylogeny will be used to address three major objectives. First, the monophyly of the genus and of the *Cryptantha* s. s. 1 and *Cryptantha* s. s. 2 clades recovered by Hasenstab-Lehman and Simpson (2012) will be tested, and phylogenetic interrelationships within *Cryptantha* will be inferred. Second, character evolution will be assessed for several of the diagnostic morphological traits that Johnston used to describe his series and sections, including nutlet number, plant duration, and evolution of cleistogamy. Third, biogeographic history will be assessed by inferring the number, timing, and direction of possible intercontinental dispersals.

MATERIALS AND METHODS

Taxon Sampling and DNA Isolation—A total of 81 taxa were used for phylogenetic analyses (Appendix 1). Samples of *Cryptantha* were obtained from both existing herbarium specimens and recent field collections. For the latter, fresh leaf material was dried in silica gel to preserve it for DNA extraction. Voucher specimens are housed at the following herbaria: CONC, GH, JEPS, MERL, MO, RSA, SBBG, SD, SDSU, SGO, SI, UC, and UCR (acronyms after Thiers 2017).

To test the monophyly of *Cryptantha*, representatives of the closely related genera of subtribe Amsinckiinae were selected based on previous phylogenetic studies of the group (Hasenstab-Lehman and Simpson 2012; Weigend et al. 2013; Cohen 2014). Taxa include representatives of *Adelinia* [formerly *Cynoglossum*], *Amsinckia*, *Andersonglossum* [formerly *Cynoglossum*], *Dasynotus*, *Greeneocharis*, *Johnstonella*, *Oreocarya*, *Pectocarya*, and *Plagiobothrys*. *Microula tibetica* Benth., of subtribe Microuleae Weigend (see Chacón et al. 2016), the clade sister to the Amsinckiinae, was used to root the tree.

From leaf material, total genomic DNA was extracted and purified using a modified three-day version of the CTAB (cetyl trimethyl ammonium bromide) protocol (Friar 2005; Doyle and Doyle 1987). RNaseA was added for degradation of single-stranded RNA for more efficient downstream analyses. Whole genomic DNA was quantified using NanoDrop spectroscopy (Thermo Fisher Scientific) and viewed for presence using gel electrophoresis, prior to submission for library preparation.

DNA Sequencing and Quality Control—Whole genomic DNA was sent to Global Biologics (Columbia, Missouri) for library preparation and barcoding for multiplexing to be used for genome skimming methods (Straub et al. 2011, 2012). High throughput sequencing was performed on an Illumina HiSeq2000 (Illumina, San Diego, California) at the Institute for Integrative Genome Biology (IIGB) Instrumentation Facilities at the University of California, Riverside or on an Illumina HiSeq2500 at Global Biologics. Runs at both facilities yielded 100 base-pair single-end reads. Quality control followed the same protocol as Ripma et al. (2014).

Assembly, Alignment, and Model Selection—De novo assemblies of the plastome were prepared using Geneious v. 8.0 (Kearse et al. 2012), with default settings on the largest read pools to recover nearly complete plastomes (Ripma et al. 2014). The de novo assembly of *Cryptantha barbigera* (A. Gray) Greene produced a 125,000 bp partial plastome sequence. To ensure this sequence was cpDNA, the annotations function in Geneious was used to transfer annotations from the *Solanum lycopersicum* L. (AM087200) sequence from GenBank (Benson et al. 2005) with 50% or greater similarity. The newly annotated, partial plastome sequence of

C. barbigera was then used for a reference guided assembly with Geneious following the protocol of Ripma et al. (2014).

Using the ITS sequence of *Cryptantha alyssoides* (D.C.) Reiche (KM213409) from GenBank, a reference guided assembly was done using Geneious with default settings and 100 iterations. To assure that the whole cistron (ETS, 18S, ITS1, 5.8S, ITS2, and the 26S) had been captured through these iterations; annotations were transferred from *Cryptantha alyssoides* (KM213409) with 50% or greater similarity for each sample. Paralogs of the cistron that may have been present due to incomplete homogenization were removed using a strict 75% matching consensus sequence requirement and removing any base pair position with an ambiguity code.

To assemble mitochondrial genes, a reference guided assembly using the *Nicotiana tabacum* L. (BA000042) mitochondrial sequence from GenBank was also performed in Geneious. Resulting consensus contigs were annotated from the *Nicotiana tabacum* (BA000042) sequence and saved as a custom BLAST database. A file of mitochondrial genes extracted from *Nicotiana* (Ripma et al. 2014) was then used to perform a sequence search on the consensus contigs. Mitochondrial genes found in all taxa were aligned and edited using the protocol described below.

After assembly, each region was aligned separately using the MAFFT plugin v. 7.017 (Katoh et al. 2002) with default settings and examined for misalignments by eye. If portions could not be realigned with confidence, they were excluded. After visual realignments, the Strip Alignments function in Geneious was used to remove any ambiguity codes. The AIC criteria (Akaike 1974) in PartitionFinder (Lanfear et al. 2012), was used to find the best model of evolution for each codon position of the plastome, coding and non-coding regions of the cistron, and each gene for the mitochondria. Any region with the same model of evolution was then grouped into the same partition.

Phylogenomic Analysis—Maximum likelihood (ML) analyses were performed using RAxML (Stamatakis 2006), implemented in Geneious for each of the three regions, separately as well as concatenated. Regions were partitioned as stated above, and statistical support was assessed with 1000 bootstrap replicates using the GTR + I + G model of evolution. Bayesian inference (BI) was made for each of the three regions separately and concatenated using BEAST v. 1.8.0 (Drummond et al. 2012), implemented through the CIPRES portal (Miller et al. 2010). For the separate analyses, each region was partitioned and run under the model of evolution as determined in PartitionFinder (Lanfear et al. 2012). Analyses were run for 100 million generations and duplicated six times. The concatenated analysis was partitioned the same as in the ML concatenated analysis using the GTR + I + G model of evolution and run for 250 million generations. Results were viewed in Tracer (Rambaut et al. 2014) to ensure convergence, then combined in LogCombiner v. 1.8.0 (Drummond et al. 2012) using a 10% burn-in, annotated in TreeAnnotator v. 1.8.0 (Drummond et al. 2012), and viewed in FigTree (Rambaut 2014). Coalescent species tree estimates were performed using the summary statistic coalescent method ASTRAL-II (Mirarab and Warnow 2015), with the 1000 bootstrapping trees from the three ML gene tree analyses used to estimate support (Seo 2008). The resulting tree was visualized in FigTree (Rambaut 2014).

Character Evolution—Character evolution was assessed in Mesquite (Maddison and Maddison 2010), using maximum likelihood ancestral state reconstruction and the resulting concatenated maximum likelihood tree as input. The concatenated maximum likelihood tree was chosen as input because it had more nodes recovered with strong support than any of the individual gene trees (see Results). The MK1 probability model was chosen as best fit for the data considering that all characters had more than two states. Characters included were 1) nutlet number per fruit: one, one to two, three to four, or four; 2) plant duration: annual, perennial, or either; and 3) reproductive biology: chasmogamous, cleistogamous, or cleistogamous with cleistogenes.

Divergence Time Estimation—For divergence time estimation, fossil calibration using three of four known fossil *Cryptantha* relatives were used in our analysis (see Fig. 1): *Cryptantha auriculata* (M.K. Elias) Segal, *Cryptantha chaneyi* (M.K. Elias) Segal, and *Cryptantha coroniformis* (M.K. Elias) Segal (Elias 1932, 1942; Segal 1964; Segal 1966). *Cryptantha chaneyi*, although it does not resemble any extant member of *Oreocarya*, does have a large size and a triangular areola at the base of the attachment scar which then narrows into a groove that does not reach the apex of the nutlet body (Segal 1966). This has been observed as a characteristic for the genus
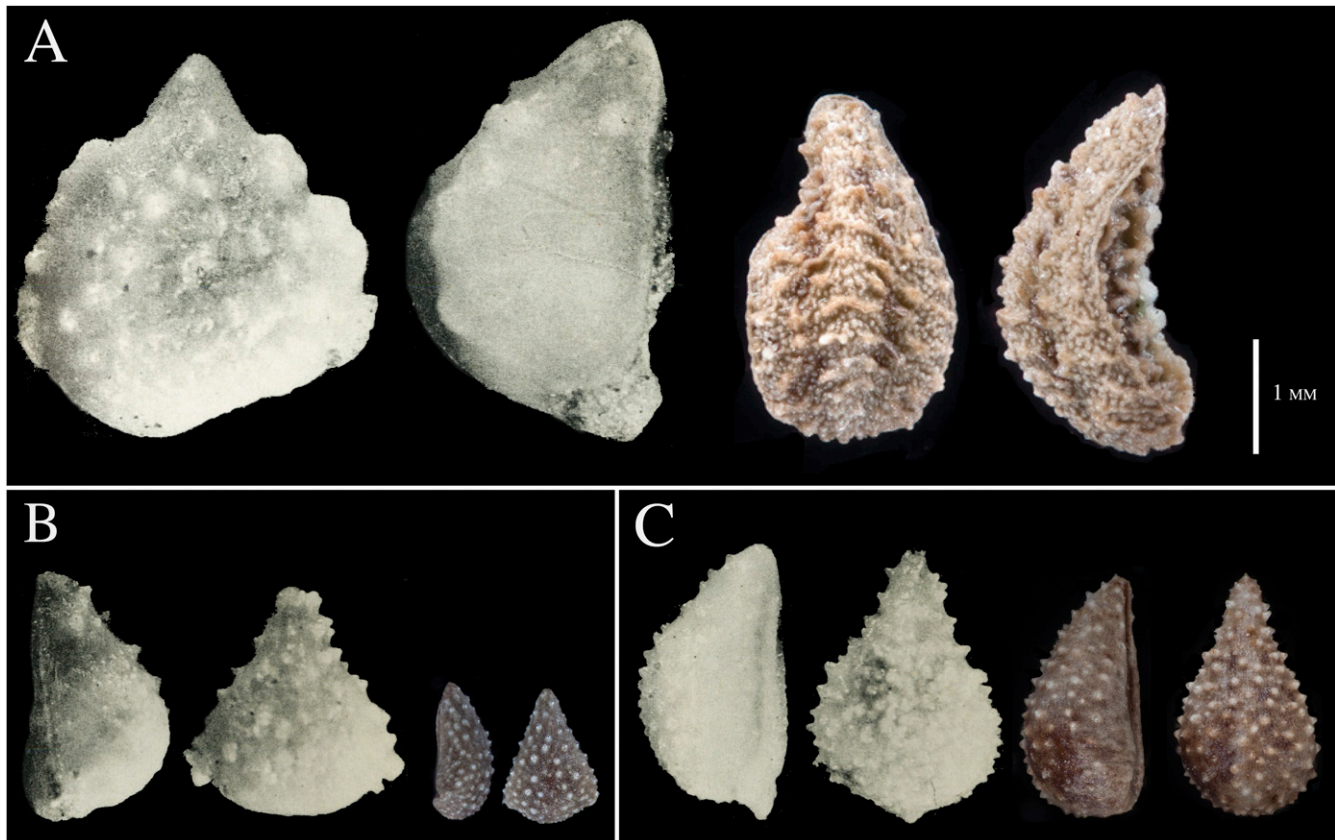

Fig. 1. Comparison of fossil Amsinckiinae used for calibration points with extant taxa. A. *Cryptantha chaneyi* (left, fossil) and *Oreocarya flavoculata* (right, extant species, SDSU 20030). B. *Cryptantha auriculata* (left, fossil) and *C. albida* (right, extant species, SD 99139). C. *Cryptantha coroniformis* (left, fossil) and *C. crassisepala* var. *elachantha* I.M. Johnston (right, extant species, small, consimilar nutlet shown, SD 64231). All photos to scale, bars = 1 mm. Fossil taxa images from Elias (1942).

Oreocarya (Simpson and Hasenstab 2009). *Cryptantha auriculata* was used to root the base of the lineage containing *C. albida*, as it has similar morphological characters to *C. albida* with its triangular shaped nutlet (Segal 1966). Lastly, *C. coroniformis* was used to root the crown node of the clade that contained the extant species *C. crassisepala* (Torrey & A.Gray) Greene and *C. minima* Rydberg, as supported by several morphological similarities noted by Segal (1966). All three fossil nutlets were all found in the Ogallala formation in Kansas, U. S. A., in Ash Hollow Rock. Boellstorff (1976, 1978) dated this formation to be from the Hemphillian period (10.3–4.9 million years ago; see also Ludvigson et al. 2009). Although we are accepting the identifications of these fossils as described, more work may be needed to confirm their relationship to *Cryptantha* and close relatives.

Approximation of divergence times of major clades was performed using treePL (Smith and O'Meara 2012), which utilizes a penalized likelihood approach. Three separate analyses were run using the fossils described above as well as, in two analyses, an additional constraint of the node leading to the crown Amsinckiinae. For the fossils, a maximum of 10.3 Ma and minimum of 4.9 Ma was set and the Amsickiinae node was constrained to a maximum of 26.9 Ma and minimum of 17.4 Ma to reflect the 95% high posterior density intervals of the date recoverd by Chacón et al. (2017) for the crown Amsickiinae in their study. The concatenated maximum likelihood tree was chosen as input because, as noted above, it had more nodes recovered with strong support than any other tree (see Results). Analyses were run using only the three fossils for calibration, only the Amsickiinae node calibration, and using all data with all four calibration points. For all three analyses, a smoothing parameter of 1000 was determined using cross-validation and priming was used to establish the best optimization scores.

Biogeographic Inference—Biogeographic analyses were performed using BioGeoBEARS (Matzke 2012, 2013) to determine patterns of dispersal. The program BioGeoBEARS evaluates phylogeography models typically used to estimate biogeography patterns. These include the DEC model of LAGRANGE (Ree and Smith 2008), a model similar to DIVA (Ronquist 1997), DIVALIKE, and a model similar to BAYAREA (Landis et al. 2013), BAYAREALIKE. BioGeoBEARS then provides a common statistical framework in order to judge which models are preferred for the input dataset. As input, the time calibrated tree using all four calibrations (three fossils and Amsinckiinae crown node) was chosen as best (see Results) and the areas were set using the global ecological zones published by the Forestry Department of the Food and Agriculture Organization of the United Nations (Davis and Holmgren 2001). These global ecological zones were described using the vegetation, climate, and physiography of the world. In North America *Cryptantha* occurs in six of the 20 Global Ecological Zones defined for that region: subtropical desert, subtropical dry forest, subtropical mountain system, subtropical steppe, temperate desert, and temperate mountain system. In South America *Cryptantha* occurs in five of the 20 Global Ecological Zones defined for that region: subtropical dry forest, subtropical mountain system, subtropical steppe, tropical desert, and tropical mountain system. To limit computational load for analyses to run, North America subtropical dry forest and subtropical mountains zones were combined into one area (termed "subtropical dry forest and mountain") and in South America, subtropical steppe and subtropical dry forest were combined (termed "subtropical steppe and dry forest"), for a total of nine areas (labeled A–I, Appendix 2). For North America, all *Cryptantha* occurring in the combined subtropical dry forest and mountain region are restricted to the westernmost, Mediterranean zone of this region, corresponding to the California Floristic Province (see Burge et al. 2016). Species ranges within these zones were determined using herbarium records and online distribution databases for South America (CONC, LP, MO, SDSU, SGO) and North America (CCH 2016; SEINet 2016; Kartesz 2014). A given species occurred in up to a maximum of five areas (Appendix 2).

Results

Sequence Matrices—Genome skimming resulted in 81 individual read pools (deposited at the Short Read Archive; see Appendix 1). *Oreocarya flavoculata* A.Nelson had the largest read pool of 7,593,640 reads. Analysis of *Microula tibetica* resulted in the smallest read pool of just 820,347 reads. Although the latter read pool had significantly fewer reads, the plastome (cpDNA), complete cistron (nrDNA), and mitochondrial (mtDNA) genes were all successfully recovered. De novo assembly of *Cryptantha barbigera* resulted in a 125,000 bp

contig that was further used as a reference for assembly of the cpDNA for all other taxa. After editing, an alignment of 119,580 bp was used for phylogenetic inference of the cpDNA, with a total of 14,728 variable and 6,964 parsimony informative characters recovered. The complete cistron sequence (5,638 bp) was recovered for all taxa. Non-coding regions contained most of the variability; however, coding regions did contribute to the total of 498 variable characters, of which 304 were parsimony informative. Lastly, the mitochondria assembly resulted in the recovery of 38 genes. Of those 38 genes, 23 of them were complete in all taxa and used for phylogenetic inference for 100% matrix occupancy. These genes ranged from 100 bp to over 1000 bp in length. Concatenation of the 23 genes resulted in a 9685 bp alignment with 1888 variable, and 1038 parsimony informative characters.

Phylogenetic Analyses—Maximum likelihood (ML) and Bayesian inference (BI) of the chloroplast DNA (cpDNA) resulted in trees with exactly the same topology (Fig. 2). In both analyses, three separate monophyletic groups of *Cryptantha* taxa were recovered. One monophyletic group consisting of the North American *C. clokeyi* I.M.Johnston, *C. maritima* var. *maritima*, *C. martirensis* M.G.Simpson & Rebman, and *C. muricata* (Hooker & Arnott) A. Nelson & J.F.Macbride var. *muricata*, plus the South American species *C. subamplexicaulis* (Philippi) I.M.Johnst. was recovered with strong support (BS = 100, PP = 1). This group we termed the *Maritimae* clade (Fig. 2), after Johnston's 1925 series by that name. A second clade containing the North American *C. albida*, *C. mexicana* I.M.Johnst., and *C. texana* Greene, plus the South American species *C. hispida* (Philippi) Reiche was recovered with strong support (BS = 100, PP = 1) as was a clade containing two species of the genus *Johnstonella* (BS = 100, PP = 1). These two clades are strongly supported as sister taxa (BS = 100, PP = 1) and are together referred to as the *Johnstonella/Albidae* clade (Fig. 2). The remaining sampled *Cryptantha* taxa form a clade of mixed support (BS = 100, PP < 0.9), termed the *Cryptantha* core clade. The *Cryptantha* core clade is sister to the *Johnstonella/Albidae* clade with mixed support (BS = 85, PP < 0.9). Within the *Cryptantha* core clade, two monophyletic groups of South America taxa were recovered, both strongly supported (BB = 100, PP = 1). We term these two clades the *Eucryptantha/Geocarya* clade, after Johnston's 1927 series names for almost all members of the group, and the *Globulifera* clade, after the earliest described species of the group, *C. globulifera* (Clos) Reiche (Fig. 2).

Both the ML and BI analyses of the cistron DNA (nrDNA) resulted in exactly the same topologies (Fig. 3). The *Maritimae* clade is recovered as monophyletic with mixed support (BS < 70, PP = 0.97) and is sister to the *Cryptantha* core clade, but with weak support. The clade containing *C. albida*, *C. mexicana*, and *C. texana* is recovered as monophyletic with strong support (BS = 93, PP = 0.93); however, *C. hispida* is more closely related to the two representatives of the genus *Johnstonella*. The entire *Johnstonella/Albidae* clade is strongly supported (BS = 100, PP = 1). The *Cryptantha* core clade is again resolved as monophyletic with strong support (BS = 89, PP = 1). Both South American clades within the *Cryptantha* core clade are recovered as monophyletic with strong support. However, relationships of the *Eucryptantha/Geocarya* and *Globulifera* clades to North American members of *Cryptantha* differ from the chloroplast (cpDNA) tree (Figs. 2, 3).

The ML and BI analyses of mitochondrial DNA (mtDNA) did not return trees with the same topology (Fig. 4, only
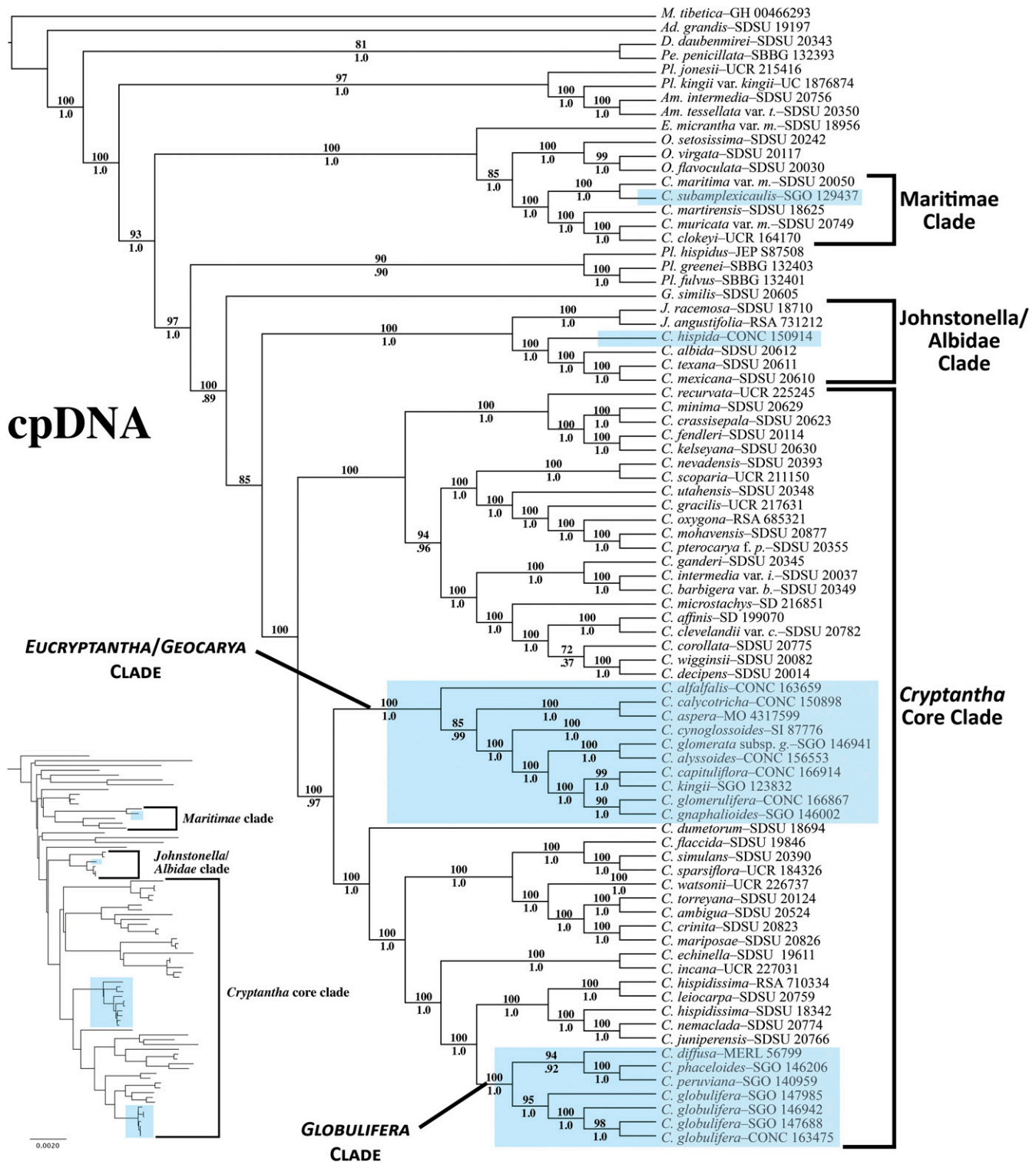
FIG. 2. Maximum likelihood tree (right) and phylogram (lower left) of the chloroplast (cpDNA). Major clades are identified and South American species are highlighted in blue. Bootstrap values above, posterior probabilities below. Genera abbreviations: *Ad.* = *Adelinia*; *Am.* = *Amsinckia*; *D.* = *Dasynotus*; *C.* = *Cryptantha*; *E.* = *Eremocarya*; *G.* = *Greeneocharis*; *J.* = *Johnstonella*; *Mi.* = *Microula*; *O.* = *Oreocarya*; *Pe.* = *Pectocarya*; *Pl.* = *Plagiobothrys*.

illustrating the ML tree). However, in both trees, all three major clades from the previous analyses are recovered as monophyletic: the *Maritimae* and *Johnstonella/Albidae* clades with strong support (BS = 80, PP = 0.98; BS = 100, PP = 1, respectively) and the *Cryptantha* core clade with weak support (BS = < 70, PP = < 0.9). In addition, the South American *Eucryptantha/Geocarya* and *Globulifera* clades were recovered

with strong (BS = 93, PP = 1.0) and mixed (BS < 70, PP = 1.0) support, respectively. The major difference between the ML and BI analyses of the mtDNA data was the placement of the other genera in relation to the aforementioned major clades. The *Cryptantha* core clade and the *Maritimae* clade are recovered as sister in both analyses with weak support, but the placement of the *Johnstonella/Albidae* clade is different in the

nrDNA

M. tibetica–GH 00466293
Pe. penicillata–SBBG 132393
Ad. grandis–SDSU 19197
D. daubenmirei–SDSU 20343
Pl. hispidus–JEP S87508
E. micrantha var. m.–SDSU 18956
O. setosissima–SDSU 20242
O. flavoculata–SDSU 20030
O. virgata–SDSU 20117
C. hispida–CONC 150914
J. angustifolia–RSA 731212
J. racemosa–SDSU 18710
C. albida–SDSU 20612
C. texana–SDSU 20611
C. mexicana–SDSU 20610

Johnstonella/ Albidae Clade

G. similis–SDSU 20605
Pl. fulvus–SBBG 132401
Pl. greenei–SBBG 132403
Pl. jonesii–UCR 215416
Pl. kingii var. k.–UC 1876874
Am. intermedia–SDSU 20756
Am. tessellata var. t.–SDSU 20350
C. subamplexicaulis–SGO 129437
C. maritima var. m.–SDSU 20050
C. martirensis–SDSU 18625
C. clokeyi–UCR 164170
C. muricata var. m.–SDSU 20749

Maritimae Clade

C. recurvata–UCR 225245
C. fendleri–SDSU 20114
C. minima–SDSU 20629
C. kelseyana–SDSU 20630
C. crassisepala–SDSU 20623
C. dumetorum–SDSU 18694
C. gnaphalioides–SGO 146002
C. cynoglossoides–SI 87776
C. calycotricha–CONC 150898
C. glomerulifera–CONC 166867
C. capituliflora–CONC 166914
C. aspera–MO 4317599
C. alyssoides–CONC 156553
C. kingii–SGO 123832
C. alfalfalis–CONC 163659
C. glomerata subsp. g.–SGO 146941

EUCRYPTANTHA/GEOCARYA CLADE

C. flaccida–SDSU 19846
C. sparsiflora–UCR 184326
C. simulans–SDSU 20390
C. torreyana–SDSU 20124
C. ambigua–SDSU 20524
C. watsonii–UCR 226737
C. crinita–SDSU 20823
C. mariposae–SDSU 20826
C. scoparia–UCR 211150
C. nevadensis–SDSU 20393
C. gracilis–UCR 217631
C. utahensis–SDSU 20348
C. mohavensis–SDSU 20877
C. pterocarya f. p.–SDSU 20355
C. oxygona–RSA 685321
C. nemaclada–SDSU 20774
C. juniperensis–SDSU 20766
C. hispidissima–RSA 710334
C. leiocarpa–SDSU 20759
C. ganderi–SDSU 20345
C. microstachys–SD 216851
C. decipens–SDSU 20014
C. intermedia var. i.–SDSU 20037
C. barbigera var. b.–SDSU 20349
C. wigginsii–SDSU 20082
C. corollata–SDSU 20775
C. affinis–SD 199070
C. clevelandii var. c.–SDSU 20782
C. hispidissima–SDSU 18342
C. incana–UCR 227031
C. echinella–SDSU 19611
C. phaceloides–SGO 146206
C. peruviana–SGO 140959
C. globulifera–SGO 146942
C. diffusa–MERL 56799
C. globulifera–SGO 147985
C. globulifera–SGO 147688
C. globulifera–CONC 163475

Cryptantha Core Clade

GLOBULIFERA CLADE

Johnstonella/ Albidae clade
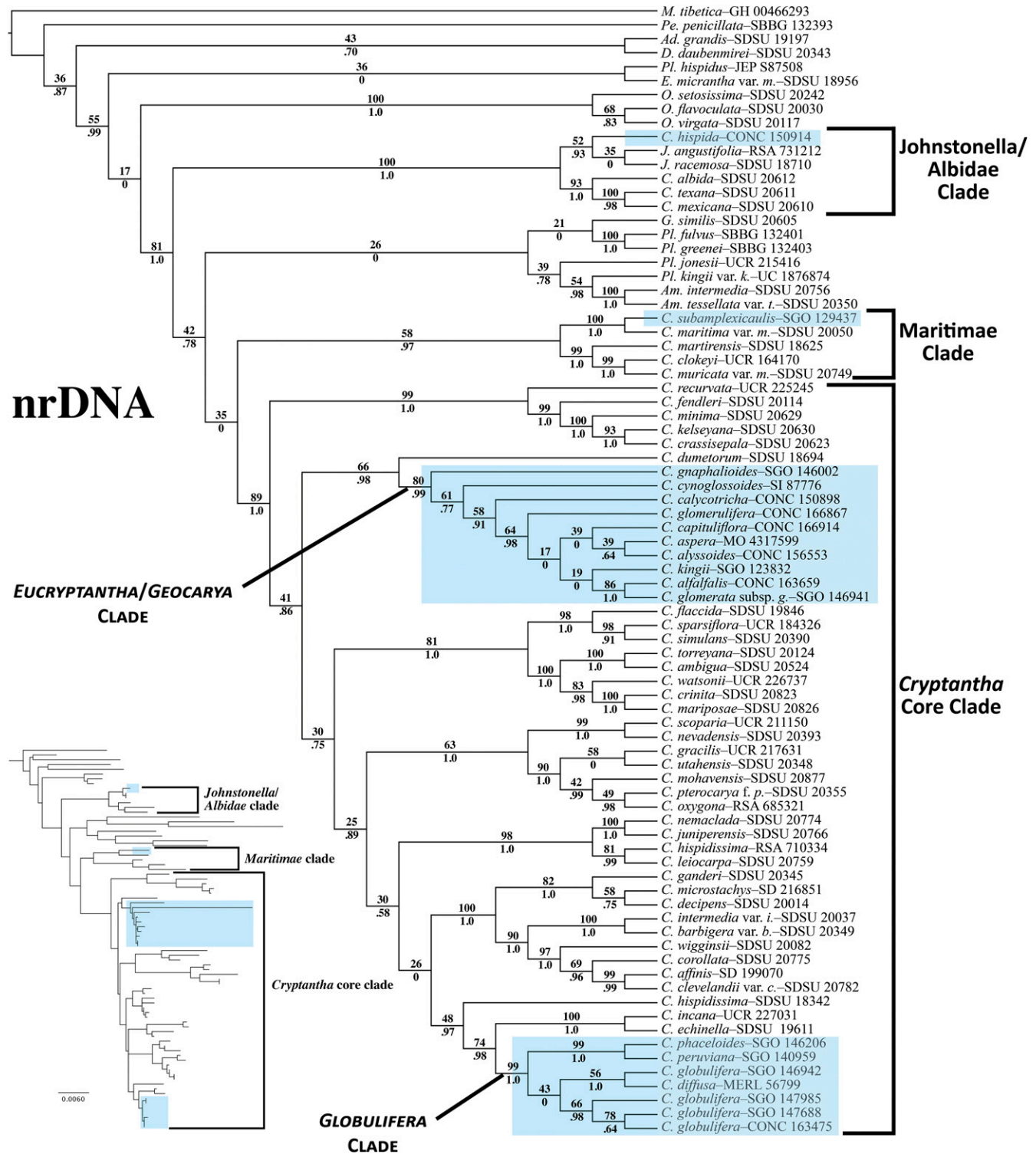Maritimae clade
Cryptantha core clade
0.0060

FIG. 3. Maximum likelihood tree (right) and phylogram (lower left) of the ribosomal cistron (nrDNA). Major clades are identified and South American species are highlighted in blue. Bootstrap values above, posterior probabilities below. Genera abbreviations as in Fig. 2.

two trees. Overall, the mtDNA tree provided relatively poor support (having the fewest number of strongly supported nodes) for the relationships of these taxa.

Phylogenetic inference using ML concatenation of all three gene regions resulted in a tree with a greater number of strongly supported nodes than any of the gene trees alone, with the BI analysis resulting in a tree with exactly the same topology. All except one node (within *Oreocarya*) are strongly

supported with a bootstrap of 80 or better (Fig. 5). The same three *Cryptantha* clades are recovered as in the gene trees. However, in the ML/BI concatenated tree, the placement of these three clades in relation to one another and in relation to other genera is resolved with higher support. The *Johnstonella/ Albidae* clade is sister to the *Cryptantha* core clade with mixed support (BS = 89, PP < 0.9), while the *Maritimae* clade is placed sister to *Oreocarya* and *Eremocarya* with strong support (BS = 100,
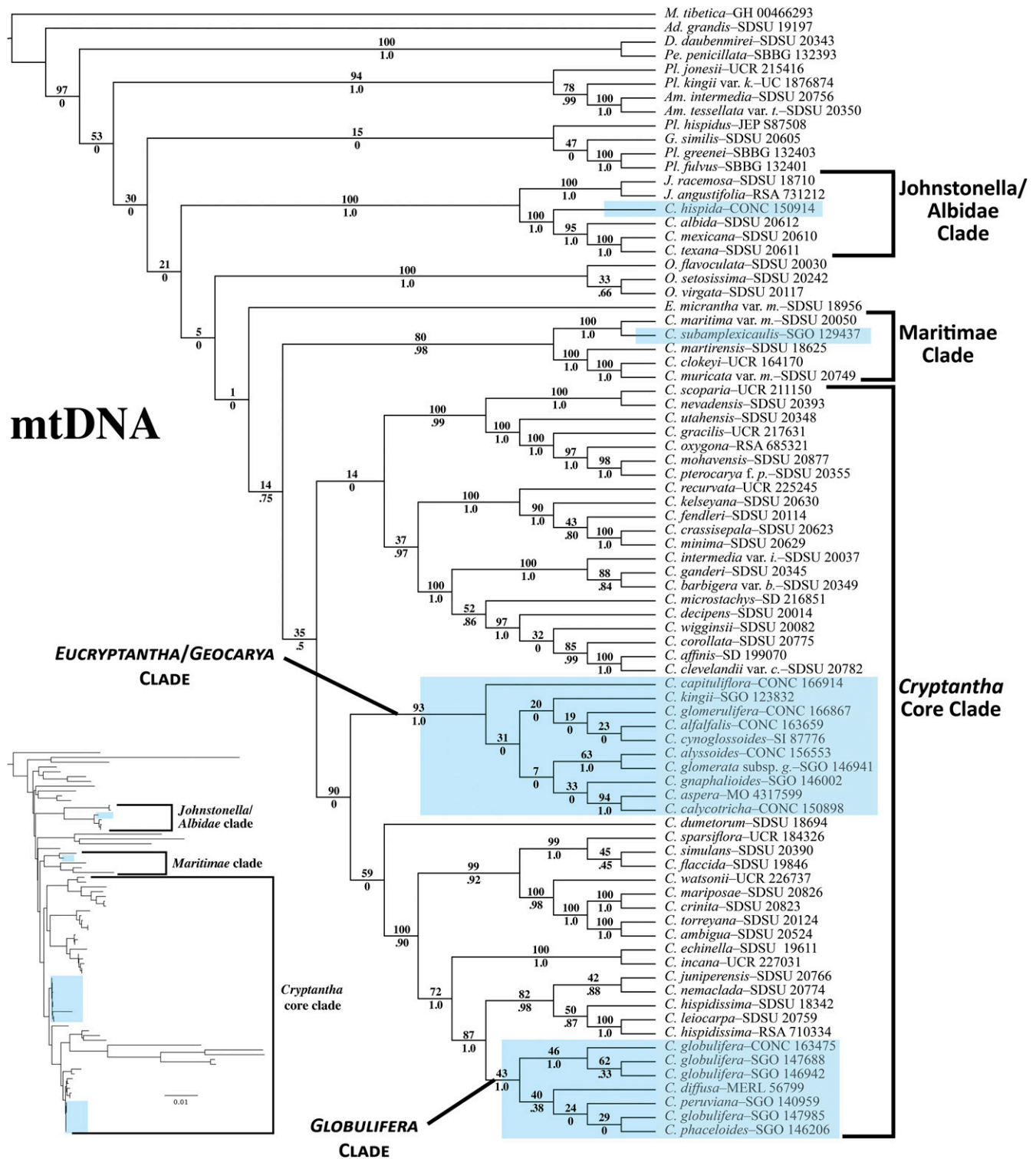
FIG. 4.   Maximum likelihood tree (right) and phylogram (lower left) of 23 concatenated mitochondrial genes (mtDNA). Major clades are identified and South American species are highlighted in blue. Bootstrap values above, posterior probabilities below. Genera abbreviations as in Fig. 2.

PP = 1.0). These relationships were also recovered in the ML and BI cpDNA analyses.

Species tree estimation using ASTRAL-II (Mirarab and Warnow 2015) produced a phylogeny more similar to the cistron tree with the *Maritimae* clade sister to the *Cryptantha* core clade, and these together, sister to the *Johnstonella/Albidae* clade (Fig. 6). Although these major clades are again recovered with strong support (BS = 100), there is no strong support for relationships among them.

Character Evolution—Using the maximum likelihood (ML) concatenated tree, character evolution for three traits were traced and evaluated. For nutlet number per fruit, there are roughly equal likelihoods for any of the states to be ancestral. However, for the South American *Eucryptantha/Geocarya* clade, the ancestral condition, possessed by virtually all members of that clade, is 1–2 nutlets per fruit (Fig. 7). For plant duration, annual is resolved as ancestral for all three major
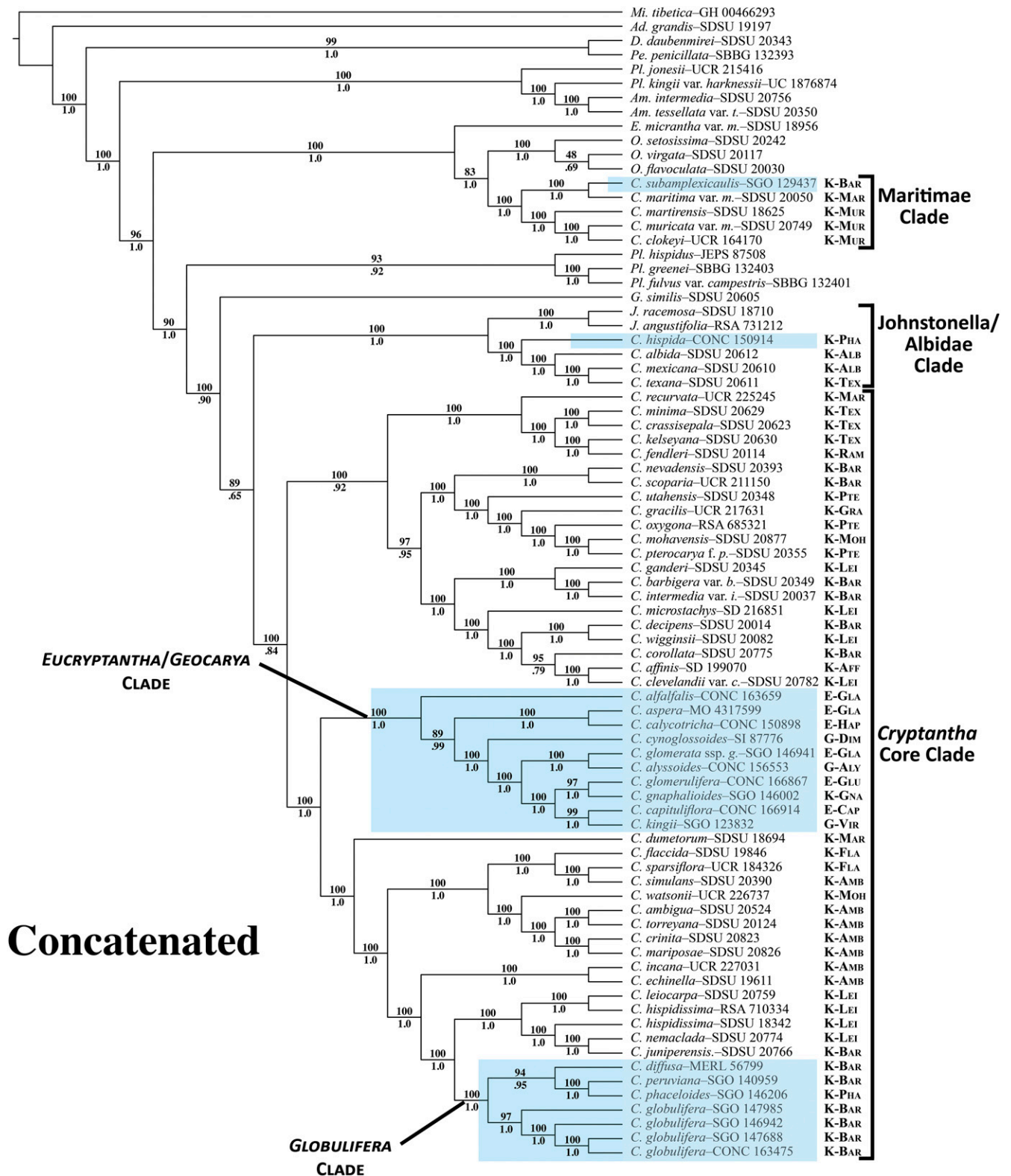
FIG. 5. Maximum likelihood tree of concatenated cpDNA (chloroplast), nrDNA (cistron), and mtDNA (mitochondrial) regions. Major clades are identified and South American species are highlighted in blue. Bootstrap values above, posterior probabilities below. Genera abbreviations as in Fig. 2. Section abbreviations: E = Sec. *Eucryptantha;* G = Sec. *Geocarya;* K = Sec. *Krynitzkia.* Series abbreviations: AFF = Ser. *Affines;* ALB = Ser. *Albidae;* ALY = Ser. *Alyssoides;* AMB = Ser. *Ambiguae;* BAR = Ser. *Barbigerae;* CAP = Ser. *Capituliflorae;* DIM = Ser. *Dimorphae;* FLA = Ser. *Flaccidae;* GLA = Ser. *Glomeratae;* GLU = Ser. *Glomeruliferae;* GNA = Ser. *Gnaphalioides;* GRA = Ser. *Graciles;* HAP = Ser. *Haplostachyae;* LEI = Ser. *Leiocarpae;* MAR = Ser. *Maritimae;* MOH = Ser. *Mohavenses;* MUR = Ser. *Muricatae;* PHA = Ser. *Phaceloides;* PTE = Ser. *Pterocaryae;* RAM = Ser. *Ramulosissimae;* TEX = Ser. *Texanae;* VIR = Ser. *Virentes.*
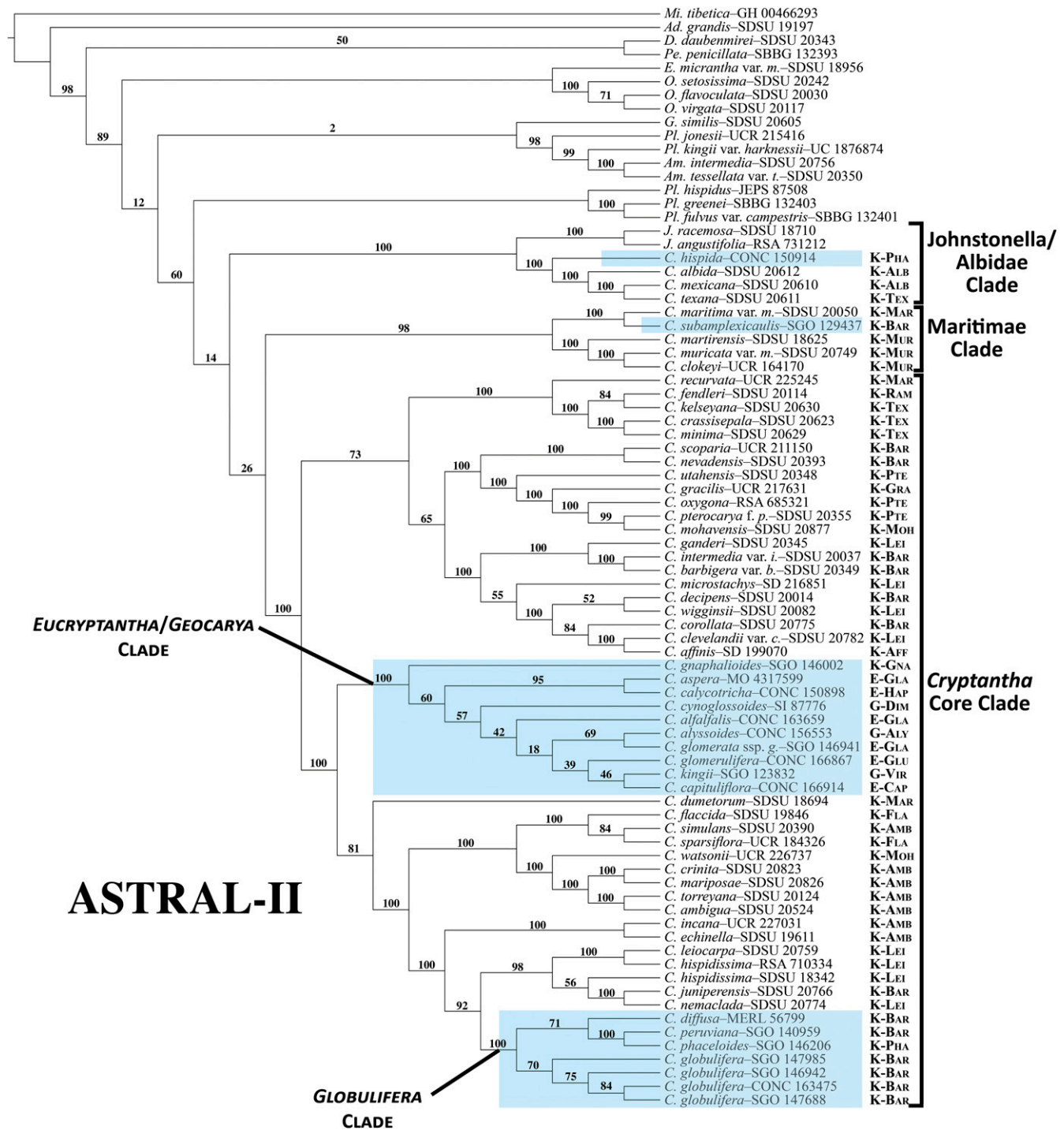
FIG. 6. Species tree estimated using ASTRAL-II of the full dataset (all 81 taxa). Major clades are identified and South American species are highlighted in blue. Bootstrap values above. Genera abbreviations as in Fig. 2. Section and series abbreviations as in Fig. 5.

clades. A perennial plant duration is found to have evolved at least once (or possibly be ancestral) in the early diverging *Adelinia* and *Dasynostus* and have been derived independently for all *Oreocarya*, *Johnstonella racemosa*, and a portion of the South American *Eucryptantha/Geocarya* clade (Fig. 8). Ancestral reconstruction for reproductive biology recovered chasmogamy as the ancestral state, with cleistogamy evolving once in the South American *Eucryptantha/Geocarya* clade (Fig. 9). The transition from cleistogamous to cleistogenes occurred as many as three times (Fig. 9). One

reversal, from cleistogamy to chasmogamy, occurred in *C. gnaphalioides* (A.DC.) Reiche (Fig. 9).

Divergence Time Estimation—Divergence time estimates using the Chacón et al. (2017) node calibration alone and estimates using the three fossils plus the Chacón et al. calibration recovered very similar dates (Table 2). However, estimates using only the three fossils as calibration returned very different dates of divergence from either of these (Table 2). Because of the similarity of dates in the aforementioned two analyses and because of the uncertainty in the placement of the
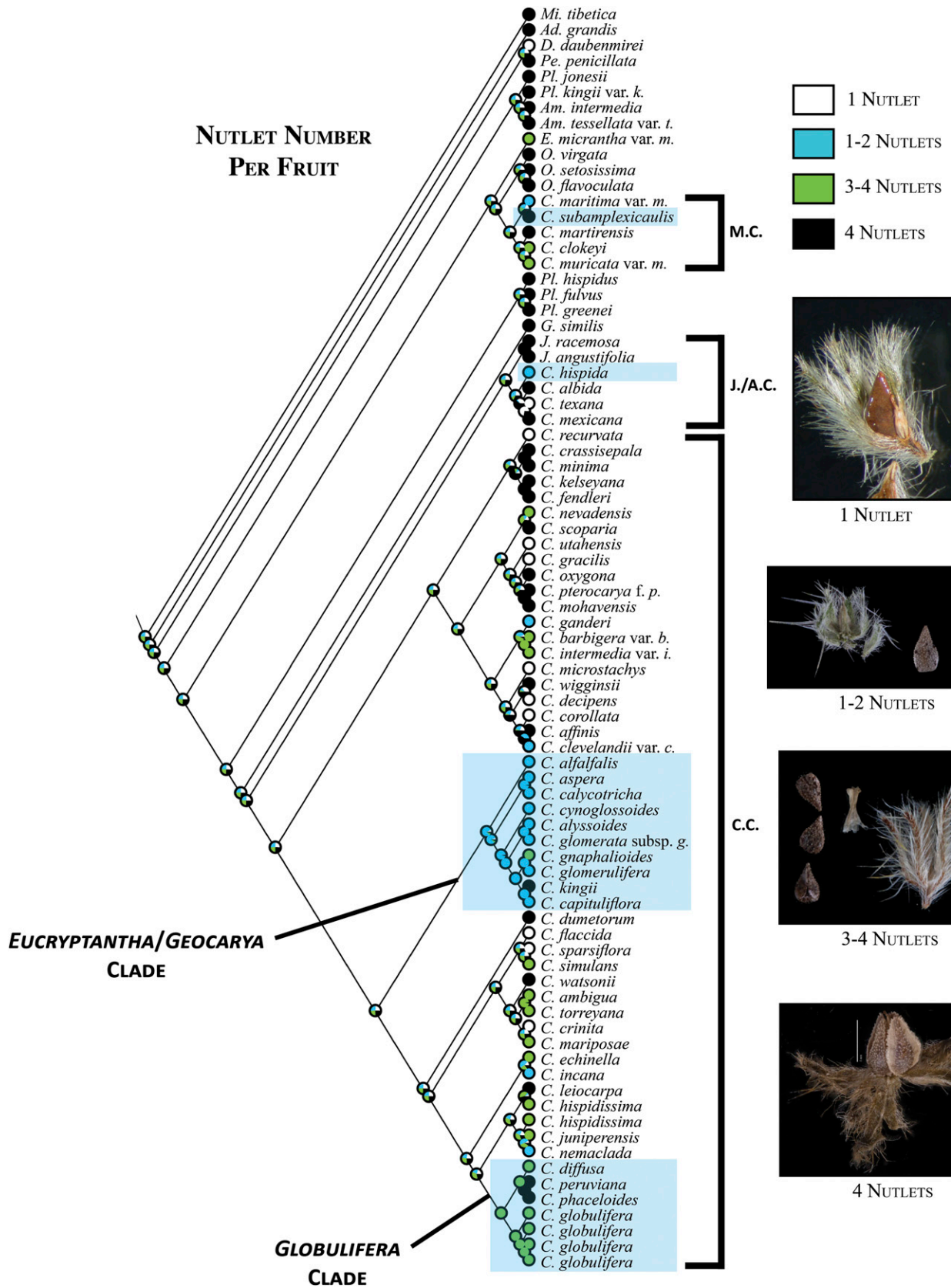
Fig. 7. Character evolution of nutlet number per fruit, using maximum likelihood tree of concatenated analysis. White = 1 nutlet/fruit, blue = 1–2 nutlets/fruit, green = 3–4 nutlets/fruit, black = 4 nutlets/fruit. Major clades are identified and South American species are highlighted in blue. J./A.C. = *Johnstonella/Albidae* core clade; C.C. = *Cryptantha* core clade; M.C. = *Maritimae* clade. Genera abbreviations as in Fig. 2.
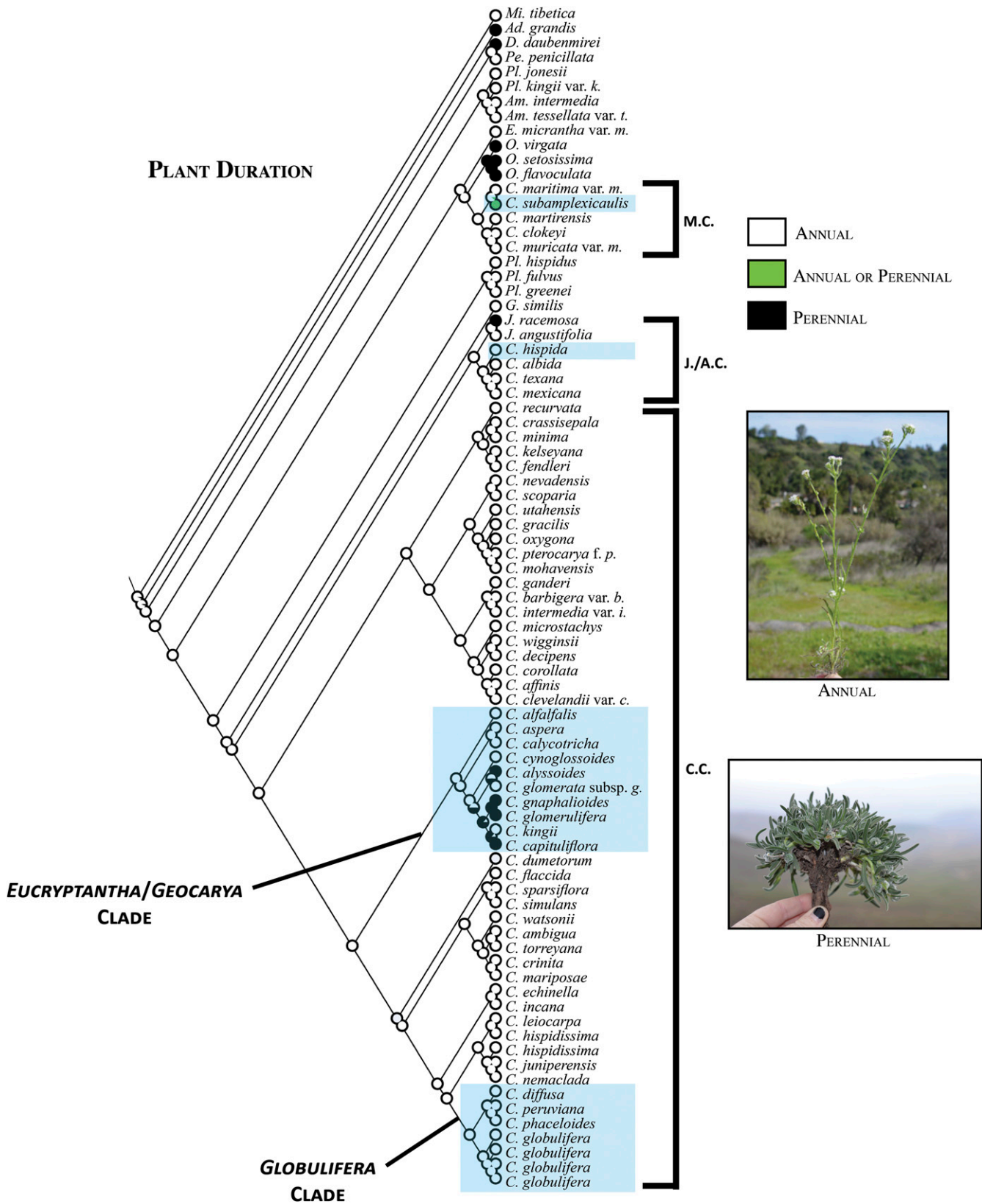
FIG. 8. Character evolution of plant duration, using maximum likelihood tree of concatenated analysis shown. White = annual; green = annual or perennial; black = perennial. Major clades and highlighted species as in Fig. 7. Genera abbreviations as in Fig. 2.

fossils alone due to limited similarities with extant taxa, we elected to use the values recovered from the fossils plus the Chacón et al. (2017) crown Amsickiinae date as the most reliable date estimates (Table 2). By these estimates, the stem node of the *Globulifera* clade diverged at about 9.20 Ma and the crown node of this clade at about 0.91 Ma. The stem node of the *Eucryptantha/Geocarya* clade diverged at about 19.26 Ma and the crown node of this clade at 5.08 Ma. The stem node of the
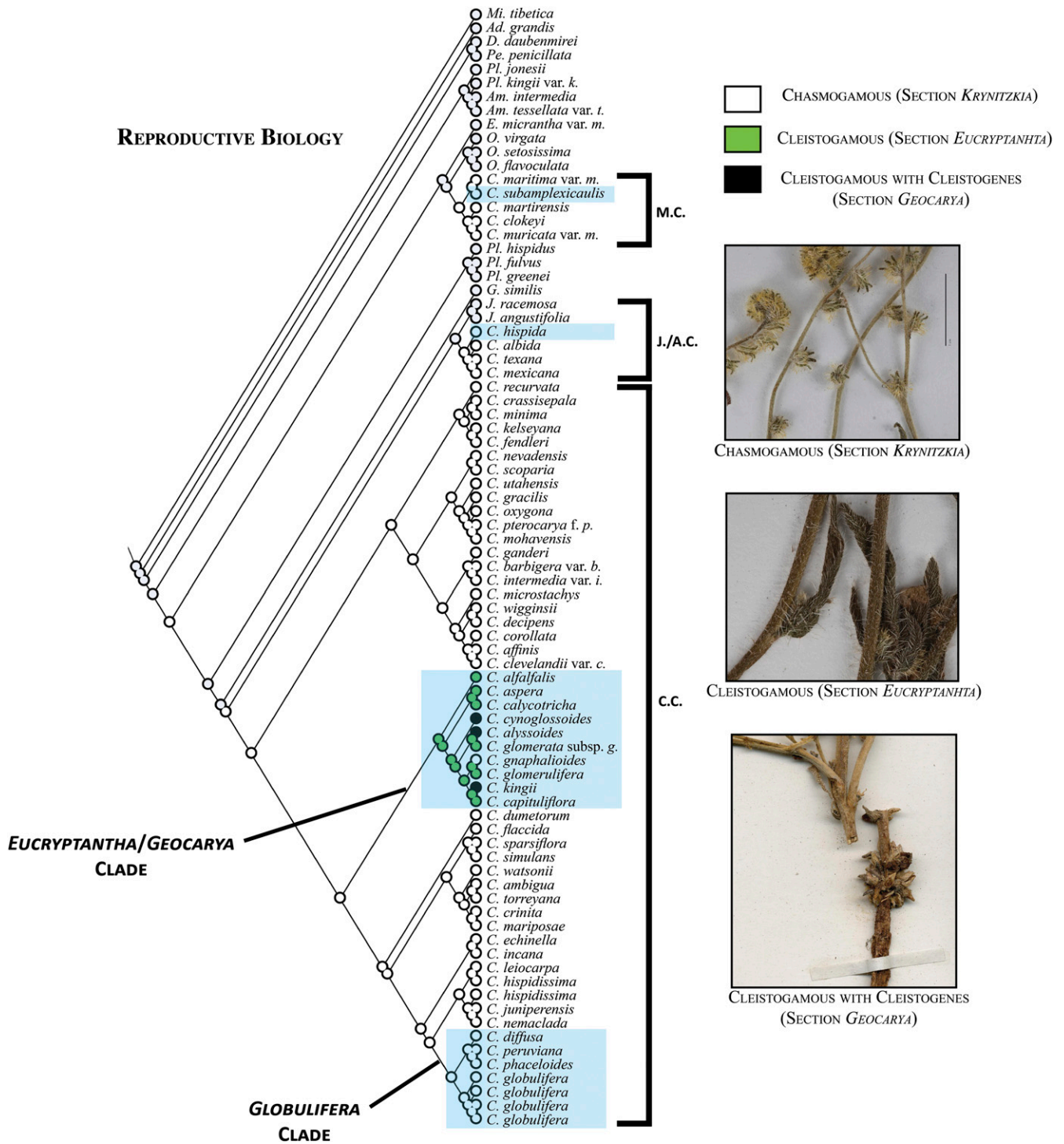
FIG. 9. Character evolution of cleistogamy, using maximum likelihood tree of concatenated analysis shown. White = chasmogamous (*Cryptantha* section *Krynitzkia*); green = cleisogamous (*Cryptantha* section *Cryptantha*); black = cleisogamous with cleistogenes (*Cryptantha* section *Geocarya*). Major clades and highlighted species as in Fig. 7. Genera abbreviations as in Fig. 2.

South America species *C. hispida*, which is nested in the *Albidae* clade, diverged at about 6.21 Ma from other North American species in this clade. Finally, the stem node of the South American *Cryptantha subamplexicaulis*, nested in the Maritimae clade, diverged at around 3.81 Ma from other North American species in this clade (Table 2).

Biogeographic Inference—The statistical analysis in BIO-GEOBEARS (Matzke 2012, 2013) returned the BAYAREALIKE+J model as the best fit for the data. This model excludes vicariance,

only allowing complete sympatric speciation to occur. The "J" function allows for jump dispersal to occur, which was hypothesized to be important for this group of plants.

A minimum of four unidirectional intercontinental dispersals was recovered. All dispersal events originated from a Mediterranean North America ancestor (the western-most Mediterranean region of the "subtropical dry forest and mountain" global ecological zone) entering a Mediterranean (the western-most "subtropical steppe and dry forest") or

TABLE 2. Comparisons of average divergence times of the four South American *Cryptantha* clades or lineages, using treePL, with fossil calibrations from three species (*Cryptantha auriculata, C. chaneyi,* and *C. coroniformis*) and the Amsinckiinae crown node calibration from Chacón et al. 2017. *Eucryp.* = *Eucryptantha; Geo.* = *Geocarya*.

Calibrations	*Globulifera*Clade Stem Node	*Globulifera*Clade Crown Node	*Eucryp./Geo.* Clade Stem Node	*Eucryp./Geo.* Clade Crown Node	*C. hispida* Stem Node	*C. subamplexicaulis* Stem Node
Only Fossils	19.37	1.91	40.93	10.72	8.58	8.01
Only Amsinckiinae Crown node Calibration	8.42	0.83	17.75	4.65	2.76	3.51
Fossils + Amsinckiinae Crown node Calibration	9.20	0.91	19.26	5.08	6.21	3.81

desert ("tropical desert") global ecological zone (Fig. 10). Within North America, one dispersal into the temperate mountain system alone (in *Oreocarya virgata* (Porter) Greene) and one dispersal into the desert region alone (*Cryptantha dumetorum*) are recovered. The *Johnstonella/Albidae* clade dispersed from the Mediterranean North America region (the western-most portion of the "subtropical dry forest and mountain" global ecological zone) to various regions, including the tropical desert region (Atacama Desert) of South America (*C. hispida* in the *Johnstonella/Albidae* clade). There is strong support for a Mediterranean North America ancestry of the *Maritimae* clade, with most of the species of this clade still found in the Mediterranean Region of western North America. In this same clade, one dispersal to the South America tropical desert (the Atacama Desert) is recovered (*C. subamplexicaulis*). Both dispersals from North to South America in the *Cryptantha* core clade had Mediterranean North America ancestors. In the *Eucryptantha/Geocarya* clade, the ancestor dispersed to the Mediterranean South America region with a later dispersal to the high elevation areas of the Andes. Also in this clade, one dispersal back to the Mediterranean region of South America is recovered in *C. gnaphalioides*. The stem node of the *Globulifera* clade dispersed from Mediterranean North America to Mediterranean South America, with a later dispersal to the tropical Andes (*C. peruviana* I.M.Johnst.).

DISCUSSION

Phylogenetic Analyses and Classification—Genome skimming methods successfully recovered nearly complete sequence data from the three major regions of the plant genome for all taxa studied. However, trees obtained using each of the separate genomes differed, mainly in relationships of major clades (Figs. 2–4). Possible reasons for the incongruence between these genomes may be related to how they are inherited. Both the chloroplast and mitochondria are uniparentally inherited, possibly confounding results by tracing evolution from only one line of descent (Rieseberg and Soltis 1991; Rieseberg and Wendel 1993). The differences between the two may be related to the fact that they do have different histories of descent, despite both being uniparentally inherited. In addition, mitochondrial DNA has a great deal of plasticity in plants, making its use in phylogenetic studies less reliable (see Knoop 2004). Problems have also been noted with regard to using the ITS regions of the cistron (nrDNA) for phylogenetic analyses (Alvarez and Wendel 2003). Although the cistron is part of the nuclear genome and is therefore biparently inherited, many plant genomes are found with several different copies of ITS sequences. These multiple copies are perhaps due to incomplete homogenization, making paralog sequence relationships potentially misleading for phylogenetic analysis (Alvarez and Wendell 2003). For this analysis, however, positions of the cistron that may have been subject to incomplete

homogenization were removed using a strict 75% matching consensus sequence requirement and removing any base pair positions with ambiguity codes.

Our analyses largely support the conclusions of Hasenstab-Lehman and Simpson (2012) to divide *Cryptantha* s. l. into five genera. In all analyses, we resolved three of their four segregate genera, *Eremocarya, Greeneocharis,* and *Oreocarya,* as monophyletic with strong support (Figs. 2–6), although our sample size for these was limited. The fourth segregate genus, *Johnstonella,* was also resolved as monophyletic with strong support in all but the nrDNA tree, in which *Cryptantha hispida* is nested within, this *Johnstonella + C. hispida* clade having mixed support (Fig. 3). Although not the focus of this study and having a limited sample size, we note that the genus *Plagiobothrys* is non-monophyletic in all of our analyses. Members of the genus consistently occur in two clades, although the relative positions of these clades differ in analyses: 1) a clade of three species, with *P. hispidus* A. Gray (section *Sonnea*) sister to a clade of *P. fulvus* (Hook. and Arn.) I.M.Johnst. var. *campestris* (Greene) I.M.Johnst. plus *P. greenei* (A.Gray) I.M.Johnst. (the latter two of *Plagiobothrys* section *Plagiobothrys*); and 2) a clade of *P. jonesii* A.Gray plus *P. kingii* (S.Watson) A.Gray (these both of *Plagiobothrys* section *Amsinckiopsis*) plus two species of *Amsinckia*. Similar results were obtained by Hasenstab-Lehman and Simpson (2012) and Simpson et al. (2017a).

In all analyses, *Cryptantha* as is currently defined (i.e. minus the four segregate genera) is recovered as triphyletic, with species occurring in one of three monophyletic groups, each of which had strong or mixed support in all or most analyses (Figs. 2–6). One *Cryptantha* clade recovered is what we term the *Maritimae* clade, consisting of North American *C. clokeyi, C. maritima, C. martirensis,* and *C. muricata,* plus the South American species *C. subamplexicaulis.* The *Maritimae* clade is compatible with *Cryptantha* s. s. 2 clade of Hasenstab-Lehman and Simpson (2012), but with additional taxa added and two not included in our analysis. A second group, the *Johnstonella/Albidae* clade, encompasses the two included species of *Johnstonella, J. angustifolia,* and *J. racemosa,* plus the North American *C. albida, C. mexicana,* and *C. texana* and the South American species *C. hispida.* None of these four *Cryptantha* taxa were included by Hasenstab-Lehman and Simpson (2012); thus, their placement with *Johnstonella* is novel (see below). Lastly, a clade of the remaining *Cryptantha* species is recovered in all analyses. This *Cryptantha* core clade is largely compatible with *Cryptantha* s. s. 1 of Hasenstab-Lehman and Simpson (2012), but with a large addition of samples and a few of their taxa not included.

The ML/BI concatenated tree (Fig. 5) provides stronger support for the placement of the three major clades in relation to one another and to other genera than any of the three separate gene trees. In our concatenated analyses the *Johnstonella/Albidae* clade is recovered as sister to the *Cryptantha*
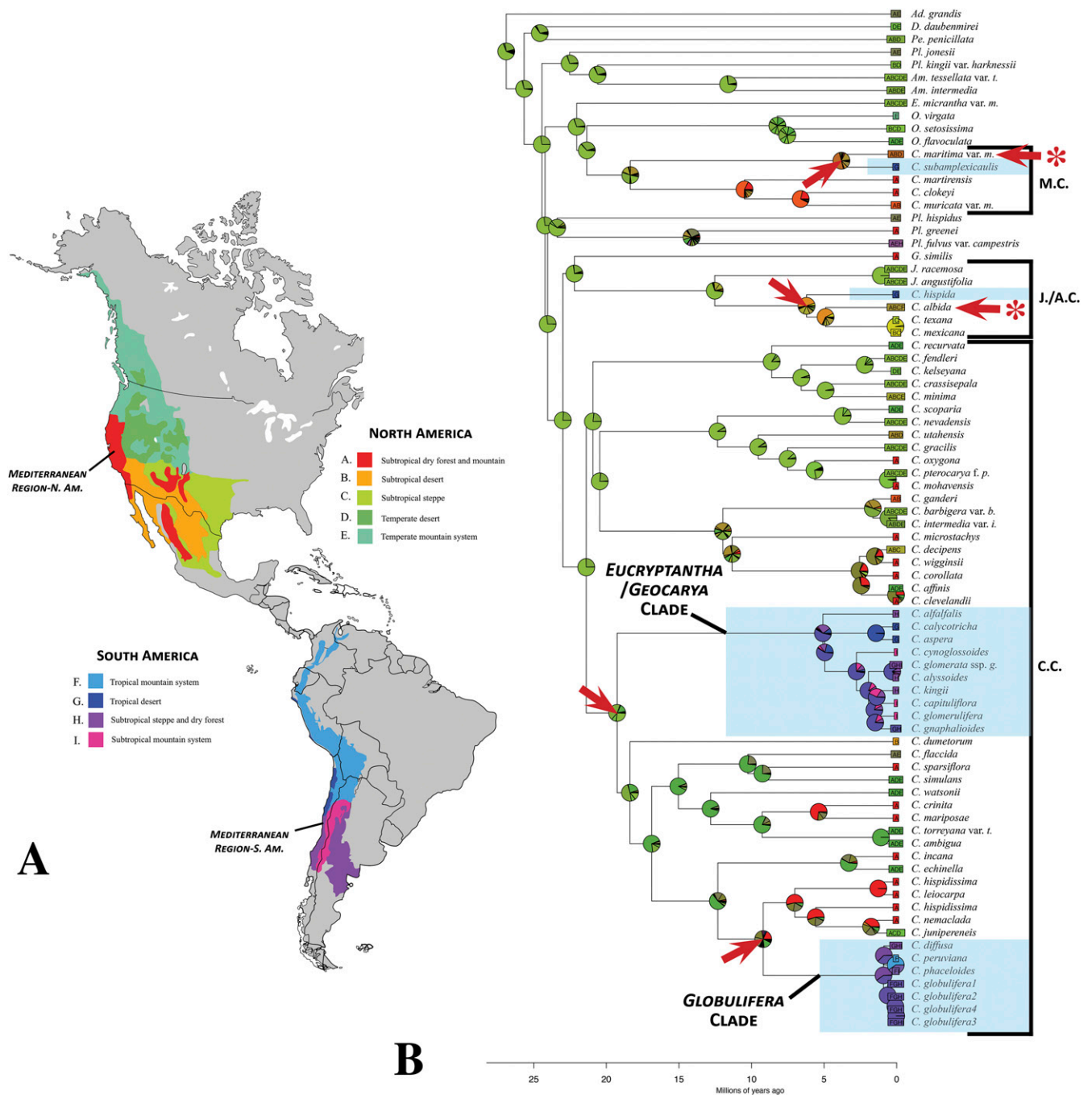
FIG. 10. A. Global Ecological Zones of North and South America (after Davis and Holmgren 2001), used for determining species boundaries for BioGeoBEARS (Matzke 2012, 2013). B. BioGeoBEARS graphical output, showing the most likely ancestral range for *Cryptantha*. Legend for ecological zones: A (red) = North America subtropical dry forest and mountain system, B (orange) = North America subtropical desert, C (light green) = North America subtropical steppe, D (green) = North America temperate desert, E (blue green) = North America temperate mountain system, F (light blue) = South America tropical mountain system, G (blue) = South America tropical desert, H (purple) = South America subtropical steppe and dry forest, I (pink) = South America subtropical mountain system. Major clades indicated are: J./A.C. = *Johnstonella/Albidae* clade; C.C. = *Cryptantha* core clade; M.C. = *Maritimae* clade. South American species are highlighted in blue. Arrows = *Cryptantha* dispersal events from North America to South America. * = Inferred very recent dispersals of *C. albida* and *C. maritima*, species that occur in both continents.

core clade with marginally mixed support (BS = 89; PP = 0.65) (Fig. 5). *Greeneocharis* is sister to these two sister groups with good support (BS = 100; PP = 0.90), and a clade of three *Plagiobothrys* species is sister to all of these with strong support (BS = 90; PP = 1.0). The *Maritimae* clade forms a strongly supported group sister to *Oreocarya* with strong support (BS = 83; PP = 1.0) and these two sister to *Eremocarya* with strong support (BS = 100; PP = 1.0). Most differences between our

study and Hasenstab-Lehman and Simpson (2012) are with regard to the placement of other Amsinckiinae genera in relation to the clades containing *Cryptantha* species. This is not surprising due to the uncertainties along the backbone of the tree in many of the analyses.

The ASTRAL-II tree, using a coalescent algorithm, recovered the same three clades, *Cryptantha* core, *Johnstonella/Albidae*, and *Maritimae*, with strong support (Fig. 6). However,

this analysis failed to find strong support for the relationships among them, especially along the backbone of the tree (Fig. 6), and shows differences in interrelationships of these and other major clades in comparison to other analyses. Given that only three gene trees were used as input, this ASTRAL-II species tree estimate may not be accurate for species tree inference. Simulations show that summary statistic coalescence methods require many gene trees (more than three) to accurately recover the true species tree (Mirarab et al. 2014).

Recovery of four species of *Cryptantha* (*C. albida*, *C. hispida*, *C. mexicana*, and *C. texana*) as part of a strongly supported *Johnstonella/Albidae* clade was an intriguing discovery. *Cryptantha albida* (the sole member of Johnston's 1925 series *Albidae*) and *C. mexicana* (which Johnston 1961 cites as similar to *C. albida*) both share morphological features with the genus *Johnstonella*. These two *Cryptantha* species have whitish tubercles and nutlets that are triangular in shape, similar to species of *Johnstonella* (Hasenstab-Lehman and Simpson 2012; Simpson et al. 2014). *Cryptantha hispida* has ovate-triangular nutlets with sharp margins; all features typical of many *Johnstonella* species (see Hasenstab-Lehman and Simpson 2012). In fact, as mentioned earlier, in the nrDNA gene tree *C. hispida* is more closely related to the two included *Johnstonella* species than to members of the *Albidae* group. *Cryptantha hispida* differs from *Johnstonella* taxa in having smooth to rugulose nutlets that are generally two per fruit. But the nutlet outline and margin shape similarities support its close relationship to the latter. Lastly, *Cryptantha texana*, which was included in Johnston's (1925) series *Texanae*, has nutlets that don't resemble those of *Johnstonella*. Nutlets of *C. texana* bear some similarities to the odd nutlet of the heteromorphic *C. minima* and to a lesser degree to *C. crassisepala*, but both of the last two taxa are nested within the *Cryptantha* core clade. In many ways, *Cryptantha texana* is unique in the genus, having solitary, densely papillate nutlets.

The species composition of the *Maritimae* clade was a little more surprising. Hasenstab-Lehman and Simpson (2012) also recovered a clade (which they termed *Cryptantha* s. s. 2) including a North American sample of *C. maritima* and samples of South American collections of *C. chaetocalyx* (Philippi) I. M.Johnst., *C. grandulosa* (Ruiz & Pavon) I.M.Johnst., and *C. maritima*. Unfortunately, the latter three samples did not pass quality control for library prep in this study and were not included in our analyses. However, we are hypothesizing that these three taxa would nest within the *Maritimae* clade and plan to include them in future studies. The placement of the North American species *C. clokeyi*, *C. martirensis*, and *C. muricata* in this clade is unexpected. These three species, not sampled by Hasenstab-Lehman and Simpson (2012), are obvious close relatives of one another. *Cryptantha clokeyi* and *C. muricata* are both members of Johnston's series *Muricatae* (Johnston 1925, 1939). *Cryptantha martirensis* is a recently described segregate species of *C. muricata* (Simpson and Rebman 2013), proposed by these authors to belong to *Muricatae*, which our study confirms. Johnston diagnosed section *Muricatae* as "Nutlets 4, verrucose or coarsely tuberculate, triangular-ovate, decidedly homomorphous, back obtuse, and bearing a suggestion of a medial ridge, with sides evidently angled and beaded; style usually surpassing the nutlets though rarely only equaling them." No distinctive morphological features are apparent between the members of *Muricatae* and the other members of our *Maritimae* clade, yet the latter is strongly supported as monophyletic in all analyses. As previously discussed, the *Maritimae* clade is sister to *Oreocarya* and these two sister to *Eremocarya* in the concatenated analysis, all with strong support (Fig. 5). However, the *Maritimae* clade is sister to the *Cryptantha* core clade in the ASTRAL-II analysis, but with weak support (Fig. 6). No uniting, non-molecular apomorphy is currently known for the *Maritimae* clade, a group warranting additional study. It is intriguing, however, that *C. maritima* of the *Maritimae* clade has a chromosome number ($2n = 20$; Las Peñas 2003) different from that of other known North American *Cryptantha*, *Eremocarya*, *Greeneocharis*, or *Oreocarya* taxa, which have a base number of $n = 6$ or $n = 12$ (Higgins 1971; Grau 1983; Sivinski 1993).

The *Cryptantha* core clade, strongly supported in all but the mtDNA analysis, exhibits no clear morphological apomorphies. With regard to the members of this clade, the sections and series proposed by Johnston (1925, 1927, 1939, 1961) and supplemented by others (Grau 1981; Simpson and Rebman 2013; see Table 1) are supported only in part by our analyses. Our *Eucryptantha/Geocarya* clade contains only members of the cleistogamous sections *Eucryptantha* and *Geocarya* of Johnston (1927) with one exception: *Cryptantha gnaphalioides*, which belongs to Johnston's *Cryptantha* section *Krynitzkia* and does not exhibit cleistogamy. Interestingly, *C. gnaphalioides* has a perennial duration, like many *Eucryptantha* and *Geocarya* species. Although additional sampling of this taxon is warranted in future studies to verify its position within the clade, a tentative hypothesis is that *C. gnaphalioides* lost cleistogamy and should be classified as a member of this group. However, within the *Eucryptantha/Geocarya* clade, neither of these two sections as defined by Johnston is monophyletic, section *Eucryptantha* being paraphyletic and section *Geocarya* polyphyletic (Figs. 2–6). Our sample size is insufficient to evaluate the series of Johnston (1927) within these two sections (Table 1).

Johnston's third section of *Cryptantha*, section *Krynitzkia* (Table 1), is paraphyletic within the *Cryptantha* core clade and polyphyletic when considering trees as a whole (agreeing with Hasenstab-Lehman and Simpson 2012), many members occurring in the *Johnstonella/Albidae* or *Maritimae* clades. This is not surprising, given that section *Krynitzkia* appears to be diagnosed by a symplesiomorphy, the absence of cleistogamous flowers as are found in sections *Cryptantha* and *Geocarya* (Johnston 1927). Johnston's (1925, 1927) series were described using only morphological characteristics, and, as suggested by the results from the character evolution analysis, many of these traits are evolutionarily plastic (see Character Evolution). Although our sample size is not large enough to propose a revised intergeneric classification of *Cryptantha*, a few taxonomic trends are worth noting. (See Figs. 5 and 6, in which we denote Johnston's sections and series on the cladograms. We do not further discuss the infrageneric classification of Brand 1931, as we found very little phylogenetic correspondence relative to our study.)

Four members of series *Ambiguae*, *C. ambigua* (A.Gray) Greene, *C. crinita* Greene, *C. mariposae* I.M.Johnst., and *C. torreyana* (A.Gray) Greene, form a strongly supported clade. Among the other three sequenced members of this series, *C. echinella* and *C. incana* are sister species of a clade, but *C. simulans* Greene is more distantly related.

Series *Barbigerae* is highly polyphyletic. However, *Cryptantha barbigera* var. *b*. and *C. intermedia* (A.Gray) Greene of this series (long considered close relatives; Johnston 1925) are sister taxa, as are *C. nevadensis* A.Nelson & P.B.Kenn. and *C. scoparia*

A.Nelson in a more distant clade. Our strongly-supported South American *Globulifera* clade contains three species of series *Barbigerae, C. diffusa* (Phil.) I.M.Johnst., *C. globulifera,* and *C. peruviana,* but other members of this series are scattered among six different lineages or clades of the *Cryptantha* core clade and the *Maritimae* clade.

Cryptantha flaccida (Douglas ex Lehm.) Greene and *C. sparsiflora* (Greene) Greene, the two sequenced species of series *Flaccidae,* form a strongly supported clade along with *C. simulans* of section *Ambiguae.* Series *Leiocarpae* is polyphyletic, with members occurring in five lineages/clades. However, *Cryptantha leiocarpa* (Fisch. & C.A.Mey.) Greene, *C. hispidissima* Greene, and *C. nemaclada* Greene of that series form a strongly supported clade along with *C. juniperensis* R.B.Kelley and M.G. Simpson of series *Barbigerae.*

The two members of series *Mohavenses, C. mohavensis* (Greene) Greene and *C. watsonii* (A.Gray) Greene, are well separated in our analyses, arguing against the integrity of this series. As previously discussed, *Cryptantha clokeyi, C. martirensis,* and *C. muricata,* the three species of series *Muricatae,* form a well-supported clade within the *Maritimae* clade. *Cryptantha hispida* and *C. phaceloides* (Clos) Reiche, the two sequenced species of series *Phaceloides,* are well-separated from one another. *Cryptantha hispida* (as previously discussed) nests within the *Johnstonella/Albidae* clade, and *C. phaceloides* nests within the *Globulifera* clade of the *Cryptantha* core clade.

Cryptantha oxygona (A.Gray) Greene, *C. pterocarya* (Torr.) Greene, and *C. utahensis* (A.Gray) Greene, the only three species of series *Pterocaryae,* form a strongly-supported clade with two other species: *C. mohavensis* (series *Mohavenses*) and *C. gracilis* Osterh. (of the monotypic series *Graciles*). Finally, *C. crassisepala, C. kelseyana* Greene, and *C. minima* of series *Texanae* form a clade along with *C. fendleri* of series *Ramosissimae.* However, *Cryptantha texana,* the other sequenced member of series *Texanae,* falls firmly within the *Johntonella/Albidae* clade.

Interestingly, *Cryptantha nevadensis* and *C. juniperensis* are well separated in our trees, supporting the recognition of the latter as a separate species, as opposed to a variety of C. nevadensis [*C. nevadensis* var. *rigida* I.M.Johnst.]; see Simpson and Kelley (2017). Similarly, *C. clevelandii* and *C. hispidissima* are well separated, supporting the recognition of the latter as a separate species as opposed to a variety of *C. clevelandii* [*C. clevelandii* var. *florosa* I.M.Johnst.].

Character Evolution—Ancestral state reconstruction for nutlet number per fruit showed no strong pattern for the ancestral condition (Fig. 7). Hasenstab-Lehman and Simpson (2012) inferred four nutlets per fruit as ancestral and results here do not conflict with that, but neither are they strongly supportive. The family Boraginaceae (sensu Luebert et al. 2016) is characterized as having four-lobed ovaries, each lobe at maturity typically developing into one unit fruit (the nutlet), containing a single seed. Many species in the subtribe Amsinckiinae consistently produce fruits with a reduced nutlet number by ovule abortion, a feature used to delimit several taxa. One finding that corroborates Hasenstab-Lehman and Simpson (2012) is that a reduced (one-two) nutlet number is found to be apormorphic for the South American cleistogamous taxa, our *Cryptantha/Geocarya* clade.

Plant duration is recovered as being ancestrally annual for all major *Cryptantha* clades (Fig. 8). A perennial duration is shown to have evolved at least twice in the South American *Euryptantha/Geocarya* clade. The advantage of a perennial plant duration may correlate with a high elevation habitat; however, more samples from South America would be needed to test this hypothesis. Early conjectures by Johnston (1925) and Higgins (1971) suggested that a perennial duration, which is found in all *Oreocarya,* was the ancestral condition for this complex. Results found here, however, agree with Hasenstab-Lehman and Simpson (2012) that a perennial duration is derived in the complex.

Cleistogamy, a specialized type of self-pollination, evolved once in *Cryptantha* (Fig. 9), an apomorphy for the *Eucryptantha/Geocarya* clade. These taxa are distinguished in having cleistogamous flowers in either the middle and lower regions of inflorescence units of the plant (*Cryptantha* section *Eucryptantha*) or near the base of the plant with modified nutlets, the cleistogenes (*Cryptantha* section *Geocarya*). (As discussed earlier, *C. gnaphalioides* is the exception in this clade, hypothesized here to have lost cleistogamy.) Maximum likelihood reconstruction strongly supports normal cleistogamy evolving prior to clestogenes. Cleistogenes evolved up to three times from this ancestral non-cleistogenic cleistogamy. Interestingly, one species in Johnston's (1927) *Cryptantha* section *Geocarya* that we included, *C. cynoglossoides,* was placed by Grau (1981) in *Cryptantha* section *Eucryptantha* [=*Cryptantha* section *Cryptantha*] along with *C. dimorpha.* His reasoning was that, although these species have ground-level cleistogamic fruits, these are similar in morphology to those that form in the cauline leaves, thus resembling other members of *Cryptantha* section *Eucryptantha* (and possibly represent a type of intermediate condition). These species may provide supporting evidence that once cleistogamy evolved, the transition to cleistogene may be more labile than previously thought. A possible advantage of cleistogamy is the ability to produce offspring without the presence of pollinators. Given that the *Eucryptantha/Geocarya* clade is the product of a single, long-distance dispersal (see below), the possible absence of pollinators in a novel environment may have constituted the selective pressure for self-pollination via cleistogamy.

Chromosome number, a character not traced in our cladograms, shows some interesting trends. Of the included members of our *Eucryptantha/Geocarya* clade for which chromosome numbers are known, all have an elevated count of $2n = 62$ (*C. kingii*), $2n = 64$ (*C. calycotricha, C. capituliflora, C. glomerata* [also $2n = 124$]), or $2n = 120$ (*C. alfalfalis*) (Grau 1983; Las Peñas 2003). This contrasts with $2n = 12$ (*C. barbigera*), $2n = 20$ (*C. maritima*), $2n = 24$ (*C. affinis, C. barbigera, C. pterocarya*) of North American *Cryptantha* and $2n = 14$ (*C. diffusa, C. globulifera*) or $2n = 56$ (*C. diffusa*) of South American *Cryptantha* in *Cryptantha* section *Krynitzkia* (Rattenbury 1959; Grau 1983; Ward 1983; Sivinski 1993; Las Peñas 2005), this second count for *C. diffusa* being the exception. Polyploidy in *Cryptantha* is only known in these South American clades and, at least in the *Eucryptantha/Geocarya* clade, may possibly be associated with the evolution of both cleistogamy and a perennial plant duration. Future evolutionary development studies may help determine if this correlation is also causal.

Biogeographical Inference and Divergence Time Estimation—Four unidirectional dispersals of *Cryptantha* taxa from North to South America are inferred from our analyses (Fig. 10). This pattern of unidirectional dispersal from north to south agrees with studies of other plant taxa that are amphitropically distributed (Raven 1963; Moore et al. 2006; Simpson et al. 2017b). In the *Cryptantha* core clade, using all three fossils and the date recovered by Chacón et al. (2017) for the Amsickiinae crown node as calibration, the

Eucryptantha/Geocarya clade diverged from North American taxa at about 19.26 Ma (clade stem node) and diversified at about 5.08 Ma (clade crown node; Table 2). The first uplift of the Andes occurred around 20–30 Ma (Houston and Hartley 2003; Rech et al. 2010), which may have resulted in the establishment of new topographic niches. Thus, dispersals into the newly uplifted Andes could have been a potential causative factor in the establishment and diversification of this clade. Heibl and Renner (2012) proposed that the Mediterranean region of Chile acted as a refuge for species unable to adapt to harsh environments such as high elevation habitats or the hyper-aridity of the Atacama Desert. The one dispersal of *C. gnaphalioides* back to the Mediterranean South America region within this clade may provide support for this hypothesis.

Also within the *Cryptantha* core clade, the *Globulifera* clade diverged from North American taxa at about 9.20 Ma (clade stem node) and diversified at around 0.91 Ma (clade crown node; Table 2). The earlier divergence date roughly corresponds with the end of the second pulse of the Andean uplift (5–10 Ma; Houston and Hartley 2003; Rech et al. 2010). The common ancestor of this clade is inferred to have occurred in what is today the Mediterranean South America region. Taxa belonging to this clade lack cleistogamic flowers and are more similar to the North American counterparts in *Cryptantha* section *Krynitzkia*. The common ancestor of two species of this clade, *C. peruviana* and *C. phaceloides*, was widespread in the Mediterranean region and tropical Andes, but subsequently went extinct in Mediterranean South America; currently extant taxa occur only in the tropical part of the Andes (Fig. 10).

Cryptantha hispida, a South American member of the *Johnstonella/Albidae* clade, diverged from North American taxa at about 6.21 Ma (stem node; Table 2). Within this clade, *C. albida* occurs in both North and South America; thus, the South American populations of this species (samples not included in our analyses) are likely indicative of another, very recent dispersal event. The distribution of the North American species of *C. albida* in deserts of North America may have pre-adapted these South American species for life in one of the driest regions of the world, the Atacama Desert.

Cryptantha subamplexicaulis, a South American member of the *Maritimae* clade, diverged from North American taxa at about 3.81 Ma (stem node; Table 2). This species is currently found in the tropical desert ecoregion (Atacama Desert) of South America. Its common ancestor with *C. maritima* could have originated from a number of North American ecoregions (Fig. 10). *Cryptantha maritima*, like *C. albida* of the *Johnstonella/Albidae* clade, occurs in both North and South America. Although we were not able to include any samples of South American populations of this species, the fact that it is present in both continents suggests that its South American counterparts are the product of a recent dispersal event. In North America *C. maritima* is present in the subtropical dry forest and mountain, subtropical desert, and temperate desert regions; in South American it occurs in the subtropical mountains.

Two other South American species (not included here) presumed to belong to the Maritimae clade, as based on Hasenstab-Lehman and Simpson 2012, occur in the Atacama region of Chile (*C. chaetocalyx*), and Peru (*C. granulosa*). Although intriguing patterns of dispersal within South America emerge, these results should be considered preliminary. The inclusion of additional South American taxa in future analyses will contribute greatly to a better understanding of the history of this group, including evaluations of the hypothesis that these diversification events correlate with the hyperaridity of the Atacama Desert 10–15 Ma (Houston and Hartley 2003; Rech et al. 2010).

The genus *Cryptantha*, even after removal of four segregate genera, is confirmed to be non-monophyletic. This study strongly supports the existence of three major clades that contain species of *Cryptantha* as it is currently defined, termed here the *Cryptantha* core clade, the *Maritimae* clade, and the *Johnstonella/Albidae* clade. The former two clades largely correspond with, respectively, the *Cryptantha* s. s. 1 and s. s. 2 groups of Hasenstab-Lehman and Simpson (2012). However, the placement of *C. albida* and relatives in the *Johnstonella/Albidae* clade is a novel discovery in our study. The placement of these three clades and other genera within the Amsinckiinae, however, varies in different analyses. Future nomenclatural changes, including the possible expansion of *Johnstonella* or the naming of new genera, will likely be needed.

Character analysis based on the phylogenetic studies indicates that the ancestral condition for *Cryptantha* was: 1) one to four nutlets per fruit; 2) plants annual in duration; and 3) flowers chasmogamous. The possible adaptive significance of the derived conditions is not always clear. However, cleistogamy (and its more specialized manifestation, cleistogenes), which occurs only in South American species, may have functioned as means of ensuring seed set in the absence of pollinators, at the time when these taxa were dispersed to their novel environment. The evolution of polyploidy in the *Cryptantha/Geocarya* subclade of the *Cryptantha* core clade is associated with, and could possibly be causally related to, cleistogamy and/or a perennial plant duration.

Four unidirectional dispersals of *Cryptantha* taxa from North to South America were recovered in the biogeographic analysis and two additional ones are inferred to represent very recent dispersals. How these plants dispersed to South America is still a matter of conjecture. No known observations of birds feeding on or near *Cryptantha* plants have been documented. However, migratory birds flying south, perhaps in a single uninterrupted flight, are still the best hypothesis to explain this pattern. Collins (1974) notes the occurrence of bristly (hispid) calyces enclosing the fruits of many Boraginaceae with the likely possibility of these propagules being transported on the feathers of birds. Collins (1974) also reviews possible bird species vectors, such as the black-bellied (gray) plover (*Pluvialis squatarola*), which migrates long distances from North America to as far south as Chile and Argentina. Lewis et al. (2014) cite the first observation of plant propagules on long-distance migratory birds, proving the possibility of their being transported. There are no known fossils of *Cryptantha* plants, nutlets, or pollen in the tropics, indicating that these species may never have occurred there or could not establish there, supporting the hypothesis that the amphitropical distribution was caused by long-distance dispersal, not vicariance of a widespread population with subsequent extinction of plants in the tropics.

Although the three major clades that include *Cryptantha* taxa are consistently recovered, their placements relative to one another and to other genera of the Amsinckiinae are unclear, varying in our different analyses. Future work must include additional representatives of all taxa in the Amsinckiinae in order to establish strong support for these relationships in order to carry out complete taxonomic revisions. This study

is a crucial first step in determining the sampling for these future studies. It also provides support of hypotheses for the dispersal patters of amphitropically distributed plants. Understanding the timing, direction, and frequency of dispersal between North and South America in *Cryptantha* gives insight to the origin of the great biodiversity of these regions and informs future studies on other species that share this distribution.

LITERATURE CITED

Akaike, H. 1974. A new look at statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

Alvarez, I. and J. F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

APGIV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.

Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and D. L. Wheeler. 2005. GenBank. *Nucleic Acids Research* 33: D34–D38.

Boellstorff, J. 1976. The succession of late Cenozoic volcanic ashes of the Great Plains—A progress report. Pp. 37–71 in *Guidebook, 24th Annual Meeting of the Midwestern Friends of the Pleistocene*, ed. C. K. Bayne.

Boellstorff, J. 1978. Chronology of some late Cenozoic deposits from the central United States and the Ice Ages. *Transactions of the Nebraska Academy of Sciences* 6: 35–49.

Brand, A. 1931. Borraginaceae-Borraginoideae-Amsinckieae. Pp. 204 in *Das Pflanzenreich* 97, ed. A. Engler. Leipzig: Verlag von Wilhelm Engelmann.

Burge, D. O., J. H. Thorne, S. P. Harrison, B. C. O'Brien, J. P. Rebman, J. R. Shevock, E. R. Alverson, L. K. Hardison, J. D. Rodrguez, S. A. Junak, T. A. Oberbauer, H. Riemann, S. E. Vanderplank, and T. Barry. 2016. Plant diversity and endemism in the California Floristic Province. *Madroño* 63: 3–206.

Chacón, J., F. Luebert, H. H. Hilger, S. Ovcinnikova, F. Selvi, L. Cecchi, C. M. Guilliams, K. Hasenstab-Lehman, K. Sutorý, M. G. Simpson, and M. Weigend. 2016. The borage family (Boraginaceae s.str.): A revised infrafamilial classification based on new phylogenetic evidence, with emphasis on the placement of some enigmatic genera. *Taxon* 65: 523–546.

Chacón, J., F. Luebert, and M. Weigend. 2017. Biogeographic events are not correlated with diaspore dispersal modes in Boraginaceae. *Frontiers in Ecology and Evolution* 5 (Article 26): 1–14.

Cohen, J. I. 2014. A phylogenetic analysis of morphological and molecular characters of Boraginaceae: Evolutionary relationships, taxonomy, and patterns of character evolution. *Cladistics* 30: 139–169.

Cohen, J. I. 2015. *Adelinia* and *Andersonglossum* (Boraginaceae), two new genera from New World species of *Cynoglossum*. *Systematic Botany* 40: 611–619.

Collins, R. S. 1974. *Mechanisms for long-distance dispersal in some amphitropically disjunct plant species*. M.A. thesis. Claremont, California: Claremont Graduate School.

Consortium of California Herbaria. 2016. Data provided by the participants of the Consortium of California Herbaria. ucjeps.berkeley.edu/consortium (last accessed 12 Aug 2017).

Davis, R. and P. Holmgren. 2001. *FRA 2000: Global ecological zoning for the global forest resources assessment 2000 Final report*. Forest Resources Assessment Programme. Rome.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.

Elias, M. K. 1932. Grasses and other plants from the Tertiary Rocks of Kansas and Colorado. *The University of Kansas Science Bulletin* 20: 333–367.

Elias, M. K. 1942. Tertiary prairie grasses and other herbs from the high plains. *Geological Society of America* Special Papers 41: 1–176.

Friar, E. A. 2005. Isolation of DNA from plants with large amounts of secondary metabolites. *Methods in Enzymology* 395: 1–12.

Grau, J. 1981. Zwei neue Arten der Gattung *Cryptantha* Lehm. (Boraginaceae) und ihre systematische Stellung. *Mitteilungen der Botanischen Staatssammlung München* 17: 511–526.

Grau, J. 1983. Life form, reproductive biology and distribution of the Californian/Chilean genus *Cryptantha*. Pp. 231–240 in *Sonderbände des Naturwissenshaftlichen Vereins in Hamburg* 7, ed. K. Kubitski. Hamburg: P. Pary.

Hasenstab-Lehman, K. E. and M. G. Simpson. 2012. Cat's eyes and popcorn flowers: Phylogenetic systematics of the genus *Cryptantha* s.l. (Boraginaceae). *Systematic Botany* 37: 738–757.

Heibl, C. and S. S. Renner. 2012. Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. *Systematic Biology* 61: 823–834.

Higgins, L. C. 1971. A revision of *Cryptantha* subgenus *Oreocarya*. *Brigham Young University Science Bulletin Biological Series* 8: 1–62.

Houston, J. and A. J. Hartley. 2003. The central Andean west-slope rainshadow and its potential contribution to the origin of hyper-aridity in the Atacama Desert. *International Journal of Climatology* 23: 1453–1464.

Johnston, I. M. 1924. Studies in the Boraginaceae II. A synopsis of the American native and immigrant Borages of the subfamily Boraginoideae. *Contributions from the Gray Herbarium of Harvard University* 70: 3–61.

Johnston, I. M. 1925. Studies in the Boraginaceae IV. The North American species of *Cryptantha*. *Contributions from the Gray Herbarium of Harvard University* 74: 1–114.

Johnston, I. M. 1927. Studies in the Boraginaceae VI. A revision of the South American Boraginoideae. *Contributions from the Gray Herbarium of Harvard University* 78: 1–118.

Johnston, I. M. 1937. Studies in the Boraginaceae XII. Novelties and critical notes. *Journal of the Arnold Arboretum* 18: 23–24.

Johnston, I. M. 1939. Studies in the Boraginaceae, XIII: New or otherwise noteworthy species, chiefly from western United States. *Journal of the Arnold Arboretum* 20: 375–398.

Johnston, I. M. 1961. Notes on some Texan borages. *Wrightia* 2: 158–162.

Kartesz, J. T. 2014. The Biota of North America Program (BONAP). North American Plant Atlas (http://www.bonap.org/MapSwitchboard. html). Chapel Hill, North Carolina [maps generated from Kartesz, J.T. 2010. Floristic Synthesis of North America, Version 1.0. Biota of North America Program (BONAP). (in press)].

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies, and A. Drummond. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Knoop, V. 2004. The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. *Current Genetics* 46: 123–139.

Landis, M., N. J. Matzke, B. R. Moore, and J. P. Huelsenbeck. 2013. Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology* 62: 789–804.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.

Långström, E. and M. W. Chase. 2002. Tribes of Boraginoideae (Boraginaceae) and placement of *Antiphytum, Echiochilon, Ogastemma* and *Sericostoma*: A phylogenetic analysis based on *atpB* plastid DNA sequence data. *Plant Systematics and Evolution* 234: 137–153.

Las Peñas, M. L. 2003. Estudios cromosómicos de dos especies argentinas de *Cryptantha* (Boraginaceae). *Kurtziana* 30: 77–79.

Las Peñas, M. L. 2005. Números cromosómicos de *Cryptantha diffusa* y *C. maritima* (Boraginaceae). *Bulletin of the Botanical Society of Argentina* 40: 283–284.

Lewis, L. R., E. Behling, H. Gousse, E. Qian, C. S. Elphick, J.-F. Lamarre, J. Bety, J. Liebezeit, R. Rozzi, and B. Goffinet. 2014. First evidence of bryophyte diaspores in the plumage of transequatorial migrant birds. *PeerJ* 2: e424.

Ludvigson, G. A., R. S. Sawin, E. K. Franseen, W. L. Watney, R. R. West, and J. J. Smith. 2009. A review of the stratigraphy of the Ogallala Formation and revision of Neogene ("Tertiary") nomenclature in Kansas. *Current Research in Earth Sciences. Bulletin* 256 (part 2): 1–9.

Luebert, F., L. Cecchi, M. W. Frohlich, M. Gottschling, C. M. Guilliams, K. E. Hasenstab-Lehman, H. H. Hilger, J. S. Miller, M. Mittelbach, M. Nazaire, M. Nepi, D. Nocentini, D. Ober, R. G. Olmstead, F. Selvi, M. G. Simpson, K. Sutorý, B. Valdés, G. K. Walden, and M. Weigend. 2016. Familial classification of the Boraginales. *Taxon* 65: 502–522.

Mabberley, D. J. 2008. *Mabberley's Plant-Book: A Portable Dictionary of the Higher Plants, Their Classification and Uses.* Ed. 3. Cambridge: Cambridge University Press.

Maddison, W. P. and D. R. Maddison. 2010. Mesquite: A modular system for evolutionary analysis. Version 2.73. http://mesquiteproject.org.

Matzke, N. J. 2012. Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in dispersal–extinction–cladogenesis (DEC) analyses. *Frontiers of Biogeography* 4: 210.

Matzke, N. J. 2013. BioGeoBEARS: BioGeography with Bayesian (and Likelihood) Evolutionary Analysis in R Scripts. University of California, Berkeley, Berkeley, CA. https://cran.r-project.org/web/packages/BioGeoBEARS/index.html (last accessed February 2015).

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 in *Proceedings of the Gateway Computing Environments Workshop (GCE).* New Orleans: Gateway Computing.

Mirarab, S., R. Reaz, M. S. Bayzid, T. Zimmerman, M. S. Swenson, and T. Warnow. 2014. ASTRAL: Genome-scale coalescent-based species tree estimation. *Bioinformatics* 30: 541–548.

Mirarab, S. and T. Warnow. 2015. ASTRAL-II: Coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. *Bioinformatics* 31: i44–i52.

Moore, M. J., A. Tye, and R. K. Jansen. 2006. Patterns of long-distance dispersal in *Tiquilia* subg. *Tiquilia* (Boraginaceae): Implications for the origins of amphitropical disjuncts and Galapagos Islands endemics. *American Journal of Botany* 93: 1163–1177.

Nazaire, M. and L. Hufford. 2012. A broad phylogenetic analysis of Boraginaceae: Implications for the relationships of *Mertensia*. *Systematic Botany* 37: 758–783.

Otero, A., P. Jiménez-Mejías, V. Valcárcel, and P. Vargas. 2014. Molecular phylogenetics and morphology support two new genera (*Memoremea* and *Nihon*) of Boraginaceae s. s. *Phytotaxa* 173: 241–277.

Payson, E. B. 1927. A monograph of the section Oreocarya of *Cryptantha*. *Annals of the Missouri Botanical Garden* 14: 211–358.

Rambaut, A. 2014. FigTree v. 1.4.2: Tree drawing tool. http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A., M. A. Suchard, D. Xie, and A. J. Drummond. 2014. Tracer v. 1.6. http://beast.bio.ed.ac.uk/Tracer.

Rattenbury, J. A. 1959. Chromosome number publication. *Madroño* 15: 49–52.

Raven, P. H. 1963. Amphitropical relationships in the floras of North and South America. *The Quarterly Review of Biology* 38: 151–177.

Rech, J. A., B. S. Currie, E. D. Shullenberger, S. P. Dunagan, T. E. Jordan, N. Blaneo, A. J. Tomlinson, H. D. Rowe, and J. Houston. 2010. Evidence for the development of the Andean rain shadow from a Neogene isotopic record in the Atacama Desert, Chile. *Earth and Planetary Science Letters* 292: 371–382.

Ree, R. H. and S. A. Smith. 2008. Lagrange (Software for likelihood analysis of geographic range evolution), v. 2. Distributed by the authors at https://code.google.com/archive/p/lagrange. (last accessed February 2015.).

Rieseberg, L. H. and D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* 5: 65–84.

Rieseberg, L. H. and J. F. Wendel. 1993. Introgression and its consequences in plants. Pp. 70–109 in *Hybrid Zones and the Evolutionary Process*, ed. R. Harrison. Oxford: Oxford University Press.

Ripma, L., M. G. Simpson, and K. Hasenstab-Lehman. 2014. Geneious! Simplified genome skimming methods for phylogenetic systematic studies: A case study in *Oreocarya* (Boraginaceae). *Applications in Plant Sciences,* doi: 10.3732/apps.1400062.

Ronquist, F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Systematic Biology* 46: 195–203.

Segal, R. 1964. Nomenclatural changes in fossil species of *Cryptantha*. *Transactions of the Kansas Academy of Science* 67: 203.

Segal, R. 1966. Taxonomic study of the fossil species of the genus *Cryptantha* (Boraginaceae). *The Southwestern Naturalist* 11: 205–210.

SEINet. 2016. Southwest Environmental Information Network. http//: swbiodiversity.org/seinet/index.php. (last accessed 4 August 2017).

Seo, T. K. 2008. Calculating bootstrap probabilities of phylogeny using multilocus sequence data. *Molecular Biology and Evolution* 25: 960–971.

Simpson, M. G. and K. E. Hasenstab. 2009. *Cryptantha* of Southern California. *Crossosoma* 35: 1–59.

Simpson, M. G., K. Hasenstab-Lehman, and R. B. Kelley. 2014. Typification of the name *Johnstonella* (Boraginaceae). *Taxon* 63: 930–931.

Simpson, M. G. and R. B. Kelley. 2017. *Cryptantha nevadensis* var. *rigida* elevated to species with a new name, *C. juniperensis. Phytotaxa* 295: 227–236.

Simpson, M. G. and J. P. Rebman. 2013. A new species of *Cryptantha* (Boraginaceae) from the Sierra de San Pedro Mártir, Baja California, Mexico. *Madroño* 60: 35–45.

Simpson, M. G., C. M. Guilliams, K. E. Hasenstab-Lehman, M. E. Mabry, and L. Ripma. 2017a. Phylogeny of the popcorn flowers: Use of genome skimming to evaluate monophyly and interrelationships in subtribe Amsinckiinae (Boraginaceae). *Taxon* 66: 1406–1420.

Simpson, M. G., L. A. Johnson, T. Villaverde, and C. M. Guilliams. 2017b. American Amphitropical Disjuncts: Perspectives from vascular plant analyses and prospects for future research. *American Journal of Botany* 104: 1600–1650.

Sivinski, R. C. 1993. Chromosome numbers for some North American *Cryptantha. Phytologia* 74: 459–463.

Smith, S. A. and B. C. O'Meara. 2012. TreePL: Divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics* 28(20): 2689–2690.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.

Straub, S. C. K., M. Fishbein, T. Livshultz, Z. Foster, M. Parks, K. Weitemier, R. C. Cronn, and A. Liston. 2011. Building a model: Developing genomic resources for common milkweed (*Asclepias syriaca*) with low coverage genome sequencing. *BMC Genomics* 12: 211.

Straub, S. C. K., M. Parks, K. Weitemier, M. Fishbein, R. C. Cronn, and A. Liston. 2012. Navigating the tip of the genomic iceberg: Next-generation sequencing for plant systematics. *American Journal of Botany* 99: 349–364.

Thiers, B. 2017. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/.

Ward, D. E. 1983. Chromosome counts for New Mexico and Southern Colorado. *Phytologia* 54: 302–308.

Weigend, M., F. Luebert, F. Selvi, G. Brokamp, and H. H. Hilger. 2013. Multiple origins for hound's tongues (*Cynoglossum* L.) and navel seeds (*Omphalodes* Mill.) – The phylogeny of the borage family (Boraginaceae s.str.). *Molecular Phylogenetics and Evolution* 68: 604–618.

Wen, J. and S. M. Ickert-Bond. 2009. Evolution of the Madrean–Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *Journal of Systematics and Evolution* 47: 331–348.

APPENDIX 1. Taxa included in the phylogenetic analyses with their corresponding country of origin, collector/collection number, herbarium accessions, and NCBI Short Read Archive accession numbers.

Outgroup Taxon: Microula: *Microula tibetica* Benth., China, *Boufford 31295* (GH 00466293), SRR5713384.

Ingroup Taxa: Adelinia: *Adelinia grandis* (Douglas ex Lehm.) J.I.Cohen, U.S.A., *Simpson 3007* (SDSU 19197), SRR5713435; **Amsinckia:** *Amsinckia intermedia2* Fisch. & C.A.Mey., U.S.A., *Mabry 65* (SDSU 20756), SRR5713430; *Amsinckia tessellata* A.Gray var. *tessellata*, U.S.A., *Mabry 29* (SDSU 20350), SRR5713421; **Cryptantha:** *Cryptantha affinis* (A.Gray)

Greene, U.S.A., *Rebman 18116* (SD 199070), SRR5713395; *Cryptantha albida* (Kunth) I.M.Johnst., U.S.A., *Kelley 1426* (SDSU 20612), SRR5713394; *Cryptantha alfalfalis* (Phil.) I.M.Johnst., Chile, *Arroyo 995313* (CONC 163659), SRR5713397; *Cryptantha alyssoides* (DC.) Reiche, Chile, *Teiller 5210* (CONC 156553), SRR5713396; *Cryptantha ambigua* (A.Gray) Greene, U.S.A., *Benet-Pierce 524* (SDSU 20524), SRR5713391; *Cryptantha aspera* (Phil.) J. Grau, Chile, *Munoz et al. 2634* (MO 4317599), SRR5713390; *Cryptantha barbigera* (A.Gray) Greene var. *barbigera*, U.S.A., *Mabry 27* (SDSU 20349), SRR5713393; *Cryptantha calycotricha* I.M.Johnst., Chile, *Luebert 3023* (CONC 150898), SRR5713392; *Cryptantha capituliflora* (Clos) Reiche, Chile, *Arroyo 991122* (CONC 166914), SRR5713389; *Cryptantha clevelandii* Greene, U.S.A., *Simpson 3733* (SDSU 20782), SRR5713388; *Cryptantha clokeyi* I.M.Johnst., U. S.A., *Andre 4153* (UCR 164170), SRR5713360; *Cryptantha corollata* (I.M. Johnst.) I.M. Johnst., U.S.A., *Mabry 83* (SDSU 20775), SRR5713361; *Cryptantha crassisepala* (Torr. & A.Gray) Greene, U.S.A., *Kelley 1997* (SDSU 20623), SRR5713362; *Cryptantha crinita* Greene, U.S.A., *Lepley s.n.* (SDSU 20823), SRR5713363; *Cryptantha cynoglossoides* (Phil.) I.M.Johnst., Argentina, *Kiesling 8083* (SI 87776), SRR5713364; *Cryptantha decipens* (M.E.Jones) A.Heller, U.S.A., *Simpson 3661* (SDSU 20014), SRR5713365; *Cryptantha diffusa* (Phil.) I.M.Johnst., Argentina, *Mendez 9862* (MERL 56799), SRR5713366; *Cryptantha dumetorum* (Greene ex A.Gray) Greene, U.S.A., *Hasenstab 57* (SDSU 18694), SRR5713367; *Cryptantha echinella* Greene, U.S.A., *Simpson 3319* (SDSU 19611), SRR5713335; *Cryptantha fendleri* (A.Gray) Greene, U.S.A., *Ripma 372* (SDSU 20114), SRR5713334; *Cryptantha flaccida* (Douglas ex Lehm.) Greene, U.S.A., *Simpson 3619* (SDSU 19846), SRR5713333; *Cryptantha ganderi* I.M.Johnst., U.S.A., *Hasenstab 40* (SDSU 20345), SRR5713332; *Cryptantha globulifera1* (Clos) Reiche, Chile, *Teillier 3845* (SGO 147985), SRR5713328; *Cryptantha globulifera2* (Clos) Reiche, Chile, *Arroyo 995294* (SGO 146942), SRR5713329; *Cryptantha globulifera3* (Clos) Reiche, Chile, *Arroyo 993602* (CONC 163475), SRR5713331; *Cryptantha globulifera4* (Clos) Reiche, Chile, *Arroyo 993602* (SGO 147688), SRR5713330; *Cryptantha glomerata* Lehmann ex G. Don ssp. *g.*, Chile, *Arroyo 995177* (SGO 146941), SRR5713337; *Cryptantha glomerulifera* (Phil.) I.M. Johnst., Chile, *Teiller 5579* (CONC 166867), SRR5713336; *Cryptantha gnaphalioides* (Phil.) Reiche, Chile, *Eggli 2983* (SGO 146002), SRR5713453; *Cryptantha gracilis* Osterh., U.S.A., *Andre 12644* (UCR 217631), SRR5713454; *Cryptantha hispida* (Phil.) Reiche, Chile, *Teillier 4754* (CONC 150914), SRR5713451; *Cryptantha hispidissima1* Greene, U.S.A., *Helmkamp 8471* (RSA 710334), SRR5713358; *Cryptantha hispidissima2* Greene, U.S.A., *Hasenstab 30* (SDSU 18342), SRR5713359; *Cryptantha incana* Greene, U.S.A., *Myers 1032* (UCR 227031), SRR5713452; *Cryptantha intermedia* (A.Gray) Greene var. *intermedia*, U.S.A., *Simpson 3686* (SDSU 20037), SRR5713457; *Cryptantha junipereneis* R.B.Kelley & M.G.Simpson, U.S.A., *Mabry 75* (SDSU 20766), SRR5713448; *Cryptantha kelseyana* Greene, U.S.A., *Kelley 2254* (SDSU 20630), SRR5713458; *Cryptantha kingii* (Phil.) Reiche, Chile, *Muñoz 2580* (SGO 123832), SRR5713455; *Cryptantha leiocarpa* (Fisch. & C.A.Mey.) Greene, U.S.A., *Mabry 68* (SDSU 20759), SRR5713456; *Cryptantha mariposae* I.M.Johnst., U.S.A., *Helmkamp 15796* (SDSU 20826), SRR5713459;

Cryptantha maritima (Greene) Greene var. *maritima*, U.S.A., *Simpson 3665* (SDSU 20050), SRR5713460; *Cryptantha martirensis* M.G.Simpson & Rebman, Mexico, *Rebman 15973* (SDSU 18625), SRR5713440; *Cryptantha mexicana* (Brandegee) I.M.Johnst., U.S.A., *Kelley 1230* (SDSU 20610), SRR5713439; *Cryptantha microstachys* (Greene ex A.Gray) Greene, U.S.A., *Rebman 21420B* (SD 216851), SRR5713442; *Cryptantha minima* Rydb., U.S.A., *Kelley 2248* (SDSU 20629), SRR5713441; *Cryptantha mohavensis* (Greene) Greene, U.S.A., *Ripma 348* (SDSU 20877), SRR5713444; *Cryptantha muricata* (Hook. & Arn.) A.Nelson & J.F.Macbr. var. *muricata*, U.S.A., *Simpson 3818* (SDSU 20749), SRR5713443; *Cryptantha nemaclada* Greene, U.S.A., *Mabry 82* (SDSU 20774), SRR5713446; *Cryptantha nevadensis* A.Nelson & P.B.Kenn., U.S.A., *Barth 913* (SDSU 20393), SRR5713445; *Cryptantha oxygona* (A.Gray) Greene, U.S.A., *Honer 811* (RSA 685321), SRR5713447; *Cryptantha peruviana* I.M.Johnst., Chile, *Teillier 4100* (SGO 140959), SRR5713426; *Cryptantha phaceloides* (Clos) Reiche, Chile, *Ackerman 211* (SGO 146206), SRR5713427; *Cryptantha pterocarya* (Torr.) Greene f. *pterocarya*, U.S.A., *Mabry 33* (SDSU 20355), SRR5713428; *Cryptantha recurvata* Coville, U.S.A., *Sanders 39404* (UCR 225245), SRR5713429; *Cryptantha scoparia* A.Nelson, U.S.A., *Andre 10360* (UCR 211150), SRR5713422; *Cryptantha simulans* Greene, U.S.A., *Hains 258* (SDSU 20390), SRR5713423; *Cryptantha sparsiflora* (Greene) Greene, U.S.A., *Sanders 34146* (UCR 184326), SRR5713424; *Cryptantha subamplexicaulis* (Phil.) Reiche, Chile, *Teillier 2620* (SGO 129437), SRR5713425; *Cryptantha texana* (A.DC.) Greene, U.S.A., *Kelley 1415* (SDSU 20611), SRR5713431; *Cryptantha torreyana* (A.Gray) Greene var. *torreyana*, U.S.A., *Ripma 377* (SDSU 20124), SRR5713432; *Cryptantha utahensis* (A. Gray) Greene, U.S.A., *Mabry 28* (SDSU 20348), SRR5713416; *Cryptantha watsonii* (A.Gray) Greene, U.S.A., *Andre 15116* (UCR 226737), SRR5713415; *Cryptantha wigginsii* I.M.Johnst., U.S.A., *Clonessy s.n.* (SDSU 20082), SRR5713414; **Dasynotus:** *Dasynotus daubenmirei* I.M.Johnst., U.S.A., *Kelley 1951* (SDSU 20343), SRR5713413; **Eremocarya:** *Eremocarya micrantha* (Torrey) Greene var. *micrantha1*, U.S.A., *Guilliams 602* (SDSU 18956), SRR5713418; **Greeneocharis:** *Greeneocharis similis* (K.Mathew & P.H.Raven) Hasenstab & M.G.Simpson, U.S.A., *Kelley 1015* (SDSU 20605), SRR5713375; **Johnstonella:** *Johnstonella angustifolia* (Torr.) Hasenstab & M.G.Simpson, U. S.A., *Boyd 11841* (RSA 731212), SRR5713371; *Johnstonella racemosa* Brand, U. S.A., *Hasenstab 68* (SDSU 18710), SRR5713338; **Oreocarya:** *Oreocarya flavoculata* A.Nelson, U.S.A., *Ripma 307* (SDSU 20030), SRR5713340; *Oreocarya setosissima* (A.Gray) Greene, U.S.A., *Kelley 1466* (SDSU 20242), SRR5713323; *Oreocarya virgata* (Porter) Greene, U.S.A., *Ripma 371* (SDSU 20117), SRR5713357; **Pectocarya:** *Pectocarya penicillata* A.DC., U.S.A., *Kelley 1967* (SBBG 132393), SRR5713355; **Plagiobothrys:** *Plagiobothrys fulvus* (Hook. & Arn.) I.M.Johnst. var. *campestris* (Greene) I.M.Johnst., U.S.A., *Guilliams 1105* (SBBG 132401), SRR5713436; *Plagiobothrys greenei* (A.Gray) I.M.Johnst., U.S. A., *Forrestal 4-15-09* (SBBG 132403), SRR5713379; *Plagiobothrys hispidus* A. Gray, U.S.A., *Oswald & Ahart 5655* (JEPS 87508), SRR5713386; *Plagiobothrys jonesii* A.Gray, U.S.A., *André & Clifton 10750* (UCR 215416), SRR5713387; *Plagiobothrys kingii* (S.Watson) A.Gray var. *harknessii* (Greene) Jeps., U.S.A., *Taylor 15044* (UC 1876874), SRR5713403.

APPENDIX 2. Taxa included for phylogenetic interference, including continent locality and global ecological zone (after Davis and Holmgren 2001). Legend for ecological zones (see Fig. 10): A = North America subtropical dry forest and mountain system; B = North America subtropical desert; C = North America subtropical steppe; D = North America temperate desert; E = North America temperate mountain system; F = South America tropical mountain system; G = South America tropical desert; H = South America subtropical steppe and dry forest; I = South America subtropical mountain system.

	North America					South America			
Taxa	A	B	C	D	E	F	G	H	I
Amsinckia intermedia	1	1	0	1	1	0	0	0	0
Amsinckia tessellata	1	1	1	1	1	0	0	0	0
Cryptantha affinis	1	0	0	1	1	0	0	0	0
Cryptantha albida	1	1	1	0	0	1	0	0	0
Cryptantha alfalfalis	0	0	0	0	0	0	0	1	0
Cryptantha alyssoides	0	0	0	0	0	0	0	1	0
Cryptantha ambigua	1	0	0	1	1	0	0	0	0
Cryptantha aspera	0	0	0	0	0	0	1	0	0
Cryptantha barbigera	1	1	1	1	1	0	0	0	0
Cryptantha calycotricha	0	0	0	0	0	0	1	0	0
Cryptantha capituliflora	0	0	0	0	0	0	0	0	1
Cryptantha clevelandii	1	0	0	0	0	0	0	0	0
Cryptantha clokeyi	1	0	0	0	0	0	0	0	0
Cryptantha corollata	1	0	0	0	0	0	0	0	0
Cryptantha crassisepala	1	1	1	1	1	0	0	0	0
Cryptantha crinita	1	0	0	0	0	0	0	0	0
Cryptantha cynoglossoides	0	0	0	0	0	0	0	0	1
Cryptantha decipens	1	1	1	1	0	0	0	0	0
Cryptantha diffusa	0	0	0	0	0	0	1	1	1
Cryptantha dumetorum	0	1	0	0	0	0	0	0	0
Cryptantha echinella	1	0	0	1	1	0	0	0	0
Cryptantha fendleri	1	1	1	1	1	0	0	0	0
Cryptantha flaccida	1	0	0	0	1	0	0	0	0
Cryptantha ganderi	1	1	0	0	0	0	0	0	0
Cryptantha globulifera	0	0	0	0	0	1	1	1	1
Cryptantha glomerata	0	0	0	0	0	0	1	1	1
Cryptantha glomerulifera	0	0	0	0	0	0	0	0	1
Cryptantha gnaphalioides	0	0	0	0	0	0	1	1	0
Cryptantha gracilis	1	1	1	1	1	0	0	0	0
Cryptantha hispida	0	0	0	0	0	0	1	0	0
Cryptantha hispidissima	1	0	0	0	0	0	0	0	0
Cryptantha incana	1	0	0	0	0	0	0	0	0
Cryptantha intermedia	1	1	0	1	1	0	0	0	0
Cryptantha juniperensis	1	0	1	1	1	0	0	0	0
Cryptantha kelseyana	0	0	0	1	1	0	0	0	0
Cryptantha kingii	0	0	0	0	0	0	0	1	0
Cryptantha leiocarpa	1	0	0	0	0	0	0	0	0
Cryptantha mariposae	1	0	0	0	0	0	0	0	0
Cryptantha maritima	1	1	0	1	0	0	0	0	1
Cryptantha martirensis	1	0	0	0	0	0	0	0	0
Cryptantha mexicana	0	1	1	0	0	0	0	0	0
Cryptantha microstachys	1	0	0	0	0	0	0	0	0
Cryptantha minima	1	1	1	0	1	0	0	0	0
Cryptantha mohavensis	1	0	0	0	0	0	0	0	0
Cryptantha muricata var. *muricata*	1	1	0	0	0	0	0	0	0
Cryptantha nemaclada	1	0	0	0	0	0	0	0	0
Cryptantha nevadensis	1	1	1	1	1	0	0	H	0
Cryptantha oxygona	1	0	0	0	0	0	0	0	0
Cryptantha peruviana	0	0	0	0	0	1	0	0	0
Cryptantha phaceloides	0	0	0	0	0	1	0	0	1
Cryptantha pterocarya f. *pterocarya*	1	1	1	1	1	0	0	0	0
Cryptantha recurvata	1	0	0	1	1	0	0	0	0
Cryptantha scoparia	1	0	0	1	1	0	0	0	0
Cryptantha simulans	1	0	0	1	1	0	0	0	0
Cryptantha sparsiflora	1	0	0	0	0	0	0	0	0
Cryptantha subamplexicaule	0	0	0	0	0	0	1	0	0
Cryptantha texana	0	0	1	0	0	0	0	0	0
Cryptantha torreyana	1	0	0	1	1	0	0	0	0
Cryptantha utahensis	1	1	0	1	0	0	0	0	0
Cryptantha watsonii	1	0	0	1	1	0	0	0	0
Cryptantha wigginsii	1	0	0	0	0	0	0	0	0

(*Continued*)

APPENDIX 2. (CONTINUED).

	North America					South America			
Taxa	A	B	C	D	E	F	G	H	I
Cynoglossum grande	1	0	0	0	1	0	0	0	0
Dasynotus daubenmirei	0	0	0	1	1	0	0	0	0
Eremocarya micrantha var. *micrantha*	1	1	1	1	1	0	0	0	0
Greeneocharis similis	1	0	0	0	0	0	0	0	0
Johnstonella angustifolia	1	1	1	1	1	0	0	0	0
Johnstonella racemosa	1	1	1	1	1	0	0	0	0
Microula tibetica	0	0	0	0	0	0	0	0	0
Oreocarya flavoculata	1	0	0	1	1	0	0	0	0
Oreocarya setosissima	0	1	1	1	1	0	0	0	0
Oreocarya virgata	0	0	0	0	1	0	0	0	0
Pectocarya penicillata	1	1	0	1	1	0	0	0	0
Plagiobothrys fulvus	1	0	0	0	1	0	0	1	0
Plagiobothrys greenei	1	0	0	0	0	0	0	0	0
Plagiobothrys hispidus	1	0	0	0	1	0	0	0	0
Plagiobothrys jonesii	1	0	0	0	1	0	0	0	0