Diversification, biogeography, and classification of Amsinckiinae (Boraginaceae), with an emphasis on the popcornflowers (*Plagiobothrys*)

By

Christopher Matthew Guilliams

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Bruce G. Baldwin, Chair
Professor David Ackerly
Professor Brent Mishler
Professor Patrick O'Grady

Summer 2015

Abstract

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Amsinckiinae is a diverse and ecologically important subtribe of annual herbaceous or perennial suffrutescent taxa with centers of distribution in western North America and southern South America. Taxa in the subtribe occur in all major ecosystems in California and more broadly in western North America, from the deserts of Baja California in the south where *Johnstonella* and *Pectocarya* are common, north to the ephemeral wetland ecosystems of the California Floristic Province where a majority of *Plagiobothrys* sect. *Allocarya* taxa occur, and east to the Basin and Range Province of western North America, where *Cryptantha* sensu stricto (s.s.) and *Oreocarya* are well represented. The subtribe minimally includes 9 genera: *Amsinckia*, *Cryptantha* s.s., *Eremocarya*, *Greeneocharis*, *Harpagonella*, *Johnstonella*, *Oreocarya*, *Pectocarya*, and *Plagiobothrys*; overall minimum-rank taxonomic diversity in the subtribe is ca. 330-342 taxa, with ca. 245--257 taxa occurring in North America, 86 in South America, and 4 in Australia.

Despite their prevalence on the landscape and a history of active botanical research for well over a century, considerable research needs remain in Amsinckiinae, especially in one of the two largest genera, *Plagiobothrys*. Taxonomic concepts in the genus have fluctuated through the 1900s and to date no critical reappraisal of the accepted taxonomy in *Plagiobothrys* has been performed using modern molecular phylogenetic methods; evolutionary patterns have been neglected as well. This dissertation focuses on evaluating patterns of diversification in the subtribe with an emphasis on *Plagiobothrys*. The objectives of the dissertation were three-fold: (1) to generate a hypothesis of evolutionary relationships among target Amsinckiinae and if necessary, revise classification in *Plagiobothrys* so that only monophyletic groups are recognized taxonomically; (2) to evaluate patterns and processes involved with the American Amphitropical Disjunction (AAD) biogeographic pattern in Amsinckiinae; and (3) to examine patterns of diversification within *Plagiobothrys* with respect to habitat affinity.

Chapter 1 examines evolutionary relationships and classification in Amsinckiinae with a focus on *Plagiobothrys*. Molecular sequence data were used to estimate a hypothesis of evolutionary relationships. *Plagiobothrys* was found to be non-monophyletic, with some members more closely related to *Amsinckia* or *Cryptantha* s.l. than to *Plagiobothrys* in the strict sense. Two new genera were established and one older genus name was recommended for usage so that only monophyletic groups are recognized taxonomically. Morphology was examined in

light of the new estimate of phylogenetic relationships. These analyses demonstrate that the trait traditionally used to delimit the large genus *Cryptantha* s.l., a grooved adaxial surface, is a shared ancestral feature and is in conflict with the evolutionary history inferred in this study.

Chapter 2 focuses on AAD, an interesting but understudied New World biogeographic pattern wherein close relatives grow in temperate North America and temperate South America, but are lacking in the intervening New World tropics. Molecular sequence data and fossil and secondary calibrations were used to generate a time-calibrated phylogram on which biogeographic patterns could be assessed using maximum likelihood and Bayesian inference approaches. This study shows that sampled members of Amsinckiinae have moved between North America and South America over 10 times, and always from north to south. Timing of inferred dispersals ranges from Plio-Pleistocene to near to the present. Statistical models suggest that taxa that dispersed from North America to other continents had smaller fruits and more highly ornamented fruits, a pattern that supports birds as the probable dispersal vector.

Chapter 3 evaluates diversification in *Plagiobothrys* with an emphasis on examining the role of habitat and ecological opportunity in promoting increased net diversification rates. Once again, molecular sequence data and fossils calibrations were used to generate a time-calibrated phylogeny of *Plagiobothrys* and the subtribe. Likelihood-based methods were used to reconstruct the ancestral habitat in the genus, in particular to assess the number of times that vernal pools and other ephemeral aquatic ecosystems were invaded. This analysis was unequivocal in estimating a single transition to ephemerally aquatic ecosystems. A character (habitat) independent diversification analysis identified a major net diversification rate shift following the invasion of vernal pools and other ephemeral aquatic ecosystems. A character dependent analysis of diversification with respect to habitat resulted in an estimate of diversification rates over three times greater in ephemeral aquatic lineages than in terrestrial lineages. These findings support the hypothesis that increased rates of diversification accompanied the invasion of vernal pool and ephemeral aquatic ecosystems in the Mediterranean-type climate regions of western North America and western South America. Diversification did not begin until these systems formed ca. 4-3 Ma, suggesting that the evolution of these ephemeral water bodies over millions of acres presented an ecological opportunity that was exploited by members of *Plagiobothrys* from the Plio-Pleistocene to the present day.

For Jo, Jaia, and Malaya

Acknowledgements

Chapter 1. Revisiting evolutionary relationships and sectional classification of *Plagiobothrys* in the context of the Amsinckiinae (Boraginaceae) phylogeny

INTRODUCTION

Classifications of organismal diversity based upon a single character may be inherently unstable due to the prevalence of evolutionary convergence, parallelism, and reversal and the more general difficulty of inferring relationships without multiple sources of phylogenetic evidence. Taxonomic reliance on a limited set of characters that may be developmentally or ecologically correlated and therefore evolutionarily non-independent, such as measured or observed dimensions of a single morphological structure (e.g., a fruit), warrant similar concern. Here we critically reexamine classification in the subtribe Amsinckiinae Brand, a clade of Boraginaceae within which historical classifications have relied heavily on external characters of the fruit.

Amsinckiinae is a diverse and ecologically important subtribe of annual herbaceous or perennial suffrutescent taxa with centers of distribution in western North America and southern South America (Johnston 1923, 1927, Hasenstab-Lehman and Simpson 2012). Members of the subtribe were among the principal groups of California plants identified by Raven and Axelrod (1978) as important examples of major diversifications hypothesized to have occurred since development of the mediterranean-type climate in California. Indeed, members of Amsinckiinae occur in all major ecosystems in California and more broadly in western North America, from the deserts of Baja California in the south where *Johnstonella* A. Brand and *Pectocarya* DC. ex Meisn. are common, north to the ephemeral wetland ecosystems of the California Floristic Province where *Plagiobothrys* Fisch. & C.A. Mey. sect. *Allocarya* I.M. Johnston occurs, and east to the Basin and Range Province of western North America, where *Cryptantha* Lehm. ex G. Don. sensu stricto (s.s.) and *Oreocarya* Greene are well represented.

The subtribe minimally includes 9 genera, which are listed here with global estimates of minimum-rank taxonomic diversity, given parenthetically (NA=North America; SA=South America): *Amsinckia* Lehm. (16--17; 15--16 NA, 2 SA), *Cryptantha* s.s. (113; 68 NA, 47 SA), *Eremocarya* Greene (2 NA), *Greeneocharis* Guerke & Harms (3 NA), *Harpagonella* A. Gray (2 NA), *Johnstonella* (15; 13 NA, 2 SA), *Oreocarya* (72 NA), *Pectocarya* (14; 9 NA, 7 SA), *Plagiobothrys* (ca. 92--102; ca. 60--70 NA, ca. 28 SA, 4 Australia); overall minimum-rank taxonomic diversity in the subtribe is ca. 330--342 taxa, with ca. 245--257 taxa occurring in North America, 86 in South America, and 4 in Australia. These estimates are conservative, and do not include the vast majority of the taxa recognized by Suksdorf (1931) in *Amsinckia* or by Piper (1920) in *Plagiobothrys* sect. *Allocarya*; some of these 300+ names may correspond to evolutionary lineages worthy of taxonomic recognition. Finally, note that a recent broad-scale phylogenetic analysis suggests that the morphologically distinctive *Dasynotus daubenmirei* (Pacific Northwest, in Idaho) nests phylogenetically within Amsinckiinae (Cohen 2013). Additionally, in an analysis by Weigend et al. (2013) some members of *Cynoglossum* were recovered in a polytomy with a monophyletic Amsinckiinae and may best be treated as members of the subtribe.

General morphological patterns in Amsinckiinae are a blend of moderately conserved vegetative traits and variable reproductive traits. Plants often have ascending to erect stems that can be branched or unbranched, and bear simple, alternate leaves. Herbage is often hairy, and

vestiture has been widely used in taxonomy. Some reproductive characters vary moderately, such as flower color, corolla size, and degree of herkogamy, but characters of the fruits are the most variable and have been used frequently by Amsinckiinae taxonomists. As in other members of the Boraginaceae s.s., fruits in Amsinckiinae are schizocarps of four mericarps, with the mericarps colloquially referred to as "nutlets".

Nutlets in Amsinckiinae are extremely variable, with relatively large differences among taxa in orientation, shape, size, and sculpturing, as well as degree of differentiation within an individual fruit, termed nutlet heteromorphism, and between fruits on a plant, termed fruit dimorphism. Figure 1 shows some of the nutlet and fruit variability in the subtribe. Nutlet features have played a key role in circumscribing taxonomic units at different ranks, from tribe, e.g., *Anchuseae* (Johnston 1924) down to subspecies and varieties, e.g., in *Pectocarya setosa* (Johnston 1924).

Johnston's (1923) circumscription of *Plagiobothrys* continues to be widely followed and includes those members of Amsinckiinae that have flowers with white corollas and nutlets with an adaxial keel distal to a well-defined attachment scar (**Figure 1. O**, showing *P. austiniae* and **Q**, showing *P. leptocladus*). Johnston (1923, page 62) states:

> "...*Plagiobothrys* is at once distinguished from its nearest relatives, *Cryptantha* and *Oreocarya*, by the lack of a pronounced longitudinal ventral groove, and the possession instead of a well developed ventral keel and a definitely circumscribed small scar. The gynobase is a pyramid or low frustum and very much shorter than the nutlets, and is not subulate and about equalling [sic] the nutlets as in *Cryptantha* and *Oreocarya*."

At the time that Johnston wrote the above, there were other alternative genus-rank classifications in Amsinckiinae, but Johnston explicitly preferred the simpler classification above based upon features he felt could be used to unambiguously support the recognized genera, as he notes here in a discussion about the recognition of the genus *Allocarya*, which he treated as part of *Plagiobothrys* (Johnston 1932, page 7):

> "That *Allocarya* is most closely related to *Plagiobothrys* has been admitted by most authors. As it is very much more closely related to *Plagiobothrys* than that genus is to any other, its recognition merely lowers generic values and standards, logically introduces further generic segregation of *Plagiobothrys*, eventually of such genera as *Cryptantha*, and so threatens these well marked and distinctive groups. With *Plagiobothrys* and *Cryptantha* defined broadly, the American borage genera are capable of very precise definition."

Circumscribed broadly by Johnston (1923), *Plagiobothrys* comprises approximately 100 taxa distributed among western North America, western South America, and Australia. Like the subtribe, the genus occupies a wide diversity of ecosystems of western North America (Fig. 2), with members ranging from the coast to inland deserts, and from sea level to high elevations (nearly 3,400 m, 11,150 ft). The genus in South America is also taxonomically rich and spans a broad range of ecological settings as well, from low elevation coastal environments to high Andean montane environments (to ca. 4,600 m, or ca. 15,100 ft, fide Horn 2000).

The still widely accepted infrageneric classification of *Plagiobothrys* (Messick 1993, Kelley 2013) also follows Johnston (1923), who divided the genus into five sections: *Allocarya*, *Amsinckiopsis* I.M. Johnston, *Echidiocarya* I.M. Johnston, *Plagiobothrys*, and *Sonnea* I.M.

Johnston. Section *Allocarya* comprises taxa with linear leaves that are opposite and connate-perfoliate basally, and pedicels that distally thickened and turbinate (Greene 1887a). Members of sect. *Allocarya* usually occur in vernal pools and other ephemeral aquatic ecosystems. Section *Amsinckiopsis* includes plants of arid ecosystems, from central Oregon south to eastern Baja California, Mexico. Comprising only 3 minimum-rank taxa*, P. jonesii, P. kingii* var. *harknessii*, and *P. kingii* var. *kingii*, this section includes plants with hispid vestiture and nutlets with an elongate attachment scar positioned along the top of an adaxial keel. As the name suggests, these plants are morphologically similar to the genus *Amsinckia*, although they lack putative synapomorphies of that genus: bifid cotyledons and yellow to orange colored corollas. Of the section, Johnston (1923, pages 58-59) himself states:

> "These species differ from those in genuine *Plagiobothrys* by having a coarse hispid pubescence and nutlet which superficially closely simulate the nutlets of *Amsinckia*...The nutlets have a submedial scar that is borne, not at or below the lower end of the ventral keel, but surrounded by and wedged in between the pericarpial margins that form the keel and consequently appearing at first glace to be borne upon it. The striking nutlet difference seems of funamental [sic] importance and were other important concomitant characters forthcoming I should feel that the group merits generic recognition."

Section *Echidiocarya* includes the two species *P. collinus* and *P. pringlei*, with the former having five recognized varieties. These species occur in shrub and grassland communities of central and southern California, Arizona, and Baja California, Mexico, with *P. collinus* also having one variety in similar ecological conditions in central Chile. Members of sect. *Echidiocarya* have distinctive nutlets, each attached to the gynobase via an elongated stipe that persists on the nutlet after it has disarticulated from the plant. In *P. pringlei*, the nutlets are sometimes paired by fusion of stipes proximally, so that pairs of nutlets fall together. Section *Plagiobothrys* are broadly distributed upland taxa, with several members that can be abundant on the landscape, such as *P. fulvus* and *P. nothofulvus*. Members of this section appear to lack a shared, distinguishing morphological feature, but often have combinations of well-developed basal rosettes of leaves, relatively robust nutlets, prominent and raised attachment scars positioned in the center of the adaxial surface, and herbage that produces bright red exudate. Section *Sonnea* comprises only two taxa, *P. glomeratus* and *P. hispidus*, which occur in arid environments along the California-Nevada border and north to Oregon. These two taxa have bracteate, glomerate inflorescences, hispid pubescence, and attachments scars positioned above the middle of the nutlet (Greene 1887c, Johnston 1923, Tiehm 2000). Greene (1887) noted that plants in this group typically lack a basal rosette of leaves. Nutlets of both lower taxa may occasionally lack an adaxial keel (Guilliams, personal observation; Tiehm 2000), one of the major features identified by Johnston (1923, quoted above) that permit members of the genus to be "at once distinguished" from close relatives.

  Previous phylogenetic work has demonstrated the monophyly of Amsinckiinae, and to varying extents provided insight into phylogenetic relationships within the subtribe (Schwarzer 2007, Hasenstab 2009, Mansion et al. 2009, Guilliams and Baldwin 2010, Weigend et al. 2010, Hasenstab-Lehman and Simpson 2012, Nazaire and Hufford 2012, Cohen 2013, Weigend et al. 2013). *Amsinckia* has been included in several phylogenetic studies (e.g., Schoen et al. 1997, Schwarzer 2007, Hasenstab 2009, Guilliams and Baldwin 2010, Hasenstab-Lehman and Simpson 2012) and has been recovered as monophyletic in all analyses that have included

sufficient outgroup sampling. Members of *Cryptantha* sensu lato (s.l.) have been included in a number of recent studies, with one focusing specifically on estimating relationships in this large genus (Hasenstab 2009, Hasenstab-Lehman and Simpson 2012). Hasenstab-Lehman and Simpson (2012) recovered a non-monophyletic *Cryptantha* s.l. with six well-supported clades, four of which correspond to previous taxonomic concepts for the genera *Eremocarya*, *Greeneocharis*, *Johnstonella*, and *Oreocarya*. The remaining two clades comprise what Hasenstab-Lehman and Simpson called *Cryptantha* s.s. 1 and *Cryptantha* s.s. 2. Relationships among these six clades of *Cryptantha* s.l. were not strongly supported. *Pectocarya* has been sampled for various studies, but this small genus has not been the primary target of a phylogenetic analysis. *Plagiobothrys* has been demonstrated to be non-monophyletic (Hasenstab 2009, Guilliams and Baldwin 2010, Hasenstab-Lehman and Simpson 2012); however, limited sampling of the genus in these previous studies did not permit the authors to evaluate phylogenetic relationships definitively. In particular, sampling of *Plagiobothrys* has been insufficient to evaluate sectional monophyly and relationships among the sections.

The goals of this study were several-fold. The primary goal was to develop a robust phylogenetic hypothesis of relationships among members of *Plagiobothrys*. Given previous findings that *Plagiobothrys* is to some degree non-monophyletic, we also included samples of all closely related genera in Amsinckiinae, and in particular greatly increased sampling of the genera *Amsinckia* and *Pectocarya* beyond what has been done in previous phylogenetic studies of Amsinckiinae. Using this phylogeny, we reassessed the monophyly of *Plagiobothrys*, as well as monophyly of the currently recognized sections in the genus. We assessed the placement of taxa of *Plagiobothrys* among Amsinckiinae, as well as examining other genus-level relationships in the subtribe. We used the phylogeny to evaluate the evolution of nutlet morphology among select members of Amsinckiinae. In particular we examined the evolution of the nutlet characters used by Johnston (1923) to circumscribe genera in Amsinckiinae. We conclude with a revision of generic, sectional, and species-level taxonomy for *Plagiobothrys* sensu Johnston.

MATERIALS AND METHODS

Taxon sampling

A list of all collections included in this study is given in Appendix 1. Within *Plagiobothrys*, we included samples of most recognized taxa (75 in total) and, where possible, we included two or three samples per minimum-rank taxon so that taxon circumscription can be evaluated, at least preliminarily. We also included samples of exemplar taxa from each of the genera of Amsinckiinae: *Amsinckia* (15 taxa), *Cryptantha* s.s. 1 (4 taxa) and *Cryptantha* s.s. 2 (1 taxon) of Hasenstab-Lehman and Simpson (2012), *Eremocarya* (2 taxa), *Greeneocharis* (1 taxon), *Harpagonella* (2 taxa), *Johnstonella* (3 taxa), *Oreocarya* (6 taxa), and *Pectocarya* (11 taxa). Nearly all sequence data were generated *de novo* for this project; however, some sequence data for five taxa outside *Plagiobothrys* were downloaded from GenBank (*Cynoglossum amabile*, *Cynoglossum magellense*, *Dasynotus daubenmirei*, *Eremocarya lepida*, and *E. micrantha*). We used two species of *Cynoglossum* as outgroups for this study. These *Cynoglossum* taxa were shown to be closely related to Amsinckiinae in three recent broad-scale analyses of the Boraginaceae (Nazaire and Hufford 2012, Cohen 2013, Weigend et al. 2013).

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from leaf fragments of herbarium specimens, silica-dried leaf material, and fresh leaf material using a modified CTAB extraction (Doyle and Doyle 1987)

or DNeasy extraction kits (Qiagen, Inc.). No further purification of the genomic DNA was necessary prior to PCR amplification.

For this study, we targeted eight regions of the nuclear and chloroplast genomes. For all regions, we used the Phire Plant Direct PCR Kit (Thermo Scientific, Inc.) and the following reagents/volumes: 10 uL 2× Phire Plant PCR Buffer, 1 uL 10uM forward primer, 1 uL 10uM reverse primer, 0.4 uL Phire Hot Start II DNA Polymerase, 7.6 uL 1:50 diluted genomic DNA in water. All reactions were performed on a PTC-200 thermal cycler (MJ Research). Although annealing temperatures varied by region, cycling generally followed the recommended parameters suggested for the Phire polymerase: 98°C for 1 min; followed by 40 cycles of 98°C for 10 s, region-specific annealing temperature (see below) for 10 s, 72°C for 30 s; and ending with a final extension of 72°C for 2 min.

In the nuclear genome, both internal and external transcribed spacer regions (ITS and ETS) of the 18S-26S ribosomal RNA cistron were amplified and sequenced. The ITS region was amplified using primers ITS4 (White et al. 1990) and ITS-I (Urbatsch et al. 2000) and an annealing temperature of 55°C, and sequenced with ITS4 and ITS5A (White et al. 1990). Primers for the 3' end of the ETS region (upstream from the 18S subunit) were developed for this study as described by Baldwin and Markos (1998). The intergenic spacer (IGS) was amplified in a long-distance PCR reaction using the universal angiosperm primers 18S-IGS and 26S-IGS (Baldwin and Markos 1998). For IGS PCR reactions, we used Advantage® Genomic LA Polymerase Mix (Clontech) following the manufacturer's recommendations for both cycle sequencing and reaction components, except that 1 µL of dimethyl sulfoxide (DMSO) was added to each 25 µL reaction volume. PCR cycles began with an initial denaturing step of 94° for 1 minute, followed by 30 cycles of 98° for 10 seconds and 68° for 15 minutes, ending with a step of 72° for 10 minutes. Following amplification and subsequent sequencing of the IGS region using the primer 18S-IGS, multiple 5' ETS primers were designed to facilitate amplification across the diverse groups of Amsinckiinae (Plagio_ETS557, 5'-TCTTTCGYGCCAAGCGGTGYT-3', Plagio_ETS511, 5'ACGGGGTGGTCTCTTGTCGACT-3', ACP_ETS470, 5'-CGWGYAGGCGCATGAGTGGT-3', Crypt_EST468, 5'-GTGYAGGCGCATGAGTGGTGT-3'). All 5' ETS primers were used in conjunction with a shortened 18S-IGS primer (18S-IGSa, 5'-GAGACAAGCATATGACTACTGGCAGGATCAAC- 3') for all subsequent PCR reactions targeting ETS using a 60°C annealing temperature.

Six non-coding regions of the chloroplast genome were used in this study: the *psbJ-petA* intergenic spacer, the *rpl16* intron, the *rps16* intron, the *3' trnK* intron, the 3' *rps16*- 5' *trnK* intergenic spacer, and the *trnL-trnF* spacer. The *psbJ-petA* intergenic spacer was amplified at an annealing temperature of 53 °C using the primers psbJ and petA (Shaw et al. 2007). The *rpl16* intron was amplified at an annealing temperature of 64 °C using the primers rpl16F71 and rpl16R1516 (Small et al. 1998). The *rps16* intron was amplified at an annealing temperature of 58 °C using the primers rps16F and rps16R (Oxelman et al. 1997). The *3' trnK* intron was amplified at an annealing temperature of 55 °C using the primers matK8 and trnK-2R (Steele and Vilgalys 1994). The 3' *rps16*-5' *trnK* intergenic spacer was amplified at an annealing temperature of 60 °C using the primers rps16x2f2 and trnK^{UUU}x1 (Shaw et al. 2007). The *trnL-trnF* intergenic spacer was amplified at an annealing temperature of 55 °C using the primers trnL and trnF (Taberlet et al. 1991).

All PCR products were cleaned using USB ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) following the manufacturer's protocol. Both strands of cleaned PCR products were

sequenced at the UC Berkeley DNA Sequencing Facility on an Applied Biosystems 3730xl DNA Analyzer.

Phylogenetic analyses

Forward and reverse sequences were assembled into contigs and contig consensus sequences were manually edited in Geneious v.6.1.6 (Biomatters, LLC). Sequence alignments for each region were made using the Geneious Aligner tool integrated in Geneious v.6.1.6 under the default settings, then adjusted manually where necessary. Models of evolution for each region were estimated using jModelTest (Posada 2008). For the primary phylogenetic analyses, we made concatenated matrices of the nuclear regions only, chloroplast regions only, and all regions combined.

We estimated phylogenies under maximum likelihood (ML) using RAxML (Stamatakis 2006) and Bayesian inference using BEAST v.1.8.0 (Drummond and Rambaut 2007) through the CIPRES Science Gateway v3.3. For the RAxML analyses, we selected the RAxML-HPC2 on XSEDE tool with our samples of *Cynoglossum* specified as the outgroup. We selected a GTR model of sequence evolution for estimating a best ML tree and performed rapid bootstrapping in each run. For Bayesian analyses, sampling from the posterior probability distribution was done in BEAST using Markov Chain Monte Carlo (MCMC) and a uniform or uninformative prior. MCMC was run for 30 million generations per analysis, sampling model parameters every $1,000^{th}$ generation. Adequate sampling from the posterior distribution of model parameters was evaluated in two ways: by examining plots for each model parameter in Tracer v.1.5, and by evaluation of Estimated Sample Size (ESS). For the former, we found the approximate generation at which parameter traces became relatively flat, while for the latter we assumed adequate sampling when ESSs for model parameters were above 200. Based on these criteria, we discarded the first 10% of the trees (3 million generations) as "burn-in". We ran separate phylogenetic analyses of the nuclear and chloroplast data, and then compared trees in an attempt to find moderately- to well-supported instances of incongruence (e.g., ML bootstrap proportions >50-70) between the best ML trees from the separate analyses of the chloroplast and nuclear datasets. We then ran an analysis of the combined nuclear and chloroplast data after our careful inspection revealed no instances of well-supported incongruence between the separate nrDNA and cpDNA analyses.

For the purpose of evaluating phylogenetic relationships, taxon circumscriptions, and classification, we employed the taxon sampling strategy described above. However, to generate a tree for use in evaluating morphological evolution in Amsinckiinae, we reduced our sampling to a single exemplar per minimum-rank taxon. We obtained this tree by randomly pruning tips from the best ML tree from the combined analysis until only one exemplar from each taxon remained. We preferred this approach rather than reducing the data matrix and generating a new tree with reduced sampling, reasoning that the most robust phylogeny is likely to result from an analysis with the broadest sampling.

Analysis of nutlet evolution in Amsinckiinae

We used the single exemplar tree described above to examine the evolution of three morphological characters of the nutlet across sampled members of Amsinckiinae: nutlet adaxial configuration, nutlet orientation within the calyx, and nutlet surface sculpturing. Nutlet adaxial configuration is the character used by Johnston (1923) to differentiate between his broadly circumscribed *Cryptantha* and *Plagiobothrys*. We coded this character as "grooved", a state corresponding to his genus *Cryptantha* but also occurring in *Amsinckia*, "keeled" a state

corresponding to his genus *Plagiobothrys* but also occurring in *Amsinckia*, and "other", here corresponding largely to groups that lack grooves and keels, e.g., *Pectocarya*, but also some taxa of *Plagiobothrys* that vary in presence or absence of keels.

Nutlet orientation describes the orientation of the long axis of the nutlet relative to the floral axis. Most Amsinckiinae have vertically oriented nutlets with the long axis parallel to the floral axis. Some taxa of Amsinckiinae such as *Pectocarya* have horizontally oriented nutlets, with the long axis perpendicular to the floral axis. A few cases of intermediate nutlet orientation exist. We coded nutlet orientation as a character with three states, "vertical", "horizontal", and "intermediate".

Nutlet ornamentation describes the abaxial surface features of the nutlet. Nutlet abaxial surfaces may be smooth, but more commonly in the sampled taxa they are roughened, with rugae, papillae, tubercles, or combinations thereof. These features are relatively small compared to the overall size of the nutlet (e.g., < 10% the size of the nutlet). Some taxa of Amsinckiinae are remarkable for their prickles and or toothed margins, which we combined into one state for simplicity. These features are significantly larger (e.g., > 25% of the size of the nutlet) and often bear further ornamentation such as retrorse barbs. Finally, some of the sampled taxa bear wings of tissue along the margin of the nutlet, and these wings are often further elaborated with teeth or bristles. We coded this nutlet ornamentation as a character with four states: "smooth", "roughened", "prickles/teeth", and "winged".

All character state data were obtained from direct observation of herbarium specimens with corroboration from the published literature. The morphological data matrix is in Table 1. Parsimony ancestral character state reconstruction was performed in Mesquite (Maddison and Maddison 2008).

RESULTS

Phylogenetic inference

For the set of analyses using combined ITS and ETS sequences for 179 samples, the dataset was 1315 base pairs (bp) in length, 604 bp of which were invariant and 489 bp of which were parsimony informative. Tree topologies from the ML and Bayesian analyses were generally similar, except in regions where support was lacking in both analyses. For the set of analyses using combined cpDNA sequences for 143 samples, the dataset was 5,849 base pairs (bp) in length, 4,474 bp of which were invariant and 425 bp of which were phylogenetically informative. As in the nrDNA analyses, tree topologies from the ML and Bayesian analyses were generally similar, except in regions where support was lacking in both analyses.

The trees generated in the separate nrDNA and cpDNA analyses were similar topologically at well-supported nodes with only one exception (discussed below in the next section on early divergences in Amsinckiinae). Given general lack of well-supported incongruence between the nrDNA and cpDNA trees, for the remainder of the paper we will discuss the results of the combined analyses. Figure 3 shows the best ML tree from the RAxML analysis of the combined dataset, partitioned by nrDNA and cpDNA, with values on branches corresponding to ML bootstrap values (BS) and posterior probabilities (PP) from the BI analysis.

Early divergences in Amsinckiinae

In the combined and nrDNA analyses, the earliest diverging lineage in Amsinckiinae was *Greeneocharis circumscissa*, although with only weak support. Following this split, the samples were divided into two major, weakly to moderately supported clades, one comprising exemplars

of *Pectocarya*, *Harpagonella*, *Oreocarya*, *Eremocarya*, *Dasynotus*, *Cryptantha* s.s., *Johnstonella*, and *Plagiobothrys* sect. *Sonnea*, and the other with the remaining members of *Plagiobothrys* and all exemplars of *Amsinckia*. In the former clade, two of three samples of *Johnstonella* formed a well-supported group sister to the rest of the clade. Members of *Cryptantha* s.s. formed a well-supported clade that includes one sample of *Johnstonella*: *J. micromeres*. Although support within *Cryptantha* s.s. was strong, this large genus was sparsely sampled here, so the recovered patterns, including the placement of *J. micromeres*, should be viewed with caution. Relationships among the other major genera in the clade sister to *Cryptantha* s.s. were poorly supported in general, with two notable exceptions: (1) the relationship between *Pectocarya* and *Harpagonella* and (2) the relationship between *Oreocarya* and *Eremocarya*.

Pectocarya and *Harpagonella*

Pectocarya and *Harpagonella* formed a clade with strong support in all analyses. Our analyses recovered *P. pusilla* (sect. *Gruvelia*) as the earliest diverging lineage among samples of both genera. Following this split, a monophyletic *Harpagonella* was sister to the remainder of *Pectocarya*. *Harpagonella*, comprising only the two varieties of *H. palmeri*, was monophyletic in all analyses, and the varieties were each monophyletic. Among the other *Pectocarya* taxa, *P. setosa* (sect. *Gruvelia*) was sister to the remaining members of *Pectocarya*, which correspond to sect. *Pectocarya*.

Dasynotus daubenmirei

Our analyses placed the monotypic *Dasynotus daubenmirei* sister to the *Pectocarya* + *Harpagonella* clade, but with no ML bootstrap or posterior probability support. The position of this taxon varied between the nrDNA and cpDNA trees as well. Support was lacking at deeper nodes immediately subtending the common ancestor of *Dasynotus*, *Pectocarya*, and *Harpagonella*, such that placement of *Dasynotus* as sister to the *Oreocarya* + *Eremocarya* clade or the *Plagiobothrys* sect. *Sonnea* clade should be considered equally plausible.

Oreocarya and *Eremocarya*

The nrDNA and combined analyses strongly supported the monophyly of the genera *Oreocarya* and *Eremocarya*, as well as the sister relationship between them. The cpDNA dataset placed *Oreocarya* sister to a clade comprising samples of *Plagiobothrys jonesii*, but with minimal support in the Bayesian analysis only (PP=0.67). The cpDNA analysis strongly supported the monophyly of both *Oreocarya* and *Eremocarya*.

Plagiobothrys sect. *Sonnea*

Plagiobothrys sect. *Sonnea*, comprising only *P. glomeratus* and *P. hispidus*, was recovered as a strongly supported monophyletic group in all analyses. Our sampling included 3 individuals of both recognized species. The well-supported phylogenetic patterns among these 6 samples were identical in all 3 analyses, with the 3 samples of *P. glomeratus* recovered as a monophyletic group nested within a paraphyletic *P. hispidus*.

Amsinckia and *Plagiobothrys* sect. *Amsinckiopsis*

The combined analysis supported a relationship between *Amsinckia* and the three members of *Plagiobothrys* sect. *Amsinckiopsis*: *P. jonesii*, *P. kingii* var. *harknessii*, and *P. kingii* var. *kingii*. *Amsinckia* was monophyletic with strong support in all three analyses. All analyses also supported *Amsinckia* as sister to samples of *P. kingii*, the samples of which constituted a

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clade when multiple exemplars are included. Samples of *P. jonesii* were monophyletic in all analyses as well. However, placement of the *P. jonesii* clade varied between the cpDNA and nrDNA analyses: in the former, it was placed sister to *Eremocarya* with poor support in the Bayesian analysis and no ML BS support; in the latter, it was recovered sister to the remainder of *Plagiobothrys*, but with negligible support in both the Bayesian or ML analysis.

Plagiobothrys sensu stricto: *P.* sects. *Allocarya*, *Echidiocarya*, and *Plagiobothrys*

All analyses recovered a monophyletic group comprising the three remaining sections of *Plagiobothrys*: sects. *Allocarya*, *Echidiocarya*, and *Plagiobothrys*. These sections contain the majority of the recognized lower taxa in the genus. For the remainder of the paper, we will refer to this group as *Plagiobothrys* s.s. Within *Plagiobothrys* s.s., sects. *Allocarya* and *Echidiocarya* were strongly supported as monophyletic in all analyses. Our analyses indicated that *Plagiobothrys* sect. *Plagiobothrys* is non-monophyletic, although relationships among *Plagiobothrys* sects. *Allocarya*, *Echidiocarya*, and the samples from sect. *Plagiobothrys* varied between the cpDNA and nrDNA analyses.

In the combined and cpDNA analyses, the earliest diverging group in *Plagiobothrys* s.s. comprised samples of *P. fulvus* vars. *campestris* and *fulvus*, and the single included sample of *P. infectivus*. These three lower taxa formed a strongly-supported clade in all analyses, although the clade was positioned sister to *P.* sect. *Echidiocarya* in the nrDNA analysis (BS<50, PP=1). Members of this clade are traditionally placed in *P.* sect. *Plagiobothrys*; *P. fulvus* is the type for the genus. Within this group, samples of *P. fulvus* formed a monophyletic group with strong support.

Within the remainder of *Plagiobothrys* s.s., all analyses found strong support for another clade of taxa typically included in *P.* sect. *Plagiobothrys*: *P. arizonicus*, *P. canescens* varieties, and *P. nothofulvus*. In the combined and cpDNA analysis, this grouping diverged after the *P. fulvus* + *P. infectivus* clade; in the nrDNA analysis this group nested more deeply within *Plagiobothrys* s.s. The varieties of *P. canescens* formed a clade sister to the clade of *P. arizonicus* and *P. nothofulvus* samples. Samples of *P. arizonicus* and *P. nothofulvus* were strongly supported as reciprocally monophyletic.

Plagiobothrys sect. *Echidiocarya* was recovered as a monophyletic group with strong support in all analyses. The section was variously placed, however; the combined and cpDNA analyses placed the section sister to *P.* sect. *Allocarya* + a third clade of *P.* sect. *Plagiobothrys* sensu Johnston (1923), while in the nrDNA analysis, *P.* sect. *Echidiocarya* as sister to the clade *P. fulvus* + *P. infectivus*. *Plagiobothrys* sect. *Echidiocarya* includes six lower taxa: the five varieties of *P. collinus* (*californicus*, *collinus*, *fulvescens*, *gracilis*, and *ursinus*) and *P. pringlei*. In all analyses, *P. pringlei* nested deeply within samples of the varieties of *P. collinus*, rendering the latter paraphyletic. Samples of *P. collinus* var. *californicus*, *P. collinus* var. *ursinus*, and *P. pringlei* each constituted taxon-specific clades in the combined analysis.

The remaining, fourth clade of *P.* sect. *Plagiobothrys* sensu Johnston (1923), with *P. shastensis*, *P. tenellus*, *P. tinctorius*, *P. torreyi* varieties, *P. uncinatus*, and *P. verrucosus*, was sister to *P.* sect. *Allocarya* in the combined and nrDNA analyses. In the cpDNA analysis, the clade was placed in a polytomy with *P.* sects. *Allocarya* and *Echidiocarya*. Phylogenetic patterns within this last clade of *P.* sect. *Plagiobothrys* varied between the analyses. Notable patterns included the resolution of a clade of *P. myosotoides* samples plus a sample of *P. tinctorius*, often treated as a nomenclatural synonym, which is sister to a clade of *P. uncinatus* samples. Also notable was the resolution of a clade comprising *P. shastensis*, *P. tenellus*, and some but not all *P. torreyi* samples.

Plagiobothrys sect. *Allocarya* was strongly supported as monophyletic in all analyses; however, support for relationships within the section is low in general. All analyses resolved a first split in the section that yielded the small clade of Australian taxa *P. elachanthus* + *P. plurisepalus* sister to the rest of the section. *Plagiobothrys elachanthus* and *P. plurisepalus* were reciprocally monophyletic in the combined analysis. Backbone relationships in the rest of the section were poor in general, so the remaining presentation of results will separately highlight important clades. Additionally, no other clades of *P.* sect. *Allocarya* received support in the cpDNA analysis, so the presentation below is further limited to only the nrDNA and combined analyses. A clade of South American perennial taxa was recovered, which includes *P. congestus*, *P. humilis*, *P. kunthii*, *P. linifolius*, and *P. macbridei*. Within this clade, a subclade comprising samples of *P. kunthii* and *P. linifolius* was sister to a clade comprising *P. congestus*, *P. humilis*, and *P. macbridei*. Samples of *P. kunthii* and *P. linifolius* were reciprocally monophyletic. Samples of *P. figuratus* are sister to *P. hirtus*. A clade comprising lower taxa superficially united in having abaxial tubercles or prickles was recovered; taxa include *P. acanthocarpus*, *P. austiniae*, *P. distantiflorus*, *P. greenei*, *P. humistratus*, and *P. hystriculus*. Finally, although not strongly placed relative to one another, samples of the following taxa formed monophyletic groups by taxon: *P. glyptocarpus* (some samples), *P. mollis* var. *mollis*, *P. parishii*, *P. procumbens,* and *P. strictus*.

Estimation of ancestral character states

Our analyses of the discrete morphological characters showed that all three characters, nutlet adaxial configuration, nutlet orientation, and nutlet surface ornamentation have derived states that evolved multiple times among the sampled Amsinckiinae lineages (Figure 4).

The common ancestor of Amsinckiinae was reconstructed under parsimony as possessing a grooved adaxial surface, which is the common condition among the sampled members of the non-monophyletic *Cryptantha* s.l. Nearly all *Plagiobothrys* s.l. possess nutlets with a keeled adaxial surface, and this feature was reconstructed at the base of the clade comprising most members of *Plagiobothrys* and *Amsinckia*. Most *Amsinckia* taxa have nutlets with a keeled adaxial surface and this analysis suggested that the common ancestor of *Amsinckia* and of *Plagiobothrys* + *Amsinckia* also had a nutlet with a keeled adaxial surface, but a few members represent evolutionary reversals to nutlets with an adaxial groove. Finally, *Dasynotus*, *Harpagonella*, *Pectocarya*, and *Plagiobothrys* sect. *Sonnea* possess nutlets that lack adaxial keels or grooves entirely, or in the case of *Plagiobothrys* sect. *Sonnea*, that lack them in some specimens.

A vertical nutlet orientation is by far the most common condition among minimum-rank Amsinckiinae taxa. This analysis showed that intermediate and horizontal nutlet orientations evolved in one small subset of Amsinckiinae genera, and these genera, at least in the combined ML analysis, clustered together in one portion of the tree. Horizontal nutlet orientation occurs in *Dasynotus*, all taxa of *Pectocarya*, and some individuals of *Plagiobothrys* sect. *Sonnea*. *Harpagonella*, nested within *Pectocarya*, has highly derived fruits (a bur-like structure comprising two nutlets surrounded by an indurated, accrescent, enveloping calyx bearing several barbed appendages) that are difficult to categorize as either vertical or horizontal.

Nutlet ornamentation appears to be a very labile character in Amsinckiinae. Roughened nutlets appear to be the ancestral condition in the subtribe, with repeated transitions from roughened to smooth (at least 2 transitions) and from roughened to toothed or with prickles (approximately 10 transitions). Also it appears that there are potentially a number of reversions

from smooth to roughened nutlets. The winged nutlet character appears to have evolved from the toothed state several times in *Pectocarya*.

DISCUSSION

Phylogenetic patterns among genera of Amsinckiinae

Despite limited sampling of *Cryptantha* s.s. and *Oreocarya* and weak support for some deep nodes in Amsinckiinae, a number of well-supported patterns in the subtribe are worthy of discussion. First, we found moderate support (BS = 54/PP = 0.98) for a clade comprising *Cryptantha* s.s., *Eremocarya*, *Oreocarya*, *Dasynotus*, *Pectocarya*, *Harpagonella*, and some members of *Johnstonella*. This large and diverse group of Amsinckiinae spans a wide range of life histories and habitats, from desert annuals to montane suffrutescent perennials. Noteworthy higher-level patterns are discussed in the sections immediately below.

Pectocarya and *Harpagonella*

These two genera were recovered together in a clade with strong support, but in our analyses a monophyletic *Harpagonella* was nested within a paraphyletic *Pectocarya*, and thus some taxonomic revision is required so that only monophyletic groups are recognized. Veno (1979) had earlier proposed the reduction of *Harpagonella* to synonymy with *Pectocarya* due to the close morphological similarity in all characters except those of the fruit (Figure 1). Veno's proposal for a broadened *Pectocarya* would suffice, but as it was made in an unpublished doctoral dissertation, it does not qualify as a valid nomenclatural action under the ICN (McNeill et al. 2012).

We favor an alternative nomenclatural solution. The majority of taxa of *Pectocarya* form a well-supported monophyletic group equivalent in circumscription to *Pectocarya* sect. *Pectocarya* and united by the distinctive morphological synapomorphy of nutlets that are divergent in pairs; only *P. setosa* and *P. pusilla* of *P*. sect. *Gruvelia* occupy positions that render *Pectocarya* s.l. non-monophyletic (depending upon which genetic compartment is evaluated in the phylogenetic analysis). Both of these taxa have combinations in the genus *Gruvelia* A.DC., with *G. pusilla* A.DC. being the type for the genus. Because these two taxa do not in any analysis form a monophyletic group, we advocate treating *P. pusilla* as *G. pusilla* in a monotypic *Gruvelia*, a change that would require no new nomenclatural action. A new genus name and new combination would be needed for *P. setosa*.

This approach is preferable to that of Veno (1979) for several reasons. The fruit of *Harpagonella* is highly derived and arguably the most distinctive in all of the Boraginaceae s.s.. *Harpagonella* has a bur-like fruit derived from the fusion of two accrescent sepals that surround the gynoecium of two nutlets and become indurated at maturity. These sepals bear 5--10 horn-like, barbellate appendages that may serve a role in epizoochoric dispersal, hence the common name "grappling hooks" for these taxa. Including the morphologically distinctive *Harpagonella* in *Pectocarya* would eliminate the similarly distinctive morphological synapomorphy of the latter genus as well. *Pectocarya pusilla* and *P. setosa* are each easily distinguished from both *Pectocarya* s.s. and *Harpagonella* using vegetative and reproductive morphological features. Our approach would result in slightly more splitting, but would permit the ready diagnosis of all derivative genera. Furthermore, the amount of nomenclatural action required would be minimal and roughly equivalent to the approach advocated by Veno. All nomenclatural changes will be made in a forthcoming study with greatly expanded sampling of both *Pectocarya* and *Harpagonella* (Guilliams et al. in prep).

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Dasynotus daubenmirei

The morphologically distinctive, monotypic *Dasynotus* was described by Johnston in 1948. In the protologue, Johnston remarks on the anomalous combination of characters that taken together preclude the placement of the new species in any genus recognized at the time. Vegetatively, *Dasynotus* approaches *Hackelia*, but in reproductive features it is very different. The relatively large flowers have corolla scales or "faucal appendages" that are long (~4 mm) and curve outward from the floral axis. The nutlets are unusual as well, with a horizontal orientation, supramedial attachment, and flat upper/abaxial surface densely covered with trichomes. Johnston speculates based on morphology that *Dasynotus* is most closely related to *Hackelia* and *Eritrichium*, although he notes that the adaxial keel above the nutlet scar evokes a superficial resemblance to *Plagiobothrys* sect. *Plagiobothrys* ("*Euplagiobothrys*").

Recent phylogenetic studies have found a close relationship between *D. daubenmirei* and Amsinckiinae. Cohen (2013) showed analyses that placed *D. daubenmirei* well within Amsinckiinae, sister to a clade comprising *Cryptantha, Greeneocharis, Pectocarya*, and *Plagiobothrys*. Weigend et al. (2013) showed an analysis that placed *D. daubenmirei* in a trichotomy at the base of what they call the "*Cryptantha* clade", here mostly equivalent to Amsinckiinae. Our analyses placed *D. daubenmirei* sister to *Pectocarya + Harpagonella*, but with negligible support. Our morphological reconstructions showed that this part of Amsinckiinae tree has some lineages with horizontally oriented nutlets, so placement of *D. daubenmirei* here is not without some corroborating morphological support. Near to *D. daubenmirei* was *Plagiobothrys* sect. *Sonnea*, which also has nutlets with a supramedial attachment and occasional horizontal orientation. Regardless of its position in Amsinckiinae, *Dasynotus* is a morphological outlier in the subtribe, with a vexing combination of characteristics that made its initial placement by Johnston difficult.

Plagiobothrys sect. *Sonnea*

Plagiobothrys sect. *Sonnea*, with only *P. glomeratus* and *P. hispidus*, is monophyletic and placed sister to the clade comprising the genera *Dasynotus, Eremocarya, Harpagonella, Oreocarya*, and *Pectocarya*. *Plagiobothrys* sect. *Sonnea* taxa are very distinctive in having hispid vestiture, glomerate inflorescences, nutlets with an attachment scar above the middle of the nutlet on the adaxial surface (Greene 1887c, Johnston 1923), and occasional nutlet reduction to two nutlets per fruit; when nutlet number is reduced, nutlets are often positioned horizontally in the calyx, with the "upper" portion of the nutlet shifted toward the floral axis and the "lower" portion of the nutlet shifted outward, away from the floral axis (Tiehm 2000). These sect. *Sonnea* taxa are both morphologically and phylogenetically distinct, and have existing combinations in the available genus name *Sonnea* Greene. Therefore, we suggest using the names *Sonnea glomerata* (A. Gray) Greene and *S. hispida* (A. Gray) Greene for these two taxa. As Greene did not suggest a type for *Sonnea* at the time of publication, we lectotypify this genus name below.

Amsinckia and *Plagiobothrys* sect. *Amsinckiopsis*

Amsinckia + Plagiobothrys sect. *Amsinckiopsis* constituted a clade with *Plagiobothrys* s.s. with moderate support (BS=56, PP=0.97). Hasenstab-Lehman and Simpson (2012) found a similar but not identical grouping in their ML molecular analysis of Amsinckiinae, although with negligible support. This grouping is supported in part by the presence of an adaxial keel in all members of *Plagiobothrys* s.s. and *P.* sect. *Amsinckiopsis*, and nearly all of *Amsinckia*.

Plagiobothrys sect. *Amsinckiopsis*, comprising only *P. jonesii* and *P. kingii* vars. *harknessii* and *kingii*, formed a paraphyletic grade at the base of *Amsinckia*. If only monophyletic

groups are to be recognized taxonomically in the subtribe, then these sect. *Amsinckiopsis* taxa must be either included in an expanded *Amsinckia* or in two new genera. As previously mentioned, Johnston (1923) recognized the distinctiveness of these three taxa, stating that "the striking nutlet difference seems of funamental [sic] importance and were other important concomitant characters forthcoming I should feel that the group merits generic recognition." Johnston also noted that *P.* sect. *Amsinckiopsis* taxa have nutlets that "closely simulate the nutlets of *Amsinckia*" (Johnston 1923). Presently, *Amsinckia* is easily circumscribed morphologically in having members with orange or yellow-orange corollas and bifid cotyledons, two synapomorphic features not found elsewhere in the subtribe to our knowledge. Including *P. jonesii* and *P. kingii* vars. *harknessii* and *kingii* in *Amsinckia* would eliminate these morphological synapomorphies for the broadened genus, although the expanded group would possess some nutlet features in common, such as a similar attachment scar. As some nomenclatural action would be required under either scenario, we advocate for retaining a narrower, more readily diagnosable *Amsinckia* and propose two new genera and new combinations for taxa of *Plagiobothrys* sect. *Amsinckiopsis* in the Taxonomic Treatment section below.

Phylogenetic patterns in *Plagiobothrys sensu stricto*

Unlike the former *Cryptantha* s.l., which was found to be polyphyletic (Hasenstab-Lehman and Simpson 2012), most currently recognized taxa in *Plagiobothrys* generally were resolved in these analyses as constituting a monophyletic group that we have referred to as *Plagiobothrys* s.s. Within *Plagiobothrys* s.s., five well-supported clades were recovered as described below.

The earliest diverging group was a clade from *P.* sect. *Plagiobothrys*, comprising *P. fulvus* and *P. infectivus*. These taxa are morphologically similar in possessing nutlets with a deeply excavated attachment scar. This feature is the basis for the name *Plagiobothrys*, which derives from the Greek *plagio* for "oblique or placed sideways" and *bothrys* for "pit or scar". This characteristic is so distinctive that it is not surprising that *P. fulvus* (the type species) and *P. infectivus* are sister. Of note is the distant placement of *P. fulvus* and *P. greenei*, a member of *P.* sect. *Allocarya*. In Johnston's redefinition of *Plagiobothrys* (1923), he used the apparently convergent nutlet morphologies of *P. fulvus* and *P. greenei* as evidence against maintaining *Allocarya* as a distinct genus. Our results show that Johnston was justified in merging the genera, but erred in this morphological comparison.

Another clade from *P.* sect. *Plagiobothrys* included *P. arizonicus*, *P. nothofulvus*, and *P. canescens*. The species in this group have long been recognized as closely related on the basis of nutlet similarities (transverse ridges on the abaxial surfaces) and putative hybrids where ranges of these taxa overlap. For example, *P. canescens* var. *catalinensis* is purported to be a possible stable hybrid between *P. canescens* and *P. arizonicus* (Kelley 2013). Unlike *P. canescens*, *P. nothofulvus* and *P. arizonicus* have circumscissile calyces, a feature otherwise not found in the genus and apparently diagnostic of a clade comprising the two species. All three species can produce red exudate from leaves and roots.

Members of *P.* sect. *Echidiocarya* formed a group with strong support. These taxa are united in having nutlets with adaxial, stipitate attachment scars, a feature very distinctive in the genus. These taxa are also somewhat geographically cohesive, with most members found in southern California and northern Baja California, Mexico. *Plagiobothrys pringlei* is found in central and southern Arizona and *P. collinus* var. *collinus* occurs as an amphitropical disjunct in Chile.

The last clade of *P.* sect. *Plagiobothrys* included *P. myosotoides*, *P. shastensis*, *P. tenellus*, *P. tinctorius*, *P. torreyi*, *P. uncinatus*, and *P. verrucosus*. These species are united in having nutlets with grooves on the abaxial surfaces, and they also generally produce basal rosettes of leaves and may produce red exudate. These species occur in western North America where some are widely distributed, e.g., *P. tenellus*, and in western South America, where *P. myosotoides* is common. The *P. torreyi* varieties represent a small radiation of montane taxa in the Sierra Nevada and Transverse Ranges of California. Some narrow endemics occur in this group as well, including *P. uncinatus* and an anomalous occurrence of plants morphologically and phylogenetically similar to *P. myosotoides*, both from the South Coast Ranges of California.

The last clade of *Plagiobothrys* s.s. that we found comprised all taxa from *P.* sect. *Allocarya*. These species are united in having conspicuously opposite leaves proximally, distally thickened pedicels, and an affinity for ephemeral aquatic settings, such as vernal pool ecosystems, intermittent streams, and meadow margins. This clade is by far the most taxon-rich in the genus, with current estimates of about 65 minimum-rank taxa. This estimate does not include all of the taxa recognized by Piper in his monograph of *Allocarya* (1920), most of which are now in synonymy with other taxa in the section. Members of sect. *Allocarya* are predominantly distributed in western North America and western South America, but are also present in Australia and Asia. Well-supported phylogenetic patterns are wanting in this clade, potentially due to a recent onset of diversification in the group, which might have resulted in both low amounts of genetic change across lineages and also incomplete lineage sorting where changes have occurred. Also complicating phylogenetic inference in this group may be the presence of polyploidy. Cronquist in Hitchcock (1959) reported a hexaploid count for *P. scouleri* (Hook. & Arn.) I.M. Johnst. ($2n = 72$), Moore (1981) reported a potential hexaploid count for *P. calandrinioides* (Phil.) I.M. Johnst. ($n = 34$), Löve (1982) reported a polyploid count for *P. scouleri* var. *penicillatus* (Greene) Cronquist ($2n = 54$), and diploid ($2n = 24$), triploid, tetraploid, and hexaploid counts were reported by Horn and references therein (2000).

Evolution of nutlet morphology in Amsinckiinae

Nutlet morphology is highly variable in Amsinckiinae and has often been used for taxonomic delimitation at ranks from genus to variety, often as one of a suite of characters. Major differences among taxa include nutlet number (Hasenstab-Lehman and Simpson 2012), nutlet orientation, nutlet heteromorphism (Veno 1979, Hasenstab-Lehman and Simpson 2012, Guilliams et al. 2013), nutlet dimorphism, nutlet shape, nutlet attachment, and nutlet ornamentation to list some major types of variation. These features are often used in conjunction with floral or vegetative characters. In distinguishing *Cryptantha* s.l. from *Plagiobothrys* s.l., however, early authors developed a classification that relied heavily upon a single nutlet character, the attachment scar, here examined as "nutlet adaxial configuration". Lacking a phylogenetic perspective, it was not possible to understand the evolutionary history of this critical character, and thus a classification was developed that was inconsistent with the evolutionary history of Amsinckiinae. Here we examine only three characters: nutlet adaxial configuration, nutlet orientation, and nutlet surface ornamentation.

Nutlet adaxial surface characteristics were examined because these features were used by Johnston (1923) in support of his broad concepts for *Cryptantha* and *Plagiobothrys*. He classified all Amsinckiinae with white flowers and nutlets with an adaxial groove as *Cryptantha* and all Amsinckiinae with white flowers and nutlets with an adaxial keel as *Plagiobothrys*. Analysis of this feature here shows that the keeled condition is derived and synapomorphic for the clade comprising *Plagiobothrys* s.s., *Plagiobothrys* sect. *Amsinckiopsis*, and *Amsinckia*.

Johnston (Johnston 1925) speculated on the closeness of *Plagiobothrys* and *Amsinckia*, suggesting that the latter developed from the former based upon nutlet similarity. A nutlet with an adaxial groove is likely the ancestral condition in the subtribe, and represents a symplesiomorphic feature that when used for classification led to a non-monophyletic *Cryptantha* s.l.

Nutlet orientation is most commonly vertical in Amsinckiinae, but appears to be either variable or trending toward horizontal in one clade comprising *Dasynotus*, *Pectocarya*, and *Plagiobothrys* sect. *Sonnea*. Phylogenetic uncertainty in this part of the tree may mean that this result is spurious, but other morphological similarities in this clade are noteworthy, such as the supramedial attachment scar feature shared by *P*. sect. *Sonnea* and *Dasynotus daubenmirei*.

Nutlet ornamentation has been a major taxonomic character in Amsinckiinae historically. Our analysis showed that nutlet ornamentation is quite variable in the subtribe. The ancestral condition is reconstructed as roughened, with multiple shifts between roughened and smooth (at least 2), and roughened and either prickles or teeth (about 10). Phylogenetic uncertainty precludes a precise quantification of these transitions. Hasenstab-Lehman and Simpson (2012) performed a similar reconstruction among the members of Amsinckiinae that they sampled. Although the taxa included in their study differ, they also concluded that roughened nutlets are the ancestral condition in the subtribe.

TAXONOMIC TREATMENT

Recognition of *Amsinckiopsis* and *Simpsonanthus* for taxa of *Plagiobothrys* sect. *Amsinckiopsis*

Plagiobothrys sect. *Amsinckiopsis* is more closely related to *Amsinckia* than to the majority of taxa of *Plagiobothrys* sensu Johnston. Furthermore, the three constituent taxa, *P. jonesii*, *P. kingii* var. *harknessii*, and *P. kingii* var. *kingii* do not form a monophyletic group. Based upon the phylogenetic evidence we present in this study, we here describe a new genus and make new combinations for these taxa referable to *P*. sect. *Amsinckiopsis* so that only monophyletic groups are recognized taxonomically. Hasenstab-Lehman and Simpson demonstrated similar but not identical patterns in an earlier study (2012).

Amsinckiopsis (I.M. Johnston) Guilliams, Hasenstab-Lehman, & B.G. Baldwin, stat. nov. Basionym: *Plagiobothrys* sect. *Amsinckiopsis* I.M. Johnston, Contributions from the Gray Herbarium of Harvard University 68: 59. 1923. TYPE: *Eritrichium kingii* S. Watson ≡ *Amsinckiopsis kingii* (Watson) Guilliams & B.G. Baldwin.

Annuals, 1--4 dm tall, hirsute and villous; taprooted. Stems ascending to erect, 1 to several. Leaves alternate, basal and cauline, simple, sessile, basal blades oblanceolate, cauline blades linear to lanceolate, 2--6 cm long, surfaces hirsute to hispid. Inflorescences circinate, scorpioid cymes, glomerate, not much elongating in fruit, basally bracteate, proximal bracts sometimes exceeding flowers. Calyces 5--6 mm long, lobes lanceolate; corolla limbs 4--7 mm in diameter. Fruits schizocarps, mericarps (nutlets) usually 4; nutlets ± ovate in outline, 2.5--3 mm long, abaxial surface sometimes with medial and lateral keels, irregularly rugose and papillate, attachment scar on adaxial surface, medial and positioned along raised adaxial keel, elongate, narrowly triangular to narrowly lanceolate, length ca. 1/2 that of the nutlet, scar apices acute.

Taxa of *Amsinckiopsis* are distinctive in Amsinckiinae in having the combination of hirsute to hispid vestiture throughout; ± glomerate, circinate, scorpioid cymes; white corollas; and nutlets with rugose and papillate sculpturing and an elongate attachment scar positioned along a raised, adaxial keel.

Amsinckiopsis is known from southeastern Oregon and eastern California to western Utah; it is most common in Nevada. Taxa usually occur on sandy and gravelly substrates, less commonly on volcanic and clay substrates, in valleys and on bajadas between approximately 1,200 and 2,100 m (4,000 and 7,000 ft) in open vegetation types. Flowering occurs from April to June (July).

Etymology: The meaning of "similar to *Amsinckia*," originally pertaining to morphological similarity, acquires a new sense given the close phylogenetic relatedness of *Amsinckia* and *Amsinckiopsis*.

Amsinckiopsis kingii var. **harknessii** (Greene) Guilliams & B.G. Baldwin, comb. nov. Basionym: *Sonnea harknessii* Greene, Pittonia 1(2): 23. 1887. *Plagiobothrys harknessii* (Greene) A. Nelson & J.F. Macbr., Botanical Gazette 62(2): 143. 1916. *Plagiobothrys kingii* (S. Watson) A. Gray var. *harknessii* (Greene) Jeps., A Manual of the Flowering Plants of California, 856. 1925. TYPE: U.S.A. California: "Mono Lake, Sierra Nevada," *H.W. Harkness s.n.*, June, 1886 (holotype: NDG).

Amsinckiopsis kingii (S. Watson) Guilliams & B.G. Baldwin comb. nov. Basionym: *Eritrichium kingii* S. Watson, United States Geological Expolration [sic] of the Fortieth Parallel. Vol. 5, Botany, 243, pl. 23, f. 3--5. 1871. *Plagiobothrys kingii* (S. Watson) A. Gray, Proceedings of the American Academy of Arts and Sciences 20: 281. 1885. TYPE: U.S.A, California: "Eastern side of the Sierra Nevada at Truckee Pass" *S. Watson, 854*, 1865. (holotype: GH).

Simpsonanthus Guilliams, Hasenstab-Lehman, & B.G. Baldwin, gen. nov. TYPE: *Plagiobothrys jonesii* A. Gray, Synoptical Flora of North America 2(1): 430. 1886. ≡ *Simpsonanthus jonesii* (A. Gray) Guilliams & B.G. Baldwin

Annuals to 50 cm tall, taprooted, usually few to several branched throughout; stems ascending to erect, hirsute to hispid and with shorter, fine, spreading to retrorse hairs. Leaves alternate, cauline, 2--10 cm long, blades oblong to elliptic, distally sometimes lanceolate, usually hispid, hairs with pustulate bases, sometimes hirsute and/or with short, fine hairs. Inflorescences circinate, scorpioid cymes, proximally bracteate; pedicels 0--1 mm long. Calyces 4--8 mm long, hirsute, lobes narrowly lanceolate to ± linear, margins often ciliate; corollas ± funnel-shaped, limb 1--3 mm in diameter, fornices minute, white. Fruits schizocarps, mericarps (nutlets) 3--4; nutlets triangular-ovoid, 2--3 mm long, abaxial surfaces tessellate, coarse tubercles absent, abaxial and lateral ridges ± poorly defined to absent, transverse ridges and grooves absent, adaxial keel present. Nutlet attachment scar medial on adaxial surface along crest of adaxial keel, narrowly lanceolate to narrowly triangular, irregular, length ca. 1/2 that of the nutlet.

Simpsonanthus is distinctive in Amsinckiinae in having the combination of hirsute to hispid vestiture throughout; non-glomerate, circinate, scorpioid cymes; white corollas; and nutlets with

tessellate abaxial nutlet sculpturing and an elongate attachment scar positioned along a raised, adaxial keel.

Simpsonanthus is known in California predominantly east of the Sierra Nevada in the central part of the state and through the Mojave and Colorado deserts. It extends through the southern half of Nevada to southern Utah and through Arizona to Mexico, where it is known from northwestern Sonora and from Isla Angel de la Guarda at Refugio Bay in the Gulf of California. It occurs on sandy, gravelly, and rocky substrates in washes and on slopes between ca. 90 and 1,770 m (30 and 5,800 ft) in vegetation types including creosote bush scrub and pinyon juniper woodland. Flowering typically occurs from March to May, sometimes in January, February, and June.

Etymology: We are pleased to name Simpsonanthus for Michael G. Simpson, professor of Biology at San Diego State University, who has contributed greatly to the study of Amsinckiinae.

Simpsonanthus jonesii (A. Gray) Guilliams & B.G. Baldwin comb. nov. Basionym: *Plagiobothrys jonesii* A. Gray, Synoptical Flora of North America 2(1): 430. 1886. TYPE: U.S.A. California: "Needles," *M.E. Jones, s.n.*, May 5, 1884. (holotype: GH).

Lectotypification of *Sonnea*

When Greene named *Sonnea* in 1887, his circumscription included five lower taxa, four of which had been previously described. Based upon our interpretation of the phylogenetic evidence presented here, we retain in *Sonnea* only *Sonnea glomerata* (A.Gray) Greene and *Sonnea hispida* (A.Gray) Greene; the other three taxa are more closely related to *Amsinckia* as described above (and see the following section).

Sonnea Greene, Pittonia 1(2): 22. 1887. LECTOTYPE (here designated): *Sonnea hispida* (A. Gray) Greene.

Sectional taxonomy in *Plagiobothrys*

This study provided evidence that the current sectional taxonomy of *Plagiobothrys* requires revision if only monophyletic groups are to be recognized. Table 2 shows the current sectional classification of Johnston (1923) with a focus on sections affected by our proposed changes. Two of Johnston's *Plagiobothrys* sections, sects. *Amsinckiopsis* and *Sonnea* are more closely related to non-*Plagiobothrys* taxa than to other taxa in *Plagiobothrys* and are best removed from *Plagiobothrys*. We provide nomenclatorial solutions to deal with those two sections below. Here we propose a sectional classification for species from the remaining three of Johnston's sections, *Allocarya*, *Echidiocarya*, and *Plagiobothrys*. *Plagiobothrys* sects. *Allocarya* and *Echidiocarya* are strongly supported as monophyletic groups in all phylogenetic analyses. We retain *P.* sects. *Allocarya* and *Echidiocarya* as previously circumscribed in our sectional treatment, but here designate lectotypes for those sections.

Plagiobothrys sect. *Allocarya* I.M. Johnston, Contributions from the Gray Herbarium of Harvard University 68: 65. 1923. LECTOTYPE (here designated): *Allocarya lithocarya* (Greene ex A. Gray) Greene, Pittonia 1(1): 12. 1887. ≡ *Plagiobothrys lithocaryus* (Greene ex A. Gray) I.M. Johnston, Contributions from the Gray Herbarium of Harvard University 68: 76. 1923.

Plagiobothrys sect. ***Echidiocarya*** I.M. Johnston, Contributions from the Gray Herbarium of Harvard University 68: 65. 1923. LECTOTYPE (here designated): *Eritrichium collinum* Phil., Linnaea 29: 17. 1858. ≡ *Plagiobothrys collinus* (Phil.) I.M. Johnston, Contributions from the Gray Herbarium of Harvard University 78: 81. 1927.

Plagiobothrys sect. *Plagiobothrys* sensu Johnston was non-monophyletic in all analyses, comprising three well-supported clades that were variously placed with respect to the other two sections. The type of *Plagiobothrys* is *Plagiobothrys rufescens* Fisch. & C. A. Mey., which is a taxonomic synonym of *P. fulvus* (Hook. & Arn.) I. M. Johnst. The clade that contains *P. fulvus* is named *P.* sect. *Plagiobothrys*, albeit reduced to just three minimum-rank taxa (Table 2). No sectional name is available for either of the other two monophyletic groups of *P.* sect. *Plagiobothrys* sensu Johnston. We describe those two new sections here (see Table 2 for lists of taxa in each section).

Plagiobothrys sect. ***Costacarya*** Guilliams & B.G. Baldwin, sect. nov. TYPE: *Plagiobothrys nothofulvus* (A. Gray) A. Gray, Proceedings of the American Academy of Arts and Sciences 20. 285. 1885.

Annuals, with a taproot. Stems many, branched from base and throughout, prostrate to decumbent or ascending, to ca. 6 dm, or 1-few, erect, branched above base, to ca. 7 dm. Leaves alternate, basal and cauline, margins and midribs producing dark reddish exudate or not, basal leaves in rosette or not, usually withering during anthesis, green to slightly gray-green, simple, sessile, blades ± linear to oblanceolate, to 10 cm, cauline leaves smaller than basal leaves and further reduced distally, narrowly oblanceolate to oblong or lanceolate to narrowly lanceolate. Inflorescences circinate, scorpioid cymes, elongating in fruit, bracteate throughout or only at proximal flower(s). Calyces 2--6 mm long, lobes erect or oriented inward over nutlets, circumscissile in fruit or not; corollas white, limb 2--9 mm in diameter, fornices pale yellow to yellow. Nutlets 2--4, widely ovate in outline, 1.6--2.7 mm long, 1.2--2.4 mm wide, abaxial surfaces usually with medial keel and narrow, transverse ribs or ridges separated by wider flat areas, adaxial surfaces with a raised, medial attachment scar ± in center of adaxial surface proximal to a prominent medial keel, scar shape irregular, scar length approximately equaling width, nutlet margins usually with lateral keels, ± abruptly incurved in distal third of nutlet proximal to an acute apex.

Taxa of *Plagiobothrys* sect. *Costacarya* are distinctive in Amsinckiinae in being non-wetland plants with the combination of white corollas with yellow fornices, nutlet abaxial surfaces with medial keel and narrow transverse ribs or ridges separated by wider flat areas, and adaxial surfaces with a raised attachment scar proximal to a medial keel. Some but not all members of the section have herbage that produces conspicuous, reddish exudate.

Plagiobothrys sect. *Costacarya* is known from northern Mexico and the western United States from Washington to California and east to New Mexico; it is most prevalent in the California Floristic Province and in the deserts of California. Taxa usually occur on a wide range of substrates, usually from sea level to approximately 1,800 m (5,900 ft), rarely to 2,250 m (7,380 ft). Flowering occurs from February to June.

Etymology: The section name *Costacarya* is from the Latin *costa* for "rib" or "ridge," and *carya* for "nut," alluding to the parallel transverse ridges on the abaxial surface of the nutlets of the species in this section.

Plagiobothrys sect. ***Striacarya*** Guilliams & B.G. Baldwin, sect. nov. TYPE: *Myosotis tenella* Nutt., Hooker's Journal of Botany and Kew Garden Miscellany 3: 295. 1851. ≡ *Plagiobothrys tenellus* (Nutt.) A. Gray, Proceedings of the American Academy of Arts and Sciences 20: 283. 1885.

Annuals, taprooted. Stems 1 to several, branched from near base or branched distally or simple, prostrate to decumbent, ascending, or erect, usually 2--35+ cm. Leaves alternate, basal and cauline, basal leaves in rosettes, withering at anthesis or persistent, usually green, simple, sessile, blades lanceolate to ovate or ellipitic, usually 0.5--3 cm, margins and midribs producing bright reddish exudate in most taxa, sparsely to densely hispidulous or strigulose, sometimes pubescent, cauline leaves smaller and further reduced distally, usually lanceolate to ovate, margins and midribs producing dark reddish exudate or not. Inflorescences circinate, scorpioid cymes, elongating in fruit, usually bracteate, sometimes not. Calyces ca. 1--3 mm long, lobes 0.5--1.5 mm; corollas white, 1.15--2.65 mm long, limbs 0.9--2.4 mm in diameter. Nutlet abaxial surfaces usually with broad, flat to rounded, sometimes smooth, transverse ridges separated by narrow transverse grooves, with medial keels or not, adaxial surfaces with a raised, medial attachment scar ± in center of adaxial surface proximal to a prominent medial keel, scar shape irregular, length ca. equaling width, nutlet margins with lateral keels or not.

Taxa of *Plagiobothrys* sect. *Striacarya* are distinctive in Amsinckiinae in being non-wetland plants with the combination of white corollas with yellow fornices, nutlet abaxial surfaces with broad, flat to rounded, sometimes smooth transverse ridges separated by narrow transverse grooves, and adaxial surfaces with a raised attachment scar positioned proximal to a medial keel. Transverse ridges are sometimes obscure or lacking in *P. uncinatus*. Some members of the section have herbage that produces conspicuous, reddish exudate.

Plagiobothrys sect. *Striacarya* is known from western North America from British Columbia, Canada, in the north to Baja CA, Mexico in the south, and extends as far east as Arizona. In South America, taxa of *P*. sect. *Striacarya* are the most common upland *Plagiobothrys* group, with *P. myosotoides* occurring in Peru, Bolivia, Chile, and Argentina. Taxa in *P*. sect. *Striacarya* occur from near sea level to 3,400 m (11,150 ft) in various ecological settings, including coarse, rapidly drained soils in chaparral and more mesic sites in meadows and floodplains. Flowering occurs from March to August.

Etymology: *Striacarya* is from the Latin *stria* for "groove" and *carya* for "nut," alluding to the parallel transverse grooves on the abaxial surface of the nutlets of plants in this section.

A new combination in *Plagiobothrys*

Based on the phylogenetic evidence presented in this paper, a new combination is required for *P. pringlei*, samples of which are phylogenetically nested among samples representing the varieties of *P. collinus*. *Plagiobothrys pringlei* was originally published under the name *Echidiocarya arizonica* A. Gray in 1876. Greene transferred it to *Plagiobothrys* as *P. pringlei* Greene (1887), choosing a new specific epithet because "*arizonica*" was already in use

for *P. arizonica* (A. Gray) Greene ex A. Gray (1885). Absence of an available name at varietal rank for *P. pringlei* permits us to use either "*arizonica*" or "*pringlei*" for the new varietal combination in *P. collinus*. Given the longstanding recognition of this taxon under the name *Plagiobothrys pringlei*, we use the epithet "*pringlei*" in our new combination.

Plagiobothrys collinus (Phil.) I.M. Johnston var. ***pringlei*** (Greene) Guilliams & B.G. Baldwin, comb. nov. Basionym: *Plagiobothrys pringlei* Greene, Pittonia 1:21. 1887. TYPE: U.S.A. Arizona: "Verde Mesa, Arizona", *Dr. Smart, s.n.*, no collection date provided. (holotype: GH).

Figure 1A-R. Nutlet and fruit diversity in Amsinckiinae. **A.** Nutlet of *Amsinckia spectabilis*, abaxial view; **B.** Nutlet of *Amsinckia spectabilis* adaxial view; **C.** Nutlet of *Amsinckia tessellata* abaxial view; **D.** Nutlet of *Amsinckia tessellata* adaxial view; **E.** Nutlet of *Amsinckia vernicosa*, abaxial view; **F.** Nutlet of *Amsinckia vernicosa*, adaxial view; **G.** Nutlet of *Cryptantha utahensis*, abaxial view; **H.** Nutlet of *Cryptantha utahensis*, adaxial view; **I.** Nutlet of *Cryptantha barbigera* var. *barbigera*, abaxial view; **J.** Nutlet of *Cryptantha barbigera* var. *barbigera*, adaxial view; **K.** Fruit of *Pectocarya setosa*, top view; **L.** Fruit of *Pectocarya peninsularis*, top view; **M.** Fruit of *Harpagonella palmeri*, lateral view; **N.** Nutlet of *Plagiobothrys austiniae*, abaxial view; **O.** Nutlet of *Plagiobothrys austiniae*, adaxial view; **P.** Nutlet of *Plagiobothrys leptocladus*, abaxial view; **Q.** Nutlet of *Plagiobothrys leptocladus*, abaxial view; **R.** Nutlet of *Plagiobothrys nothofulvus*, abaxial view.
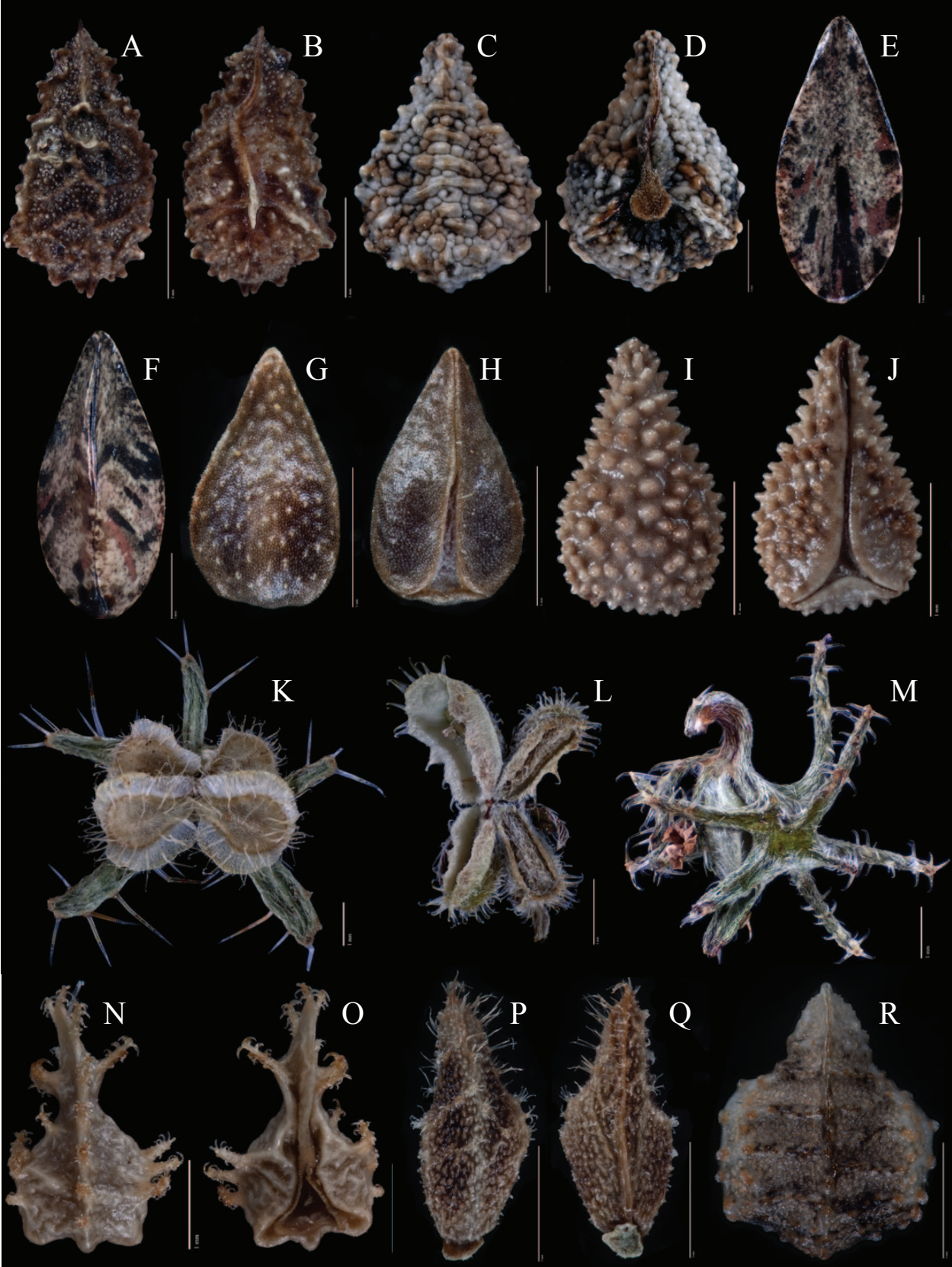
Figure 2A-G. Diverse natural settings where *Plagiobothrys* taxa occur. **A.** *Plagiobothrys fulvus* in an open grassland with other wildflowers; **B.** *Plagiobothrys figuratus* dominant in a marshy area; **C.** *Plagiobothrys collinus* var. *californicus* common in shrub openings in Coastal Sage Scrub vegetation in southern California; **D.** *Plagiobothrys jonesii*, a desert annual; **E.** A close up of *Plagiobothrys parishii*, a plant of wet areas in the desert; **F.** Rabbit Springs, one location where *Plagiobothrys parishii* occurs; **G.** A large vernal pool or lake where *Plagiobothrys undulatus* is common as an aquatic winter annual.
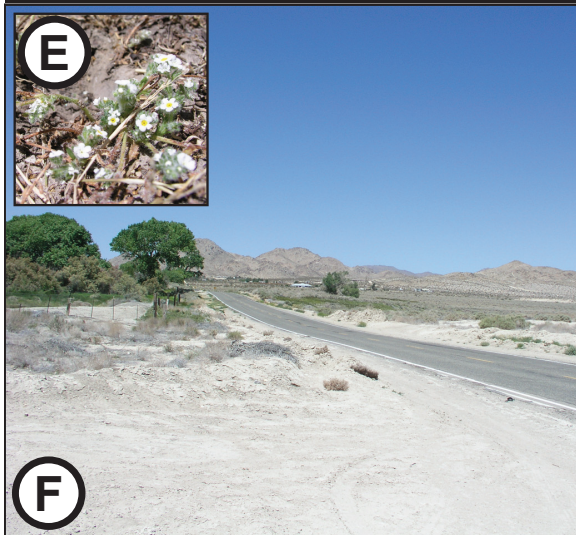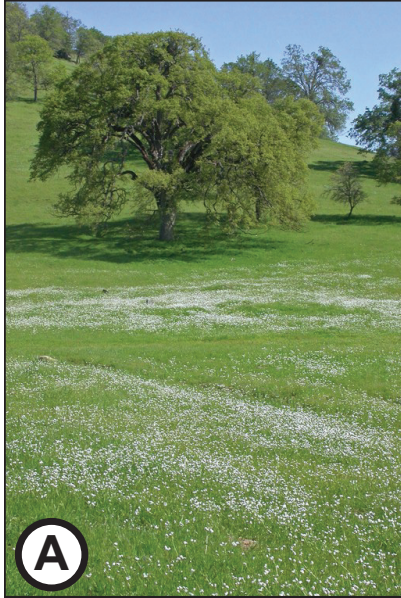
Figure 3A-B. Phylogenetic tree of *Plagiobothrys* and other Amsinckiinae based on combined and partitioned nrDNA and cpDNA data. The tree is the highest likelihood tree from the maximum likelihood analysis in RAxML. Numbers above branches are maximum likelihood bootstrap proportions followed by Bayesian posterior probabilities. **A.** Lower part of the phylogenetic tree; **B.** Upper part of the phylogenetic tree.

B

Plagiobothrys_corymbosus_SA_cmg331
Plagiobothrys_undulatus_cmg414
Plagiobothrys_orientalis_cmg477
Plagiobothrys_stipitatus_var_micranthus_cmg17
Plagiobothrys_cognatus_cmg101
Plagiobothrys_undulatus_cmg12
Plagiobothrys_polycaulis_cmg326
Plagiobothrys_albiflorus_cmg271
Plagiobothrys_calandrinioides_cmg333
Plagiobothrys_scriptus_cmg18
Plagiobothrys_bracteatus_cmg120
Plagiobothrys_hispidulus_cmg86
Plagiobothrys_cusickii_cmg20
Plagiobothrys_cusickii_cmg97
Plagiobothrys_orientalis_cmg478
Plagiobothrys_magellanica_cmg328
Plagiobothrys_scriptus_cmg207
Plagiobothrys_leptocladus_cmg15
Plagiobothrys_humistratus_cmg93
Plagiobothrys_chorisianus_var_chorisianus_cmg102
Plagiobothrys_leptocladus_cmg464
Plagiobothrys_cognatus_cmg19
Plagiobothrys_stipitatus_var_micranthus_cmg309
Plagiobothrys_chorisianus_var_hickmanii_cmg118
Plagiobothrys_chorisianus_var_chorisianus_cmg4
Plagiobothrys_strictus_cmg479
Plagiobothrys_strictus_cmg480
Plagiobothrys_parishii_cmg273
Plagiobothrys_parishii_cmg37
Plagiobothrys_stipitatus_var_stipitatus_cmg92
Plagiobothrys_stipitatus_var_stipitatus_cmg64
Plagiobothrys_glyptocarpus_var_glyptocarpus_cmg72
Plagiobothrys_glyptocarpus_var_modestus_cmg88
Plagiobothrys_colonetensis_cmg470
Plagiobothrys_bracteatus_cmg258
Plagiobothrys_hispidulus_cmg10
Plagiobothrys_trachycarpus_cmg257
Plagiobothrys_tener_var_tener_cmg7
Plagiobothrys_trachycarpus_cmg11
Plagiobothrys_greenei_cmg62
Plagiobothrys_greenei_cmg104
Plagiobothrys_humistratus_cmg13
Plagiobothrys_acanthocarpus_cmg121
Plagiobothrys_acanthocarpus_cmg1
Plagiobothrys_hystriculus_cmg14
Plagiobothrys_greenei_cmg63
Plagiobothrys_glyptocarpus_var_glyptocarpus_cmg5
Plagiobothrys_austiniae_cmg105
Plagiobothrys_austiniae_cmg61
Plagiobothrys_distantiflorus_cmg87
Plagiobothrys_distantiflorus_cmg89
Plagiobothrys_colonetensis_cmg465
Plagiobothrys_diffusus_cmg71
Plagiobothrys_reticulatus_var_reticulatus_cmg16
Plagiobothrys_scouleri_cmg67
Plagiobothrys_mollis_var_mollis_cmg91
Plagiobothrys_mollis_var_mollis_cmg9
Plagiobothrys_figuratus_cmg266
Plagiobothrys_figuratus_cmg265
Plagiobothrys_hirtus_cmg267
Plagiobothrys_macbridei_cmg462
Plagiobothrys_congestus_cmg455
Plagiobothrys_congestus_cmg463
Plagiobothrys_humilis_cmg457
Plagiobothrys_kunthii_cmg426
Plagiobothrys_kunthii_cmg433
Plagiobothrys_linifolius_cmg461_460
Plagiobothrys_linifolius_cmg459
Plagiobothrys_calandrinioides_cmg334
Plagiobothrys_pulchellus_cmg321
Plagiobothrys_pratensis_cmg345
Plagiobothrys_oppositifolius_cmg344
Plagiobothrys_uliginosus_cmg320
Plagiobothrys_procumbens_cmg323
Plagiobothrys_procumbens_cmg330
Plagiobothrys_polycaulis_cmg327
Plagiobothrys_elachanthus_cmg235
Plagiobothrys_elachanthus_cmg451
Plagiobothrys_plurisepalus_cmg452
Plagiobothrys_plurisepalus_cmg237

Plagiobothrys
sect. *Allocarya*

Figure 3A

51/-
53/-
67/0.89
-/1
100/1
80/1
95/1
62/-
88/1
71/1
78/1
60/-
100/1
59/-
100/1
-/0.64
-/0.76
99/1
99/1
85/1
-/0.55
61/0.96
98/1
72/0.91
76/0.99
86/1
61/1
75/1
54/0.82
-/0.64
65/0.92
-/0.59
92/0.89
63/1
78/0.67
97/1

0.01

27

Figure 4A-C. Phylogenetic trees showing ancestral character state estimates for nutlet morphological characters. **A.** Nutlet adaxial configuration; **B.** Nutlet orientation; **C.** Nutlet surface sculpturing.

Character: Adaxial Surface Parsimony reconstruction (Unordered) [Steps: 7]

Grooved
Keeled
Other

Character: Nutlet Orientation Parsimony reconstruction (Unordered) [Steps: 6]

Vertical
Intermediate
Horizontal

Character: Nutlet ornamentation Parsimony reconstruction (Unordered) [Steps: 32]

Smooth
Roughened
Prickles/Teeth
Winged

TABLES

Table 1. Morphological data matrix used in ancestral character state estimation of nutlet morphology

Taxon/Sample	Nutlet adaxial configuration	Nutlet orientation	Nutlet abaxial sculpturing
Amsinckia calycina cmg514	Keeled	Vertical	Roughened
Amsinckia eastwoodiae cmg175	Keeled	Vertical	Roughened
Amsinckia furcata cmg227	Grooved	Vertical	Smooth
Amsinckia grandiflora cmg226	Grooved	Vertical	Smooth
Amsinckia inepta cmg485 486	Keeled	Vertical	Roughened
Amsinckia lycopsoides cmg511	Keeled	Vertical	Roughened
Amsinckia menziesii var intermedia cmg173	Keeled	Vertical	Roughened
Amsinckia menziesii var menziesii cmg223	Keeled	Vertical	Roughened
Amsinckia retrorsa cmg176	Keeled	Vertical	Roughened
Amsinckia spectabilis cmg179	Keeled	Vertical	Roughened&Prickles/Teeth
Amsinckia tessellata var gloriosa cmg482	Keeled	Vertical	Roughened
Amsinckia tessellata var tessellata cmg508	Keeled	Vertical	Roughened
Amsinckia vernicosa cmg225	Grooved	Vertical	Smooth
Cryptantha flaccida cmg228	Grooved	Vertical	Smooth
Cryptantha gracilis cmg233	Grooved	Vertical	Smooth
Cryptantha maritima cmg125	Grooved	Vertical	Smooth&Roughened
Cryptantha muricata cmg126	Grooved	Vertical	Roughened
Cryptantha simulans cmg127	Grooved	Vertical	Roughened
Cynoglossum amabile	Other	Intermediate	Prickles/Teeth
Cynoglossum magellense	Other	Intermediate	Prickles/Teeth
Dasynotus daubenmirei	Other	Horizontal	Smooth
Eremocarya lepida	Grooved	Vertical	Smooth&Roughened
Eremocarya micrantha	Grooved	Vertical	Smooth&Roughened
Greeneocharis circumscissa cmg230	Grooved	Vertical	Roughened
Harpagonella palmeri var arizonica cmg370	Other	Vertical	Smooth
Harpagonella palmeri var palmeri cmg376	Other	Vertical	Smooth
Johnstonella costata cmg124	Grooved	Vertical	Roughened
Johnstonella micromeres cmg234	Grooved	Vertical	Smooth&Roughened
Johnstonella racemosa cmg232	Grooved	Vertical	Roughened
Oreocarya cana cmg366	Grooved	Vertical	Roughened
Oreocarya celosioides cmg368	Grooved	Vertical	Smooth
Oreocarya cinerea var jamesii cmg369	Grooved	Vertical	Smooth
Oreocarya confertiflora cmg229	Grooved	Vertical	Smooth
Oreocarya flavoculata cmg231	Grooved	Vertical	Roughened
Oreocarya nubigenia cmg367	Grooved	Vertical	Smooth
Pectocarya anisocarpa cmg384	Other	Horizontal	Winged
Pectocarya anomala cmg359	Other	Horizontal	Prickles/Teeth
Pectocarya heterocarpa cmg391	Other	Horizontal	Prickles/Teeth
Pectocarya linearis var ferocula cmg383	Other	Horizontal	Prickles/Teeth
Pectocarya linearis var linearis cmg355	Other	Horizontal	Prickles/Teeth

Taxon/Sample	Nutlet adaxial configuration	Nutlet orientation	Nutlet abaxial sculpturing
Pectocarya penicillata cmg99	Other	Horizontal	Winged
Pectocarya peninsularis cmg436	Other	Horizontal	Winged
Pectocarya platycarpa cmg389	Other	Horizontal	Prickles/Teeth
Pectocarya pusilla cmg363	Other	Intermediate	Winged
Pectocarya recurvata cmg382	Other	Horizontal	Prickles/Teeth
Pectocarya setosa cmg74	Other	Horizontal	Winged
Plagiobothrys acanthocarpus cmg121	Keeled	Vertical	Prickles/Teeth
Plagiobothrys albiflorus cmg271	Keeled	Vertical	Roughened
Plagiobothrys arizonicus cmg169	Keeled	Vertical	Roughened
Plagiobothrys austiniae cmg61	Keeled	Vertical	Prickles/Teeth
Plagiobothrys bracteatus cmg120	Keeled	Vertical	Roughened
Plagiobothrys calandrinioides cmg333	Keeled	Vertical	Roughened
Plagiobothrys canescens var canescens cmg22	Keeled	Vertical	Roughened
Plagiobothrys canescens var catalinense cmg36	Keeled	Vertical	Roughened
Plagiobothrys chorisianus var chorisianus cmg4	Keeled	Vertical	Roughened
Plagiobothrys cognatus cmg101	Keeled	Vertical	Roughened
Plagiobothrys collinus var californicus cmg119	Keeled	Vertical	Roughened
Plagiobothrys collinus var collinus cmg341	Keeled	Vertical	Roughened
Plagiobothrys collinus var fulvescens cmg24	Keeled	Vertical	Roughened
Plagiobothrys collinus var gracilis cmg25	Keeled	Vertical	Roughened
Plagiobothrys collinus var ursinus cmg240	Keeled	Vertical	Roughened
Plagiobothrys colonetensis cmg470	Keeled	Vertical	Roughened
Plagiobothrys congestus cmg463	Keeled	Vertical	Roughened
Plagiobothrys corymbosus SA cmg331	Keeled	Vertical	Smooth
Plagiobothrys cusickii cmg20	Keeled	Vertical	Roughened
Plagiobothrys diffusus cmg71	Keeled	Vertical	Roughened
Plagiobothrys distantiflorus cmg89	Keeled	Vertical	Roughened
Plagiobothrys elachanthus cmg235	Keeled	Vertical	Roughened
Plagiobothrys figuratus cmg266	Keeled	Vertical	Roughened
Plagiobothrys fulvus var campestris cmg95	Keeled	Vertical	Roughened&Prickles/Teeth
Plagiobothrys fulvus var fulvus cmg336	Keeled	Vertical	Roughened&Prickles/Teeth
Plagiobothrys glomeratus cmg311	Grooved&Other	Vertical&Horizontal	Smooth
Plagiobothrys glyptocarpus var glyptocarpus cmg72	Keeled	Vertical	Roughened
Plagiobothrys glyptocarpus var modestus cmg88	Keeled	Vertical	Roughened
Plagiobothrys greenei cmg62	Keeled	Vertical	Prickles/Teeth
Plagiobothrys hirtus cmg267	Keeled	Vertical	Roughened
Plagiobothrys hispidulus cmg86	Keeled	Vertical	Prickles/Teeth
Plagiobothrys hispidus cmg301	Grooved&Other	Vertical&Horizontal	Smooth

Taxon/Sample	Nutlet adaxial configuration	Nutlet orientation	Nutlet abaxial sculpturing
Plagiobothrys humilis cmg457	Keeled	Vertical	Roughened
Plagiobothrys humistratus cmg13	Keeled	Vertical	Prickles/Teeth
Plagiobothrys hystriculus cmg14	Keeled	Vertical	Prickles/Teeth
Plagiobothrys infectivus cmg103	Keeled	Vertical	Roughened&Prickles/Teeth
Plagiobothrys jonesii cmg211	Keeled	Vertical	Roughened
Plagiobothrys kingii var harknessii cmg29	Keeled	Vertical	Roughened
Plagiobothrys kingii var kingii cmg30	Keeled	Vertical	Roughened
Plagiobothrys kunthii cmg433	Keeled	Vertical	Roughened
Plagiobothrys leptocladus cmg464	Keeled	Vertical	Smooth&Prickles/Teeth
Plagiobothrys linifolius cmg459	Keeled	Vertical	Roughened
Plagiobothrys macbridei cmg462	Keeled	Vertical	Prickles/Teeth
Plagiobothrys magellanica cmg328	Keeled	Vertical	Roughened
Plagiobothrys mollis var mollis cmg9	Keeled	Vertical	Roughened
Plagiobothrys myosotoides SA cmg317	Keeled	Vertical	Roughened
Plagiobothrys nothofulvus cmg65	Keeled	Vertical	Roughened
Plagiobothrys oppositifolius cmg344	Keeled	Vertical	Roughened
Plagiobothrys orientalis cmg477	Keeled	Vertical	Roughened
Plagiobothrys parishii cmg37	Keeled	Vertical	Roughened
Plagiobothrys plurisepalus cmg237	Keeled	Vertical	Roughened
Plagiobothrys polycaulis cmg326	Keeled	Vertical	Roughened
Plagiobothrys pratensis cmg345	Keeled	Vertical	Roughened
Plagiobothrys pringlei cmg300	Keeled	Vertical	Roughened
Plagiobothrys procumbens cmg323	Keeled	Vertical	Prickles/Teeth
Plagiobothrys pulchellus cmg321	Keeled	Vertical	Prickles/Teeth
Plagiobothrys reticulatus var reticulatus cmg16	Keeled	Vertical	Roughened
Plagiobothrys scouleri cmg67	Keeled	Vertical	Roughened
Plagiobothrys scriptus cmg18	Keeled	Vertical	Prickles/Teeth
Plagiobothrys shastensis cmg32	Keeled	Vertical	Roughened
Plagiobothrys stipitatus var micranthus cmg309	Keeled	Vertical	Roughened
Plagiobothrys stipitatus var stipitatus cmg64	Keeled	Vertical	Roughened
Plagiobothrys strictus cmg480	Keeled	Vertical	Roughened
Plagiobothrys tenellus cmg263	Keeled	Vertical	Roughened
Plagiobothrys tener var tener cmg7	Keeled	Vertical	Roughened
Plagiobothrys tinctorius cmg324	Keeled	Vertical	Roughened
Plagiobothrys torreyi var diffusus cmg34	Keeled	Vertical	Roughened
Plagiobothrys torreyi var perplexans cmg260	Keeled	Vertical	Roughened
Plagiobothrys torreyi var torreyi cmg90	Keeled	Vertical	Roughened
Plagiobothrys trachycarpus cmg257	Keeled	Vertical	Roughened&Prickles/Teeth
Plagiobothrys uliginosus cmg320	Keeled	Vertical	Roughened

Taxon/Sample	Nutlet adaxial configuration	Nutlet orientation	Nutlet abaxial sculpturing
Plagiobothrys uncinatus cmg262	Keeled	Vertical	Roughened
Plagiobothrys undulatus cmg414	Keeled	Vertical	Roughened
Plagiobothrys verrucosus cmg319	Keeled	Vertical	Roughened

Table 2. Current and proposed sectional classification of *Plagiobothrys*

Johnston (1923)	This Paper
Plagiobothrys* § *Allocarya See Johnston (1923) for lower taxa	***Plagiobothrys* § *Allocarya*** Circumscription unchanged, although lower taxa included differs due to changing views toward lower taxa
Plagiobothrys* § *Euplagiobothrys *P. arizonicus* (A. Gray) Greene ex A. Gray *P. canescens* Benth. *P. catalinensis* (A. Gray) J.F. Macbr. *P. fulvus* (Hook. & Arn.) I.M. Johnst. var. *campestris* (Greene) I.M. Johnston *P. fulvus* (Hook. & Arn.) I.M. Johnst. var. *fulvus* *P. nothofulvus* (A. Gray) A. Gray *P. shastensis* Greene ex. A. Gray *P. tenellus* (Nutt.) A. Gray var. *colorans* (Greene) I.M. Johnston *P. tenellus* (Nutt.) A. Gray var. *parvululs* (Greene) I.M. Johnston *P. tenellus* (Nutt.) A. Gray var. *tenellus* *P. tinctorius* (Ruiz & Pav.) A. Gray *P. torreyi* (A. Gray) A. Gray var. *diffusus* I.M. Johnston *P. torreyi* (A. Gray) A. Gray var. *perplexans* I.M. Johnston *P. torreyi* (A. Gray) A. Gray var. *torreyi*	***Plagiobothrys* § *Plagiobothrys*** *P. fulvus* (Hook. & Arn.) I.M. Johnst. var. *campestris* (Greene) I.M. Johnston *P. fulvus* (Hook. & Arn.) I.M. Johnst. var. *fulvus* *P. infectivus* I.M. Johnston ***Plagiobothrys* § *Costacarya*** *P. arizonicus* (A. Gray) Greene ex A. Gray *P. canescens* Benth. var. *canescens* *P. canescens* Benth. var. *catalinensis* (A. Gray) Jeps. *P. nothofulvus* (A. Gray) A. Gray ***Plagiobothrys* § *Striacarya*** *P. shastensis* Greene ex A. Gray *P. tenellus* (Nutt.) A. Gray *P. tinctorius* (Ruiz & Pav.) A. Gray *P. torreyi* (A. Gray) A. Gray var. *diffusus* I.M. Johnston *P. torreyi* (A. Gray) A. Gray var. *perplexans* I.M. Johnston *P. torreyi* (A. Gray) A. Gray var. *torreyi* *P. uncinatus* J.T. Howell
Plagiobothrys* § *Amsinckiopsis *P. harknessii* (Greene) Nels. & Macbr. *P. jonesii* A. Gray *P. kingii* (S. Watson) A. Gray	***Amsinckiopsis*** *A. kingii* (S. Watson) Guilliams & B.G. Baldwin var. *harknessii* (Greene) Guilliams & B.G. Baldwin *A. kingii* (S. Watson) Guilliams & B.G. Baldwin var. *kingii* ***Simpsonanthus*** *S. jonesii* (A. Gray) Guilliams & B.G. Baldwin
Plagiobothrys* § *Echidiocarya *P. californicus* var. *fulvescens* I.M. Johnston *P. californicus* var. *genuinus* I.M. Johnston *P. californicus* var. *gracilis* I.M. Johnston *P. californicus* var. *ursinus* (A. Gray) I.M. Johnston *P. pringlei* Greene	***Plagiobothrys* § *Echidiocarya*** *P. collinus* (Phil.) I.M. Johnston var. *californicus* (A. Gray) L.C. Higgins *P. collinus* (Phil.) I.M. Johnston var. *collinus* *P. collinus* (Phil.) I.M. Johnston var. *fulvescens* (I.M. Johnston) L.C. Higgins *P. collinus* (Phil.) I.M. Johnston var. *gracilis* (I.M. Johnston) L.C. Higgins *P. collinus* (Phil.) I.M. Johnston var. *ursinus* (A. Gray) L.C. Higgins *P. collinus* (Phil.) I.M. Johnston var. *pringlei* Guilliams & B.G. Baldwin
Plagiobothrys* § *Sonnea *P. glomeratus* A. Gray *P. hispidus* A. Gray var. *foliaceus* (Greene) I.M. Johnston *P. hispidus* A. Gray var. *genuinus* I.M. Johnston	***Sonnea*** *S. glomerata* (A. Gray) Greene *S. hispida* (A. Gray) Greene

Chapter 2. Pattern and process of American Amphitropical Disjunction in Amsinckiinae

INTRODUCTION

American amphitropical disjunction (AAD) is a biogeographic pattern that has long intrigued botanists and biogeographers. Gray and Hooker (1880) were perhaps the first to describe this pattern, wherein related plants are found in extratropical North America and South America, but are absent in the intervening tropics. Early work on AAD was largely descriptive, tending to focus on the taxonomy and biogeography of amphitropically disjunct taxa, and few studies examined the pattern across many taxonomic groups. In 1963, the entire second volume of the Quarterly Review of Biology was devoted to this topic. Research during that time was important for enumerating lists of amphitropically distributed taxa in the Americas and elucidating distinct biogeographic subpatterns within AAD.

Genera, "species groups" (putative close relatives within a genus), and species displaying AAD have been tabulated in the literature (e.g., Gray and Hooker 1880, Constance 1963, Raven 1963, Thorne 1972). Extant genera were usually placed in the category of AAD if lower taxa are amphitropically distributed and either 1) relationships within the genus are insufficiently resolved to identify the lineage involved in the disjunction or 2) there are only 2 lower taxa. "Species groups" were identified as such when morphological, phylogenetic, or other evidence permitted the more precise identification of the lineage within a genus where a disjunction occurred. Species may be amphitropically distributed in the New World, with infraspecific taxa recognized on each continent or not. In the most complete list to date, Raven (1963) listed 160 AAD genera, species groups or pairs, and species.

In a summary paper on AAD in that volume, Raven recognized three subcategories of AAD: mediterranean-mediterranean disjunctions, desert-desert disjunctions, and bipolar disjunctions (Raven 1963, Thorne 1972). A fourth pattern of ADD is often discussed for high elevation taxa that are disjunct between North America and South America but extend toward the equator from one or both continents along the somewhat continuous chain of mountains that extends from western North America through portions of Central America to western South America. Figure 1 shows hypothetical examples of the first three of these subcategories on a map of the western hemisphere. While these categories may be useful summary devices, in nature, many of the previously recognized AAD taxa fail to conform to these general patterns (Raven 1963).

The most interesting and perhaps the most challenging aspect of AAD concerns the mechanism whereby taxa may become amphitropically distributed. A number of explanations for the many observed disjunct distributions between North America and South America have been suggested in the literature. Raven (1963) considered only two potential explanations of AAD worthy of consideration, 1) North American and South American temperate region congeners or conspecifics became isolated following the historical fragmentation of a continuous range that spanned the areas now occupied by the American Tropics, which we call the "range fragmentation" hypothesis and 2) a taxon that arose in either North America or South America and then subsequently crossed the tropics to the other hemisphere in one or more steps, which we call the "long-distance dispersal" hypothesis. Recent human activity has also resulted in the exchange of species between North America and South America, but such transport is not further considered here.

The range fragmentation hypothesis has not been much examined in the literature. Raven (1963) did not focus on this pattern in his review and to our knowledge it has not been explored in detail since its early formulation by Johnston (1940) in a paper describing the pattern in desert shrubs shared between North America and South America (but see Simpson et al. 2005 and references therein). Nevertheless, it is clearly the case that AAD taxa identified in the literature to date, especially those that are herbaceous or shrubby, are overwhelmingly those without close relatives in the American tropics.

The long-distance dispersal hypothesis has been favored in the literature on AAD since receiving a unanimous endorsement by all contributors to the 1963 issue of the Quarterly Review on this topic. Figure 2 provides images of some features and habitats associated with the long-distance dispersal hypothesis. This hypothesis can be further elaborated based on putative dispersal vector. Although nearly all studies examined for the present paper favor a hypothesis of bird dispersal, one recent unpublished study suggested that large mammals may have facilitated dispersal of plant propagules following the closure of the Isthmus of Panama during the Great American Biotic Interchange (GABI; Biro and Whittall 2015). Although empirical studies demonstrating the efficacy of any animal vector in dispersing propagules between North America and South America are lacking (but see Lewis et al. 2014), the vast numbers of migratory birds travelling annually between North America and South America along the Pacific Flyway would seem to offer ample opportunity for occurrence of this low-probability event. Furthermore, Raven (1963) noted that many AAD plant groups occur in habitats that would bring them into contact with migratory birds, such as coastal areas, vernal pools, wetlands, and "open weedy grasslands". Some studies of AAD plant groups noted that propagules have morphological features consistent with dispersal by animals, such as barbed appendages, hooked trichomes, and gelatinous seeds (Constance 1963, Carlquist 1983). One study advocating long-distance dispersal by large mammals during the GABI (Biro and Whittall 2015) provided three lines of evidence in support of their hypothesis: 1) some portion of the branch subtending the AAD plant clades examined fell within the period of time that GABI has been hypothesized to have occurred (there, given as the range ca. 3.2-1.4 Ma), 2) inferred directionality of dispersals was predominantly from the north to the south, which they asserted is consistent with the primary direction of mammal dispersals during GABI, and 3) propagules of the AAD lineages examined were largely without morphological features that would promote bird dispersal. Element three in the preceding list would seem to be at odds with the observations of some authors that AAD lineages possess fruit features with putative adaptations for epizoochory, such as barbs and hooked trichomes. Note that recent evidence has largely overturned the historically dominant view that GABI occurred in four major pluses of migration (GABI 1, 2.6-2.4 Ma; GABI 2, 1.8 Ma; GABI 3, 0.8 Ma; and GABI 4, 0.125 Ma) following the closure of the Isthmus of Panama between 3.5 and 3 Ma (Woodburne 2010). The emerging view of an early and protracted intermingling of the long-isolated biotas of North America and South America is based upon recent geological evidence pointing toward an earlier (middle Miocene) closure of the Isthmus (15-13 ma; Montes et al. 2015), as well as fossil evidence (Woodburne 2010 and references therein) and an increasingly large number of molecular phylogenetic studies that suggested much earlier biotic connections (e.g., Cody et al. 2010, Pinto-Sanchez et al. 2012, Bacon et al. 2013, Parada et al. 2013, Leite et al. 2014, Wilson et al. 2014, Bacon et al. 2015, Barker et al. 2015). Given the broad interval of geologic time within which plant dispersals may have occurred via GABI, we found it difficult to formulate a meaningful test of the GABI hypothesis of AAD and thus will focus on other aspects of this biogeographic pattern.

Following a successful dispersal event, a disseminule must establish in the new location if a population is to be founded. It has long been hypothesized that breeding system may affect establishment following a long-distance dispersal event. Baker (1955) suggested that plants with the ability to self-pollinate would be more likely to establish after dispersal since only a single disseminule would be required to found a new population, an idea now codified as Baker's Law or Rule (Stebbins 1957). Baker's Rule was first described in pan-tropical Plumbaginaceae, where autogamy tended to correlate with long-distance dispersal (Baker 1948). Raven (1963) listed several instances of AAD where Baker's Rule may apply, most of which involve North American clades of predominantly out-crossing taxa represented in South America by autogamous taxa. There have been no recent phylogenetically-based biogeographic studies that have examined the role of Baker's Rule with respect to AAD.

Statistical hypothesis testing in biogeography has generally been lacking historically (Crisp et al. 2010, Gillespie et al. 2012) and this is true for studies of the AAD biogeographic pattern as well. Only a handful of recent studies have examined the AAD pattern in a phylogenetic context, and of these, only three to four attempted to examine aspects of biogeography beyond assessing number and direction of dispersal events (e.g., timing of dispersal), and none evaluated hypotheses regarding the vector of dispersal. Hypothesis testing in these studies may have been deemphasized because there are generally too few transitions between North America and South America to permit it; essentially, the sample size is too small. One recent review paper (Wen and Ickert-Bond 2009) aggregated results from recent phylogenetic studies of AAD taxa and found that 65 percent of dispersals were from North America to South America, and of those that estimated timing of dispersals, most inferred dispersals were between 8.4 Ma and within a few thousand years ago. Although the study did not evaluate the hypothesis using a statistical test, their meta-analysis did examine the issue of timing of dispersals in a way that permits qualitative evaluation of patterns. What is still needed is a study of AAD across all angiosperms in the context of phylogeny; absent that study, a good first step is a study of AAD in a group with a sufficiently large number of transitions between North America and South America to allow for hypothesis testing. Amsinckiinae (Boraginaceae) qualifies as such a group.

Subtribe Amsinckiinae (Boraginaceae s.s.) comprises approximately 330-342 minimum-rank, annual and perennial herbaceous taxa that are native to North America, South America, and Australia (Guilliams, Chapter 1). There are nine recognized genera in the subtribe, six of which are amphitropically distributed in the Americas. In North America, Amsinckiinae taxa may occur in a variety of habitats from near sea level at the coast to above tree line in the interior mountains of western North America, and have been heralded by Raven and Axelrod as being emblematic of the diverse California flora (Raven and Axelrod 1978). In South America, members of the subtribe appear to occupy a similar range of conditions, but there are fewer minimum-rank taxa in general (ca. 245--257 in North America as compared to ca. 86 in South America). Outside the New World, three to four species of the genus *Plagiobothrys* are native to Australia.

With high taxonomic richness in both North America and South America and AAD present in many genera, subtribe Amsinckiinae may be an ideal candidate for examining the AAD pattern in a hypothesis-testing framework. Amsinckiinae possess other desirable attributes as well. First, the Boraginaceae s.s. including Amsinckiinae have a fruit with four subunits, called "nutlets", and these nutlets have a wide variety of surface ornamentation patterns (Figure 3). Taxa in the subtribe have nutlet outer surfaces that range from smooth to transversely ridged to appendaged with spine-like processes with retrorse barbs distally. Nutlets also differ in size

and shape. This diversity of nutlet features may provide the opportunity to test for correlations between AAD and morphological features of the nutlets that may serve as adaptations that facilitate animal dispersal. Second, members of Amsinckiinae are found in many ecological settings, which may allow examination of Raven's hypothesis (1963) regarding the relationship between AAD, habitat (e.g., marshes, vernal pools), and bird migration/dispersal. Finally, breeding system diversity has been reported in Amsinckiinae (e.g. Ray and Chisaki 1957, Higgins 1969, Moldenke 1976), which may permit the assessment of Baker's Rule in this system.

This paper had several objectives thematically centering on the goal of examining AAD, using target members of Amsinckiinae as a case study. We inferred a phylogeny of the subtribe using sequence data, which we fossil-calibrated to convert branch lengths to estimates of absolute time. Using this calibrated phylogeny we estimated the number, directionality, and timing of inferred range shifts between North America and South America with a primary focus on *Plagiobothrys* and secondary foci on *Amsinckia* and *Pectocarya*. We performed statistical model-fitting under maximum likelihood (ML) to determine the best fitting biogeographic model given the data, and explicitly evaluate the importance of founder-event speciation in this system, a reasonable biogeographic scenario in island and certain continental settings. We also inferred biogeographic states under ML over the Bayesian posterior distribution of trees, an approach that while not explicitly modeling biogeography does account in some way for phylogenetic uncertainty.

We then formally tested or examined several aspects of the long-distance dispersal hypothesis of AAD in Amsinckiinae. Two statistical tests concerned the timing of dispersal between North America and South America using the 95% HPD interval (HPD) of node age estimates subtending inferred dispersals. We examined whether the estimated distributions of node ages are statistically similar or if one or more means differ. A non-significant test statistic means that estimated node ages are not statistically different, which may suggest a response among the sampled lineages of Amsinckiinae to single, common causative agent. A significant test statistic means that one or more node age estimate(s) are statistically significantly different, which we would consider to be consistent with an on-going (low probability) process such as rare, long-distance dispersal by animals. We then qualitatively examined node age estimates with respect to the onset of summer drying at ca. 15 Ma (Jacobs et al. 2004).

We then examined the relationship between AAD and three morphological features of the nutlets of Amsinckiinae. For these tests we developed a morphological dataset that includes measurements of fruit size and fruit ornamentation for target Amsinckiinae. We used this morphological dataset in a phylogenetic context across the subtribe to test for a relationship between values of these features and dispersal. We hypothesized a negative relationship between fruit size and AAD, under the reasoning that smaller fruits would be more likely to be transported long distances without being dislodged from a migrating animal. We hypothesized a positive relationship between degree of fruit ornamentation and AAD, under the reasoning that more highly ornamented fruits would adhere more strongly to a migrating animal.

Finally, we considered the potential contribution of breeding system in AAD in Amsinckiinae through a non-exhaustive greenhouse survey of automatic selfing in the absence of pollinators.

MATERIALS AND METHODS

Taxon sampling

A list of all accessions included in this study is given in Appendix 2. Within *Plagiobothrys*, we included one sample each of most recognized taxa (75 in total). We also included exemplar taxa from each of the genera of Amsinckiinae: *Amsinckia* (15 taxa), *Cryptantha* s.s. 1 (4 taxa) and *Cryptantha* s.s. 2 (1 taxon) of Hasenstab and Simpson (2012), *Eremocarya* (2 taxa), *Greeneocharis* (1 taxon), *Harpagonella* (2 taxa), *Johnstonella* (3 taxa), *Oreocarya* (6 taxa), and *Pectocarya* (11 taxa). All sequence data for this study were generated for a previous study (Guilliams, Chapter 1). Some sequence data for five non-*Plagiobothrys* taxa were downloaded from Genbank (*Cynoglossum amabile*, *Cynoglossum magellense*, *Dasynotus daubenmirei*, *Eremocarya lepida*, and *E. micrantha*). We used two *Cynoglossum* species as outgroups for this study. These *Cynoglossum* taxa were shown to be closely related to Amsinckiinae in three recent broad-scale analyses of the Boraginaceae (Nazaire and Hufford 2012, Cohen 2013, Weigend et al. 2013).

DNA extraction, amplification, and sequencing

Methods of genomic DNA isolation, fragment amplification, and sequencing were all described in Chapter 1. No new DNA sequence data were added for Chapter 2.

Phylogenetic inference and divergence time estimation in Amsinckiinae

We simultaneously estimated phylogenetic relationships and divergence times using BI in BEAST v.1.8.0 (Drummond and Rambaut 2007) through the CIPRES Science Gateway v3.3. Sampling from the posterior probability distribution was done in BEAST using Markov Chain Monte Carlo (MCMC) under a GTR + Γ model of sequence evolution. MCMC was run for 100 million generations per analysis, sampling model parameters every 10,000[th] generation. Adequate sampling from the posterior distribution of model parameters was evaluated in two ways: by examining plots for each model parameter in Tracer v.1.5, and by evaluation of Estimated Sample Size (ESS). For the former, we found the approximate generation at which parameter traces became relatively flat, while for the latter we assumed adequate sampling when ESSs for model parameters were above 200 (Rambaut and Drummond 2007). Based on these criteria, we discarded the first 10% of the trees (10 million generations) as "burn-in". The maximum clade credibility tree (MCCT) was found using Tree Annotator v. 1.7.4. We ran separate phylogenetic analyses of the nuclear and chloroplast data, then ran an analysis of the combined nuclear and chloroplast data after a careful inspection of congruence between the separate nrDNA and cpDNA analyses.

Branch lengths were scaled to absolute time in two ways. First, we applied a fossil calibration at the crown of our sampled *Oreocarya* taxa based on a separate morphological analysis of fossil Amsinckiinae (Guilliams unpublished data). These *Cryptantha* s.l. fossils are from what have historically been referred to as the Ash Hollow sediments of the Ogallala formation of Kansas, the lower and upper bounds of which have been dated to between 10.6 ± 1 Ma and 6.8 ± 0.3 Ma, respectively (Thomasson 1979 and references therein). Nutlets of fossil *Cryptantha* taxa are known from a specific region in the lower Ash Hollow sediments and have never been located outside of the Ash Hollow sediments. Therefore, we applied a prior on the node age of a constrained monophyletic *Oreocarya* using a normal distribution with a mean of 8.7 Ma and a standard deviation of 0.97. This prior permits node heights for crown *Oreocarya* to be drawn from a normal distribution centered on the middle of the Ash Hollow and spanning the

entire Ash Hollow sediments and slightly beyond. We applied a secondary calibration at the crown of Amsinckiinae based upon a reanalysis of the broad study of divergence times and biogeography in the Boraginales (= Boraginaceae s.l.) by Nazaire and Hufford (2014). In this study, the authors used BEAST to infer a phylogenetic tree of target Boraginaceae, which employed 20 fossil calibrations across the angiosperms to scale the branch lengths to absolute time. Of these fossils, 2 were used to calibrate nodes within the Boraginaceae, while the remaining 18 were used to calibrate deeper nodes in the tree. Within the Boraginales, Nazaire and Hufford used the same fossils described above to calibrate "the Great Plains cryptanthas", using a minimum age constraint of 2.6 Ma based upon an earlier, erroneous estimate of the age of the Ash Hollow sediments (Elias 1932). For this present study, we obtained the Nazaire and Hufford dataset from the authors and modified the inference parameters to exclude their fossil calibration in Amsinckiinae for two reasons. First, we wish to use these same fossils in the manner indicated above to calibrate our phylogenetic analysis. To use both the *Cryptantha* fossils and the secondary calibration from the Nazaire and Hufford analysis, the latter must not include the former to avoid circularity. Second, recent revised age estimates of the now defunct "Ogallala Formation" and the Ash Hollow sediments suggest that these fossils are much older than 2.6 Ma, at least 6.8 ± 0.3 Ma and perhaps as old as 10.6 ± 1 Ma. While underestimating the age of these fossils for the broader analysis of the Boraginaceae might have a negligible effect on node age estimates in general, we suspect that this prior would have a strong local effect in Amsinckiinae. Therefore, we performed a reanalysis of their dataset using identical parameters (see Nazaire and Hufford 2014) except we eliminated the fossil constraint on the "Great Plains cryptanthas". In our reanalysis of the Nazaire and Hufford dataset, Amsinckiinae crown had a mean node age of 11.07 Ma and 95% HPD interval of 15.7 Ma to 7.28 Ma. Therefore, in our analysis of Amsinckiinae, we constrained the root height of sampled Amsinckiinae using a uniform prior with upper and lower bounds set to 7.54 Ma and 16.2 Ma, respectively, to encompass the entire range of node age estimates.

Biogeographic analysis

Biogeographic analysis was performed using the R package BioGeoBEARS (Matzke 2014) and under ML in Mesquite (Maddison and Maddison 2008). BioGeoBEARS simultaneously performs two analyses. First, the program performs biogeographic analyses under a range of biogeographic models. Available models include the parsimony model DIVA (which under likelihood in BioGeoBEARS is called "DIVALIKE"), the likelihood based Dispersal Extinction Cladogenesis (DEC) model as implemented in the program Lagrange (Ree et al. 2005, Ree and Smith 2008), and an extension of all models using parameter "J", which permits the inference of founder-event speciation events. Founder-event speciation has not been available as an option under previous likelihood-based biogeographic analyses. Previous studies have demonstrated the importance of founder-event speciation in both island systems, where founder-event speciation may be the rule, as well as in continental systems where it may be common. Following biogeographic inference under many models, BioGeoBEARS then finds the optimal biogeographic model using the Akaike Information Criterion (AIC). BioGeoBEARS therefore allows for the direct comparison of biogeographic models using a standard model comparison metric. Prior to BioGeoBEARS, models and results from different programs could not be compared directly, which could have resulted in increased subjectivity during the analysis of results. A drawback of both Lagrange and BioGeoBEARS is that analyses must be performed on a single phylogenetic tree, which may be undesirable if the best tree available has clades of low

support. In that case, it may be preferable to perform analyses across a set of trees, e.g., a Bayesian posterior distribution of trees, to allow for phylogenetic uncertainty.

To partially account for phylogenetic uncertainty, ancestral character estimation of biogeographic areas was performed across a sample from the posterior distribution of trees resulting from the BEAST analysis under ML in Mesquite. Inferred states at each node were then summarized onto the MCCT as marginal likelihoods.

Examining hypotheses relating to timing of dispersal

The biogeographic analyses identified important biogeographic transitions between North America and South America. As the trees on which these biogeographic analyses were conducted were calibrated to absolute time, we had previously obtained a posterior distribution of age estimates (the 95% HPD interval) associated with these biogeographic range shifts. We used these distributions of node ages to evaluate two hypotheses.

First, node age estimates across inferred biogeographic transitions may be similar or different. Similarity of node age estimates among inferred biogeographic transitions may suggest a single event acting on those lineages concurrently, while at the same time reducing support for hypotheses such as bird dispersal that would be expected to yield a pattern of biogeographic splits that accumulate over time. Alternatively, a finding of different node age estimates among the inferred biogeographic transitions may lend support to on-going, low-probability events such as dispersal by birds, while reducing support for the single-event explanation. We hypothesize that birds have been the primary vector associated with movement of propagules between North America and South America. If this has been the case, we would predict that node age estimates among biogeographic transitions will be statistically dissimilar. We examine the null hypothesis that node age estimates are statistically similar using the non-parametric Kruskal-Wallis in R. We then performed Bonferroni-corrected pairwise post-hoc comparisons using the non-parametric Wilcoxon rank sum test. Second, we qualitatively compare the inferred biogeographic transitions to the timing of the onset of summer drought in western North America, which began following the middle Miocene Climatic Optimum ca. 15.5 Ma.

Testing factors hypothesized to be important in promoting long-distance dispersal and establishment in American mediterranean-type climates (including Australia)

Comparative phylogenetic tests were performed to evaluate the relationship between inferred dispersals between North America and South America and four morphological features that may correlate with animal dispersal: fruit size (here represented by fruit length) and three aspects of fruit ornamentation. To do this, we constructed a large morphological dataset of some members of Amsinckiinae. A fruit/nutlet collection was made by removing one nutlet per specimen for usually between 10 and 30 specimens per taxon. Nutlets were affixed to labeled microscope slides using double-stick tape. Digital images of each nutlet in adaxial, lateral, and abaxial views were taken using a Nikon D90 digital camera attached to a Leica Wild MZ8 dissecting microscope. Characters were measured from digital images of adaxial nutlet surfaces on a computer using ImageJ (Abramoff et al. 2004).

Due to the paucity of collections of many South American taxa, the collection of nutlet samples was mostly from North American taxa. For some South American taxa that were difficult or impossible to obtain on loan, we obtained measurement data from high quality scanning electron micrographs or high-resolution nutlet images provided by collaborators. In these cases, micrographs were taken from the dissertation of Horn (2000) and high-resolution images were obtained from Michael G. Simpson (unpublished images).

A rich dataset of 29 directly-measured continuous characters was developed, from which an additional 20 composite characters (e.g., ratio characters) were created. For this study we examine two directly measured characters and two composite characters: fruit size (mm), prickle length (mm), relative prickle length (unitless), and average relative tubercle extent (unitless). Fruit size was obtained through direct measurement of nutlet length (mm) and prickle length was obtained through direct measurement of the distance from the base of a subjectively chosen representative prickle to the apex. Relative prickle length was calculated by dividing prickle length (mm) by nutlet size (=nutlet length (mm)). Average relative tubercle extent is intended to measure the extent of nutlet sculpturing, which may include surface features such as papillae, tubercles, and rugae. The extent of these features was measured on the medial line of the abaxial surface (= medial tubercles (mm)) as well as along the lateral perimeter, taken from the abaxial surface view (=lateral tubercles (mm)). A composite variable was calculated by adding the values for medial tubercles and lateral tubercles, then dividing by nutlet perimeter (mm).

The relationship between long-distance dispersal, which we coded here as a binary variable, and these four continuous variables, was estimated using three approaches: 1) phylogenetic generalized linear mixed models for binary characters, 2) phylogenetic logistic regression, and 3) non-phylogenetically-corrected generalized linear models. Earlier biogeographic analyses (Guilliams and Baldwin 2011) and those presented here suggested North America as the source of disseminules for long-distance dispersal in the subtribe. As these analyses examining the relationship between long-distance dispersal and morphology were focused on long-distance dispersal irrespective of the continent to which disseminules were conveyed (South America or Australia), we coded this character as a binary variable. Based on biogeographic analyses suggesting a North American origin for the subtribe, those taxa from North America were coded as non-dispersed (0), while those in South America and Australia were coded as dispersed (1). The four continuous morphological predictor variables were standardized to mean = 0, variance = 1. Phylogenetic generalized linear mixed models were estimated using the approach of Ives and Helmus (2011), implemented using the *binaryPGLMM* function in the APE version 3.3 package (Paradis et al. 2004) in R using default settings. Phylogenetic logistic regression for binary variables was performed following the approach of Ives and Garland (2010), implemented using the *phyloglm* function in the phylolm version 2.2 package (Ho and Ane 2014) in R using default settings with the bound on the linear predictor increased from 10 to 15. Standard generalized linear models were estimated using the *glm* function in R. For each analysis type, we estimated individual models for each variable, then estimated joint models incorporating three of the four predictor variables; we eliminated raw prickle length but retained relative prickle length as the potentially more biologically-meaningful use of the prickle length data.

Finally, we gathered preliminary data on rates of nutlet production in the absence of pollinators for several Amsinckiinae taxa. Nutlets of eight *Plagiobothrys* taxa spanning three sections were germinated under sterile conditions and transplanted to 4" pots that were kept in a pollinator-free environment. Pollinators were excluded by placing the plants under netting, although even outside the netting pollinators were rare in this greenhouse setting. Plants were gathered when they had accumulated > 25 post-anthesis flowers/fruits or when they showed signs of senescence. For each plant, the nutlets produced in 25 fruits were tallied, with a maximum number of nutlets possible = 100. We were not able to gather data from all plant taxa and these data are therefore not sufficient for a formal statistical test. These are nevertheless

useful findings for examining qualitative relationships between rates of selfing and biogeographic distribution, and pointing the way to future work.

RESULTS

Phylogenetic inference

The phylogenetic analysis in BEAST yielded a posterior distribution of trees that is summarized on the MCCT shown in Figure 4. The 95% HPD intervals for node ages are shown as bars associated with each node. Support values throughout the tree are high in general and, importantly, are high in most regions of the tree where biogeographic shifts are inferred to have occurred (discussed in the section that follows). In general, phylogenetic relationships inferred in this analysis are consistent with those inferred by Guilliams (Chapter 1), a study with greater taxon sampling and which performed phylogenetic inference under both ML and BI. The primary goal of that earlier study was to examine and revise genus-level taxonomy in Amsinckiinae outside of *Cryptantha* s.l., as well as sectional taxonomy in *Plagiobothrys* in light of inferred evolutionary history. Therefore, we do not focus on those topics here.

Divergence time estimation in Amsinckiinae

Divergence time estimates in the form of node-associated 95% HPD intervals are shown on Figure 4, which is displayed on an axis with units in millions of years. Major clades in Amsinckiinae have stem lineages that originate in the late Miocene, in all cases after 11 Ma. Most clades appear to have begun diversifying after 5 Ma in the Pliocene. Many of the species-level divergences occurred much more recently, and in rare cases splits have 95% HPD intervals that extend to the present.

Biogeographic inference

The results of biogeographic inference using BioGeoBEARS and ML in Mesquite are shown on the time-calibrated MCCT in Figure 5 and Figure 6. On the tree from each biogeographic analysis, the inferred biogeographic state(s) are shown at nodes and inferred dispersals between North America, South America, and Australia are illustrated graphically on the right side of the figure.

The results of biogeographic inference in BioGeoBEARS are shown in Figure 5. Table 1 summarizes the results of biogeographic model fitting using this approach. These results include model fitting under DEC and DEC+J, DIVALIKE and DIVALIKE+J, and BAYAREALIKE and BAYAREALIKE+J models. Inference under these different classes of models were similar, so here we focus only on the DEC and DEC+J models. In the comparisons of nested, likelihood-based models (e.g., DEC vs. DEC+J), the model that incorporates founder-event speciation (i.e., "+J") was a better fit to the data (p = 9.7e-10). Inferred parameters of the BioGeoBEARS models show the relative unimportance of the range expansion ("d") and range contraction ("e") parameters of the model, and the larger, positive contribution of the founder-event jump dispersal parameter ("J"). Under the DEC+J model, there were approximately 13 dispersal-cladogenesis events in Amsinckiinae. Of the cases where directionality can be assessed unequivocally, nine cases were from North America to South America. There is one case of dispersal to Australia, but in this analysis it is not clear if the common ancestor of the Australian clade was from North America or South America. Dispersals where directionality cannot be assessed numbered three or four (with and without the Australian dispersal). Most of these dispersals occurred within the *Plagiobothrys* s.s. clade (unequivocal cases include six North America to South America dispersals, and likely one North America to Australia dispersal).

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Within the *Pectocarya* clade, there were two inferred dispersals from North America to South America, and within the *Amsinckia* clade there was one inferred dispersal from North America to South America.

The results of the ML estimation in Mesquite are shown in Figure 6. Although this approach is not explicitly spatial, it may be useful for estimating biogeographic states at nodes given the inferred importance of founder-event speciation in Amsinckiinae. Furthermore, this approach recovers estimates of biogeographic states at ancestral nodes that are similar to the results of the BioGeoBEARS analysis, but is not limited to a single input tree, thereby allowing for phylogenetic uncertainty. Considering only those nodes where a single state has high marginal likelihoods, this approach estimates minimally nine dispersals from North America to South America and one dispersal from North America to Australia across Amsinckiinae. Ignoring phylogenetic uncertainty, a maximum of 12 dispersals across the subtribe are inferred. In the *Plagiobothrys* s.s. clade, six dispersals from North America to South America are inferred, and one dispersal from North America to Australia is inferred. In the *Pectocarya* clade, two dispersals from North America to South America are inferred. In the *Amsinckia* clade, one dispersal from North America to South America is inferred.

Directionality of dispersals was resolved similarly in both biogeographic analyses. In all unequivocal cases of dispersal between North America and South America among the sampled Amsinckiinae lineages, direction of dispersal was inferred to be from North America to South America. Similarly, in the case of the disjunction between North America and Australia in the *Plagiobothrys* s.s. clade, the inferred direction of dispersal was from North America to Australia in the Mesquite analysis but was equivocal in the BioGeoBEARS analysis.

Testing hypotheses relating to timing of dispersal

We tested two biogeographic hypotheses relating to the timing of dispersal between North America and South America using the distribution of node age estimates within the 95% HPD intervals for the nodes directly subtending the inferred dispersals. As the BioGeoBEARS analysis favored a model that included a high likelihood of founder-event speciation, we reason that these node age estimates also represent the best estimates of the timing of dispersal between biogeographic regions.

Estimated mean ages of dispersals between biogeographic regions were between 3.350 Ma and 0.298 Ma (**Table 2, Figure 7**). The oldest estimated dispersal among sampled Amsinckiinae occurred in *Plagiobothrys* in the *P. collinus* clade, with a 95% HPD interval spanning from 4.870 Ma to 1.982 Ma. The most recent estimated dispersal also occurred in *Plagiobothrys*, in the *P. fulvus* clade, with a 95% HPD interval spanning from 662 KYA to essentially present day (38 KYA ago).

We first tested if independent dispersals happened during approximately the same period of time or during different time periods using the non-parametric Kruskal-Wallis rank sum test with differences between each pairwise combination of dispersals examined using Bonferroni-corrected Wilcoxon rank sum tests. The Kruskal-Wallis rank sum test was highly significant (chi-squared = 94,627.16, df = 11, p=2.2e-16), suggesting the dispersals likely happened at different times from the late Miocene to near to the present day. Pairwise Wilcoxon rank sum tests were each highly significant (p<2e-16). We then examined the relationship between estimated dispersals and the onset of summer drought and the mediterranean-type climate in North America and South America, which began after the middle Miocene Climatic Optimum at ca. 15.5 Ma. All inferred dispersals occurred well after the onset of summer drying during the middle Miocene, which in this context means that: 1) dispersals occurred during a geologic time

period after Mediterranean-type climates had developed (or were in the process of developing) globally, and 2) that the middle Miocene Climatic Optimum itself does not appear to correlate with dispersals *per se* (but full summer drought, the timing of which has not been established, may).

Testing factors hypothesized to be important in promoting long-distance dispersal and establishment in American mediterranean-type climates (including Australia)

We used three statistical approaches to examine the relationship between long-distance dispersal and four nutlet characters that we hypothesized to be potentially important in facilitating long-distance dispersal by way of zoochory. Results of these statistical tests are shown in **Table 3**. Under a binary phylogenetic generalized linear mixed model, only relative tubercle extent, a measure of nutlet surface sculpturing, was significant at alpha=0.05 (Z=2.7391, p=0.0062) in single variable models. Relative tubercle extent had a strong positive relationship with dispersal as predicted (coefficient=1.3457), meaning that lineages that dispersed had significantly greater surface sculpturing than those lineages that did not disperse. Under single variable PGLMM models, nutlet length, a proxy for nutlet size, was negatively associated with dispersal as predicted (coefficient= -1.4095), but not significantly so at alpha=0.05 (Z=-1.6437, p=0.1002). Individual PGLMM models showed no relationship between dispersal and prickle length. The PGLMM model of dispersal as a function of nutlet length, relative prickle length, and relative tubercle extent showed similar results to the individual models, although 1) the test statistic for nutlet length increased in magnitude (Z= -1.6598, p=0.0969) and the predicted negative relationship strengthened (coefficient= -1.5769), and similarly 2) the test statistic for relative tubercle extent increased in magnitude (Z= 2.9495, p=0.0032) and the predicted positive relationship strengthened (coefficient= -1.6397). The relationship between dispersal and prickle length remained insignificant. Results of model estimation using phylogenetic generalized logistic models were similar to those under PGLMM (Table 3). Models including only a single predictor variable showed significant relationships between dispersal and both nutlet length (Z= -1.9767, p=0.0481) and relative tubercle extent (Z=2.2249, p=0.0261). The direction and strength of the association was consistent with the PGLMM (coefficients= -0.8024 and 0.6311, respectively). The phylogenetic logistic regression model of dispersal as a function of nutlet length, relative prickle length, and relative tubercle extent showed similar results to the individual models and to the PGLMM full model. Both nutlet length (Z= -2.2650, p=0.0235) and relative tubercle extent (Z=2.4374, p=0.0148) remained statistically significant, the direction of association was similar, but the strength of the relationship increased in both cases (coefficients= -1.2238 and 0.8817, respectively). The unphylogenetically corrected GLM results are qualitatively identical to the phylogenetically corrected statistical models, although the estimated relationships between dispersal and both nutlet length and relative tubercle extent are greater in all models. Results are presented in **Table 3**, but will not be further discussed here.

Self-Pollination Rates in Selected Amsinckiinae

All Amsinckiinae taxa examined for ability to self-pollinate in the absence of pollinators proved capable of self-pollination by producing viable nutlets. **Table 4** summarizes the results of these limited trials. In total we examined self-pollination rates in 18 individuals from 8 *Plagiobothrys* taxa, spanning 3 *Plagiobothrys* sections. Nutlet production in the absence of pollinators ranged from 28 percent in 100 percent among individual plants examined. Plants of sections *Allocarya*, *Echidiocarya*, and *Plagiobothrys* had average self-pollination rates of 88.9%, 54.5%, and 41.2% respectively.

DISCUSSION

Phylogeny

The phylogeny inferred in this study is nearly identical to that of our previous study (Guilliams, Chapter 1), which included multiple exemplars for many of the sampled taxa and inference under both ML and BI estimation approaches. Importantly, the MCCT inferred here and the best ML tree from our previous study are identical with respect to clades within which dispersals are inferred to have occurred and differ only in minor ways that do not affect the outcome of this study.

Biogeographic inference

Amsinckiinae have dispersed between North America and South America more than any other studied vascular plant group of comparable age or rank (10-13 times among the sampled lineages, depending upon the analysis and phylogenetic uncertainty). This figure is even more impressive when one considers 1) that there are at least two other AAD events among sampled genera that were undetected here due to unsampled lower taxa in *Amsinckia* (*A. tessellata* var. *tessellata*) and *Pectocarya* (*P. pusilla*) and 2) that the largest genus of the subtribe, the amphitropically distributed *Cryptantha* s.s., has not been sampled to assess biogeographic history. Furthermore, although molecular phylogenetic analyses focusing on this biogeographic pattern are few in number, it is clear that most AADs in other comparable groups can be explained by relatively few dispersal events. In only a few cases do AADs surpass two or three, e.g., with three in *Hordeum* (Poaceae; Blattner 2006), four to five in *Tiquilia* (Ehretiaceae; Moore et al. 2006, Moore and Jansen 2006), four in *Hoffmannseggia* (Fabaceae; Simpson et al. 2005), and three in *Castilleja* (Plantaginaceae; Tank and Olmstead 2009). The highest previously reported subfamilial clade-level estimate of AAD events was in the Muhlenbergiinae, with either eight or nine dispersals from North America to South America required to explain biogeographic patterns above the species level (Poaceae; Peterson et al. 2010). Why has this group of western American plants dispersed between the continents a conservatively estimated 10-13 times when many comparable vascular plant groups, in fact most such groups, do not display this pattern? We attempted to address this question in this paper.

Our study of the AAD biogeographic pattern began with model testing in BioGeoBEARS to evaluate the contribution of founder-event speciation to the biogeographic model. We found strong support for a model that includes founder-event speciation, meaning that the likeliest scenario in all cases was that dispersal between the continents resulted in a cladogenesis event. While this is a highly reasonable inference, the contribution of founder-event speciation generally has not been as rigorously tested as is possible now with BioGeoBEARS, a program that specifically parameterizes this potential pattern and permits standard model-testing through likelihood ratio tests and by AIC. This study is among the first to employ BioGeoBEARS to study biogeography of angiosperms, aside from the reanalysis of the *Psychotria* dataset by the program author (Matzke 2014). In that paper, Matzke speculated on the potential prevalence of founder-event speciation in explaining divergences and biogeographic patterns, especially on islands or certain island-like continental settings. Our findings in the present study support his viewpoint. Founder-event speciation may be especially important in seed plants, which often possess features seemingly ideal for long-distance dispersal and establishment. Seed plants have tough propagules containing a nutrient-provisioned embryo. Seeds and fruits are often ornamented with bristles, hooks, barbs, and other morphological features (e.g., gelatinous seed coats in *Navarretia*) that are thought to facilitate animal dispersal (Carlquist 1983). Perhaps most

importantly, many seed plant lineages are capable of self-pollination, a life history feature that may permit the establishment of a new population with the dispersal of only a single seed (Baker 1955).

With dispersal the most likely explanation of the observed biogeographic pattern in Amsinckiinae, we could then evaluate the directionality of dispersals between North America and South America. In all cases, South American Amsinckiinae lineages are embedded within North American clades. Both BioGeoBEARS and the ML reconstructions in Mesquite infer dispersals from North America to South America. This is consistent with the overall trend noted by Wen and Ickert-Bond (2009) in their recent review of this biogeographic pattern, with approximately 65 percent of tabulated AAD stemming from North America to South America dispersals.

Divergence time estimation

Fossil and secondary calibration of the Amsinckiinae phylogeny allowed us to better understand the timing of dispersal between North America and South America/Australia. Our results show that dispersals between North America and South America were asynchronous and likely occurred throughout recent geological history. The earliest dispersal occurred approximately 3.35 Ma, with subsequent dispersals spread through the Pliocene to the present. This asynchronous pattern of dispersal timing argues against any hypothesis for AAD that would require parallel and synchronous development of amphitropically disjunct taxa, such as any vicariance-based hypothesis. This point was made by Moore et al. (2006), and gains considerable weight here from multiple, simultaneously inferred dispersals within a common divergence time analysis.

All dispersals between North America and either South America or Australia occurred after the onset of summer drying following the mid-Miocene thermal optimum ca. 15.5 Ma.

Hypothesized factors promoting long-distance dispersal and establishment and AAD

We found strong and statistically significant associations between long-distance dispersal among the sampled Amsinckiinae and two of the four examined variables: 1) nutlet length and 2) relative tubercle extent. These associations were qualitatively identical among the three statistical approaches we attempted. We hypothesized that long-distance dispersal by birds would require a negative relationship between dispersal and disseminule size (here represented in proxy by nutlet length). A negative relationship between these variables was estimated in all models, and was statistically significant in two of three; this relationship approached statistical significance in the PGLMM. These results are consistent with dispersal by birds, wherein smaller disseminules would be advantageous due to weight savings as well as reduced likelihood of detection/elimination by the bird.

We hypothesized a positive relationship between long-distance dispersal and nutlet surface ornamentation under the reasoning that more complex, three-dimensional surface shapes should increase the likelihood of affixing to a dispersal vector either through tangling in feathers or in mud. There was a strong and statistically significant positive association between long-distance dispersal and nutlet surface ornamentation in all models.

Interestingly, there was no statistical association between long-distance dispersal and either variable involving prickle length (prickle length and relative prickle length). Prickles with retrorse barbs are highly effective at attaching nutlets and other disseminules to passing animals (e.g., socks of humans) and their role in dispersal at some spatial scale would seem to be beyond

question. In the case of long-distance dispersal in Amsinckiinae, however, there appears to be no association between dispersal and these fruit features.

Taxa that are capable of self-pollination tend to occur more frequently on oceanic islands than their self-incompatible close relatives (Baker 1955), a pattern that has been formalized under the name Baker's Law or Rule (Stebbins 1957). It is reasonable to assume that this pattern exists in continental settings as well, in cases where the distribution of suitable habitat for a given taxon is island-like and separated by large distances. Disjunct temperate, mediterranean-type, and polar regions of North America and South America could be considered continental islands separated by ecologically dissimilar tropical environments at lower latitudes. Therefore, when considering the impact of self-compatibility on dispersal between North America and South America, our expectation was to find a relationship between self-compatibility and dispersal in Amsinckiinae. We found that all Amsinckiinae taxa examined for self-compatibility were capable of automatic self-pollination and self-fertilization to some degree. This finding accords with other published accounts of AAD, where lineage(s) that dispersed from North America to South America were nearly entirely those that were self-compatible (see Raven 1963 and references therein for a thorough list). For example, in *Camissonia*, a genus nearly completely confined to North America, the only member to occur in South America is self-compatible *C. dentata* Reiche (Moore and Raven 1970). As all studied members of Amsinckiinae were capable of self-pollination and self-fertilization, it was not possible to perform a statistical test to examine the strength of the relationship between self-pollination/fertilization and AAD using this dataset. However, it is noteworthy that of all Amsinckiinae taxa that have dispersed from North America to South America, all are hypothesized to be or demonstrated to be self–compatible (e.g., *Amsinckia tessellata*). Conversely, those Amsinckiinae taxa with breeding system modifications that would facilitate outcrossing, e.g., heterostyly, are all strictly North American (e.g., some *Amsinckia* and *Oreocarya*). A broader comparative analysis examining the strength of the association between breeding system and AAD at the family-level or among all angiosperms would be illuminating, but until then we consider results of this study to be consistent with the importance of Baker's Rule in long-distance dispersal and establishment.

SUMMARY AND CONCLUSIONS

The AAD biogeographic pattern has intrigued biologists and biogeographers since the time of Gray and Hooker (1880) yet, despite this long history of interest, several important aspects of this pattern have been understudied. The subtribe Amsinckiinae is an excellent taxonomic group for use in studying this biogeographic pattern due to having: 1) an amphitropical distribution, 2) high taxon richness in both North America and South America, 3) described fossil taxa that permit fossil calibration, 4) natural history features potentially associated with animal dispersal, and 5) the potential for multiple long-distance dispersals between North America and South America that would permit hypothesis testing.

Ancestors of the sampled extant members of subtribe Amsinckiinae have dispersed between North America and South America 11-13 times, which is more often than nearly all other clades of living things of similar rank (except Muhlenbergiinae, and acknowledging that taxa at a given rank are often not of comparable age). Biogeographic analysis suggests that dispersals usually correspond to cladogenesis events, in keeping with the great distances involved in these dispersals and the genetic isolation and ecological displacement that would result. This study is among the first in angiosperms (other than the exemplar case study) to use

BioGeoBEARS, and our analysis corroborates the findings of the author regarding the potential importance of founder-event speciation as an evolutionary model, even in continental settings (Matzke 2014). Directionality of the inferred dispersals were entirely from North America to South America, a pattern consistent with the general trend noted by Raven (1963) and Wen and Ickert-Bond (2009),.

Estimation of dispersal chronology reveals that dispersals happened at different times, beginning in the early Pliocene (when considering the HPD intervals, ca. 4.87 Ma) and continuing through the Pleistocene to near the present day. We suggest that this pattern of timing of dispersals eliminates any single-cause hypothesis for the origin of AAD and perforce we must consider alternatives, such as low probability but continually-operating processes like animal dispersal.

Examination of the factors involved with AAD events has yielded intriguing results that are consistent with some but not all of our hypotheses. We hypothesized that we would detect an association between factors that may promote dispersal by animals and AAD in Amsinckiinae. We found a strong and significant negative relationship between dispersal and nutlet size, and a strong and significant positive relationship between dispersal and nutlet surface ornamentation. While each of the variables we examined would seem to be important in facilitating dispersal at some scale, only nutlet size and surface ornamentation seemed to matter at the intercontinental scale. Finally, we note that all Amsinckiinae species that we examined for the potential to produce viable nutlets in the absence of pollinators were capable of automatic self-pollination and self-fertilization. Although this positive but invariant finding did not allow us to perform a statistical test of the association between ability to self-pollinate and long-distance dispersal, we add these observations to the long list of self-pollinating lineages that have successfully made the long trip between North America and South America (Raven 1963). These results further strengthen the general pattern formalized under the name Baker's Rule, namely that self-compatible taxa are more likely to establish following a long-distance dispersal event.

Finally, we suggest that the best available explanation for AAD is long-distance dispersal by birds. All evidence from this study and those before it is consistent with that explanation and no evidence that we know of contradicts it. Of the animal dispersal hypotheses, only dispersal by birds occurs with the frequency and on time scales necessary to explain the observed phylogenetic and biogeographic patterns in Amsinckiinae and most other AAD plant groups. Yet, it would be highly desirable to have direct evidence of long-distance dispersal by birds between North America and South America. We suggest that fruitful research may include direct tagging of birds using miniature light-level geolocators, such as in the studies of songbirds underway by Deluca et al. (2015) and Peterson et al. (2015), to demonstrate the temporally rather immediate zoological connection between the landscapes of western North America and western South America. Of course, no form of evidence would be more conclusive than the observation of the propagule of a North American plant taxon on a bird captured in South America, but given the low likelihood of these dispersal events, we likely will be constrained to simpler empirical studies and historical inference, while staying mindful of the importance of improbable events in nature.

FIGURES

Figure 1. Map of the Americas showing three examples of American Amphitropical Disjunction, Mediterranean-Mediterranean (light blue), desert-desert (red), and boreal-boreal (dark blue).
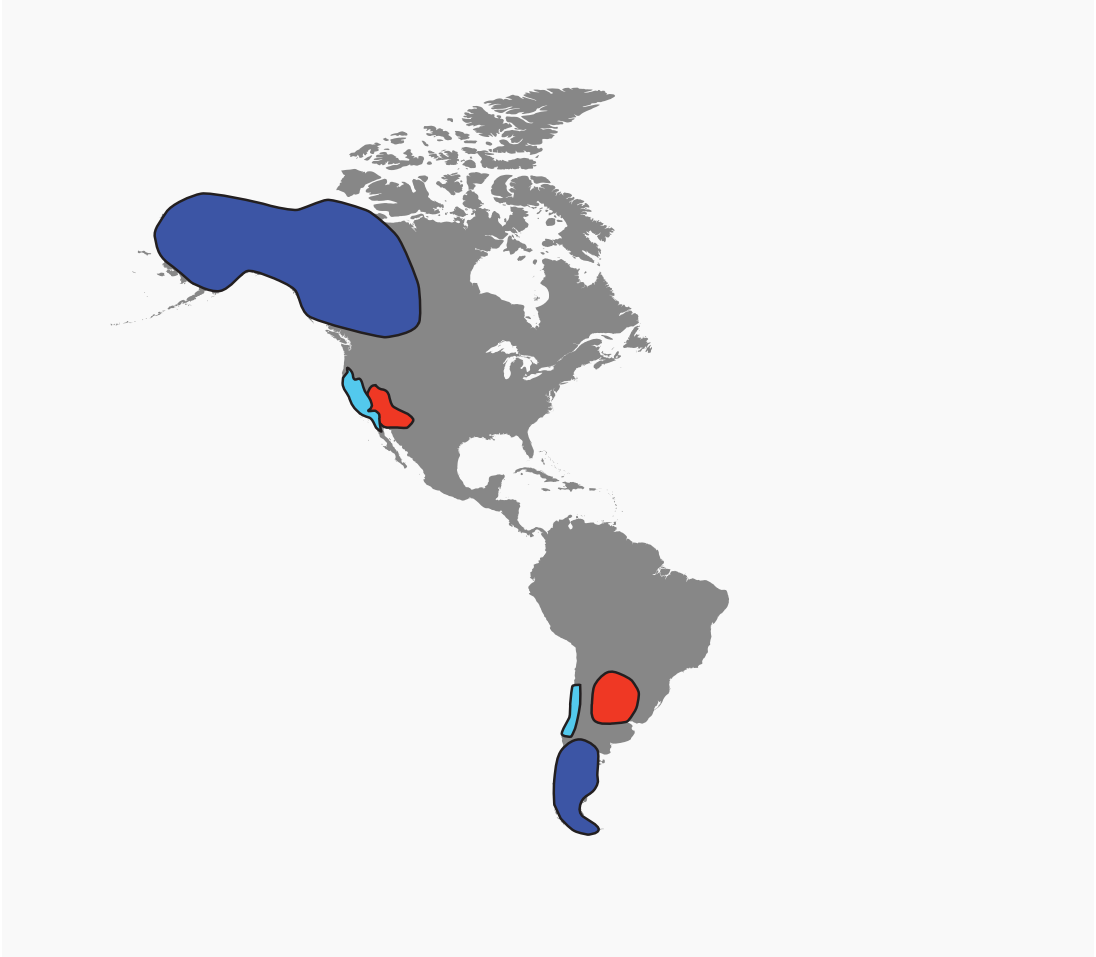
Figure 2A-E. Plate of images showing natural history features hypothesized to be important in explaining patterns of American Amphitropical Disjunction. **A.** *Sanicula crassicaulis* in flower; **B.** *Sanicula crassicaulis* in fruit, showing hooked bristles on fruits that may serve to attach mericarps to feathers and/or fur of animals; **C.** Genera with high diversity in vernal pool ecosystems of North America: *Downingia* and *Lasthenia*; **D.** Water fowl utilize vernal pools and marshes extensively during migration along the Pacific Flyway; **E.** Taxa from some habitats such as wildflower fields appear to be dispersed between North America and South America disproportionately more often than some other habitat types.

Figure 3A-I. Plates of images showing abaxial nutlet surfaces of some members of Amsinckiinae. **A.** *Plagiobothrys undulatus*; **B.** *Plagiobothrys arizonicus*; **C.** *Plagiobothrys austiniae*; **D.** *Amsinckia furcata*; **E.** *Amsinckia douglasiana*; **F.** *Amsinckia spectabilis*; **G.** *Pectocarya linearis* var. *ferocula*; **H.** *Oreocarya crassipes*; **I.** *Cryptantha hooveri*.
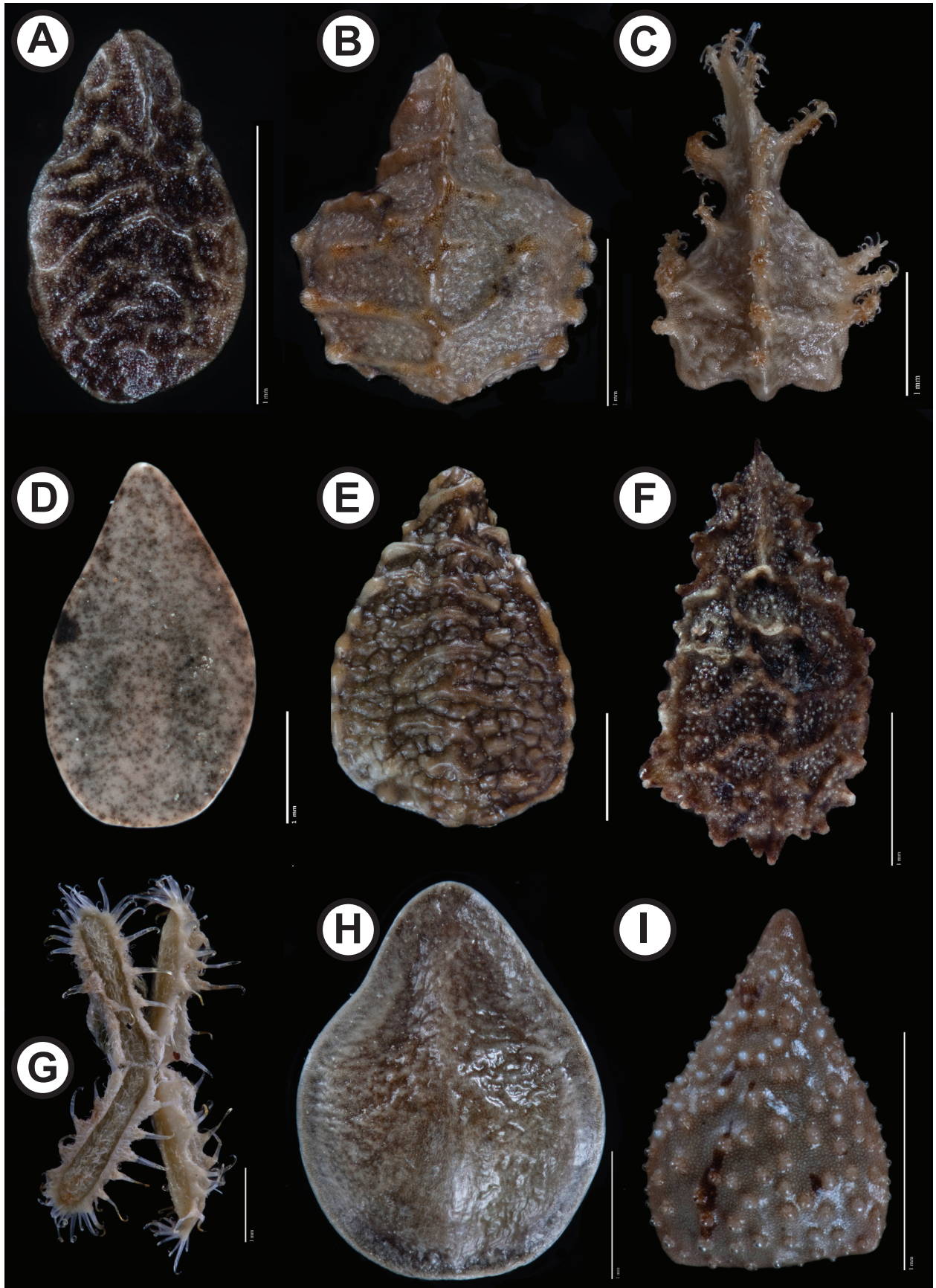
Figure 4A-B. Fossil calibrated phylogenetic hypothesis of relationships in Amsinckiinae from BEAST analysis. **A.** Lower portion of tree; **B.** Upper portion of tree.
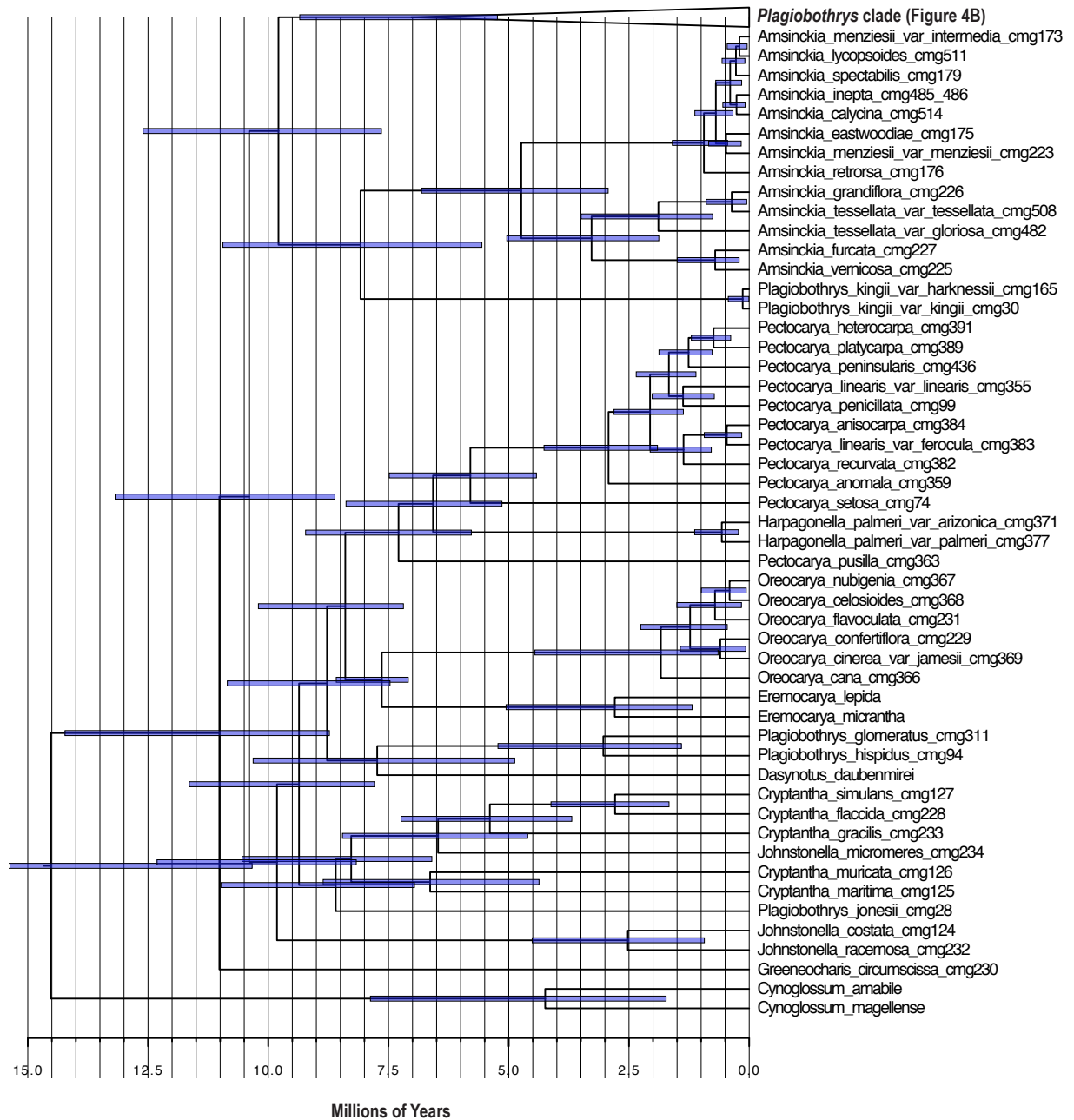
Figure 4B
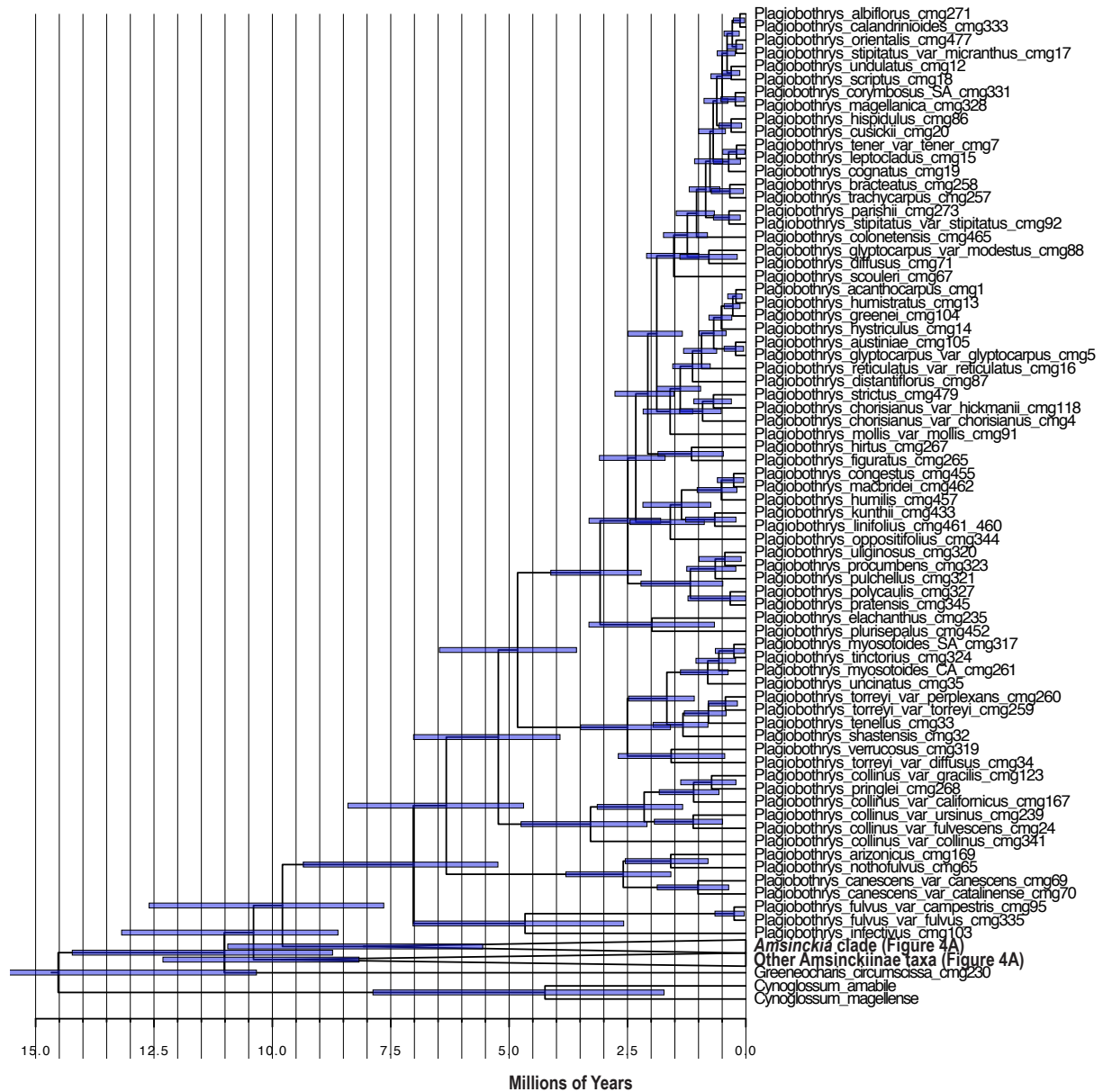
Figure 5. Phylogenetic tree from BEAST analysis showing results of biogeographic analysis in BioGeoBEARS. Inferred maximum likelihood biogeographic states at splits and descendant lineages shown as proportional likelihood pie charts. Transitions between regions based on this analysis are shown in a column on the right side of diagram.

BioGeoBEARS DEC+J on Borages M0_unconstrained
ancstates: global optim, 3 areas max. d=0; e=0; j=0.0282; LnL=−54.29

Common ancestor of the
Amsinckiinae occurred
in North America

Common ancestor of
Plagiobothrys occurred
in North America

North America
South America
Australia
Equivocal
Equivocal

15   10   5   0

Millions of years ago

62
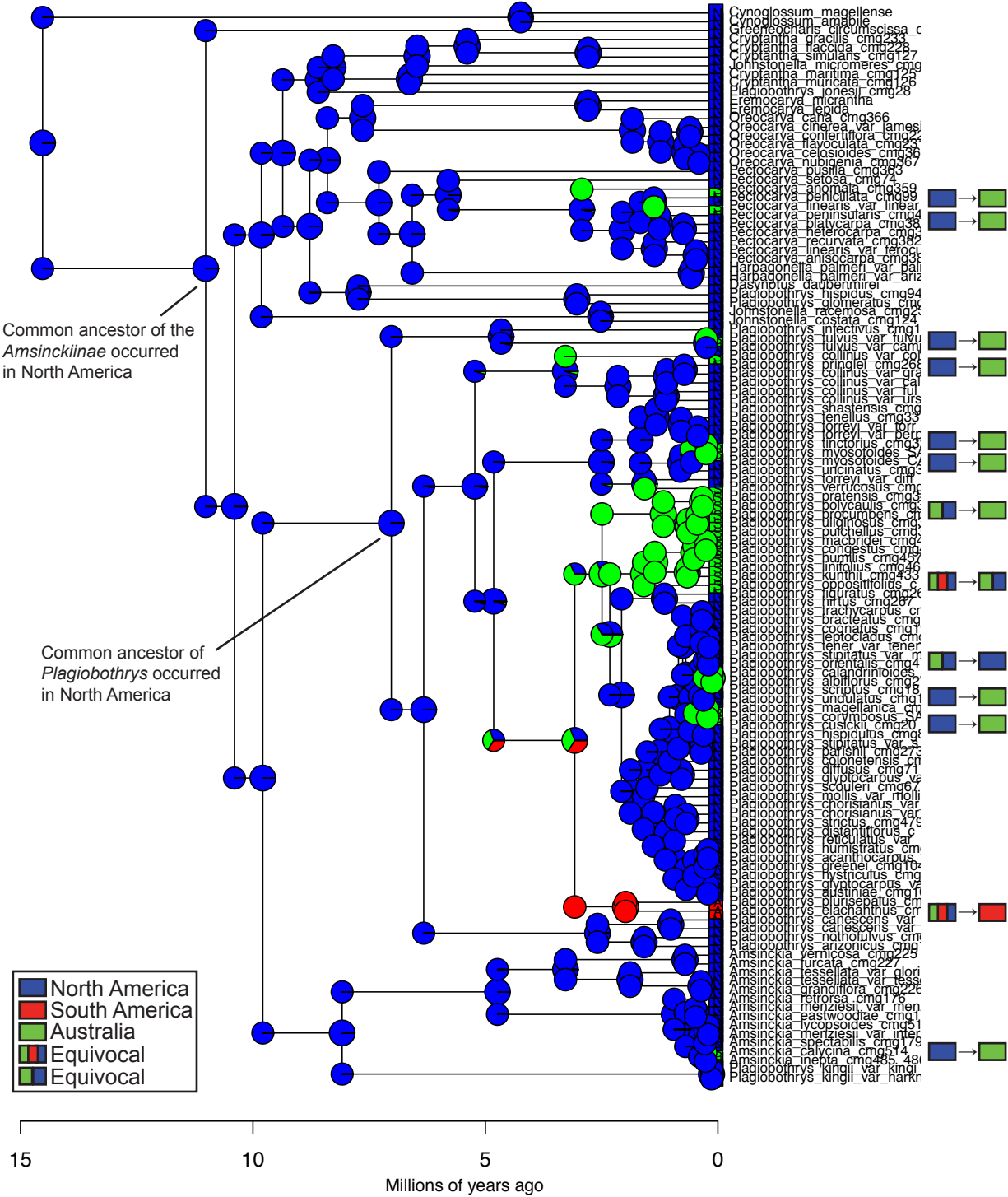
Figure 6. Phylogenetic tree from BEAST analysis showing results of biogeographic analysis in Mesquite. Inferred maximum likelihood biogeographic states summarized across the sample of trees from the posterior distribution of trees shown as pie charts at nodes. Transitions between regions based on this analysis are shown in a column on the right side of diagram.

The common ancestors of all major *Amsinckiinae* lineages occurred in North America, and all dispersals in the subtribe were from North America to either South America or to Australia.

Amsinckia: 1 dispersal NA → SA
Plagiobothrys: 6-8 dispersals NA → SA
                 1 dispersal NA → Australia
Pectocarya: 2 dispersals NA → SA
Cryptantha s.l.: No SA lineages sampled
                 for this study

Common ancestor of *Plagiobothrys* occurred in North America

Common ancestor of the *Amsinckiinae* occurred in North America

1 or 2 shifts due to phylogenetic uncertainty

1 or 2 shifts due to reconstruction uncertainty

North America
South America
Australia
Node Absent
Equivocal

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Figure 7. Box and whisker plots of 95% Highest Posterior Density interval values for fossil calibrated node ages of American Amphitropical Disjunction groups in Amsinckiinae.
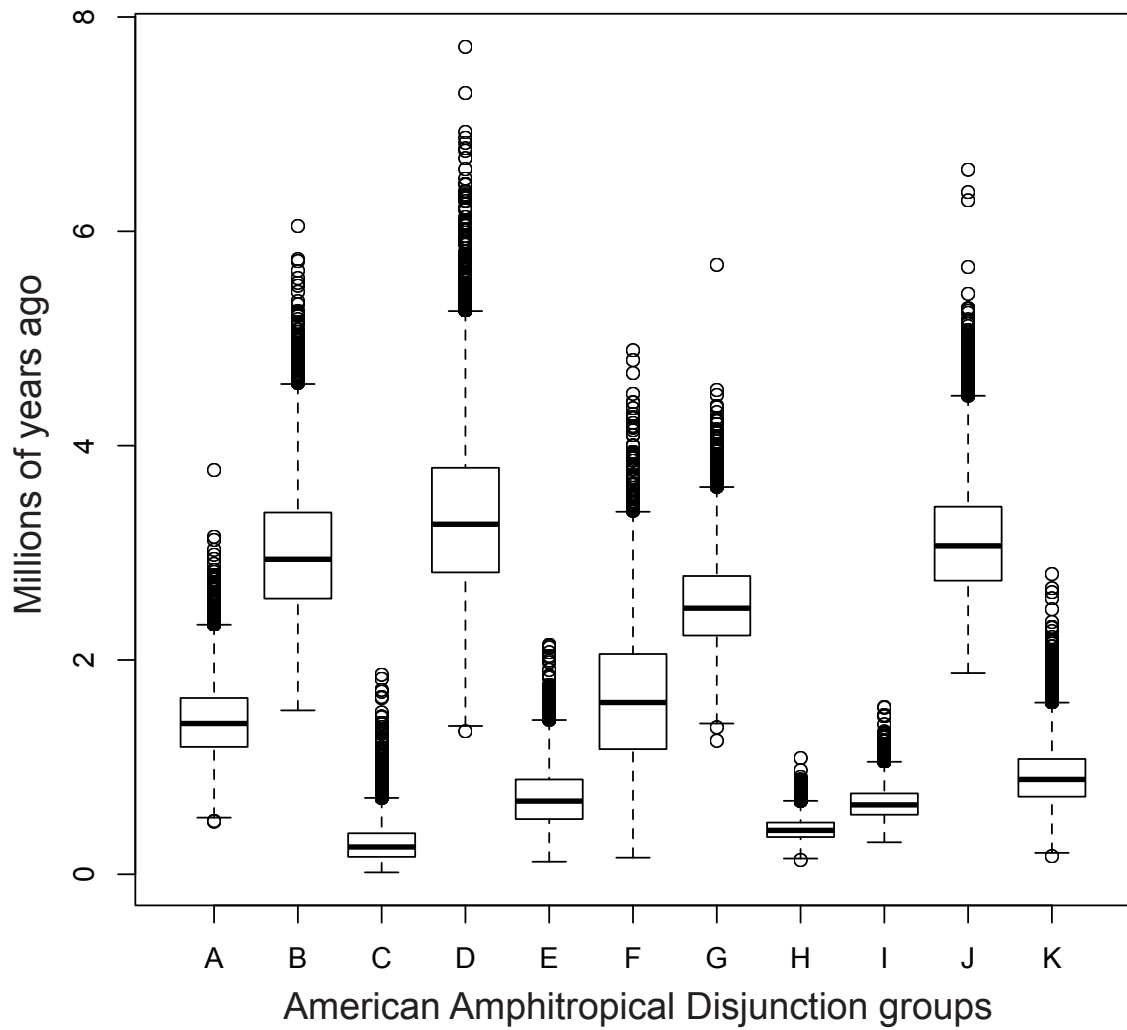
A - *Pectocarya linearis* group
B - *Pectocarya anomala* group
C - *Plagiobothrys fulvus* group
D - *Plagiobothrys collinus* group
E - *Plagiobothrys myosotoides* group
F - *Plagiobothrys verrucosus* group
G - *Plagiobothrys* early *Allocarya* group
H - *Plagiobothrys* mid *Allocarya* group 1
I - *Plagiobothrys* mid *Allocarya* group 2
J - *Plagiobothrys* Australian group
K - *Amsinckia calycina* group

TABLES

Table 1. Results of biogeographic model testing using BioGeoBEARS.

Model	LnL	Number of parameters	d	e	j
DEC	-72.98429	2	1.46462E-02	1.00000E-12	0.00000000
DEC + J	-54.29481	3	1.00000E-12	1.00000E-12	0.02818858
DIVALIKE	-73.97044	2	1.92131E-02	1.00000E-12	0.00000000
DIVALIKE + J	-54.29532	3	1.00000E-12	1.00000E-12	0.02805695
BAYAREALIKE	-123.69746	2	1.96001E-02	4.80805E-02	0.00000000
BAYAREALIKE + J	-54.29542	3	1.00000E-07	1.00000E-07	0.02766801

alt	null	LnL alt	LnL null	Dfalt	Dfnull	DF	D statistic	pval	AIC 1	AIC 2
DEC+J	DEC	-54.29	-72.98	3	2	1	37.38	9.7E-10	114.6	150
DIVALIKE+J	DIVALIKE	-54.3	-73.97	3	2	1	39.35	3.5E-10	114.6	151
BAYAREALIKE +J	BAYAREA LIKE	-54.3	-123.7	3	2	1	138.8	4.9E-32	114.6	251.4

Table 2. Dispersal directionality and timing for American Amphitropical Disjunction groups in Amsinckiinae.

	Group	Directionality	Mean Node Age Estimate	95% HPD lower	95% HPD upper
A	*Pectocarya linearis* group	N to S	1.4317	0.8178	2.1564
B	*Pectocarya anomala* group	N to S	3.0141	1.917	4.2456
C	*Plagiobothrys fulvus* group	N to S	0.2979	3.80E-02	0.6622
D	*Plagiobothrys collinus* group	N to S	3.3503	1.9816	4.8697
E	*Plagiobothrys myosotoides* group	N to S	0.719	0.2258	1.2388
F	*Plagiobothrys verrucosus* group	N to S	1.64	0.4426	2.8223
G	*Plagiobothrys* early *Allocarya* group	N to S?	2.5272	1.7634	3.3821
H	*Plagiobothrys* mid *Allocarya* group 1	N to S	0.6653	0.3867	0.9641
I	*Plagiobothrys* mid *Allocarya* group 2	N to S	0.422	0.2328	0.631
J	*Plagiobothrys* Australian group	N to S	3.1219	2.1255	4.1642
K	*Amsinckia calycina* group	N to S	0.9224	0.396	1.4944

Table 3. Results of generalized linear models examining relationship between morphological variables and dispersals.

binary PGLMM					
Model	Var	Coefficient	SE	Z	P value
Dispersal ~ NL	NL	-1.4095	0.8575	-1.6437	0.1002
Dispersal ~ PL	PL	-0.2609	0.4770	-0.5470	0.5844
Dispersal ~ RPL	RPL	-0.1629	0.4248	-0.3835	0.7014
Dispersal ~ RTL	RTL	**1.3457**	0.4913	2.7391	**0.0062****
Dispersal ~ NL + RPL + RTL	NL	**-1.5769**	0.9501	-1.6598	**0.0970***
	RPL	-0.6192	0.8363	-0.7403	0.4591
	RTL	**1.6397**	0.5559	2.9495	**0.0032****

phyloGLM					
Model	Var	Coefficient	SE	Z	P value
Dispersal ~ NL	NL	**-0.8024**	0.4059	-1.9767	**0.0481****
Dispersal ~ PL	PL	0.0017	0.1597	0.0109	0.9913
Dispersal ~ RPL	RPL	0.0013	0.1621	0.0080	0.9936
Dispersal ~ RTL	RTL	**0.6311**	0.2836	2.2249	**0.0261****
Dispersal ~ NL + RPL + RTL	NL	**-1.2238**	0.5403	-2.2650	**0.0235****
	RPL	-0.0011	0.2186	-0.0048	0.9962
	RTL	**0.8817**	0.3617	2.4374	**0.0148****

uncorrected GLM					
Model	Var	Coefficient	SE	Z	P value
Dispersal ~ NL	NL	**-1.4687**	0.5434	-2.7030	**0.0069*****
Dispersal ~ PL	PL	-0.2420	0.3458	-0.7000	0.4840
Dispersal ~ RPL	RPL	-0.0253	0.2599	-0.0970	0.9220
Dispersal ~ RTL	RTL	**1.1697**	0.3242	3.6080	**0.0003******
Dispersal ~ NL + RPL + RTL	NL	**-1.7188**	0.5799	-2.9640	**0.0030*****
	RPL	-0.4100	0.5958	-0.6880	0.4914
	RTL	**1.5720**	0.4163	3.7760	**0.0002******

Table 4. Results of pollinator exclusion trials to examine self-pollination ability in selected Amsinckiinae.

Section	Taxon	Plant ID	Number of fruits counted	Number viable nutlets	Percent viable	Section average
§ *Allocarya*	*Plagiobothrys austiniae*	1020B_A	25	93	93.0	
§ *Allocarya*	*Plagiobothrys austiniae*	1020B_B	25	93	93.0	
§ *Allocarya*	*Plagiobothrys greenei*	1888A_4	25	64	64.0	
§ *Allocarya*	*Plagiobothrys trachycarpus*	2358B_1	25	98	98.0	
§ *Allocarya*	*Plagiobothrys trachycarpus*	2358B_2	25	100	100.0	
§ *Allocarya*	*Plagiobothrys trachycarpus*	2358B_3	25	100	100.0	**88.9**
§ *Allocarya*	*Plagiobothrys undulatus*	1126A	25	85	85.0	
§ *Allocarya*	*Plagiobothrys undulatus*	1126C	25	94	94.0	
§ *Allocarya*	*Plagiobothrys undulatus*	1126D	25	96	96.0	
§ *Allocarya*	*Plagiobothrys undulatus*	1126E	25	97	97.0	
§ *Allocarya*	*Plagiobothrys undulatus*	1126G	25	87	87.0	
§ *Allocarya*	*Plagiobothrys undulatus*	1126I	25	60	60.0	
§ *Echidiocarya*	*Plagiobothrys collinus* var. *ursinus*	1061B_1	25	43	43.0	**54.5**
§ *Echidiocarya*	*Plagiobothrys collinus* var. *ursinus*	1418A_1	25	66	66.0	
§ *Plagiobothrys*	*Plagiobothrys fulvus* var. *campestris*	1105A_2	20	23	28.8	
§ *Plagiobothrys*	*Plagiobothrys fulvus* var. *campestris*	1105B_3	25	33	33.0	**41.2**
§ *Plagiobothrys*	*Plagiobothrys nothofulvus*	1143	13	23	44.2	
§ *Plagiobothrys*	*Plagiobothrys shastensis*	1017A_1	17	40	58.8	

Chapter 3. Dated phylogenies reveal increased net diversification in *Plagiobothrys* (Amsinckiinae, Boraginaceae) with invasion of vernal pools and other ephemeral aquatic ecosystems

INTRODUCTION

Lineage diversity can be affected by intrinsic and extrinsic factors. When intrinsic factors result in increased diversification, they are sometimes referred to as "key innovations". Examples of intrinsic factors described as key innovations include the nectar spur in the genus *Aquilegia* (Whittall and Hodges 2007) and floral bilateral symmetry in angiosperms (Sargent 2004), both features associated with relatively specialized pollination and elevated diversity (see Armbruster 2014). When extrinsic factors result in increased diversification, they are sometimes called "key opportunities" (Vamosi and Vamosi 2011). An example of an extrinsic factor leading to increased net diversification rates is the contemporaneous radiation of the world's major succulent plant lineages in response to aridification in the late Miocene (Arakaki et al. 2011).

Regions of the world that support a disproportionately large number of minimum-rank taxa (MRT) given their area are ideal for studying patterns and processes of diversification, including the role of key opportunities in promoting lineage diversification. Among the areas best studied by botanists are the five regions of the world with Mediterranean-type climates, all of which are considered to be global biodiversity hotspots (Myers 1990). Mediterranean-type climate regions share the following four attributes: 1) they occur between 30 and 40 degrees latitude, 2) they are positioned along the western margins of continents, 3) adjacent oceans with cold water currents moderate temperatures during winter and summer, and 4) yearly precipitation falls during the mild winter months and summer months are warm and dry. These extrinsic oceanic and climatological features correlate with the patterns of biodiversity encountered in these regions (Dallman 1998).

Landscape heterogeneity has also been implicated as an important extrinsic factor contributing to the observed high levels of biodiversity in the Mediterranean-type climate regions of the world, although the mechanism by which landscape heterogeneity results in high levels of biodiversity may vary (Raven and Axelrod 1978, Lancaster and Kay 2013). Under one common argument, landscape heterogeneity provides opportunity for niche partitioning and subsequent lineage diversification, or may provide discontinuous suitable habitat such that allopatric speciation could occur following dispersal. In these cases, one might infer increased rates of speciation (lambda) and higher net diversification rates as compared to an area with lower landscape heterogeneity. Alternatively, landscape heterogeneity may provide a buffering effect during periods of environmental instability, e.g., during the Pleistocene glacial-interglacial cycles when changing climate may have caused some plant taxa into refugia; by this reasoning, landscape heterogeneity permits taxa to track their suitable ecological niche parameters and avoid local extirpation. In this case, one might infer lower rates of extinction (mu) as compared to an area with lower landscape heterogeneity.

Vernal pools are unique seasonal aquatic ecosystems of the Mediterranean-type climate regions of the world that contribute to overall landscape heterogeneity in these areas (Figure 1; Thorne 1984, Keeley and Zedler 1998). In these regions, winter rains may stand as temporary ponds in places where an impervious soil layer precludes the percolation of water. Vernal pools typically initiate with a soil wetting phase in late fall and early winter. Depending upon precipitation patterns, vernal pools may become inundated in early winter, with ponding

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extending through early to late spring. Pools dry down in the spring and summer as water is lost to evaporation. In late summer and fall, pools are completely dry. Note that there are other ephemeral seasonal aquatic ecosystems with hydrology similar to vernal pools but which lack some of the features commonly used to define vernal pools in North America. For example, seasonally intermittent streams and the seasonally fluctuating margins of shallow lakes and reservoirs sometimes support populations of species usually considered to be vernal pool plants, or support congeners of vernal pool endemic clades. In this study we attempt to be clear when we are describing vernal pools, "other ephemeral aquatic ecosystems" as described above, or both.

The yearly vernal pool hydrological cycle of aquatic conditions followed by extended hot and dry conditions present a challenge to the pool biota. This yearly cycle tends to preclude the establishment of truly aquatic, long-lived organisms, as these plants and animals usually fail to persist during the hot, dry summer months. Conversely, sessile, non-aquatic terrestrial organisms perish during the period of inundation. Some life history characteristics of vernal pool biota may mitigate against the harsh conditions, including an annual life cycle with a dormant seed phase or, alternatively, perennating structures in herbaceous perennials (e.g., *Eryngium*); rapid ontological development; stem and leaf aerenchyma; heterophylly; and CAM photosynthesis (Keeley 1984, 1990, Stone 1990, Zedler 1990).

Vernal Pools of North America and South America

Vernal pools and other ephemeral aquatic ecosystems occur extensively in the regions of western North America and western South America with a Mediterranean-type climate, and were likely much more extensive historically (Bliss et al. 1998, Holland 2011). In North America, vernal pools occur primarily in the California Floristic Province (CA-FP), the part of western North America roughly corresponding to the region of the continent with a Mediterranean-type climate and long recognized for its unique floristic composition (Howell 1957, Ackerly et al. 2014). Spanning a broad latitudinal gradient from southwestern Oregon to northwestern Baja California, Mexico, the CA-FP has been recognized by scientists and conservation groups as a biodiversity hotspot due to extremely high levels of biological diversity and endemism coupled with extensive habitat loss to agricultural, commercial, and residential land uses in the region (Myers 1990). Vernal pool ecosystems, themselves biologically unique and critically endangered (Holland and Jain 1981, Bauder and McMillan 1998), have long been considered ideal habitats for biological diversification because of their island-like geographical distribution (Holland and Jain 1981, Bauder and McMillan 1998, Simovich 1998) and within-pool vertical zonation into ecologically and floristically distinct microenvironments (Linhart 1974, Bauder 1987, Holland and Dains 1990, Emery et al. 2009, Emery et al. 2012).

In South America, vernal pools have been documented throughout the Mediterranean-type climate region of central Chile, but they have been much less studied than their North American counterparts. In the first focused survey of vernal pools in central Chile, Bliss et al. (1998) encountered vernal pools from Puerto Oscuro (ca. 31°S, 23') in the north to the Chol Chol Valley (ca 38°N, 36') in the south. At the time of their survey effort (1994, 1995), most vernal pool habitat had been lost to agriculture and that which remained was lightly to intensively grazed by cattle and goats.

The study of the flora of American vernal pools mirrors that of vernal pools in general, with the vernal pools of the North American CA-FP having received significantly more botanical study than the pools of South America. In the CA-FP of North America, vernal pools have long captivated botanists due to their conspicuous floral displays and high degree of plant endemism (Hoover 1937, Stebbins and Major 1965, Raven and Axelrod 1978); some of this endemic

diversity is thought to have arisen following the invasion of vernal pool ecosystems. Some California native plant groups that are hypothesized to have diversified in the vernal pools of the CA-FP are listed here, followed parenthetically by the family, the total number of minimum-rank taxa (or species, when indicated) recognized in the group, and the number of minimum-rank taxa occurring in CA-FP vernal pools: *Downingia* (Campanulaceae, 18, ~17), *Eryngium* (Apiaceae, 230+ species, 14+), *Lasthenia* (Asteraceae, 19, 8), *Limnanthes* (Limnanthaceae, 19, 18), *Navarretia* (Polemoniaceae, 40+ species, ~9), subtribe Orcuttiinae (*Neostapfia*, *Orcuttia*, and *Tuctoria*; Poaceae, 9, 8), and *Pogogyne* (Lamiaceae, 9--12, 8--11). Other plant clades have members that are native to the CA-FP and commonly found in vernal pools but which are also found in other ecological settings, e.g.: *Alopecurus* (Poaceae, 36 species, 5), *Centromadia* (Asteraceae, 9, 4--5), *Isöetes* (Isoetaceae, 200+ species, 2--3), *Pleuropogon* (Poaceae, 6, 2), and *Psilocarphus* (Asteraceae, 6, ~4). Taxonomic study of the vernal pool flora of Chile has been limited, possibly due to the greater proportion of widespread wetland species and smaller number of both vernal pool obligate and vernal pool endemic taxa (Raven 1963, Bliss et al. 1998). Specialist and/or endemic vernal pool plants of Chile include *Blennosperma chilense* (Asteraceae), *Lasthenia kunthii* (Asteraceae), *Downingia pusilla* (Campanulaceae), *Legenere valdiviana* (Campanulaceae), *Deschampsia danthonioides* (Poaceae), *Myosurus apetalus* (Ranunculaceae), and *Plectritis samolifolia* (Valerianaceae). Interest in vernal pools in South America appears to be increasing of late, however, as there have been a number of recent studies of the vegetation of the vernal pools and other ephemeral aquatic ecosystems of South America (e.g., Deil et al. 2007, Deil et al. 2011, San Martín et al. 2013), as well as some focused studies of vernal pool plant species (e.g., Alvarez et al. 2012). Notable among vernal pool endemic groups on both continents is a clade of subtribe Amsinckiinae (Boraginaceae), *Plagiobothrys* sect. *Allocarya*, with at least 60 MRT in vernal pools and other ephemeral aquatic ecosystems of both North America and South America.

Amsinckiinae is a diverse and ecologically important subtribe of annual herbaceous or perennial suffrutescent taxa with centers of distribution in western North America and southern South America (Johnston 1923, 1927, Hasenstab-Lehman and Simpson 2012). Members of the subtribe were among the principal groups of California plants identified by Raven and Axelrod (1978) as important examples of major diversifications hypothesized to have occurred since development of the Mediterranean-type climate in California. Indeed, members of Amsinckiinae have diversified into all major ecosystems in California and more broadly in western North America, from the deserts of Baja California in the south, where *Johnstonella* A. Brand and *Pectocarya* DC. ex Meisn. are common, north to the ephemeral wetland ecosystems of the CA-FP, where *Plagiobothrys* Fisch. & C.A. Mey. sect. *Allocarya* I.M. Johnston occurs, and east to the Basin and Range Province of western North America, where *Cryptantha* Lehm. ex G. Don. sensu stricto (s.s.) and *Oreocarya* Greene are well represented. The subtribe minimally includes 9 genera, which are listed here with global estimates of minimum-rank taxonomic diversity, given parenthetically (NA=North America; SA=South America): *Amsinckia* Lehm. (16--17; 15--16 NA, 2 SA), *Cryptantha* s.s. (113; 68 NA, 47 SA), *Eremocarya* Greene (2 NA), *Greeneocharis* Guerke & Harms (3 NA), *Harpagonella* A. Gray (2 NA), *Johnstonella* (15; 13 NA, 2 SA), *Oreocarya* (72 NA), *Pectocarya* (14; 9 NA, 7 SA), and *Plagiobothrys* (ca. 92--102; ca. 60--70 NA, ca. 28 SA, 4 Australia); overall minimum-rank taxonomic diversity in the subtribe is ca. 330--342 taxa, with ca. 245--257 taxa occurring in North America, 86 in South America, and 4 in Australia.

Phylogenetic study of Amsinckiinae provides an ideal opportunity to examine the patterns of diversification of the most diverse group of American vernal pool plants in the broader context of diversification of the subtribe throughout its range. In particular, phylogenetic study permits evaluation of the role of vernal pools and specifically the formation of vernal pool ecosystems on patterns of diversification in the subtribe. Both the Mediterranean-type climate and vernal pools are of recent (Neogene) origin in the Americas and have undoubtedly contributed to patterns of diversification. Vernal pool ecosystems, with their uniquely adapted flora, likely could not occur absent a Mediterranean-type climate with mild wet winters and warm dry summers. This consideration, coupled with the observed high levels of taxon richness among vernal pool endemic clades, suggests an origin of vernal pools after, and possibly well after, the onset of summer drying at the middle Miocene Climatic Optimum (Axelrod and Schorn 1994, Jacobs et al. 2004). Dating of some surfaces on which vernal pools occur suggest a maximum age for vernal pool landscapes of 4--3 Ma (Arkley 1962, Marchand and Allwardt 1981), although some landforms on which vernal pools occur are much younger. Such a recent origin of vernal pools --- across millions of acres in the CA-FP alone (Holland 1978) --- could have presented a tremendous ecological opportunity for lineage diversification. The present study, conducted on the backdrop of a well-sampled phylogenetic hypothesis of the subtribe, allows us to examine the role of vernal pool formation in promoting diversification, addressing the question: did the formation of vernal pools constitute a "key opportunity"?

In this study we examined the contribution of niche specialization on vernal pool and other ephemeral aquatic ecosystems to the overall patterns of diversification in *Plagiobothrys* and other selected Amsinckiinae. In particular, we sought to address the following questions:

1) How many times have sampled members of Amsinckiinae invaded vernal pools and other ephemeral aquatic ecosystems of Mediterranean-type climates in western North America and western South America?

2) Is timing of invasion consistent with putative, recent evolution of vernal pools and other ephemeral aquatic ecosystems of Mediterranean-type climates?

3) Are there statistically significant diversification rate shifts in *Plagiobothrys* and the other sampled members of Amsinckiinae?

4) Are any diversification rate shifts associated with invasion of vernal pools and other ephemeral aquatic ecosystems? If shifts in net diversification rates are identified, are they driven by increased rates of speciation or decreased rates of extinction?

To answer these questions, we used DNA sequence data from the nuclear and chloroplast genomes from Chapter 1 and used these data plus evidence from the fossil record to infer a time-calibrated phylogenetic hypothesis of relationships in Amsinckiinae. Using this chronogram, we examined the evolutionary history of habitat preference in Amsinckiinae to assess the number of times that vernal pools and other ephemeral aquatic ecosystems have been invaded among sampled members of the subtribe, as well as the timing of these invasions. We then looked for trait-independent shifts in diversification rates across the subtribe, as well as habitat-associated differences in parameters of diversification.

MATERIALS AND METHODS

Evolutionary relationships and divergence times in Plagiobothrys and Amsinckiinae

Taxon sampling

  A list of all accessions included in this study is given in Appendix 2. Within *Plagiobothrys*, we included one sample each of most recognized taxa (75 in total). We also included exemplar taxa from each of the genera of Amsinckiinae: *Amsinckia* (15 taxa), *Cryptantha* s.s. 1 (4 taxa) and *Cryptantha* s.s. 2 (1 taxon) of Hasenstab and Simpson (2012), *Eremocarya* (2 taxa), *Greeneocharis* (1 taxon), *Harpagonella* (2 taxa), *Johnstonella* (3 taxa), *Oreocarya* (6 taxa), and *Pectocarya* (11 taxa). All sequence data for this study were generated for a previous study (Guilliams, Chapter 1). Some sequence data for five non-*Plagiobothrys* taxa were downloaded from Genbank (*Cynoglossum amabile*, *Cynoglossum magellense*, *Dasynotus daubenmirei*, *Eremocarya lepida*, and *E. micrantha*). We used two *Cynoglossum* species as outgroups for this study. These *Cynoglossum* taxa were shown to be closely related to Amsinckiinae in three recent broad-scale analyses of the Boraginaceae (Nazaire and Hufford 2012, Cohen 2013, Weigend et al. 2013).

Sequence acquisition

  Methods of genomic DNA isolation, fragment amplification, and sequencing were all described in Chapter 1. No new DNA sequence data were added for Chapter 3.

Phylogenetic inference and divergence time estimation

  Methods of phylogenetic inference and divergence time estimation were identical to those described in Chapter 2.

Estimation of ancestral habitat preference

  Ancestral habitat preference was estimated under maximum likelihood (ML) in the program Mesquite (Maddison and Maddison 2008). Habitat preference was coded for extant members of Amsinckiinae using published floras validated by direct observation. We assigned all members of the subtribe to one of two states, corresponding to terrestrial, non-aquatic ecosystems and ephemeral aquatic ecosystems. The majority of Amsinckiinae taxa occur in terrestrial, non-aquatic habitats. We performed this analysis over the post-burn-in posterior distribution of trees generated in the BEAST phylogenetic analysis using the Trace Over Trees option. The results were displayed on the maximum clade credibility tree (MCCT).

Among lineage diversification rate analyses

  We initially explored branching time data by constructing lineage through time (LTT) plots using the APE package (Paradis et al. 2004) in R (R Development Core Team 2008). As our taxon representation was incomplete across Amsinckiinae, we also constructed LTT plots using just that clade corresponding to *Plagiobothrys* s.l. (not including *Plagiobothrys* sect. *Sonnea*) + *Amsinckia*, for which we did have representation of nearly all currently recognized taxa. Departures from expected branching times across Amsinckiinae were examined using the gamma statistic (Pybus and Harvey 2000) for both the full sampling as well as with sampling reduced to *Plagiobothrys* s.l. + *Amsinckia* as described above.

  We tested for significant net diversification rate shifts across Amsinckiinae using the MEDUSA approach of Alfaro et al. (2009) implemented in the GEIGER package (Harmon et al.

2008) in R. Incomplete taxon representation of some non-*Plagiobothrys* Amsinckiinae genera, phylogenetic uncertainty within sampled members of *Cryptantha* s.s. + *Johnstonella micromeres* in our analyses, and general lack of backbone support in previously published phylogenies that include taxa from *Cryptantha* s.s. required a conservative approach in assigning values of unsampled taxonomic diversity for this analysis. We effectively collapsed poorly supported nodes and assigned clade diversity values as follows. We represented the five included samples of *Cryptantha* s.s. + *J. micromeres* by a single terminal called "*Cryptantha_muricata_*cmg126". This terminal was given an overall diversity of 114 (113 for number of minimum-rank taxa in *Cryptantha* s.s. + 1 for *J. micromeres*). A similar approach was taken with the genus *Oreocarya*. For this genus, we represented our six sampled lineages with one terminal called "*Oreocarya_cana_*cmg366". We assigned this terminal an overall diversity of 72. For *Johnstonella*, we represented the two monophyletic samples in our analysis with one terminal called "*Johnstonella_racemosa_*cmg232", which we assigned an overall diversity of 14.

Character-associated diversification rate analyses

We tested for character-associated shifts in diversification rates using the BiSSE approach of Maddison et al. (2007) in the Diversitree package (FitzJohn 2012) in R. There have been criticisms of inferring diversification dynamics, especially speciation and extinction rates, using a phylogeny of only extant taxa (Liow et al. 2010, Quental and Marshall 2010, Morlon et al. 2011). These criticisms did not discourage us from applying this approach, however, as our interest was in examining changes in net diversification, rather than speciation and extinction *per se*, and our expectation for this phylogenetically shallow clade was that extinction would contribute in only a minor way to net diversification.

The original implementation of the BiSSE approach requires a phylogeny with representation of all taxa in the clade of interest. As we did not have such a comprehensively sampled phylogeny of Amsinckiinae, we modified the inferred MCCT to include polytomies for the *Cryptantha* s.s. + *J. micromeres* clade with 114 terminals, the *Oreocarya* clade with 72 terminals, and the *Johnstonella* clade with 14 terminals. We used the *clades.from.polytomies* function in Diversitree to collapse these polytomies to "clades" in the Diversitree sense. As this approach requires that character-associated values are included for every terminal, including those unsampled and collapsed into "clades", we assigned habitat values for all remaining Amsinckiinae (all other taxa were terrestrial, non-aquatic). We fit four total models. The full, six-parameter model included estimates of character-associated speciation (lambda) and extinction (mu) rates, and transition rates between character states (q). To test for significant differences in rate estimates, we constructed three reduced models and compared them to the full model. Reduced models included: 1) a five-parameter model with equal speciation rates, 2) a five-parameter model with equal extinction rates, and 3) a five-parameter model with equal transition rates. The full model was compared to each of the reduced models using likelihood ratio tests (LRTs), with a significance level of alpha<0.05.

RESULTS

Evolutionary relationships and divergence times in Plagiobothrys and Amsinckiinae

Inferring evolutionary relationships in *Plagiobothrys* and Amsinckiinae and assessing these findings in light of classification is the topic of Chapter 1 and will not be discussed further here. Results of divergence time estimation show that Amsinckiinae began to diversify in the mid to late Miocene, ca. 11.02 Ma (95% HPD interval = 14.23-8.23 Ma), while *Plagiobothrys*

began to diversify in the late Miocene, ca. 7.02 Ma (95% HPD interval = 9.35-5.24 Ma). *Plagiobothrys* sect. *Allocarya* began to diversify approximately 3.08 Ma (95% HPD interval = 4.12-2.21 Ma).

Estimation of ancestral habitat preference

Estimation of ancestral habitat preference among sampled Amsinckiinae is shown on **Figure 2**. Most members of Amsinckiinae occur in non-aquatic, terrestrial habitats. The transition from terrestrial settings to vernal pools and other ephemeral aquatic ecosystems occurred a single time in *Plagiobothrys* in the lineage subtending *Plagiobothrys* sect. *Allocarya*. Despite localized phylogenetic uncertainty among trees in the posterior distribution of trees used for this analysis, the ML estimation for the node representing the common ancestor of *Pl.* sect. *Allocarya* is unequivocal.

Among lineage diversification rate shifts

Trees and associated LTT plots for both the full taxon dataset (n=122) and the dataset reduced to the clade comprising *Plagiobothrys* s.l. (minus *Plagiobothrys* sect. *Sonnea*) + *Amsinckia* (n=86) are given in **Figure 3**. Both LTT plots show a roughly linear relationship between log lineage accumulation and time, with slight uptick in lineage accumulation toward the present. The gamma statistic for the full analysis was 3.715, while the gamma statistic for the reduced analysis was 2.496. Both are non-significant, which suggests that rates of diversification have been constant through time when these larger clades are considered.

The analysis of diversification rate shifts in MEDUSA identified three portions of Amsinckiinae tree with significant rate shifts (**Table 1**) based upon the AICc threshold of 4.998329 for a tree with 111 tips. The largest diversification rate shift occurred at the stem of the lineage representing the common ancestor of North American + South American *Pl.* sect. *Allocarya*, a shift that represents a change in log likelihood of 11.8624 and in AICc of 21.6693 (**Figure 4**). Net diversification across *Pl.* sect. *Allocarya* was 1.13547 lineages per million years, while average net diversification across the subtribe was 0.0942 lineages per million years. Two other significant rate shifts were identified, one near the base of the tree and one significant rate decrease at the node representing the common ancestor of *Pl.* sect. *Sonnea* + *Dasynotus*.

Character-associated diversification rate analyses

The analysis of character-associated differences in diversification rates revealed differences in all three rate categories between terrestrial and ephemeral wetland habitats. The speciation rate estimate for lineages occurring in terrestrial habitats (lambda 0) was 0.4698, while the speciation rate estimate for ephemeral wetland habitats (lambda 1) was 1.0351. The extinction rate estimate for lineages occurring in terrestrial habitats (mu 0) was 0.1273, while the extinction rate estimate for ephemeral wetland habitats (mu 1) was effectively 0. Net diversification rates calculated from these speciation and extinction rates by habitat show that net diversification in ephemeral aquatic ecosystems is 3.0222 times greater than net diversification rates in terrestrial settings. The transition rate estimate from terrestrial to ephemeral wetland habitats (q01) was 0.0013, while the transition rate estimate from ephemeral wetland habitats to terrestrial (q10) was effectively 0.

Comparisons of full and constrained models by rate category were used to assess if these estimated differences within rate category of the full model were statistically significant. **Table 2** shows the results of model comparison. The full model has a log likelihood of -209.48 with six degrees of freedom. Of the nested constrained models, only the model with speciation rates

constrained to be equal was a significantly worse fit to the data than the full model (chi-squared value=7.5424, p=0.006026). The models with extinction rates or transition rates constrained to be equal were not statistically different from the full, six-parameter model.

DISCUSSION

Phylogeny of Amsinckiinae

Phylogeny estimation in Amsinckiinae resulted in a hypothesis of evolutionary relationships in the subtribe scaled to absolute time that resolved an association between shifts in habitat and diversification rate, and unequivocal estimation of ancestral habitat preference. These topics are discussed below, while examination of tree topology and biogeography are treated elsewhere (Chapter 1 and Chapter 2, respectively).

Divergence time estimation in Plagiobothrys and Amsinckiinae

Diversification of subtribe Amsinckiinae and many of the nested genera such as *Plagiobothrys* in mid to late Miocene is consistent with a hypothesis of diversification in response to decreased summer rainfall following the middle Miocene Climatic Optimum. Decreased summer rainfall and especially development of full summer drought have been hypothesized to be important factors in shaping the flora of the CA-FP in general (Raven and Axelrod 1978) with most CA-FP crown groups estimated to have begun diversifying since the mid-Miocene onset of summer drying (see Baldwin 2014). The results presented here reinforce that hypothesis for a particularly taxon-rich clade of predominantly annual plants that Raven and Axelrod (1978), who referred to Amsinckiinae as "the Boraginaceae", noted as exemplifying the remarkable diversity of some groups of California native plants.

How old are vernal pool ecosystems?

Establishing the age of vernal pools and ephemeral aquatic ecosystems is a critical component to evaluating the ecological opportunity represented by the development of these ecosystems, yet this is a difficult task. Ephemeral aquatic ecosystems of Mediterranean-type climates of the world, especially those with a highly endemic flora, are likely of an age younger than the onset of summer drought in these regions, corresponding roughly to the age of the middle Miocene Climatic Optimum (ca. 17-15 Ma). Note that in this context, the middle Miocene Climatic Optimum marks only the approximate beginning of the reduction in summer rainfall in Mediterranean-type climates and does not necessarily correspond to conditions such as full summer drought that would promote the development of vernal pools as ecosystems. It is clear that vernal pools as features of the landscape could not have predated the landforms on which they occur, and by similar reasoning, the diversification of the many vernal pool endemic lineages probably do not predate the origin of vernal pools. While establishing a precise date for the presentation of these ephemeral water bodies on the landscape is likely impossible, but it may be possible to designate an approximate upper age for North American vernal pools, with two caveats. First, vernal pools occur widely across western North America and the landforms on which they occur are of different ages, even at a local scale. For example, vernal pools occur across the Merced area chronoseries despite the very different ages of these terraces, ranging from ca. 4-3 my to <0.01 my (Arkley 1962, Marchand and Allwardt 1981). Second, the age of the origin of a particular landscape feature (e.g., a coastal terrace or river terrace) does not necessarily coincide with the development of vernal pools on that feature. Available vernal pool landscape age estimates (Arkley 1962, Marchand and Allwardt 1981) coupled with rough estimates of the age of full summer drought in the Mediterranean-type climate region of western

North America (Axelrod 1973) suggest a recent age for the development of vernal pools, likely within the last 3-4 million years. The upper bound of this age estimate comes from the extensively studied Pliocene age China Hat member of the Laguna Formation, the age of which has been estimated correlatively using K-Ar dating of basalt clasts to 3.76 Ma ± 0.4 Ma (see Marchand and Allwardt 1981 and references therein).

Number and timing of invasion of vernal pools and other ephemeral aquatic ecosystems

Vernal pools and other ephemeral aquatic ecosystems were evidently invaded a single time in Amsinckiinae, apparently along the lineage representing the common ancestor of *Pl*. sect. *Allocarya*. This finding of a single invasion of vernal pools and other ephemeral aquatic ecosystems is consistent with the results of some but not all published studies of plant groups that are wholly or partly endemic to vernal pools and ephemeral aquatic ecosystems. Vernal pool endemic lineages with published phylogenetic hypotheses include *Lasthenia* (Asteraceae), *Downingia* (Campanulaceae), *Pogogyne* (Lamiaceae), *Limnanthes* (Limnanthaceae), the Orcuttiinae (Poaceae), and *Navarretia* (Polemoniaceae). Of these taxa, *Pogogyne*, the Orcuttiinae, and *Navarretia* have apparently invaded vernal pools and ephemeral aquatic ecosystems a single time and then diversified within these ecosystems (Spencer and Porter 1997, Boykin et al. 2010, Silveira and Simpson 2013). On the opposite extreme, a recent study of *Lasthenia* (Emery et al. 2012) found four transitions from terrestrial to terrestrial/aquatic, and at least three transitions from terrestrial/aquatic to vernal pool, a remarkable number of transitions overall for a genus of 21 minimum rank taxa.

Divergence time estimates of the node subtending the vernal pool and ephemeral wetland clade, *Pl*. sect. *Allocarya* (95% HPD interval = 4.12-2.21 my, average = 3.08 my), are strikingly similar to the oldest estimates of vernal pool landscapes in the Central Valley of California (the China Hat formation, ca. 4--3 Ma). It seems likely that the onset of diversification of *Pl*. sect. *Allocarya* occurred after vernal pools became more common on the landscape and after the common ancestor developed traits sufficient to allow for the invasion and exploitation of this novel habitat.

Rate shifts in *Plagiobothrys* sect. *Allocarya*

Many previous studies of vernal pool and ephemeral wetland lineages describe vernal pool clades as having undergone rapid diversification (e.g., Stebbins 1976, Spencer and Porter 1997, Meyers et al. 2010, Silveira and Simpson 2013). However, to examine the hypothesis of rapid diversification following invasion of vernal pools, the context of rate patterns outside of the vernal pool clade must be investigated concurrently. The present study is the first to sample broadly enough outside of the vernal pool clade so that shifts in rates of diversification can be evaluated.

The character-independent diversification analysis in MEDUSA indicated a major rate shift near the base of the crown group of *Pl*. sect. *Allocarya* (excluding only the Australian members of this section). As this analysis uses only the shape of the tree and branch length information (and not, e.g., habitat-related information), it is informative that the largest rate shift in this diverse subtribe occurs just after the node at which the ecological transition to vernal pools and ephemeral wetlands evolved. We interpret the placement of this inferred rate shift just after the inferred transition to vernal pools and other ephemeral aquatic ecosystems as consistent with the hypothesis that invasion of vernal pools led to increased net diversification rates in *Pl*. sect. *Allocarya*.

Increased diversification rates in vernal pool and wetland lineages

 The character-dependent diversification rate analysis in BiSSE estimated that vernal pool and ephemeral wetland lineages have a net diversification rate that is over three times higher than in terrestrial plant lineages. This separate analysis corroborates the inferences of ancestral terrestrial ecology from the ML analysis and of accelerated diversification at the base of *Pl*. sect. *Allocarya* from the character-independent analysis in MEDUSA, lending further support to the hypothesis that invasion of vernal pools and other ephemeral aquatic ecosystems promoted diversification in *Pl*. sect. *Allocarya*. Although estimates of speciation and extinction must be interpreted with caution, the character-dependent BiSSE analysis indicates that the observed net increase in diversification rates between wetland and terrestrial lineages is due to increased speciation rates rather than lower extinction rates.

Summary

 Diversification of subtribe Amsinckiinae of Boraginaceae evidently postdates the onset of summer drying in western North America, following the middle Miocene Climatic Optimum, and thus is a particularly taxon-rich example of a clade that has been estimated to have evolved in association with the development of Mediterranean-type climate. *Plagiobothrys* sect. *Allocarya*, long hypothesized to be a natural group of vernal pool and wetland plants, was strongly supported as a monophyletic group, and therefore the estimation of ancestral habitat preference was unequivocal in placing the shift from terrestrial habitat to vernal pool and ephemeral wetland habitat along the lineage leading to the common ancestor of the section. The diversification time analysis estimates a mean age for the section at ca. 3 my, which is just after the putative origin of landscapes supporting vernal pools in the Central Valley of California. The character-independent diversification rate analysis showed that net rates of diversification shifted dramatically along the branch leading to the common ancestor of American *Pl*. sect. *Allocarya*, while the character-dependent diversification rate analysis shows a net diversification rate in vernal pool and ephemeral wetland lineages that is over three times higher than in closely related terrestrial plant lineages. Taken together, we believe these findings support the hypothesis that the development of vernal pools and other ephemeral wetland habitats in the Mediterranean-type climate region of western North America and western South America provided a key opportunity that permitted the rapid diversification of *Pl*. sect. *Allocarya* and likely other vernal pool endemic lineages as well.

Figure 1A-G. Vernal pools and some associated plant groups. **A.** Calistoga popcornflower, *Plagiobothrys strictus*; **B.** Mexican mesa-mint, *Pogogyne* "mexicana"; **C.** goldfields (yellow; *Lasthenia*) and downingia (purple; *Downingia*); **D.** Close-up of *Downingia* flowers; **E.** Vernal pools at Jepson Prairie in the Central Valley, CA, U.S.A; **F.** Vernal pools on Mesa el Descanso in Baja California, Mexico; **G.** Vernal pool-swale complex near Middletown, CA, U.S.A.

Figure 2. Vernal pool and ephemeral wetland habitat reconstruction over a sample of trees from the posterior distribution of trees in Mesquite.

Vernal pool and ephemeral wetland clade

Plagiobothrys kingii var harknessii cmg165
Plagiobothrys kingii var kingii cmg30
Amsinckia inepta cmg485 486
Amsinckia calycina cmg514
Amsinckia spectabilis cmg179
Amsinckia menziesii var intermedia cmg173
Amsinckia lycopsoides cmg511
Amsinckia eastwoodiae cmg175
Amsinckia menziesii var menziesii cmg223
Amsinckia retrorsa cmg176
Amsinckia grandiflora cmg226
Amsinckia tessellata var tessellata cmg508
Amsinckia tessellata var gloriosa cmg482
Amsinckia furcata cmg227
Amsinckia vernicosa cmg225
Plagiobothrys arizonicus cmg169
Plagiobothrys nothofulvus cmg65
Plagiobothrys canescens var canescens cmg69
Plagiobothrys canescens var catalinense cmg70
Plagiobothrys elachanthus cmg235
Plagiobothrys plurisepalus cmg452
Plagiobothrys austiniae cmg105
Plagiobothrys glyptocarpus var glyptocarpus cmg5
Plagiobothrys hystriculus cmg14
Plagiobothrys greenei cmg104
Plagiobothrys acanthocarpus cmg1
Plagiobothrys humistratus cmg13
Plagiobothrys reticulatus var reticulatus cmg16
Plagiobothrys distantiflorus cmg87
Plagiobothrys strictus cmg479
Plagiobothrys chorisianus var hickmanii cmg118
Plagiobothrys chorisianus var chorisianus cmg4
Plagiobothrys mollis var mollis cmg91
Plagiobothrys scouleri cmg67
Plagiobothrys glyptocarpus var modestus cmg88
Plagiobothrys diffusus cmg71
Plagiobothrys colonetensis cmg465
Plagiobothrys parishii cmg273
Plagiobothrys stipitatus var stipitatus cmg92
Plagiobothrys hispidulus cmg86
Plagiobothrys cusickii cmg20
Plagiobothrys corymbosus SA cmg331
Plagiobothrys magellanica cmg328
Plagiobothrys undulatus cmg12
Plagiobothrys scriptus cmg18
Plagiobothrys albiflorus cmg271
Plagiobothrys calandrinioides cmg333
Plagiobothrys orientalis cmg477
Plagiobothrys stipitatus var micranthus cmg17
Plagiobothrys tener var tener cmg7
Plagiobothrys leptocladus cmg15
Plagiobothrys cognatus cmg19
Plagiobothrys bracteatus cmg258
Plagiobothrys trachycarpus cmg257
Plagiobothrys hirtus cmg267
Plagiobothrys figuratus cmg265
Plagiobothrys oppositifolius cmg344
Plagiobothrys kunthii cmg433
Plagiobothrys linifolius cmg461 460
Plagiobothrys humilis cmg457
Plagiobothrys congestus cmg455
Plagiobothrys macbridei cmg462
Plagiobothrys pulchellus cmg321
Plagiobothrys uliginosus cmg320
Plagiobothrys procumbens cmg323
Plagiobothrys polycaulis cmg327
Plagiobothrys pratensis cmg345
Plagiobothrys verrucosus cmg319
Plagiobothrys torreyi var diffusus cmg34
Plagiobothrys uncinatus cmg35
Plagiobothrys myosotoides CA cmg261
Plagiobothrys myosotoides SA cmg317
Plagiobothrys tinctorius cmg324
Plagiobothrys torreyi var perplexans cmg260
Plagiobothrys torreyi var torreyi cmg259
Plagiobothrys tenellus cmg33
Plagiobothrys shastensis cmg32
Plagiobothrys collinus var ursinus cmg239
Plagiobothrys collinus var fulvescens cmg24
Plagiobothrys collinus var californicus cmg167
Plagiobothrys collinus var gracilis cmg123
Plagiobothrys pringlei cmg268
Plagiobothrys collinus var collinus cmg341
Plagiobothrys fulvus var campestris cmg95
Plagiobothrys fulvus var fulvus cmg335
Plagiobothrys infectivus cmg103
Johnstonella costata cmg124
Johnstonella racemosa cmg232
Plagiobothrys glomeratus cmg311
Plagiobothrys hispidus cmg94
Dasynotus daubenmirei
Harpagonella palmeri var arizonica cmg371
Harpagonella palmeri var palmeri cmg377
Pectocarya anisocarpa cmg384
Pectocarya linearis var ferocula cmg383
Pectocarya recurvata cmg382
Pectocarya heterocarpa cmg391
Pectocarya platycarpa cmg389
Pectocarya peninsularis cmg436
Pectocarya linearis var linearis cmg355
Pectocarya penicillata cmg99
Pectocarya anomala cmg359
Pectocarya setosa cmg74
Pectocarya pusilla cmg363
Oreocarya nubigenia cmg367
Oreocarya celosioides cmg368
Oreocarya flavoculata cmg231
Oreocarya confertiflora cmg229
Oreocarya cinerea var jamesii cmg369
Oreocarya cana cmg366
Eremocarya lepida
Eremocarya micrantha
Plagiobothrys jonesii cmg28
Cryptantha muricata cmg126
Cryptantha maritima cmg125
Johnstonella micromeres cmg234
Cryptantha simulans cmg127
Cryptantha flaccida cmg228
Cryptantha gracilis cmg233
Greeneocharis circumscissa cmg230
Cynoglossum amabile
Cynoglossum magellense

Legend:
- Non-wetland habitats
- Vernal pools and ephemeral wetlands
- Node Absent
- Equivocal

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Figure 3A-B. Phylogenetic trees and associated lineage through time plots. **A.** Phylogenetic tree and lineage through time plot for Amsinckiinae; **B.** Phylogenetic tree and lineage through time plot for that portion of the Amsinckiinae tree with near-complete sampling of minimum-rank taxa.

A

B

Figure 4. Phylogenetic tree showing results of MEDUSA analysis.

Diversification rate shifts
identified by MEDUSA:

Shift	r	E	AICC
1	1.135	NA	407.6
2	0.433	NA	399.1
3	0.540	NA	393.8

VP = invasion of
vernal pools
and ephemeral
wetlands

Non-*Plagiobothrys*
Amsinckiinae

Plagiobothrys s.s.

Plagiobothrys
sect. *Allocarya*
in red

Non-*Plagiobothrys*
Amsinckiinae

12.5 10.0 7.5 5.0 2.5 0.0
Millions of years ago

92
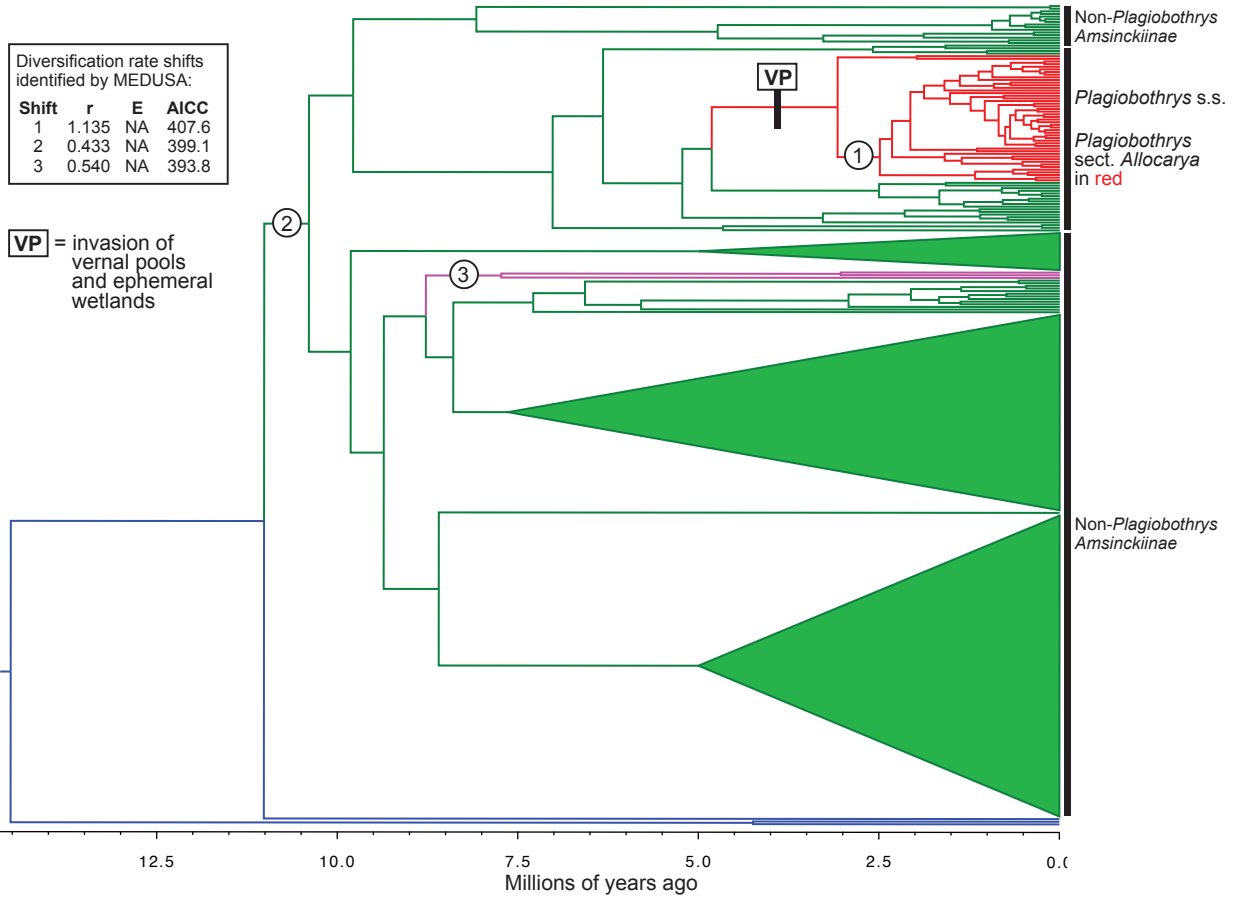
TABLES

Table 1. Results of trait-independent diversification analysis in MEDUSA.

Appropriate aicc-threshold for a tree of 111 tips is: 4.998329.

Step 1:	lnLik=-212.6229	aicc=429.3009	model=bd			
Step 2	lnLik=-200.7605	aicc=407.6316	shift at node 139	model=yule	cut=stem	# shifts=1
Step 3:	lnLik=-194.4068	aicc=399.0926	shift at node 114	model=yule	cut=stem	# shifts=2
Step 4:	lnLik=-189.644	aicc=393.8138	shift at node 203	model=yule	cut=node	# shifts=3

Model ID	Shift Node	Cut At	Model	Ln.Lik.part	r epsilon	r.low	r.high
1	112	node	yule	-8.735208	0.0942651	0	0.2252959
2	139	stem	yule	-39.28201	1.13547	0.835256	1.5002459
3	114	stem	yule	-137.7089	0.433287	0.3659434	0.5128886
4	203	node	yule	-3.917921	0.0540456	0	0.2379147

Table 2. Results of trait-dependent diversification analysis in BiSEE.

Model	Df	lnLikelihood	AIC	ChiSq	p-value
Full	6	-209.48	430.97		
Constrained - equal speciation rates	5	-213.25	436.51	7.5424	**0.006026**
Constrained - equal extinction rates	5	-209.74	429.47	0.5034	0.478010
Constrained - equal transition rates	5	-209.54	429.01	0.1056	0.745182

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Appendices

APPENDIX 1. SPECIMENS USED FOR PHYLOGENETIC ANALYSIS IN CHAPTER 1

Amsinckia calycina (Moris) Chater: cmg514, Escobar 118, Santiago, Chile. *Amsinckia eastwoodiae* J.F. Macbr.: cmg175, Helmkamp, G.K. & Helmkamp, E.A. 3265, Kern County, California, USA. *Amsinckia furcata* Suksd.: cmg227, Taylor, D.W. 11673, San Benito County, California, USA. *Amsinckia grandiflora* (A. Gray) Kleeb ex Greene: cmg226, Heckard, L.R. 3671, San Joaquin County, California, USA. *Amsinckia inepta* J.F. Macbr.: cmg486. *Amsinckia intermedia* Fisch. & C.A. Mey.: cmg173, Guilliams, C.M. 868. *Amsinckia lycopsoides* Lehm.: cmg511, Ahart, L. 10064, Butte County, California, USA. *Amsinckia menziesii* (Lehm.) A. Nelson & J.F. Macbr.: cmg223, Guilliams, C.M. 943. *Amsinckia retrorsa* Suksd.: cmg176, Rebman, J.P. & Gregory, J. 8354, San Diego County, California, USA. *Amsinckia spectabilis* Fisch. & C.A. Mey. var. *spectabilis:* cmg179, Cain, I., et al. 369, San Diego County, California, USA. *Amsinckia tessellata* A. Gray var. *gloriosa* (Eastw. ex Suksd.) Hoover: cmg482, Keil, D. 29188, San Luis Obispo County, California, USA. *Amsinckia tessellata* A. Gray var. *tessellata:* cmg508, Ertter, B. & Hosley, L. 11532, Alameda County, California, USA. *Amsinckia vernicosa* Hook. & Arn.: cmg225, Taylor, D.W. 8739, San Luis Obispo County, California, USA. *Cryptantha flaccida* (Douglas ex Lehm.) Greene: cmg228, Ahart, L. & Dittes, J. 12022, Siskiyou County, California, USA. *Cryptantha gracilis* Osterh.: cmg233, Thorne, R.F. & Henrickson, J. 45365, San Bernardino County, California, USA. *Cryptantha maritima* (Greene) Greene: cmg125, Guilliams, C.M., Simpson, M.G., Hasenstab, K. 554, San Diego County, California, USA. *Cryptantha muricata* (Hook. & Arn.) A. Nelson & J.F. Macbr.: cmg126, Guilliams, C.M., Franklin, J., Bergman, E. 365, San Diego County, California, USA. *Cryptantha simulans* Greene: cmg127, Guilliams, C.M., Frankin, J., Santos, L., Bergman, E. 346, San Diego County, California, USA. *Cynoglossum amabile* Stapf & J.R. Drumm.: Genbank accession number JF976215. *Cynoglossum magellense* Ten.: Genbank accession number FJ789861. *Dasynotus daubenmirei* I.M. Johnst.: Genbank accession number KF287969. *Eremocarya lepida* (A. Gray) Greene: Genbank accession number JQ513427. *Eremocarya micrantha* (Torr.) Greene: Genbank accession number JQ513427. *Greeneocharis circumscissa* (Hook. & Arn.) Rydb.: cmg230, Gowen, D. 410, Kern County, California, USA. *Harpagonella palmeri* A. Gray var. *arizonica* I.M. Johnst.: cmg370, Tedford, J. 599, Pima County, Arizona, USA. *Harpagonella palmeri* A. Gray var. *arizonica* I.M. Johnst.: cmg371, Van Devender, T.R. 88-54, Pima County, Arizona, USA. *Harpagonella palmeri* A. Gray var. *palmeri:* cmg376, Rebman, J.P. 8348, San Diego County, California, USA. *Harpagonella palmeri* A. Gray var. *palmeri:* cmg377, Rebman, J.P. 8031, San Diego County, California, USA. *Johnstonella costata* (Brandegee) Hasenstab & M.G. Simpson: cmg124, Guilliams, C.M., Marshall, J. 630, Imperial County, California, USA. *Johnstonella micromeres* (A. Gray) Hasenstab & M.G. Simpson: cmg234, Taylor, D.W. 6884, Inyo County, California, USA. *Johnstonella recemosa* (S. Watson ex A. Gray) Brand: cmg232, Taylor, D.W. 6364B, Inyo County, California, USA. *Oreocarya cana* A. Nelson: cmg366, USA. *Oreocarya celosioides* Eastw.: cmg368, USA. *Oreocarya cinerea* Greene var. *jamesii:* cmg369, USA. *Oreocarya confertiflora* Greene: cmg229, Buck, R.E. 1336, Inyo County, California, USA. *Oreocarya flavoculata* A. Nelson: cmg231, Taylor, D.W. 7991, Mono County, California, USA. *Oreocarya nubigenia* Greene: cmg367, USA. *Pectocarya anisocarpa* Veno: cmg384, Kelley, R. 1976, USA. *Pectocarya anomala* I.M. Johnst.: cmg359, SPECIMEN NOT FOUND. *Pectocarya heterocarpa* (I.M. Johnst.) I.M. Johnst.: cmg391, Kelley, R. 1987, USA. *Pectocarya linearis* (Ruiz & Pav.) DC. var. *ferocula* I.M. Johnst.: cmg383, Kelley, R. 1974, USA. *Pectocarya linearis* (Ruiz & Pav.) DC. var. *linearis:* cmg355, Kalin & Humaña 993615, Santiago, Chile. *Pectocarya penicillata* (Hook. & Arn.) A. DC.: cmg99, Gowen, D. 204, Kern County,

California, USA. *Pectocarya peninsularis* I.M. Johnst.: cmg436, USA. *Pectocarya platycarpa* (Munz & I.M. Johnst.) Munz & I.M. Johnst.: cmg389, Kelley, R. 1983, USA. *Pectocarya pusilla* A. Gray: cmg363, Taylor, D.W., Clifton, G.L., Andre, J. 10682, Shasta County, California, USA. *Pectocarya recurvata* I.M. Johnst.: cmg382, Kelley, R. 1975, USA. *Pectocarya setosa* A. Gray: cmg74, Taylor, D.W. 10246, Inyo County, California, USA. *Plagiobothrys "colonetensis"* unpublished: cmg465, Guilliams, C.M. 2350, MX. *Plagiobothrys "colonetensis"* unpublished: cmg470, Guilliams, C.M. 2184, MX. *Plagiobothrys acanthocarpus* (Piper) I.M. Johnst.: cmg1, Gowen, D. 172, Stanislaus County, California, USA. *Plagiobothrys acanthocarpus* (Piper) I.M. Johnst.: cmg121, Guilliams, C.M., Guilliams, J.D. 510, San Diego County, California, USA. *Plagiobothrys albiflorus* R.L. Pérez-Mor.: cmg271. *Plagiobothrys arizonicus* (A. Gray) Greene ex A. Gray: cmg21, Wilken, D.H., Painter, E. 15436, Inyo County, California, USA. *Plagiobothrys arizonicus* (A. Gray) Greene ex A. Gray: cmg169, Guilliams, C.M. 870, USA. *Plagiobothrys austiniae* (Greene) I.M. Johnst.: cmg61, Ahart, L., Lansdown, R. 13696, Tehama County, California, USA. *Plagiobothrys austiniae* (Greene) I.M. Johnst.: cmg105, Ahart, L., Lansdown, R. 13696, Tehama County, California, USA. *Plagiobothrys bracteatus* (Howell) I.M. Johnst.: cmg120, Bauder, E. 30V95A, San Diego County, California, USA. *Plagiobothrys bracteatus* (Howell) I.M. Johnst.: cmg258, Guilliams, C.M. 936, USA. *Plagiobothrys calandrinioides* (Phil.) I.M. Johnst.: cmg333, Teneb 649, Magallanes, Chile. *Plagiobothrys calandrinioides* (Phil.) I.M. Johnst.: cmg334, Dominguez 472, U Esperanza, Chile. *Plagiobothrys canescens* Benth. var. *canescens:* cmg22, Ahart, L., Dittes, J., Guardino, J. 13605, Tehama County, California, USA. *Plagiobothrys canescens* Benth. var. *canescens:* cmg69, Ahart, L. 13718, Tehama County, California, USA. *Plagiobothrys canescens* Benth. var. *catalinensis* (A. Gray) Jeps.: cmg36, Junak, S.A. SC-3053, Santa Barbara County, California, USA. *Plagiobothrys canescens* Benth. var. *catalinensis* (A. Gray) Jeps.: cmg70, Heckard, L.R. 4583, Kern County, California, USA. *Plagiobothrys chorisianus* (Cham.) I.M. Johnst. var. *chorisianus:* cmg4, Taylor, D.W. 14173, Santa Cruz County, California, USA. *Plagiobothrys chorisianus* (Cham.) I.M. Johnst. var. *chorisianus:* cmg102, Taylor, D.W. 14173, Santa Cruz County, California, USA. *Plagiobothrys chorisianus* (Cham.) I.M. Johnst. var. *hickmanii* (Greene) I.M. Johnst.: cmg118, Howe, D.F. 4342, San Mateo County, California, USA. *Plagiobothrys cognatus* (Greene) I.M. Johnst.: cmg19, Taylor, D.W. 17030, Madera County, California, USA. *Plagiobothrys cognatus* (Greene) I.M. Johnst.: cmg101, Ahart, L. 9231, Plumas County, California, USA. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *californicus* L.C. Higgins: cmg119, Guilliams, C.M., Ross, J. 536, Riverside County, California, USA. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *californicus* L.C. Higgins: cmg167, Guilliams, C.M. 865, USA. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *collinus:* cmg341, Squeo 87054, Elqui, Chile. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *fulvescens* L.C. Higgins: cmg24, Rebman, J.P. & Hollingsworth, B. 7284, San Diego County, California, USA. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *fulvescens* L.C. Higgins: cmg122, Simpson, M.G., Estrella, D., Lauri, R. 2358, San Diego County, California, USA. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *gracilis* L.C. Higgins: cmg25, Junak, S.A. SC-3074, Santa Barbara County, California, USA. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *gracilis* L.C. Higgins: cmg123, Rebman, J.R. 7257, San Diego County, California, USA. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *ursinus* L.C. Higgins: cmg239, Guilliams, C.M. 1061, USA. *Plagiobothrys collinus* (Phil.) I.M. Johnst. var. *ursinus* L.C. Higgins: cmg240, Guilliams, C.M. 1067, USA. *Plagiobothrys congestus* (Wedd.) I.M. Johnst.: cmg455, Liberman, M. 289, La Paz, Aroma, Bolivia. *Plagiobothrys congestus* (Wedd.) I.M. Johnst.: cmg463, Beck,

S.G. 343, La Paz, Aroma, Bolivia. ***Plagiobothrys corymbosus*** I.M. Johnst.: cmg331, Montero 4066, Cautin, Chile. ***Plagiobothrys cusickii*** (Greene) I.M. Johnst.: cmg20, USA. ***Plagiobothrys cusickii*** (Greene) I.M. Johnst.: cmg97, Ahart, L. & Dittes, J. 11954, Lassen County, California, USA. ***Plagiobothrys diffusus*** (Greene) I.M. Johnst.: cmg71, Buck, R.E. & West, J.A. 527, San Mateo County, California, USA. ***Plagiobothrys distantiflorus*** (Piper) I.M. Johnst.: cmg87, Taylor, D.W. 16334, Tuolumne County, California, USA. ***Plagiobothrys distantiflorus*** (Piper) I.M. Johnst.: cmg89, York, D., Schierenbeck, K., Fauver, T. 443, Fresno County, California, USA. ***Plagiobothrys elachanthus*** NOT IN TROPICOS: cmg235, Australia. ***Plagiobothrys elachanthus*** NOT IN TROPICOS: cmg451, Australia. ***Plagiobothrys figuratus*** I.M. Johnst. ex M. Peck: cmg265, USA. ***Plagiobothrys figuratus*** I.M. Johnst. ex M. Peck: cmg266, USA. ***Plagiobothrys fulvus*** (Hook. & Arn.) I.M. Johnston var. ***campestris*** (Greene) I.M. Johnst.: cmg95, Ahart, L. 13777, Yuba County, California, USA. ***Plagiobothrys fulvus*** (Hook. & Arn.) I.M. Johnston var. ***campestris*** (Greene) I.M. Johnst.: cmg205, Guilliams, C.M. 926, USA. ***Plagiobothrys fulvus*** (Hook. & Arn.) I.M. Johnston var. ***fulvus***: cmg335, Tome 222, Santiago, Chile. ***Plagiobothrys fulvus*** (Hook. & Arn.) I.M. Johnston var. ***fulvus***: cmg336, Tome 221, Santiago, Chile. ***Plagiobothrys glomeratus*** A. Gray: cmg310, USA. ***Plagiobothrys glomeratus*** A. Gray: cmg311, USA. ***Plagiobothrys glomeratus*** A. Gray: cmg314, USA. ***Plagiobothrys glyptocarpus*** (Piper) I.M. Johnst. var. ***glyptocarpus***: cmg5, Ahart, L. 10140, Shasta County, California, USA. ***Plagiobothrys glyptocarpus*** (Piper) I.M. Johnst. var. ***glyptocarpus***: cmg72, Ahart, L. 9597, Nevada County, California, USA. ***Plagiobothrys glyptocarpus*** (Piper) I.M. Johnst. var. ***modestus*** I.M. Johnst.: cmg88, Ahart, L. s.n., Butte County, California, USA. ***Plagiobothrys greenei*** (A. Gray) I.M. Johnst.: cmg62, Taylor, D.W. 17115, Madera County, California, USA. ***Plagiobothrys greenei*** (A. Gray) I.M. Johnst.: cmg63, Ruygt, J. 837, Napa County, California, USA. ***Plagiobothrys greenei*** (A. Gray) I.M. Johnst.: cmg104, Taylor, D.W. 16250, Madera County, California, USA. ***Plagiobothrys hirtus*** (Greene) I.M. Johnst.: cmg267, Zika, P. 10239, Douglas County, Oregon, USA. ***Plagiobothrys hispidulus*** (Greene) I.M. Johnst.: cmg10, Ahart, L. 12926, Plumas County, California, USA. ***Plagiobothrys hispidulus*** (Greene) I.M. Johnst.: cmg86, Ahart, L. & Dittes, J. 14092, Plumas County, California, USA. ***Plagiobothrys hispidus*** A. Gray: cmg94, Arnett, M. 8322, Madera County, California, USA. ***Plagiobothrys hispidus*** A. Gray: cmg301, Halse, R.R. 2559, Deschutes County, Oregon, USA. ***Plagiobothrys hispidus*** A. Gray: cmg315, USA. ***Plagiobothrys humilis*** (Ruiz & Pav.) I.M. Johnst.: cmg456, Sagástegui, A. 6387, Guzmango-Cruz Grande; Prov. Contumazá Ladera, Peru. ***Plagiobothrys humilis*** (Ruiz & Pav.) I.M. Johnst.: cmg457, Montesinos, D.B. 2510, Distrito Ichuña. Ichuña; Cachilaya, Apacheta, Peru. ***Plagiobothrys humistratus*** (Greene) I.M. Johnst.: cmg13, Taylor, D.W. 16251, Madera County, California, USA. ***Plagiobothrys humistratus*** (Greene) I.M. Johnst.: cmg93, Taylor, D.W. 17120, Madera County, California, USA. ***Plagiobothrys hystriculus*** (Piper) I.M. Johnst.: cmg14, Preston, R.E. 2386, Solano County, California, USA. ***Plagiobothrys infectivus*** I.M. Johnst.: cmg103, Hendrix, T.M. 124, San Luis Obispo County, California, USA. ***Plagiobothrys jonesii*** A. Gray: cmg28, Silverman, D. 3991, San Bernardino County, California, USA. ***Plagiobothrys jonesii*** A. Gray: cmg166, Barth, J., Gregory, J., Hendrickson, L., Mulligan, M., Rich, K. 409, San Diego County, California, USA. ***Plagiobothrys jonesii*** A. Gray: cmg211, USA. ***Plagiobothrys kingii*** (S. Watson) A. Gray var. ***harknessii*** (Greene) Jeps.: cmg29, Taylor, D.W. 16839, Mono County, California, USA. ***Plagiobothrys kingii*** (S. Watson) A. Gray var. ***harknessii*** (Greene) Jeps.: cmg165, Kelley, W.A., Baer, F. 8724, Mono County, California, USA. ***Plagiobothrys kingii*** (S. Watson) A. Gray var. ***kingii***: cmg30, Honer, M. 92, Mono County, California, USA.

Plagiobothrys kunthii (Walp.) I.M. Johnst.: cmg426, NR 6483. *Plagiobothrys kunthii* (Walp.) I.M. Johnst.: cmg433, Wood 19922. *Plagiobothrys leptocladus* (Greene) I.M. Johnst.: cmg15, Gowen, D. 208, Kern County, California, USA. *Plagiobothrys leptocladus* (Greene) I.M. Johnst.: cmg464, Guilliams, C.M. 2349, USA. *Plagiobothrys linifolius* (Willd. ex Lehm.) I.M. Johnst.: cmg459, Holm-Nielsen, L.B., et al. 5293, Carchi, Ecuador. *Plagiobothrys linifolius* (Willd. ex Lehm.) I.M. Johnst.: cmg460, Øllgaard, B. & Balslev, H. 8021, Napo, Ecuador. *Plagiobothrys macbridei* I.M. Johnst.: cmg462, Nee, M.H. & Solomon, J.C. 36645, Cochabamba, Tapacari, Bolivia. *Plagiobothrys magellanica* NOT IN TROPICOS: cmg328, Ricardi & Matthei 246, Tierra Del Fuego, Chile. *Plagiobothrys mollis* (A. Gray) I.M. Johnst. var. *mollis*: cmg9, Ahart, L. 9211, Plumas County, California, USA. *Plagiobothrys mollis* (A. Gray) I.M. Johnst. var. *mollis*: cmg91, Ahart, L. 14484, Butte County, California, USA. *Plagiobothrys myosotoides* (Lehm.) Brand: cmg261, Gowen, D. 1029, California, USA. *Plagiobothrys myosotoides* (Lehm.) Brand: cmg317, Kalin & Humaña 995209, Santiago, Chile. *Plagiobothrys myosotoides* (Lehm.) Brand: cmg318, Tome 223, Santiago, Chile. *Plagiobothrys nothofulvus* (A. Gray) A. Gray: cmg31, Blakley, E.R. 7557, Tuolumne County, California, USA. *Plagiobothrys nothofulvus* (A. Gray) A. Gray: cmg65, Ahart, L., Dittes, J., Guardino, J. 13615, Tehama County, California, USA. *Plagiobothrys oppositifolius* I.M. Johnst.: cmg344, Gunckel 21711, Cautin, Chile. *Plagiobothrys orientalis* I.M. Johnst.: cmg477. *Plagiobothrys orientalis* I.M. Johnst.: cmg478. *Plagiobothrys parishii* I.M. Johnst.: cmg37, Taylor, D.W. 16759, Mono County, California, USA. *Plagiobothrys parishii* I.M. Johnst.: cmg273, DeDecker, M. 5369, Inyo County, California, USA. *Plagiobothrys plurisepalus* NOT IN TROPICOS: cmg237, Australia. *Plagiobothrys plurisepalus* NOT IN TROPICOS: cmg452, Australia. *Plagiobothrys polycaulis* (Phil.) I.M. Johnst.: cmg326, Enriquez s.n., Concepción, Chile. *Plagiobothrys polycaulis* (Phil.) I.M. Johnst.: cmg327, Garcia, N. 3775, Chacabuco, Chile. *Plagiobothrys pratensis* I.M. Johnst.: cmg345, Levi 1865, Osorno, Chile. *Plagiobothrys pringlei* Greene: cmg268, USA. *Plagiobothrys pringlei* Greene: cmg300, USA. *Plagiobothrys procumbens* (Colla) Colla: cmg323, Teillier 4864, Choapa, Chile. *Plagiobothrys procumbens* (Colla) Colla: cmg330, Bravo 183, Santiago, Chile. *Plagiobothrys pulchellus* I.M. Johnst.: cmg321, Kunzagk s.n, Biobio, Chile. *Plagiobothrys reticulatus* (Piper) I.M. Johnst. var. *reticulatus*: cmg16, Taylor, D.W. 17478, Mendocino County, California, USA. *Plagiobothrys scouleri* (Hook. & Arn.) I.M. Johnston: cmg67, Joyal, E. & Mehlman, D. 1287, Modoc County, California, USA. *Plagiobothrys scriptus* (Greene) I.M. Johnst.: cmg18, Ahart, L. 10715, Butte County, California, USA. *Plagiobothrys scriptus* (Greene) I.M. Johnst.: cmg207, Guilliams, C.M. 906, California, USA. *Plagiobothrys shastensis* Greene ex A. Gray: cmg32, Ahart, L. 10124, Butte County, California, USA. *Plagiobothrys stipitatus* (Greene) I.M. Johnst. var. *micranthus* (Piper) I.M. Johnst.: cmg17, Ahart, L. 10141, Shasta County, California, USA. *Plagiobothrys stipitatus* (Greene) I.M. Johnst. var. *micranthus* (Piper) I.M. Johnst.: cmg309, USA. *Plagiobothrys stipitatus* (Greene) I.M. Johnst. var. *stipitatus*: cmg64, Heckard, L.R. & Hickman, J.C. 5831, Yolo County, California, USA. *Plagiobothrys stipitatus* (Greene) I.M. Johnst. var. *stipitatus*: cmg92, Ertter, B., Jokerst, J., Magney, D. 8120, Contra Costa County, California, USA. *Plagiobothrys strictus* (Greene) I.M. Johnst.: cmg479, Guilliams, C.M. 2284, USA. *Plagiobothrys strictus* (Greene) I.M. Johnst.: cmg480, Guilliams, C.M. 2285, USA. *Plagiobothrys tenellus* (Nutt.) A. Gray: cmg33, Gowen, D. 179, Santa Clara County, California, USA. *Plagiobothrys tenellus* (Nutt.) A. Gray: cmg263, Ertter, B. 19895, USA. *Plagiobothrys tener* (Greene) I.M. Johnst. var. *tener*: cmg7, Ruygt, J. 2498, Napa County, California, USA. *Plagiobothrys tinctorius* (Ruiz & Pav.) A. Gray: cmg324, Rodriguez & Landero 2005,

Concepción, Chile. ***Plagiobothrys torreyi*** (A. Gray) A. Gray var. ***diffusus*** I.M. Johnst.: cmg34, Buck, R.E. 576, Madera County, California, USA. ***Plagiobothrys torreyi*** (A. Gray) A. Gray var. ***perplexans*** I.M. Johnst.: cmg260, Gowen, D. 1048, USA. ***Plagiobothrys torreyi*** (A. Gray) A. Gray var. ***torreyi***: cmg90, Colwell, A.E. & Coulter, C. AC4-16, Mariposa County, California, USA. ***Plagiobothrys torreyi*** (A. Gray) A. Gray var. ***torreyi***: cmg259, Gowen, D. 1054, USA. ***Plagiobothrys trachycarpus*** (A. Gray) I.M. Johnst.: cmg11, Yadon, V. s.n., Monterey County, California, USA. ***Plagiobothrys trachycarpus*** (A. Gray) I.M. Johnst.: cmg257, Guilliams, C.M. 948, USA. ***Plagiobothrys uliginosus*** I.M. Johnst.: cmg320, Rodriguez, R. 567, Nuble, Chile. ***Plagiobothrys uncinatus*** J.T. Howell: cmg35, Taylor, D.W. 17427, Monterey County, California, USA. ***Plagiobothrys uncinatus*** J.T. Howell: cmg262, Gowen, D. 1007, USA. ***Plagiobothrys undulatus*** (Piper) I.M. Johnst.: cmg12, Taylor, D.W. 17442, Santa Cruz County, California, USA. ***Plagiobothrys undulatus*** (Piper) I.M. Johnst.: cmg414, Guilliams, C.M. 1138, USA. ***Plagiobothrys verrucosus*** (Phil.) I.M. Johnst.: cmg319, Teillier & Romero 6053, General Carrera, Chile.

APPENDIX 2. SPECIMENS USED FOR PHYLOGENETIC ANALYSIS IN CHAPTERS 2 AND 3

Amsinckia calycina (Moris) Chater: cmg514, Escobar 118, Santiago. *Amsinckia eastwoodiae* J.F. Macbr.: cmg175, Helmkamp, G.K. & Helmkamp, E.A. 3265, Kern County, California. *Amsinckia furcata* Suksd.: cmg227, Taylor, D.W. 11673, San Benito County, California. *Amsinckia grandiflora* (A. Gray) Kleeb ex Greene: cmg226, Heckard, L.R. 3671, San Joaquin County, California. *Amsinckia inepta* J.F. Macbr.: cmg486. *Amsinckia intermedia* Fisch. & C.A. Mey.: cmg173, Guilliams, C.M. 868. *Amsinckia lycopsoides* Lehm.: cmg511, Ahart, L. 10064, Butte County, California. *Amsinckia menziesii* (Lehm.) A. Nelson & J.F. Macbr.: cmg223, Guilliams, C.M. 943. *Amsinckia retrorsa* Suksd.: cmg176, Rebman, J.P. & Gregory, J. 8354, San Diego County, California. *Amsinckia spectabilis* Fisch. & C.A. Mey. var. *spectabilis:* cmg179, Cain, I., et al. 369, San Diego County, California. *Amsinckia tessellata* A. Gray var. *gloriosa* (Eastw. ex Suksd.) Hoover: cmg482, Keil, D. 29188, San Luis Obispo County, California. *Amsinckia tessellata* A. Gray var. *tessellata:* cmg508, Ertter, B. & Hosley, L. 11532, Alameda County, California. *Amsinckia vernicosa* Hook. & Arn.: cmg225, Taylor, D.W. 8739, San Luis Obispo County, California. *Cryptantha flaccida* (Douglas ex Lehm.) Greene: cmg228, Ahart, L. & Dittes, J. 12022, Siskiyou County, California. *Cryptantha gracilis* Osterh.: cmg233, Thorne, R.F. & Henrickson, J. 45365, San Bernardino County, California. *Cryptantha maritima* (Greene) Greene: cmg125, Guilliams, C.M., Simpson, M.G., Hasenstab, K. 554, San Diego County, California. *Cryptantha muricata* (Hook. & Arn.) A. Nelson & J.F. Macbr.: cmg126, Guilliams, C.M., Franklin, J., Bergman, E. 365, San Diego County, California. *Cryptantha simulans* Greene: cmg127, Guilliams, C.M., Frankin, J., Santos, L., Bergman, E. 346, San Diego County, California. *Cynoglossum amabile* Stapf & J.R. Drumm.: GB. *Cynoglossum magellense* Ten.: GB. *Dasynotus daubenmirei* I.M. Johnst.: GB. *Eremocarya lepida* (A. Gray) Greene: GB. *Eremocarya micrantha* (Torr.) Greene: GB. *Greeneocharis circumscissa* (Hook. & Arn.) Rydb.: cmg230, Gowen, D. 410, Kern County, California. *Harpagonella palmeri* A. Gray var. *arizonica* I.M. Johnst.: cmg371, Van Devender, T.R. 88-54, Pima County, Arizona. *Harpagonella palmeri* A. Gray var. *palmeri*: cmg377, Rebman, J.P. 8031, San Diego County, California. *Johnstonella costata* (Brandegee) Hasenstab & M.G. Simpson: cmg124, Guilliams, C.M., Marshall, J. 630, Imperial County, California. *Johnstonella micromeres* (A. Gray) Hasenstab & M.G. Simpson: cmg234, Taylor, D.W. 6884, Inyo County, California. *Johnstonella recemosa* (S. Watson ex A. Gray) Brand: cmg232, Taylor, D.W. 6364B, Inyo County, California. *Oreocarya cana* A. Nelson: cmg366. *Oreocarya celosioides* Eastw.: cmg368. *Oreocarya cinerea* Greene var. *jamesii*: cmg369. *Oreocarya confertiflora* Greene: cmg229, Buck, R.E. 1336, Inyo County, California. *Oreocarya flavoculata* A. Nelson: cmg231, Taylor, D.W. 7991, Mono County, California. *Oreocarya nubigenia* Greene: cmg367. *Pectocarya anisocarpa* Veno: cmg384, Kelley, R. 1976. *Pectocarya anomala* I.M. Johnst.: cmg359. *Pectocarya heterocarpa* (I.M. Johnst.) I.M. Johnst.: cmg391, Kelley, R. 1987. *Pectocarya linearis* (Ruiz & Pav.) DC. var. *ferocula* I.M. Johnst.: cmg383, Kelley, R. 1974. *Pectocarya linearis* (Ruiz & Pav.) DC. var. *lineari*s: cmg355, Kalin & Humaña 993615, Santiago. *Pectocarya penicillata* (Hook. & Arn.) A. DC.: cmg99, Gowen, D. 204, Kern County, California. *Pectocarya peninsularis* I.M. Johnst.: cmg436. *Pectocarya platycarpa* (Munz & I.M. Johnst.) Munz & I.M. Johnst.: cmg389, Kelley, R. 1983. *Pectocarya pusilla* A. Gray: cmg363, Taylor, D.W., Clifton, G.L., Andre, J. 10682, Shasta County, California. *Pectocarya recurvata* I.M. Johnst.: cmg382, Kelley, R. 1975. *Pectocarya setosa* A. Gray: cmg74, Taylor, D.W. 10246, Inyo County, California. *Plagiobothrys* **"colonetensis"** unpublished: cmg465, Guilliams, C.M. 2350. *Plagiobothrys acanthocarpus* (Piper) I.M. Johnst.: cmg1, Gowen, D. 172, Stanislaus County, California. *Plagiobothrys albiflorus* R.L. Pérez-Mor.: cmg271. *Plagiobothrys*

arizonicus (A. Gray) Greene ex A. Gray: cmg169, Guilliams, C.M. 870. ***Plagiobothrys austiniae*** (Greene) I.M. Johnst.: cmg105, Ahart, L., Lansdown, R. 13696, Tehama County, California. ***Plagiobothrys bracteatus*** (Howell) I.M. Johnst.: cmg258, Guilliams, C.M. 936. ***Plagiobothrys calandrinioides*** (Phil.) I.M. Johnst.: cmg333, Teneb 649, Magallanes. ***Plagiobothrys canescens*** Benth. var. ***canescens***: cmg69, Ahart, L. 13718, Tehama County, California. ***Plagiobothrys canescens*** Benth. var. ***catalinensis*** (A. Gray) Jeps.: cmg70, Heckard, L.R. 4583, Kern County, California. ***Plagiobothrys chorisianus*** (Cham.) I.M. Johnst. var. ***chorisianus***: cmg4, Taylor, D.W. 14173, Santa Cruz County, California. ***Plagiobothrys chorisianus*** (Cham.) I.M. Johnst. var. ***hickmanii*** (Greene) I.M. Johnst.: cmg118, Howe, D.F. 4342, San Mateo County, California. ***Plagiobothrys cognatus*** (Greene) I.M. Johnst.: cmg19, Taylor, D.W. 17030, Madera County, California. ***Plagiobothrys collinus*** (Phil.) I.M. Johnst. var. ***californicus*** L.C. Higgins: cmg167, Guilliams, C.M. 865. ***Plagiobothrys collinus*** (Phil.) I.M. Johnst. var. ***collinus***: cmg341, Squeo 87054, Elqui. ***Plagiobothrys collinus*** (Phil.) I.M. Johnst. var. ***fulvescens*** L.C. Higgins: cmg24, Rebman, J.P. & Hollingsworth, B. 7284, San Diego County, California. ***Plagiobothrys collinus*** (Phil.) I.M. Johnst. var. ***gracilis*** L.C. Higgins: cmg123, Rebman, J.R. 7257, San Diego County, California. ***Plagiobothrys collinus*** (Phil.) I.M. Johnst. var. ***ursinus*** L.C. Higgins: cmg239, Guilliams, C.M. 1061. ***Plagiobothrys congestus*** (Wedd.) I.M. Johnst.: cmg455, Liberman, M. 289, La Paz, Aroma. ***Plagiobothrys corymbosus*** I.M. Johnst.: cmg331, Montero 4066, Cautin. ***Plagiobothrys cusickii*** (Greene) I.M. Johnst.: cmg20. ***Plagiobothrys diffusus*** (Greene) I.M. Johnst.: cmg71, Buck, R.E. & West, J.A. 527, San Mateo County, California. ***Plagiobothrys distantiflorus*** (Piper) I.M. Johnst.: cmg87, Taylor, D.W. 16334, Tuolumne County, California. ***Plagiobothrys elachanthus*** NOT IN TROPICOS: cmg451. ***Plagiobothrys figuratus*** I.M. Johnst. ex M. Peck: cmg265. ***Plagiobothrys fulvus*** (Hook. & Arn.) I.M. Johnston var. ***campestris*** (Greene) I.M. Johnst.: cmg95, Ahart, L. 13777, Yuba County, California. ***Plagiobothrys fulvus*** (Hook. & Arn.) I.M. Johnston var. ***fulvus***: cmg335, Tome 222, Santiago. ***Plagiobothrys glomeratus*** A. Gray: cmg311. ***Plagiobothrys glyptocarpus*** (Piper) I.M. Johnst. var. ***glyptocarpus***: cmg5, Ahart, L. 10140, Shasta County, California. ***Plagiobothrys glyptocarpus*** (Piper) I.M. Johnst. var. ***modestus*** I.M. Johnst.: cmg88, Ahart, L. s.n., Butte County, California. ***Plagiobothrys greenei*** (A. Gray) I.M. Johnst.: cmg104, Taylor, D.W. 16250, Madera County, California. ***Plagiobothrys hirtus*** (Greene) I.M. Johnst.: cmg267, Zika, P. 10239, Douglas County, Oregon. ***Plagiobothrys hispidulus*** (Greene) I.M. Johnst.: cmg86, Ahart, L. & Dittes, J. 14092, Plumas County, California. ***Plagiobothrys hispidus*** A. Gray: cmg94, Arnett, M. 8322, Madera County, California. ***Plagiobothrys humilis*** (Ruiz & Pav.) I.M. Johnst.: cmg457, Montesinos, D.B. 2510, Distrito Ichuña. Ichuña; Cachilaya, Apacheta. ***Plagiobothrys humistratus*** (Greene) I.M. Johnst.: cmg13, Taylor, D.W. 16251, Madera County, California. ***Plagiobothrys hystriculus*** (Piper) I.M. Johnst.: cmg14, Preston, R.E. 2386, Solano County, California. ***Plagiobothrys infectivus*** I.M. Johnst.: cmg103, Hendrix, T.M. 124, San Luis Obispo County, California. ***Plagiobothrys jonesii*** A. Gray: cmg28, Silverman, D. 3991, San Bernardino County, California. ***Plagiobothrys kingii*** (S. Watson) A. Gray var. ***harknessii*** (Greene) Jeps.: cmg165, Kelley, W.A., Baer, F. 8724, Mono County, California. ***Plagiobothrys kingii*** (S. Watson) A. Gray var. ***kingii***: cmg30, Honer, M. 92, Mono County, California. ***Plagiobothrys kunthii*** (Walp.) I.M. Johnst.: cmg433, Wood 19922. ***Plagiobothrys leptocladus*** (Greene) I.M. Johnst.: cmg15, Gowen, D. 208, Kern County, California. ***Plagiobothrys linifolius*** (Willd. ex Lehm.) I.M. Johnst.: cmg460, Øllgaard, B. & Balslev, H. 8021, Napo. ***Plagiobothrys macbridei*** I.M. Johnst.: cmg462, Nee, M.H. & Solomon, J.C. 36645, Cochabamba, Tapacari. ***Plagiobothrys magellanica*** NOT IN TROPICOS: cmg328, Ricardi & Matthei 246, Tierra Del Fuego.

Plagiobothrys mollis (A. Gray) I.M. Johnst. var. ***mollis***: cmg91, Ahart, L. 14484, Butte County, California. ***Plagiobothrys myosotoides*** (Lehm.) Brand: cmg261, Gowen, D. 1029, California. ***Plagiobothrys myosotoides*** (Lehm.) Brand: cmg317, Kalin & Humaña 995209, Santiago. ***Plagiobothrys nothofulvus*** (A. Gray) A. Gray: cmg65, Ahart, L., Dittes, J., Guardino, J. 13615, Tehama County, California. ***Plagiobothrys oppositifolius*** I.M. Johnst.: cmg344, Gunckel 21711, Cautin. ***Plagiobothrys orientalis*** I.M. Johnst.: cmg477. ***Plagiobothrys parishii*** I.M. Johnst.: cmg273, DeDecker, M. 5369, Inyo County, California. ***Plagiobothrys plurisepalus*** NOT IN TROPICOS: cmg452. ***Plagiobothrys polycaulis*** (Phil.) I.M. Johnst.: cmg327, Garcia, N. 3775, Chacabuco. ***Plagiobothrys pratensis*** I.M. Johnst.: cmg345, Levi 1865, Osorno. ***Plagiobothrys pringlei*** Greene: cmg268. ***Plagiobothrys procumbens*** (Colla) Colla: cmg323, Teillier 4864, Choapa. ***Plagiobothrys pulchellus*** I.M. Johnst.: cmg321, Kunzagk s.n, Biobio. ***Plagiobothrys reticulatus*** (Piper) I.M. Johnst. var. ***reticulatus***: cmg16, Taylor, D.W. 17478, Mendocino County, California. ***Plagiobothrys scouleri*** (Hook. & Arn.) I.M. Johnston: cmg67, Joyal, E. & Mehlman, D. 1287, Modoc County, California. ***Plagiobothrys scriptus*** (Greene) I.M. Johnst.: cmg18, Ahart, L. 10715, Butte County, California. ***Plagiobothrys shastensis*** Greene ex A. Gray: cmg32, Ahart, L. 10124, Butte County, California. ***Plagiobothrys stipitatus*** (Greene) I.M. Johnst. var. ***micranthus*** (Piper) I.M. Johnst.: cmg17, Ahart, L. 10141, Shasta County, California. ***Plagiobothrys stipitatus*** (Greene) I.M. Johnst. var. ***stipitatus***: cmg92, Ertter, B., Jokerst, J., Magney, D. 8120, Contra Costa County, California. ***Plagiobothrys strictus*** (Greene) I.M. Johnst.: cmg479, Guilliams, C.M. 2284. ***Plagiobothrys tenellus*** (Nutt.) A. Gray: cmg33, Gowen, D. 179, Santa Clara County, California. ***Plagiobothrys tener*** (Greene) I.M. Johnst. var. ***tener***: cmg7, Ruygt, J. 2498, Napa County, California. ***Plagiobothrys tinctorius*** (Ruiz & Pav.) A. Gray: cmg324, Rodriguez & Landero 2005, Concepción. ***Plagiobothrys torreyi*** (A. Gray) A. Gray var. ***diffusus*** I.M. Johnst.: cmg34, Buck, R.E. 576, Madera County, California. ***Plagiobothrys torreyi*** (A. Gray) A. Gray var. ***perplexans*** I.M. Johnst.: cmg260, Gowen, D. 1048. ***Plagiobothrys torreyi*** (A. Gray) A. Gray var. ***torreyi***: cmg259, Gowen, D. 1054. ***Plagiobothrys trachycarpus*** (A. Gray) I.M. Johnst.: cmg257, Guilliams, C.M. 948. ***Plagiobothrys uliginosus*** I.M. Johnst.: cmg320, Rodriguez, R. 567, Nuble. ***Plagiobothrys uncinatus*** J.T. Howell: cmg35, Taylor, D.W. 17427, Monterey County, California. ***Plagiobothrys undulatus*** (Piper) I.M. Johnst.: cmg12, Taylor, D.W. 17442, Santa Cruz County, California. ***Plagiobothrys verrucosus*** (Phil.) I.M. Johnst.: cmg319, Teillier & Romero 6053, General Carrera.