

INVITED PAPER

For the Special Issue: Patterns and Processes of American Amphitropical Plant Disjunctions: New Insights

Memoirs of a frequent flier: Phylogenomics reveals 18 long-distance dispersals between North America and South America in the popcorn flowers (Amsinckiinae)¹

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PREMISE OF THE STUDY: American amphitropical disjunction (AAD) is an important but understudied New World biogeographic pattern in which related plants occur in extratropical North America and South America, but are absent in the intervening tropics. Subtribe Amsinckiinae (Boraginaceae) is one of the richest groups of plants displaying the AAD pattern. Here, we infer a time-calibrated molecular phylogeny of the group to evaluate the number, timing, and directionality of AAD events, which yields generalizable insights into the mechanism of AAD.

METHODS: We perform a phylogenomic analysis of 139 samples of subtribe Amsinckiinae and infer divergence times using two calibration schemes: with only fossil calibrations and with fossils plus a secondary calibration from a recent family level analysis. Biogeographic analysis was performed in the R package BioGeoBEARS.

KEY RESULTS: We document 18 examples of AAD in the Amsinckiinae. Inferred divergence times of these AAD examples were strongly asynchronous, ranging from Miocene (17.1 million years ago [Ma]) to Pleistocene (0.33 Ma), with most (12) occurring <5 Ma. Four events occurred 10–5 Ma, during the second rise of the Andes. All AAD examples had a North America to South America directionality.

CONCLUSIONS: Second only to the hyperdiverse Poaceae in number of documented AAD examples, the Amsinckiinae is an ideal system for the study of AAD. Asynchronous divergence times support the hypothesis of long-distance dispersal by birds as the mechanism of AAD in the subtribe and more generally. Further comparative phylogenomic studies may permit biogeographic hypothesis testing and examination of the relationship between AAD and fruit morphology, reproductive biology, and ploidy.

KEY WORDS Boraginaceae; *Cryptantha*; Great American Biotic Interchange; *Oreocarya*; Pacific Flyway; *Pectocarya*; *Plagiobothrys*; zoochory

AMERICAN AMPHITROPICAL DISJUNCTION IN PLANTS

The 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 New World tropics. Since Gray and Hooker's early paper, botanists have attempted

to better understand the pattern by tabulating the taxa and clades that display AAD (e.g., Constance, 1963; Raven, 1963; Thorne, 1972; Simpson et al., 2017b, in this issue). The most comprehensive early list was presented by Raven (1963), which included 160 AAD taxa (species or infraspecies) or taxon groups (e.g., species pairs) thought to be each other's closest relatives.

Continued study of the floras of North America and South America has revealed additional AAD taxa and clades in vascular plants as well as in bryophytes and lichens. The most recent tabulation of vascular plant AADs by Simpson et al. (2017b, in this issue) counts 238 examples, including 133 conspecific AADs, 57 species-species pairs, and 48 clade-level disjunctions (including species-clade and clade-clade AADs). According to this summary, the AAD pattern is most common in Poaceae (51 AADs), followed by Boraginaceae s.s. (19 AADs), Fabaceae (16 AADs), Asteraceae (15 AADs), Cyperaceae (15 AADs), Polemoniaceae (14 AADs; Johnson and Porter, 2017, in this issue), and Apiaceae

¹ Manuscript received 11 September 2017; revision accepted 23 October 2017.

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<https://doi.org/10.3732/ajb.1700369>

(9 AADs). The most recent tabulation of bryophytes and lichens by Lewis et al. (2014a) lists 250 AAD taxa, including 66 species of mosses, 24 species of liverworts, and 160 species of lichens. Note that even these recent lists, much improved by further study as well as advances in DNA sequencing and phylogenetic methods, likely under-report clade-level disjunctions at deeper phylogenetic depths of divergence, which are difficult to detect without a broad phylogeographic study. As a result, we anticipate that these lists will continue to grow as progress is made estimating the tree of life in clades that are taxonomically diverse in the New World.

POTENTIAL EXPLANATIONS FOR AMERICAN AMPHITROPICAL DISJUNCTION

The most interesting and perhaps the most challenging aspect of AAD concerns the mechanism whereby taxa become amphitropically distributed. A small number of explanations for the 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) individuals of an ancestral North American or South American plant lineage crossed the tropics to the other hemisphere in one or more steps, which we call the “long-distance dispersal” hypothesis, and (2) 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 (a vicariance-based hypothesis). For simplicity here, our concept of long-distance dispersal includes both direct dispersal and stepwise dispersal along the Andes range. It also includes two nonmutually exclusive subhypotheses: dispersal by birds and dispersal by large mammals during the Great American Biotic Interchange (GABI). Finally, recent human activity has also resulted in the exchange of plants between North America and South America. As we are concerned with naturally occurring examples of AAD, we do not consider these anthropogenic AAD examples further here. Owing to the different possible explanations for the AAD pattern, as well as the differing phylogenetic depths of divergence at which AAD may occur, for simplicity we will refer to examples of AAD at any rank or depth of divergence as AAD “events”.

Long-distance dispersal by birds—The hypothesis of long-distance dispersal by birds 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. 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., 2014b), the vast numbers of migratory birds traveling annually between North America and South America along the Pacific Flyway would seem to offer ample opportunity for occurrence of this low-probability event, via either epizoochory or endozoochory. Some studies of plant groups displaying AAD noted that propagules have morphological features consistent with epizoochorous dispersal by animals, such as barbed appendages, hooked trichomes, and gelatinous seeds (Constance, 1963; Carlquist, 1983; Vargas et al., 1998). 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”.

Dispersal by large mammals during the Great American Biotic Interchange—One study advocating long-distance dispersal by large mammals during the GABI as the explanation for AAD (Biro and Whittall, 2015) provided three lines of evidence in support of their hypothesis: (1) some portion of the branch subtending one of the AAD events 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 million years ago [Ma]), (2) inferred directionality 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.

Recent evidence has questioned 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 (mid-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-Sánchez et al., 2012; Bacon et al., 2013, 2015; Parada et al., 2013; Leite et al., 2014; Wilson et al., 2014; Barker et al., 2015).

Range fragmentation—The range fragmentation hypothesis, a vicariance-based explanation for AAD, 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 seems to be the case that AAD events identified in the literature to date, especially AAD events that include herbaceous or shrubby plant groups, are overwhelmingly those without close relatives in the American tropics. Lack of relict tropical populations would seem to weaken this hypothesis.

AAD EVENT DIRECTIONALITY

A number of studies have examined the AAD pattern and inferred directionality in a phylogenetic context. The recent review of AAD (Simpson et al., 2017a, this issue) summarizes inferred directionality, finding 100% inferred North America to South America directionality in AAD events involving bipolar taxa ($n = 7$), 53.8% inferred North America to South America directionality in AAD events involving desert taxa ($n = 26$), and 92.1% inferred North America to South America directionality in AAD events involving temperate taxa ($n = 63$). Ten AAD events had equivocal inferred AAD directionality. Summing across regions, we find that 82.3% of

nonequivocal AAD events have an inferred North America to South America directionality. These findings are consistent with, but greater than those reported in an earlier review by Wen and Ickert-Bond (2009), who found that 65% of AAD events had a North America to South America directionality.

Why should there be an asymmetry in directionality between North America and South America? Raven (1963) speculated that the asymmetry could be explained under the long-distance dispersal by birds hypothesis from the observed overlap in timing between the fall bird migration along the Pacific Flyway and timing of fruit and seed maturation in the northern hemisphere. Two new explanations have emerged more recently. Using bird observation data from the eBird platform, Park (2016) offered an explanation for the directional asymmetry, also under the long-distance dispersal by birds hypothesis, based on the different flight routes used by select migratory birds during the fall and spring migrations. During the fall migration, the empirical data seem to show that for a small suite of potential seed-dispersing birds, the migration corridor between North America and South America is relatively narrow. Migratory birds pass rapidly between ecologically similar temperate regions, e.g., the Mediterranean-type climate regions found along the western margins of each continent. In contrast, during the spring migration, these select bird species fly from south to north in a much wider migratory corridor, with a seemingly reduced likelihood of departing and arriving in ecologically similar environments. These different seasonal migratory paths would result in a greater probability of dispersing plant propagules from north to south during the winter migration than from south to north during the spring migration.

The GABI hypothesis would seem to account for the directional asymmetry in plants owing to the primary direction of animal dispersal during this protracted biotic exchange (Biro and Whittall, 2015). However, there are major conceptual differences between the two long-distance dispersal hypotheses. Individuals of some migratory bird species travel between the continents twice yearly and number in the millions. Many birds make this long journey with few to no intermediate stops. Therefore, the avian biotic connection between North America and South America is both demonstrable and temporally immediate (Lewis et al., 2014b; Park, 2016; but see Cruden, 1966). The same cannot be said of dispersing mammals during the GABI. With the shrinking and final closure of the Isthmus of Panama, terrestrial vertebrates north of the Isthmus would have encountered new opportunities for dispersal southward. These individuals would be drawn from local populations and would have dispersal limitations typical of terrestrial vertebrates. As such, it is difficult to conceive of a scenario whereby a propagule of a plant species adapted to, e.g., a Mediterranean-type climate located 1000s of kilometers to the north, could be effectively conveyed between continents by locally dispersing animals.

TIMING OF DISPERSAL

Temporal patterns in the timing of AAD events may provide evidence for one or more of the explanations for the AAD biogeographic pattern. A temporal pattern of clustered AAD events, for example, might suggest a common explanation for AAD across these plant groups. Clustered time estimates might lend support to the range fragmentation hypothesis and possibly the long-distance dispersal by mammals (GABI) hypothesis. Nonclustered

or asynchronous time estimates might be viewed as supporting a continual but low-probability phenomenon, such as long-distance dispersal by birds.

A recent review of published estimates of AAD event timing suggests that most occurred from the Middle Miocene (ca. 16–15 Ma) to the present (Simpson et al. 2017b, this issue). Only four published estimates of AAD events have mean values older than 16–15 Ma, but each of these has a broad range. For example, the oldest estimated AAD event was in the genus *Ephedra* (Ephedraceae), which has a mean value of 24.78 Ma, but a range of 41.53–8.84 Ma (Ickert-Bond et al., 2009); the most recent nonanthropogenic dispersals have estimated dispersal times measured in 1000s of years (Simpson et al., 2017b, this issue).

AMERICAN AMPHOTROPICAL DISJUNCTION IN SUBTRIBE AMSINCKIINAE (BORAGINACEAE)

With high minimum-rank taxonomic richness (i.e., richness in species, subspecies, and varieties combined) in both North America and South America and AADs present in many genera, the Amsinckiinae is an ideal focal group for further study of the AAD biogeographic pattern. Amsinckiinae, a subtribe of the flowering plant family Boraginaceae s.s., comprises approximately ~290 species and ~333 minimum-rank, annual and perennial herbaceous taxa that are native to temperate North America, South America, and Australia (Hasenstab-Lehman and Simpson, 2012; Guilliams, 2015; Chacón et al., 2016; Simpson et al., 2017a). There are 13 recognized genera in the subtribe, six of which are amphitropically distributed in the New World. In North America, Amsinckiinae taxa occur in diverse ecological settings 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 (1978) as being emblematic of the diversification of the iconic California flora. In South America, members of the subtribe occupy a similar range of ecological conditions, but there are fewer minimum-rank taxa in general (72 taxa in South America, 248 taxa in North America, 10 taxa in both North America and South America). Outside of the New World, three to four species of the genus *Plagiobothrys* are native to Australia.

Members of subtribe Amsinckiinae share many morphological features common to the Boraginaceae s.s. of Chacón et al. (2016), hereafter referred to simply as the Boraginaceae. Plants in this family have hirsute to hispid vestiture, a circinate scorpioid cyme, actinomorphic flowers, a deeply four-lobed ovary, a gynobasic style position, and a fruit that is a schizocarp of four mericarps (nutlets). In the family and especially the subtribe, nutlet morphology is highly variable and taxonomically important, often serving as a primary source of evidence for delimiting taxa at ranks from genus to variety (Fig. 1).

This paper has several objectives thematically centering on the goal of examining AAD, using a broad sampling of Amsinckiinae in a phylogenomic context. First, we infer a phylogeny of the subtribe using high throughput genome skimming data. We then use two calibration schemes to convert resulting branch lengths to units of absolute time, one based on primary fossil calibrations and the other using fossil calibrations and a secondary calibration from a recent, broad dating study of Boraginaceae (Chacón et al., 2017). Using these time-calibrated phylogenies, we estimate the number, directionality, and timing of inferred AAD events and consider the

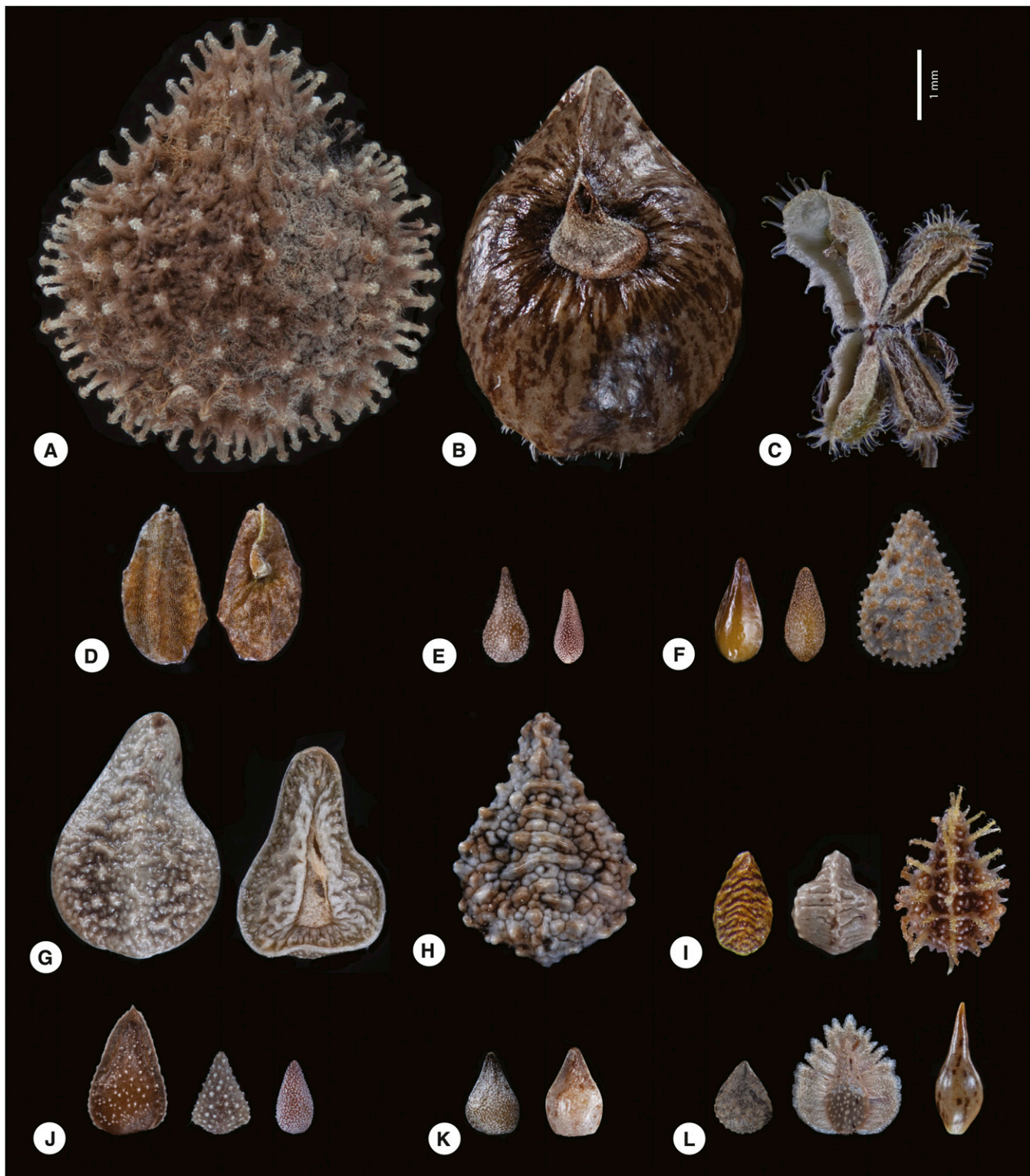


FIGURE 1 Images showing nutlets (dorsal view unless otherwise indicated, all to the same scale) of exemplars of the major clades of subtribe Amsinckiinae, after Simpson et al. (2017a). (A) *Andersonglossum* clade: *A. virginianum*. (B) *Dasynotus daubenmirei*. (C) *Pectocarya/Harpagonella* clade: *Pectocarya peninsularis*. (D) *Plagiobothrys* sect. *Sonnea* clade: *P. hispidus* (dorsal view, left; ventral view, right). (E) *Eremocarya* clade: *E. lepida* (left); *E. micrantha* var. *micrantha* (right). (F) *Maritimae* clade: *Cryptantha maritima* (left, showing two, heteromorphic nutlets); *C. muricata* var. *muricata* (right). (G) *Oreocarya* clade: *O. hoffmannii* (dorsal view, left; ventral view, right). (H) *Amsinckia/Plagiobothrys* sect. *Amsinckiopsis* clade: *Amsinckia tessellata*. (I) *Plagiobothrys* s.s. clade: *P. undulatus* (left); *P. torreyi* (middle); *P. acanthocarpus* (right). (J) *Johnstonella/Albidae* clade: *J. holoptera* (left); *Cryptantha albida* (middle); *J. angustifolia* (right). (K) *Greeneocharis* clade: *G. circumscissa* var. *circumscissa* (left); *G. similis* (right). (L) *Cryptantha* s.s. clade: *C. glomerata* (left); *C. pterocarya* var. *pterocarya* (middle); *C. flaccida* (right).

impact of these results on potential explanations for the AAD biogeographic pattern. We conclude by placing these results in the context of AAD across the angiosperms and provide directions for future research.

MATERIALS AND METHODS

Phylogenetic inference—Taxon sampling—We used 143 samples of Boraginaceae in this study, as in appendix 1 of Simpson et al. (2017a). Briefly, four outgroup taxa, *Cynoglossum creticum* Mill., *Hackelia micrantha* (Eastw.) J.L.Gentry, *Microula tibetica* Benth., and *Myosotis laxa* Lehm., as well as 139 Amsinckiinae ingroup samples were included. These samples represented 12 genera (all but the monotypic *Oncaglossum*), 123 species (43% of the subtribe), and 127 minimum-ranked taxa (38% of the subtribe).

Tree inference—Alignments of the plastome, cistron, and mitochondrial sequence data generated by Simpson et al. (2017a; <http://purl.org/phylo/treebase/phyloids/study/TB2:S21212>) were concatenated together for downstream inference. This concatenated alignment was partitioned by codon position for the plastome, by gene and noncoding spacer regions (ETS, 18S, ITS1, 5.8S, ITS2, and 26S) for the nuclear ribosomal cistron, and by exons for the mitochondria. The best-scoring maximum likelihood (ML) tree was inferred RAxML version 8 (Stamatakis, 2014) using the GTR+G model of evolution and designating *Hackelia micrantha* as the outgroup. Analyses were run on the CIPRES Science Gateway (Miller et al., 2010). The final tree was viewed in FigTree (Rambaut, 2006–2014).

Divergence time estimation—A penalized likelihood approach using treePL (Smith and O’Meara, 2012) was used to estimate divergence times. This method was preferable to other methods such as BEAST (Drummond et al., 2012) due to the large size of the data set. A smoothing parameter of 1000 was determined using the cross-validation option, and priming was used to determine the best optimization scores.

We performed separate divergence time analyses using two different calibration schemes, which we refer to as the “fossils only” scheme and the “fossils+secondary calibration” scheme. In the “fossils only” scheme, we used three Amsinckiinae fossil outlets as primary calibrations: *Cryptantha auriculata* (M.K.Elias) Segal, *C. chaneyi* (M.K.Elias) Segal, and *C. coroniformis* (M.K.Elias) Segal (Elias, 1932, 1942; Segal, 1964, 1966). These three fossil calibrations were placed subjectively through morphological comparison to extant taxa. *Cryptantha chaneyi* was used to calibrate the most recent common ancestor of *Oreocarya crymophila* (I.M.Johnst.) Jeps. & Hoover and *O. nubigena* Greene “granite” population. This placement was chosen due to its size and triangular areola at the base of the attachment scar, which then narrows into a groove that does not reach the apex of the nutlet body (Segal, 1966). These features are present in extant members of the genus *Oreocarya* (Simpson and Hasenstab, 2009; Fig. 1G). *Cryptantha auriculata* was placed at the node representing the most recent common ancestor of the clade containing *C. albida* (Kunth) I.M.Johnst. and *C. texana* Greene, due to its triangular shape and similarity in other morphological characters (Segal, 1966). Last, *C. coroniformis* was placed at the node representing the most recent common ancestor of the clade containing *C. minima* Rydberg and *C. crassisejala* (Torr. & A.Gray) Greene due to the many morphological

similarities noted by Segal (1966). All three fossil outlets were all found in the Ogallala Formation in Kansas, United States, in Ash Hollow deposits. Boellstorff (1976, 1978) dated this formation to the Hemphillian period (10.3–4.9 Ma; see also Ludvigson et al., 2009). Therefore, a maximum of 10.3 Ma and a minimum of 4.9 Ma was used in constraining these nodes.

In a second divergence time analysis, we employed the “fossils+secondary calibration” scheme. In this scheme, in addition to using the three Amsinckiinae fossils as primary calibrations, we also used a secondary calibration on the crown node of Amsinckiinae based on a date estimated for the crown node of the subtribe in a broad analysis of the Boraginaceae (26.9–7.4 Ma; Chacón et al., 2017). All other parameters were as in the analysis employing the “fossils only” scheme.

For nine amphitropical species or infraspecies lacking South American samples in these molecular analyses, we provide or calculate divergence times using one of two approaches. For five of these amphitropical species or infraspecies—*Amsinckia tessellata* A.Gray var. *tessellata*, *Cryptantha maritima* (Greene) Greene var. *maritima*, *Pectocarya pusilla* (A.DC.) A.Gray, *Plagiobothrys fulvus* (Hook. & Arn.) I.M.Johnst. var. *fulvus*, and *Plagiobothrys myosotoides* (Lehm.) Brand—divergence time estimates were available from previous studies with broader sampling inclusive of both North American and South American populations (Hasenstab-Lehman and Simpson, 2012; Williams, 2015). We provide these estimates directly here. For the remaining four amphitropical species or infraspecies lacking South American samples—*Cryptantha albida*, *Greeneocharis circumscissa* (Hook. & Arn.) Rydb. var. *circumscissa*, *Pectocarya linearis* (Ruiz & Pav.) DC. var. *ferocula* I.M.Johnst., *Plagiobothrys collinus* (Phil.) I.M.Johnst. var. *ursinus*—we provide a rough approximation of the timing of the AAD event by calculating one-half of the estimated age of the stem node of the North American exemplar of that taxon. For one additional species, the North American *Cryptantha minima* Rydb., we hypothesized a sister relationship with the morphologically very similar South American species *Cryptantha mendocina* I.M.Johnst., and by the same reasoning, roughly approximate the divergence time between them as one-half of the estimated stem node age of North American *C. minima*.

Biogeographic inference—Biogeographic analysis was performed using the R package BioGeoBEARS (Matzke, 2013). After comparison of the results of divergence time estimation, we used the “fossils+secondary calibration” scheme tree as the input tree and areas were defined as North America, South America, Australia, or China (this last only for one of the outgroups, *Microula tibetica*). Species were determined to be present in these areas by using herbarium records and online distribution databases for South America (CONC, LP, MO, SDSU, SGO) and North America (Kartesz, 2015; Consortium of California Herbaria, 2017; SEINet, 2017).

Our BioGeoBEARS run performed two analyses. First, the program performed biogeographic analyses under a range of biogeographic models. Available models included 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), BAYAREALIKE, and an extension of all models using parameter “J”, which permits the inference of founder-event speciation. Following biogeographic inference under many models, BioGeoBEARS then determined the optimal biogeographic model using the Akaike information criterion (AIC).

RESULTS

Phylogenetic inference—The total alignment lengths, number of parsimony informative characters, and number of uninformative characters for the three data sets used in our concatenated analysis are reported by Simpson et al. (2017b, in this issue; Table 1) and are available at TreeBase for download (<http://purl.org/phylo/treebase/phylo/study/TB2:S21212>). The resulting tree is illustrated in Fig. 2. Almost all nodes had strong support (bootstraps >90%), with the exception of some backbone nodes (Fig. 2). Support at backbone nodes was similarly weak in the study of Simpson et al. (2017a), which the authors attribute to a rapid diversification of major lineages within the subtribe. The same major clades are recovered as in the analyses of Simpson et al. (2017a). For a complete discussion of phylogenetic patterns inferred using this sequence data set, especially as these patterns relate to current classification in the subtribe, refer to Simpson et al. (2017a). In particular, Simpson et al. discuss findings on nonmonophyly in the genera *Cryptantha* and *Plagiobothrys*, highlighting changes that would be necessary in these genera to achieve a classification in which only monophyletic groups are recognized taxonomically. Formal nomenclatural changes in *Plagiobothrys* s.l. will be addressed in a forthcoming study (C. M. Williams and B. G.

TABLE 1. Estimated mean divergence times for all known examples of American amphitropical disjunct events in the Amsinckiinae, Boraginaceae. *Abbreviations:* NA = distributed in North America; SA = distributed in South America. *SA population not sequenced, divergence time calculated as one-half age of NA stem node; **Divergence time listed or calculated from Williams (2015) or (for *Cryptantha maritima*) from Hasenstab-Lehman and Simpson (2012); ***SA taxon not sequenced, but relationship to NA taxon assumed from morphology, divergence time calculated as one-half age of NA stem node.

NA-SA divergence node	Divergence time (Ma)	Dispersal direction
1 <i>Pectocarya pusilla</i> (NA, SA)	0.72**	N→S
2 <i>Pectocarya linearis</i> var. <i>ferocula</i> (NA, SA)	0.59*	N→S
3 <i>Cryptantha subamplexicaulis</i> – <i>Johnstonella echinosepala</i> , <i>Cryptantha maritima</i> CLADE	5.53	N→S
4 <i>Cryptantha maritima</i> var. <i>pilosa</i> (NA, SA)	0.92**	N→S
5 <i>Amsinckia tessellata</i> var. <i>tessellata</i> (NA, SA)	0.31**	N→S
6 <i>Plagiobothrys fulvus</i> var. <i>campestris</i> (NA) – <i>P. fulvus</i> var. <i>fulvus</i> (SA)	0.30**	N→S
7 <i>Plagiobothrys collinus</i> vars. <i>californicus</i> , <i>fulvescens</i> , <i>gracilis</i> , <i>ursinus</i> (NA) – <i>P. collinus</i> var. <i>collinus</i> (SA)	1.48*	N→S
8 <i>Plagiobothrys myosotoides</i> (NA, SA) [<i>P. verrucosus</i>]	0.72**	N→S
9–10: Two possibilities (a or b):		
9a <i>Plagiobothrys austinae</i> , <i>P. glyptocarpus</i> , <i>P. undulatus</i> , <i>P. leptocladus</i> , <i>P. mollis</i> , <i>P. congestus</i> , <i>P. linifolius</i> (NA) CLADE – <i>P. polycaulis</i> (SA), <i>P. gracilis</i> (SA), <i>P. greenei</i> (NA) CLADE	3.03	N→S
10a <i>Plagiobothrys greenei</i> (NA) – <i>P. gracilis</i> (SA)	0.97	S→N
or		
9b <i>Plagiobothrys greenei</i> (NA) – <i>P. gracilis</i> (SA) CLADE – <i>P. polycaulis</i> (SA)	1.37	N→S
10b <i>Plagiobothrys greenei</i> (NA) – <i>P. gracilis</i> (SA)	0.97	N→S
11 <i>Plagiobothrys mollis</i> (NA) – <i>P. congestus</i> , <i>P. linifolius</i> (SA) CLADE	1.45	N→S
12 <i>Johnstonella parviflora</i> (SA) – <i>J. angelica</i> (NA)	3.38	N→S
13 <i>Cryptantha albida</i> , <i>C. mexicana</i> , <i>C. texana</i> (NA) CLADE – <i>C. hispida</i> , <i>Johnstonella diplotricha</i> (SA) CLADE	6.01	N→S
14 <i>Cryptantha albida</i> (NA, SA)	2.45*	N→S
15 <i>Greeneocharis circumscissa</i> var. <i>circumscissa</i> (NA, SA)	0.33*	N→S
16 <i>Cryptantha minima</i> (NA) – <i>C. mendocina</i> (SA)	2.45***	N→S
17 Stem node of SA <i>Eucryptantha</i> – <i>Geocarya</i> CLADE	17.11	N→S
18 Stem node of SA <i>Globulifera</i> CLADE	7.39	N→S

Baldwin [University of California, Berkeley], unpublished manuscript).

The current concatenated analysis is almost identical with regard to placement of these major clades to the plastome (cpDNA) analysis of Simpson et al. (2017a), differing only in the relative placements of *Plagiobothrys* section *Sonnea* and the *Amsinckia*/*Plagiobothrys* sect. *Amsinckiopsis* clade (see below). In the current analysis, *Adelinia grandis* (Douglas ex Lehm.) J.I.Cohen is sister to the two species of *Andersonglossum* and this *Andersonglossum*–*Adelinia* clade is sister to all other examined members of the Amsinckiinae (Fig. 2). The monotypic *Dasynotus daubenmirei* I.M.Johnst. is sister to a *Harpagonella*–*Pectocarya* clade, and these three genera are sister to the remainder of the subtribe (Fig. 2). *Plagiobothrys* sect. *Sonnea* is sister to the remainder of the subtribe. *Eremocarya* and the “*Maritimae* clade” are sister groups and form a clade with *Oreocarya*, this clade sister to the remainder of the subtribe. Two *Plagiobothrys* species of section *Amsinckiopsis* plus the genus *Amsinckia* comprise a clade, which is sister to *Plagiobothrys* s.s., and this larger clade is sister to the rest of the subtribe, which consists of the *Johnstonella*/*Albidae* clade, a *Greeneocharis* clade, and a large *Cryptantha* s.s. clade, the last two sister taxa (Fig. 2).

Divergence time estimations—In the treePL analysis, the date of the crown node of the Amsinckiinae is estimated at 24.97 Ma (Fig. 2). The “fossils only” calibration scheme (not illustrated) resulted in nearly identical divergence dates (to within 3–4 significant figures). As our calibration schemes resulted in nearly identical divergence date estimates, we arbitrarily selected the “fossils+secondary calibration” scheme for all subsequent analyses.

Biogeographic inference—The results of biogeographic inference in BioGeoBEARS are shown in Fig. 2. The DIVALIKE+J analysis, illustrated in Fig. 2, received the highest likelihood score (Appendices S1 and S2, see the Supplemental Data with this article). A total of 18 AAD events were inferred. Dates of divergence range from 17.1–0.33 Ma (mid-Miocene to Pleistocene) using the “fossils+secondary calibration” scheme (see Table 1; Fig. 2). A plot of increasing AAD event divergence times (Fig. 3) shows a rough correlation with the “taxonomic categories” of Simpson et al. (2017b, this issue), with some exceptions. This biogeographic analysis shows North America to South America directionality for 17 AAD events, with one equivocal scenario (Fig. 2; Table 1).

DISCUSSION

American amphitropical disjunction in the Amsinckiinae—Number of AAD events in the Amsinckiinae—The Amsinckiinae contain a greater number of inferred AAD events than any other vascular plant group

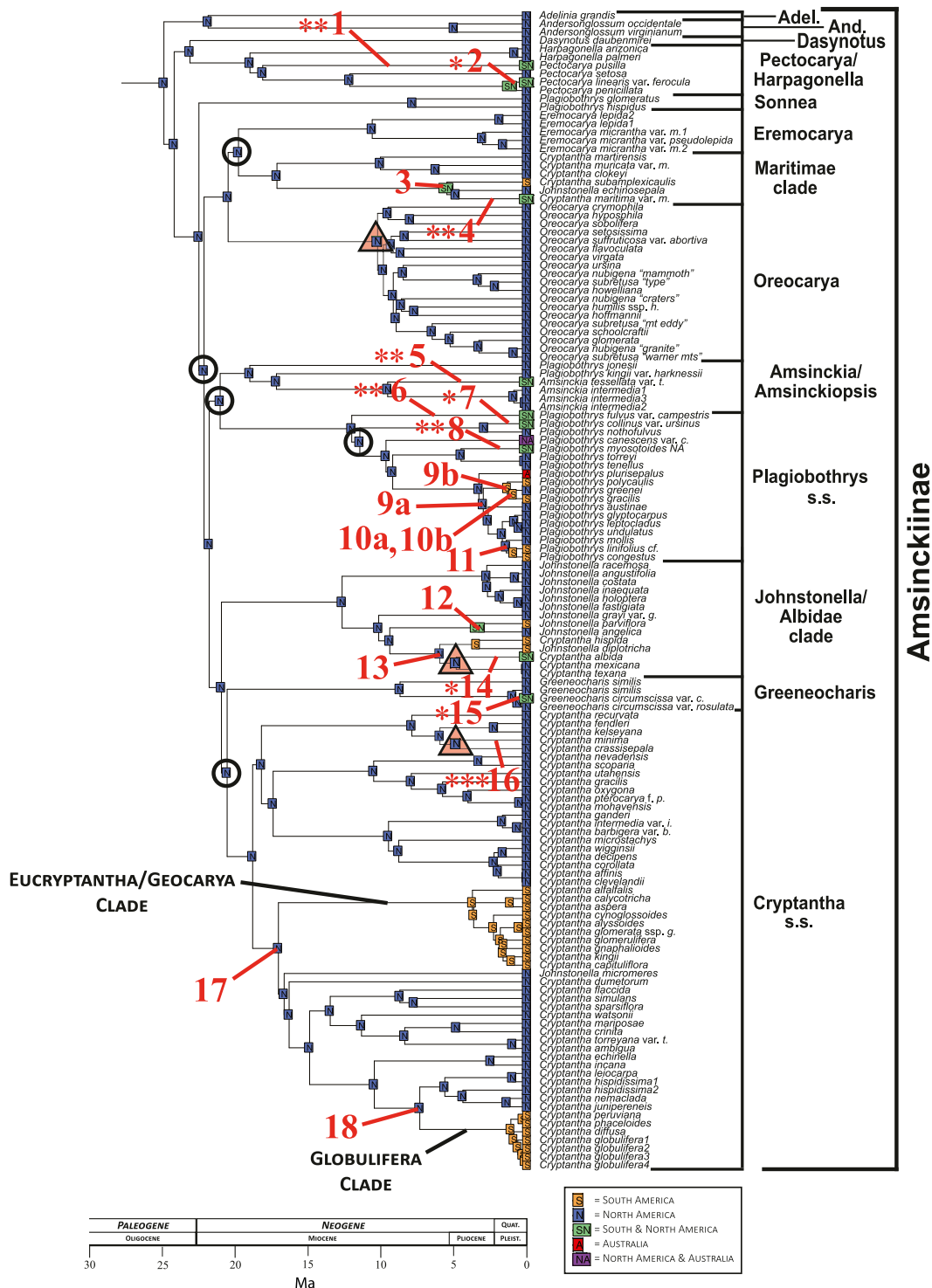


FIGURE 2 Ultrametric phylogram of subtribe Amsinckiinae, inferred using concatenated genome skimming data from Simpson et al. (2017b), under the “fossils+secondary calibration” scheme, with genera and clade designations after that publication. Circles indicate nodes of major clades lacking strong support from bootstrap analysis. Triangles indicate nodes on which primary fossil constraints were placed. Most likely ancestral ranges from BioGeoBEARS analysis shown at nodes. Legend for distributions: blue = North America; orange = South America; green = North America and South America; purple = North America and Australia; red = Australia. Lines point to unique AAD events, numbered from top to bottom. *Single species or infraspecies lacking samples from South America, with divergence date calculated as one-half age of NA stem node (see text). **Single species or infraspecies lacking samples from South America, but with directionality and divergence dates listed or calculated from Guilliams (2015) or (for *Cryptantha maritima*) from Hasenstab-Lehman and Simpson (2012). ****Cryptantha minima*, assumed to be a sister species to *C. mendocina*, the latter not included in the analysis. Geologic time scale in millions of years shown on the x-axis.

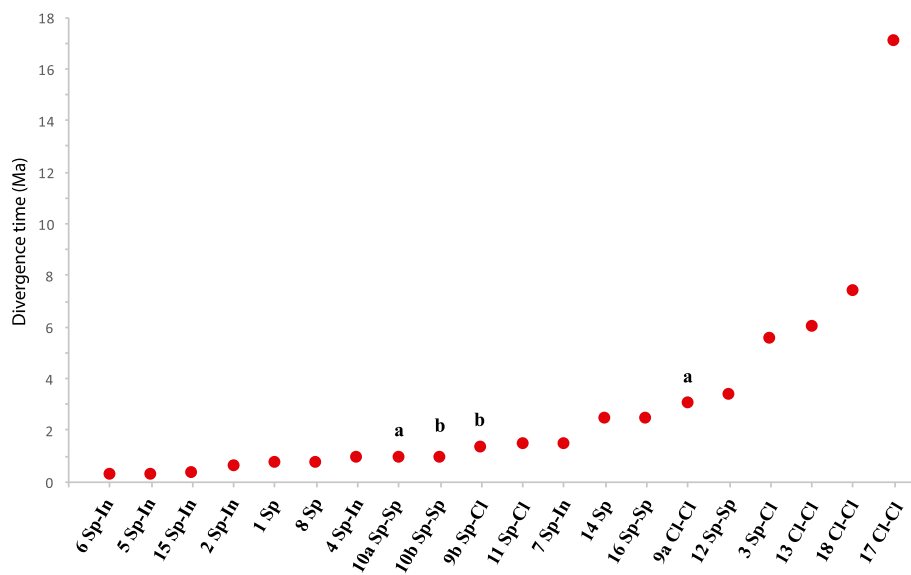


FIGURE 3 Graph of divergence times for each unique American amphitropical disjunct event, numbers corresponding to Table 1. *Abbreviations:* Sp = single species; Sp-In = infraspecies of a single species; Sp-Sp = sister species; Sp-Cl = species + clade; Cl-Cl = clade + clade. Alternative possibilities for “8” and “9” events indicated as “a” or “b” on graph.

of comparable age, a total of 18 times among the sampled lineages, representing 94.7% of the number of AADs in Boraginaceae. Only the hyperdiverse Poaceae have a greater number of inferred AAD events (51; Simpson et al., 2017b, this issue), but the grasses are both four times older than the Amsinckiinae (83 vs. 21.9 Myr old; Stevens, 2001 and references therein; Chacón et al., 2017) and with ca. 39 times the species-level taxonomic diversity (11,337 vs. ~290 species; Stevens, 2001 and references therein; Simpson et al., 2017b, this issue). The Amsinckiinae has a comparable number of AAD events to the families Fabaceae (with 16 events), Asteraceae (with 15 events), and Cyperaceae (with 15 events), as tallied by Simpson et al. (2017b, this issue), and Polemoniaceae (with 14 events; Johnson and Porter, 2017, this issue). Although molecular phylogenetic analyses focusing on this biogeographic pattern are modest in number, it is clear that most AADs in other groups can be explained by many fewer AAD events. In only a few cases, do AADs in a focal group 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 (Poaceae), with 12–13 events required to explain the observed biogeographic patterns under our criteria for AAD (see Peterson et al., 2010).

The analyses presented here describe 10 AAD examples for which we lack samples from both North America and South America. Our assessment that these cases represent examples of AAD was guided by several lines of evidence. First, in these cases, the single sample of each amphitropically distributed taxon was robustly placed among nonamphitropically distributed congeners with strong support. Second, a long history of study of these species or species groups has yielded relatively stable taxonomic concepts on the basis of morphology. While morphological convergence

between more distantly related congeners in North America and South America is a possibility, we do not find it to be the most likely explanation for the patterns we observe in these taxa. Third, in five of these cases, we have evidence from other phylogenetic studies that include samples of these taxa from both North America and South America that corroborate both current taxonomic concepts (e.g., a sister relationship between varieties of *Plagiobothrys fulvus*) and directionality (Hasenstab-Lehman and Simpson, 2012; Williams, 2015). While we intend to broaden our current genome skim sampling to include North American and South American samples for all AAD examples, we do not anticipate that the addition of these data will change the results presented here with respect to number and directionality of AAD events in the Amsinckiinae.

Timing—The two divergence time calibration schemes applied to the Amsinckiinae phylogeny provided nearly identical divergence time estimates and allowed us to have greater confidence in interpreting the timing

of AAD events between North America and South America. Inferred AAD events in the Amsinckiinae were strongly asynchronous and likely occurred throughout recent geological history, with most divergence time estimates ranging from the mid-Miocene (ca. 16–15 Ma) to the mid-Pleistocene (ca. 300 ka). This asynchronous pattern of event timing argues against any hypothesis for AAD that would require parallel and synchronous development of amphitropically disjunct taxa, such as the range fragmentation hypothesis. This point was made by Moore et al. (2006) and gains considerable weight here from multiple, simultaneously inferred AAD events within a common divergence time analysis. Furthermore, strong asynchronicity coupled with the broad range of inferred dates among the 18 AAD Amsinckiinae weakens the hypothesis that long-distance dispersals were effected by terrestrial vertebrates during the GABI, especially under the formulation of this hypothesis by Biro and Whittall (2015) that requires dispersals in the range 2.6–0.125 Ma. Recent evidence for a more protracted mingling between the biotas of North America and South America dating to the early Miocene would permit the range of inferred dispersal times we present here, but the logical problems with this hypothesis mentioned earlier (e.g., terrestrial vertebrate dispersal limitation) persist.

The relationship between “taxonomic category” of Simpson et al. (2017b, this issue) and timing of AAD events (Fig. 3) confirms the general pattern that coninfraspecific or single species examples tend to have more recent divergence times, whereas species-clade or clade-clade examples tend to have older divergence times, although we note some exceptions. This relationship is to be expected, given that an earlier AAD event would allow more time for the evolutionary divergence of lineages into clades. We note that the AAD events that occurred at deeper phylogenetic depths of divergence, e.g., the three clade-clade AAD events, may have been difficult to detect without the broad taxonomic sampling employed in this study. This finding corroborates the idea that clade-level

AAD events may be under-reported in the literature, especially for taxonomically diverse groups that have not been the subjects of phylogenetic study using a large sample size.

The timing of major geological and climatological events may have, in turn, affected the timing of AAD events. Almost all dispersals between North America and South America occurred well after the onset of summer drying following the mid-Miocene climatic optimum ca. 16 Ma, with one AAD event (that resulting in the South American *Eucryptantha/Geocarya* clade) occurring slightly before. Changing global climate following the mid-Miocene climatic optimum had profound effects on the world's biota (Flower and Kennett, 1994) and may have promoted the diversification of plant groups such as the Amsinckiinae that possess certain favorable life history traits (e.g., annual duration, rapid maturation; Raven and Axelrod, 1978). Four AAD events occurred during the second uplift of the Andes range, ca. 10–5 Ma (Houston and Hartley, 2003; Rech et al., 2010), these possibly related to the opening of new niches brought about by this major geologic event. The other AAD events occurred after 5 Ma, with two <1 Ma. Although there are different perspectives in the literature on the timing of the establishment the world's Mediterranean-type climate regions (e.g., Axelrod, 1973; Suc, 1984; Baldwin and Sanderson, 1998; Ackerly, 2009; Lancaster and Kay, 2013), this largest group of AAD events likely occurred near or after the establishment of full summer drought in these regions. Clearly, these geological and climatological events resulted in broad-scale changes in North America and South America, including the development of new ecological niches. We speculate that these global changes triggered the diversification of major lineages of Amsinckiinae, members of which were then dispersed between the newly arising Mediterranean-type climate and desert regions of the western Americas.

Directionality—In this study, 17 of 18 AAD events in the Amsinckiinae had a North America to South America directionality (with one equivocal; see Table 1), which agrees with the most common pattern for AAD vascular plants (Raven, 1963; Simpson et al., 2017b, this issue). The majority (14 of 18) of Amsinckiinae AAD examples were classified as “temperate” disjunctions Simpson et al. (2017b, this issue), while four were classified as “desert” disjunctions. These desert AAD examples also had inferred North America to South America directionality, despite the fact that a South America to North America directionality is most common desert AADs as a whole.

The importance of founder-event speciation in biogeographic studies involving long-distance dispersal—Our study of the AAD pattern included biogeographic 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 cladogenesis. While this is a reasonable finding, and with successful establishment on a new continent it could scarcely be otherwise, the contribution of founder-event speciation was impossible to test before the development of BioGeoBEARS (Matzke, 2013), a program that specifically parameterizes this potential pattern and permits standard model-testing through likelihood ratio tests and under the AIC.

Matzke (2013, 2014) speculated on the potential prevalence of founder-event speciation in explaining biogeographic patterns,

especially on islands or certain island-like continental settings. Our findings 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 can 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 AAD Polemoniaceae) that are thought to facilitate animal dispersal (Raven, 1963; 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; Stebbins, 1957).

Propagule morphology—Nutlet morphology may in part explain why has this group of western American plants has a larger number of AAD events than other groups of comparable size. Although a comprehensive analysis of morphological evolution in the subtribe is beyond the scope of this paper, in a separate comparative phylogenetic analysis of AAD in the Amsinckiinae, Guilliams (2015) found a statistically significant negative relationship between nutlet size and inferred long-distance dispersal and a significant positive relationship between degree of nutlet sculpturing and inferred long-distance dispersal. These findings indicate that taxa with smaller, rougher nutlets were more likely to be present in South America, lending support to the hypothesis that long-distance dispersal by birds is responsible for many of the AADs in the subtribe. We are broadening this comparative phylogenetic study to investigate whether this pattern is generalizable when all AAD events in the subtribe are included.

Insights into potential explanations for American amphitropical disjunction—The data we present here along with a recent tabulation of estimated AAD divergence times (Simpson et al., 2017b, this issue) show that AAD examples throughout the angiosperms have become established via multiple asynchronous “events” throughout the last several million years, with most estimated examples occurring within the last 5 Myr. This temporal pattern argues against any vicariance-based hypothesis (e.g., the range fragmentation hypothesis). We suggest that asynchronous timing of dispersal supports a low-probability, continual process as the primary explanation for this biogeographic pattern.

Examples of AAD are weighted toward lineages capable of self-pollination (Raven, 1963; Simpson et al., 2017b, this issue), highlighting the potential validity of Baker's Rule even in certain continental settings (Baker, 1955). Baker's Rule posits that plants capable of self-pollination will be more likely than obligately outcrossing plants to successfully establish new populations on islands following a long-distance dispersal (but see Carlquist, 1966 for an alternative viewpoint). The observation of island floras with a preponderance of plant lineages capable of self-pollination supports Baker's hypothesis (Baker, 1953, 1955; Stebbins, 1957). The same reasoning holds for regions of similar climate that are amphitropically separated, such as the Mediterranean-type climate regions of North America and South America. Assuming a north to south directionality among AAD examples, Raven (1963) noted that AAD plant examples in the recipient flora of South America are overwhelmingly capable of self-pollination. Published studies (Ray and Chisaki, 1957; Moldenke, 1976) and unpublished observations of Amsinckiinae taxa (C. M. Guilliams, unpublished data) suggest

that many or perhaps most Amsinckiinae taxa are also capable of self-pollination. The disproportionate number of AAD plants capable of self-pollination in recipient “island-like” continental settings such as the Mediterranean-type climate region of Chile suggests a strong role for long-distance dispersal as a mechanism for AAD rather than the fragmentation of a once continuous range.

The majority of AAD events in Amsinckiinae were inferred to have occurred in the last 5 Myr. This time window encompasses a critical period of earth history on both continents, including the development of the full Mediterranean-type climate regions of both North America and South America, major landform evolution (e.g., uplift of the Andes), and possibly the closure of the Isthmus of Panama. These events are hypothesized to have triggered the diversification on both continents and greater biotic interchange between them. Although we doubt that AAD plant lineages were dispersed overland by terrestrial vertebrates, it is nevertheless the case that boreal, desert, and Mediterranean-type climate adapted lineages have been repeatedly conveyed between ecologically similar regions in North America and South America.

We join with authors of previous studies in suggesting 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 the time scales necessary to explain the observed phylogenetic and biogeographic patterns in Amsinckiinae and most other AAD plant groups.

Future directions—The Amsinckiinae serves as a model group for the study of the AAD biogeographic pattern. Given the large number of examples of AAD in the subtribe, continued studies with additional sequence data (particularly nuclear genes) and an increased sample size would allow further refinement of the results obtained here. More nuanced biogeographic analyses (as done by Mabry and Simpson, in press; see Matzke, 2013), incorporating detailed locality information and defined ecoregions (as done by Davis and Holmgren, 2001; Josse et al., 2003) may give insight into common ecological processes in AAD source and sink regions and/or common ecological requirements of AAD plants.

With the large number of AAD examples in the subtribe, study of morphological evolution in a comparative phylogenetic framework may allow formal hypothesis testing to elucidate the relationship between features such as nutlet morphology (including heteromorphism, number per fruit, and sculpturing), polyploidy, and reproductive biology (including compatibility mechanisms and cleistogamy) on the one hand and the likelihood of dispersal between the continents on the other (see Simpson et al., 2017a).

It would be highly desirable to have direct evidence of long-distance dispersal of angiosperm propagules 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 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, it may be best to be content with simpler empirical studies, historical inference, and awe for the improbable nature of life on the planet.

ACKNOWLEDGEMENTS

The authors thank two anonymous reviewers for their thoughtful reviews of this manuscript, which greatly improved its quality. The authors thank the following individuals for assistance in the field: Gina Arancio, Victor Ardilles, Ron Kelley, Roberto Kiesling, Melica Muñoz, Michael Park, Lee Ripma, Gloria Rojas, Rosita Scherson, and Rob Schlising. The authors thank the staff of the herbaria that provided material for this study: University of Arizona (ARIZ), Universidad de Concepción (CONC), Duke University (DUKE), Gray Herbarium (GH), Instituto Argentino de Investigaciones de las Zonas Áridas (MERL), Missouri Botanical Garden (MO), National Herbarium of New South Wales, Australia (NSW), Rancho Santa Ana Botanical Garden (RSA), Santa Barbara Botanic Garden (SBBG), San Diego Natural History Museum (SD), San Diego State University (SDSU), Museo Nacional de Historia Natural (SGO), Instituto de Botánica Darwinion, Argentina (SI), University of California and Jepson Herbaria (UC, JEPS), and University of Riverside (UCR), and Utah State University (UTC). They are grateful to the following sources of funding for this project: American Society of Plant Taxonomists, California Native Plant Society, Joshua Tree National Park, National Geographic Society grant (9533-14 to Michael Simpson), and San Diego State University Travel Grants. This material is based in part upon work supported by the National Science Foundation under grant no. DEB-1210833 (B. G. Baldwin and C.M.G.). Finally, they thank Lee Simpson for his assistance in taking the Amsinckiinae nutlet images in Fig. 1.

DATA ACCESSIBILITY

The sequence data used in this paper are publicly available on the NCBI Short Read Archive. See Simpson et al. (2017a) for the NCBI Short Read Archive accession numbers for each sample used in this study.

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Appendix S1. BioGeoBEARS Results Table.

	LnL	numparams	d	e	j
DEC	-98.97399	2	0.00474	0.00084	0
DEC+J	-91.09945	3	0.00228	1e-12	0.01189
DIVALIKE	-96.91804	2	0.00528	1e-12	0
DIVALIKE+J	-90.86931	3	0.00300	1e-12	0.00989
BAYAREALIKE	-139.21230	2	0.00510	0.01381	0
BAYAREALIKE+J	-95.07830	3	0.00170	1e-07	0.01557

Appendix S2. BioGeoBEARS Test Table.

	alt	null	LnLalt	LnLnull	DFalt	DFnull	DF	Dstatistic	pval	test
1	DEC+J	DEC	-91.1	-98.97	3	2	1	15.75	7.2e-05	chi-squared
2	DIVALIKE+J	DIVALIKE	-90.87	-96.92	3	2	1	12.1	0.0005	chi-squared
3	BAYAREALIKE+JBAYAREALIKE	BAYAREALIKE	-95.08	-139.2	3	2	1	88.27	5.7e-21	chi-squared

	tail	AIC1	AIC2	AICwt1	AICwt2	AICweight_ratio_model1	AICweight_ratio_model2
1	one-tailed	188.2	201.9	1.00	0.0010	967.3	0.0010
2	one-tailed	187.7	197.8	0.99	0.0064	155.8	0.0064
3	one-tailed	196.2	282.4	1	1.8e-19	5.41e+18	1.8e-19