**INVITED PAPER**

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

# Parallel Pleistocene amphitropical disjunctions of a parasitic plant and its host[1]

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**PREMISE OF THE STUDY:** *Aphyllon* is a clade of holoparasites that includes closely related North American and South American species parasitic on *Grindelia*. Both *Aphyllon* (Orobanchaceae) and *Grindelia* (Asteraceae) have amphitropical disjunctions between North America and South America; however, the timing of these patterns and the processes to explain them are unknown.

**METHODS:** Chronograms for the Orobanchaceae and *Grindelia* and their relatives were constructed using fossil and secondary calibration points, one of which was based on the inferred timing of horizontal gene transfer from a papilionoid legume into the common ancestor of *Orobanche* and *Phelipanche*. Elevated rates of molecular evolution in the Orobanchaceae have hindered efforts to determine reliable divergence time estimates in the absence of a fossil record. However, using a horizontal gene transfer event as a secondary calibration overcomes this limitation. These chronograms were used to reconstruct the biogeography of *Aphyllon*, *Grindelia*, and relatives using a DEC+J model implemented in RevBayes.

**KEY RESULTS:** *Aphyllon* had two amphitropical dispersals from North America to South America, while *Grindelia* had a single dispersal. The dispersal of the *Aphyllon* lineage that is parasitic on *Grindelia* (0.40 Ma) took place somewhat after *Grindelia* began to diversify in South America (0.93 Ma). Using a secondary calibration based on horizontal gene transfer, we infer more recent divergence dates of holoparasitic Orobancheae than previous studies.

**CONCLUSIONS:** Parallel host–parasite amphitropical disjunctions in *Grindelia* and *Aphyllon* illustrate one means by which ecological specialization may result in nonindependent patterns of diversity in distantly related lineages. Although *Grindelia* and *Aphyllon* both dispersed to South America recently, *Grindelia* appears to have diversified more extensively following colonization. More broadly, recent Pleistocene glaciations probably have also contributed to patterns of diversity and biogeography of temperate northern hemisphere Orobancheae. We also demonstrate the utility of using horizontal gene transfer events from well-dated clades to calibrate parasite phylogenies in the absence of a fossil record.

**KEY WORDS**  amphitropical disjunction; *Aphyllon*; biogeography; chronogram; divergence time; *Grindelia*; horizontal gene transfer; host–parasite ecology; long-distance dispersal; Orobanchaceae

Interactions between biogeography and ecology, that is, the interrelationship between species distributions and interspecific interactions, may strongly influence patterns of biodiversity (Sexton et al., 2009; Wiens, 2011). Parasites, like other organisms that engage in obligate antagonistic or symbiotic interactions, require the presence of other species to complete their lifecycles. In such cases, strong links between host and parasite biogeography, ecology, and perhaps also diversification are expected (Vanhove et al., 2016).

The Orobanchaceae Vent. comprise a diverse lineage (~2000 species) found on every continent except Antarctica, with the greatest diversity in Eurasia (Wolfe et al., 2005). The large majority of species are root parasites, either relying entirely on other plants for their energetic demands (holoparasites) or, more commonly, able to supplement host-derived resources to varying degrees by photosynthesis (hemiparasites). In the western hemisphere, multiple dispersals from North America to South America of hemiparasitic lineages have resulted in several notable radiations, such as in the genera *Bartsia* L. (Uribe-Convers and Tank, 2015) and *Castilleja* Mutis ex. L.f. (Tank and Olmstead, 2009). A recent phylogenetic study of the holoparasitic genus *Aphyllon* Mitch., which contains at least two dozen western hemisphere species formerly treated under *Orobanche* L. s.l.,

found that all South American *Aphyllon* fall within the predominantly temperate North American *A. ludovicianum* complex, but do not form a single monophyletic group (Schneider et al., 2016). Rather, these taxa represent two separate clades, nested among temperate North American ancestors. In one of these instances, the South American clade composed of *A. chilense* and *A. tacnaense* is most closely related to two species native to the central United States, *A. ludovicianum* and *A. multiflorum*. Together with the remaining two North American taxa of the *A. ludocivianum* complex and the third sampled South American species, *A. tarapacanum*, all species of this larger clade parasitize perennial Asteraceae.

Within the *Aphyllon ludovicianum* complex, even finer scale phylogenetic affinities exist between the hosts of North American and South American parasites. For example, species in the New World genus *Baccharis* L. have been reported as hosts for *A. ludovicianum* (e.g., *B. salicina* Torr. & A.Gray; *Collins 1323*, WIS), and for several collections of South American *Aphyllon* (*B. polyantha* Kunth; *Vargas 3161*, MO; *Vargas 12880*, US), as well as occasional hosts of several other *Aphyllon* species. Similarly, *A. tarapacanum* has been reported on South American *Franseria* Cav. (e.g., *Hutchison & Wright 7189*; UC, US). Nuclear ribosomal and plastid DNA support different phylogenetic placements of *A. tarapacanum* (Schneider et al., 2016), but in either case, close North American relatives parasitize genera that are also in the Ambrosiinae (Asteraceae). Specifically, *A. riparium* (supported by nrDNA evidence as a close relative) primarily parasitizes *Ambrosia artemisiifolia* L. and *Xanthium strumarium* L. (Collins et al., 2009), and *A. cooperi* (cpDNA) commonly parasitizes *Ambrosia dumosa*.

Most striking, however, is the *Aphyllon*–*Grindelia* Willd. host–parasite relationship. The primary hosts of *A. chilensis* are *G. chiloensis* (Cornel.) Cabrera and *G. ventanensis* Adr.Bartoli & Tortosa (Burkart, 1942), and its presumed closest North American relative, *A. ludovicianum*, commonly parasitizes *G. squarrosa* (Pursh) Dunal. *Grindelia* is also a host of the more distantly related *A. californicum* subsp. *californicum*.

The genus *Grindelia* (Asteraceae, Astereae), like *Aphyllon*, is restricted to the New World and has an amphitropical distribution. Fine-scale taxonomic limits are somewhat controversial, with 25 to 50 recognized species native to North America and 26 recognized species native to South America (Bartoli and Tortosa, 1999; Moore et al., 2012). This distribution appears to be the result of a single amphitropical dispersal event in *Grindelia*, with subsequent diversification in both North America and South America (Moore et al., 2012), although neither the timing of the dispersal and subsequent diversification nor the direction of dispersal were resolved in that study.

This current study was motivated by a desire to test this apparent case of parallel host–parasite American amphitropical disjunction and infer the relative timing of colonization. American amphitropical disjunctions (AADs) are one of the most important and well-studied biogeographic patterns in plants (Raven, 1963, 1972; Wen and Ickert-Bond, 2009; Simpson et al., 2017). Some disjunctions, such as those involving Eurasia and North America, likely were a result of range expansion across Beringia, the North Atlantic land bridge, or other large areas where the habitat is now unsuitable (Wen and Ickert-Bond, 2009). In contrast, AADs seem to be largely the result of long-distance dispersal because a more or less continuous band of suitable habitat does not seem to have been available for most groups involved when the dispersal event must have occurred (e.g., Moore et al., 2006), although there are some exceptions (e.g., Seavey and Raven, 1977; Ellison et al., 2006). Specifically, in this study we (1) infer divergence times of major clades within *Grindelia* as well as *Aphyllon* and its

holoparasitic close relatives in the Orobanchaceae, employing a novel approach of using a host-to-parasite horizontal gene transfer event as a secondary calibration, (2) test the hypothesis of two independent colonizations of South America by North American *Aphyllon* and one colonization by *Grindelia* by modeling historical biogeography of these clades, and (3) compare the timing of these colonization events.

## MATERIALS AND METHODS

***Orobanchaceae phylogeny***—SUMAC version 1 (Freyman, 2015) was used to download all Orobanchaceae and Paulowniaceae sequences from GenBank release 204 and to assemble a 4-gene supermatrix (external transcribed spacer [ETS] and internal transcribed spacer region [ITS] of the nuclear ribosomal DNA, and low-copy nuclear genes phytochrome A and phytochrome B). Sampling within major clades outside the clade of holoparsites that contains *Aphyllon* (tribe Orobancheae sensu McNeal et al., 2013) was arbitrarily reduced to decrease computational time. Additional ETS and ITS sequences for both subspecies of *Aphyllon epigalium*, *A. purpureum*, and the undescribed clade of *A. fasciculatum* that parasitizes hosts in genera other than *Artemisia* L. were added manually for a total alignment of 160 minimum-ranked terminals. Final sequence alignments were generated using the program MAFFT v7.123b (Katoh and Standley, 2013) and trimmed to form an aligned matrix of 5569 bp. GenBank accession numbers for sequences used in constructing the Orobanchaceae supermatrix are presented in Appendix S1 (see Supplemental Data with this article) and as a .csv file in the Dryad database (data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.6hm4p).

A chronogram of Orobanchaceae (including Rehmanniaceae, Angiosperm Phylogeny Group, 2016) + Paulowniaceae was estimated using an uncorrelated lognormal relaxed clock model and a GTR+Γ substitution model for each gene partition, implemented in the program RevBayes v. 1.0.4 (Höhna et al., 2014). Rate variation across sites was modeled under a gamma distribution approximated by four discrete rate categories. The constant rate birth–death sampling tree prior (Stadler, 2009) was used with the probability of sampling species at the present ($\rho$) set to 0.08. The value $\rho$ was calculated by dividing the number of extant species sampled in the supermatrix (160) by the estimated total number of species in Orobanchaceae (~2000), though sampling density was substantially higher within the Orobancheae compared to other lineages. Branch rates were drawn from a lognormal prior with the mean and standard deviation given exponentially distributed hyperpriors with rate parameters of 0.25. Tree topology was fixed to the result of a maximum likelihood analysis performed on the same partitioned 4-gene data set using the program RAxML v. 8.2.9 (Stamatakis, 2014), which was generally consistent with topologies inferred by more thorough studies (McNeal et al., 2013; Schneider et al., 2016).

The Paulowniaceae + Orobanchaceae root age was calibrated based on the stem age of Paulowniaceae reported by Magallón et al. (2015) as part of a 5-gene, 792-taxon study of angiosperms with 137 node calibrations. For incorporating the uncertainty of the root age in our analysis, the root node was given a uniform calibration density with a range of 57.02–28.08 Ma, representing the 95% highest posterior density interval (HPD) reported by Magallón et al. (2015), who used an uncorrelated lognormal Bayesian analysis run in BEAST.

Divergence times within Orobanchaceae were estimated using two independent stochastic nodes (i.e., variables specified as a distribution;

Fig. 1). The calibration used to date the crown node of *Orobanche* s.l. (including the genera *Aphyllon, Diphelypaea* D.H.Nicolson, and *Phelipanche* Pomel) was based on a dated tree of the *albumin1* gene, which originated in parasitic Orobanchaceae through horizontal gene transfer from a papilionoid legume host approximately 16 Ma, before the divergence of *Orobanche* and *Phelipanche* 11 Ma (95% HPD: 16–6 Ma; Zhang et al., 2013). How long before this divergence event is unclear, because the authors who discovered the xenologous gene did not test for its presence or absence in more distantly related Orobancheae lineages. Therefore, we calibrated the crown node of *Orobanche* + *Phelipanche* (+ *Aphyllon*) based on their posterior distribution of dates for this node, which is the earliest to appear in both our phylogeny and their gene tree. Specifically, we modeled this node using a normal prior with a mean of 11 Ma and a standard deviation of 2.55. Unlike a fossil or geological calibration that may represent a hard bound on the age of the lineage it is calibrating, the inferred timing of the HGT is more variable, based indirectly on the host fossils used to time-calibrate the gene tree and the branch lengths of the tree itself. Moreover, a fossil may be, but more likely is not, a direct ancestor, while the HGT event definitely occurred in a direct ancestor somewhere along an internal branch of the tree.

The other calibration was based on *Cistanche* Hoffmanns. ex Link pollen from a Neogene formation in Sebkha el Mellah, Algeria (Beucher, 1971). Muller (1981) reported the fossil as Pliocene, so we conservatively fixed the fossil age at 2.58 Ma, which represents the end of that epoch. Because the Miocene–Pliocene boundary is not well defined in this formation (see Beucher, 1971, pp. 295, 398–399), the fossil could also be substantially older. We treated the crown age of *Cistanche* as a stochastic node with the waiting time to fossilization modeled by a lognormal prior with a mean of 2 and a standard deviation of 0.5. The waiting time to fossilization represents the time elapsed between the node being calibrated and the fossilization of a particular specimen in that lineage, as the probability of sampling the actual common ancestor is vanishingly small. In summary, the sampled prior ages had a median of 10.0 Ma, mode of 8.3 Ma, and 95% were between 5.35 Ma and 22 Ma, reflecting the large uncertainty associated with this fossil date.

Four independent replicates of a Markov chain Monte Carlo (MCMC) were run for 5000 iterations as a pre-burnin to tune the proposals, then sampled every 10 iterations for 82,500 iterations, with the first 750 samples (9%) discarded as burnin. Each iteration consisted of 467 moves randomly scheduled from 369 different possible moves. Results were considered reliable when effective sampling size for the following parameters exceeded 200, as measured by the program Tracer v. 1.6.1 (Rambaut et al., 2014): posterior, prior, each GTR rate parameter, speciation and extinction rates, root time, and clade age of *Orobanche* s.l.

***Grindelia phylogeny***—The ITS and ETS alignment from Moore et al. (2012) was used for the analysis, with GenBank sequences from four additional outgroup taxa added and aligned by eye to allow for secondary time calibration: *Eurybia divaricata* (L.) G.L.Nesom (ITS: AY772423.1, ETS: AY772437), *E. radulina* (A.Gray) G.L.Nesom (ITS: EU200208.1, ETS: EU196489.1), *Symphyotrichum adnatum* (Nutt.) G.L.Nesom (ITS: JQ360444.1, ETS: JQ360214.1), and *S. drummondii* (Lindl.) G.L.Nesom var. *texanum* (E.S.Burgess) G.L.Nesom (ITS: U97603.1, ETS: AF516069.1). A starting tree was constructed with the GTR+Γ model of sequence evolution using RAxML v. 7.3.0, then made ultrametric using the chronos command in the APE package (Paradis et al., 2004) in R (R Core Team, 2016).

We gave the root age of the tree [the divergence of *Eurybia* (Cass.) Cass. from all other species] a uniform probability from 9.0–3.0 Ma (Fig. 2). This range is the estimated divergence time between *Eurybia* and *Symphyotrichum* Nees plus *Grindelia* in the fossil-calibrated phylogeny of the Asteraceae reconstructed by Huang et al. (2016). No fossil or geological calibration points exist for internal nodes of the *Grindelia* phylogeny.

RevBayes was used to produce a time-calibrated phylogeny, as described above for the Orobanchaceae. It was run for 10,000 iterations and sampled every 2 iterations, with a pre-burnin of 5000 iterations and $\rho$ set to 0.20. Each iteration consisted of 285 moves randomly scheduled from 297 different moves.

***Inferring historical biogeography***—Ancestral geographic ranges were inferred using a DEC+J model implemented in RevBayes, which allows for sympatric speciation, allopatric speciation, and jump dispersal events. These reconstructions were performed across the time-calibrated maximum clade credibility (MCC) trees inferred above. The Orobanchaceae tree was pruned to include only members of the clade Orobancheae (*Aphyllon* and holoparasitic relatives) to prevent bias due to undersampling.

For the Orobancheae analysis, we used six nonoverlapping biogeographic regions: (A) The California Floristic Province (CFP), (B) western North America (except the CFP) to the Mississippi River basin and Hudson Bay, including Mexico and Central America, (C) eastern North America, (D) Eurasia and northern Africa, (E) Australia, and (F) South America (Fig. 3). Four independent MCMC replicates were run for 750 iterations as a pre-burnin to tune the proposals, then sampled every 10 iterations for 8000 iterations, with the first 160 samples (20%) discarded as burnin. Each iteration consisted of three moves randomly scheduled from 11 different moves. Results were considered reliable when the ESS for each parameter was >200 in each replicate run. When the four runs were combined, the ESS of all parameters was >1600.

For the *Grindelia* analysis, we used the same five regions delimited by Moore et al. (2012): (A) California and Oregon west of the Cascade–Sierra Nevada axis, (B) the remainder of North America west of the crest of the Rocky Mountains, (C) North America east of the crest of the Rocky Mountains, (D) South America west of the crest of the Andes, and (E) South America east of the crest of the Andes (Fig. 4). Four independent MCMC replicates were run for 500 iterations as a pre-burnin to tune the proposals, then sampled every 10 iterations for 2000 iterations. Each iteration consisted of five moves randomly scheduled from 12 different moves. The first 500 samples of each run were discarded as burnin.

Sequence alignments, RevBayes scripts, sampled trees from the MCMC analyses, summary tree files used in Figs. 1–4, and a .csv version of Appendix S1 is available in the Dryad database (data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.6hm4p).

## RESULTS

***Biogeography and divergence times in Orobanchaceae***—Diversification of extant Orobanchaceae was relatively recent, with the family originating approximately 30 Ma, the origin of parasitism evolving shortly thereafter in the late Oligocene, and all but the earliest-diverging lineages arising in the Neogene (Table 1, Fig. 1). Among the three clades of holoparasites in Orobanchaceae, tribe
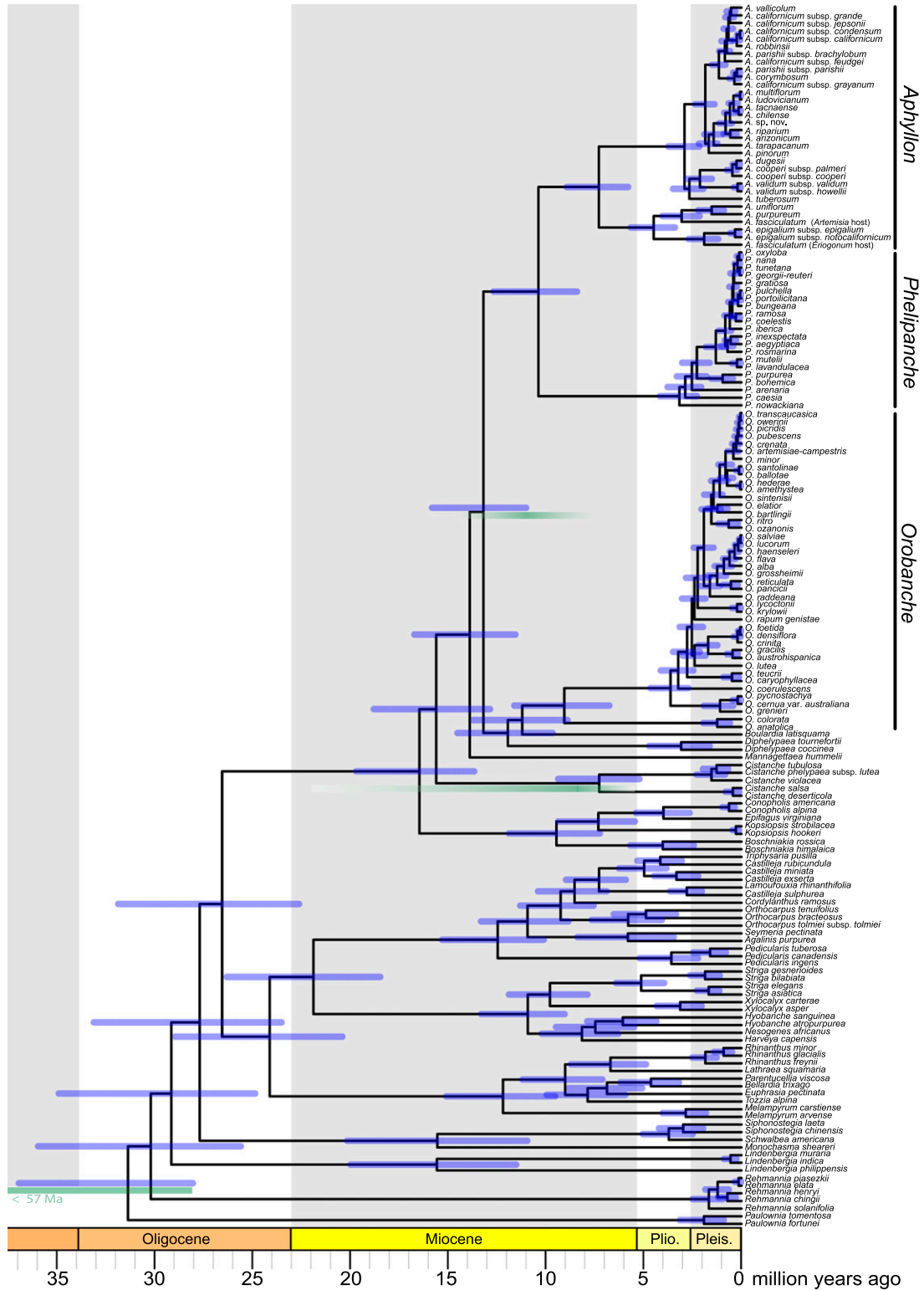
**FIGURE 1** Bayesian inference maximum a priori chronogram (maximum likelihood topology) of Orobanchaceae. Blue bars represent 95% HPD confidence intervals of node ages. Green bars represent priors on the root age (uniform), crown *Cistanche* (lognormal), and the most recent common ancestor of *Orobanche* and *Phelipanche* (normal).

Orobancheae (sensu McNeal et al., 2013) is the oldest and most species-rich. We estimate a mean stem age of 26.6 Ma (95% HPD interval of 22.6–31.9 Ma) and a mean crown age of 16.5 Ma (13.7–19.7 Ma) and infer that it arose from a widespread ancestor in the northern hemisphere (Eurasia/Northern Africa + Western North America + Eastern North America, PP = 0.43, Table 1).

G. squarrosa 3
G. squarrosa 1
G. havardii 2
G. aphanactis 3
G. subalpina
G. squarrosa 6
G. squarrosa 2
G. squarrosa 4
G. oxylepis
G. aphanactis 1
G. nana 3
G. revoluta 1
G. squarrosa 8
G. squarrosa 5
G. revoluta 2
G. aphanactis 2
G. squarrosa 9
G. nana 4
G. squarrosa 7
G. lanceolata 4
G. arizonica
G. pusilla
G. grandiflora
G. lanceolata 3
G. lanceolata 2
G. lanceolata 1
G. havardii 1
G. scabrascabra
G. ciliata 2
G. ciliata 1
G. adenodonta 2
G. adenodonta 1
G. oolepis 2
G. oolepis 1
G. greenmanii
G. robinsonii
G. ctenella
G. inuloides
G. nana 2
G. nana 1
G. sstricta 2
G. splatyphylla 2
G. splatyphylla 1
G. hirsutula
G. procera
G. hallii
G. camporum 2
G. camporum 1
G. camporum 3
G. sstricta 2
G. integrifolia
G. howellii
G. nana5
G. sstricta 3
G. fraxinipratensis 3
G. fraxinipratensis 2
G. fraxinipratensis 1
G. fastigiata 1
G. decumbens 2
G. decumbens 1
G. fastigiata 2
G. fastigiata 3
G. laciniata
G. nuda 2
G. nuda 1

**North American Grindelia**

G. chiloensis 3
G. anethifolia 2
G. chiloensis4
G. coronensis 2
G. chiloensis 2
G. mendocina
G. chiloensis 1
G. anethifolia 1
G. coronensis 1
G. patagonica
G. tarapacana
G. glutinosa
G. covasii 2
G. pygmaea
G. pruneloides
G. boliviana 2
G. boliviana 1
G. buphthalmoides 2
G. buphthalmoides 1
G. covasii 1
G. pulchella 1
G. globularifolia
G. orientalis
G. pulchella 2
G. brachystephana 3
G. brachystephana 2
G. scorzonerifolia
G. brachystephana 1

**South American Grindelia**

Isocoma menziesii 2
Isocoma menziesii 1
Isocoma acradenia
Rayjacksonia phyllocephala 2
Rayjacks phyllocephala 1
Xanthocephalum gymnospermoides
H. glutinosus 2
H. glutinosus 1
H. glutinosus 3
H. paucidentatus
H. anthylloides
H. velutinus
H. uncinatus
H. macrocephalus
H. setigerus
H. undulatus
H. marginalis
H. multifolius 2
H. multifolius 1
H. foliosus

**Haplopappus**

Lessingia glandulifera
Lessingia arachnoidea
Corethrogyne filaginifolia 2
Corethrogyne filaginifolia 1
Hazardia squarrosa 2
Hazardia squarrosa 1
Hazardia detonsa
Benitoa occidentalis
Hazardia whitneyi
Pyrrocoma racemosa
Pyrrocoma apargioides
Pyrrocoma lanceolata
Xanthisma spinulosum 2
Xanthisma spinulosum 1
Machaeranthera tagetina
Xylorhiza tortifolia
Dieteria canescens
Symphyotrichum drummondii
Symphyotrichum adnatum
Eurybia radulina
Eurybia divaricata

< 9 Ma

| Miocene | Pliocene | Pleistocene |

7  6  5  4  3  2  1  0 million years ago

**FIGURE 2** Bayesian inference maximum a priori chronogram (maximum likelihood topology) of *Grindelia* and relatives (Asteraceae), reconstructed using RevBayes. Blue bars represent 95% HPD confidence intervals of node ages. Green bar represents the uniform prior on the root age.

Most early diversification of Orobancheae took place in Eurasia/Northern Africa (Fig. 3) with origination (stem ages) of the genera *Boulardia* F.W.Schultz, *Diphelypaea*, *Orobanche* s.s., *Phelipanche*, *Cistanche*, and *Mannagettaea* Harry Sm. all between 9.1 and 15.6 Ma, although crown ages for *Phelipanche* and the clade of *Orobanche* that excludes *O. anatolica* and *O. colorata* are much more recent (*Phelipanche*: 3.2 Ma [0.3–4.2 Ma]; *Orobanche*: 3.6 Ma [2.7–4.7 Ma]). The genus *Aphyllon* represents a single colonization from Eurasia/northern Africa 7.3 Ma (5.8–8.9 Ma) into the CFP (PP = 0.40) or elsewhere in western North America (PP = 0.41), with only recent, repeated colonizations of eastern North America. The other species of Orobancheae in eastern North America, in the genera *Conopholis* Wallr. and *Epifagus* Nutt., also arrived there from range expansion of a common western North American ancestor, albeit much earlier, in the Pliocene or late Miocene (Fig. 3).

Other *Aphyllon* lineages moved into the CFP from western North America and diversified in situ, such as the clade that includes *A. vallicolum*, *A. robbinsii*, *A. parishii* subsp. *parishii*, and all subspecies of *A. californicum* except subsp. *grayanum* (PP = 0.99; crown age 0.75 Ma [0.58–1.13 Ma]). Another example is the most recent common ancestor of *A. tuberosum* and *A. validum* (PP = 0.88; crown age 2.7 Ma [2.0–3.5 Ma]), which diversified in the CFP as well as dispersed back out to form the lineage that became *A. cooperi* and *A. dugesii* in the deserts of southwestern North America (PP = 0.97, Fig. 3).

We infer three amphitropical disjunctions caused by recent long-distance dispersal events: two temperate American disjunctions in *Aphyllon*, and a third, of the rare southern Australian native *Orobanche cernua* var. *australiana* from a northern hemisphere Eurasian (or northern African) ancestor within the last 0.46 million years (mean jump-dispersal date of 0.19 Ma [0.002–0.46 Ma], biogeography PP = 0.77). The two American amphitropical disjunctions resulted in two separate South American lineages from western North American ancestors. The first dispersal occurred ca. 1.4 Ma (1.0–1.9 Ma, PP = 0.74) and resulted in modern-day *A. tarapacanum*. The second occurred ca. 0.40 Ma (0.19–0.65, PP = 0.71) in the lineage that subsequently diversified into *A. tacnaense* and *A. chilense*.

***Biogeography and divergence times of Grindelia***—*Grindelia* appears to have arisen very recently, with a crown age, which also represents the divergence of North American and South American *Grindelia*, of approximately 1.40 Ma (0.71–2.43 Ma; Table 1, Fig. 2). *Grindelia* was reconstructed, with high probability (PP = 0.92; Fig. 4), as having arisen in North America, east of the Continental Divide. The genus reached eastern South America sometime before the first branching event in the South American clade ca. 0.93 Ma (0.41–1.36 Ma; biogeography PP = 1.0). The diversification in North America began at approximately the same time, ca. 1.18 Ma (0.57–2.04 Ma) in eastern North America, with a subsequent dispersal to western North America ca. 0.63 Ma (0.26–1.17 Ma) and from there over the Sierra Nevada/Cascades to the Pacific Coast ca. 0.16 Ma (0.04–0.25 Ma).

## DISCUSSION

***Parallel American amphitropical disjunctions***—The difficulty of a successful long-distance dispersal event is particularly acute for holoparasites or other extreme specialists, whose propagules must traverse a long distance and then also make contact with a host plant. The probability of successful colonization is therefore the product of the probabilities of both dispersal and establishment of the parasite and the probabilities of prior dispersal and establishment of suitable hosts. Yet in the eons of evolutionary time, unlikely events are commonplace.
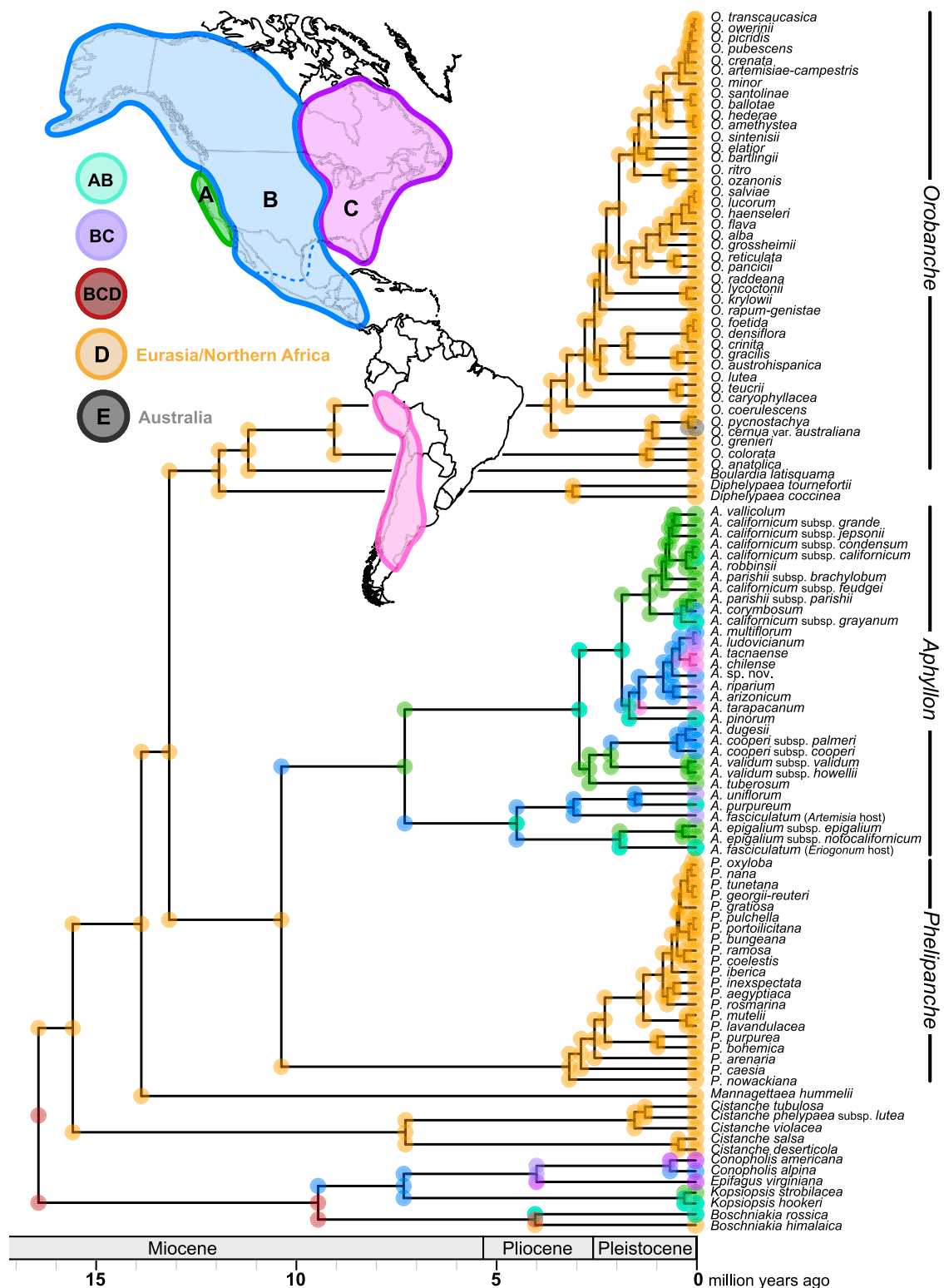
**FIGURE 3** Bayesian inference chronogram (maximum likelihood topology) of Orobancheae biogeography, reconstructed using a DEC+J model implemented in RevBayes. Colored circles at tips represent the biogeographical range of each taxon. Circles on each node represent the most probable reconstructed ancestral area of the most recent common ancestor of the two daughter lineages; circles on either side of the node show the reconstructed areas of daughter lineages immediately following cladogenesis. Each color represents a different biogeographical region or combination of regions as indicated by the map and legend in the upper left. Well-supported alternative biogeographical hypotheses of important nodes are presented in Table 1. The blue dashed line represents the southernmost extent of *Aphyllon* in North America.
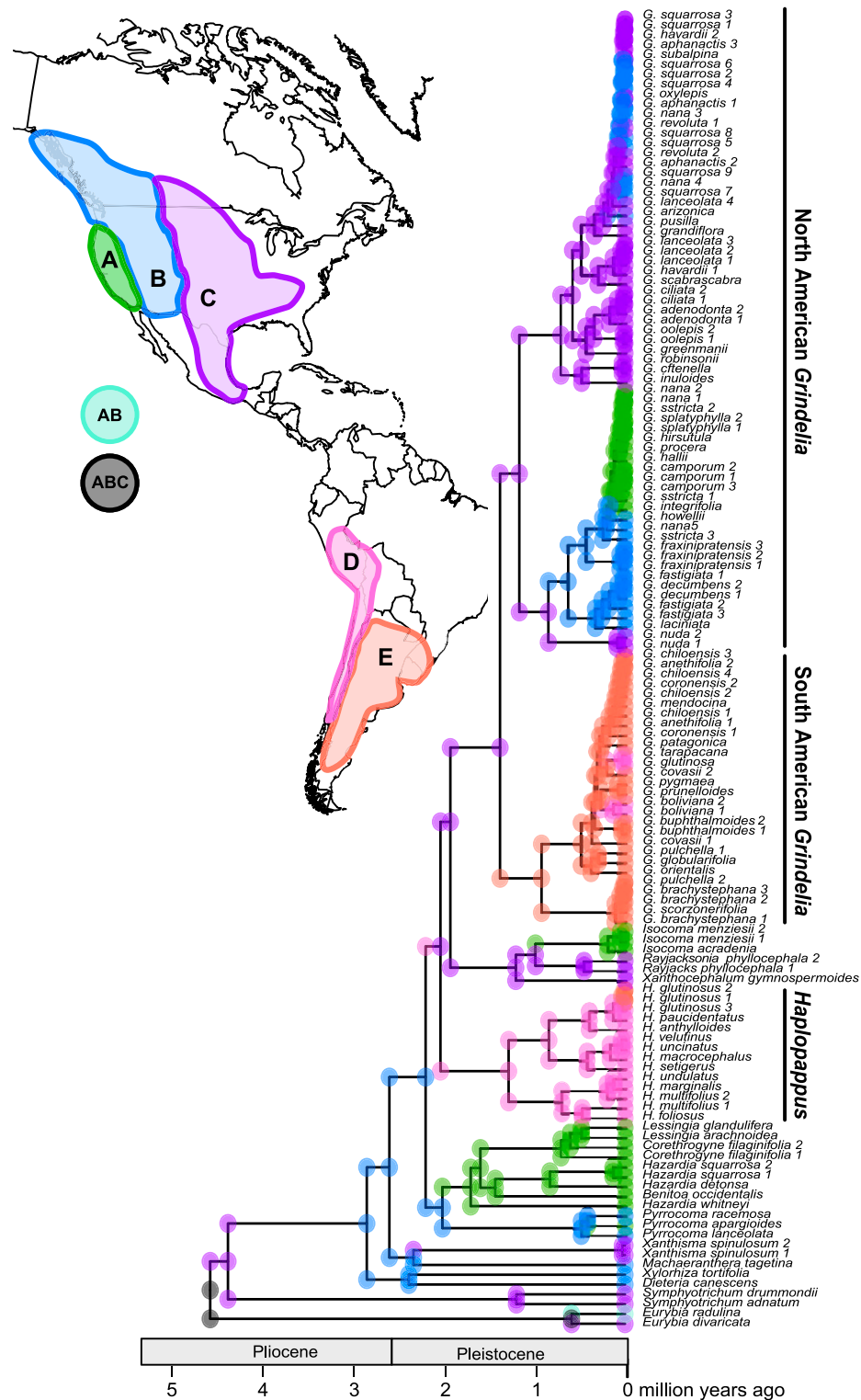
**FIGURE 4** Bayesian inference chronogram (maximum likelihood topology) of *Grindelia* and relatives showing biogeography, reconstructed using a DEC+J model implemented in RevBayes. Colored circles on each node represent the reconstructed ancestral area of the most recent common ancestor of the two daughter lineages; circles on either side of the node show the reconstructed areas of daughter lineages immediately following clado-genesis. Each color represents a different biogeographical region or combination of regions as indicated by the map and legend.

**TABLE 1.** Divergence times with credible intervals (95% highest posterior density [HPD]) and inferred historical biogeography of selected well-supported clades within the Orobanchaceae and *Grindelia*. For a map defining the biogeographical regions, see Figs. 3 or 4.

| Clade | Crown age (Ma) | | Ancestral Biogeography | |
|---|---|---|---|---|
| | Mean | 95% HPD | Region | Posterior probability |
| Orobanchaceae (incl. Rehmanniaceae) | 30.2 | 25.6–36.0 | – | – |
| Parasitic Orobanchaceae | 27.7 | 23.5–33.1 | – | – |
| Holoparasitic Orobancheae | 16.5 | 13.7–19.7 | Eurasia/ Northern Africa + Western North America + Eastern North America | 0.43 |
| | | | Eurasia/Northern Africa + Eastern North America | 0.31 |
| *Orobanche* s.l. (incl. *Diphelypaea*) | 13.9 | 11.0–15.8 | Eurasia/ Northern Africa | 0.95 |
| *Aphyllon + Phelipanche* | 10.4 | 8.4–12.7 | Eurasia/ Northern Africa | 0.61 |
| *Aphyllon* | 7.3 | 5.8–8.9 | California Floristic Province | 0.33 |
| | | | Western North America | 0.33 |
| | | | California Floristic Province + Western North America | 0.24 |
| *Aphyllon* sect. *Aphyllon* | 4.49 | 3.40–5.66 | California Floristic Province + Western North America | 0.43 |
| | | | Western North America | 0.36 |
| *Aphyllon* sect. *Nothaphyllon* | 2.92 | 2.15–3.74 | California Floristic Province + Western North America | 0.62 |
| | | | California Floristic Province | 0.30 |
| *A. ludovicianum* complex[a] | 1.43 | 0.99–1.90 | Western North America | 0.74 |
| *A. chilense + A. tacnaense + A. ludovicianum + A. multiflorum* | 0.40 | 0.19–0.65 | Western North America | 0.71 |
| *A. chilense + A. tacnaense* | 0.14 | 0.02–0.28 | South America | 1 |
| *Grindelia* | 1.40 | 0.71–2.43 | Eastern North America | 0.92 |
| North American *Grindelia* | 1.18 | 0.57–2.04 | Eastern North America | 0.98 |
| Western North American *Grindelia* | 0.63 | 0.26–1.17 | Western North America | 1 |
| California and Oregon *Grindelia* | 0.16 | 0.04–0.25 | Pacific Coast | 1 |
| South American *Grindelia* | 0.93 | 0.41–1.36 | Eastern South America | 1 |

[a] The *A. ludovicianum* complex comprises *A. chilense, A. tacnaense, A. ludovicianum, A. multiflorum, Aphyllon* sp. nov., *A. arizonicum, A. riparium,* and *A. tarapacanum* (see Schneider et al., 2016).

In this study, we report strong support for two independent dispersals of *Aphyllon* from western temperate North America to temperate South America within the last 1.9 million years (Table 1, Fig. 3). The more recent case, of the common ancestor of *A. chilense* and *A. tarapacanum* was slightly preceded by the North to South American dispersal and subsequent diversification of *Grindelia* less than 2.5 Ma, a common host of South American *A. chilense* and closely related North American *Aphyllon* (Table 1, Fig. 4). In contrast, the host associations of the earlier-dispersing *Aphyllon* lineage are less clear, but likely were also asteraceous shrubs. The timing and direction of dispersal in both clades is consistent with the recent timing and north-to-south direction of many other reported temperate AADs (Wen and Ickert-Bond, 2009). The apparent lack of long-distance dispersal ability in *Aphyllon* may explain the lag time between host and parasite colonization of South America. However, it also likely reflects the time necessary for the *Grindelia* to establish a sufficient range within South America, or perhaps more importantly density (Tsai and Manos, 2010), to support the subsequent establishment of *Aphyllon* (assuming no other plants were suitable hosts.).

Recent historical biogeographic studies of holoparasitic plants have generally not considered the biogeography of potential hosts (e.g., Wolfe et al., 2005; Bendiksby et al., 2010; Moore et al., 2010). One reason may be that the phylogenetic breadth of their host associations due to frequent host switching at deeper phylogenetic levels, or host generalism in the case of many hemiparasites, would not be expected to provide a biogeographic constraint. However,

the geographically and temporally parallel amphitropical disjunctions in *Aphyllon* and *Grindelia* illustrate a link between the ecology and biogeography of parasites and their host.

***Historical biogeography of Orobancheae***—Although holoparasitism likely emerged in the Miocene (Fig. 1), the origination of most extant diversity in the Orobancheae appears to have taken place in the last several million years, with the stem ages of several large clades, most notably *Orobanche* s.s. (excluding *O. anatolica* and *O. colorata*) and *Phelipanche*, 2–3 times older than the crown ages (Fig. 3). One process that may result in this "stemminess" is a high extinction rate, with internal nodes clustered near the present representing lineages that have not yet had time to go extinct. However, differentiating a high background extinction rate from variable or shifting diversification rates in the absence of a fossil record can be difficult (Nee et al., 1994; Rabosky, 2010).

Nonetheless, a high background extinction rate in the Orobancheae is an attractive hypothesis given the extreme specialization of holoparasitic Orobancheae to their hosts. Ecological specialization has been noted as an important contributor to extinction risk in many systems, although susceptibility to extinction and evolutionary trajectories more broadly may also depend on type of specialization (host–parasite, plant–pollinator, fundamental niche of plants, etc.; Munday, 2004; Jiguet et al., 2007; Colles et al., 2009; Eskildsen et al., 2015).

In addition to intrinsic risk of extinction due to specialization, the dynamic climate of the Quaternary may have also played a role in the

relative paucity of long-branch terminal taxa in *Orobanche*, *Phelipanche*, and to a lesser extent *Aphyllon*. Since the beginning of the Pleistocene, a series of five major ice sheet advances and retreats have swept across North America (Balco and Rovey, 2010), resulting in alternating episodes of plant species range contraction into refugia, followed by relatively unimpeded expansion or migration during interglacial periods (Shafer et al., 2010; Lumibao et al., 2017). Contemporaneous glaciations in South America may have also limited available habitat for colonization of hosts or parasites (Sérsic et al., 2011; Fraser et al., 2012). These changes could have contributed to increased extinction rates by reducing population size or disrupting host–parasite associations. On the other hand, the glacial–interglacial cycles may have also promoted speciation, as new opportunities for host-switching arose due to changes in plant assemblages. For example, the genus *Kopsiopsis* (Beck) Beck became isolated in California and the Pacific Northwest following a host shift to Ericaceae (Fig. 3). The remainder of this New World lineage, that is, the genera *Epifagus*, *Conopholis*, and *Boschniakia* C.A.Mey. ex Bong., has been present in eastern North America since the late Miocene or Pliocene, likely retreating into southeastern North America during the Pleistocene glaciations (Tsai and Manos, 2010; Rodrigues and Stefanović, 2016).

Such environmental changes undoubtedly shaped the ranges of extant species, perhaps most obviously *Aphyllon uniflorum*. This species is primarily found in eastern North America, but also on the Alaska Peninsula and in far southwestern British Columbia, a vicariant pattern perhaps explained by recent glaciation. Historical species ranges, such as those inferred from population genetic analyses or pollen cores, are not taken into account in the ancestral state reconstructions performed here.

Other eastern North American *Aphyllon* species such as *A. fasciculatum* and *A. riparium* are also nested in the phylogeny, with western North America (either including or excluding the CFP; Table 1) as the inferred origin of *Aphyllon*, which is also where extant taxonomic diversity is highest. Within western North America, we infer relatively frequent dispersal into and out of the CFP, in addition to recent in situ diversification in both the CFP and the rest of western North America (Fig. 3). This frequency of dispersal likely contributes to our difficulty in inferring the historical biogeography at these earlier nodes, or in being able to distinguish among alternative biogeographic hypotheses of California origins, western North American origins outside of California, or widespread ranges in both regions, in spite of the Sierra Nevada crest being a relatively strong biogeographical barrier today. One other important limitation in these analyses is uncertainty in tree topology. Incongruence between trees inferred using plastid and nuclear data at some deeper nodes (Schneider et al., 2016) likely point to a more complicated phytogeographic history of *Aphyllon* in western North America than can be inferred simply from extant distributions.

Finally, although the biogeographical patterns of species in Eurasia and Africa are not the main focus of this study, these regions contain the majority of extant taxonomic and phylogenetic diversity within Orobancheae, particularly in the genera *Orobanche*, *Phelipanche*, and to a lesser extent *Cistanche*. As in North America, cooling and drying during the Pliocene followed by glacial–interglacial cycles likely greatly affected the diversification and extant ranges of the Orobancheae. Two centers of extant *Orobanche* and *Phelipanche* diversity, the Mediterranean and Caucasus, have been hypothesized as refugia for both plant and animal lineages during Pleistocene ice ages (Taberlet et al., 1998; Hewitt, 1999; Lumibao et al., 2017), although other processes may also have contributed to the high diversity now seen in those regions (Feliner, 2011). A more precise study of historical biogeography of Old World Orobancheae, which would require additional sampling of Eurasian taxa, would shed additional light on the range of the common ancestor of holoparasitic Orobancheae and, by extension, how this lineage dispersed across the northern hemisphere, and eventually into sub-Saharan Africa and southern Australia.

***Historical biogeography of Grindelia***—Our analyses indicate that *Grindelia* diversified extremely recently in both North America and South America, more so than many of the other plants with which it co-occurs. For example, the estimated diversification time of the CFP species (that is, the species that are sister to *G. integrifolia* DC.) is 0.16 Ma, which is much more recent than almost all of the other groups that have significant ecological diversity in the CFP (Baldwin, 2014). Similarly, compared to many other North American–South American disjuncts that show extensive diversification on the continent to which they dispersed, *Grindelia* arrived in South America through a long-distance dispersal event much more recently, around 1.4 Ma (Fig. 4; Wen and Ickert-Bond, 2009). In contrast, other recent disjuncts, such as *Aphyllon*, have only one or two described species on the continent to which they dispersed.

Our finding of recent diversification for North American *Grindelia* is consistent with several lines of evidence from previous studies. First, North American species that have been tested are capable of hybridization with one another (although some species are separated by chromosomal rearrangements and only produce sterile hybrids; Dunford, 1964, 1986). Second, many taxonomic species sampled in phylogenetic analyses are resolved as paraphyletic, which is likely due to a combination of incomplete lineage sorting, recent hybridization, and lack of sequence variation in the sampled regions (Moore et al., 2012). Third, there are some taxonomic difficulties, especially in the North American species, likely due to ecological diversification and potential reproductive isolation in the field occurring more rapidly than sorting of morphological variation (Strother and Wetter, 2006; Moore et al., 2014).

Despite these independent lines of evidence for recent diversification of *Grindelia*, it is possible that better calibrations in future analyses could change our age estimates. The only available calibration for this *Grindelia* phylogeny was a secondary calibration of the root node, based on a larger fossil-calibrated phylogeny of the Asteraceae as a whole (Huang et al., 2016). These authors used several fossil calibration points, but these calibration points were also all from relatively deep in the tree. Thus, it is possible that our 95% HPD intervals do not reflect the full uncertainty in the dating of these nodes, in which case *Grindelia* may have been in South America for a longer period before establishment of *Aphyllon*.

***Inferring divergence times by horizontal gene transfer***—The lack of fossils in the Orobanchaceae has required previous studies to rely on molecular-clock methods to infer divergence times (Wolfe et al., 2005). However, parasitic plants, which include nearly all members of the Orobanchaceae, have accelerated rates of molecular evolution in the nuclear, plastid, and mitochondrial genomes (Bromham et al., 2013). Therefore, a molecular clock calibrated using a nonparasitic lineage, or a mixture of nonparasitic and parasitic plants, should be systematically biased toward earlier parasite divergence times, which is likely a major reason our results support more recent diversification than previous authors have proposed (Wolfe et al., 2005).

At the same time, these biases are a double-edged sword. Our Orobanchaceae calibration points are only within relatively nested clades

of holoparasites, i.e., ones with higher rates of molecular evolution. Therefore, the inferred ages of autotrophic lineages, such as crown Orobanchaceae, *Rehmannia* and *Lindenberghia* are likely underestimates, since the elevated rate of molecular evolution was erroneously applied to those lineages when converting sequence variation into time. Fossil evidence from a number of sources (e.g., Call and Dilcher, 1992; Kar, 1996; Conran and Christophel, 2004), indicate that the Lamiales had already diversified extensively by the Eocene. These and other fossils formed the basis of several recent large angiosperm-wide molecular dating analyses that support an earlier mean root age than we infer (Zanne et al., 2014; Magallón et al., 2015), although our distribution of root ages do fall within the 95% HPD credible interval reported by Magallón et al. (2015), per our a priori constraint. Within the Orobanchaceae, previous phylogenetic studies have also inferred older divergence dates, beginning with Wolfe et al. (2005) who averaged two previously inferred dates that they considered reasonable upper and lower bounds for the true age, one from the fossil record and the other from a molecular clock study of Lamiales. More recently, in a study of the mostly hemiparasitic Rhinantheae (Orobanchaceae), Uribe-Convers and Tank (2015) cross-validated the age estimate of Wolfe et al. (2005), using the most recent uplift of the Andes to calibrate the highly nested *Bellardia–Neobartsia* split. With the caveat that the Rhinantheae were not the focus of our study, we do infer a more recent split of *Bellarida trixago* and *Parentucellia* (=*Bellarida*) *viscosa* (the closest node sampled in both studies). However, there is some limited overlap between our 95% HPD estimate of this node age (3.2–6.2 Ma) and theirs (5.1–12.8 Ma).

While the age estimates of *Orobanche* s.l. (including *Aphyllon*) are likely not to suffer from biases introduced by rate heterogeneity because they are constrained by the timing of the HGT event, estimates of other node ages, particularly those closer to the root, should be interpreted as a lower bound, just as the dates inferred by Wolfe et al. (2005) are a reasonable upper bound on the true age. To fully resolve the conflicts between the two calibrations, we suggest an expanded study that includes dense sampling throughout the Orobanchaceae, as well as close relatives in the Lamiales. Minimum-age fossil calibrations of early Lamiales could constrain earlier nodes in the tree more strongly and accurately than our broad uniform prior, and divergence times of more nested clades could be estimated using calibrations based on horizontal gene transfer, biogeography, or geology (Schenk, 2016). Moreover, further work to develop evolutionary models that explicitly incorporate horizontal transfer events could be used to more rigorously test evolutionary hypotheses and infer historical biogeography of Orobanchaceae and other lineages of parasitic plants, nine of which have documented host-to-parasite horizontal gene transfers (Davis and Xi, 2015).

## CONCLUSIONS

Together, the patterns and timing of diversification in *Aphyllon* and *Grindelia* illustrate the role that host–parasite ecology can play in shaping biogeographical patterns. Among the three amphitropical disjunctions in the Orobancheae (one in Old World *Orobanche* and two temperate American disjunctions in *Aphyllon*), the phylogenetic and host relationships are most congruent in the *Aphyllon–Grindelia* system. Although these two genera appear to have taken different evolutionary trajectories following dispersal to South America (with only four described South American taxa in *Aphyllon* originating from two dispersal events, and 26 taxa of *Grindelia* originating from a single dispersal), the timing of their dispersals to South America are relatively congruent, with the dispersal of *Grindelia* occurring only slightly

before the dispersal of *Aphyllon.* In the northern hemisphere, recent Pleistocene glaciations probably have also contributed to diversity and biogeography of temperate Orobancheae. Given the preponderance of host switches that have occurred in *Aphyllon*, it is uncertain whether the existence of *Grindelia* directly enabled this lineage of *Aphyllon* to successfully colonize South America, but host availability should not be ignored in the context of parasite biogeography.

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