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Phylogeny and historical biogeography of Hydrophyllaceae and Namaceae, with a special reference to *Phacelia* and *Wigandia*

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This study aimed to examine the systematic position of South American species of *Phacelia* (Hydrophyllaceae) and *Wigandia* (Namaceae) and the historical biogeography of Hydrophyllaceae and Namaceae using molecular dating and ancestral area reconstruction. To this end, we constructed two datasets, one with a plastid (*ndhF*) and one with a nuclear marker (ITS), using previously published and newly generated sequences. We inferred the phylogeny of Hydrophyllaceae and Namaceae implementing both likelihood and Bayesian methods. We also estimated divergence times and ancestral areas for all major clades using a relaxed Bayesian uncorrelated molecular clock and the dispersal-extinction-cladogenesis (DEC) approach, respectively. The South American representatives of *Phacelia* are placed in three different clades of the genus and two colonizations of South America by North American species took place in the Miocene and at least one in the late Pliocene. *Wigandia* forms a well-supported monophylum with interspecific relationships partly unresolved. Within Namaceae a colonization of South America by North American species occurred during the Oligocene–Miocene transition. The MRCA of *Wigandia* was distributed in North and Central America in late Oligocene. Long-distance dispersal may have been necessary for the colonization of South America by Namaceae during the Oligocene–Miocene transition, when North and South America were not connected.

Key words: Amphitropical disjunction, ancestral area reconstruction, Boraginales II, molecular dating, South America

Introduction

Hydrophyllaceae and Namaceae are two new world families mainly distributed in western North and South America and a few species in Central America. Mostly herbaceous Hydrophyllaceae comprise 12 genera and ∼240–260 species. *Phacelia* Juss. is the largest and most diverse member of the family (∼207 species; Hofmann et al., 2016). Namaceae are a smaller family of herbs, shrubs, or small, soft-wooded trees. They comprise four genera with ∼75 species in total and most species in *Nama* L. (∼50 spp.).

Hydrophyllaceae and Namaceae are successively sister families to the remainder of the Boraginales II clade (including also Coldeniaceae, Cordiaceae, Ehretiaceae, Heliotropiaceae, Hoplestigmataceae, and Lennoaceae) based on plastid data (Luebert et al., 2016;

Stull et al., 2015; Weigend et al., 2014). Phylogenetic studies over the last two decades have resolved the relationships in these two to families: Hydrophyllaceae falls into three major clades (Ferguson, 1998; Walden, 2010). The first clade includes *Phacelia* and *Romanzoffia* Cham. (equivalent to Romanzoffieae, Walden et al., 2014), the second clade comprises *Hydrophyllum* L., *Pholistoma* Lilja, *Nemophila* Nutt., *Emmenanthe* Benth., *Ellisia* L. and *Eucrypta* Nutt. (equivalent to Hydrophylleae; Walden, 2015), and the third clade includes *Draperia* Torr., *Tricardia* Torr. ex S. Watson, *Howellanthus* (Constance) Walden & R. Patt. and *Hesperochiron* S. Watson (unnamed; Luebert et al., 2016; Walden et al., 2014). Namaceae falls into two clades: a *Nama* clade (about 50 spp.; Hofmann et al., 2016), and a clade comprising woody *Wigandia* Kunth (6 spp.; Hofmann et al., 2016), *Eriodictyon* Benth., and *Turricula* J.F. Macbr. plus two species of *Nama* (rendering *Nama* polyphyletic Ferguson, 1998; Luebert et al., 2016; Taylor, 2012).

Most species of Hydrophyllaceae and Namaceae are found in western North America. Only one genus of Hydrophyllaceae (*Phacelia*) and two of Namaceae (*Nama*, *Wigandia*) have representatives in South America. *Phacelia* and *Nama* thus represent amphitropical disjunctions, the latter also with one species in Hawaii (Hofmann et al., 2016). *Wigandia* is present in south-western North America, Central America and its South American distribution is restricted to the northern part of that continent.

Phacelia has been subject to several molecular phylogenetic studies with plastid and nuclear sequence data (Gilbert et al., 2005; Hansen et al., 2009; Walden et al., 2014). These studies aimed at evaluating existing classifications of the genus based on a broad sampling. However, none of them included South American species.

In *Nama*, one dissertation (Taylor, 2012) addressed the phylogenetic relationships of its species based on plastid and nuclear sequence data and a sampling that included one out of three South American species. However, this study and its sequence data have not been published so far.

In *Wigandia*, there is no specific phylogenetic study available and only few large-scale phylogenies (e.g., Ferguson, 1998; Walden et al., 2014) have included one or two species of the genus from North America.

Therefore, the systematic placement of the South American species of Hydrophyllaceae and Namaceae remains to be assessed with molecular data. As a consequence, the biogeographic history of these genera, across their ranges, has not been investigated.

Late Eocene divergence times of the crown node of Hydrophyllaceae and Namaceae were recovered in the first historical biogeography study of the order by Luebert et al. (2017). The study further retrieves a North American ancestral area for both families and subsequent dispersal into Central and South America. Due to low sampling density in the families specific questions about the biogeographic history were not addressed.

The present study uses previously published sequence data and supplements them with a broad sampling of South American representatives of *Phacelia* and *Wigandia* to address the following questions: (1) What is the phylogenetic position of South American representatives of *Phacelia* and *Wigandia*? (2) When and where did these genera originate and diversify? (3) How many dispersal events between North and South America took place and in which directions? To address these questions, we inferred the phylogenetic relationships of Hydrophyllaceae and Namaceae with an expanded taxon sampling for *Phacelia* and *Wigandia*

and estimated their divergence times and ancestral ranges based on molecular clock analyses and the dispersal-extinction-cladogenesis (DEC) approach to historical biogeography.

Materials and methods
Phylogenetic analyses

Taxon sampling and outgroup selection. We built two datasets, one with plastid NADH dehydrogenase F (*ndhF*) and one with nuclear Internal transcribed spacer (ITS). In both cases we combined newly generated and published sequences. Newly generated sequences include seven out of 10 South American species of *Phacelia* and four species of *Wigandia*. The *ndhF* dataset consisted of 241 accessions, 117 Hydrophyllaceae (110 taxa), 29 Namaceae (23 taxa), and includes representatives from all major clades of each family of Boraginales (see Luebert et al., 2017; Weigend et al., 2014 for details). All *ndh* genes are absent in the chloroplast of the holoparasitic Lennoaceae (Schneider et al., 2018), so they could not be included in the analyses. Four taxa from the Gentianales were chosen as an outgroup, which is likely the sister order to Boraginales (Leebens-Mack et al., 2019; Stull et al., 2015). The ITS dataset consisted of 131 accessions, 110 Hydrophyllaceae (107 taxa), and 19 Namaceae (19 taxa). One species each of Ehretiaceae and Heliotropiaceae were used as outgroups, because a further expansion of the outgroup sampling made the alignment equivocal. Sampling within *Phacelia* for both markers included representatives of all subgenera and sections recognized by Walden and Patterson (2012). New sequences were deposited in GenBank. Voucher information and GenBank accession numbers are given in Appendix S1.

DNA extraction, amplification, and sequencing. DNA was extracted from samples of silica-gel-dried leaves or herbarium material with a modified CTAB method (Doyle & Doyle, 1987). PCR amplifications were performed in a Trio-Thermoblock thermal cycler (Biometra, Göttingen, Germany) and a Mastercycler ep (Eppendorf AG, Hamburg) in a 25 µl volume containing 0.5 U of Taq Polymerase, 5 µl 5× Taq Buffer, 25 mM MgCl$_2$, 1.25 mM of each dNTP, 0.2 mM of each primer, and about 50 ng of genomic DNA. Amplification primers and cycling conditions followed Moore and Jansen (2006). PCR products were purified according to Werle et al. (1994). Sanger sequencing was performed by GATC Biotech (Eurofins Genomics Germany GmbH). The resulting sequences were assembled and aligned

automatically using Geneious v.8.1.9 (https://www.gene-ious.com). Manual adjustments followed using PhyDE 0.9971 (Müller et al., 2010).

Phylogenetic inference. Previous studies showed that nuclear and plastid markers are significantly heterogeneous in Hydrophyllaceae and Namaceae and should therefore be analysed separately (Ferguson, 1998; Hansen et al., 2009; Moore & Jansen, 2006; Nazaire & Hufford, 2012; Taylor, 2012; Walden et al., 2014; Weeks et al., 2010). Maximum likelihood (ML) analysis for each marker was conducted through CIPRES Science Gateway (Miller et al., 2010) using RAxML-HPC BlackBox (8.2.12) tool (Stamatakis, 2014) on XSEDE (Extreme Science and Engineering Discovery Environment). The substitution model $GTR + \Gamma$ was used in both analyses (*ndhF* and ITS), with 1,000 bootstrap replicates and best tree search. Bayesian inferences (BI) were conducted in MrBayes 3.2.6 using XSEDE through the CIPRES portal using default parameters, random starting trees, and four independent runs. The number of generations was set to 1,000,000 using four chains and sampling every 1,000 generations. The $GTR + \Gamma$ model was chosen and the number of substitution types was set to 6.

Historical biogeography

Fossil record and node calibration. The phylogeny was calibrated by fixing the age of the Boraginales stem node (Boraginales & Gentianales) with a secondary calibration from Magallón et al. (2015), namely a normal calibration with a mean of 88.25 Ma and SD of 11 Ma. Following Luebert et al. (2017), four fossil calibrations for internal nodes of Ehretiaceae, Heliotropiaceae, and Boraginaceae s.str. were set as lognormal distributions with an offset of the minimum age of the fossil and with a lognormal mean so that the median age falls in the middle of the time period assigned to the fossil. Fossils used for calibration are listed in Table 1.

Estimating divergence times. To estimate divergence times in Boraginales the *ndhF* dataset was used. The alignment of ITS across Boraginales and Gentianales is unreliable due to extensive sequence variation. We used Gentianales as a root of the tree and a sister group of Boraginales based on previous phylogenetic studies (Stull et al., 2015). Uncorrelated Bayesian dating was conducted with the relaxed molecular clock model implemented in BEAST v1.8.4. (Drummond et al., 2012). Following previous analysis (Luebert et al., 2017), an uncorrelated lognormal relaxed-clock and the $GTR + \Gamma + I$ model were selected

and a Yule prior (Gernhard, 2008) was assigned to the branching process. One run of 100,000,000 chains with sampling every 10,000 generations was performed and convergence was assessed by analysing the results in Tracer v1.7.1 (Rambaut et al., 2018). After removing 10,000,000 burn-in samples, median ages and 95% High Posterior Density (HPD) intervals with a posterior probability limit of 0.95 were summarized with TreeAnnotator v1.8.4 (https://beast.community/treeannotator), prior to visualization in FigTree v1.4.4 (https://beast.community/figtree).

Biogeographic analysis. Biogeographic analyses were performed separately for Hydrophyllaceae and Namaceae. Three biogeographic areas were defined according to the extant distribution patterns of the two families: A: North America, B: Central America, C: South America. Hydrophyllaceae are found in North or South America only, whereas for Namaceae all three biogeographic areas are represented. The chronogram obtained from the BEAST analysis was first edited using the R-package 'ape' (Paradis & Schliep, 2019), in order to extract the clades corresponding to Hydrophyllaceae and Namaceae. Using the R-package 'BioGeoBEARS' 1.1.2 (Matzke, 2013), the Dispersal-Extinction-Cladogenesis approach (DEC) described by Ree and Smith (2008), was implemented. Maxareas was set to the number of areas in the respective analyses (i.e., two for Hydrophyllaceae and three for Namaceae). The coded areas correspond to the distributions at species level, except for *Wigandia*, for which we coded the distribution of the specimens due to uncertainties in the species delimitations and distributions and a lack of up-to-date taxonomic revision.

Results
Phylogenetic analysis and age estimates

The aligned *ndhF* dataset consisted of 2,228 sites, with 1,347 distinct alignment patterns and the aligned ITS dataset was 889 sites in length with 517 distinct alignment patterns. Separate analyses for the plastid and nuclear datasets resulted in similar tree topologies, with the same well-supported major clades in terms of Bayesian posterior probabilities (BPP) and ML bootstrap values (BS; Figs 1, 2).

Hydrophyllaceae and Namaceae were retrieved as monophyletic (1 BPP, 100% BS and 1 BPP, 94% BS respectively for *ndhF*; 0.98 BPP, 75% BS and 0.99 BPP, 90% BS respectively for ITS).

In Hydrophyllaceae, *Draperia* forms a clade with *Tricardia*, *Howellanthus*, and *Hesperochiron* (1 BPP, 92% BS for *ndhF*; 0.96 BPP, 60% BS for ITS; hereafter

Table 1. List of fossils used for calibration.

Family	Taxon	Fossil type	Collectors	Epoch	Region	Estimated age (Ma)	Offset (Ma)	Mean (Ma)	Median age (Ma)
1. Boraginaceae s.str.	*Cryptantha* Lehm ex. Fisch & C.A. Mey	Nutlets	Thomasson (1987); Gabel et al. (1998)	Upper Miocene	USA	13.6–10.3	10.3	0.5	11.95
2. Boraginaceae s.str.	*Ogastemma* Brummitt	Nutlets	Hammmouda et al. (2015)	Eocene	SW Algeria	56–41.2	41.2	2	48.6
3. Heliotropiaceae	*Tournefortia* L. sect. *Tournefortia*	Pollen grains	Graham & Jarzen (1969)	Lower Oligocene	Puerto Rico	33.9–28.1	28.6	1.1	31.1
4. Ehretiaceae	*Ehretia* P. Browne	Fruit parts	Reid & Chandler (1933); Chandler (1961)	Lower Eocene	London Clay	56–47.8	47.8	1.1	51.8

DHHT clade). The *ndhF* dataset supports that DHHT clade is sister to Hydrophylleae + Romanzoffieae (1 BPP, 100% BS). The ITS dataset on the other hand shows the DHHT clade as sister to Hydrophylleae with low support (0.53 BPP). The genera *Emmenanthe*, *Eucrypta*, *Ellisia*, *Hydrophyllum*, *Pholistoma*, and *Nemophila* form a clade (Hydrophylleae; 0.98 BPP, 63% BS for *ndhF*; 1 BPP, 91% BS for ITS) sister to *Phacelia* + *Romanzoffia* for the *ndhF* dataset (Romanzoffieae; 0.83 BPP, 55% BS). The placement of *Romanzoffia* was incongruent between the plastid and the nuclear topologies: the ITS tree (Fig. 2) recovers *Phacelia* as monophyletic (0.97 BPP, 72% BS) and sister to *Romanzoffia* (0.99 BPP, 83% BS), while the *ndhF* tree (Fig. 1) retrieves *Romanzoffia* nested in *Phacelia* (0.99 BPP, 94% BS).

Most of the currently recognized taxonomic groups of *Phacelia* were retrieved as monophyletic with high support values in both plastid and nuclear analyses. The only exceptions in the majority-rule consensus tree (BI) derived from *ndhF* were *Phacelia* subg. *Pulchellae*, *Phacelia* sect. *Euglypta*, and *Phacelia* sect. *Miltitzia* (Fig. 1). In the majority-rule consensus tree (BI) derived from ITS, *Phacelia* sect. *Whitlavia* was retrieved as paraphyletic (Fig. 2), resulting in a non-monophyletic *Phacelia* subg. *Phacelia*.

The South American species of *Phacelia* were not retrieved as monophyletic, but are rather placed in three different clades within *Phacelia* in both analyses. Andean *P. secunda* J.F. Gmel. and south-temperate *P. brachyantha* Benth. belong to *Phacelia* sect. *Phacelia* clade and the ITS analysis suggests that they are sister taxa (0.78 BPP, 80% BS) and deeply nested in a North American clade. *P. artemisioides* Griseb., *P. pinnatifida* Griseb. Ex. Wedd., *P. setigera* Phil., and *P. viscosa* Phil. belong to *Phacelia* sect. *Glandulosae.* They form a well-supported polytomy along with *P. vossii* N.D. Atwood for *ndhF* (1 BPP, 95% BS) and a well-supported clade for ITS (1 BPP, 100% BS) deeply nested in a large clade of western North American species. In the *ndhF* tree *P. affinis* A. Gray is sister to all other species of the *Phacelia* sect. *Euglypta* + *Miltitzia* clade, including South American *P. cumingii* (Benth.) A. Gray (1 BPP, 100% BS). The ITS analysis suggests that *P. affinis* and *P. cumingii* are direct sister taxa in the *Phacelia* sect. *Euglypta* + *Miltitzia* clade (1 BPP, 90% BS).

In Namaceae, most *Nama* species are grouped together in one clade (1 BPP, 100% BS for *ndhF*; 1 BPP, 100% BS for ITS), except for *Nama rothrockii* A. Gray and *Nama lobbii* A. Gray. These two species are retrieved in the second clade with *Eriodictyon*, *Turricula*, and *Wigandia* (1 BPP, 98% BS for *ndhF*;
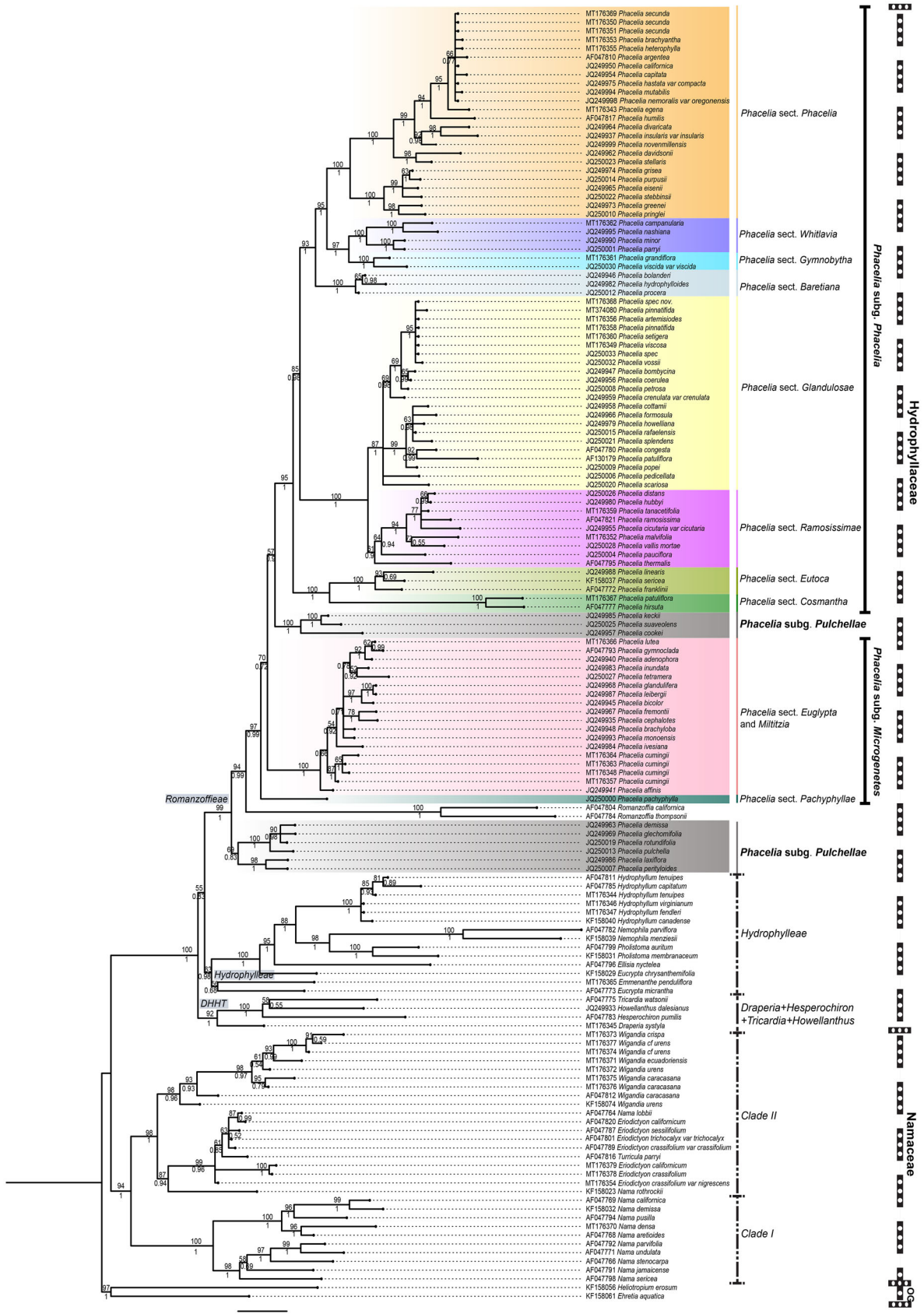
0.99 BPP, 93% BS for ITS). *Wigandia* is recovered as monophyletic with high support in both analyses (0.96 BPP, 98% BS for *ndhF*; 1 BPP, 100% BS for ITS).

Age estimates for Hydrophyllaceae and Namaceae are shown in Table 2. The median ages estimated for the nodes are placed within the range of the 95% HPD intervals. Our analysis suggests that Hydrophyllaceae and Namaceae started their diversification almost simultaneously, between late Cretaceous and middle Paleogene. Hydrophylleae is estimated as the oldest clade of Hydrophyllaceae, with a median age in the Paleocene. The crown node of Romanzoffieae was dated in early Eocene and the crown node of the DHHT clade in middle Eocene, albeit with large 95% HPD intervals. Among the South American *Phacelia* species, the crown node of south temperate to Mediterranean *P. cumingii* is retrieved with the highest age (4.3 Ma). The essentially Andean species of *Phacelia* sect. *Phacelia* follow with a crown node median age estimate of 3.3 Ma, whereas the deserticolous clade in *Phacelia* sect. *Glandulosae* is dated to a median age of 3.2 Ma for the crown node. Slightly younger ages are suggested for most well-supported clades of Namaceae (see Table 2). The crown node age of *Wigandia* is 25.4 Ma.

Ancestral area reconstructions of Hydrophyllaceae

The ancestral area reconstructions of Hydrophyllaceae resulting from the DEC analysis are presented in Fig. 3. The most recent common ancestor (MRCA) of Hydrophyllaceae probably occurred in North America. North America is suggested as the ancestral area of all three major clades of the family and virtually all major subclades (see Table 3). At least three dispersal events from North to South America were inferred. South America was first colonized by representatives of the basal grade of *Phacelia* sect. *Euglypta* and *Miltitzia*. *Phacelia affinis* as a lineage (current distribution in North America) had split from *P. cumingii* (current distribution in South America) ~8.6 Ma. Our ancestral area reconstruction indicates that the dispersal from North to South America occurred before the inferred split. South American representatives of *P.* sect. *Glandulosae* (*P. artemisioides*, *P. pinnatifida*, *P. setigera*, and *P. viscosa*) form a well-supported clade which split from its North American sister group in the late Miocene (~6.8 Ma). The most recent colonization of South America occurred in *P.* sect. *Phacelia*

during late Pliocene (~3.6 Ma) and could be the result of a single dispersal event. *P. brachyantha* occurs only in South America while *P. secunda* in both North and South America. Due to lack of resolution in the *ndhF* topology and low sample size in the ITS dataset, the exact number of dispersal events remains to be confirmed.

Ancestral area reconstructions of Namaceae

The ancestral area reconstructions of Namaceae resulting from the DEC analysis are presented in Fig. 4. The MRCA of Namaceae probably occurred in North America. Both the MRCA of clade I and clade II likely occurred in North America (see Table 4). The MRCA of *Wigandia* occurred in North and Central America in the Oligocene (~25 Ma) and the colonization of South America took place during the Oligocene–Miocene transition.

Discussion
Phylogenetic analysis

Our results largely confirm previous studies on Hydrophyllaceae and Namaceae. We recovered similar *ndhF* topologies as Ferguson (1998) and Walden et al. (2014), and similar ITS topologies and support values as Gilbert et al. (2005), Hansen et al. (2009) and Walden et al. (2014). The plastid analysis resulted in better resolution regarding the sister relationships between major clades, whereas the nuclear analysis resulted in better resolution within the individual clades. Weaker support for deeper nodes in the ITS trees was also shown by Hansen et al. (2009) and Walden et al. (2014). However, divergent taxon sampling between the plastid and the nuclear analyses in this study may also explain some of the topological differences.

Hydrophyllaceae is divided into three major clades as shown before (Ferguson, 1998; Luebert et al., 2016; Walden, 2010). However, some of our results differ from previous studies. *Draperia* is well-supported sister to the DHHT clade in both analyses, which is probably a result of the increased sample size compared with Walden et al. (2014). The placement of *Emmenanthe* is incongruent between the ITS and *ndhF* phylogenies. In the ITS analysis *Emmenanthe* is sister to the remaining Hydrophylleae clade, whereas in the *ndhF* analysis it forms a monophylum with *Eucryptha micrantha* (Torr.) A. Heller with low support (0.68 BPP, 56% BS), which

Fig. 1. Phylogram obtained from the Bayesian analysis based on *ndhF*. Only the Hydrophyllaceae and Namaceae clades and their sister genera *Heliotropium* and *Ehretia* (OG = outgroup) are shown. The remaining clades were pruned. Numbers above branches are ML bootstrap values (≥50) and below branches Bayesian posterior probabilities (BPP). Scale bar = mean number of nucleotide substitutions per site. Major clades of *Phacelia* are indicated with different colours.
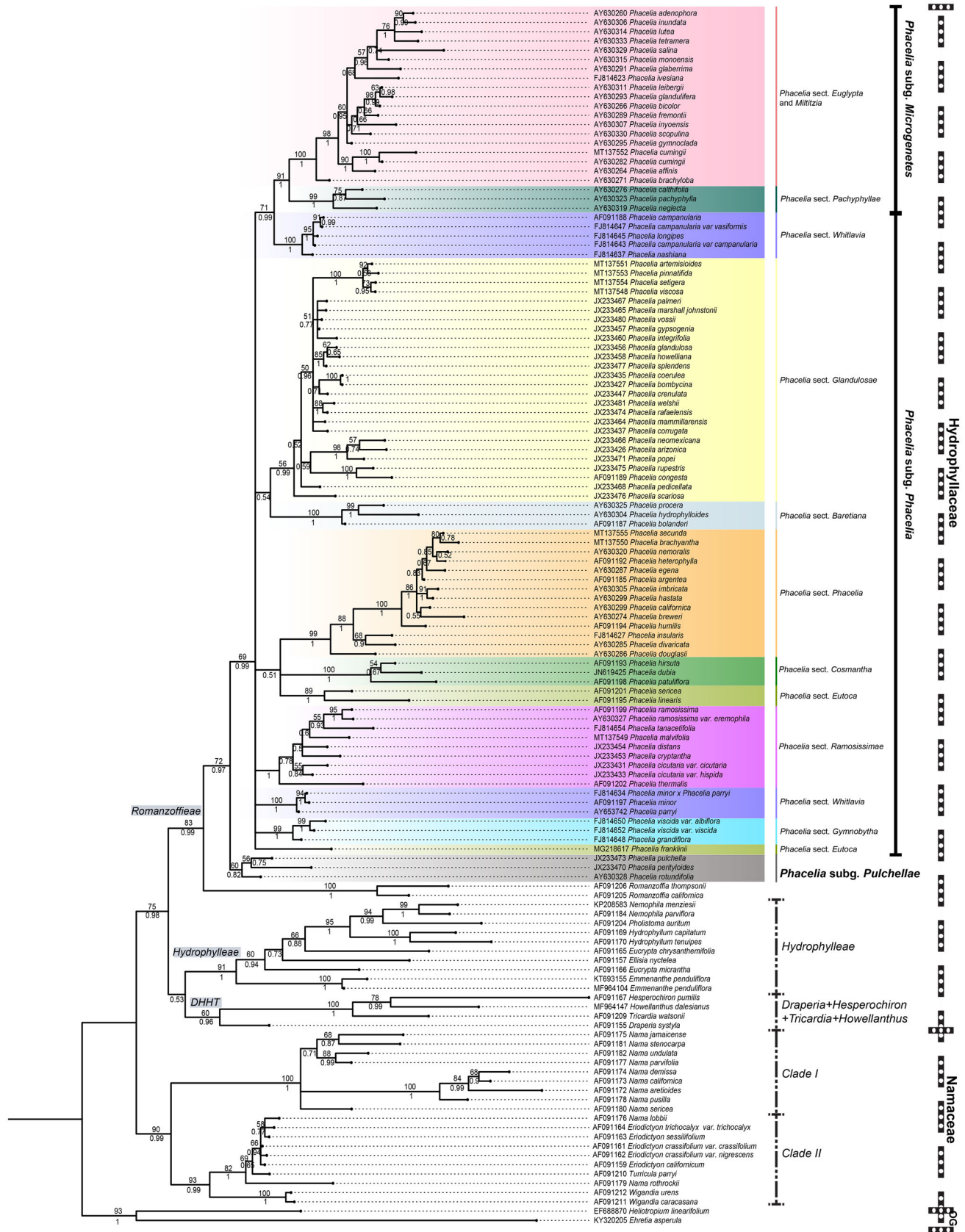
Table 2. Crown (C) and stem (S) node age estimates in major groups of Boraginales phylogeny. Time is given in millions of years (Ma) before present and in parentheses are noted the 95% highest posterior density intervals.

Node	Age estimates (Ma)
(C) *Hydrophyllum+Pholistoma+Nemophila+Ellisia+Eucrypta+Emmenanthe* (Hydrophylleae)	58.2 (43.5-73.9)
(C) *Phacelia+Romanzoffia* (Romanzoffieae)	54.9 (41.6-68.5)
(C) *Draperia+Howellanthus+Hesperochiron+Tricardia* (DHHT)	47.9 (26.3-68.8)
(C) Hydrophyllaceae	66.1 (51.7-83.1)
(C) *Nama* (Clade I)	42.2 (27.5-59.8)
(C) *Wigandia* clade (Clade II)	25.4 (14.3-39.4)
(S) *Wigandia* clade (Clade II)	38.6 (23.9-58.3)
(C) *Nama+Eriodictyon+Turricula* (Clade II)	30.3 (15.6-47.4)
(C) Namaceae	66.8 (46-88)

is then sister to the remaining Hydrophylleae (Walden et al., 2014; see also Walden, 2015). *Eucrypta* is retrieved as paraphyletic (Ferguson, 1998; Walden, 2015). The relationship between *Phacelia* and *Romanzoffia* remains incongruent between the two markers. The monophyly of the genus *Phacelia* with *Romanzoffia* sister to it, is supported only by the ITS analysis (Gilbert et al., 2005; Hansen et al., 2009; Walden et al., 2014), whereas in the *ndhF* phylogeny *Romanzoffia* is nested in *Phacelia*.

Phacelia is currently subdivided in three subgenera, 11 sections and 12 subsections (Figs 1, 2; Walden & Patterson, 2012). The majority of these units were retrieved as monophyletic in both analyses. The paraphyly of *Phacelia* subg. *Pulchellae* in the *ndhF* analysis could not be confirmed in the ITS tree: no ITS-sequences were available for the relevant species *P. keckii* Munz & I.M. Johnst, *P. suaveolens* Greene and *P. cookei* Constance & Heckard. Sections *Euglypta* and *Miltitzia* were not monophyletic (see also Ferguson, 1998; Gilbert et al., 2005; Hansen et al., 2009; Walden et al., 2014), but they were retrieved in one clade exactly as in Walden et al. (2014). Section *Whitlavia* was paraphyletic in the nuclear analysis and consequently *Phacelia* subg. *Phacelia* was not retrieved as monophyletic (see also Hansen et al., 2009; Walden et al., 2014). The monophyly of section *Whitlavia* is supported in the *ndhF* analysis (also in Walden et al., 2014) and additionally in a combined *rpl16* intron + ITS dataset in Hansen et al. (2009). The placement of section *Baretiana* was incongruent in plastid and nuclear analyses, as in Walden et al. (2014). Section *Eutoca* was monophyletic only in the *ndhF* tree. Walden et al. (2014) retrieved a monophyletic section *Eutoca* for ITS without sampling *P. franklinii*, which in our case did not group together with the other two representatives of the section.

The South American *Phacelia* species are not monophyletic, but are placed in three different clades in both phylogenies. *P. viscosa*, *P. artemisioides*, *P. pinnatifida*, and *P. setigera* are placed in *Phacelia*. sect. *Glandulosae*. *Phacelia brachyantha* belongs to *Phacelia* subsect. *Phacelia* (not *Phacelia* subsect. *Humiles* as suggested by Walden and Patterson (2012)). The placement of *P. cumingii* in *Phacelia* sect. *Euglypta* and its sister relationship to the North American *P. affinis* is in congruence with previous studies (Gilbert et al., 2005; Hansen et al., 2009; Walden et al., 2014).

The topology of Namaceae retrieved in the present study is congruent with previous phylogenetic analyses (Ferguson, 1998; Luebert et al., 2016; Taylor, 2012). Our extensive sampling of *Wigandia* confirmed its monophyly.

Wigandia urens (Ruiz & Pav.) Kunth and *Wigandia caracasana* Kunth were retrieved as non-monophyletic in the *ndhF* tree. Cornejo (2006, 2007) recognizes six species in *Wigandia*, treating *W. caracasana* as a synonym of *W. urens* s.l., but this would still not render *W. urens* monophyletic. A critical revision of this widespread genus is urgently required.

The odd placement of two species of *Nama* (*Nama rothrockii* and *N. lobbi*) further underlines the need for generic alignments in Namaceae. Taylor (2012) already indicated the isolation of the latter two species, with *N. lobbii* (= *Eriodictyon lobbii* Greene) nested in *Eriodictyon* and *Nama rothrockii* as sister to *Eriodictyon*. An expansion of the genus *Eriodictyon* to include both species, as well as *Tirricula parryi* which appears nested within *Eriodictyon* clade (Figs 1, 2), would thus be the simplest solution to resolve the taxonomy.

Divergence times and historical biogeography

We obtained similar age estimates to previous studies (Bremer et al., 2004; Luebert et al., 2011, 2017) for the

Fig. 2. Phylogram obtained from the Bayesian analysis based on ITS. Hydrophyllaceae and Namaceae clades and their sister genera *Heliotropium* and *Ehretia* (OG = outgroup) are shown. Numbers above branches are ML bootstrap values (≥50) and below branches Bayesian posterior probabilities (BPP). Scale bar = mean number of nucleotide substitutions per site. Major clades of *Phacelia* are indicated with different colours.
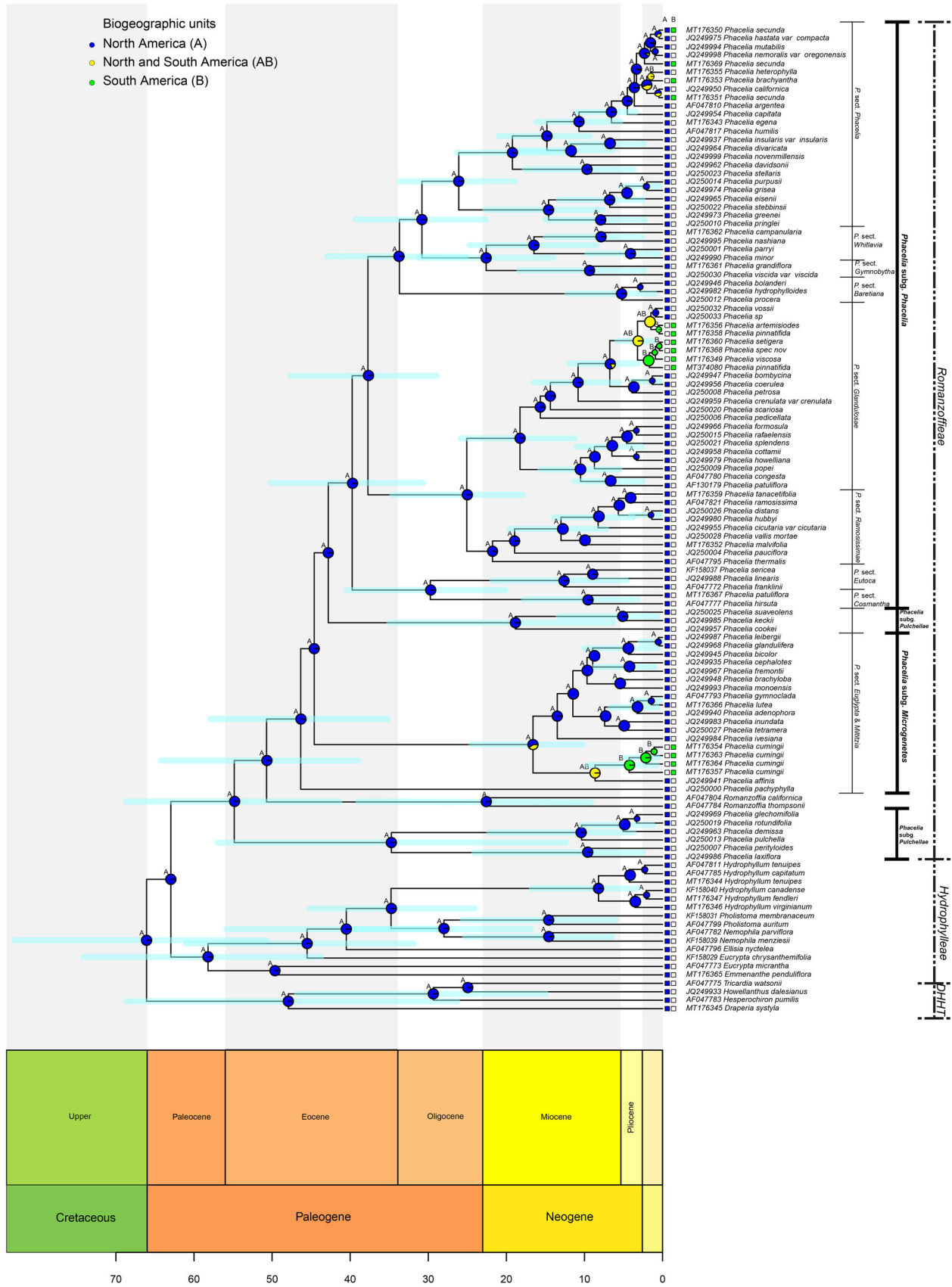
Table 3. Results of the Dispersal-Extinction-Cladogenesis (DEC) analysis for the major crown (C) or stem (S) nodes of the phylogeny of Hydrophyllaceae. The log-likelihood of the analysis is lnL = −43. Letters indicate the ancestral areas at the node and the numbers in parentheses are their relative probabilities. Displayed are only reconstructions with relative probabilities (RP) ≥ 0.05. A: North America, B: South America.

Node	Unconstrained, total area lnL = −43
(C) Hydrophyllaceae	A (0.99)
(C) *Draperia+Howellanthus+Hesperochiron+Tricardia* (DHHT)	A (1)
(C) Hydrophylleae	A (0.99)
(C) Romanzoffieae	A (0.99)
(S) Romanzoffieae	A (0.99)
(C) *Phacelia* sect. *Miltitzia* & *Euglypta*	A (0.6); AB (0.4)
(C) *P. affinis* & *P. cumingii* clade	AB (1)
(C) *P. cumingii* clade	B (1)
(C) *Phacelia* sect. *Glandulosae*	A (1)
(C) *P. vossii, P. artemisiodes, P. pinnatifida, P. setigera, P. viscosa* clade	AB (0.99)
(C) *P. viscosa* & *P. setigera* clade	B (1)
(C) *P. vossii, P. artemisiodes, P. pinnatifida* clade	AB (1)
(C) *Phacelia* sect. *Phacelia*	A (1)
(C) *P. brachyantha* & *P. heterophylla* clade	AB (1)

stem node of Boraginales (112.7 (95% HPD 96.4–129 Ma)). The age estimates for the crown nodes of Hydrophyllaceae (66.1 (95% HPD 51.7–83.1 Ma)) and Namaceae (66.8 (95% HPD 46–88 Ma)) are dated to the early Paleocene whereas Luebert et al. (2017) suggested that both families may have diversified in parallel during the Middle Eocene. This difference is probably due to the expanded taxon sampling for these families in our study, or due to the use of a single marker (*ndhF*). Reduced ingroup sampling has been shown to lead to lower age estimates (Linder et al., 2005; Luebert et al., 2017; Soares & Schrago, 2015).

Our expanded sampling of Hydrophyllaceae and Namaceae provides the first detailed insights into their historical biogeography. They both appear to have originated in North America. Our results are consistent with the general trend that amphitropical disjunctions are mainly the outcome of long distance dispersal (LDD) during the Miocene to Holocene and that the most common directionality is from North to South America (Raven, 1963; Simpson et al., 2017; Wen & Ickert-Bond, 2009). Direct dispersal by migratory birds may have occurred by epizoochory or endozoochory.

Hydrophyllaceae first colonized South America during the early to middle Miocene (*Phacelia* sect. *Euglypta*) and at least two more dispersal events occurred later, one in the late Miocene (*Phacelia* sect. *Glandulosae*) and one in the Pliocene (*Phacelia* sect. *Phacelia*). *P. vossii*, which occurs in North America,

groups together with the South American species of *P.* sect. *Glandulosae* with low support and no resolution, indicating the possibility of a back-dispersal to North America. The phylogenetic relationships of *P. secunda* and *P. brachyantha* in the clade of *P.* sect. *Phacelia* are not well-resolved and South American divergence and subsequent back-dispersal cannot be excluded. *P. secunda* could be an ancestral paraphyletic species or a species complex with cryptic taxa embedded.

Independent of the individual timing and the taxa involved, long-distance dispersal must be assumed for the exchange between North and South America. Although some species of *Phacelia* show no obvious dispersal mode (Heckard, 1963), a range of dispersal mechanisms are realized in Hydrophyllaceae and Namaceae, including myrmecochory (Hofmann et al., 2016) and epizoochory (Heckard, 1963; Schenk & Saunders, 2017). However, very little is known about the real dispersal mechanisms in Hydrophyllaceae and Namaceae in general, although many species, especially in Namaceae (*Wigandia, Eriodictyon*) have dust-fine, wind-dispersed seeds (Hofmann et al., 2016). Especially *Wigandia* and many species of *Nama* and *Phacelia* are early colonizing species with naturally small, disjunct populations, depending on regular dispersal for their preservation. *Wigandia* is thus common on road-cuttings in the Andes and *Phacelia viscosa* is commonly found on recent landslides and scree-slopes. *Nama dichotomum* is an extremely widespread weed on roadsides and

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Fig. 3. Divergence time estimates for the phylogeny of Hydrophyllaceae. Major clades are indicated. The distribution corresponding to each species is indicated by the coloured squares next to the tips. Pie charts at the nodes depict relative probabilities of areas as estimated from the Dispersal-Extinction-Cladogenesis (DEC) analysis with BioGeoBEARS (unconstrained, single-area analysis). Significant areas (relative probability > 0.05) are also indicated next to the pie charts (for details see Table 3). Bars around nodes are 95% highest posterior density intervals.
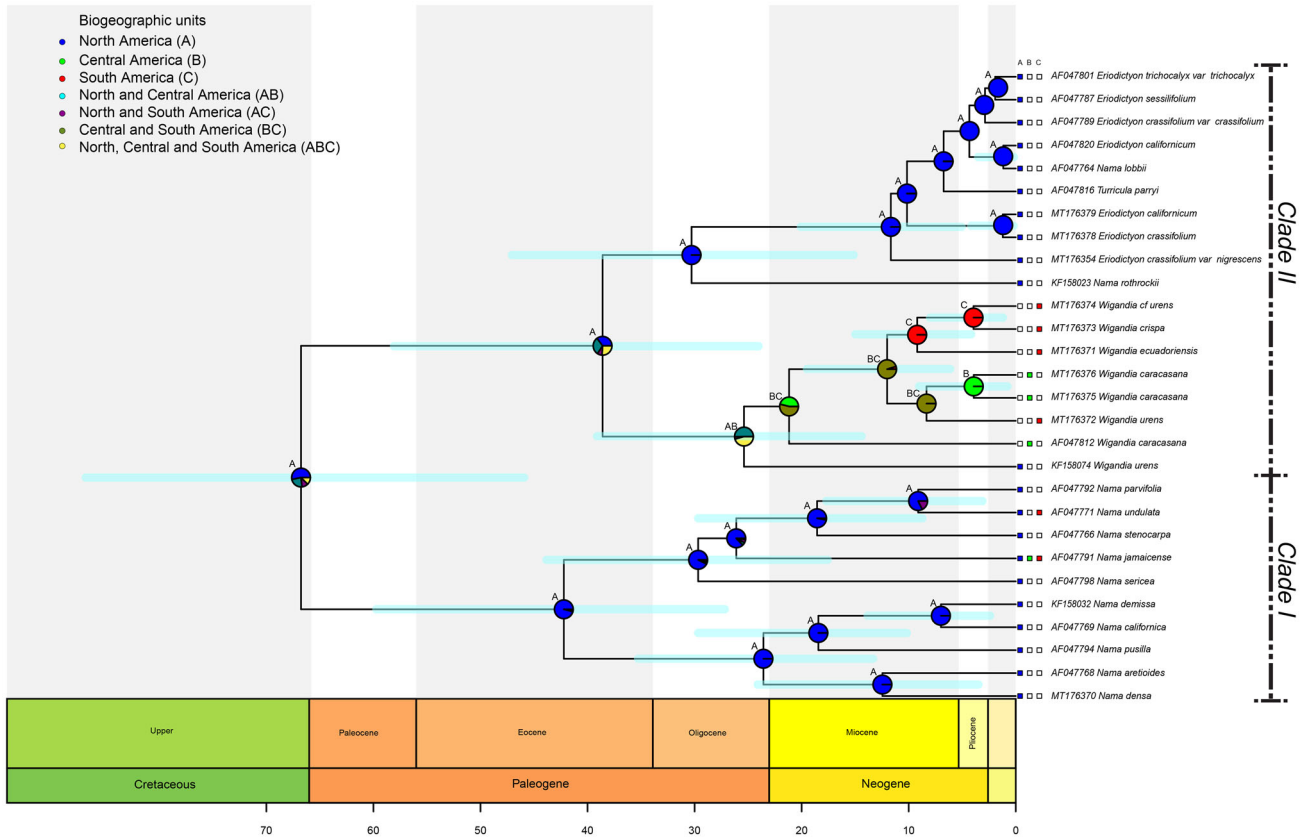
Fig. 4. Divergence time estimates for the phylogeny of Namaceae. Major clades are indicated. The distribution corresponding to each species is indicated by the coloured squares next to the tips. Pie charts at the nodes depict relative probabilities of areas as estimated from the Dispersal-Extinction-Cladogenesis (DEC) analysis with BioGeoBEARS (unconstrained, single-area analysis). Significant areas (relative probability > 0.05) are also indicated next to the pie charts (for details see Table 4). Bars around nodes are 95% highest posterior density intervals.

Table 4. Results of the Dispersal-Extinction-Cladogenesis (DEC) analysis for the major crown nodes (C) of the phylogeny of Namaceae. The log-likelihood of the analysis is lnL = −20.93. Letters indicate the ancestral areas at the node and the numbers in parenthesis are their relative probabilities. Displayed are only reconstructions with relative probabilities (RP) ≥ 0.05. A: North America, B: Central America, C: South America.

Node	Unconstrained, total area lnL = −20.93
(C) Namaceae	A (0.53); AB (0.23); AC (0.11); ABC (0.13)
(C) Clade I (*Nama* species)	A (0.96)
(C) Clade II (Shrubby genera)	A (0.35); AB (0.30); AC (0.08); ABC (0.26)
(C) *Wigandia*	AB (0.50); AC (0.04); ABC (0.46)
(C) *Eriodictyon*, *Nama*, *Tirricula*	A (1)

fallows. All these species evidently have high dispersibility. Due to its broad ecological range and tropical nature, stepping stone dispersal across much of its current range is thus likely for *Wigandia* – which is fully tropical and present in tropical Central America – ever since the development of the Isthmus of Panama (15–13 Ma; Coates & Stallard, 2013; Hoorn & Flantua, 2015; or even earlier: Bacon et al., 2013) and possibly also for *Nama dichomtomum*. Based on ecological preferences of extant species, this can be ruled out for *Phacelia*, which today has a large distribution gap in

Central America. For this genus, only epizoochory can be invoked (Heckard, 1963; Schenk & Saunders, 2017).

Phacelia shows an amphitropical disjunct distribution and has been listed as an example of temperate disjunctions (Raven, 1963; Simpson et al., 2017; Wen & Ickert-Bond, 2009), although desert and temperate American amphitropical disjunctions (AAD) are occasionally difficult to differentiate (Simpson et al., 2017). We suggest that the case of *P. affinis* and *P. cumingii* represents a desert disjunction (dating to 8.64 Ma BP) based on current distribution ranges (see details about

AAD bioregions in Simpson et al., 2017) and divergence times. The mean divergence time of desert AAD has been found to be 5.96 Ma, whereas that of temperate AAD is 2.76 Ma (Simpson et al., 2017), which correlates to our dates for the respective clades in *Phacelia* (*P.* sect. *Euglypta* and *Miltitzia* ∼8.64, *P.* sect. *Glandulosae* ∼6.8 Ma, *P.* sect. *Phacelia* ∼3.6 Ma).

Colonization of South America by Namaceae occurred earlier, during the Oligocene-Miocene transition or later. Species of the genus *Nama* have also been listed as examples of temperate disjunctions for which LDD has been suggested (Raven, 1963; Simpson et al., 2017; Wen & Ickert-Bond, 2009). For species in Nama with continuous distributions in the Neotropics, stepwise migration is a possible explanation.

Wigandia also shows a continuous distribution range from northern Mexico to north-western South America and it is found in subtropical and tropical areas and in semiarid situations (Hofmann et al., 2016). The timing of the diversification in *Wigandia* coincides with the timing of colonization of South America during the Oligocene–Miocene transition, indicating that LDD may have to be invoked.

Luebert et al. (2017) suggested a minimum of one dispersal event in each Hydrophyllaceae and Namaceae. The present study demonstrates several dispersal events in the two families, at least three in Hydrophyllaceae and at least three in Namaceae, which took place across a wide chronological range and must be explained in the context of different biogeographic processes, with LDD as the overruling mechanism. Future research should focus on filling the sampling gaps that still exist in the phylogenies of both families and increasing phylogenetic resolution by adding more markers or employing next-generation sequencing. Most importantly, the taxonomic uncertainties, especially in South American *Phacelia* and *Wigandia*, need to be addressed in order to design an adequate sampling strategy.

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Disclosure statement

Supplemental data

Supplemental data for this article can be accessed here: https://doi.org/doi/10.1080/14772000.2020.1771471.

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