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Historical biogeography of Haloragaceae: An out-of-Australia hypothesis with multiple intercontinental dispersals

Ling-Yun Chen [a], Shu-Ying Zhao [a], Kang-Shan Mao [b], Donald H. Les [c], Qing-Feng Wang [a,*], Michael L. Moody [d]

[a] Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, Hubei, PR China
[b] Key Laboratory for Bio-resources and Eco-environment, College of Life Sciences, Sichuan University, Chengdu 610064, PR China
[c] Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269-3043, USA
[d] Department of Biological Sciences, University of Texas at El Paso, 500 W. University Ave, El Paso, TX 79968, USA

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ABSTRACT

Haloragaceae are a cosmopolitan plant family with its centre of diversity in Australia. Here, we investigate the historical biogeography of the family and the role of vicariance or dispersal in shaping its current distribution. DNA sequences from ITS, *matK* and the *trnK* 5′ and *trnK* 3′ introns were obtained for 102 species representing all 8 genera of Haloragaceae for use in Bayesian molecular dating. Molecular dating was conducted using two macrofossils as calibration points for the analyses. Biogeographic history was investigated using a Bayesian dispersal-vicariance analysis and a dispersal-extinction-cladogenesis model. The results suggest that the earliest diversification of the extant Haloragaceae occurred in Australia during the Eocene (37.3–56.3 Ma). Early diversification of the family in the Southern Hemisphere is inferred as resulting from vicariance events among Australia, South America and New Zealand. The results also indicate multiple out of Australia dispersal routes, primarily including (1) from Australia to Asia during the Miocene, with subsequent dispersal to Europe and North America; (2) from Australia to New Zealand, then to South America during the Miocene and Pliocene. Most of the inferred dispersal events occurred throughout the Miocene and later, and are biased towards the aquatic Haloragaceae lineages.

1. Introduction

The wide distributional patterns that characterise various organisms have intrigued biogeographers for hundreds of years, including Darwin, who discussed the origin of widely distributed species and factors influencing their distribution (Darwin, 1872). Although such early accounts were highly circumstantial, the development of techniques like phylogenetic reconstruction (Edwards, 1963), molecular clock analysis (Zuckerkandl and Pauling, 1965) and biogeographic analyses such as dispersal-vicariance analysis (DIVA, Ronquist (1997), and dispersal-extinction-cladogenesis (DEC) model (DEC, Ree and Smith (2008)) have provided contemporary biogeographers with more empirical approaches for studying the history of broadly distributed organisms. Today these techniques, in combination with advanced analytical methods, computational tools and a growing and more accurate fossil record have made it possible to uncover hypotheses of a multi-faceted biogeographic history for a number of widely-distributed plant taxa (e.g., Cupressaceae, Mao et al. (2012); Arecaceae, Baker and Couvreur (2013)). However, relatively few studies have presented a biogeographic history for a cosmopolitan plant group with it's centre of diversity in Australia.

Haloragaceae are a dicotyledonous family in the order Saxifragales (Stevens, 2012). It includes 8 genera and about 138 species with a cosmopolitan distribution (Moody and Les, 2007). APG III (2009) recognises either Haloragaceae s.l. (including Aphanopetalaceae, Penthoraceae and Tetracarpaeaceae) or Haloragaceae s.s. excluding these families. For clarity we will use Haloragaceae throughout to refer to the latter. The centre of distribution is Australia, where 6 of the 8 genera and about 70% of the species occur (Orchard, 1990; Moody and Les, 2007). Life forms vary widely in the family including both terrestrial (small trees, shrubs, subshrubs, and annuals) and aquatic (or semiaquatic) genera (perennial or annual herbs) (Moody and Les, 2007). Some species of the aquatic genus *Myriophyllum* are highly invasive in several countries (Moody and Les, 2002; Thum et al., 2011) including the most costly for aquatic plant management in North America (*M. spicatum*, Pimentel (2009)), whereas some also are being used widely to remediate polluted waterbodies (Ridvan Sivaci et al., 2004; Fawzy et al.,

* Corresponding author.
E-mail address: qfwang@wbgcas.cn (Q.-F. Wang).

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2012; Souza et al., 2013). The terrestrial *Gonocarpus chinensis* is of medicinal importance in China (Chen and Funston, 2008).

Although phylogenetics and evolution of floral characters and habitats of Haloragaceae have been studied thoroughly by Moody and Les (2007, 2010), historical biogeography and patterns of diversification remain poorly understood. Fossilised pollen and vegetative organs attributed to Haloragaceae have been found in Tertiary deposits of Australia, Burma, Europe, New Zealand and the Americas (Praglowski, 1970; Friis, 1979; Muller, 1981). The oldest macrofossils discovered have been from Haloragaceae in the Late Cretaceous beds of Northern Mexico (Hernandez-Castillo and Cevallos-Ferriz, 1999), while pollen deposits in the south of France have been attributed to Haloragaceae genera during this same time period. A previous study estimated the stem node age for Haloragaceae at ca. 56 Ma (Jian et al., 2008), however, the family was represented by only two species. Improved and more comprehensive divergence time estimates among Haloragaceae clades can be obtained by more inclusive sampling in combination with the incorporation of fossil calibrations (e.g., Ramirez et al., 2007; Feldberg et al., 2013).

The current cosmopolitan distribution of Haloragaceae does not reveal a clear pattern of historic biogeography. The primary diversity in the family is found in the southern hemisphere with the majority of species in Australia (Fig. 1), but lineages occur on all major land masses notably including those thought to be part of the Gondwana supercontinent. Therefore, diversification might be related to vacariance facilitated by the Gondwanan breakup. There is also extensive distribution of Haloragaceae in the Northern Hemisphere predominantly comprising *Myriophyllum*, *Gonocarpus* and *Proserpinaca*, none of which are closely related in the family, but all of which share a most recent common ancestor (MRCA) in Australia (Moody and Les, 2007). The pathway to diversification in the Northern Hemisphere may thus be out of Australia. If so, the role of vicariance through the breakup of Gondwanan land masses in the late Cretaceous or intercontinental dispersal is unclear.

In order to clarify the historical biogeography of Haloragaceae, we sampled 102 of the 138 known extant species in this family. A divergence timescale of the family was estimated using DNA sequence data from four gene regions along with two calibration points; additional analyses were performed to investigate its biogeography. Specifically, we set out to: (1) test an "out of Australia" hypothesis for extant Haloragaceae by determining when and where the family and major lineages originated geographically as well as divergence times and origins of all taxa not currently found in Australia; (2) evaluate possible vicariance or dispersal patterns in shaping the current distribution and diversification of the family.

2. Materials and methods

2.1. Taxon sampling, DNA sequencing and sequence alignment

In the present study, 102 out of the 138 species in all 8 Haloragaceae genera were sampled (see Table A. 1 in Supplementary material). All genera within the family were sampled for all species, except *Gonocarpus*, *Haloragis*, *Myriophyllum* and *Laurembergia*.

Fig. 1. Distribution of Haloragaceae. For each genus, the number of species sampled in the present study/total species number is shown.

Myriophyllum quitense, *M. sibiricum* and *Gonocarpus chinensis* were represented by two different samples representing their disjunct distribution. The number of species in each genus and their distributions are illustrated in Fig. 1. We incorporated all of the other five species of Haloragaceae s.l. (Penthoraceae, Tetracarpaeaceae, Aphanopetalaceae) as outgroups. In addition, 7 species representing 7 genera from other closely related families, Crassulaceae, Saxifragaceae and Iteaceae (Jian et al., 2008), were also included as outgroups (see Table A. 1 in Supplementary material), as more outgroups can improve discrimination power in these analyses (Graham et al., 2002).

DNA sequences of four regions, the nuclear ITS, and chloroplast *trnK* 5′, *matK* and *trnK* 3′ were collected. In total, 388 sequences were obtained from GenBank, and 69 new sequences for 16 species were generated according to the methodology in Chen et al. (2012) and Moody and Les (2010) (see Table A. 1 in Supplementary material). Sequences were aligned for each marker separately using MAFFT (Katoh et al., 2009) with E-INS-i strategy and default settings. The aligned sequences were inspected manually, and ambiguously aligned regions of ITS were excluded (Moody and Les, 2007).

An incongruence length difference (ILD) test between the nrITS and cpDNA was performed in PAUP v4.0b10 (Swofford, 2002) with 100 replicates and indicated significant differences between data partitions ($P < 0.01$). Preliminary maximum likelihood analyses were conducted for the two data sets respectively using RAxML v7.4.2 (Stamatakis, 2006) with the GTR + G model and 100 rapid bootstraps (BS). The results (not shown) indicated that incongruence exists primarily in the phylogenetic position of *Proserpinaca* and *Meionectes* which had low BS support under analyses of either data set. In general, ITS provides less resolution and low support for relationships among early diverging lineages. It has been shown that ILD can recommend non-combinability of data even where data performed better when partitions were combined (Barker and Lutzoni, 2002; Yoder et al., 2003). A number of reasons involving variation in rates of molecular evolution, as would be expected between *matK* and ITS, can lead to rejection of combinability (Barker and Lutzoni, 2002). We followed the recommendation of Moody and Les (2007, pg. 2013) in combining these data sets using separate nucleotide substitution model for nrITS and cpDNA data. The final data matrix comprised 3,102 nucleotides.

2.2. Molecular dating analyses

Molecular dating analyses were performed in BEAST package v1.7.5 (Drummond and Rambaut, 2007) using the combined data matrix. The analysis was conducted with separate models ('Unlink Subst. Models' in BEASTi) for nrITS and cpDNA. Mrmodeltest v2.3 (Nylander, 2004) was used to determine the best fit nucleotide substitution model for nrITS and cpDNA respectively. The GTR + I + G model was the best fit for each of the two data sets. A relaxed clock (uncorrelated lognormal) was selected, as preliminary likelihood-ratio test (LRT) (Huelsenbeck and Rannala, 1997) rejected the strict molecular clock hypothesis for our data ($P < 0.01$). A Yule Process tree prior with a randomly generated starting tree was used. Six independent Bayesian Markov chain Monte Carlo (MCMC) chains were run for 40,000,000 generations, sampling every 10,000 generations. The effective sample size (ESS) scores for all relevant estimated parameters were checked to ensure values above 250 using Tracer v1.5. The first 25% of generations were discarded as burn-in, and the remaining trees were combined. TreeAnnotator v1.7.5 was used to generate a summary tree including the topology, 95% credibility intervals and mean ages of Haloragaceae.

To minimise error we used a conservative approach choosing fossil calibration points following recommendations from Gandolfo et al. (2008) and Parham et al. (2012). Two fossil calibration points were accepted. (1) The extinct *Tarahumara sophiae*, representing the oldest known macrofossil record for Haloragaceae, is from the Maastrichtian-Campanian period (70.0 Ma) in northern Mexico (Hernandez-Castillo and Cevallos-Ferriz, 1999). It was sister to *Haloragis* and *Myriophyllum* in a cladistic analysis incorporating morphological characters with molecular data (Hermsen et al., 2006) and reported to have a mosaic of morphological similarities to *Haloragodendron* and *Myriophyllum*. We calibrated the crown node (Haloragaceae + Penthoraceae) using a lognormal prior (mean = 0.9, stdev = 1.0, offset = 70.0). We set the offset to correspond to the minimum age of the node, then specified the mean and standard deviation that resulted in 95% of the distribution falling between the age of the fossil and upper age of the period of the fossil found. (2) Two Iteaceae species *Divisestylus brevistamineus* and *D. longistamineus* (Hermsen et al., 2003), and one Altingiaceae species *Microaltingia apocarpelata* (Zhou et al., 2001), represent the oldest fossils of Saxifragales (Cracraft et al., 2004) represented by macrofossils from the Upper Cretaceous (ca. 90 Ma) in New Jersey (USA). Their hypothesised phylogenetic relationships were determined by Hermsen et al. (2006). However, the age of stem Iteaceae was around 35–81 Ma (Wikström et al., 2001; Bell et al., 2010; Jian et al., 2008). We defined 90 Ma as the lower boundary for the root age of our tree (offset = 90.0) with mean and standard deviation falling between the age of the fossil and upper age of the period of the fossil found (mean = 0.8, stdev = 0.9). The data set incorporated calibration points is present (see Data matrix 1 in Supplementary material).

We did not calibrate the split between Iteaceae and its close relative Saxifragaceae using the *Divisestylus* fossil, as we cannot exclude the possibility of error in specific taxonomic identification and Bell et al. (2010) suggested crown age of the core Saxifragales ca. 91 Ma. Detailed fossil information is provided in Table A. 2 (Supplementary material). The two calibrated nodes were constrained to be monophyletic.

The geologic time scale designations follow those recommended by the International Chronostratigraphic Chart 2013 (http://www.stratigraphy.org).

2.3. Biogeographic analysis

Eight operational areas (Fig. 2a) were defined for our analyses: A. North America (including Caribbean and Central America), B. South America; C. Europe (including North Africa), D. Africa, (including Madagascar), E. northern Asia; F. southern Asia (including the Malay Archipelago), G. Australia; and H. New Zealand. Distributional information was compiled from several sources (Orchard, 1975, 1990; Chen and Funston, 2008; Stevens, 2012). Haloragaceae and the outgroups Penthoraceae, Tetracarpaeaceae and Aphanopetalaceae were included in the analyses. Other outgroup families were excluded as they have poor representation and the result can be biased.

Two approaches were used for the biogeographical analyses. Firstly, a Bayes-DIVA analysis was performed using RASP v2.1b (also referred to as S-DIVA (statistical dispersal-vicariance analysis), Yu et al., 2013). To account for phylogenetic uncertainty, we inferred the distributions by integrating 2000 trees from the BEAST analysis, keeping the maximum of areas for each node at 3. Secondly we used the DEC model in LAGRANGE v. 20130526 (Ree and Smith, 2008) with the summary tree (included only Haloragaceae s.l.) resulting from BEAST analysis. We defined the DEC model using five time scales: (1) 83–70 Ma, (2) 70–45 Ma, (3) 45–30 Ma, (4) 30–5 Ma, 5–0 Ma (Mao et al., 2012; Baker and Couvreur, 2013). Dispersal rates over time among the eight geographical areas were scaled (very low or no dispersal = 0.01, low dispersal = 0.25, medium dispersal = 0.5, high dispersal = 0.75; contiguous (or close)

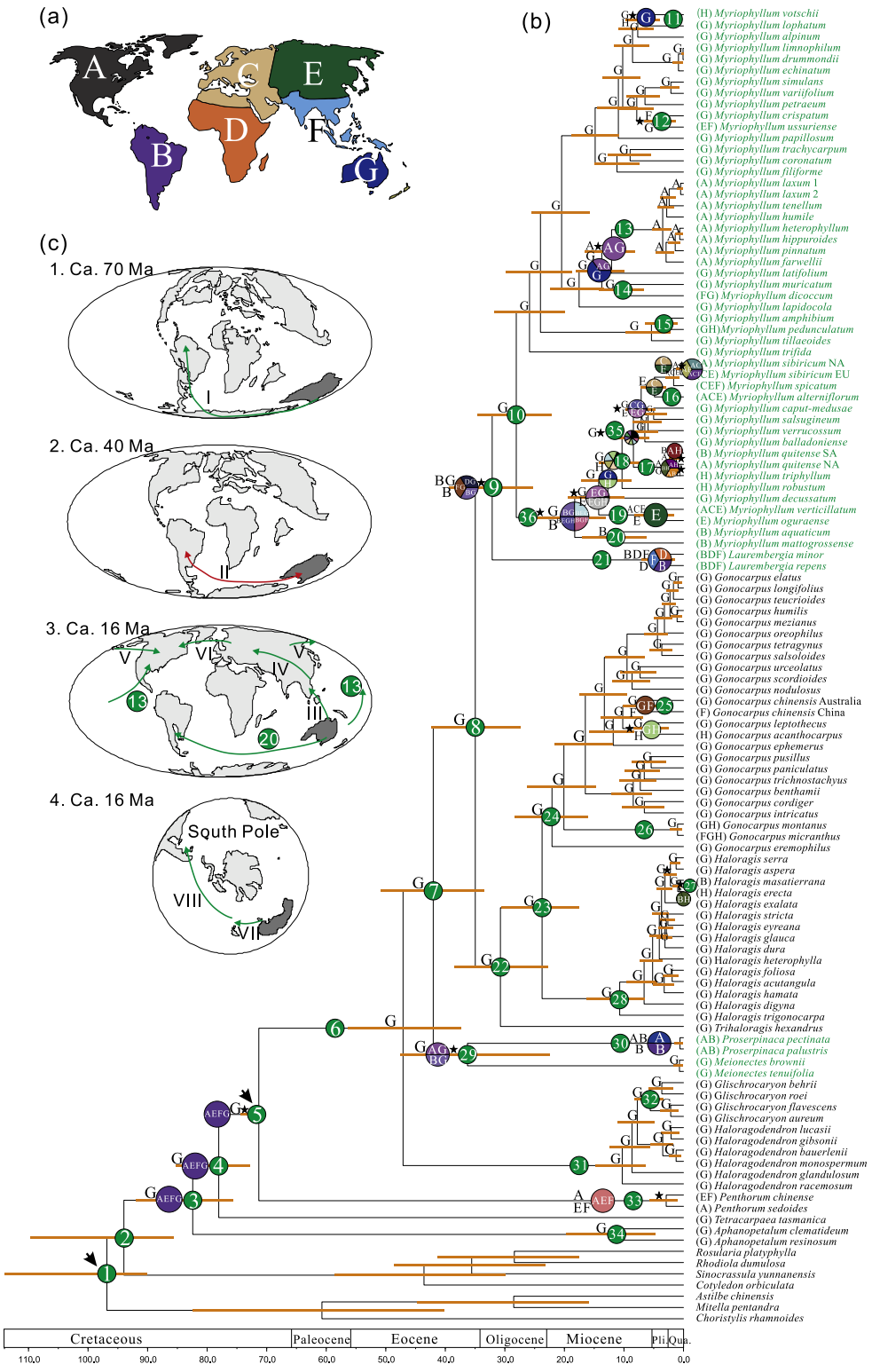

Fig. 2. Histroical biogeography of Haloragaceae. (a) Chronogram of Haloragaceae obtained under a Bayesian relaxed clock model using combined ITS + *trnK* 5′ intron + *matK* + *trnK* 3′ intron DNA sequence data. Arrows above nodes indicate the two calibrated nodes. Orange coloured bars at nodes indicate the 95% credibility intervals of age estimates. The numbers within circles on the tree refer to nodes in Table 1. Distribution for each species is listed in brackets before the species name. The characters at each node indicate the ancestral areas with the highest likelihood inferred from DEC analysis. The coloured pie charts indicate the results of Bayes-DIVA analysis which is different from DEC analysis. Dark asterisks indicate the vicariance events suggested by Bayes-DIVA. The green coloured names indicate the aquatic (or semiaquatic) genera within Haloragaceae. (b) Area delimitation in the biogeographic analysis. (c) Main dispersal routes of Haloragaceae. Maps indicate the position of plates in different periods (based on Markwick, 2011). Red arrows on maps refer to the vicariance event and green arrows refer to dispersal events of Haloragaceae discussed in the main text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

areas = 1; see Table A. 3 in Supplementary material for details). The maximum number of areas at each node was constrained at 6. In all, only the 20 most likely area combinations (AB, AC, ACE, AE, AEF, AG, BD, BDF, BF, BG, CD, CE, CEF, DF, DG, EF, EG, FG, FGH, GH) were allowed in analyses, because other area combinations (e.g., AD, ABC) do not make empirical sense (Ree and Smith, 2008) for Haloragaceae.

3. Results

3.1. Divergence time estimations

The divergence time estimation (Fig. 2b; Table 1) indicated that the MRCA of extant Haloragaceae occurred during the Eocene and Paleocene (crown node age, mean: 47.1 Ma, 95% HPD: 37.3–56.3 Ma). Haloragaceae split into two major clades: *Glischrocaryon* + *Haloragodendron* (crown node age, 95% HPD: 6.4–14.8 Ma), and *Proserpinaca* + *Meionectes* + *Myriophyllum* + *Laurembergia* + *Gonocarpus* + *Haloragis* + *Trihaloragis* (crown node age, 95% HPD: 33.5–50.9 Ma). Divergence of most lineages occurring outside Australia from an Australian MRCA occurred in the Miocene-Quaternary (Table 1; Fig. 2b).

3.2. Biogeographical analyses

The results inferred from the Bayes-DIVA and DEC are shown in Fig. 2b. Both analyses showed that extant Haloragaceae (node 6) began to diversify in Australia. All genera within the family were inferred to have their MRCA in Australia, except *Laurembergia* and *Proserpinaca*, which do not currently occur in Australia and have no known fossil record there.

In total 38 dispersal events and 16 vicariance events (Fig. 2b) were inferred from Bayes-DIVA. Vicariance and dispersal are equally probable at two nodes, viz. nodes 35 and 36. Origin of *Proserpinaca* and *Laurembergia* can be attributed to the vicariance among Australia and South America. Based on divergence times and distribution of Haloragaceae, we also can inferred some "out of Australia" dispersals (Table 1), including: (1) A dispersal of *Myriophyllum* from Australia to North America in the Miocene (Fig. 2b, node 13). (2) Dispersal during the Miocene for *Myriophyllum ussuriense* and *Gonocarpus chinensis* (nodes 12 and 25; Southeast Asia), *M. votschii* and *M. robustum* (nodes 11 and 18; New Zealand), *M. triphyllum* + *M. quitense* (node 17; New Zealand/South America), *M. alterniflorum* + *M. spicatum* + *M. sibiricum* and *M. verticillatum* + *M. oguraense* (node 16 & 19; Asia/Europe/North America). (3) Dispersal directly from Australia to South America during the Miocene was inferred for the ancestor of *Myriophyllum aquaticum* and *M. mattogrossense* (Fig. 2b, node 20).

4. Discussion

4.1. Divergence time estimation

The divergence times of extant Haloragaceae in the present study is estimated using Bayesian molecular dating with two fossil calibration points, one is based on fossils of the extinct species

Table 1
Divergence times of Haloragaceae.

Node No.	Description of node	Priors for calibration points		Divergence	
		Distribution	95% HPD (Ma)	Mean (Ma)	95% HPD (Ma)
1	Crown of all the taxa	Lognormal	90.5–99.8	96.8	90.1–114.0
2	Stem of Crassulaceae			94.0	85.6–109.7
3	Stem of Aphanopetalaceae			82.4	75.7–92.0
4	Stem of Tetracarpaceaceae			78.1	72.9–85.3
5	Stem of Penthoraceae	Lognormal	70.5–82.7	71.3	70.1–74.4
6	Crown of Haloragaceae.s.s.			47.1	37.3–56.3
7	Stem of *Proserpinaca* + *Meionectes*			42.0	33.5–50.9
8[a]	Crown of (*Myriophyllum*, *Laurembergia*, *Gonocarpus*, *Haloragis*, *Trihaloragis*)			35.0	27.4–42.4
9	Stem of *Laurembergia*			32.1	25.3–39.4
10[a]	Crown of *Myriophyllum*			28.1	22.1–34.5
11[a]	Split: *M. lophatum and M. votschii*			6.8	3.9–9.7
12[a]	Split: *M. crispatum and M. ussuriense*			3.6	1.3–6.6
13[a]	Stem of North American endemic *Myriophyllum* species clade			8.6	8.1–16.6
14[a]	Split: *M. muricatum and M. dicoccum*			10.2	6.6–14.1
15[a]	Split: *M. amphibium and M. pedunculatum*			3.3	1.0–6.4
16[a]	Crown of (*M. spicatum, M. sibiricum NA, M. sibiricum EU, M. alterniflorum*)			4.9	3.0–7.3
17	Crown of (*M. quitense SA, M. quitense NA, M. triphyllum*)			2.0	0.8–3.7
18[a]	Stem of *Myriophyllum robustum*			10.3	7.1–14.2
19[a]	Crown of *M.verticillatum* + *M. oguraense*			4.2	1.6–8.2
20	Crown of *M.aquaticum* + *M. mattogrossense*			11.4	6.3–17.1
21	Crown of *Laurembergia*			3.7	1.4–7.0
22	Split: *Trihaloragis hexandrus and* (*Gonocarpus* + *Haloragis*)			30.7	22.8–38.5
23	Split: *Gonocarpus and Haloragis*			23.8	17.6–30.5
24	Crown of *Gonocarpus*			22.1	16.1–28.4
25[a]	Split: *G. chinensis* China and *G. chinensis* Australia			6.2	2.7–10.1
26[a]	Split: *G. montanus* + *G. micranthus*			1.0	0.3–2.3
27	Split: *Haloragis masatierrana* + *H. erecta*			1.0	0.3–1.9
28	Crown of *Haloragis*			10.7	6.6–16.3
29	Crown of *Proserpinaca* + *Meionectes*			36.3	22.5–47.6
30[a]	Crown of *Proserpinaca*			0.6	0.1–1.5
31	Crown of *Haloragodendron* + *Glischrocaryon*			10.3	6.4–14.8
32	Crown of *Glischrocaryon*			5.6	3.4–8.2
33	Crown of *Penthorum*			2.9	1.0–5.8
34	Crown of *Aphanopetalum*			11.0	4.7–19.6
35[a]				8.4	5.8–11.7
36[a]				18.3	13.1–24.6

Node numbers refer to nodes in Fig. 2a.
[a] Indicate the nodes where out of Australia dispersal occurred.

Tarahumara sophiae, one is based on fossils of *Divisestylus brevistamineus*, *D. longistamineus* and *Microaltingia apocarpelata*. In the phylogeny of Saxifragales (Jian et al., 2008), which incorporated two Haloragaceae genera and several well-characterised fossil relatives of the family, the crown age of Haloragaceae was estimated to be ca. 20 Ma. Our results support a crown age of extant Haloragaceae to be 37.3–56.3 Ma, which is earlier than that suggested by Jian et al. (2008). However, both our estimates and those of Jian et al. (2008) provide a fairly narrow range of probable earliest divergence time for Haloragaceae.

The stem node ages of *Myriophyllum* and *Haloragis* in this study (95% HPD: 22.1–34.5 and 6.6–16.3 Ma, respectively) were estimated using more taxa and are earlier than those (ca. 20 Ma) from Jian et al. (2008) which only included a single species from these two genera to represent the family. The ages of the two genera estimated by our analyses were younger than the oldest fossil pollen attributed to them, which were recorded from the Upper Paleocene and Lower Eocene of the Paris basin respectively (Gruas-Cavagnetto and Praglowski, 1977). Gandolfo et al. (2008) suggested caution should be used in utilizing fossil pollen alone for occurrence of taxon, given the few characteristics available and lack of taxonomic specificity. Muller (1984) also noted taxonomic identification to extant lineages can become ambiguous the older the age of the pollen fossil. Notably pollen fossils associated with the well preserved extinct *Tarahumara* macrofossil were also determined to be similar to *Haloragis* or *Myriophyllum* (Hernandez-Castillo and Cevallos-Ferriz, 1999), further suggesting caution in attributing pollen fossils of Haloragaceae to specific extant lineages.

Additionally, we have conducted preliminary BEAST analysis with four fossil calibration points, viz. two points based on *Haloragagis* and *Myriophyllum* separately (stem node *Haloragis*: 41.6–51.8 Ma; stem node *Myriophyllum*: 56.7–65.1 Ma), as well as the two points mentioned in Materials and methods section (see Data matrix 2 in Supplementary material). This resulted in the mean age of the root to be 159.3 Ma (95% HPD: 125.3–200.0 Ma) (Fig. A. 1 in Supplementary material), which is much earlier than the crown node age of the core Saxifragales (ca. 90 Ma) accepted in previous studies (Bell et al., 2010). Thus, we chose not to use the pollen fossil dates for calibration of *Myriophyllum* and *Haloragis* stem nodes in our final analyses.

With the two fossil calibration points, based on well characterised marofossils, our results are also congruent with other lines of evidence: (1) The root of all the taxa (Fig. 2b, node 1) in our analysis was supported at ca. 96.8 Ma, consistent with the age (ca. 98 Ma) suggested by Jian et al. (2008); (2) The stem node age of *Proserpinaca* was 22.5–47.6 Ma (95% HPD), which is consistent with its oldest fossil from the Oligocene (Friis, 1979), (3) The divergence between *Penthorum sedoides* and *P. chinense* (Fig. 2b, node 33) was 1.0–5.8 Ma (mean age: 2.9 Ma), consistent with the age of 4.21 Ma obtained from allozyme data (Lee et al., 1996).

4.2. Biogeographical patterns

Our biogeographic analyses indicated that the MRCA of the extant Haloragaceae occurred in Australia during the late Cretaceous, when there was still a connection between southern land masses (notably Australia, Antarctica, New Zealand and South America). Some diversification of lineages, especially in the South Hemisphere can be attributed to vicariance with the subsequent breakup of these southern land masses.It is clear dispersal events out of Australia also played an important role in the current distribution of the family and that these occurred multiple times primarily throughout the Miocene and later (Fig. 2b; Table 1). We were able to reconstruct several highly probable vicariance events and dispersal routes out of Australia, which have shaped the current distribution of this family.

4.2.1. Vicariance and dispersal: pre-oligocene

Biotic exchanges between Australia and South America via Antarctica during the Late Cretaceous through the Eocene are believed to have occurred commonly (Briggs, 1995; Morley, 2003; Almeida et al., 2012). However, it must be noted that full division between Australia and South America, which was facilitated by the changing climate that eliminating the connection through the Antarctic passage, did not occur until ca. 34 Ma (Barker et al., 2007; Thorn and DeConto, 2006). The oldest Haloragaceae fossils were reported from ca. 70 Ma in Northern Mexico and the Paleocene of Europe (Hernandez-Castillo and Cevallos-Ferriz, 1999; Praglowski, 1970; Gruas-Cavagnetto and Praglowski, 1977), whereas the MRCA of extant Haloragaceae was suggested to have occurred in Australia during the Eocene. Thus, as has been hypothesised for other Angiosperm taxa such as *Fuscospora* (Knapp et al., 2005) and *Peumus*/*Palmeria* (Renner et al., 2010), Haloragaceae ancestors might have moved across this passage between the now well-separated continents (Fig. 2c–1, arrow I). However, the extinct *Tarahumara* fossil may represent an ancestral Haloragaceae rather than an extant genus, as was also postulated by Hernandez-Castillo and Cevallos-Ferriz (1999).

Continental isolation occurred after the divergence of the only two Haloragaceae genera not occurring in Australia, *Proserpinaca* and *Laurembergia*, which have stem nodes dating to ca. 36.3 and 32.1 Ma respectively. *Proserpinaca* is now distributed and widespread in eastern North America with highly localised South American populations in Colombia and southeastern Brazil (Fig. 1; Wood, 1972; Moody and Les, 2007). However, the genus had a much wider past distribution, as evidenced by the documentation of macrofossils from as early as the Oligocene (Mai, 1989; Hernandez-Castillo and Cevallos-Ferriz, 1999). The stem node age is consistent with vicariance hypothesis for the current distribution of these taxa as supported by Bayes-DIVA (Fig. 2c–2, arrow II).

Laurembergia is found throughout much of South America, but also Africa and Southern Asia, suggesting a possible historical vicariance origin similar to *Proserpinaca*, but remaining primarily in the southern hemisphere (Fig. 1). The modern continent South America and Africa have been separated by oceans since at least ca. 105 Ma (Davis et al., 2002). Morley (2003) noted several apparent dispersals between the continents among Angiosperm lineages dating mostly through the Eocene based on fossil pollen. Renner (2004) also attributed trans-Atlantic disjunctions at genus level to dispersals. More recent phylogeographic studies support this as a likely route to explain distributions similar to that of *Laurembergia* (e.g., Richardson et al., 2004; Dick et al., 2007). Morley (2003) postulated this pattern as attributable to remnants of the Walvis Ridge/Rio Grand Rise creating stepping-stones between Brazil and Angola through the late Eocene. The ancestral divergence time and current distribution of *Laurembergia* correspond with this time scale.

4.2.2. Out of Australia: Oligocene-Quaternary dispersals

4.2.2.1. Australia to North America in the Miocene. Australia is distant from North America, and the continents have not been adjacent since the establishment of Pangaea (Markwick, 2011). Relatively few studies have addressed dispersal of plant species directly between North America and Australia, with the exception of several aquatic lineages (Les et al., 2003), although numerous studies have reported dispersal between Asia and North America (e.g., Lu et al., 2011; Xiang et al., 2000; Romaschenko et al., 2013) and Asia and Australia (e.g., Sniderman and Jordan (2011), Baker and Couvreur (2013)). Our results clearly indicate a disjunct distribution from Australia to North America sometime during the

Miocene, which involved the North American endemic *Myriophyllum* subsection *Spondylastrum* (Fig. 2b, node 13; Fig. 2c–3, arrow 13).

However, Les et al. (2003) noted that the most likely route for this disjunction found for multiple aquatic plant lineages was through the Southeast Asia Bering Sea area via bird dispersal, as the distance between North America and Australia was too far for any plausible dispersal agent. While it is conceivable that the many islands that have existed in the Pacific basin since the Oligocene, e.g., the Hawaiian archipelago, Fiji islands (Price and Clague, 2002; Howarth et al., 2003), could have facilitated migration between Australia and North America (Harbaugh et al., 2009), the lack of any relict populations over this great distance make this route improbable.

4.2.2.2. Australia to Asia, and from Asia to Europe and North America. At least five dispersal events to Asia from an Australian MRCA have been revealed from our results (Table 1, Fig. 2c-3, arrow III) including *Myriophyllum dicoccum* (Fig. 2b, node 14), *M. ussuriense* (Fig. 2b, node 12), *Gonocarpus micranthus* (Fig. 2b, node 26) and *G. chinensis* (Fig. 2b, node 25), all occurring no earlier than the mid-Miocene. These events come well after the emergence of the Malay Archipelago from the Early Miocene which likely plays an important role for dispersal in the region (van Welzen et al., 2005; Hall, 2009). Dispersal dates between Southeast Asia and Australia are becoming well documented, often from the late Miocene or later, for other groups (e.g., *Alocasia*, Nauheimer et al. (2012); *Cucumis*, Sebastian et al. (2010); Simaroubaceae, Clayton et al. (2009)), but in most cases the opposite direction (Asia to Australia). This result provides strong evidence for these unusual long distance dispersals in the Miocene and later from Australia to Asia.

The Bering Land Bridge (BLB), which was present from at least the early Paleocene until ca. 7.4–4.8 Ma (Marincovich and Gladenkov, 1999; Tiffney and Manchester, 2001), and the North Atlantic Land Bridge (NALB), which connected North America and Europe from the Paleocene to Miocene (Denk et al., 2010) have long been considered as major dispersal routes between the northern continents. This route has been suggested for many aquatic lineages (Les et al., 2003) and most likely facilitated dispersal of the circumboreal *Myriophyllum* (*M. alterniflorum*, *M. sibiricum* (Fig. 2b, node 16) and *M. verticillatum* (Fig. 2b, node 19)) from Eurasia to North America via the BLB and/or NALB (Fig. 2c–3, arrow V and VI) following earlier Miocene dispersion out of Australia.

4.2.2.3. Australia to New Zealand, and to South America. Several Haloragaceae are endemic to New Zealand (*Myriophyllum votschii*, *M. propinquum*, *M. triphyllum*, *M. robustum*, *Gonocarpus aggregatus*, *G. incanus*, *Haloragis erecta*), and others (*M. pedunculatum*, *G. micranthus*, *G. montanus*) exhibit an Australia–New Zealand disjunct. Our results support divergence of the New Zealand taxa sampled here from the lower Miocene through the Pliocene due to multiple independent dispersal events from an Australian MRCA. This directional dispersal from Australia to New Zealand is well documented and generally recognised as more frequent than in the reverse direction (Sanmartin et al., 2007; Crisp et al., 2009). It is widely accepted that New Zealand broke away from Australia ca. 80 Ma (Sanmartin and Ronquist, 2004), therefore, the New Zealand taxa likely originated from a trans-Tasman dispersal of ancestors from the Australia continent (Fig. 2c-4, arrow VII). This dispersal route may have been facilitated by the eastward Antarctic Circumpolar Current (ACC) and West Wind Dispersal (WWD), which has flowed clockwise around Antarctica since the Miocene and has been hypothesised as a mechanism for out of Australia dispersal (Barker and Burrell, 1982; Winkworth et al., 2002; Sanmartin et al., 2007). This is supported by lack of evidence of dispersal in the reverse direction (from New Zealand to Australia) for any Haloragaceae in this study, which would have to occur against the direction of this current.

Alternatively, Winkworth et al. (2002) hypothesise that birds are the most likely dispersal agent of plant propagules in this region. The Australia to New Zealand bias could then be explained by the availability of habitats for establishment, as new habitats would have been more prominent in New Zealand due to environmental instability during the late tertiary and early Quaternary. Importantly, the role of the ACC and WWD in facilitating plant dispersal is dependent on dispersal mechanisms (Winkworth et al., 2002; Sanmartin et al., 2007). Wind or oceanic dispersal, are not easily accommodated by *Myriophyllum* or *Gonocarpus* fruit or seed morphology, although slightly winged fruit are found for *Haloragis erecta*. If birds are the likely dispersal vector, wind currents would affect flight patterns (Sanmartin et al., 2007), thus WWD in combination with bird dispersal could have a role. As more evidence accrues a better understanding of the bias of dispersal from Australia to New Zealand rather than the reverse direction is developing.

Myriophyllum aquaticum, *M. mattagrossense*, *M. quitense* and *Haloragis masatierrana* are native to South America, although the latter is not found on the mainland, but is endemic to the Juan Fernandez Islands off the west coast of Chile (Sanders et al., 1982; Ceska et al., 1986; Moody and Les, 2007). The two later species are sister taxa of the New Zealand endemic *M. triphyllum* and *H. erecta* respectively and we inferred that the split of all these taxa from an Australian MRCA occurred during the Miocene (Fig. 2b, nodees 17 and 27). Our results are consistent with an eastward dispersal for the latter two taxa from Australia to New Zealand with subsequent migration to South America during the Pliocene (Fig. 2c-4, arrow VII and VIII). For *H. masatierrana* this likely involved a stepping stone model through South Pacific Islands as *H. stokesii* (not sampled here) from Rapa Island (part of the Austral Islands located about midway between New Zealand and the Juan Fernandez Islands) (Orchard, 1975). For the *Myriophyllum* disjuncts we lack evidence for stepping stones as there are no records of *Myriophyllum* from the South Pacific Islands. Les et al. (2003) invoked long distance dispersal for *M. aquaticum*, which can't be rejected here and has been reported for other taxa with similar divergence times and distribution (e.g., *Nothofagus*, Knapp et al. (2005); Monimiaceae, Renner et al. (2010)).

A general pattern of dispersal from Australia or New Zealand throughout Pacific islands is becoming better understood using phylogeographic techniques (Keppel et al., 2009; Harbaugh et al., 2009) with successful plant dispersal between New Zealand and South America during the Miocene and Quaternary well documented (Winkworth et al., 2002; Sanmartin et al., 2007; Crisp et al., 2009). For example, Harbaugh and Baldwin (2007) found that several events have led to *Santalum* dispersal out of Australia, mostly more recent than 1.5 Ma, including the dispersal of *Santalum fernandezianum* in the Juan Fernandez Islands from Australia. While ACC or WWD may have a role in New Zealand to South America dispersal, Sanmartin et al. (2007) found attribution of dispersal to ACC or WWD equivocal. Bird dispersal is considered a more likely vector for much of the South Pacific (Carlquist, 1996; Winkworth et al., 2002; Sanmartin et al., 2007; Harbaugh and Baldwin, 2007).

4.2.3. Terrestrial vs. aquatic lineage dispersal

Dispersal out of Australia for Haloragaceae is biased to the aquatic lineages. Only three dispersal events involving terrestrial taxa out of Australia are recognised here (*Gonocarpus chinensis*, *G. micranthus*, *Haloragis erecta* + *H. masatierrana*). This is particularly remarkable given that there are nearly equal numbers of terrestrial and aquatic species in the family (Moody and Les, 2007). The general bias of widespread distribution of aquatic plants has been recognised since de Candolle (1855) and Darwin (1872). Schenck

(1886) attributed this to a uniformity of habitat at a greater geographic scale than terrestrial habitats. Others have attributed this distribution to the high level of bird dispersal among aquatic plants (Sculthorpe, 1967; Hutchinson, 1975; Les et al., 2003).

Les et al. (2003) in a comprehensive biogeographical study of discontinuous aquatic plants concluded that most of the disjunct aquatic lineages in their study were due to long distance dispersal and the best explanation for this was bird dispersal. In this study we find that most dispersal events were well after the final break-up of the southern continents, although dispersal via the Australia–Antarctica–South America connection is likely for three aquatic lineages. Therefore, most dispersal out of Australia was long distance. The aquatic lineages do not have fruit or seed that is conducive to wind dispersal as fruits lack wings and seeds are retained in small nuts that break into nutlets in *Myriophyllum* generally falling directly into the water (Orchard, 1975), but *Myriophyllum* and *Proserpinaca* fruits are also eaten by waterfowl (Sculthorpe, 1967). While other dispersal agents may have a role, long distance dispersal by birds likely was significant in forming the dispersal patterns we see in the family.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.04.030.

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