Age and origin of enigmatic megaherbs from the subantarctic islands

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Biogeographic relationships in the southern hemisphere have puzzled biologists for the last two centuries[1,2]. Once joined to form the supercontinent Gondwana, Africa, Antarctica, Australia, New Zealand and South America are widely separated by the Pacific and Indian oceans. Sir Joseph Hooker was the first to suggest that Antarctica served as a corridor for plant migration not unlike the land-bridges in the northern hemisphere[7,8,9,10]. While the Antarctic flora was largely erased by glaciation during the Pleistocene, at least some of these Antarctic plant communities found refuge on the subantarctic islands. Here we provide support for the hypothesis that giant herbs persisted in the subantactic islands prior to the onset of Pleistocene glaciation, then dispersed northward in response to glacial advance. Our findings provide further evidence that Antarctica has played a pivotal role in shaping southern hemisphere biogeography.

During the early 1800s voyages of discovery brought many explorers to the southern oceans. Much of the early botanical work was completed by European taxonomists whose interpretations were influenced by their familiarity with the Old World flora[1,2]. They thought southern hemisphere plants had diversified from northern hemisphere ancestors, and this has likely been the case in large genera such as *Veronica*, *Ranunculus* and *Epilobium*[3,4]. However, some plant groups such as *Nothofagus*[5] and

Agathis[6] have evolved in the southern hemisphere then dispersed in a northerly direction.

The floras of North America and Eurasia were united by land bridges that existed during much of the Tertiary[7,8]. However, the southern hemisphere continents have been isolated by the southern oceans since the break-up of Gondwana at the end of the Cretaceous. During much of the Tertiary the now widely separated southern continents – South America, Africa, Australia and New Zealand – faced a continuous Antarctic coastline that was clothed in diverse forest vegetation. The abundance of fossils in Antarctica suggests that it may have served as a corridor for plant migration until late in the Tertiary[9,10]. The environmental conditions were very different at this time. The climate was much warmer, and because of their positions at high latitudes, Antarctic ecosystems experienced long periods of complete darkness followed by short growing seasons with almost continuous but low-angle daylight. These environmental conditions supported diverse open woodland vegetation[10]. The Antarctic ice cap grew from the Oligocene (35 Myr) culminating in several episodes of glaciation during the Pleistocene. During major glaciations the polar ice sheets spread considerably and temperature, marine and vegetation zones were compressed towards the equator[8]. During the glacial maxima the Antarctic vegetation was almost completely eliminated, though surely a few remnants of this once lush Antarctica found refuge to the north in isolated circum-Antarctic island archipelagos.

The massive Antarctic ice sheets that persist to the present day have imposed a limit on our ability to interpret past environments before their formation and during their repeated waxing and waning[11]. Given the increasing interest in the role the subantarctic islands have played in southern hemisphere biogeography, there is a growing need for phylogenetic studies of taxa endemic to these islands. Such analyses

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can partly overcome the problems of interpretation that arise from extinctions associated with past glaciation events[12,13].

The subantarctic islands fall into two groups, older islands with a continental origin whose biota is shared by nearby land masses and younger volcanic islands, where the history of the biota remains enigmatic[12]; the New Zealand subantarctic islands fall within this latter category. The New Zealand subantarctic islands were formed by volcanic activity during the Tertiary, but interbedded limestone and basement rocks are found in restricted areas[14]. The islands now lie in a region of cool surface waters, and the weather is dominated by strong westerly winds, cool temperatures, persistent cloudiness and high rainfall. In the Tertiary the winds and circum-polar current were not as strong and dispersal by windblown propagules north to the islands was more easily accomplished[15]. Nutrient inputs are mostly marine in origin from nesting seabirds, penguins and seals and this coastal eutrophication has occurred at least for the past 10,000 years[12]. Such nutrients, long summer day-lengths, and adequate water lead to high primary production in the subantarctic islands. In association with anaerobic conditions this has created extensive peat deposits. The New Zealand subantarctic islands were granted World Heritage status by UNESCO in 1998 because they encapsulate a diverse set of natural heritage values, many of which are unique[16].

These natural heritage values were already recognized by early naturalists. Sir Joseph Hooker was struck by the unique vegetation particularly the extensive meadows dominated by lush perennial herbs with large leaves. He wrote in his notes on the Ross Expedition[17]: "The most extraordinary of the megaherbs is the *Pleurophyllum* meadow, a community dominated by the large-leafed herbaceous composite, producing a floral display second to none outside the tropics." Megaherbs have adapted not only to the harsh climate and short growing season, but also to the highly acidic, peaty soil. The megaherb growth habit has evolved independently in diverse species from a range of

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different genera and families such as *Gunnera*, *Plantago, Stilbocarpa* and the composite *Pleurophyllum* that was admired by Hooker (see above).

We tested the hypothesis that the endemic genus *Pleurophyllum* is a geographically isolated relict of a once lush Antarctic flora that then dispersed northward in response to glacial advance and survived the LGM in refugia on the subantarctic islands of New Zealand. *Pleurophyllum* is a genus of three species, restricted to Auckland, Campbell, Antipodes and Macquarie islands. In some areas on Campbell Island, *P. speciosum* with its large coriaceous leaves is the dominant life form. The other two species (*P. criniferum* and *P. hookeri*) often grow sympatrically. *Pleurophyllum speciosum* forms an enormous rosette, up to 1.2 m across, of huge, broad pleated leaves. Its spectacular capitula, about 6 cm in diameter (including the long ray-florets), are arranged in subcorymbose racemes, about 60 cm tall. The flowers (Fig. 1) show considerable colour variation, probably due to the mineral content of the soil or the degree of acidity. The colouration seems to vary from bluish pink to pink and even white. The other two related species, *P. hookeri* and *P. criniferum*, with scapes up to 60 cm and 2 m respectively, have silvery lance-shaped leaves and maroon florets. Their ray-florets are very inconspicuous. The two may be distinguished by the narrow smooth silvery leaves in *P. hookeri* being pointed and erect, compared with the broad rounded heavily veined greenish leaves of *P. criniferum* that form a rosette close to the ground.

Previous researchers have suggested that *Damnamenia* and *Olearia* are closely related to *Pleurophyllum*[18,19]. The monotypic genus *Damnamenia*, a segregate from *Celmisia*[18], is also endemic to Campbell and Auckland islands. In alpine scree, immense drifts of *D. vernicosa* carpet the ground. It grows to 35 cm tall and the capitula are 5 cm wide. *Olearia* is Australasia's largest genus of Compositae. Drury[19] has already pointed out that one group of *Olearia*, the macrocephalous olearias [*O. chathamica*, *O. colensoi, O. lyallii*, *O. oporina* (incl. *O. angustifolia*), and *O. semidentata*] are more similar to

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Pleurophyllum than to other olearias. All members of the group are characterized by terminal, solitary or racemose capitula, long villose achenes, subduplex subrufescent pappus, and large, flat woolly haired leaves. Their floret colour, leaf venation and leaf hair-type also distinguishes them from the other olearias. With the exception of *O. colensoi*, all of them occur in the southern part of New Zealand (Fig. 2): *O. lyallii* is restricted to the Snares and Auckland islands, *O. semidentata* and *O. chathamica* to the Chatham Islands, and *O. oporina* to Stewart Island and southernmost South Island. *Olearia colensoi* grows in montane to subalpine scrub from lat. 38° southwards (North, South, Stewart and Solander islands), descending to sea level in the southernmost part of its range.

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An analysis of the phylogeny of the large woody Australasian genus *Olearia* based on ITS data[20] demonstrated the polyphyly of the genus and also provided some support for each of the above conclusions, in that the sole macrocephalous *Olearia* species included in the study, *O. chathamica*, was placed with *Pleurophyllum*, and *Damnamenia* was also included in the clade.

Our analysis of the ITS and ETS sequences is that these are largely congruent. This suggests the two data sets are converging on the same evolutionary tree. The ETS sequences provide strong support for a clade comprised of *Damnamenia*, *Pleurophyllum* and the macrocephalous *Olearia* species (bootstrap 92%) whereas this relationship is not recovered by ITS sequences. The analysis of the combined data provides even greater support and resolution (bootstrap 96%). Maximum likelihood analysis of the combined data set (Fig. 3) again recovered the clade comprised of *Damnamenia*, *Pleurophyllum*, and the macrocephalous *Olearia* species (the macrocephalous clade, Fig. 2). *Damnamenia* is sister to the other members of this clade. The three species of *Pleurophyllum* form a clade (bootstrap 64%), as do *Olearia angustifolia* and *O. oporina*

(bootstrap 100%), *O. semidentata* and *O. chathamica* (bootstrap 99%), and *O. colensoi* and *O. lyallii* (bootstrap 100%).

Our results reveal significant rate variation across the lineages: the annual species *Senecio vulgaris* is evolving at a faster rate than the arborescent *Dasyphyllum dicanthoides*. We therefore used a rate-smoothing procedure to transform the maximum likelihood tree. We calibrated the rate for the combined analyses using a fossil date of 38 million years, which represents the stem age of the Asteroideae in our analysis (Fig. 4). The mean substitution rates estimated for *Pleurophyllu*m are similar to those calculated for *Abrotanella*[21], *Robinsonia*[22] and *Dendroseris* [23] despite the different methods calibration (see Table 1). However they differ substantially from the mean value for 21 angiosperm families calculated by Kay *et al*.[24]. They note that long-lived woody plants appear to have a slower substitution rate; the fastest rate was recorded in *Gentianella*, whereas the slowest rate was in the Winteraceae. Andreasen and Baldwin[25] noted a similar pattern in which the nucleotide substitution rate was faster in annual species of *Sidalcea* than in perennial species, and the substitution rates in ITS and ETS, though different, were correlated.

Our findings suggest that the stem age of many lineages of Asteraceae predate the late Tertiary extinction of plants in Antarctica; similar results were reported by Kim *et al*.[26]. The combined maximum likelihood analysis suggests the *Damnamenia*, *Pleurophyllum*, and macrocephalous *Olearia* clade diverged during the mid-Pliocence about 4.1 Myr ago. The crown radiation occurred at the boundary between the Pliocene and Pleistocene. Independent analyses of the ITS and ETS sequences provided similar divergence estimates. The late Tertiary was a time of intense environmental upheaval in New Zealand brought about by the uplift of the Southern Alps, glaciation, and volcanism[27]. Glaciers nearly completely covered the Auckland Islands and a considerable portion of Campbell Island during the last glacial maximum, while

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Macquarie Island remained unglaciated[14]. The retreat of the glaciers on Auckland and Campbell islands occurred around 15,000 years ago. Pollen profiles from post-glacial peat profiles indicate megaherbs such as *Pleurophyllum* were present in abundance at the end of the last glaciation maximum (LGM) and survived close to the glacial terminus.

The ancestors of megaherb clade must have survived the LGM in the New Zealand subantarctic islands. There has subsequently been at least one instance of dispersal to the mainland of New Zealand and to the Chatham Islands, about 800 km to the east of New Zealand. There are a number of equally plausible area reconstructions, provided our phylogenetic inference is correct. Most of these involve either several independent dispersal events from the subantarctic islands to the mainland of New Zealand, Stewart Island and the Chatham Islands or dispersal in a more stepwise fashion from the New Zealand subantarctic islands to the mainland, and then subsequent dispersal from the New Zealand mainland to the Chatham Islands and back to the New Zealand subantarctic islands.

The megaherb growth form is not unique to the subantarctic islands and has evolved independently in several distinct lineages of flowering plants. The large-leaved buttercup *Ranunculus lyallii* is common on the mainland of New Zealand and *Mysotidium hortensia*, a large forget-me-not, is endemic to the Chathams Islands. Elsewhere the Chilean *Gunnera*, the Hawaian silverswords, *Argyroxiphium*, and the Kenyan *Dendrosenecio* and *Lobelia* are notable examples. However, the megaherbs of the subantarctic islands are unique with corrugated leaves, stereome tissue, hairy and occasionally coriaceous lamina, a rosette growth form, fleshy root system and colourful flowers[28]. The megaherb growth form may confer a selective advantage in subantarctic environments[28]. Nutrient availability may be limiting as it varies most with moisture-holding capacity and the acidity of the peat. Large leaves may intercept nutrients from

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marine aerosols channelling nutrients directly to the stem base and roots. The large overlapping leaves could act as solar panels focusing radiant energy towards the growing apex of the plant. Hence the detrimental effects of cold temperatures and decreases in wind speed would be moderated with the rosette growth form further reducing reductions in water loss by transpiration. Light is also limiting and the big leaves of *Pleurophyllum* suppress competitors with their dense and compact growth form.

Present biogeographic interpretations of the Asteraceae neglect to consider the importance of Antarctica as a corridor for migration during the early diversification of the family[29]. The northern hemisphere circumboreal flora was once linked by land bridges during the Tertiary. We would like to suggest a similar scenario of a circumantarctic flora that flourished until the late Tertiary. Pulses of dispersal occurred to the north during glacial advance with retreat and intermingling during interglacial periods. Unlike the northern hemisphere, the circum-Antarctic vegetation was nearly completely eliminated during the last ice age. The distinctive flora of the subantarctic islands may harbour some of the last remnants of this once diverse flora, and plants such as *Pleurophyllum* may be the key to resolving this puzzle.

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METHODS

We included sequences from the three species of *Pleurophyllum*, *Damnamenia vernicosa* and representatives of all of the macrocephalous species of *Olearia*. More distant outgroups were five representatives from most of the other tribes of Asteroideae recognized by Bremer[30] with greater sampling within tribe Astereae. We rooted the analysis on the long branch leading to *Dasyphyllum*, which is placed in subfamily Barnadesioideae.

Voucher information along with GenBank (http://www.ncbi.nlm.nih.gov) accession numbers is detailed in the Appendix. The complete data sets are available on request from the first author and were deposited in TreeBASE (http://www.treebase.org/treebase) study accession number = ????? and matrix accession numbers = ??????.

DNA extraction, amplification and sequencing. Total DNA was extracted from leaves following a modification of the CTAB method of Doyle and Doyle[31]. The amplification and sequencing procedure for the ITS region has been described previously[32]. We also amplified the ETS region following the procedure of Baldwin and Marcos[33]. DNA samples were labelled with fluorescent dyes (Big Dye Chemistry) and then sequenced by the Allan Wilson Centre (Massey University) DNA sequencing facility. In all instances both the forward and reverse DNA strands were sequenced.

Data analysis. The sequences were initially aligned using ClustalX[34] and gaps were inserted in the data matrix. The resulting alignments were then visually inspected and minor changes were made manually to ensure positional homology prior to the phylogenetic analyses. The phylogenetic analyses were accomplished using PAUP* version 4.0b10 [35] with both parsimony and maximum likelihood selected as optimality criteria. The parsimony analysis was conducted with the PAUP* settings TBR branch-swapping, MULPARS, RANDOM ADDITION with 1,000 replicates. Duplicate trees were eliminated using the condense trees option collapsing branches with a maximum length of zero. The characters were unordered and equally weighted and gaps were treated as missing data. The most appropriate maximum likelihood model and parameter estimates were determined by the Akaike Information Criterion test (AIC) implemented in Modeltest vers. 3.06 [36]. Congruence of the data matrices was assessed using the ILD test of Farris *et al*. [37,38] with 100 data partition replicates with uninformative positions deleted/excluded [39,40,41]. Support for clades was estimated by bootstrap analyses[42] with

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1,000 replications excluding uninformative sites; starting trees were obtained by RANDOM ADDITION with one replication for each bootstrap replication, TBR branch-swapping, MULPARS in effect and a MAXTREE limit of 1,000.

The likelihood ratio test (LR = −2 log LR; where LR is the difference between the –ln likelihood of the tree, with and without enforcing a molecular clock and the X2 distribution, with *n*–2 degrees of freedom, where *n* is the number of taxa) was used to determine whether the data satisfied the assumptions of a molecular clock[43]. In the absence of a molecular clock, we used the semiparametric rate smoothing by penalized likelihood approach to accommodate rate heterogeneity across lineages[44,45]. This procedure is implemented in the program r8s[46] and uses a likelihood model combined with a smoothing parameter estimated by cross-validation to estimate divergence times. The checkGradient command was implemented to provide an additional assessment of the divergence estimates. We calculated substitution rates for each gene and for the combined data matrix using a calibration date of 38 Myr BP determined from the fossil record[47,48]. In our analysis this is the split between subfamily Barnadesioideae and the remaining members of the Asteraceae and represents the stem age of subfamily Asteroideae. Confidence intervals were calculated by applying this procedure to 100 bootstrap trees. The influence of different tree topologies on the divergence estimates was tested using the topological constraint option in PAUP*.

Present distributions were mapped on the inferred phylogeny and the most parsimonious reconstructions of ancestral areas were determined using MacClade vers. 4.07[49].

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature

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Table 1 Comparison of nucleotide substitution rates

Taxon	Genomic region	Nucleotide substitution rate	Calibration	Reference
Pleurophyllum	ETS	9.59×10^{-9} per site per year	First appearance of modern Asteraceae in the fossil record – (DeVore & Stuessy 1995; Graham 1996)	This paper
Pleurophyllum	ITS	4.98×10^{-9} per site per year	First appearance of modern Asteraceae in the fossil record – (DeVore & Stuessy 1995; Graham 1996)	This paper
Abrotanella	ITS	8.19×10^{-9} per site per year	First appearance of modern Asteraceae in the fossil record – (DeVore & Stuessy 1995; Graham 1996)	Wagstaff et al. (2006)
Dendroseris	ITS	6.06×10^{-9} per site per year	Age of Masatierra in Juan Fernandez Islands approximately 4 Myr; age of Masafurera approximately 1–2 Myr	Sang et al. (1994) Schmidt & Schilling (2000)
Robinsonia	ITS	7.83×10^{-9} per site per year	Age of Masatierra in Juan Fernandez Islands approximately 4 Myr; age of Masafurera approximately 1–2 Myr	Sang et al. (1995)
Tarweeds/Hawaiian silverswords	ITS	3.00×10^{-9} per site per year	Aridification accompanying uplift of	Baldwin & Sanderson

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			the California mts	(1998)
21 Angiosperm families	ITS	2.86 ×10^{-9} per site per year	Average of 28 rates representing 21 different angiosperm families	Kay et al. (2006)

Abrotanella, *Dendroseris*, *Robinsonia* and the Hawaiian silversword alliance are members of the Asteraceae that have diversified on islands.

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Figure 1 Subantarctic island herbfields. A. Panoramic view of Campbell Island. **B**. Lyall Ridge herbfield community dominated by *Pleurophyllum speciosum*. **C**. Inflorescence of *P. speciosum*. Photos by Colin Meurk.

Figure 2 Distribution of the macrocephalous clade. *Damnamenia vernicosa*: monotypic genus endemic to Campbell and Auckland islands. *Pleurophyllum*: genus of three species, *P speciosum*, *P. hookeri*, and *P. criniferum*, endemic to Auckland, Campbell, Antipodes and Macquarie islands. *Olearia lyallii*: endemic to the Snares and Auckland islands. *O. colensoi*: North, South, Stewart and Solander islands. *O. angustifolia*: confined to Stewart Island, the South Island shores of Foveaux Strait and surrounding smaller islands. *O. oporina*: South Island, sounds of Fiordland from Martins Bay southward. *O. chathamica*: endemic to the Chatham Islands, known from the southern tablelands, Pitt, Mangere and South-East islands. *O. semidentata*: endemic to the Chatham Islands.

Figure 3 Maximum likelihood analysis of the combined data set. The macrocephalous clade was again recovered, with *Damnamenia* sister to the other members of this clade. The three species of *Pleurophyllum* form a clade as do *Olearia angustifolia* and *O. oporina*, *O. semidentata* and *O. chathamica*, and *O. colensoi* and *O. lyallii*.

Figure 4 Calibrated rate for the combined analyses using a fossil date of 38 million years. Our results suggest the macrocephalous clade diverged

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during the mid-Pliocene about 4.1 million years ago. Independent analyses of the ITS and ETS sequences provided similar divergence estimates. The crown radiation occurred at the boundary between the Pliocene and Pleistocene. This was a time of intense environmental upheaval in New Zealand brought about by the uplift of the Southern Alps, glaciation, and volcanism.

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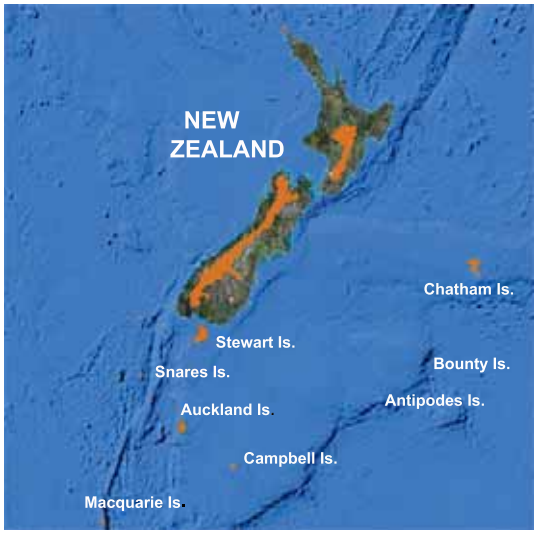
NEW
ZEALAND
Chatham Is.
Stewart Is.
Snares Is.
Bounty Is.
Antipodes Is.
Auckland Is
Campbell Is.
Macquarie Is.

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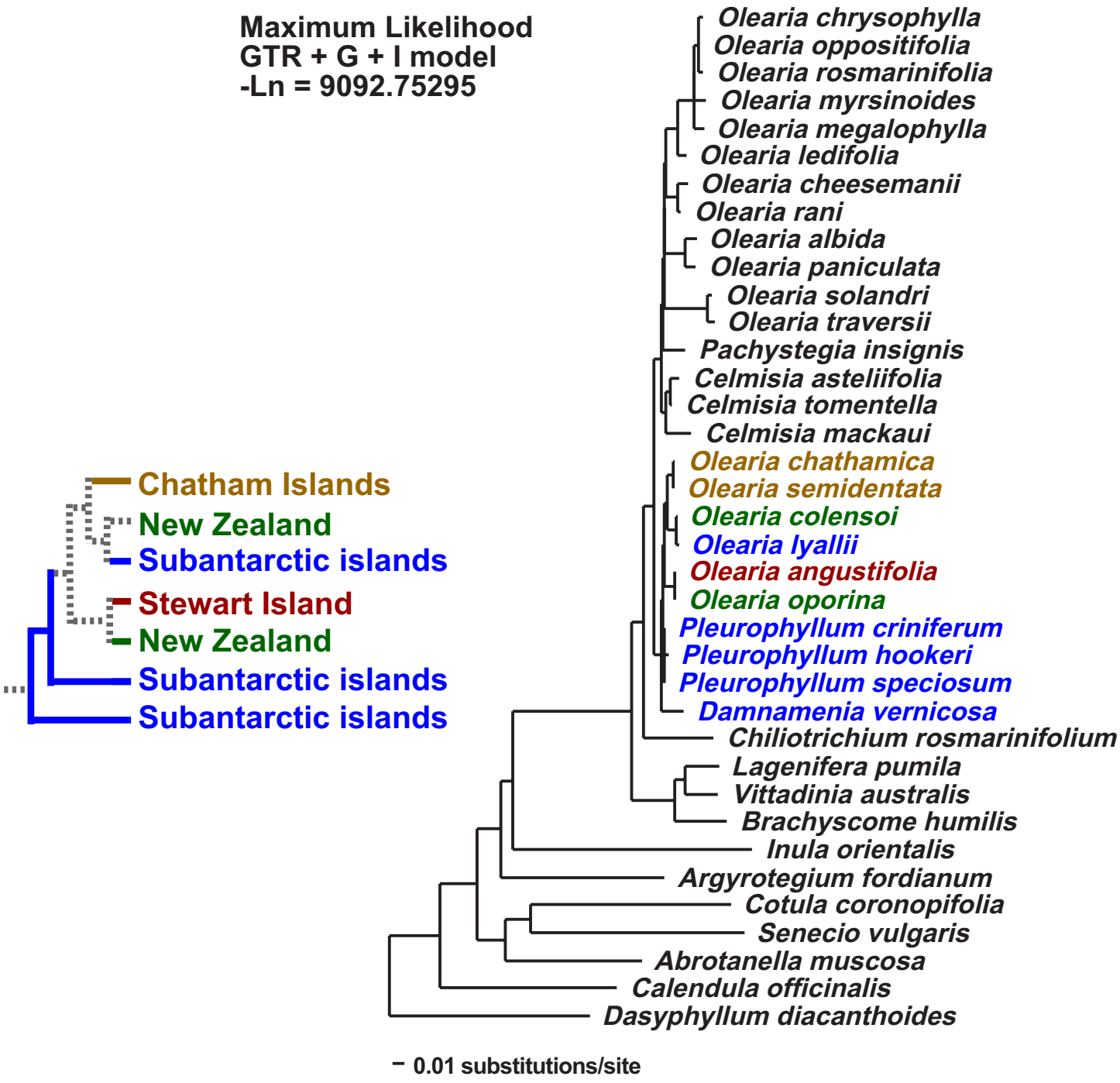
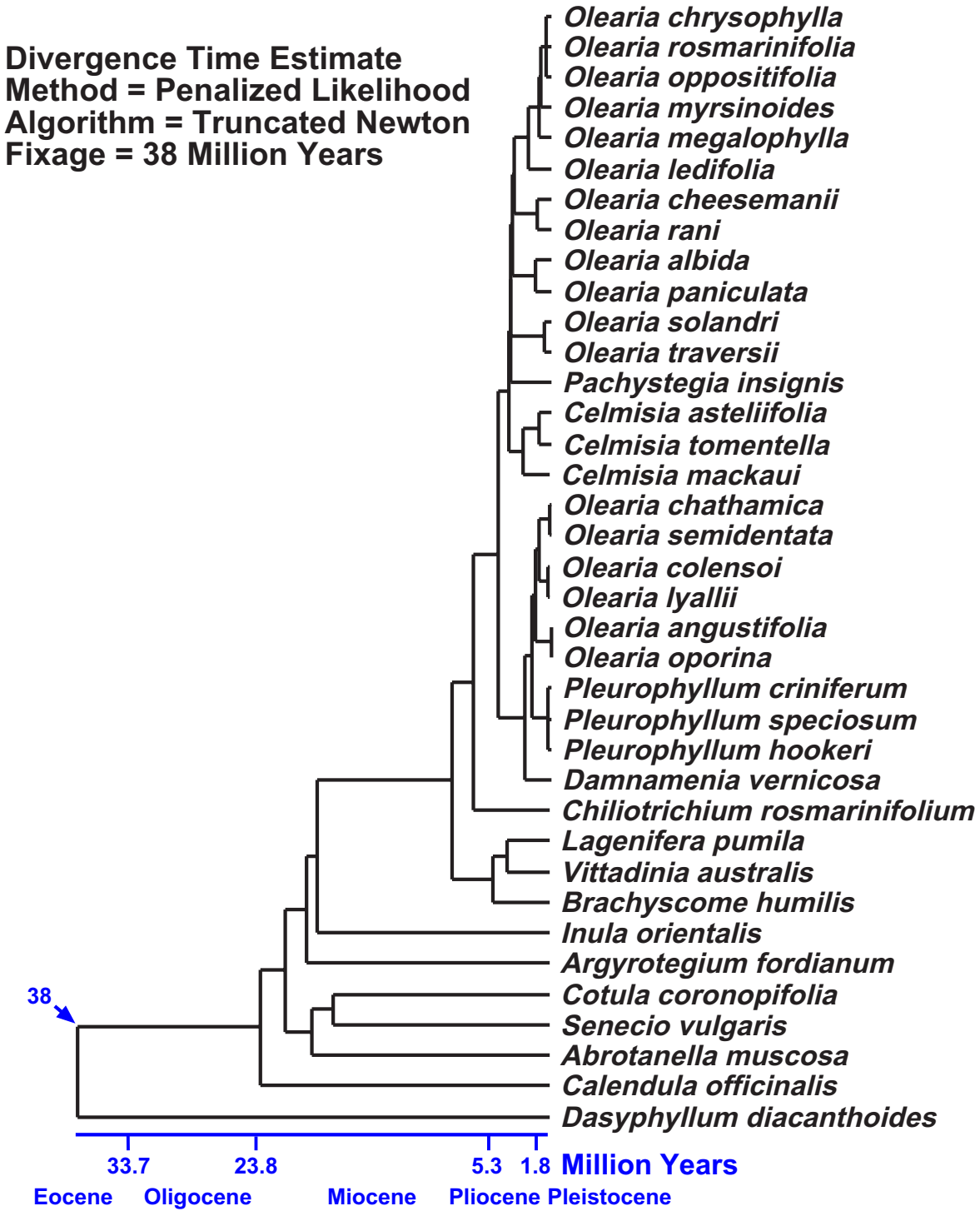
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