

Supporting Information

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SI Results

DNA Sequences. Between 17 and 151 aligned positions were lacking for 36 accessions in the plastid *trnS^{GCU}-trnG^{UUC}* region due to poor sequence overlap. The full-length sequences contained no variation in that region. For the nuclear *RPC2* region, direct sequencing of the PCR products revealed high levels of within-individual sequence polymorphism. All tetraploid *Empetrum atropurpureum*, one diploid *E. cf. atropurpureum*, almost all tetraploid *E. nigrum*, and nearly half of the diploid *E. nigrum* plus two diploid *E. eamesii* accessions contained more than one distinct sequence. In total, 25 accessions contained two or more sequences and were therefore subjected to cloning. These accessions are indicated in Table S1 by having two or more GenBank accession numbers. The cloning failed for three diploid accessions showing polymorphic sequences, and these were excluded from subsequent analyses due to polymorphisms in parsimony-informative characters (indicated with a “p” in Table S1). A total of 252 *RPC2* clones were sequenced, and 57 consensus sequences were constructed (Table S1). The final *RPC2* matrix consisted of 71 sequences from a total of 34 accessions and 925 aligned positions.

For the nuclear *RPB2-I* region, 19 accessions showed one or more polymorphisms in direct sequenced PCR products. Accessions for which only a single polymorphism in a parsimony-informative character was detected were included in the analyses, and eight more polymorphic accessions were removed from the final analyses (indicated with a “p” in Table S1). Five accessions, including both diploids and tetraploids, were cloned and contributed two or more sequences to the matrix. A total of 168 *RPB2-I* clones were sequenced and 13 consensus sequences were constructed (Table S1). The final *RPB2-I* matrix consisted of 36 sequences from a total of 28 accessions and 2,511 aligned positions. GenBank accession numbers for the sequences produced by direct sequencing of PCR products as well as for consensus sequences produced from cloned PCR products are given in Table S1.

SI Materials and Methods

DNA Extraction, PCR, and Sequencing. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions except for the following modifications. Silica-dried leaf tissue was mechanically ground in 2.0-mL tubes with two tungsten carbide beads for ~2 min at 15 Hz in a mixer mill (MM301; Retsch). The samples were frozen at -80 °C for at least 10 min after adding 400 μ L AP1 buffer and thawed at 65 °C before adding 4 μ L RNase.

The plastid-encoded *trnFM^{CAU}-trnS^{UGA}* region was amplified using primers *trnFM^{CAU}* and *trnS^{UGA}* (1) and a 5-min initial denaturation at 80 °C followed by 35 cycles of 95 °C 1 min, 61 °C 1 min, and 72 °C 2 min, and a final extension at 72 °C for 10 min. The plastid-encoded *trnS^{GCU}-trnG^{UUC}* region was amplified using the primers *trnS^{GCU}** and *trnG^{UUC}** as described (2). PCR was performed with 0.4 U Taq DNA polymerase (Applied Biosystems) and 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM Mg²⁺, 0.4 μ M each primer, 1 mM dNTP mix (Applied Biosystems), and 0.04% BSA.

The nuclear RNAP gene family consists of three large DNA-dependent RNA polymerase multisubunit enzymes (RNA poly-

merases I–III) in most eukaryotes and a fourth member (RNA polymerase IV) that appears to be unique to plants (3, 4). The second-largest subunits (i.e., *RPA2*, *RPB2*, *RPC2*, and *RPD2*) of each of the four multisubunit enzymes are encoded on chromosomes 1, 4, 5, and 3, respectively, in *Arabidopsis thaliana* and are thus unlinked (3). All four second-largest subunits have successfully been used as phylogenetic markers in plant studies (5–20). In this study, we sequenced a region spanning exons 31–32 in the *RPC2* gene encoding the second-largest subunit of RNA polymerase III, and a region spanning exons 2–6 from the *RPB2-I* gene encoding the second-largest subunit of RNA polymerase II.

Exons 31–32 in the *RPC2* gene were amplified and cloned using the nested PCR approach outlined in ref. 10. A set of specific primers, EmpRPC2-F1 (5'-TTTGATTTGGTTCAATATTAC-TAGA-3') and EmpRPC2-R1 (5'-ACTGCCATAGTGAAACT-TACC-3'), were subsequently designed and used to amplify the region in a single PCR. PCR was performed with 0.2 U DYNzymeII DNA polymerase and 1 \times buffer (Finnzymes), 0.2 mM dNTP mix (Applied Biosystems), and 0.3 μ M each primer.

Exons 2–6 in the *RPB2-I* gene were amplified as one or two fragments. The proofreading DNA polymerase Phusion (0.2 U in 1 \times buffer; Finnzymes), 0.2 mM dNTP mix (Applied Biosystems), and 0.5 μ M primers 2F and 6R (11) were used to amplify the region in one piece. PCR cycling started with a 3-min initial denaturation at 98 °C followed by 35 cycles of 98 °C 10 s, 62 °C 30 s, and 72 °C 1.5 min, and a final extension at 72 °C for 10 min. The region was amplified in two parts with the Taq protocol described above using primer pairs 2F/3R and 4F/6R (11) with a 3-min initial denaturation at 95 °C followed by 35 cycles of 95 °C 30 s, 56–60 °C 30 s, and 72 °C 2 min, and a final extension at 72 °C for 10 min.

All PCR amplifications were performed in 10- μ L reactions with 3- μ L template DNA of unknown concentration. PCR cycling was performed with a GeneAmp 3700 (Applied Biosystems) or PTC100 or PTC200 (MJ Research) thermocycler.

Cloning was performed with the TOPO TA Cloning Kit (Invitrogen) according to the manufacturer's manual, except that only half the volumes recommended for the reactions were used. Colonies were PCR-screened for correct fragment length using the universal T7 and M13R primers. Generally, 8 and 16 clones were sequenced for diploids and tetraploids, respectively.

PCR products were purified using 2 μ L ExoSAP-IT (USB) diluted 1:10 for 5 μ L of PCR product and incubated for 45 min at 37 °C.

PCR primers were used for sequencing. To achieve full coverage of the *trnS^{GCU}-trnG^{UUC}* region, we used *trnG2G* and *trnG2S* as internal sequencing primers (21). The single-fragment *RPB2-I* region was in addition to PCR primers also sequenced with internal primers 3F, 4F, 3R, and 4R (11). Cycle sequencing was performed using the Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 1.1 (Applied Biosystems) in 1/20 10- μ L reactions according to the manufacturer's manual with initial denaturing at 96 °C for 1 min followed by 25–35 cycles of 96 °C 10 s, 50 °C 5 s, and 60 °C min and a final extension at 60 °C for 10 min.

The sequences were assembled and edited using either GeneTool (BioTools) or Aligner v. 3.0.1 (CodonCode). One or more consensus sequences from each of the cloned accessions were constructed as described (12). All sequences were manually aligned in Se-Al v. 2.0a.11 (22).

1. Demasure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol Ecol* 4:129–131.

2. Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *Am J Bot* 94:275–288.

3. Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796–815.
4. Luo J, Hall BD (2007) A multistep process gave rise to RNA polymerase IV of land plants. *J Mol Evol* 64:101–112.
5. Denton AL, McConaughy BL, Hall BD (1998) Usefulness of RNA polymerase II coding sequences for estimation of green plant phylogeny. *Mol Biol Evol* 15:1082–1085.
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8. Oxelman B, et al. (2004) *RPB2* gene phylogeny in flowering plants, with particular emphasis on asterids. *Mol Phylogenet Evol* 32:462–479.
9. Pfeil BE, Brubaker CL, Craven LA, Crisp MD (2004) Paralogy and orthology in the MALVACEAE *rpb2* gene family: Investigation of gene duplication in hibiscus. *Mol Biol Evol* 21:1428–1437.
10. Popp M, Oxelman B (2004) Evolution of a RNA polymerase gene family in *Silene* (Caryophyllaceae)-incomplete concerted evolution and topological congruence among paralogues. *Syst Biol* 53:914–932.
11. Goetsch L, Eckert AJ, Hall BD (2005) The molecular systematics of *Rhododendron* (Ericaceae): A phylogeny based upon *RPB2* gene sequences. *Syst Bot* 30:616–626.
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16. Popp M, Oxelman B (2007) Origin and evolution of North American polyploid *Silene* (Caryophyllaceae). *Am J Bot* 94:330–349.
17. Vilatersana R, Brysting AK, Brochmann C (2007) Molecular evidence for hybrid origins of the invasive polyploids *Carthamus creticus* and *C. turkestanicus* (Cardueae, Asteraceae). *Mol Phylogenet Evol* 44:610–621.
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22. Rambaut A (1996) Se-AL: Sequence Alignment Editor (<http://tree.bio.ed.ac.uk/software/seal/>).

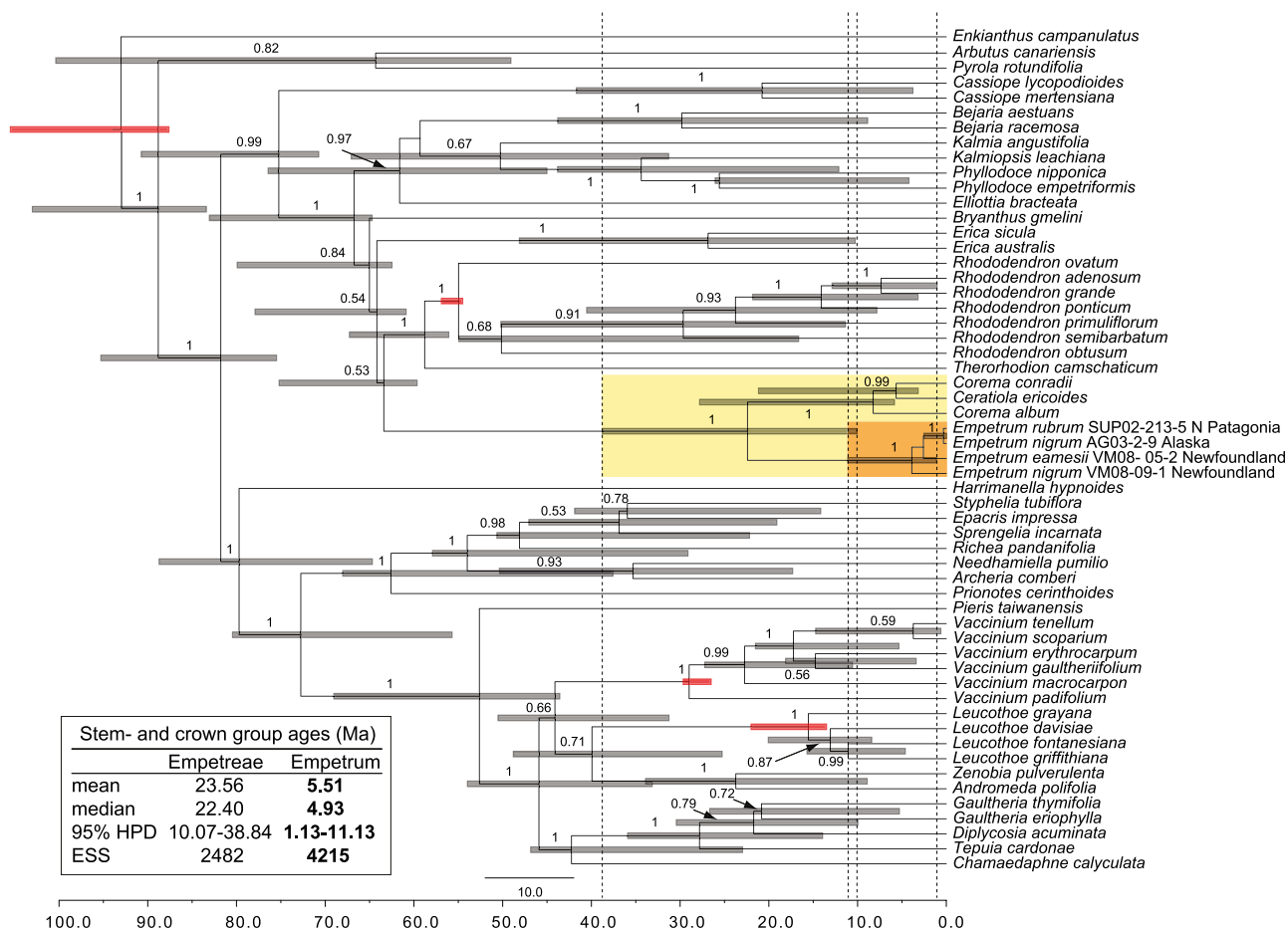


Fig. S1. Phylogeny and age estimates of groups inferred from 56 *matK* sequences representing species from all eight subfamilies and 21 of the 24 tribes in Ericaceae. Empetreae (yellow box) and *Empetrum* (orange box) are highlighted, and the 95% highest posterior probability density (HPD) of the age estimates is marked with dotted lines. Node bars indicate the 95% HPD interval; fossil-calibrated nodes are indicated with red node bars. Numbers associated with nodes indicate posterior probabilities. ESS, effective sample size. (Scale bar, Ma.)

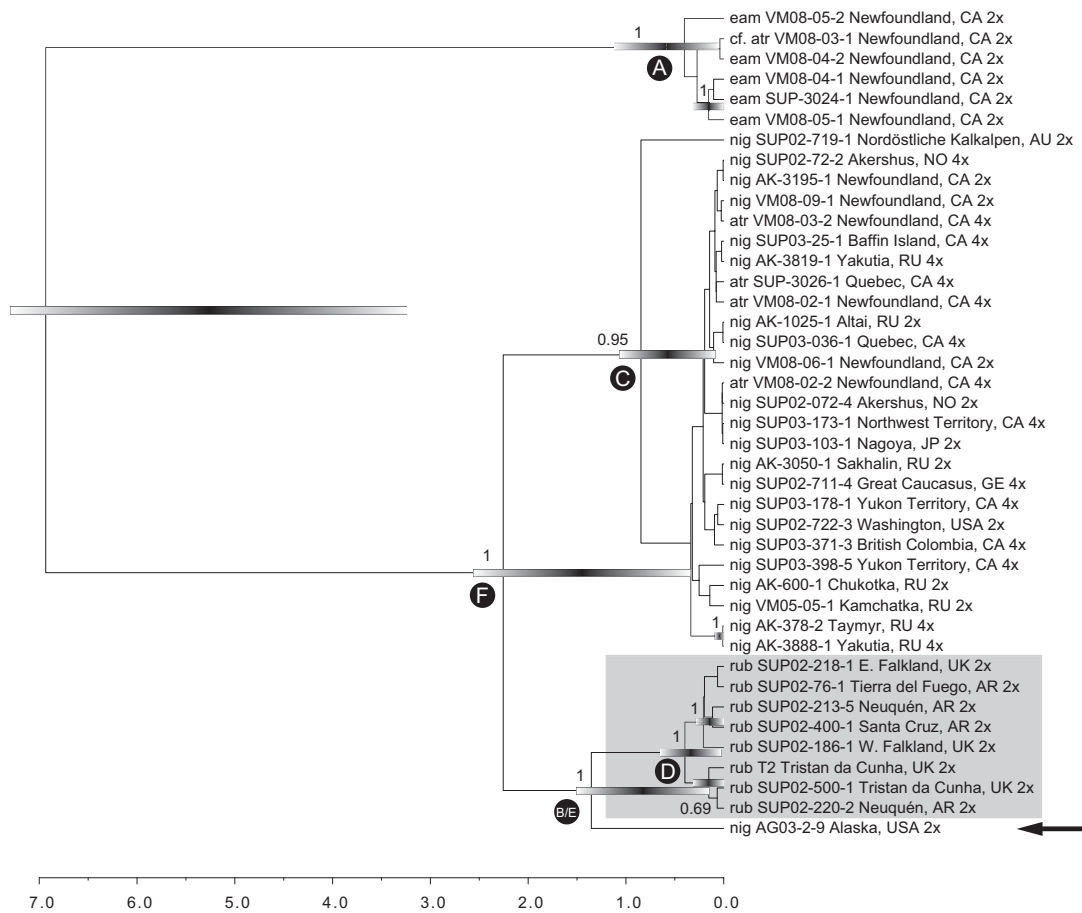


Fig. S2. Phylogeny and 95% HPD age interval estimates of nodes inferred from the combined *trnFM^{CAU}-trnS^{UGA}* and *trnS^{GCU}-trnG^{UUC}* plastid DNA regions. Node bars indicate the 95% HPD interval, and numbers associated with nodes indicate posterior probabilities. (Scale bar, Ma.) Clades designated A–F are discussed in the main text. The gray box highlights southern hemisphere diploid plants (*E. rubrum*) and the black arrow indicates the most closely related northern hemisphere diploid *E. nigrum*. Note that the B and E clades coincide in this tree. Terminal names are abbreviated as follows: atr, *E. atropurpureum*; eam, *E. eamesii*; nig, *E. nigrum*; rub, *E. rubrum*, and followed by sample identification, geographic information, and DNA ploidy level. AR, Argentina; AU, Austria; CA, Canada; GE, Georgia; JP, Japan; NO, Norway; RU, Russia; UK, United Kingdom; USA, United States of America.

Table S1. Plant material used in the study

Taxon, population ID, collection year, collector, coordinates, province, country, GenBank accession number *trnS^{GCU}-trnG^{UUC}; trnFM^{CAU}-trnS^{UGA}; RPC2; RPB2-I* (*P*, polymorphic sequence; na, no amplification)

Empetrum atropurpureum, SUP-3026-1, 2004, I. Greve Alsos & A. Krag Brysting, 48.99, -65.93, Quebec, Canada, HM146928; HM146969; HM147054, HM147055, HM147056, HM147057; HM147019

E. atropurpureum, VM08-02-1, 2008, J. Maunder, 49.69, -54.80, Newfoundland, Canada, HM146929; HM146970; HM147058; na

E. atropurpureum, VM08-02-2, 2008, J. Maunder, 49.69, -54.80, Newfoundland, Canada, HM146930; HM146971; HM147059, HM147060, HM147061, HM147062; HM147047p

E. atropurpureum, VM08-03-2, 2008, J. Maunder, 47.55, -52.70, Newfoundland, Canada, HM146931; HM146972; HM147063, HM147064, HM147065; HM147049p

E. cf atropurpureum, VM08-03-1, 2008, J. Maunder, 47.55, -52.70, Newfoundland, Canada, HM146932; HM146973; HM147066, HM147067; HM147048p

E. eamesii, SUP-3024-1, 2004, I. Greve Alsos & A. Krag Brysting, 49.59, -57.80, Newfoundland, Canada, HM146933; HM146974; HM147068; HM147018

E. eamesii, VM08-04-1, 2008, J. Maunder, 47.33, -52.74, Newfoundland, Canada, HM146934; HM146975; HM147069, HM147070; na

E. eamesii, VM08-04-2, 2008, J. Maunder, 47.33, -52.74, Newfoundland, Canada, HM146935; HM146976; HM147071; HM147050

E. eamesii, VM08-05-1, 2008, J. Maunder, 47.55, -52.70, Newfoundland, Canada, HM146936; HM146977; na; na

E. eamesii, VM08-05-2, 2008, J. Maunder, 47.55, -52.70, Newfoundland, Canada, HM146937; HM146978; HM147072, HM147073; HM147051p

E. nigrum, AG03-2-9, 2003, R. Elven & H. Solstad, 60.43, -151.27, Alaska, USA, HM146938; HM146979; HM147074, HM147075; HM147010

E. nigrum, AK-1025-1, 2003, A. Tribsch, 49.52, 88.02, Altai, Russia, HM146939; HM146980; HM147076; HM147011

E. nigrum, AK-3050-1, 2004, B. Kantz, 46.99, 142.84, Sakhalin, Russia, HM146940; HM146981; na; HM147012

E. nigrum, AK-3195-1, 2004, I. Greve Alsos & A. Krag Brysting, 49.71, -57.94, Newfoundland, Canada, HM146941; HM146982; p; HM147013

E. nigrum, AK-378-2, 2003, I. L. Chuprova, 69.41, 86.25, Taymyr, Russia, HM146942; HM146983; HM147077, HM147078, HM147079, HM147080, HM147081; HM147014

E. nigrum, AK-3819-1, 2004, R. Elven & H. Solstad, 66.71, 123.40, Yakutia, Russia, HM146943; HM146984; HM147082, HM147083, HM147084; HM147015

E. nigrum, AK-3888-1, 2004, R. Elven & H. Solstad, 71.05, 127.54, Yakutia, Russia, HM146944; HM146985; HM147085, HM147086; HM147016

E. nigrum, AK-600-1, 2002, V. Razzhivin, 64.78, 176.97, Chukotka, Russia, HM146945; HM146986; p; HM147017

E. nigrum, SUP02-072-2, 2002, S. Kjølner, 59.80, 10.99, Akershus, Norway, HM146946; HM146987; HM147087, HM147088, HM147089; HM147020, HM147021, HM147022, HM14702

E. nigrum, SUP02-072-4, 2002, S. Kjølner, 59.80, 10.99, Akershus, Norway, HM146947; HM146988; HM147090; HM147024

E. nigrum, SUP02-711-4, 2002, G. M. Schneeweiss, A. Tribsch, M. Staudinger, P. Schönswetter, 42.66, 44.58, Great Caucasus, Georgia, HM146948; HM146989; HM147091, HM147092, HM147093; HM147029, HM147030

E. nigrum, SUP02-719-1, 2002, A. Tribsch, 47.69, 15.69, Nordöstliche Kalkalpen, Austria, HM146949; HM146990; HM147094, HM147095; HM147031

E. nigrum, SUP02-722-3, 2002, P. Schönswetter & A. Tribsch, 48.48, -121.04, Washington, USA, HM146950; HM146991; HM147096, HM147097; HM147032, HM147033

E. nigrum, SUP03-025-1, 2003, C. Mallory, 63.73, -68.50, Baffin Island, Canada, HM146951; HM146992; HM147098, HM147099; HM147035p

E. nigrum, SUP03-036-1, 2003, R. Elven & A. Elven, 48.84, -68.87, Quebec, Canada, HM146952; HM146993; HM147100, HM147101, HM147102; HM147036p

E. nigrum, SUP03-103-1, 2003, T. Masuzawa, 35.99, 138.37, Nagoya, Japan, HM146953; HM146994; HM147103, HM147104; HM147037, HM147038

E. nigrum, SUP03-173-1, 2003, R. Elven & H. Solstad, 68.00, -133.33, Northwest Territory, Canada, HM146954; HM146995; HM147105, HM147106; HM147039

E. nigrum, SUP03-178-1, 2003, R. Elven & H. Solstad, 60.18, -134.70, Yukon Territory, Canada, HM146955; HM146996; HM147107, HM147108, HM147109; HM147040p

E. nigrum, SUP03-371-3, 2003, R. Elven & H. Solstad, 58.45, -129.98, British Columbia, Canada, HM146956; HM146997; HM147110, HM147111; HM147041, HM147042, HM14704

E. nigrum, SUP03-398-5, 2003, R. Elven & H. Solstad, 67.05, -136.25, Yukon Territory, Canada, HM146957; HM146998; HM147112, HM147113, HM147114, HM147115; HM147044

E. nigrum, VM05-05-1, 2005, Eriksen & Andersson, 52.86, 156.30, Kamchatka, Russia, HM146958; HM146999; p; HM147046

E. nigrum, VM08-06-1, 2008, J. Maunder, 47.549, -52.70, Newfoundland, Canada, HM146959; HM147000; HM147116; HM147052p

E. nigrum, VM08-09-1, 2008, J. Maunder, 47.76, -52.74, Newfoundland, Canada, HM146960; HM147001; HM147117; HM147053

E. rubrum, SUP02-186-1, 2002, E. de Vilder, -51.28, -60.57, W. Falkland, U.K., HM146961; HM147002; na; na

E. rubrum, SUP02-213-5, 2002, A. Alvarez, -36.45, -70.63, Neuquén, Argentina, HM146962; HM147003; HM147118; HM147025

E. rubrum, SUP02-218-1, 2002, E. de Vilder, -51.70, -57.82, Falkland, U.K., HM146963; HM147004; HM147119; HM147026

E. rubrum, SUP02-220-2, 2002, A. Alvarez, -36.82, -71.11, Neuquén, Argentina, HM146964; HM147005; HM147120; HM147027

E. rubrum, SUP02-400-1, 2002, V. Mirré, -49.24, -72.95, Santa Cruz, Argentina, HM146965; HM147006; HM147121; na

E. rubrum, SUP02-500-1, 2003, J. Cooper, -37.10, -12.30, Tristan da Cunha, U.K., HM146966; HM147007; HM147122; HM147028

E. rubrum, SUP02-76-1, 2002, V. Mirré, -54.85, -68.48, Tierra del Fuego, Argentina, HM146967; HM147008; HM147123; HM147034

E. rubrum, T2, 2002, N. Gremen, -37.08, -12.28, Tristan da Cunha, U.K., HM146968; HM147009; HM147124; HM147045

Table S2. GenBank accession numbers for *matK* sequences used to date the stem and crown group ages of *Empetrum*

Andromeda polifolia AF124569.1 (1)
Arbutus canariensis U61345.1 (2)
Archeria comberi AF015632.1 (3)
Bejaria aestuans DQ002346.1 (4)
Bejaria racemosa U61327.1 (2)
Bryanthus gmelini AF440413.1 (5)
Cassiope lycopodioides AB012754.1 (6)
Cassiope mertensiana U61346.1 (2)
Ceratiola ericoides AF519563.1 (7)
Chamaedaphne calyculata AF015630.1 (3)
Corema album AF519566.1 (7)
Corema conradii AF519567.1 (7)
Diplycosia acuminata AF124563.1 (1)
Elliottia bracteata U61339.1 (2)
Empetrum eamesii HQ115641 (this study)
Empetrum nigrum HQ115642 (this study)
Empetrum nigrum HQ115639 (this study)
Empetrum rubrum HQ115640 (this study)
Enkianthus campanulatus U61344.2 (2)
Epacris impressa AF015636.1 (3)
Erica australis U61329.1 (2)
Erica sicula U61341.1 (2)
Gaultheria eriophylla U61317.2 (8)
Gaultheria thymifolia FJ010614.1 (9)
Harrimanella hypnoides U61315.2 (8)
Kalmia angustifolia U61348.2 (2)
Kalmiopsis leachiana U61323.1 (2)
Leucothoe davisiae FJ010617.1 (9)
Leucothoe fontanesiana AF124570.1 (1)
Leucothoe grayana FJ010621.1 (9)
Leucothoe griffithiana FJ010616.1 (9)
Needhamiella pumilio AF539984.1 (5)
Phyllodoce empetriformis DQ002358.1 (4)
Phyllodoce nipponica DQ002359.1 (4)
Pieris taiwanensis AM296063.1, unpublished
Prionotes cerinthoides AF015642.1 (3)
Pyrola rotundifolia U61328.1 (2)
Rhododendron adenosum EU087326.1 (10)
Rhododendron grande DQ002360.1 (4)
Rhododendron obtusum U61350.1 (2)
Rhododendron ovatum AB012729.1 (6)
Rhododendron ponticum AB012732.1 (6)
Rhododendron primuliflorum AB012740.1 (6)
Rhododendron semibarbatum AB012733.1 (6)
Richea pandanifolia AF539986.1 (5)
Sprengelia incarnata AF015645.1 (3)
Styphelia tubiflora AY372670.1 (11)
Tepuia cardonae AF124566.1 (1)
Therorhodium camschaticum U61322.1 (2)
Vaccinium erythrocarpum AF419710.1 (12)
Vaccinium gaultheriifolium AF382806.1 (13)
Vaccinium macrocarpon U61316.2 (8)
Vaccinium padifolium AF382812.1 (13)
Vaccinium scoparium AF419716.1 (12)
Vaccinium tenellum AF382818.1 (13)
Zenobia pulverulenta AF124571.1 (1)

1. Kron KA, Judd WS, Crayn DM (1999) Phylogenetic analyses of Andromedeae (Ericaceae subfam. Vaccinioideae). *Am J Bot* 86:1290–1300.
2. Kron KA (1997) Phylogenetic relationships of Rhododendroideae (Ericaceae). *Am J Bot* 84:973–980.
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12. Powell EA, Kron KA (2002) Hawaiian blueberries and their relatives—A phylogenetic analysis of *Vaccinium* sections *Macropelma*, *Myrtillus*, and *Hemimyrtillus* (Ericaceae). *Syst Bot* 27:768–779.
13. Kron KA, Powell EA, Luteyn JL (2002) Phylogenetic relationships within the blueberry tribe (Vaccinieae, Ericaceae) based on sequence data from matK and nuclear ribosomal ITS regions, with comments on the placement of *Satyria*. *Am J Bot* 89:327–336.