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Multiple origins of circumboreal taxa in *Pyrola* (Ericaceae), a group with a Tertiary relict distribution

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• **Background and Aims** In the Northern Hemisphere, Tertiary relict disjunctions involve older groups of warm affinity and wide disjunctions, whereas circumboreal distributions in Arctic-Alpine taxa tend to be younger. Arctic-Alpine species are occasionally derived from Tertiary relict groups, but *Pyrola* species, in particular, are exceptional and they might have occurred multiple times. The aim of this study was to reconstruct the biogeographic history of *Pyrola* based on a clear phylogenetic analysis and to explore how the genus attained its circumboreal distribution.
• **Methods** Estimates of divergence times and ancestral geographical distributions based on neutrally evolving DNA sequence variation were used to develop a spatio-temporal model of colonization patterns for *Pyrola*.
• **Key Results** *Pyrola* originated and most diversification occurred in Asia; North America was reached first by series *Scotophyllae* in the late Miocene, then by sub-clades of series *Pyrola* and *Ellipticae* around the Pliocene. The three circumboreal taxa, *P. minor*, *P. chlorantha* and the *P. rotundifolia* complex, originated independently of one another, with the last two originating in Asia.
• **Conclusions** Three circumboreal *Pyrola* lineages have arisen independently and at least two of these appear to have originated in Asia. The cool, high-altitude habitats of many *Pyrola* species and the fact that diversification in the genus coincided with global cooling from the late Miocene onwards fits a hypothesis of pre-adaptation to become circumboreal within this group.

Key words: Adaptation, Arctic-Alpine taxa, biogeography, circumboreal, Ericaceae, *Pyrola*, species distribution, Tertiary relict.

INTRODUCTION

Two major categories of Northern Hemisphere intercontinental disjunctions are Tertiary relict disjunctions and circumboreal distributions. Tertiary relict disjunctions tend to be older and involve groups from warm temperate to sub-tropical regions, reflecting the warm climates of the Tertiary epoch (Tiffney, 1985; Wen, 1999; Milne and Abbott, 2002). Conversely, circumboreal distributions typically involve cold temperate to Arctic-Alpine species, and tend to be younger, reflecting the recent development of these biomes due to global cooling over the past 5 million years (Hultén, 1937; Abbott and Brochmann, 2003). Hence, the former generally involve disjunctions at the genus level (Wen, 1999; Donoghue *et al.*, 2001; Milne and Abbott, 2002; Milne, 2006), whereas the latter typically involve single species (Abbott *et al.*, 2000; Brochmann and Brysting, 2008; Eidesen *et al.*, 2013). Because of this, when a genus or clade contains taxa exhibiting both Tertiary relict and circumboreal distributions, the latter tends to be a single lineage that is young and in a derived position, for example *Circaea alpina* subsp. *alpina* (Xie *et al.*, 2009), *Arctostaphylos alpina* (Hileman *et al.*, 2001), *Cornus suecica* (Xiang *et al.*, 2006) and *Juniperus communis* (Mao *et al.*, 2010). Such single

origins for ecological transitions into colder biomes make it difficult to infer whether a genus was pre-adapted for such a shift. In genera where multiple circumboreal species exist, steppe habitats have been identified as a likely source in *Artemisia* (Tkach *et al.*, 2008*a*, *b*), whereas others are large and lack complete phylogenetic trees (e.g. *Carex*, *Oxytropis* and *Vaccinium*).

Pyrola (commonly called wintergreens) is a small and well-defined genus of evergreen herbs in the family Ericaceae, comprising approx. 30 species. Most *Pyrola* species are distributed in North America or eastern Asia, exhibiting a Tertiary relict distribution. Of these, the *P. rotundifolia* complex, *P. minor* and *P. chlorantha* are circumboreal taxa and have been placed in different series (Dorr, 1995; Liu *et al.*, 2011). Hence, there could have been up to three separate origins of circumboreal lineages in *Pyrola*. If so, *Pyrola* would be an ideal study group for examining the origin of cold-tolerant, circumboreal taxa. Although historical biogeographic patterns of selected *Pyrola* species have been examined previously based on a combination of floristic patterns, current geography and palaeoecological patterns (Hultén, 1958, 1968), a modern interpretation of historical biogeography for the entire genus has not been advanced. In the current study, a Bayesian relaxed molecular clock approach was used to calculate the ages of major diversification events

in *Pyrola*. The ancestral area of *Pyrola* and subsequent range expansion are inferred and discussed in the context of the molecular dating results and palaeoclimatic evidence to learn more about the origin of circumboreal *Pyrola* species, specifically when, where and how often they arose.

MATERIALS AND METHODS

Details concerning DNA isolation, primer specification, PCR amplification and DNA sequencing are as described in Liu *et al*. (2010).

Molecular dating

Analysis D1: Ericaceae-wide phylogenetic analysis with direct fossil calibration. Because there are no published *Pyrola* fossil records that we know of, we used fossils of other Ericaceae for calibration. For this analysis, we could only use a marker for which sequences were available across the whole family, and so *matK* was chosen. This analysis included 14 accessions from Pyroleae and 77 of other Ericaceae, representing 23 of the 24 tribes in Ericaceae and all eight subfamilies. In addition, three accessions of *Actinidia* (Actinidiaceae) were used as outgroups in this analysis, making 94 accessions in total. These sequences were downloaded from GenBank, except for three *Pyrola* species (*P. forrestiana, P. renifolia* and *P. atropurpurea*) and two accessions of *Moneses uniflora* which were newly sequenced for *matK* for this analysis. These are referred to as dataset 1 (D1) below (Fig. 1; Supplementary Data Table S1).

Seven fossils were used to calibrate this phylogenetic tree. Flowers of *Paleoenkianthus sayrevillensis*, which are inferred to be the oldest known fossils [90 million years ago (Mya)] with ericaceous affinities (Nixon and Crepet, 1993), were used as a prior for the stem group age of Ericaceae. Fossil remains of *Rhododendron newburyanum* dated from the upper Paleocene (Collinson, 1978) were taken to represent the minimum age of *Rhododendron* (60 Mya, following Milne, 2004). Leaves of *Vaccinium creedensis* and *Leucothoe nevadensis* dated to 26·5 and 13–14 Mya (Axelrod, 1987, 1995), respectively, were used as priors for the minimum age of *Vaccinium* and *Leucothoe*. Macrofossils of *Cyathodophyllum novae-zelandiae* and *Richeaphyllum waimumuensis* documented at the Oligocene/ Miocene boundary (23·3 Mya; Jordan *et al*., 2010) were used as priors for the minimum age of Stphelieae and Richeeae, respectively. A fossil species, *Arctostaphylos masoni* dated to 15·8 Mya, was used to constrain the stem age of *Arctostaphylos* (Wolfe, 1964).

A relaxed molecular clock as implemented in BEAST version 1.8.0 (Drummond and Rambaut, 2007) with uncorrelated lognormal-distributed substitution rates for each branch of the phylogenetic tree was used to estimate age divergence within Ericaceae. Using MrModeltest version 2.2 (Nylander, 2004), the general time-reversible substitution model and invariant sites and gamma distribution (GTR + I + G) was estimated as the best-fitting model of nucleotide substitution from the data. The tree was rooted with three *Actinidia* species (Actinidiaceae, Ericales) by constraining all other taxa to form a monophyletic group. Priors for the most recent common ancestor were set to lognormal distributions with logmean = 1·0, lognormal s.d. = 0·5 and offset set to 55·6, 26·2, 23·3, 23·3, 15·8 and 13·0 Mya

for *Rhododendron*, *Vaccinium*, Richeeae, Stphelieae, ancestor of *Arbutus* and *Arctostaphylos*, and *Leucothoe*, respectively. The lognormal distribution for the priors thus fixed the minimum ages of the calibrated nodes, but allowed for maximum ages to be sampled following a lognormal distribution with no hard bound. Because Nixon and Crepet (1993) placed *Paleoenkianthus sayreville* near or in Ericaceae, the root age prior was set to a normal distribution with mean = 90 Mya and s.d. = 5·0. Both the Yule speciation process and a birth/death speciation process were used as tree priors in separate analyses. The defaults in BEAUti version 1.8.0 (Drummond and Rambaut, 2007) were used for all other parameters. Four independent Markov chains were run for 50 million generations and parameters were sampled every 2000 generations. The parameter estimates from each independent analysis were checked for stationarity and convergence using Tracer v. 1.5. Results were considered reliable once the effective sampling size (ESS) for all parameters exceeded 200 as suggested in the program manual. After discarding 20 % of the saved trees as burn-in, a maximum clade credibility (MCC) tree based on the remaining trees was produced using TreeAnnotator 1.8.0 (Drummond and Rambaut, 2007) with a posterior probability (PP) limit of 0·5 and mean node heights.

Analysis D2: Pyroleae phylogenetic analysis with secondary calibration. The markers used here, i.e. internal transcribed spacer (ITS), *atpB-rbcL*, *trnL-trnF* and *trnS-trnG* were those used in our previous study (Liu *et al*., 2010). Because these DNA regions sequenced for *Pyrola* species were not available for most of the Ericaceae species in D1, molecular dating of the full *Pyrola* phylogenetic tree was done with secondary calibration. After exclusion of the putative hybrid species *P. media* and *P. fauriana*, inclusion of which might cause incongruence between molecular markers, an incongruence length difference (ILD) test (Farris *et al*., 1995) indicated that the results from these four sequences are sufficiently similar to warrant combining the loci in a single matrix for analysis ($P > 0.05$).

Dataset 2 (D2) contained 32 accessions, of which 25 were *Pyrola* and four were other Pyroleae; two *Enkianthus* and one *Arctostaphylos* species were selected as outgroups (Supplementary Data Table S2). Because each of the three circumboreal *Pyrola* taxa, i.e. *P. rotundifolia* complex, *P. minor* and *P. chlorantha*, has been identified as monophyletic (Liu *et al.*, 2011), we can randomly select one accession from each species in the following analyses. The four DNA regions partitioned in BEAUti 1.8.0 with the appropriate substitution model were used to estimate the time of lineage splits within *Pyrola*. Substitution model selection was performed as described above for *matK*, resulting in a symmetrical model with a gamma distribution (SYM + G), general time-reversible model with invariant sites (GTR + I), and general time-reversible model with a gamma distribution (GTR + G) for ITS, *atpB-rbcL* and *trnL-trnF*, and *trnS-trnG*, respectively Both Yule speciation process and a birth/death speciation process were specified as tree prior in independent analyses. The prior crown time of Pyroleae, *Pyrola* and the *Moneses – Chimaphila* clade were set to a normal distribution around the mean = 50·7, 20·7 and 28·2 Mya estimated from analysis D1, and s.d. = 11·0, 6·5 and 7·5, approximating a distribution in which values formed upper and lower limits of the 95 % probability interval, respectively
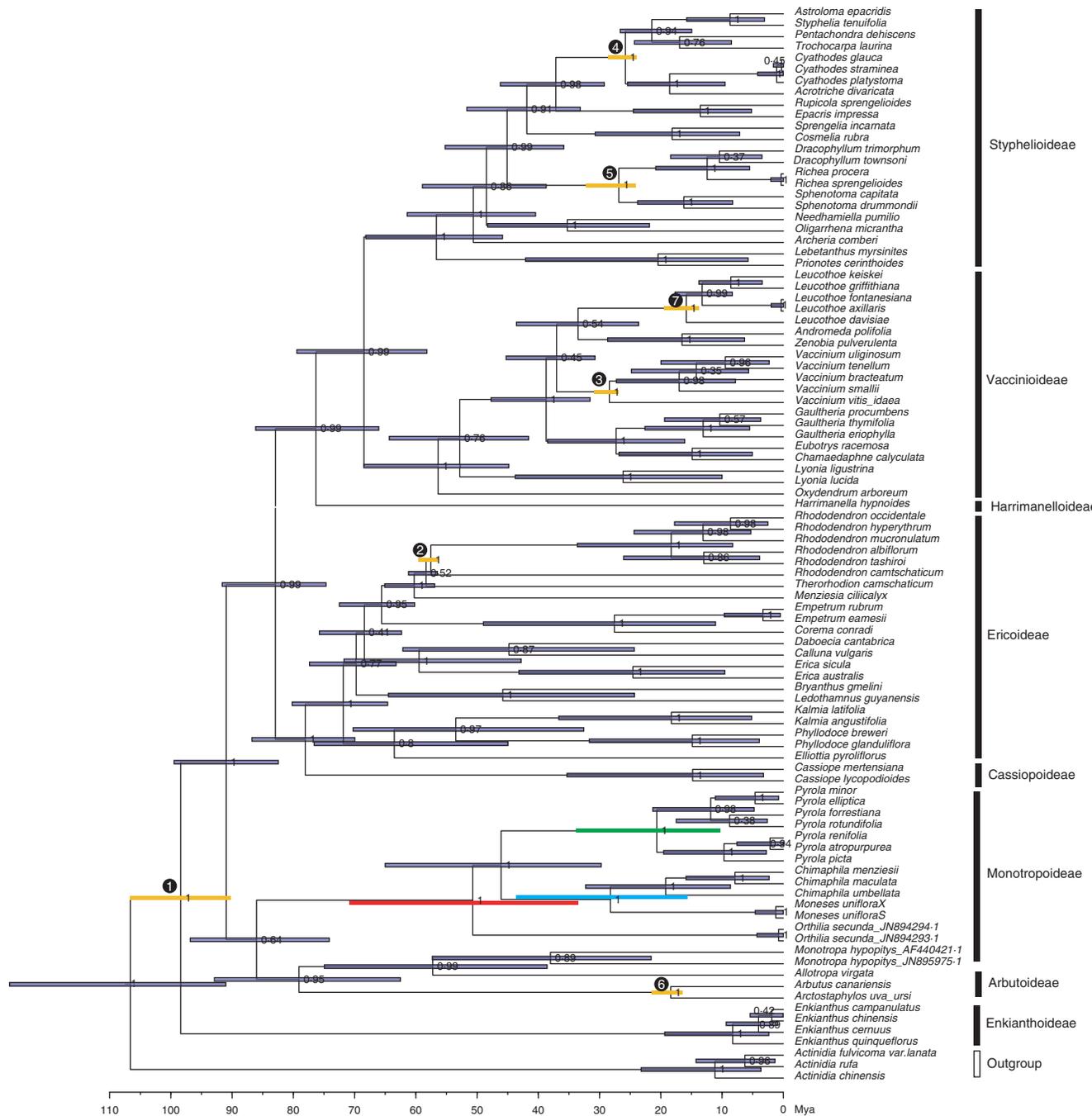
FIG. 1. Phylogenetic relationships and age estimates of groups inferred from 94 *matK* sequences representing species from all eight subfamilies and 23 of the 24 tribes of Ericaceae (dataset 1, D1), using BEAST. Fossil-calibrated nodes are indicated with brown node bars (node 1 to node 7). Nodes used to provide secondary calibration are highlighted as follows: crown radiation of Pyroleae (red node bar), crown radiation of *Pyrola* (green node bar) and divergence of *Chimaphila* from *Moneses* (blue node bar). Node bars indicate the 95 % highest posterior density interval. Numbers associated with nodes indicate posterior probabilities.

(Fig. 1). Markov chains were run as described for the *matK* analysis. BEAST analyses were run on the University of Oslo Bioportal (http://www.bioportal.uio.no).

Historical biogeography

Ancestral area reconstruction (AAR) and inference of spatial patterns of geographical diversification were done using two methods: a Bayesian approach to dispersal–vicariance analysis (Bayes-DIVA) (Ronquist, 1997) implemented in S-DIVA (Yu *et al.*, 2010) and a likelihood method using the dispersal–extinction–cladogenesis (DEC) model implemented in Lagrange version 20120508 (Ree and Smith, 2008). A dataset including *Pyrola* and relatives was used for the biogeographical analyses. Five areas of endemism were delimited based on the distributions of extant *Pyrola* species: A, Asia; W, western North America;

E, eastern North America; S, southern North America; and U, Europe. The areas were delimited following the map in Sessa *et al.* (2012). The exact boundary of Asia and Europe used was not critical as all species examined occurred either in Asia only or throughout northern Eurasia, or were absent from Eurasia altogether. In both analyses, the maximum number of areas within ancestral ranges was not constrained. For the Bayes-DIVA analyses, ancestral areas were estimated independently on 1000 randomly chosen post-burn-in trees obtained from BEAST. Relative frequencies of ancestral areas estimated for each node were recorded and plotted onto the MCC tree. In the likelihood ancestral area analysis, Python scripts were generated using the online Lagrange configurator (http://www.reelab.net/lagrange/configurator). The maximum credibility tree estimated in BEAST was used as the input tree; we assumed a dispersal probability of 1·0 for neighbouring regions, and a probability of 0·1 for all other areas.

RESULTS

Summary of phylogenetic tree

Pyrola consisted of two clades, corresponding to sections *Pyrola* and *Ampliosepala*. The former comprised series *Pyrola*, *Rugosae* and *Ellipticae*, and the latter comprised series *Japonicae*, *Scotophyllae* and *Chloranthae* (Figs 2 and 3).
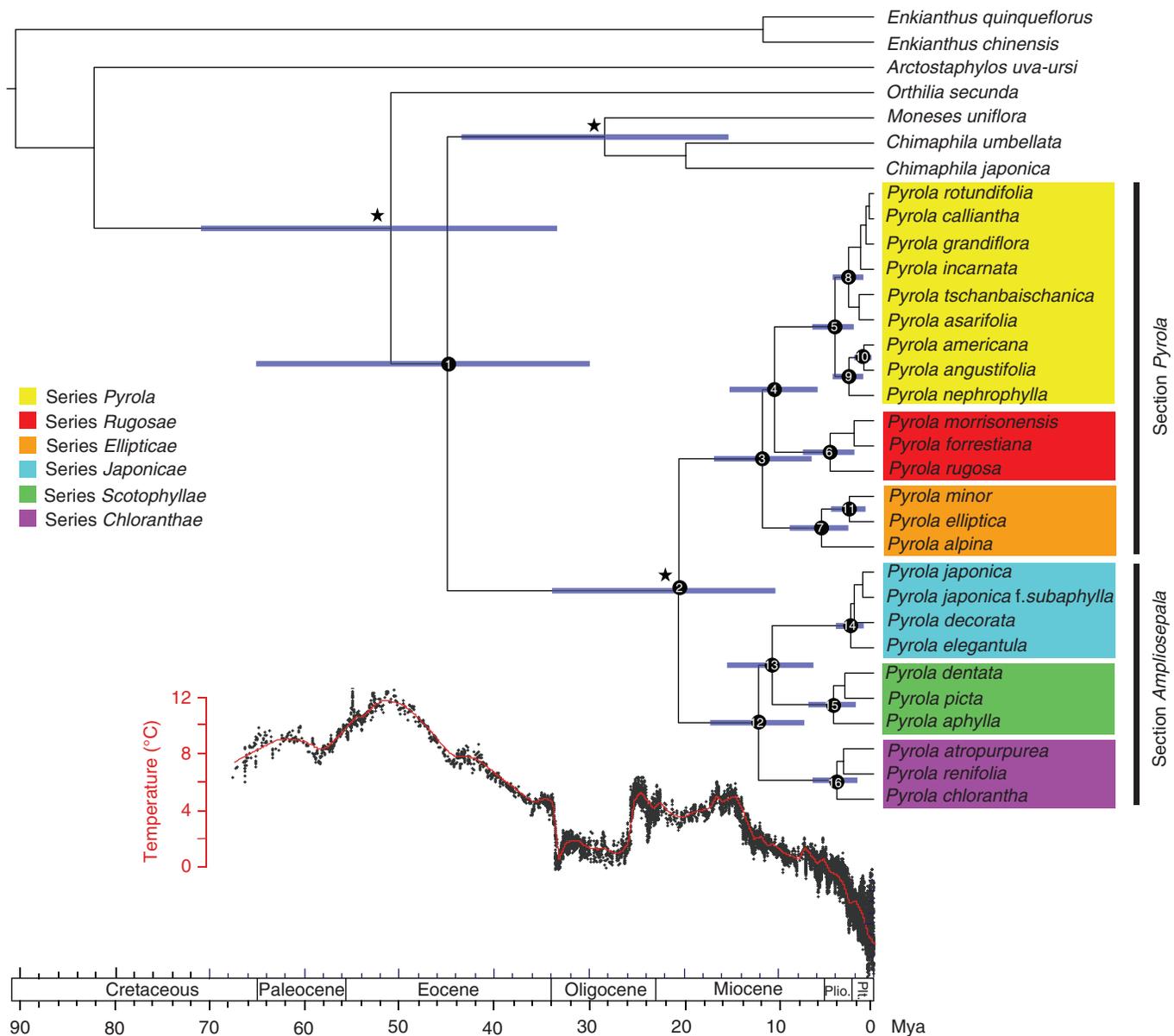
FIG. 2. Maximum clade credibility chronogram for *Pyrola* and Pyroleae inferred from a BEAST dating analysis of dataset 2 (D2) using combined data from ITS, *atpB-rbcL*, *trnL-trnF* and *trnS-trnG*. Nodes 1–16 are nodes of interest, with light blue horizontal bars indicating the 95 % highest posterior density interval. Stars indicate the three secondary calibration points inferred from D1. Global temperature changes are shown by the curve adapted from Zachos *et al.* (2001). A geological time scale showing major epochs is provided at the bottom for reference. Plio, Pliocene; Plt, Pleistocene.
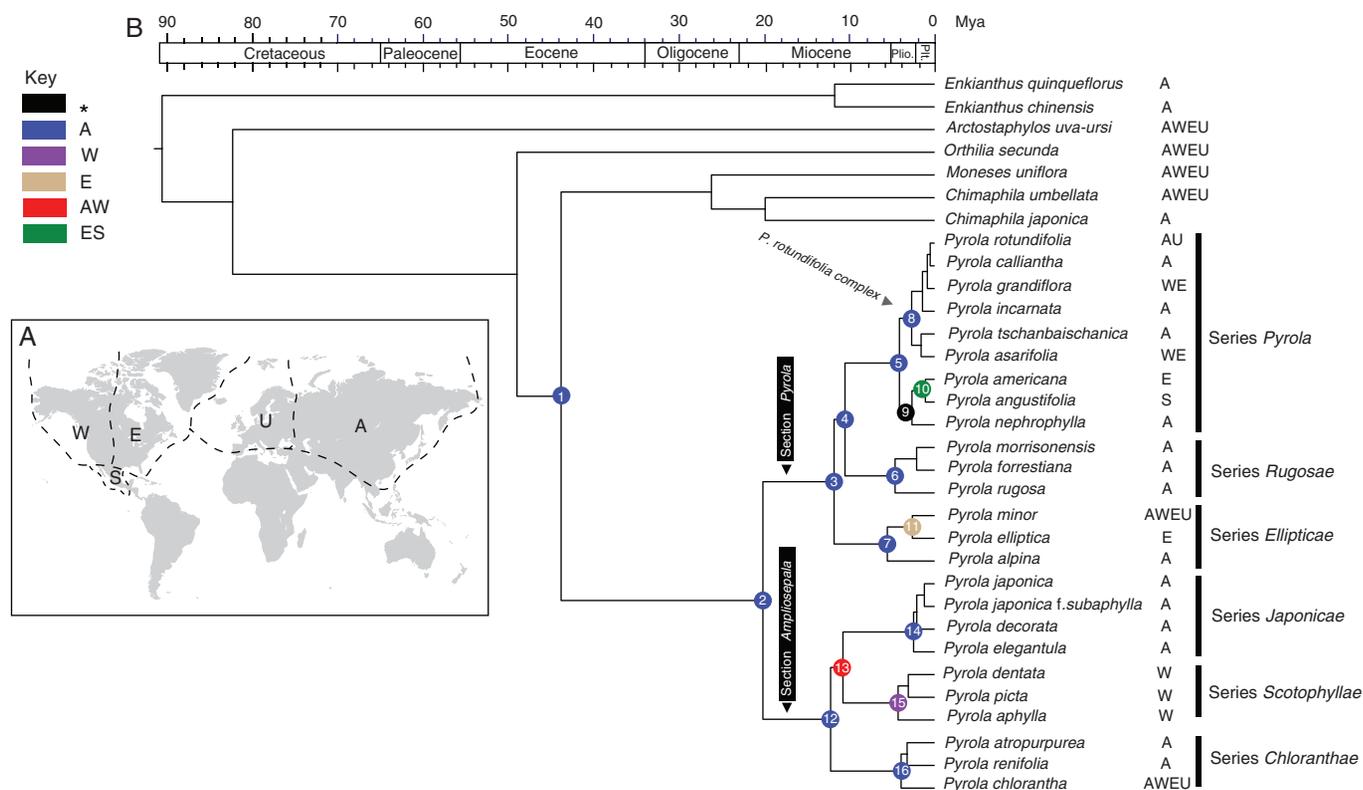
FIG. 3. (A) Map of the world showing the five areas of interest in the analysis of *Pyrola* biogeography: Asia (A); Europe (U); western North America (W); eastern North America (E); and southern North America (S). (B) A Bayesian approach to dispersal–vicariance analysis (S-DIVA), based on phylogenetic analysis D2 (Fig. 2). Pie charts at each node show probabilities of alternative ancestral ranges. Colour keys show possible ancestral ranges at different nodes; black with an asterisk represents uncertain estimation. The area coding is shown following each species name.

These phylogenetic relationships were similar to those recovered by Liu *et al.* (2010).

Direct estimation of major divergence events in Pyroleae (analysis 1)

Differences between the Yule and birth/death tree priors were evaluated by comparison of Bayes factors ($\log_{10}$ Bayes factor $<$ 0·5), which were of approximately the same magnitude and not likely to bias time estimation using Ericaceae *matK* data. Here, we only report and discuss the mean ages and 95 % highest posterior density (HPD) inferred from the Yule tree prior. Our results suggest that *Pyrola* split from the *Chimaphila–Moneses* clade during the mid Eocene (46·1 Mya; 95 % HPD 65·0–29·7 Mya; node 1 in Fig. 2 and Table 1) and began to diversify into sections *Pyrola* and *Ampliosepala* (*sensu* Liu *et al.*, 2010) in the early Miocene (20·7 Mya; 95 % HPD 33·9–10·4 Mya; node 2 in Fig. 2 and Table 1).

Estimation of divergence events in Pyrola using secondary calibration (analysis 2)

Within *Pyrola*, there is, again, no significant difference between the Yule and birth/death models for time inference ($\log_{10}$ Bayes factor $<$ 0·5), and only the mean ages and 95 % HPD inferred from the Yule tree prior were reported and discussed. It is shown that all six series (*Pyrola*, *Ellipticae*,

Rugosae, *Japonicae*, *Scotophyllae* and *Chloranthae*) in the genus diversified recently relative to divergence of genus *Pyrola* from the remainder of tribe Pyroleae, during the transitional phase between the late Miocene and early Pliocene (nodes 5–7 and 14–16 in Fig. 2; Table 1). Of these, the disjunction between series *Japonicae* in eastern Asia and *Scotophyllae* in western North America appears to have been established during the late Miocene at 10·7 (15·5–6·3) Mya (node 13 in Fig. 2; Table 1). Divergence between series *Chloranthae* and the *Japonicae–Scotophyllae* clade is estimated at 12·1 (17·2–7·3) Mya (node 12 in Fig. 2; Table 1), and similar divergence times within section *Pyrola* were estimated at approx. 11·7 (16·9–6·5) Mya (node 3 in Fig. 2; Table 1). Series *Pyrola*, containing North American and Eurasian members, is estimated to have diverged from series *Rugosae* approx. 10·5 (15·2–5·9) Mya (node 4 in Fig. 2; Table 1). The three circumboreal taxa (the *P. rotundifolia* complex, *P. minor* and *P. chlorantha*) arose more recently at 3·9–2·6 Mya in the Pliocene (nodes 8, 11 and 16 in Fig. 2; Table 1).

Biogeographic reconstructions

Ancestral area reconstruction under Bayesian optimization (Table 1; Fig. 3) or maximum likelihood (Table 1; Supplementary Data Fig. S1) yielded similar results. The origin of *Pyrola* is indicated to be Asia in both S-DIVA and Lagrange analyses (node 1 in Fig. 3 and Fig. S1). Crown

TABLE 1. *Posterior age distributions of major nodes of* Pyrola, *with results of ancestral reconstruction using S-DIVA and Lagrange*

Nodes	Age estimates		S-DIVA [MP]	Likelihood-DEC [RP]*
	Mean (Mya)	95 % HPD (Mya)		
1. Origin of *Pyrola*	46·1	65·0–29·7	A 100	[A|A] 0·48
2. Diversification of *Pyrola*	20·7	33·9–10·4	A 100	[A|A] 0·37
3. Crown of section *Pyrola*	11·7	16·9–6·5	A 100	[A|A] 0·42
4. Split between series *Pyrola* and *Rugosae*	10·5	15·2–5·9	A 100	[A|A] 0·76
5. Crown of series *Pyrola*	4·1	6·5–2·1	A 100	[A|A] 0·13
6. Crown of series *Rugosae*	4·6	7·5–2·0	A 100	[A|A] 1·00
7. Crown of series *Ellipticae*	5·5	8·8–2·7	A 100	[A|A] 0·19 [AWEU|A] 0·18 [AU|A] 0·10 [AEU|A] 0·10
8. Node of *P. rotundifolia* complex	2·6	4·3–1·1	A 100	[A|A] 0·37 [AW|A] 0·18 [W|W] 0·10
9. Node of *P. nephrophylla, P. americana* and *P. angustifolia*	2·6	4·3–1·1	Ambiguous	[WES|A] 0·23 [W|A] 0·12 [ESU|A] 0·11 [WS|A] 0·10 [WE|A] 0·10
10. Split between *P. americana* and *P. angustifolia*	1·0	2·0–0·2	ES 100	[E|S] 0·64 [E|WS] 0·12 [WE|S] 0·12
11. Split between *P. elliptica* and *P. minor*	2·6	4·5–0·8	E 100	[AWEU|E] 0·49 [AEU|E] 0·17 [AWE|E] 0·13
12. Crown of sect. *Ampliosepala*	12·1	17·2–7·3	A 100	[A|A] 0·30 [AW|A] 0·14
13. Split between series *Japonicae* and *Scotophyllae*	10·7	15·5–6·3	AW 100	[A|W] 0·49 [A|A] 0·19 [W|W] 0·13
14. Crown of series *Japonicae*	2·4	4·0–1·0	A 100	[A|A] 0·98
15. Crown of series *Scotophyllae*	4·2	6·9–1·9	W 100	[W|W] 0·92
16. Crown of series *Chloranthae*	3·9	6·4–1·7	A 100	[A|AWEU] 0·27 [A|AWU] 0·16 [A|A] 0·14

Node numbers are as in Fig. 2.

S-DIVA, statistical dispersal–vicariance analysis; DEC, dispersal–extinction–cladogenesis model; HPD, highest posterior density date range; Mya, million years ago; MP, marginal probability; RP, relative probability.

*The vertical bar separates the inferred ancestral range for the upper branch (left letter) from that reconstructed for the lower branch (right letter) descending from the node. Values represent the relative probability of that inference. Alternative ancestral area reconstructions that fall within two log-likelihood units of the optimal scenario and have a relative probability ≥0·1 are given.

radiations of section *Pyrola* (node 3), and series *Pyrola* (node 5) and *Rugosae* (node 6) therein, are indicated to be in Asia (Fig. 3 and Fig. S1); crown radiation for series *Ellipticae* (node 7) is indicated as Asia by S-DIVA (Fig. 3), but could be Asia and another area according to Lagrange (Fig. S1). The crown split of section *Ampliosepala* (node 12) was probably also in Asia (Fig. 3 and Fig. S1), although Lagrange analysis reveals a smaller possibility that it could have been a vicariance event between an Asia-only and Asia + western North America lineage (Table 1). Of the series therein, *Japonicae* (node 14) and *Scotophyllae* (node 15) clearly originate in Asia and western North America, respectively, suggesting that they diverged by vicariance (Fig. 3 and Fig. S1). The crown divergence event in series *Chloranthae* (node 16) occurred in Asia (Fig. 3), although Lagrange results show a lineage from Asia splitting from one of uncertain initial distribution, but not with high possibility (Table 1).

North America appears to have been colonized at least five times (Table 1; Fig. 3; Supplementary Data Fig. S1). Only two of these events do not involve circumboreal species; these were series *Scotophyllae* (node 15), and the common ancestor of *P. americana* and *P. angustifolia* in series *Pyrola* (node 10). A third event involved circumboreal range expansion of *P. chlorantha* (node 16), almost certainly from an Asian origin. North America was similarly reached via circumboreal range expansion from an Asian ancestor in the *P. rotundifolia* complex (node 8), at least once and possibly twice. One more colonization event involved the common ancestor of the eastern North American *P. elliptica* and the circumboreal *P. minor* (node 11). This could reflect either a third case of North America being reached via a circumboreal taxon (if *P. elliptica* is derived from *P. minor*) or that the common ancestor reached North America first and one descendent became circumboreal from there.

DISCUSSION

Our results show that Asia may be a cradle for the evolution of *Pyrola* (Table 1; Fig. 3; Supplementary Data Fig. S1). Given that all taxa that are endemic to Asia occur only in eastern

Asia, that region is strongly indicated to be the area of origin for both the genus and many of its sub-groups. Our data further show that each of the three circumboreal taxa examined originated independently (nodes 8, 11 and 16 in Fig. 3 and Fig. S1; Table 1). Including these three lineages, the genus colonized North America at least five times. If one excludes the circumboreal lineages, the biogeographical pattern within the genus is broadly that of a Tertiary relict group, with a concentration of species in eastern Asia and North America, but none in Europe (see also *Panax*, *Symplocarpus*, *Boykinia* and *Calycanthus*, Xiang and Soltis, 2001). The genus appears to have been slowly diversifying since the Eocene period, although the dates calculated for divergence events within the genus must be treated with caution because limitations in fossil data prevented cross-calibration of nodes.

Biogeographic history of Pyrola

Diversification in Pyroleae appears to have begun with the divergence of *Orthilia* at 50·7 (95 % HPD 70·9–33·5) Mya around the first half of the Tertiary period (Figs 1 and 2). It might therefore have coincided with a time when the climate in the northern hemisphere is thought to have begun cooling in a series of steps (Miller *et al.*, 1987; Miller, 1992; Zachos *et al.*, 2001), leading to a major shift of vegetation pattern in much of Eurasia and North America from thermophilic, evergreen woody taxa to herbaceous plants (Collinson, 1992; Manchester, 1999). *Pyrola* itself then diverged from the common ancestor of *Chimaphila* and *Moneses* in the middle Eocene (46·1 Mya; 95 % HPD 65·0–29·7 Mya; node 1 in Fig. 2). Diversification among extant *Pyrola* did not begin until the early Miocene at 20·7 (95 % HPD 33·9–10·4) Mya (node 2 in Fig. 2) and thus possibly began during a period of climatic cooling in the Northern Hemisphere, fitting the appearance of cold, xeric habitats (Prothero and Berggren, 1992). At this time, the ancestors of sections *Pyrola* and *Ampliosepala* began to diversify in Asia (node 2 in Fig. 3 and Supplementary Data Fig. S1). Around the middle Miocene, when global temperatures began to fall dramatically (Zachos *et al.*, 2001; Graham, 2011), each of the two sections in *Pyrola* diversified into three series. This is estimated to have occurred across a similar time frame within each section, between 10·7 (15·5–6·3) and 12·1 (17·2–7·3) Mya (nodes 3, 4, 12 and 13 in Fig. 2; Table 1). All four of these speciations are inferred either to have occurred in Asia, or in once case to have been a vicariance event between Asia and western North America (Table 1; Fig. 3; Fig. S1). This implies that Asia may have been a cradle for *Pyrola* diversification.

In section *Ampliosepala*, the second divergence event was between the east Asian series *Japonicae* and western North American series *Scotophyllae*, forming a classic trans-Beringian disjunction (node 13 in Fig. 3 and Supplementary Data Fig. S1). This divergence event occurred at 10·7 (15·5–6·3) Mya (Table 1), and hence fits a hypothesis of vicariance due to local extinction in Beringia, caused by local climatic cooling in the region, which began 15 Mya (White *et al.* 1997; Tiffney and Manchester, 2001). *Scotophyllae* are relatively xeric in their habitat preference (Haber, 1987; Jolles and Wolfe, 2012), and consequently extend further east to 103°W than many Tertiary relict groups with a western North American distribution. Despite this, and being probably the first *Pyrola* series to reach North America, *Scotophyllae* did not reach the eastern side, testifying to the strength of the middle of the continent as a barrier to Tertiary relict taxa. The only other lineage of section *Ampliosepala* to reach North America was the circumboreal *P. chlorantha*; the timing of this is not clear, but it will have been after this species diverged, hence <5 Mya and after the breaking of the Bering Land Bridge (BLB; Gladenkov *et al.*, 2002). Otherwise, series *Japonicae* and *Chloranthae* each seems to have undergone local allopatric speciation events within eastern Asia, giving rise to north-east Asian *P. japonica*, southwest Chinese *P. decorata*, south-east Chinese *P. elegantula* and Taiwanese *P. albo-reticulata* in *Japonicae*, and south-west Chinese *P. atropurpura* and north-east Asian *P. renifolia* in *Chloranthae* (Liu *et al.*, 2010). In section *Pyrola*, series *Rugosae* occurs only in high elevation forest (1500–4000 m) in south-west China and Taiwan (Qin and Stevens, 2005), exhibiting a disjunction also seen in series *Japonicae* and numerous other genera such as *Moneses*, *Chimaphila*, *Cunninghamia* and *Taiwania* (Ying and Hsu, 2002). Rapid uplift of the Qinghai–Tibetan Plateau in the Late Miocene (Harrison *et al.*, 1992; Guo *et al.*, 2002; Spicer *et al.*, 2003) and associated climate shifts might have contributed to the formation of this disjunction. In contrast, series *Pyrola* and *Ellipticae* each includes Asian, North American and circumboreal species, indicating that their ancestor may have been more northern and/or cold adapted than that of series *Rugosae*. In series *Pyrola*, an ancestor from Asia appears to have given rise to both the widespread *P. rotundifolia* complex and a clade containing the North American species *P. americana* and *P. angustifolia*; hence this series alone has twice reached North America. Vicariance between *P. nephrophylla* and *P. americana*/*P. angustifolia* occurred around 3 Mya (node 9, Fig. 2), well after the BLB breakage. Considering its dispersal ability with dust-like seeds and that movement through western North America into eastern and southern North America occurred recently, one of these areas might have been reached directly via long-distance dispersal.

Origin and spread of the circumboreal taxa

Of the three circumboreal taxa, *P. chlorantha* certainly originated in Asia (Table 1; Fig. 3; Supplementary Data Fig. S1). This species is absent from Greenland and much of north-western Europe, but is present in Kamchatka, which is consistent with it having spread east and west from a starting point in a higher latitude region of Asia. The other two are more complex. The common ancestor of *P. minor* and the North American *P. elliptica* appears to have crossed the BLB then underwent a north/south split in North America, following which the southern vicariant moved into eastern North America, and the northern vicariant, *P. minor*, returned to Eurasia. However, if *P. elliptica* was derived from *P. minor*, then it is possible to infer only one movement between Eurasia and North America of a *P. minor*-like common ancestor that was simultaneously dispersing across Eurasia. A third, less parsimonious possibility is that divergence happened in Asia, and that the two lineages arrived in North America at different times. The *P. rotundifolia* group is more widespread than the other circumboreal taxa, extending further south to the Himalayan areas. This wide climatic range might reflect greater diversification, as the group has been divided into six segregate species each with distinct ranges.

Furthermore, ecotypes within regions such as Britain and Ireland (*P. rotundifolia* subsp. *maritima*) also reflect ecological diversity within the group. Despite this, there is little genetic (Liu *et al.*, 2010) or morphological (Haber, 1983; Haber and Takahashi, 1988) differentiation within the group. The group again originated in Asia, but might have reached North America twice (though the support values are too low to confirm this). For all three circumboreal taxa, therefore, we have information about their area of origin but little concerning their subsequent spread. A detailed phylogenetic analysis of each, using rapidly evolving markers, might reveal a similarly complex history to that of certain other species that have been investigated (Abbott *et al.*, 2000; Brochmann and Brysting, 2008; Eidesen *et al.*, 2013).

Conditions on America–Eurasia land bridges became increasingly cold as the Tertiary progressed, restricting migration to cool-tolerant and deciduous taxa, and ultimately to boreal taxa (Basinger *et al.*, 1994; McIver and Basinger, 1999). Evergreen taxa such as *P. minor*, the *P. rotundifolia* complex and *P. chlorantha* can thrive in or near arctic regions at present (Křísa, 1966; Hultén, 1968), and they exhibit a small, herbaceous habit and some adaptations to cold environments similar to other Ericaceous Arcto-Tertiary taxa (Landhäusser *et al.*, 1997). Notably, mycoheterotrophy in *Pyrola* allows them to acquire carbon and other essential elements via fungal symbionts from surrounding autotrophic plants, with carbon gain through this process increasing as available light decreases (Zimmer *et al.*, 2007; Matsuda *et al.*, 2012), as in *Cephalanthera* (Preiss *et al.*, 2010). This may be part of the reason why *Pyrola* taxa are so prevalent at high latitudes where light is scarce for much of the year. Tolerance of cold and winter darkness might have assisted *Pyrola* in moving between continents and repeatedly supplying species into the Arctic flora. Comparisons of the degree of mycoheterotrophy between species might indicate the degree to which it has assisted these processes.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford journals.org and consist of the following. Table S1: GenBank accession numbers used to estimate age divergence within Ericaceae (dataset 1, D1). Table S2: voucher information and GenBank accession numbers used to estimate age divergence within Pyroleae (dataset 2, D2). Figure S1: map of the world showing the five areas of interest in the analysis of Pyrola biogeography, together with the maximum likelihood reconstruction of geographical range evolution under the dispersal–extinction–cladogenesis model, based on phylogenetic analysis D2.

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