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Diversification of almonds, peaches, plums and cherries – Molecular systematics and biogeographic history of *Prunus* (Rosaceae)

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ABSTRACT

Most previous molecular phylogenetic studies of *Prunus* have been conducted primarily with crop species and their close relatives. As the center of crop diversity of the genus is in Eurasia, the geographic origin of *Prunus* has inevitably been inferred to be Eurasia as well. The lesser-known tropical *Prunus* species have not been well represented in previous phylogenetic reconstructions; therefore, their effects on inferences about the phylogenetic structure and geographic origin of *Prunus* are uncertain. In this study, we examined the phylogeny of *Prunus*, including an expanded sampling of species from tropical regions in Southeast Asia and the Americas, using sequences from four plastid markers and the nuclear ribosomal ITS region. A penalized likelihood method was used to estimate the absolute age of *Prunus* and the timing of infrageneric cladogenic events. The geographic origin of *Prunus* and ancestral sites of cladogenesis were inferred using the Bayes-DIVA approach. Our results indicate that the modern genus appeared ~61 Myr in eastern Asia and that diversification of all major lineages may have been triggered by the global warming period of the early Eocene. In addition, our molecular dating estimates suggest that the crown clade that includes the temperate deciduous crop species is older than the one that includes the tropical evergreen species, while incongruence between plastid and nuclear phylogenies suggests that the latter lineage originated via an ancient hybridization event. The most recent common ancestor (MRCA) of the temperate crop species was a component of the continuous boreotropical forests of the Northern Hemisphere, while the MRCA of the tropical species represented the last remains of the boreotropical elements and subsequently radiated throughout the Old and New World tropics from refugial areas at lower latitudes. Complex biogeographic histories leading to the present global distribution of the genus were driven by several geologic events, climatic oscillations, and independent dispersals across continents via the Bering and the North Atlantic Land Bridges during different geologic time periods.

1. Introduction

Prunus L. (Rosaceae) consists of over 200 species of deciduous and evergreen trees and shrubs with several members that are economically important fruit and nut crops. These include peach (*P. persica* (L.) Batsch), apricot (*P. armeniaca* L.), almond (*P. dulcis* D.A.Webb), and sweet cherry (*P. avium* (L.) L.). The genus is widely distributed both in the temperate zone of the Northern Hemisphere (Rehder, 1940) and in the subtropical and tropical forests of Asia, Africa, South America and Australia (Kalkman, 1965).

The most widely accepted infrageneric classification of *Prunus* by Rehder (1940) consists of five subgenera: *Amygdalus* (peaches and almonds), *Cerasus* (cherries), *Prunus* (plums), *Laurocerasus* (evergreen laurel-cherries), and *Padus* (deciduous bird-cherries). Mason's (1913) subgenus *Emplectocladus* (six species from arid areas in North America) is considered closely related to subgenus *Amygdalus* (Rehder, 1940). All previous molecular phylogenetic studies of *Prunus* using plastid (*trnL-L-F*, *ndhF* and *trnS-S-G*) and nuclear ribosomal ITS (nrITS) sequences have clearly supported the monophyly of the genus (Bortiri et al., 2001, 2006; Chin et al., 2013; Lee and Wen, 2001; Shaw and Small, 2004; Wen et al., 2008) and shown that the small genus *Maddenia* (five Asian species) is included (Chin et al., 2010; Liu et al., 2013). All of these studies have also refuted previous circumscriptions of subgenera, as they were all shown to be either paraphyletic or polyphyletic, with the exception of subgenus *Emplectocladus*. These earlier phylogenies of the genus as exemplified by plastid DNA reveal three

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well-supported clades – the *Amygdalus–Prunus–Emplectocladus* clade, the *Cerasus* clade, and the *Laurocerasus–Padus* clade (Bortiri et al., 2001; Chin et al., 2010, 2013; Shaw and Small, 2004; Wen et al., 2008). In contrast, phylogenies based on nrITS DNA data have supported only the *Amygdalus–Prunus–Emplectocladus* clade, while *Cerasus* was closely associated with some members of *Laurocerasus*, *Padus* and all former *Maddenia* species (Chin et al., 2010, 2013; Wen et al., 2008). This incongruence has led to suggestions of a hybrid origin of subgenus *Cerasus* (Chin et al., 2010).

The phylogenetic position of *Prunus* within Rosaceae has not been firmly established. Traditionally, the circumscription of subfamily Amygdaloideae included *Oemleria*, *Exochorda*, *Prinsepia*, *Maddenia*, *Pygeum* and *Prunus* (Schulze-Menz, 1964; Takhtajan, 1997). However, this group is not supported as monophyletic (Potter et al., 2007). The most recent classification of Rosaceae proposed by Potter et al. (2007) classified *Prunus* (including *Maddenia* and *Pygeum*) as the only genus of tribe Amygdaleae within an expanded subfamily Spiraeoideae; the name of that subfamily has since been corrected to Amygdaloideae based on recent changes to the International Code of Nomenclature for Algae, Fungi, and Plants (McNeill et al., 2012).

The unresolved relationships of *Prunus* to other genera have contributed to uncertainty about its geographic origin, which has been generally speculated to be Eurasia based on the center of crop diversity in this region for many domesticated species (Watkins, 1976) and molecular phylogenetic studies that were based mostly on crop species of *Prunus* and their wild relatives (e.g., Bortiri et al., 2001). However, the phylogenetic position of *Prunus* within Rosaceae may affect inferences about its area of origin; for example, a North American origin is equally parsimonious with a Eurasian origin when *Oemleria* (native of North America) is placed as the sister group to *Prunus* (Bortiri et al., 2001). Moreover it is uncertain how the inclusion in *Prunus* of Old World species formerly assigned to *Pygeum*, proposed by Kalkman (1965), *Maddenia* (Chin et al., 2010; Shi et al., 2013; Wen and Shi, 2012) as supported by previous molecular phylogenetic analyses (Chin et al., 2010, 2013; Wen et al., 2008), along with increased sampling of neotropical species, will impact reconstruction of the geographic origin of *Prunus*, since none of these taxa were included in Bortiri et al.'s (2001) analysis or mentioned by Watkins (1976).

Intercontinental disjunctions among closely related plant species have been well documented (Li, 1952; Thorne, 1972). In particular, eastern Asian-eastern North American disjunctions have been actively investigated (e.g., Wen, 1998, 1999; Wen et al., 2010; Wood, 1972; Wu, 1983; Xiang et al., 2000). These disjunctions have generally been attributed to fragmentation of a once continuous belt of mixed mesophytic broadleaf-evergreen and subtropical vegetation (i.e., the boreotropical flora, Lavin and Luckow, 1993; Tiffney, 1985a,b; Wolfe, 1975) in the Northern Hemisphere. Remnants of the boreotropical floristic elements occur today in eastern Asia and eastern North America. The lineages that once grew in other areas became extinct by the Oligocene due to a combination of climatic and geologic changes (e.g., Li, 1952; Wen, 1999; Wen et al., 2010; Wolfe, 1975). There are two land bridges that potentially served as routes for floristic exchanges between Eurasia and North America: (A) the North Atlantic Land Bridges (NALB), which connected Europe to eastern North America via Greenland, and (B) the Bering Land Bridge (BLB), which connected northeastern Asia and western North America (Fig. 1). The relative importance of these two land bridges has been contentious as each of them may have been significant to plant migration independently and at different times (Wen et al., 2010). The BLB has been favored by most botanists as an important dispersal route because this land bridge remained open until ~3.5 Myr compared to the NALB which closed in the early Eocene (50 Myr) (Tiffney, 1985a,b; Tiffney and Manchester, 2001; Wen, 1999; Wen et al., 2010). The presence of the Turgai Straits, which divided Asia from Europe and persisted until the early Miocene (30 Myr), certainly could have limited floristic exchanges between Asia and Europe. Nonetheless, dispersal across the Turgai Straits from Asia to Europe was still possible through 'island-hopping' across the Tethys Seaway (Tiffney, 1985a,b; Wolfe, 1975). Therefore, the relative importance of the NALB and the BLB in accounting for the historical distribution of *Prunus* in the Northern Hemisphere depends on both the location of origin and the timing of cladogenesis events among species.

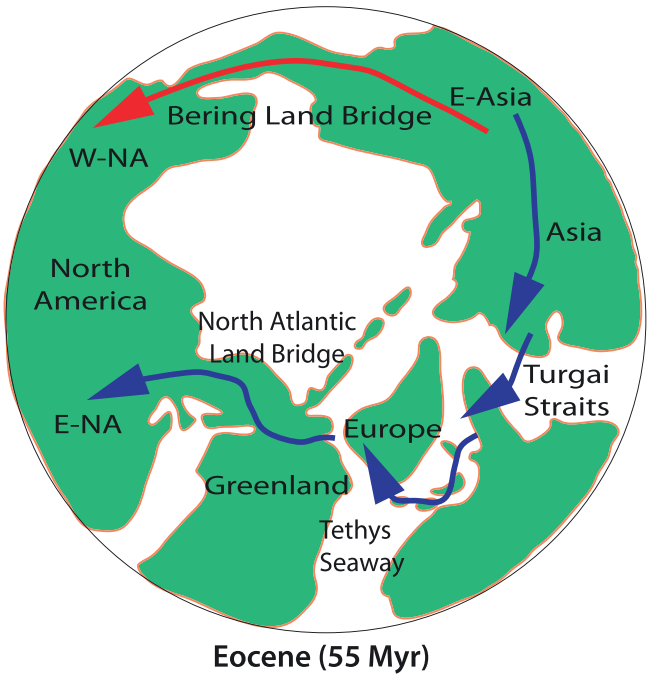

Fig. 1. Diagrammatic representation of the positions for two major land bridges during early Eocene period (55 Myr). Direct migration via the Bering Land Bridge is plausible between eastern Asia and western North America. Conditional migration between Asia and eastern North America via the North Atlantic Land Bridges is possible via 'island-hopping' across the Tethys seaways.

The main goal of this study is to obtain a well-resolved and thoroughly sampled phylogeny for *Prunus*, which will be used to address how past geologic and climatic changes have affected the evolutionary and biogeographic patterns in the genus. A good understanding of the phylogenetic relationships and divergence times of taxa that display intercontinental disjunct patterns in conjunction with understanding the fossil record is imperative to understand their biogeographic histories (Benedict et al., 2011; Wen et al., 2013; Xiang et al., 2000). Thus, there are three specific objectives of this study. Our first objective is to reconstruct the phylogeny of *Prunus* with an expanded sampling of Paleotropical species formerly assigned to the genus *Pygeum* and of Neotropical species that were poorly sampled in earlier molecular phylogenetic studies. As pointed out above, it is uncertain how the inclusion of tropical *Prunus* species will affect reconstruction of the geographic origin of *Prunus*. Our second objective is to determine the molecular divergence times of *Prunus* and its major lineages from phylogenetic analyses at the family and infrageneric levels based on plastid sequences using a penalized likelihood approach (Sanderson, 2002). Previous phylogenetic analyses with *Prunus* have been performed with up to two plastid markers. This study aims to resolve the relationships among lineages using four plastid loci as well as the nrITS region. Lastly, we seek to infer the geographic origin and biogeographic diversification histories of *Prunus* based on known paleontological evidence, paleobotanical records and our estimated divergence times for each lineage.

2. Materials and methods

2.1. Taxon sampling and outgroup selection

Taxa sampled for this study, voucher information for all plant accessions, and sequence information are listed in the Appendix A. Eighty-one *Prunus* spp. representing all five subgenera recognized by Rehder (1940) as well as subgenus *Emplectocladus* (Mason, 1913) were sampled for the phylogenetic analysis. In addition, two outgroup species, *Oemleria cerasiformis* and *Physocarpus opulifolius*, were selected based on previous phylogenetic studies of Rosaceae (Potter et al., 2007).

2.2. DNA extraction, amplification, sequencing and phylogenetic analysis

2.2.1. Prunus plastid DNA and nrITS phylogeny

DNA was extracted from leaf tissues using a modified CTAB method (Doyle and Doyle, 1987). Primer sequences and PCR amplification of the plastid regions *rbcL*, *matK*, *trnL-L-F* and *trnS-S-G* follow Shaw and Small (2004) and Shaw et al. (2005), while amplification of the nuclear ribosomal ITS (nrITS) region was performed according to Bortiri et al. (2001) and Wen et al. (2008). The cleaned amplicons of both nrITS and plastid markers were sequenced on an ABI PRISM 373l capillary DNA analyzer (Applied Biosystems, Forster City, CA, USA). The sequences were edited in Sequencher™ 4.12 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned using CLUSTAL X (Thompson et al., 1997). Minor manual editing of the aligned sequences was performed using Se-AL 2.0 (Rambaut, 1996).

2.2.2. Rosaceae plastid DNA phylogeny

The plastid DNA phylogeny of Rosaceae was inferred using three plastid markers (*trnL-L-F*, *matK* and *rbcL*) from 33 taxa covering the three recognized subfamilies Amygdaloideae, Rosoideae, and Dryadoideae, in addition to the 81 *Prunus* taxa. The sequences for other Rosaceae taxa besides *Prunus* were obtained from Potter et al. (2007), while all sequences for *Prunus* were obtained from the procedures described above. The optimal model of molecular evolution for the nrITS and plastid sequences was determined by the Aikaike Information Criterion (AIC) using Modeltest ver. 3.7 (Posada and Buckley, 2004; Posada and Crandall, 1998). The GTR + G + I model was selected and used for all subsequent analyses. Bayesian inference, implemented in MrBayes (Huelsenbeckn and Ronquist, 2001) and additional maximum likelihood bootstrap support, implemented in the RAxML Blackbox web server (Stamatakis et al., 2008) were conducted according to Chin et al. (2013).

2.3. Construction of Bayesian hypotheses

In this study, all polytomies were interpreted as soft polytomies, i.e., all possible resolutions were considered equally probable. Phylogenetic hypotheses were generated and employed to depict the range of possible evolutionary scenarios among lineages for two major polytomies: (A) among lineages in Clade A (Fig. 2) of the Rosaceae plastid DNA phylogeny; and (B) among lineages in the 'Racemose' clade of the *Prunus* plastid DNA phylogeny (Fig. 3). Construction of the hypotheses for these two groups followed a general framework: (1) the desired constraint was first generated using MacClade (Maddison and Maddison, 2005); (2) the constraint was used to filter all post-stationary posterior Bayesian trees generated as above in PAUP* (Swofford, 2001). All constraints that were generated are summarized in Appendix B ('Rosaceae') and Appendix C ('*Prunus*'). Three major hypotheses were generated to represent the cladogenesis of the four lineages (tribes Maleae, Spiraeeae, Sorbarieae, and the genus *Prunus*) in Clade A. In addition, an 'overall' construct (Appendix B), where all the four lineages in Clade A are shown to join in a polytomy, was used to filter the post-stationary trees; a total of 685 trees was obtained from this construct (Appendix B). Fifteen constraints were generated to resolve the polytomy of the 'Racemose' clade in *Prunus* and this resulted in 5639 trees (Appendix C).

2.4. Molecular dating

Molecular dating analyses were separately performed on the three-gene plastid concatenated dataset (Rosaceae phylogeny) and the four-gene plastid concatenated dataset (*Prunus* phylogeny). To test whether the two separate plastid DNA datasets had evolved in a clocklike fashion, a likelihood ratio test was performed with the best ML tree obtained from RAxML (described above). Likelihoods of the data with and without a molecular clock constraint were calculated in PAUP* and the likelihood ratio statistic was compared using χ^2 critical values with either 81 (*Prunus* phylogeny) or 116 (Rosaceae phylogeny) degrees of freedom (Felsenstein, 1981). The test rejected the molecular clock assumption ($p < 0.001$) for both datasets. Hence, we applied the penalized likelihood (PL, Sanderson, 2002) method of rate smoothing as implemented in r8s v1.71 (Sanderson, 2003) to correct for rate heterogeneity. The optimum smoothing parameter value of 3.2 was determined using cross-validation procedure in r8s, based on the 50% majority-rule consensus Bayesian tree. This value was applied to subsequent analyses below.

2.4.1. Dating the Prunus crown age

To derive the age of the *Prunus* crown from the Rosaceae plastid gene phylogeny, we applied a fixed age with two values for the Rosales crown, node Z in Fig. 2. The two calibration ages (90 and 96 Myr) applied to this node are the minimum and maximum ages obtained from Wang et al. (2009), who used a combined data set of ten plastid and two nuclear genes and seven fossil calibration points. In addition, we selected six fossils from each subfamily (Rosoideae, Dryadoideae and Amygdaloideae) and set them as a minimum age constraint to the MRCA node of the fossil for calibration (Table 1, Fig. 2). Fossil selection was largely based on whether the MRCA node was strongly supported in our phylogenetic analyses (BP > 70/PP > 90). The age of each fossil used in our calibration of the MRCA node was obtained from our best knowledge for the oldest fossil from a survey of literature (e.g., Benedict et al., 2011; DeVore and Pigg, 2007; Li et al., 2011) and online resources (Paleobiology Database, <http://paleobiodb.org>). Hence, besides testing the hypothesis of evolution, we also account for variation in topology and branch lengths. The two Rosales crown ages were treated as secondary calibrations; we therefore needed to include the uncertainty associated with using those ages. Hence, we first calculated the 95% credibility interval from the resulting ultrametric trees for each fixed age (minimum or maximum) setting. The age range for each node in the Rosaceae tree was finally summarized as the minimum age from the 95% credibility interval of the low fixed calibration and the maximum age from the 95% credibility interval of the high fixed calibration. This age interval represents the 'overall' 95% credibility interval that was used in the study.

2.4.2. Dating the Prunus lineages

The two estimated absolute ages ('overall' 95% credibility interval) for the *Prunus* crown clade were applied as fixed calibration points to derive the absolute age for all nodes within the *Prunus* plastid DNA phylogeny. Cross-validation determined a smoothing factor of 1.0 for the penalized likelihood analysis. The overall 95%

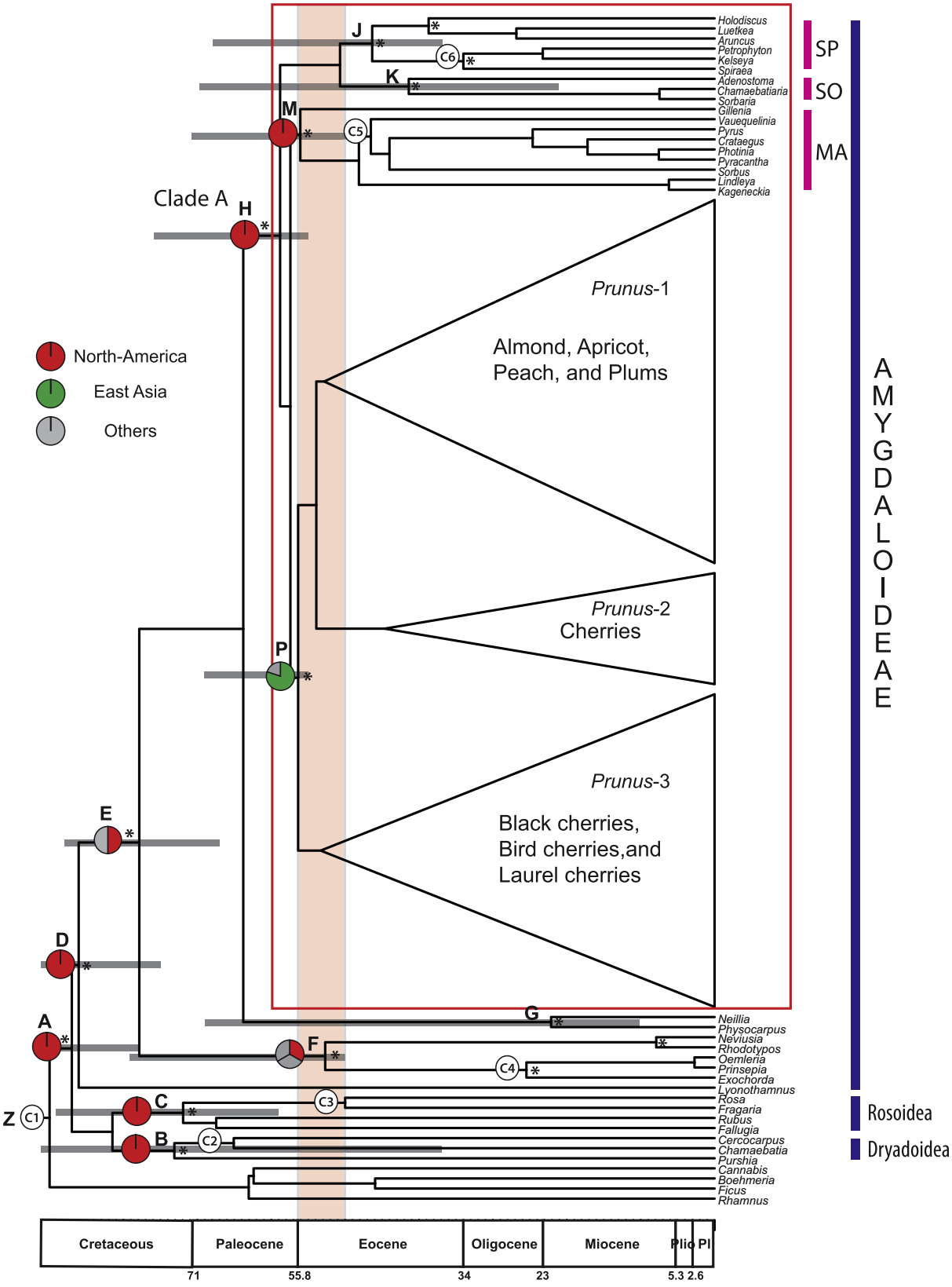

Fig. 2. Chronogram and ancestral areas for Rosaceae. The chronogram is based on the 50% majority-rule consensus tree generated by Bayesian analysis using the three-gene plastid sequences. Node marked with asterisk (*) represents support values of ML BP > 70/PP > 0.95. Labeled node bar represents the minimum and maximum values for most common recent ancestor (MRCA) of the clade summarized from both Low and High calibrations. The ancestral area for each Rosaceae lineage is inferred by Bayesian dispersal-vicariance analysis implemented in S-DIVA. Each pie chart at labeled node represents the frequency of each ancestral area. The pink bar between Paleocene-Eocene periods denotes the Paleocene-Eocene Thermal Maximum (PETM) event. Biogeographic regions: 'Others' denotes alternate ancestral areas that are less than or equal to 50% frequency. Definition of North America and eastern Asia follows Fig. 5. Abbreviations for infrafamilial rank: tribe – SP, Spiraeae, SO, Sorbariae, MA, Maleae; Calibration points c1, c2, c3, c4, c5, c6 follows Table. 1.

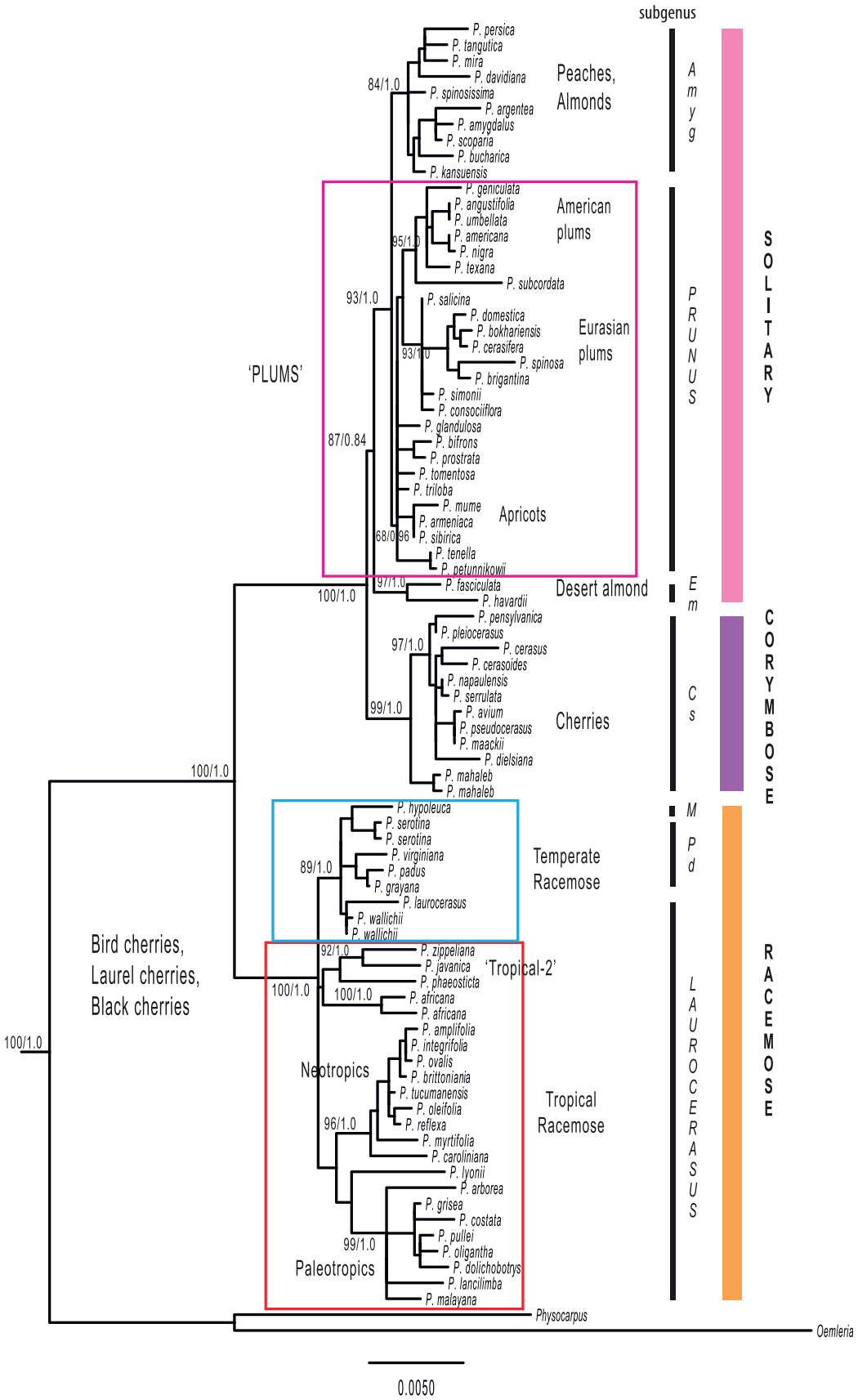

Fig. 3. Maximum likelihood tree of the four-gene concatenated plastid DNA sequences for phylogeny of *Prunus*. Numbers above or below branches indicate maximum likelihood bootstrap support/bayesian posterior probabilities. Abbreviations for subgenus: *Amyg*, *Amygdalus*; *Em*, *Emplectocladus*; *Cs*, *Cerasus*; *Lc*, *Laurocerasus*, *Pd*, *Padus*; *M*, '*Maddenia*'.

Table 1
Macrofossils of Rosaceae used as calibration points for molecular dating. Each fossil was selected to represent the oldest fossils known for each taxon. Age of each selected fossil was constrained as minimum age in the penalized likelihood analysis. Node labels correspond to Fig. 2.

Node	Anchor fossil	Geologic period, Myr	Assigned date, Myr	Geographic origin	Reference
C1*	Rosales crown	Cretaceous	Fixed (90, 96)		Wang et al. (2009)
C2	*Cercocarpus myricaefolius*	Late Eocene	34.07	North America, USA, Colorado	MacGinite (1953), Evanhoff et al. (2001)
C3	*Rosa germerensis*	Early Eocene	50	North America, USA, Idaho	Edelman (1975)
C4	*Oemleria janhartfordae*	Early Eocene	50	North America, USA, Washington	DeVore and Pigg (2007), Benedict et al. (2011)
C5	*Vauquelinia comptonifolia*	Middle Eocene	46	North America, USA, Wyoming	MacGinite (1969)
C6	*Spiraea sp.*	Early Eocene	50	North America, USA, Washington	DeVore and Pigg (2007)
C7	*Prunus wutuensis*	Early Eocene	55	East Asia, China, Shandong Province	Li et al. (2011)

credibility interval was calculated from the resulting ultrametric trees as described above.

2.5. Biogeographic inference

Seven biogeographic areas were defined based on the distributions of extant *Prunus* species: (A) North America; (B) Europe; (C) West Asia; (D) eastern Asia; (E) Southeast Asia; (F) South-Central America; and (G) Africa. We used a Bayesian approach to DIVA analysis (Nylander et al., 2008; Ronquist, 1997) implemented in S-DIVA 1.9b program (Yu et al., 2010) to infer the biogeographic patterns in *Prunus*. The analysis used maxareas = 2. Relative frequencies of ancestral areas reconstructed for each node were summarized and plotted onto one of the ultrametric trees.

3. Results

3.1. Diversification of Prunus: molecular phylogenetics and dating

3.1.1. Phylogeny of Rosaceae

The monophyly of *Prunus* and its inclusion within subfamily Amygdaloideae (Spiraeoideae of Potter et al., 2007) are strongly supported (PP = 1.0) by the three-gene plastid dataset, Fig. 2. A unifying clade, Clade A, comprising lineages of *Prunus* as well as members of tribes Maleae (MA), Sorbarieae (SO), and Spiraeeae (SP) can be observed (Fig. 2). However, it is not clear which of the latter three lineages is sister to *Prunus*. Therefore, besides the consensus topology (Maleae as sister), two other alternate evolutionary scenarios (Spiraeeae or Sorbarieae as sister) were constructed to examine the impacts of each sister lineage on ancestral area reconstruction for *Prunus* (see Section 2). Varying the sister group to *Prunus* did not affect the crown age of the genus qualitatively (*results not shown*). The absolute age for *Prunus* is between 56.7 and 67.4 Myr (95% credibility interval) as estimated by penalized likelihood in r8s v1.71 (Sanderson, 2002, 2003). The absolute age for each selected node is shown in Table 2 and the ages are plotted as a chronogram in Fig. 2.

3.1.2. Phylogeny of Prunus

The maximum likelihood (ML) tree topology is largely congruent with the 50% majority-rule consensus tree from the Bayesian analysis. For ease of discussion, we only present results obtained from the ML tree (Fig. 3). Three distinct clades can be discerned by the concatenated four-gene plastid dataset. Following Chin et al. (2013), those clades are here designated according to inflorescence structure, as 'Solitary', 'Corymbose' and 'Racemose'. Those designations, in turn, reflect the ancestral inflorescence type for each of these groups according to the analysis of Bortiri et al. (2006), although Chin et al. (2013) incorrectly referred to the 'Corymbose' group as the 'Umbels' group and that error has been corrected here. The 'Solitary' clade comprises deciduous species mainly from the subgenera *Prunus*, *Amygdalus* and *Emplectocladus* while the 'Corymbose' clade is composed of deciduous species mainly from subgenus *Cerasus*. Lastly, the 'Racemose' clade comprises both evergreen subgenus *Laurocerasus* and deciduous subgenus *Padus*. The tropical species from Southeast Asia (the former '*Pygeum*') and those from South and Central America form two distinct monophyletic groups, labeled as 'Paleotropics' and 'Neotropics', respectively. All species from subgenus *Padus* (*P. serotina*, *P. virginiana*, *P. padus* and *P. grayana*) together with former '*Maddenia*' species (*P. hypoleuca*) and the evergreen species *P. laurocerasus* and *P. wallichii* are nested in a group labeled 'Temperate-racemose'. Within that group, three subclades are resolved: (1) *P. laurocerasus* – *P. wallichii*; *P. serotina* – *P. hypoleuca;* (2) *P. virginiana* – *P. padus*, *P. grayana*; and (3) a group labeled as 'Tropical-2,' comprising evergreen species *P. zippeliana*, *P. javanica* and *P. phaeosticta*. Relationships among clades within the 'Racemose' clade are not resolved, and they are all joined in a polytomy (Fig. 3).

The ML and Bayesian analyses of nuclear ITS (nrITS) follow Chin et al. (2013). Their results showed that the phylogenetic resolution of *Prunus* with nrITS is poor overall and the topology is incongruent with inferences based on plastid data (Fig. 3), particularly with respect to the position of the 'Corymbose' and 'Temperate-racemose' clades (labeled with arrows in the nrITS phylogenetic tree, Fig. 4). The 'Corymbose' clade is weakly allied (BS = 46/PP = 0.79) with the 'Temperate-racemose' group in the nuclear data as opposed to a sister relationship to the 'Solitary' clade in the plastid DNA phylogeny. Moreover, without the inclusion of *P. serotina*, the composition of the 'Temperate-racemose' group differs from the plastid DNA phylogeny. Because of this evident incongruence in topology observed, the nrITS and plastid datasets were not concatenated to generate a combined gene phylogeny for *Prunus*.

3.1.3. Molecular dating

The unresolved 'Racemose' clade in the plastid DNA phylogeny (Fig. 3) poses challenges to the inference of biogeographic history and molecular dating. Therefore, we constructed 15 different hypotheses of evolution to depict the range of evolutionary histories among lineages in the 'Racemose' clade for the plastid dataset (see Section 2). Each of the two *Prunus* crown ages of 56.7 and 67.4 Myr was applied as a fixed calibration age to derive absolute crown ages for major lineages based on the plastid dataset. The absolute ages are summarized in Table 3.

The incongruence between the nrITS and plastid analyses suggests that the evolution of the 'Racemose' clade involved hybridization between a paternal *Cerasus* lineage and an unknown maternal lineage X (see Section 4 below). In that case, the most recent common ancestor (MRCA) of the 'Racemose' clade should have diverged from its two parental lineages at the time of hybridization. Since plastid DNA is generally maternally inherited in the *Prunus* (Brettin et al., 2000), we should expect the time when the 'Racemose' clade originated to be the time when the 'Racemose' MRCA inherited the maternal plastid genome from lineage X. Hence, we can infer that the hybridization event can be assumed

Table 2
Divergence times for Rosaceae inferred by penalized likelihood. Absolute ages for each numbered node inferred using a 90 Myr (Low) or 96 Myr (High) fixed at node Z, Rosales crown age (Fig. 2) estimated by Wang et al. (2009). The 95% credibility interval for each low and high calibration is calculated from a profile of 685 ultrametric trees. The overall 95% credibility interval is summarized as the minimum value of the 95% credibility interval from the low calibration and maximum value of the 95% credibility interval from the high calibration.

Node	Mean (min–max), Low calibration, Myr	95% Credibility interval, Myr	Mean (min max), High calibration, Myr	95% Credibility interval, Myr	95% Credibility inteval Mean (min, low–max, high), Myr
Z (Rosales crown)	90		96		
A (Rosaceae)	85.7 (80.6–88.2)	84.2–87.2	90.8 (83.6–93.9)	88.8–92.8	88.3 (84.2–92.8)
B (Dryadoideae)	61.5 (34.07–87.8)	44.7–78.3	64.7 (34.07–93.6)	46.3–83.2	63.1 (44.7–83.2)
C (Rosoideae)	69.8 (60.0–84.9)	64.3–75.3	72.4 (60.7–93.6)	46.3–83.2	63.1 (44.7–83.2)
D (Spiraeoideae)	84.6 (77.3–88.0)	82.8–86.4	89.5 (80.5–93.7)	87.2–91.8	87.0 (82.8–91.8)
E (*Exochorda–Holodiscus*)	78.5 (71.0–83.4)	76.3–80.7	82.5 (74–88.5)	79.7–85.3	80.5 (76.3–85.3)
F (*Neviusia–Exochorda*)	63.4 (57.0–75.7)	60.3–66.5	65.5 (57.9–80.0)	61.8–69.2	64.5 (60.3–69.2)
G (Neilliea)	38.6 (15.4–74.0)	29.4–47.8	40.4 (16.5–78.0)	30.6–50.2	39.5 (29.4–50.2)
H (Clade A)	66.6 (58.4–73.5)	63.7–69.5	68.9 (59.1–77.4)	65.4–72.4	67.8 (63.7–72.4)
J (Spiraeae)	58.6 (52.7–68.6)	56.0–61.2	59.8 (53.1–71.8)	56.7–62.9	59.2 (56.0–62.9)
K (Sorbariae)	46.2 (23.2–67.6)	35.7–56.7	47.7 (23.7–71.0)	36.2–58.6	47.0 (35.7–58.6)
M (Maleae)	59.8 (48.0–72.1)	55.0–64.6	61.4 (48.1–75.4)	55.7–67.1	60.6 (55.0–67.1)
P (*Prunus*)	60.7 (55.0–70.3)	56.4–64.7	62.4 (55.0–73.4)	57.4––67.4	61.5 (56.7–67.4)

as the divergence time of the 'Racemose' clade which is ~55 Myr (mean age).

3.2. Historical biogeography of Prunus

3.2.1. Geographic origin of Prunus

The ancestral areas reconstructed for selected Rosaceae lineages are plotted onto the 50% majority-rule consensus Bayesian topology in Fig. 2. Bayes-DIVA suggests that Rosaceae and each of its three subfamilies (Rosoideae, Dryadoideae and Amygdaloideae) most likely evolved in North America. As pointed out in the previous section, *Prunus* is nested in Clade A and its sister group among those lineages (tribe Maleae, Sorbarieae and Spiraeeae) is unresolved. Therefore, as outlined in the previous section and in Section 2, we evaluated the impacts of the uncertain sister group on the inference of biogeographic origin of *Prunus*. Our results from Bayes-DIVA strongly supported an eastern Asian origin for *Prunus* regardless of whether tribe Maleae, Sorbarieae or Spiraeeae is the sister group.

3.2.2. Diversification of Prunus lineages

Several dispersal and vicariance events are invoked to explain the current cosmopolitan distribution of *Prunus* from an eastern Asian origin. The ancestral areas for all major lineages are summarized in Fig. 5 and listed in Table 4. Both the MRCA of 'Solitary' and 'Corymbose' clades (nodes 3 and 13 in Fig. 5, respectively) are suggested to have widespread distributions: the first between North America and eastern Asia and the second between West Asia and eastern Asia. In contrast, the MRCA of the 'Racemose' clade (node 14, Fig. 5) had a narrow distribution in eastern Asia.

4. Discussion

4.1. Molecular phylogenetics of Prunus

The phylogeny of *Prunus*, analyzed here with a larger set of plastid markers and expanded sampling that includes better representation of tropical species from Southeast Asia and South and Central America ('Paleotropics and Neotropics in Fig. 3), is congruent with what has been reported in previous phylogenetic analyses of *Prunus* (Bortiri et al., 2001, 2006; Chin et al., 2010, 2013; Lee and Wen, 2001; Shaw and Small, 2004; Wen et al., 2008). Additional sampling of tropical *Prunus* species (from both 'Paleotropics' and 'Neotropics') from subgenus *Laurocerasus* has added support to the 'Temperate-racemose' group, first identified as the '*Maddenia*-composite' complex in Chin et al. (2010). None of the subgenera (*Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunus*) as recognized by Rehder (1940) was supported to be monophyletic in any of the previous analyses, and that conclusion is confirmed by this study. For example, species that are classified in subgenus *Cerasus* section *Microcerasus* (*P. prostrata*, *P. tomentosa* and *P. glandulosa*) are nested within the 'Plums' clade of the plastid DNA and nrITS DNA phylogenies. Moreover, Bortiri et al. (2006) also determined that most of the morphological traits that were used to define the infrageneric groups by Rehder (1940) were homoplasious. Hence, results from this study as well as from previous analyses strongly call for a revised infrageneric classification of *Prunus* that is based on both morphological traits and phylogenetic evidence.

Ancient hybrid origin for the racemose lineage: The base chromosome number for *Prunus* is 8. Most of the economically important species such as peach, *P. persica*; almond, *P. dulcis*; and sweet cherry, *P. avium* have a ploidy of $2n = 2x = 16$. On the other hand, species from the racemose subgenera *Laurocerasus* and *Padus* have been reported to have higher ploidy levels (Watkins, 1976) e.g., *P. laurocerasus*, $2n = 22x = \sim 176$, and *P. lusitanica*, $2n = 8x = 64$.

Incongruent topological patterns observed between nuclear and chloroplast DNA phylogenies most likely result from hybridization between species (Rieseberg and Soltis, 1991; Rieseberg, 1997). The discordant phylogenetic positions of the 'Corymbose' group as inferred from nrITS phylogenetic analysis (sister to the 'Temperate-racemose' group) and also supported by another nuclear marker, *s6pdh* (Bortiri et al., 2002), relative to plastid DNA (sister to the 'Solitary' group) have been interpreted as evidence of an ancient hybrid origin of the 'Corymbose' group (Bortiri et al., 2006; Chin et al., 2010). The close alliance of subgenus *Cerasus* ('Corymbose') with some racemose species in the nuclear ITS phylogeny led Chin et al. (2010) to further propose that the 'Temperate-racemose' or the '*Maddenia*-composite' group was the potential pollen donor in this hybridization event. However, on the basis that species within the 'Temperate-racemose' group have high ploidy levels and most species in subgenus *Cerasus* are diploid, we have developed an alternative hypothesis based on evidence from ploidy levels and phylogenies. We propose that the racemose lineage originated via allopolyploidization, involving an ancestral *Cerasus* as the paternal parent and another early-diverging lineage on the maternal side. This can be reconciled with the topology of the nrITS phylogeny by assuming that some clades have retained the paternal copy of nrITS (e.g., the Temperate-racemose clade) while others have retained the maternal copy of the nrITS. This hypothesis is further supported by the single maternal origin of the 'Racemose' group, as indicated by the monophyly of 'Racemose' in the plastid

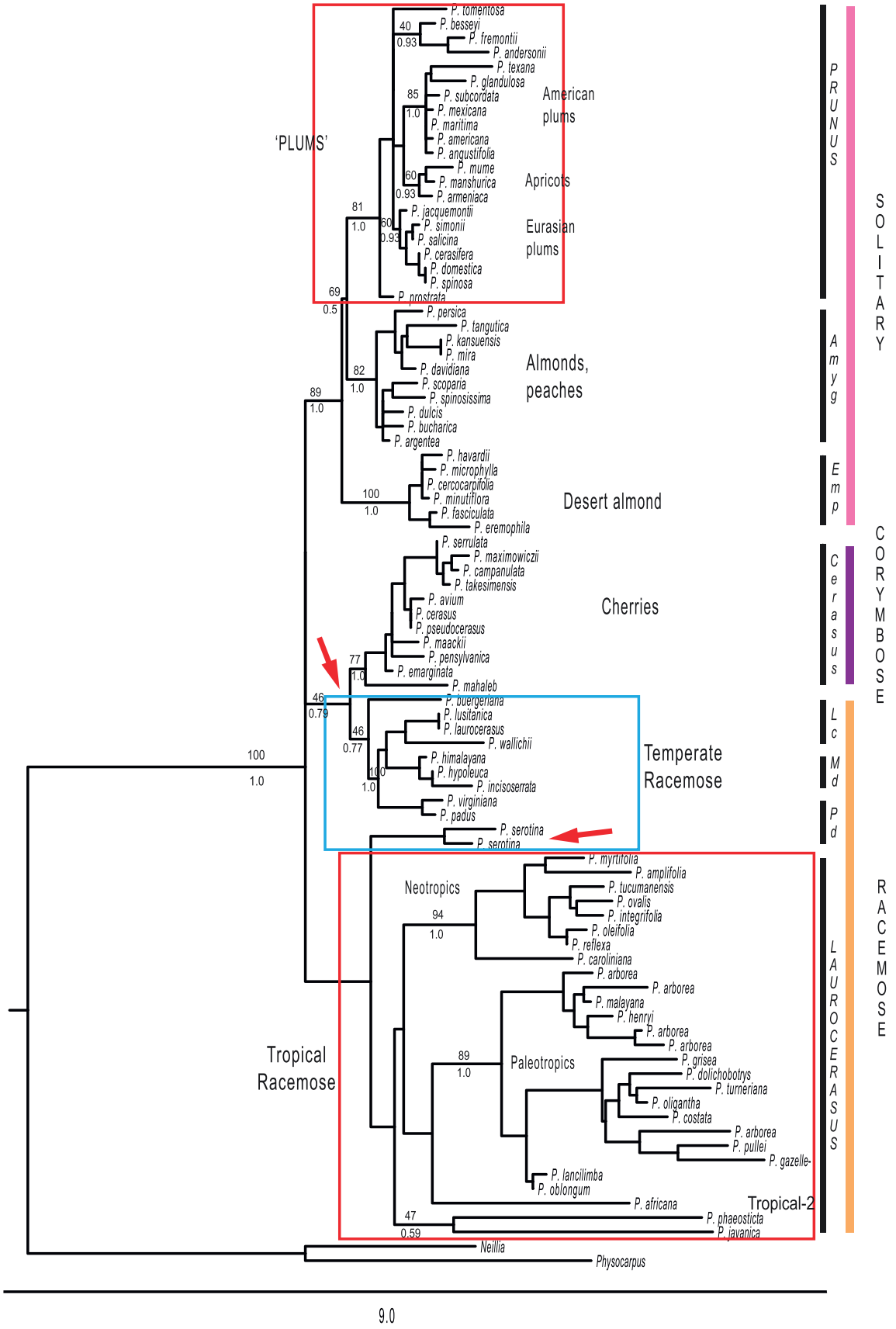

Fig. 4. Maximum likelihood tree of the nuclear ribosomal ITS region for phylogeny of *Prunus*. Numbers above and below branches indicate maximum likelihood bootstrap support and bayesian posterior probabilities respectively. Arrow indicates incongruent phylogenetic position observed in nrITS DNA phylogeny relative to plastid DNA phylogeny. Abbreviations for subgenus: *Amyg*, *Amygdalus*; *Emp*, *Emplectocladus*; *Lc*, *Laurocerasus*, *Pd*, *Padus*; *Md*, '*Maddenia*'.

Table 3
Divergence times for *Prunus* lineages inferred by penalized likelihood. Absolute ages for each node estimated using a 56.7 Myr (Low) or 67.4 Myr (High) fixed age applied at node A. The 95% credibility interval for each low and high calibration is calculated from a profile of 1500 ultrametric trees. The overall 95% credibility interval (last column) is summarized as the minimum value of the 95% credibility interval from the low calibration and maximum value of the 95% credibility interval from the high calibration. Lineages referred in text but not labeled on Fig. 5 are indicated with *.

Node label	Mean (min–max), Low calibration, Myr	95% Credibility interval, Myr	Mean (min–max), High calibration, Myr	95% Credibility interval, Myr	95% Credibility interval Mean (min, low–max, high), Myr
1 (*Prunus*)	60.7 (55.0–70.3)	56.4–64.7	62.4 (55.0–73.4)	57.4–67.4	61.5 (56.7–67.4)
2 ('Solitary' + 'Corymbose')	53.2 (46.8–56.2)	51.6–54.8	63.1 (44.3–66.8)	61.0–65.2	58.2 (51.6–65.2)
3 ('Solitary')	51.2 (43.0–56.0)	49.0–53.4	60.7 (39.8–66.5)	57.9–63.5	56.0 (49.0–63.5)
4 (*Emplectocladus*)	20.2 (2.8–34.5)	14.5–25.7	24.0 (3.2–41.4)	17.1–30.9	22.1 (14.5–30.9)
5 (*Amygdalus*)	44.7 (30.4–54.0)	41.0–48.4	53.1 (36.0–64.0)	48.8–57.4	48.9 (41.0–57.4)
6 ('Almond')	43.2 (22.5–55.3)	37.3–49.1	51.3 (26.9–65.8)	44.3–58.3	47.3 (37.3–58.3)
7 ('Peach')	32.3 (10.8–52.2)	23.2–41.4	38.4 (12.5–61.8)	27.8–49.0	35.3 (23.2–49.0)
8 ('Plums' + Amygdalus)	50.0 (36.7–55.5)	47.5–52.5	59.3 (39.6–66.0)	56.3–62.3	54.6 (47.5–62.3)
9 ('Plums')	46.4 (32.1–54.6)	42.5–50.3	55.1 (27.6–64.8)	50.4–59.8	50.8 (42.5–59.8)
10 ('Asian Plums' + 'American Plums')	34.6 (18.8–45.0)	29.8–39.4	41.2 (23.8–53.0)	35.7–46.7	37.9 (29.8–46.7)
11 ('Asian Plums')	28.4 (14.7–44.4)	22.2–34.6	33.8 (16.9–52.2)	26.5–41.1	31.1 (22.2–41.1)
12 ('American Plums')	32.0 (17.1–42.8)	27.3–36.7	38.2 (21.0–50.4)	32.9–43.5	35.1 (27.3–43.5)
13 ('Corymbose')	49.6 (30.3–55.7)	45.9–53.3	58.9 (36.7–66.2)	54.6–63.2	54.2 (45.9–63.2)
14 ('Racemose')	50.4 (37.3–56.7)	45.1–55.7	60.3 (45.6–67.4)	54.3–66.3	55.4 (45.1–66.3)
15 ('Temperate-racemose')	33.5 (14.5–54.5)	25.2–41.8	40.9 (18.6–64.8)	31.5–50.3	37.2 (25.2–50.3)
16 ('Neotropics')	30.4 (12.4–53.4)	22.4–38.4	37.2 (16.2–63.6)	28.2–46.2	33.8 (22.4–46.2)
17 ('Paleotropics')	26.2 (10.0–53.4)	16.7–35.7	31.7 (11.7–63.5)	20.5–42.9	29.0 (16.7–42.9)
18 ('Tropical-2')	25.8 (10.5–48.4)	18.8–32.8	30.8 (12.4–57.4)	22.8–38.8	28.3 (18.8–38.8)
19 (*P. africana*)	12.3 (0.40–33.7)	5.90–18.7	15.7 (0.50–41.2)	7.50–23.9	14.0 (5.90–23.9)
*Cherry–core	44.6 (24.6–54.9)	39.0–50.2	53.0 (29.5–65.3)	46.5–59.5	48.8 (39.0–59.5)
**P. laurocerasus–P. wallichii*	13.2 (0.10–43.0)	4.20–22.2	16.5 (0.10–51.9)	5.5–27.0	14.9 (4.20–27.0)

phylogeny. However, we cannot reject alternative complex scenarios that involve an autopolyploid origin of the 'Racemose' group and a homoploid hybrid origin of *Cerasus* with a maternal 'Solitary' lineage and a diploid paternal 'Racemose' lineage. Nonetheless, we favor the hypothesis of an allopolyploid origin of the 'Racemose' lineage and we offer the following insights based on our biogeographic and molecular dating analyses.

Plastid DNA is maternally inherited in most angiosperms, including *Prunus* (Brettin et al., 2000). Therefore, the maternal parent that contributed to the evolution of 'Racemose' lineages can be inferred from the plastid DNA phylogeny. Moreover, the time of the incipient formation of the racemose lineage can be estimated by the divergence time of the 'Racemose' clade based on the assumption that plastid DNA is transmitted linearly by the maternal parent. Therefore, we estimate the hybridization event is likely to be ~55 Myr. The plastid genes topology supports a sister relationship between the 'Racemose' clade and the 'Solitary – Corymbose' clade. The ancestor of the latter clade (~58 Myr) predates the hybridization event (~55 Myr). Furthermore, both the site of hybridization and the ancestral range of the 'Solitary – Corymbose' clade are inferred to be in eastern Asia; hence we postulate that the maternal parent is likely to have been an early lineage from the 'proto-*Prunus*'. Given our sampling of the racemose species is non-exhaustive, we are not certain if the diploid ancestor is unsampled or if it represents an extinct lineage. The period of hybridization (~55 Myr) in eastern Asia overlaps closely with the emergence of the 'Corymbose' group (~54 Myr) during the Early Eocene as it began diversification and spread towards West to Central Asia. Interestingly, this period of hybridization also coincides rather closely with the major tectonic collision of India plate with Eurasia in the early Eocene (e.g., Aitchison et al., 2007; Kent and Muttoni, 2008; Meng et al., 2012; Rowley, 1996). It is tempting to posit that the tectonic collision brought about disturbance to the ancestral ranges of these two lineages, plausibly attributed by orogenic events such as uplifts of the Tibetan plateau and the resultant changes to the palaeoclimatic patterns (Kent and Muttoni, 2008; Meng et al., 2012; Wen et al., 2014); hence promoted hybridization at the contact zones.

4.2. Molecular dating and biogeographic history

Prunus is an element of the boreotropical forest (65–34 Myr): Our results support the biogeographic origin and rapid diversification of Rosaceae during the Cretaceous in North America, as has been suggested in other studies (e.g., Potter et al., 2007). The molecular crown age for Rosaceae of 76 Myr (Wikström et al., 2001) has been used in several molecular dating studies as the root age to determine the divergence times for infrafamilial lineages, e.g., tribe Maleae (Lo and Donoghue, 2012), *Potentilla* (Dobes and Paule, 2010), and *Crataegus* (Lo et al., 2009). However, this estimated age is much younger than the molecular crown age of 90 Myr reported in Crepet et al. (2004). The molecular crown age for Rosaceae reported in this study (84.2–92.8 Myr) was older than the molecular age of 76 Myr (Wikström et al., 2001), but lies closer to the molecular age of 90 Myr for Rosaceae reported in Crepet et al. (2004). The discrepancy of the molecular age from Wikström et al. (2001) with that reported here could partially be attributed to a lower minimum constrained fossil age (44 Myr for Prunoideae = Amygdaloideae) used in calibrating their tree or effects of limited taxon sampling within Rosaceae that potentially decrease branch lengths and hence underestimate the divergence times (Sanderson, 1990).

Based on our Bayes-DIVA analyses, the MRCA for the Clade A composite, Fig. 2, (*Prunus* and tribes Maleae, Spiraeeae and Sorbarieae) was present in North America from 63.7 to 72.4 Myr (Paleocene period), plausibly after the K–T extinction event (65 Myr). From the Late Cretaceous to the Middle Eocene, the climate in the Northern Hemisphere was much warmer and more humid than today, i.e., a paratropical climate (Morley, 2000). This facilitated the formation of a continuous forest belt known as the boreotropical flora or the northern megathermal forest from Asia to North America across the Beringia (e.g., Lavin and Luckow, 1993; Morley, 2000; Tiffney, 1985a,b; Wolfe, 1975; Xiang and Soltis, 2001). Hence, we can speculate that the MRCA of Clade A was part of the boreotropical flora that emerged in North America. Divergence of *Prunus* was estimated to occur between 56.7 and 67.4 Myr in eastern Asia. Interestingly, a comparison with all other lineages in Clade A reveals that the ancestor of *Prunus* could be the earliest

lineages that have colonized eastern Asia from the opposite end of the continuous belt of boreotropical forest (Fig. 2). Floristic exchanges between North America and eastern Asia could have occurred by either the North Atlantic Land Bridges (NALB) or the Bering Land Bridge (BLB), as pointed out in the Introduction. Bayes-DIVA analyses suggest that the most likely ancestral area for the ancestor of Clade A was western North America; this implies the dispersal of 'proto-*Prunus*' from western North America to eastern Asia was most likely via the Bering Land Bridge (BLB). The likelihood of the BLB as the main dispersal corridor is also supported by the high density of lineages of Amygdaloideae and their fossil records in western North America, e.g., *Physocarpus*, *Neviusia*, *Oemleria*, and *Prunus* from the early Eocene Okanogan Highlands floras of northeastern Washington state (Republic) and related floras of British Columbia, Canada (Benedict et al., 2011; DeVore and Pigg, 2007; Wehr and Hopkins, 1994) and *Lyonothamnus* from the early Miocene floras of California, Nevada, and Oregon (Erwin and Schorn, 2000).

4.3. Diversification of Prunus lineages

Our analyses suggest that early diversification of *Prunus* lineages in eastern Asia began around 58 Myr as the ancestor of the 'Solitary – Corymbose' clade first emerged. This conclusion can be supported by the fact that the earliest recorded fossilized endocarps of *Prunus* (*Prunus wutuensis*) were recovered from the early Eocene (55 Myr) in eastern Asia (Shandong Province), Wutu formation of Northern China (Li et al., 2011). According to the authors, they found that the endocarp of *P. wutuensis* most closely resembled the carpological characteristics to extant *P. yedoensis* (subgenus *Cerasus*) though it is not certain whether this endocarp morphotype represents the early or derivative lineages.

Our support for an eastern Asian origin of *Prunus*, which is evidently driven by the high extant diversity of the genus in that region, is somewhat tempered by the rich fossil record of *Prunus* from the early Eocene throughout western North America. The latter suggests the importance of western North America in the early diversification of *Prunus* or even a possible scenario of a western North American origin for *Prunus* which subsequently migrated to eastern Asia and gave rise to the rest of the modern lineages. In any case, the preponderance of Eocene fossil records for woods in Clarno Nut Beds of Oregon and Yellowstone National Park in Wyoming (Wheeler et al., 1978; Cevallos-Ferriz and Stockey, 1990; Wheeler and Manchester, 2002), anatomically preserved endocarps in Princeton Chert and Clarno Nut Beds of Oregon (Cevallos-Ferriz and Stockey, 1990; Manchester, 1994), *Prunus* flowers in Republic Flora of Okanogan Highlands (Benedict et al., 2011) as well as leaves and pollen grains (reviewed in Benedict et al., 2011 and DeVore and Pigg, 2007) may lend credence to constant floristic exchanges between eastern Asia (postulated site of incipient diversification) and western North America across a belt of boreotropical forests via the BLB. Notably, a fossilized flower specimen – *Prunus cathybrownae* uncovered from late early Eocene (50 Myr) Republic Flora, described in Benedict et al. (2011) was found to contain several morphological features that resemble basal characters to extant *Prunus* species as suggested by ancestral character reconstructions by Bortiri et al. (2006). As morphological characters in *Prunus* were found to be highly homoplastic (Bortiri et al., 2006), further examination and characterization of extant morphological characters is required to determine the relationship of *P. cathybrownae* to extant *Prunus* lineages. Nonetheless, the slightly younger age reported for *Prunus cathybrownae* (50 Myr,) compared to *Prunus wutuensis* (55 Myr) is consistent with our hypothesis of early dispersal of the genus from eastern Asia to western North America. Our Bayes-DIVA analyses further suggest that an early offshoot of the 'Solitary' lineage migrated to North America and gave rise to the 'Desert-almond' lineage.

Thus, it is pertinent and exciting to further examine how early Eocene fossilized *Prunus* records (pollen, flowers, leaf, and wood) from western North America (e.g., Wheeler and Manchester, 2002; Benedict et al., 2011; DeVore and Pigg, 2007) compare with the eastern Asian and European records (see reviews in Benedict et al., 2011; DeVore and Pigg, 2007; Li et al., 2011). Such comparisons can further help shed light on the morphological evolution, lineage diversification, and biogeographic history of *Prunus*.

Our divergence time estimates, suggesting that diversification of *Prunus* had occurred from the early Eocene is considerably earlier than the previously ascribed Middle Eocene age of 44 Myr (Oh and Potter, 2005). Based on our estimated timing for the earliest lineage diversification of *Prunus* (51.6–65.2 Myr), the period coincides closely with the significant global warming episode known as Paleocene-Eocene Thermal Maximum (PETM) and Early Eocene Climatic Optima (EECO) events reported between 55 and 50 Myr (McInerney and Wing, 2011; Zachos et al., 2001). Impacts of PETM on floristic changes due to range shifts and rapid radiations have been well documented (e.g., Wing et al., 2005; Wing and Currano, 2013). Therefore, it is also tempting to suggest that climatic change was likely a driver for diversification in *Prunus* during the PETM. In addition, tectonic collision of India plate with Eurasia at ~50 Myr brought about orogenic uplifts of the Tibetan plateau that could likely contributed strong vicariance forces in shaping the diversification process. Moreover, interplay of several evolutionary pressures (climactic and vicariance) would most likely be invoked to explain the rapid diversification of *Prunus* lineages (less than 1 Myr between lineage splits): 'Solitary' (~56 Myr), 'Corymbose' (~54 Myr) and a putative maternal 'Racemose' (~55 Myr). Nonetheless, rapid radiation events were highly evident among Rosids lineages; and it has been explained to account for radiation of major Rosids lineages over a period of <15 Myr or within a window period of 4–5 Myr (Wang et al., 2009).

In summary, our Bayes-DIVA analyses suggest an eastern Asian origin for *Prunus*. The common ancestor of the 'Solitary' lineage is postulated to have occupied a widespread North America – eastern Asia range (Fig. 6A), consistent with the rich fossil records recovered in western North America (e.g., the Okanogan Highlands sites including the Princeton Chert and Republic Flora, Washington) and eastern Asia during the early Eocene (see Section 4 above). The early 'Corymbose' lineage developed from eastern Asia to West Asia (Fig. 6C), and lastly the early 'Racemose' lineage diversified within eastern Asia (Fig. 6D). Plausible hypothetical biogeographic scenarios to explain some of the significant infrageneric diversification events will be discussed below.

4.3.1. Eastern Asian – eastern North American floristic disjunctions in the plums

The eastern Asian – eastern North American floristic disjunct pattern has been well documented in several angiosperm taxa (e.g., Li, 1952; Tiffney, 1985a; Wen, 1999; Wen et al., 2010) and can also be observed among *Prunus* species. Examples include the groupings of Eurasian (e.g., *P. salicina* and *P. spinosa*) plums with eastern North American plums (e.g., *P. americana* and *P. subcordata*), Eurasian bird cherries (e.g., *P. padus* and *P. grayana*, former *Maddenia*) with eastern North American bird-cherries (*P. virginiana*, and *P. serotina*), and possibly some Eurasian cherries (e.g., *P. pleiocerasus*) with eastern North American cherries (e.g., *P. pensylvanica*). As the resolution among cherry species is poor, sister relationships between geographically disjunct species in this group require further investigations.

Our results implicate the BLB as an important dispersal/migration corridor for the eastern Asian and eastern North American plum disjunction. First, our plastid DNA phylogeny supports a

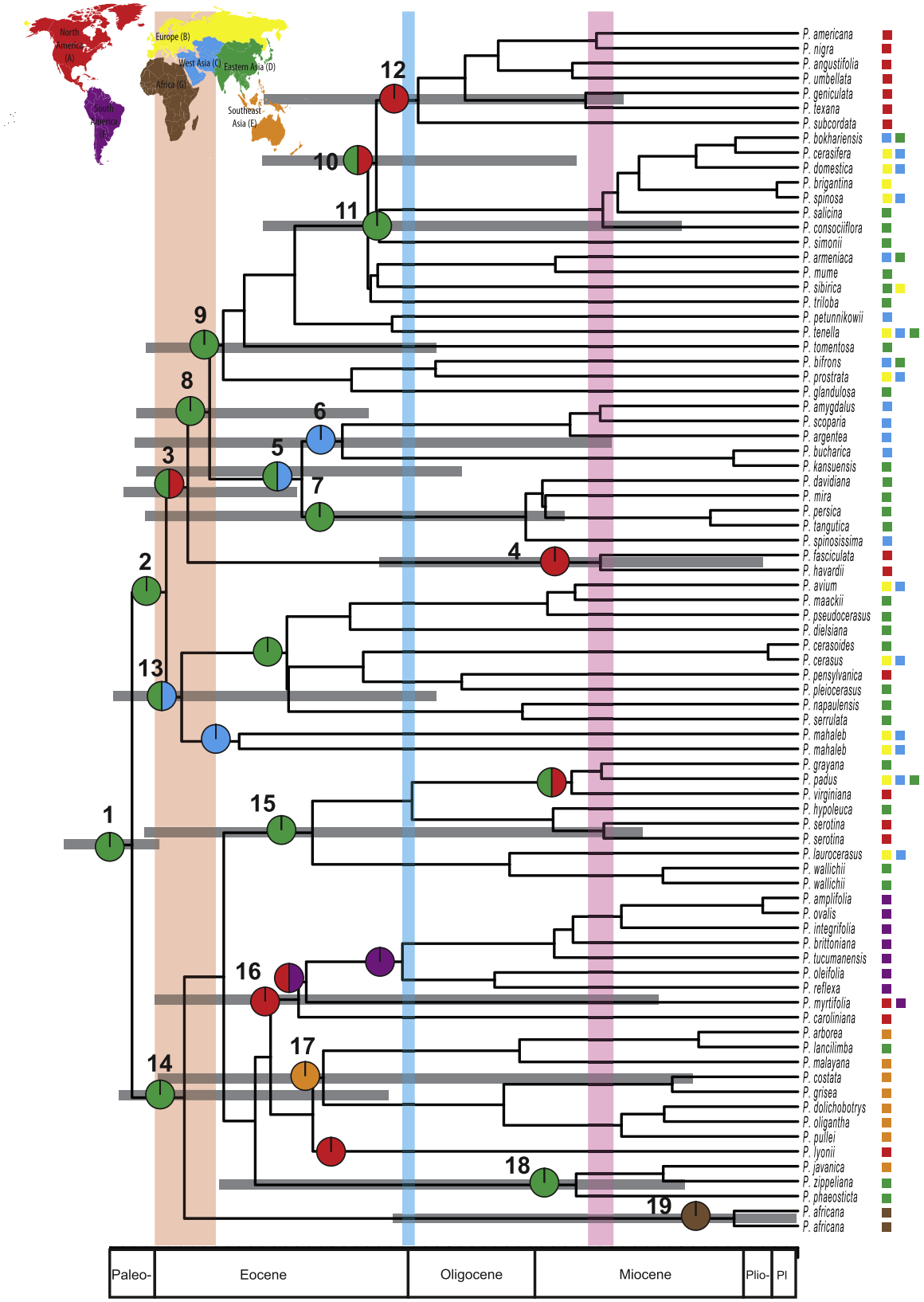

Fig. 5. Chronogram and ancestral areas for *Prunus*. The chronogram is based on one of the ultrametric tree with high fixed calibration (67.4 Myr) generated by r8s v1.71 using the four-gene plastid sequences. Node marked with asterisk (*) represents support values of ML BP > 70/PP > 0.95. Each numbered node bar represents the minimum and maximum ranges of dates for most recent common ancestor (MRCA) summarized from both Low and High calibrations. The ancestral area for each *Prunus* lineage is inferred by Bayesian dispersal-vicariance analysis implemented in S-DIVA. The distribution for each taxon (square box), as delimited in the World Map, is summarized to the right of the taxon names. Each pie chart (with solid color) at internal nodes represents the ancestral area with the highest frequency. Pie chart with two tones represents widespread region between two possible ancestral areas. The pink bar between Paleocene-Eocene periods denotes the Paleocene-Eocene Thermal Maximum (PETM) event, blue bar at the Oligocene-Eocene boundary represents the onset of Global Cooling Event, red bar at Miocene period denotes Middle Miocene Climatic Optimum event. Biogeographic regions: (A), North America; (B), Europe; (C) West Asia; (D), eastern Asia; (E), Southeast Asia; (F), South America; (G), Africa. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Geographic origin and ancestral areas of diversification in *Prunus*. Ancestral areas inferred by Bayes-DIVA based on a profile of 5639 trees. Maxareas = 2. Multiple areas expressed by frequency in percent. Single area denotes 100% frequency. Single letter denotes one area, two letters denotes widespread between both areas. Biogeographic areas: A, North America; B, Europe; C, West Asia; D, eastern Asia; E, Southeast Asia; F, South America; G, Africa.

Node label	Ancestral area
1 (*Prunus*)	D
2 ('Single-flowers' + 'Umbel')	D
3 ('Single-flowers')	AD (*West)
4 (*Emplectocladus*)	A (*West)
5 (*Amygdalus*)	CD
6 ('Almond')	C (62%), CD (38%)
7 ('Peach')	D
8 ('Plums' + *Amygdalus*)	D
9 ('Plums')	D
10 ('Asian Plums' + 'American Plums')	AD (*West)
11 ('Asian Plums')	D
12 ('American Plums')	A (*West)
13 ('Corymbs')	CD (50%), BD (50%)
14 ('Racemose')	D
15 ('Temperate-racemose')	D
16 ('Neotropics')	*A (East)
17 ('Paleotropics')	E
18 ('Tropical-2')	D
19 (*P. africana*)	G

* Indicates the specific region of North America (western or eastern) reconstructed by a separate Bayes-DIVA analysis.

western North America species (*P. subcordata*) as the sister lineage for the American plums (Fig. 3) and this is in agreement with Shaw and Small (2004). In addition, the MRCA of Asian and American plums was suggested by Bayes-DIVA to have had a widespread distribution between western North America and eastern Asia ~38 Myr. This period coincides closely with the onset of a global cooling event at the Eocene–Oligocene boundary (35 Myr), when warm-adapted elements of the boreotropical forests were being forced to recede towards lower latitudes. Consequently, the climatic change could have resulted in vicariance between the two populations across the BLB and subsequently brought about diversification of the North American and eastern Asian plum lineages. Our molecular evidence is consistent with this scenario, as the ancestor of American plums was inferred to appear ~35 Myr in western North America. Therefore, our results support a BLB migration and subsequent fragmentation of their geographic ranges to explain the eastern Asian – eastern North American disjunction in the plums.

4.3.2. Diversification of Prunus in Eurasia

The center of diversity for the major *Prunus* crop species is Eurasia (Watkins, 1976), a region with complex paleogeographic and climatic histories. The Eurasian continent/Palaeoarctic was divided into eastern and western halves by the Turgai Straits from the mid-Jurassic (180 Myr) to the Oligocene (30 Myr) (Fig. 1; see Sanmartín et al. (2001) for a short review of the geologic history to Eurasia). Though the Turgai Straits have been argued by many botanists as a dispersal barrier, several palaeobotanists have suggested that floristic exchanges between Europe and Asia could still have persisted via 'island-hopping' along the shores of the Tethys Seaway (Tiffney, 1985a,b). Below we discuss the significant geologic events that might have contributed to the biogeographic patterns for some of the major Eurasian *Prunus* lineages.

The significance of the Turgai Straits in shaping the diversification and biogeographic history of *Prunus* in Europe can be evident as suggested by the fossil records of *Prunus* (wood and, endocarps) in Europe – the fossils records were sparse from the late Eocene but became more apparent during the Miocene (~14 Myr) and Pliocene, ~3 Myr (see Benedict et al., 2011; DeVore and Pigg, 2007; Li et al., 2011; Wheeler et al., 1978). Incidentally, this trend in temporal distribution of fossil records might reflect the presence of the Turgai Straits as a dispersal barrier between Europe and eastern Asia between 55 and 35 Myr, but dispersal became possible after the disappearance of the Turgai Straits.

Eurasian cherries: Both the plastid (Fig. 3) and nrITS DNA (Fig. 4) phylogenies reveal that the 'Corymbose' clade can be divided into two groups, one comprising the fruiting and flowering cherries (hereafter termed 'cherry – core') and another leading to the basally diverging *P. mahaleb* lineage (section *Mahaleb*). The ancestor of the 'Corymbose' clade appeared ~54 Myr and it is postulated by Bayes-DIVA to have had a widespread distribution, either between eastern Asia and West Asia (50%) or from eastern Asia to Europe (50%). This uncertainty in ancestral range is affected by how we code the biogeographic areas for *P. mahaleb*. The extant native distribution of *P. mahaleb* includes Europe, Morocco, Central Asia and Pakistan. If the biogeographic region is coded as only Central Asia based on the broad assumption that floristic exchange between Europe and eastern Asia was limited during the Eocene (55–35 Myr) due to the presence of the Turgai Straits, the ancestral range of 'Corymbose' becomes eastern Asia to West Asia. However, as pointed out above, dispersal across the Turgai Straits via 'island-hopping' to Europe was also possible. Hence, if the range of *P. mahaleb* is coded to extend into Europe, then the ancestral range will include all of Eurasia. As only two accessions of *P. mahaleb* were used in this study, it remains uncertain whether plastid haplotypes of *P. mahaleb* from Europe represent the first wave of dispersal from more mesic ancestors of eastern Asia via the Tethys seaway or a later dispersal from the more xeric-adapted ancestor of Central Asia. Future phylogeographic studies with more extensive sampling of *P. mahaleb* accessions from throughout its current native range should shed light on this controversial biogeographic question.

European plums: The diversification of the entire clade of Eurasian plums began ~31 Myr (Oligocene). Hence, it is most likely that the extant European lineages *P. spinosa*, *P. cerasifera* and *P. domestica* descended from an ancestor that migrated from eastern Asia after the disappearance of the Turgai Straits.

Almonds and peaches: The monophyly of subgenus *Amygdalus* (peaches and almonds), excluding *P. triloba*, *P. petunnikowii* and *P. tenella*, is supported by both plastid and nrITS DNA phylogeny. The MRCA of subgenus *Amygdalus* was inferred by Bayes-DIVA analyses to be widespread from West Asia to eastern Asia (China) ~49 Myr with subsequent diversification into almonds and peaches in West Asia and eastern Asia, respectively. A plausible scenario that brought about the diversification of almonds and peaches could be explained by the tectonic collision of India with Asia from the early Eocene period, 50 Myr (e.g., Aitchison et al., 2007; Kent and Muttoni, 2008; Meng et al., 2012; Rowley, 1996) which predated the cladogenesis event (~47 Myr). This massive geologic event has been attributed as the mechanism for the uplifts of the Himalayan – Tibetan plateau and subsequent global paleoclimatic changes (e.g., Kent and Muttoni, 2008; Meng et al., 2012; Wen et al., 2014). Watkins (1976) suggested that peach and almond evolved separately following the mountain upheavals in Central Asia, e.g., Tian Shan, and the Himalayas. Moreover, our molecular dating has also added support to the occurrence of such a geologic event as having caused divergence of these two groups. Hence, we can envisage that evolution of almonds and peaches provides a classical example of allopatric speciation via vicariance.

4.3.3. Diversification of the racemose species

The racemose species of *Prunus* can be broadly divided into either deciduous or evergreen. All deciduous species are temperate in distribution and have been classified under subgenus *Padus* (Rehder, 1940), while nearly all evergreen species are tropical in

distribution and grouped under subgenus *Laurocerasus*. As shown by both plastid and nrITS phylogenetic analyses, relationships among the well-supported lineages of the racemose group are mostly poorly resolved. The ambiguity in relationships among the lineages could plausibly be the result of either a rapid radiation during the warming period of the Eocene, followed by extensive extinctions due to the deterioration of the climate at the end of the Eocene, or simply limited sampling of species from refugial areas like South China or Meso-America (Morley, 2000). Moreover, the divergence times estimated for each racemose lineage from this study should be interpreted with caution as the absolute age for each lineage represents the mean age of the fifteen evolutionary scenarios that we hypothesized for the entire racemose clade. Evidently, the large range of values at each node in Fig. 5 also indicates the high level of uncertainty for each lineage. Furthermore, the biogeographic relationship of subgenus *Padus* was recently reconstructed using ITS and plastid markers (Liu et al., 2013). Hence below, we tentatively discuss the plausible biogeographic histories, emphasizing the evergreen taxa.

4.3.4. Diversification of evergreen subtropical racemose species

Within the mostly deciduous 'Temperate racemose' clade, another intercontinental disjunction between East and West Eurasia comprised of the evergreen *P. laurocerasus* (Europe), *P. lusitanica* (Europe) and *P. wallichii* (Vietnam to Southeast Asia) can be observed based on the plastid and nuclear datasets. Based on our estimated divergence time for this group, it was inferred to appear ~15 Myr during the transient warming period known as the Middle Miocene thermal optimum 18–16 Myr. This transient thermal optimum period saw expansion of moist tropical forests from equatorial regions (Morley, 2000, 2007). Although the phylogenetic relationship of this disjunct group with the rest of the members of 'Temperate-racemose' clade is unclear, we can assume that the ancestor of the European species most likely dispersed from some refugial areas in South China or Central Asia. This idea can be supported based on the extant distribution of *P. laurocerasus* which also includes Central Asia. Future phylogeographic studies that include more accessions across its range are needed to decipher the biogeographic history of *P. laurocerasus*.

The ancestor for the Afromontane evergreen *P. africana* was estimated to appear ~13 Myr (Miocene). However, its relationship with the most geographically proximal European species, *P. laurocerasus* and *P. lusitanica*, is remote. This likely suggests the occurrence of two independent evolutionary events (long distance dispersal or vicariance) that brought the Afro-Eurasian racemose species (*P. laurocerasus*, and *P. africana*) to their current geographic locations. Kadu et al. (2011) proposed that *P. africana* could have followed a similar route as several Afroalpine species that colonized Africa from Asia through the coastal mountain ranges of the Arabian Peninsula during the Pleistocene. Given that *P. africana* is not extant in the Arabian Peninsula and that there is no paleobotanical evidence of its presence there, their conclusion is rather dubious. Moreover, the appearance of *Prunus africana* morphologically resembles *P. pygeoides*, a native of India and Yunnan, South China (Kalkman, 1965). *Prunus pygeoides*, according to Kalkman (1965), occupies a central position that plausibly links all section *Laurocerasus* species, including *P. laurocerasus*, *P. lusitanica*, *P. zippeliana*, and *P. africana*, together. Therefore, future studies that include *P. pygeoides* and more sampling around South China or other plausible refugial sites will be needed to ascertain the phylogenetic relationships among these species as well as their purportedly complex biogeographic histories.

4.3.5. Diversification of evergreen wet-tropical racemose Prunus

The extant amphi-Pacific disjunct tropical *Prunus* racemose species ('Paleotropics' and 'Neotropics') most likely derived from the

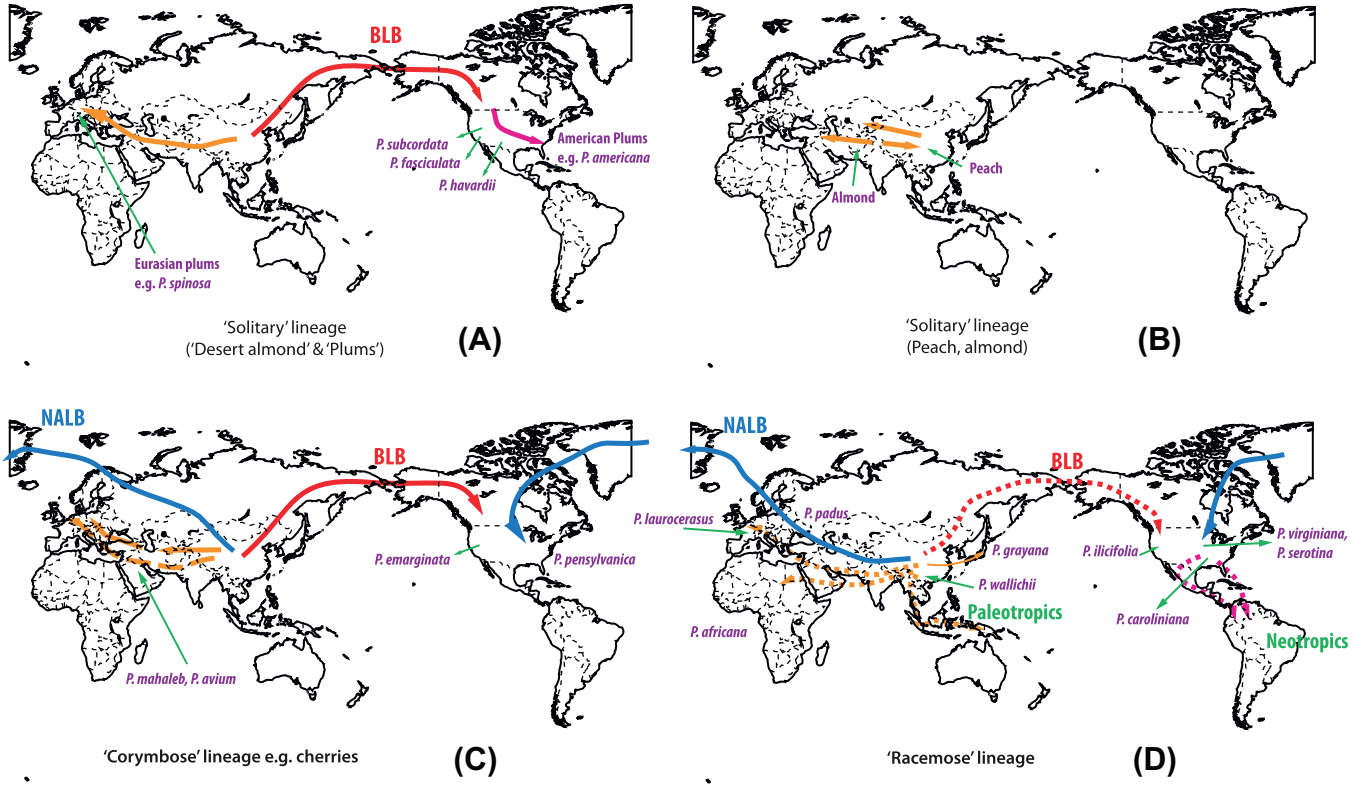

Fig. 6. Historical biogeographic tracks of *Prunus*. Hypothetical migration routes undertaken by *Prunus* lineages according to ancestral areas inference: (A) 'Solitary' lineage – 'Desert-almonds' and 'Plums'; (B) 'Solitary' lineage – 'Peaches, Almonds'; (C) 'Corymbose' lineage – 'Cherries'; (D) 'Racemose' lineages. Broken line in (D) indicates evergreen racemose species while solid line refers to the deciduous species. Definition of each lineage is in Fig. 3 or 4.

warm-temperate boreotropical elements. Based on the estimated divergence times in this study, MRCA of the Paleotropics (~29 Myr) and Neotropics (~34 Myr) groups appeared during the icehouse period of the Oligocene (34–23 Myr), which consequently also marked the end of the warm-temperate boreotropical forests in the Northern Hemisphere as the climate became cooler and drier. The extant distribution of these groups at low tropical latitudes of Southeast Asia, Mesoamerica and South America can be possibly explained as a result of disintegration of the boreotropical flora in higher latitudes and subsequent diversification from refugial areas in lower latitudes of China, Micronesia, Mesoamerica, and Central America (Morley, 2000; also see Li and Wen, 2013).

The limited sampling of tropical species in this study limits our understanding of the relationships between the Paleotropics and Neotropics groups, which were only weakly supported by the plastid DNA phylogeny. Nevertheless, this result adds further support to the hypothesis that they were once part of the extensive boreotropical forests in higher latitudes but now remain as two disjunct groups across the Pacific. Increased sampling of taxa, especially in refugial areas of South China, Meso- and South America, will be needed to clarify their relationships. Nonetheless, our results offer a first indication that current Neotropical diversity in South America evolved from a northern ancestor that dispersed to the south either through the Panama or Antilles, as supported by the basally diverging position of *P. caroliniana*, a native of SE-North America. Similarly, the warm-temperate ancestor of Paleotropics probably resided in the refugial mountains of South China and Vietnam as a result of deterioration of boreotropical forests and subsequently radiated rapidly in the equatorial regions during the early Miocene as Southeast Asia experienced a pronounced climate change from a seasonally dry to an everwet type.

5. Conclusions

A sound reconstruction of a molecular phylogeny for a speciose and cosmopolitan genus such as *Prunus* is a highly challenging task as it requires massive sampling of both taxa and variable characters because of past and present hybridization events. However, our strategic sampling of key taxa and appropriately variable characters has shed light in this large group. Our results have provided an indication of the significance of an expanded sampling of tropical species and characters towards understanding of the evolutionary history of *Prunus*. This includes providing better resolution of the relationship of the mostly tropical to subtropical 'Racemose' lineage to the temperate members.

The centers of diversity for *Prunus* crop lineages e.g., plums, cherries, peaches and almonds, are found chiefly in Eurasia. Our results have provided strong clarification that *Prunus* originated in eastern Asia and reached its current distribution via independent dispersal and vicariant events within and between both Old and New Worlds. At least two distinct intercontinental disjunctions were displayed among *Prunus* lineages. The eastern Asian – eastern North American disjunction was notably demonstrated in the American – Eurasian plum group. In addition, a pantropical disjunction can be inferred between the 'Paleotropics' and 'Neotropics' clades.

The global distribution of *Prunus* from its point of origin was shaped by a complex interplay of geologic tectonic events and climatic oscillations from the early Eocene period, which either facilitated or obstructed migration into both the Old and New Worlds at different episodic periods of geologic time. Moreover, we have provided evidence to support the hypothesis of hybrid origin of the primarily tropical 'Racemose' clade. Future studies with further increased sampling of some lineages and of additional characters, especially additional nuclear markers, will be needed to test these hypotheses rigorously. Nonetheless, incorporation of the extensive *Prunus* fossil records uncovered in western North America, Asia and Europe will continue to yield better understanding of the morphological diversification mechanisms as well as better refined the molecular age of *Prunus* diversification.

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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.2014.02.024.

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