Detecting climatically driven phylogenetic and morphological divergence among spruce species (*Picea*) worldwide

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Abstract

This study aimed to elucidate the relationship between climate and the phylogenetic and morphological divergence of spruces (*Picea*) worldwide. Climatic and georeferenced data were collected from a total of 3388 sites distributed within the global domain of spruce species. A phylogenetic tree and a morphological tree for the global spruces were reconstructed based on DNA sequences and morphological characteristics. The spatial evolutionary and ecological vicariance analysis (SEEVA) method was used to detect the ecological divergence among spruces. A divergence index (D) with (0, 1) scaling was calculated for each climatic factor at each node for both trees. The results indicated that the annual mean values, extreme values and annual range of the climatic variables were among the major determinants for spruce divergence. The ecological divergence was significant (P<0.001) for 185 of the 279 comparisons at 31 nodes in the phylogenetic tree and for 196 of the 288 comparisons at 32 nodes in the morphological tree. Temperature parameters and precipitation parameters tended to be the main driving factors for the primary divergence of spruce phylogeny and morphology, respectively. Generally, the D_{max} of the climatic variables was smaller in the basal nodes than in the remaining nodes. The primary divergence of morphology and phylogeny among the investigated spruces tended to be driven by different selective pressures. Given the future climate of severe and widespread drought during the next 30-90 years over land areas, our findings shed light on the prediction of spruce distributions under future climate change.

Keywords

23 Temperature, precipitation, natural selection, niche conservatism, parallel evolution,
24 speciation

1 Introduction

Environmental conditions play an important role in speciation (Mayr, 1947; Darnell and Dillon, 1970; Wiens, 2004; Givnish, 2010; Schemske, 2010). However, quantitative investigations of environmental influences on the origin and divergence of species are less common than expected, especially in plants (Givnish, 2010). For example, although taxonomic and phylogenetic studies have explicitly addressed phylogenetic and morphological divergence among spruces (Farjón, 1990; Sigurgeirsson and Szmidt, 1993; Fu et al., 1999; Ran et al., 2006; Li et al., 2010; Lockwood et al., 2013), ecological differentiation among sister groups or species remains unknown. Ecological vicariance differs from geographical vicariance (Wiley, 1988) and indicates the ecological differentiation among sister groups or sister species within taxa, which provides important information and ecological interpretations for the phylogenetic and morphological divergence among taxa (Escudero et al., 2009; Struwe et al., 2011).

Spruce (*Picea* A. Dietrich) is an important component of boreal vegetation and subalpine coniferous forests and has a wide geographical range that covers the Northern Hemisphere and extends from the Eurasian continent to North America (Farjón, 2001; Spribille and Chytry, 2002). Nearly thirty-four species are recognized in the genus *Picea* worldwide (Farjón, 2001). Although taxonomic schemes of *Picea* based on morphological characteristics differ slightly among authors, a consensus has been reached for the criterion to determine the first several subdivisions (Liu, 1982; Farjón, 1990; Taylor, 1993; Fu et al., 1999). Accordingly, several sections within *Picea* have been classified based on morphological similarity. For example, section *Picea* and

47 section *Casicta* are characterized by quadrangular leaves and flattened leaves,
48 respectively (Farjón, 1990). Alternatively, spruce species can be classified into
49 phylogenetically distinct clades, namely clade-1, a Eurasian clade; clade-2, a North
50 American clade; and clade-3, an Asian clade with one North American species (Ran et
51 al., 2006; Lockwood et al., 2013). These chloroplast DNA sequence data-based
52 classification schemes have the potential to reveal the phylogenetic affinity among
53 spruces. We aimed to elucidate the ecological differentiations between sister groups or
54 species identified based on their phylogenetic affinity and morphological similarity.

55 A species' ecological niche depends on both the species' adaptation to its present
56 habitat and the legacy of its ancestors (Wiens, 2004). Although species tend to retain
57 similar ecological niches as their immediate ancestors, which is generally called
58 phylogenetic niche conservatism, natural selection of ecologically important traits is
59 the key process that determines the successful adaptation of incipient species (Peterson
60 et al., 1999; Webb et al., 2002; Wiens and Graham, 2005). In addition, speciation tends
61 to occur in geographic dimensions, whereas ecological differences evolve over time
62 (Peterson et al., 1999). Thus, there should be tradeoff between niche conservatism and
63 ecological differences among splits in the phylogeny of given taxa over evolutionary
64 time scales. Spruces likely originated in the early Tertiary or late Cretaceous era. The
65 fossil spruce species *Picea burtonii* Klymiuk et Stockey is regarded as the earliest fossil
66 record for *Picea* and dates to approximately 136 Ma (Klymiuk and Stockey, 2012). The
67 ancestor of extant spruces dates to the Oligocene (Sigurgeirsson and Szmidt, 1993;
68 LePage, 2001; Ran et al., 2006; Lockwood et al., 2013). The divergence times of extant

69 spruces occurred over a long time scale, with a range of approximately 28 Ma to 1.8
70 Ma from the basal nodes to the terminal nodes (Lockwood et al., 2013). We hypothesize
71 that there should be a relationship between the time since separation and the magnitude
72 of ecological divergence or niche conservatism. Specifically, we expect to observe an
73 increasing magnitude in terms of ecological divergence among sister groups from the
74 basal nodes (older) to the terminal nodes (younger) on the evolutionary time scales
75 because natural selection would favor species with high levels of ecological adaptation.
76 Although phylogenetically close species are likely to be similar in appearance to one
77 another, differences in the rate of evolution may substantially obscure these similarities
78 (Baum et al., 2005). In the genus *Picea*, none of the morphology-based classification
79 schemes are congruent with or supported by the schemes derived from cpDNA-based
80 phylogenies. Therefore, spruce species within a taxonomic section are not always more
81 similar in phylogenetic relatedness than those between sections or subsections,
82 indicating that parallel evolution, i.e., the repeated appearance of similar characteristics
83 that occur among distantly related species (Went, 1971; Hoekstra and Price, 2004;
84 Schluter et al., 2004; Orr, 2005), occurs in *Picea*. Therefore, we hypothesize that the
85 divergence of morphology and phylogeny among the investigated spruce species may
86 be subject to different selective pressures under parallel evolution.
87 Evolutionary trees indicate historical relationships among organisms (Baum et al.,
88 2005). This "tree-thinking" approach has been used in almost all branches of biology
89 to detect relatedness among organisms (Baum and Offner, 2008) and to examine
90 ecological divergence between sister clades or species (Struwe et al., 2011). In this

study, tree-thinking methods were used to examine the ecological divergence among spruce species worldwide by reconstructing a phylogenetic tree and a morphological tree. A dataset of spruce species was compiled to test our hypothesis by answering the following three questions: are the climatic variables extracted from the current spruce locations correlated with the divergence among spruces? If so, is there a relationship between the time since separation and the magnitude of ecological divergence? Lastly, is the morphological and phylogenetic divergence among spruce species subject to different selective pressures?

2 Materials and Methods

2.1 Distribution data

The sampling sites were selected from within the entire natural range of spruce species in the Northern Hemisphere, extending over a wide geographical range (latitude: 22.8-69.9°N; longitude: 53-165°W, 5-155°E; altitude: 103-4700 m a.s.l.) and exhibiting a steep climatic gradient, varying from a cold-temperate zone to a subtropical zone (Figure 1).

Nearly 34 species are included in the genus *Picea* (Farjón, 2001). The global spruce checklist used in this study was primarily based on Farjón (2001) but refined according to the flora of China (Fu et al., 1999). Specifically, because two species distributed in western China according to Farjón (1990), *Picea retroflexa* and *P. aurantiaca*, were treated as a synonym and a variety of *P. asperata,* respectively, in the flora of China. We followed the Chinese classification. Accordingly, the checklist used for this study contained 33 spruce species.

Georeferenced data for the 33 spruce species was partially downloaded from the Global Biodiversity Information Facility (GBIF), an international open data infrastructure. Original data in the GBIF are derived from various sources, such as natural history explorations (specimens or records) collected over the past 300 years, current observations and automated monitoring programs (GBIF, 2015). We carefully verified the original data downloaded from GBIF by excluding those data points with geolocations outside of the natural distribution ranges (either horizontally or vertically or both). As a result, 2397 point locations from the GBIF remained after the verification, and they primarily represented spruce species in North America, Europe and East Asia (Japan and Korea Peninsula). Additional data for the spruce species from mainland China and Taiwan (991 locations for 16 species) were obtained from georeferenced herbarium collection records (490 sites) (Li et al., 2016) from the herbarium of the Institute of Botany, Chinese Academy of Sciences; recent fieldwork (370 sites, unpublished); and published sources (41 sites) (Tseng, 1991; Yang et al., 2002). As a result, 3388 point locations for the 33 spruce species were available for this analysis.

2.2 Climatic variables

A total of 19 climatic variables (Table 1) with a resolution of approximately 1 km^2 for the 3388 locations were acquired and downloaded from WorldClim V. 1.4 (http://www.worldclim.org) (Hijmans et al., 2005). The values of each climate variable at each site were extracted using the QGIS software package (http://qgis.osgeo.org), and the final data were exported to an Excel worksheet for subsequent analysis.

2.3 Data analysis

DNA sequences were retrieved from the NCBI GenBank (www.ncbi.nlm.nih.gov) to reconstruct a phylogenetic tree of the 33 spruce species (Figure 2). This phylogenetic tree was constructed based on 3 plastid (trnL-trnF, trn-psbA, and trnS-trnG) and 2 mitochondrial (nad5 intron1 and nad1 intron 2) DNA sequences, and it was equivalent to that of Lockwood et al. (2013), who proposed an improved phylogeny of *Picea*. In addition, we reconstructed a morphological tree of the 33 spruce species (Figure 3) based on Farjón (1990), Taylor (1993), and Fu et al. (1999). The first several splits in the tree primarily revealed divergence in the shape of the leaf cross section, the position of the stomatal line on the leaf surface, and the texture and arrangement of the seed scale, whereas traits such as the size of the leaf, seed cone and seed scale and the hairiness of the leaf or twig are important indicators for subsequent splits in the trees. The morphological tree and the phylogenetic tree contained 32 and 31 nodes, respectively.

To detect ecological divergence among sister groups or species in the above-mentioned trees, we used the spatial evolutionary and ecological vicariance analysis (SEEVA, Struwe et al., 2011), which can incorporate climatic data with phylogenetic data and morphological data using statistical methods to investigate ecological vicariance in speciation. The SEEVA compares the differences between each of the climatic variables for each node. A divergence index (D) with (0,1) scaling was calculated for each climatic factor at each node. D=0 indicates no difference between sister clades or groups, whereas D=1 indicates a maximum difference. Fisher's exact test (Fisher, 1958), which generally provides a better P-value for tests with small

157 sample sizes, was performed to determine the significance of *D*. Because 31 and 32
158 independent tests were conducted for each of the climatic variables, a *P*-value less than
159 0.0016 indicated a significant difference in the ecological features for splits at a given
160 node after performing a Bonferroni correction, i.e., $\alpha=0.05/31$ or $32\approx0.0016$. Details on
161 the calculations are available in Struwe et al. (2011). The SEEVA software can be
162 downloaded from http://seeva.heiberg.se.

163 A factor analysis was conducted to eliminate the redundant climatic variables, and a
164 principal component analysis (PCA) of the climatic variables was performed using the
165 SPSS statistical package (SPSS, Chicago, IL, USA). In addition, SEEVA was
166 performed by taking all 16 climate factors into account. To illustrate the results, we
167 focused on how the mean and extreme values of the individual climate factors
168 influenced spruce divergence. The selected climatic variables were required to have
169 higher divergence indices for the first split on the phylogeny tree and the morphology
170 tree and relatively higher loading on the five component axes. As a result, we mapped
171 eight climate factors in the histograms, including four temperature variables (annual
172 mean temperature (AMT), minimum temperature of the coldest month (MTCM),
173 maximum temperature of the warmest month (MTWM) and annual temperature range
174 (ATR)) and four precipitation variables (annual precipitation (AP), precipitation of the
175 wettest month (PWM), precipitation of the driest month (PDM) and precipitation of the
176 coldest quarter (PCQ)). In addition, elevation as a spatial variable was also used to
177 detect the ecological vicariance among sister groups because spruce is an elevation-
178 sensitive taxon (Farjón, 1990; Taylor, 1993; Fu et al., 1999).

We compared the means of the nine abiotic variables among sister groups at several key splits (i.e., the first two split levels) of both trees using a one-way analysis of variance (ANOVA) to further interpret the observed ecological divergence.

3 Results

3.1 Variation in climatic variables

A factor analysis of the climatic variables across sampling sites revealed five dominant climatic gradients that accounted for 94.06% of the variance (Table 1). The first component, which had an eigenvalue of 8.27 and accounted for 43.52% of the variance, was a gradient characterized by variations in temperature variables. The second component, which had an eigenvalue of 3.60 and accounted for 18.93% of the variance, was a gradient characterized by variations in precipitation variables. The third, fourth and fifth components, which accounted for 13.21%, 11.89% and 6.51% of the variance, respectively, were characterized by variations in the precipitation of the driest month or quarter and precipitation seasonality; maximum temperature of the warmest month or quarter; and mean temperature of the wettest quarter and precipitation of the coldest quarter, respectively.

3.2 Ecological divergence among sister groups or species in the phylogeny of *Picea*

Ecological divergence as indicated by the (0, 1) scaled index of *D* was significant ($P<0.0016$) for 185 of the 279 comparisons at 31 nodes in the phylogeny of *Picea* (see Table S1 in Appendix S1). The first split, which yielded node-2 (clade-1) and node-14 (clade-2 and clade-3), was significant for all 9 environmental variables. The annual temperature range ($D=0.26$*) showed higher divergence, and it was followed by

elevation (*D*=0.25*) and precipitation of the driest month (*D*=0.20*). The spruce species in clade-1 tended to occur in climates with a lower annual temperature range and lower precipitation than the spruce species in node-14. The divergence within node-14 and between node-15 (clade-2) and node-22 (clade-3) was also significant for all 9 environmental variables. The parameters precipitation of the coldest quarter, precipitation of the driest month and precipitation of the wettest month had relatively high divergence (*D*=0.66* to 0.42*), elevation exhibited substantial divergence (*D*=0.46*), whereas the temperature variables showed lower divergence (*D*=0.13* to 0.31*). Compared with clade-3, clade-2 occurred in climates with lower precipitation levels and a higher annual temperature range. Node-2 represented a split within clade-1 (the Eurasian clade) between a subclade at a higher elevational zone (in Caucasian area and Japan) with a warmer and wetter climate and a subclade at a lower elevational zone (esp. in boreal area) with a cold and dry climate. The elevation and temperature features showed relatively higher divergence (*D*=0.17* to 0.38*) than the precipitation variables (*D*=0.03* to 0.23*) (Figure 2, Table 2).

The ecological divergence for the subsequent 28 splits in the phylogeny of *Picea*, i.e., from node-3 to node-13 and from node-15 to node-31, was significant for approximately 63% of the comparisons. However, a universal pattern was not observed in terms of the ecological divergence for the remaining splits, which varied according to the sister groups or species. This finding suggests that a particular combination of environmental features is important for particular splits among sister groups or species (Figure 2, Table 2).

3.3 Ecological divergence among sister groups or species in the morphology of *Picea*

Ecological divergence was significant (P<0.0016) for 196 of the 288 comparisons at 32 nodes in the morphology tree of *Picea* (see Table S2 in Appendix S1). Of the 32 nodes, we focused on three splits that represent several key morphological divergence in *Picea*. Specifically, the split of node-1 represents divergence in the shape of the leaf cross section and the position of the stomatal line on the leaf surface, whereas the split of node-2 and node-25 represents divergence in the texture and seed scale arrangement. The remaining 29 splits, i.e., from node-3 to node-24 and from node-26 to node-32, reflect divergence in the leaf size, seed cone size, hairiness (pubescent vs. glabrous) and branchlet color, and these differences were significant for approximately 65% of the comparisons (Figure 3).

The first split of the morphology-defined topology tree (Figure 3) yielded node-2 (leaf quadrangular) and node-25 (leaf flattened) and was significant for all 9 environmental variables. Precipitation features (D=0.16*-0.54*), predominantly precipitation of the wettest month, showed much stronger divergence than temperature features (D=0.05*-0.18*), with elevation showing a moderate divergence (D=0.30*). Spruce species with quadrangular leaves tended to be favored by drier habitats with higher temperature annual ranges in lower elevational zones, which is inconsistent with the habitats for spruces with flattened leaves (Table 2). Such an overall pattern, however, does not always hold true for the sister groups or species that present different leaf cross sections (flattened vs. quadrangular) but close phylogenetic relationships. Sister groups

or species at node-10, node-13, node-18, node-26 and node-31 in the phylogeny tree are relevant examples (Figure 3). For example, although elevation was important for the divergence between *P. jezoensis* and *P. glehnii* (node-10), temperature parameters were important for the divergence between *P. wilsonii* and *P. purpurea* (node-31).

The second-level splits in the morphological tree (Figure 3) yielded two pairs of sister groups, namely node-3 vs. node-24 (within node-2) and node-26 vs. node-29 (within node-25). These two pairs of spruce sister groups collectively indicated divergence in the seed scale characteristics, i.e., closely arranged seed scales with a rigid woody texture vs. loosely arranged seed scales with a thin, flexible, leathery or papery texture. For the split within node-2, elevation showed the highest divergence (D=0.51*) and was followed by annual temperature range (D=0.48*) and precipitation of the driest month (D=0.35*), whereas the remaining climatic variables had significant but relative low divergence (D=0.06*-0.25*). Compared with the results for node-24 (loosely arranged seed scales), the species in node-3 (closely arranged seed scales) tended to occur in lower elevational zones with higher precipitation of the driest month and a wider variation of annual temperature range (Table 2). For the split within node-25, both the minimum temperature of the coldest month (D=0.46*) and precipitation of the driest month (D=0.43*) showed substantial divergence, with a moderate divergence for elevation (D=0.35*). Compared with the results for node-26 (loosely arranged seed scales), the species in node-29 (closely arranged seed scales) tended to occur in lower elevational zones with higher temperature and greater precipitation in the coldest quarter (Table 2).

3.4 Magnitude of ecological divergence and time since separation

Nine levels of splits occurred in the phylogenetic tree. From level 1 to 3, the (0,1) scaled index of divergence (*D*) tended to increase in terms of the median value, maximum value and interquartile range. From level 3 to 9, the maximum value of *D* for most cases (except level 8) was approximately 1, whereas the median and the interquartile range were less structured (Figure 4a). There were 10 levels of splits in the morphological tree. The maximum value of *D*, which was even slightly higher for level 1 (D=0.54) than level 2 (D=0.48), was approximately 1 for the remaining levels. The median tended to increase from level 1 to 7 and then decrease from level 7 to 10. The interquartile range tended to increase from level 1 to 9 (Figure 4b).

4 Discussion

4.1 Climatic data extracted from current spruce locations captures the ecological divergence among spruces

In this study, we used climatic data extracted from the current locations of spruce populations to examine the ecological divergence among spruce species at various time scales from approximately 28 Ma to 1.8 Ma. Our results showed significant divergence for the ecological niches among sister groups throughout the phylogenetic tree and the morphological tree, which indicated the overall relevance of the climatic data on spruce ecological divergence at various time scales. However, the magnitude of ecological divergence (as indicated by the divergence index (*D*)) decreased with the time since the separation of species and became much more specific, i.e., variation of *D* among the nine environmental variables was larger in the more recent splits than in the basal splits.

289 This finding is likely associated with the incompatibility of the time scale between
290 environmental data and ecological divergence because the environmental data extracted
291 from the current locations tended to be more relevant to the divergence of younger
292 nodes than older nodes. The low ecological divergence observed at the first split in both
293 trees should be an indicator of high ecological niche conservatism (Struwe et al., 2011);
294 however, it is likely related to the strong species interactions that obscure the splits. The
295 higher divergence observed for the younger sister groups or sister species might suggest
296 a strong selective effect of climate on extant spruce species derived from more recent
297 splits and might also be associated the fact that the fewer species of the sister groups
298 within each node in the more recent splits have relatively less complicated trait
299 compositions and hence weak interactions. Our first hypothesis is largely verified by
300 the findings of our study and those of a previous case study (Struwe et al., 2011).
301 Exceptions to the above-mentioned trend were observed for a few sister groups or
302 species in the phylogenetic tree. Specifically, within clade-3, significant ecological
303 divergence was not detected for the split (node-29 in Figure 2) between *P. spinulosa*
304 and *P. brachytyla*. These two sister species are distributed in the Circum-Tibetan Plateau
305 and their geographical ranges are adjacent. *P. spinulosa* is distributed in the Mt.
306 Himalaya region and has a narrow range (S Xizang, Bhutan, Nepal and Sikkim),
307 whereas *P. brachytyla* is distributed in the SE to E Tibetan Plateau and has a wide range.
308 These differences suggest that instead of ecological divergence, geographical isolation
309 caused by the deep valleys and high mountain peaks in this area, which act as barriers
310 to gene flow between species, might have played a major role in the speciation of these

two sister species (Li et al., 2010). In addition, we cannot rule out the possibility that the selected climate parameters and their relatively coarse resolution (approximately 1 km) do not adequately describe the climatic determinants of spruce distributions.

In mountainous regions, a distance of 1 km may cover an elevation interval of hundreds of meters, introducing several degrees of difference in temperature, which may give rise to uncertainties in the detection of subtle variations, such as within-species variation, or among elevation-sensitive species. In this case, based on Fig. 1 (a, b), instead of the elevation gradient, the geographical distributions of the three phylogenetic clades and the morphological groups (quadrangular leaves versus flattened leaves) are largely determined by horizontal gradients (latitude and longitude). Therefore, although the 1-km resolution climate data should be robust to interpret this large-scale pattern, further research that focus on the driving force underlying the variation of within-species or among elevation-sensitive species should use high-resolution climate data.

4.2 Temperature features tend to be the main driving factors of the primary divergence of spruce phylogeny

Of the 31 splits in the phylogeny tree of *Picea*, the first split is much more important than the subsequent splits because it represents "the primary trigger" that led to the divergence of the genus. Temperature parameters showed higher divergence for the first split of the spruce phylogeny, although moisture factors were not negligible. The first split of the spruce phylogeny occurred at approximately 28 Ma in a period with severe oscillations of global temperature, which sharply declined at the end of the Eocene and

then warmed during the late Oligocene and early Miocene (Lockwood et al., 2013). This oscillation may provide an explanation for the higher divergence of temperature features. The divergence among the nine environmental variables for the subsequent splits, however, varied according to the sister groups or species.

It is well established that the variations in the historical climate associated with the advancement and retreat of ice sheets during the late Tertiary and Quaternary periods played an important role in determining plant distributions (Walker, 1986; Hewitt, 2000). In this process, old taxa became extinct or survived in refugia, whereas derived taxa dispersed to new locations and underwent severe selection by climate (Hewitt, 2000; Hampe and Petit, 2010). Therefore, the formation of biogeographical plant patterns is a product of interactions among these processes (Wolf et al., 2001).

In fact, considerable variations in geology and climate have occurred since spruce originated in the late Oligocene. For example, the earliest spruce pollen fossil is from the late Oligocene to the early Miocene in Asia and was found on the Tibetan Plateau (Wu et al., 2007), and spruce pollen has frequently been found in sediments originating from the late Pliocene and the Pleistocene in northern, eastern and southwestern China (Xu et al., 1973; Xu et al., 1980; Shi, 1996) and Taiwan (Tsukada, 1966). A higher proportion of spruce pollen in specific sediments is generally assumed to indicate a cold period, whereas a lower proportion of spruce pollen indicates a warmer period (Xu et al., 1980). The proportion of spruce pollen in the sediments varied substantially with the geological age of the sediments, suggesting that spruce underwent frequent expansion and retreat during glacial cycles. In North America, fossils of Brewer spruce

(*P. breveriana*) have been observed in northeastern Oregon in Miocene deposits that date to more than 15 Ma years ago; however, the present distribution of Brewer spruce is different from the distribution of the fossil locations, indicating that expansion and retreat occurred in the past (Waring et al., 1975). It is difficult to match all the details of paleo-geological or paleo-climatic events to the ecological divergence observed for specific nodes, although our findings offer a quantitative interpretation with respect to the influence of climate on spruce speciation.

4.3 Precipitation features tend to be the main driving factors of the primary divergence of spruce morphology

The morphological tree in this study was based on spruce taxonomic schemes and highlights the divergence between leaf cross sections in spruce. Although this morphological tree is an artificial scheme, our results indicate that precipitation features were "the primary trigger" of the divergence between quadrangular leaves and flattened leaves among spruce species. A universal pattern was not observed for the climatic variables with respect to the ecological divergence of spruce morphology, which varied according to the specific nodes or splits.

The first split of the basal node of the morphological tree was based on the leaf cross section (i.e., quadrangular vs. flattened); however, each sister group is actually a combination of multiple traits, including the size, shape, color and pubescent/glabrous state of the seed cones, seed scales, bud scales, leaves, leaf apex, and first- and second-year branchlets (Farjón, 1990; Sigurgeirsson and Szmidt, 1993; Fu et al., 1999). The morphological and morphometric traits of spruce species have been demonstrated to

produce strong climatic signals; however, specific traits for different species do not always exhibit the same response to specific environmental gradients (Wang et al., 2015; Li et al., 2016). This inconsistency in response is likely due to parallel evolution because morphological similarity among species does not always coincide with the phylogenetic relatedness of species (Went, 1971; Orr, 2005). Accordingly, spruce species with similar morphological characteristics but distant phylogenetic relatedness may differ because of the tradeoff between niche conservatism and ecological divergence. In addition, the composition of traits within a species is also species specific. For example, the shape of the leaf cross section co-varies along with the stomatal line position on the leaf surface, seed scale arrangement and seed scale texture. However, evidence in support of the co-evolution between the leaf cross section (quadrangular (Q) vs. flattened (F)) and seed scale arrangement (closely (C) vs. loosely (L)) has not been observed. Trait combinations such as Q+C, Q+L, F+C and F+L are found in 22, 2, 4 and 5 of the 33 species in *Picea,* respectively (Farjón, 2001). Therefore, without providing additional details, a universal pattern of ecological divergence cannot be predicted for the entire morphological tree of *Picea*.

4.4 Divergence of morphology and phylogeny among spruce species is affected by different selective pressures under parallel evolution

Closely related species in a phylogenetic tree tend to be similar in appearance, although this may not be so under parallel evolution (Hoekstra and Price, 2004; Baum et al., 2005; Orr, 2005), and both cases can be observed in spruce. First, of the three clades in the phylogenetic tree, most of the spruce species (19 of 22) in clade-1 and clade-2 tended

399 to have quadrangular leaves, whereas nearly half of the spruce species (6 of 11) in clade-
400 3 tended to have flattened leaves. In addition, two North American species, *P. rubens*
401 and *P. mariana*, are sister species in both constructed trees. Accordingly, the
402 morphological divergence and phylogenetic divergence of these species are subject to
403 the same selective pressures. Second, cases of parallel evolution are quite obvious. For
404 example, two Asian species, *P. purpurea* and *P. wilsonii*, are sister species in the
405 phylogenetic tree but are located in different sections of the morphological tree; this
406 scenario is also observed for another two North American species, *P. glauca* and *P.*
407 *engelmannii*. As a result, the morphological and phylogenetic divergences for these
408 species pairs are subject to different selective pressures, which suggests that the
409 divergence of morphology and phylogeny among the species in question may or may
410 not be subject to different selective pressures depending on the process of speciation.

411 **4.5 Significance and implications of the findings of this research in relation to**
412 **future climate change**

413 According to the 1997 UNPE standard climate zone classification (Middleton and
414 Thomas, 1997), 8 spruce species are in arid and semi-arid areas, 11 in dry sub-humid
415 areas, and 14 in humid areas. Global climate change predictions have shown that severe
416 and widespread droughts are expected during the next 30-90 years due to either
417 decreased precipitation or increased evaporation, and the significant increases in aridity
418 are expected occur in many subtropical and adjacent humid regions (Dai, 2012; Greve
419 and Seneviratne, 2015). When overlapping the spruce sampling point to the future
420 aridity change map (data not shown), nearly all spruce species whose original

421 distribution encompasses sub-humid and humid areas is subject to drought stress.
422 Therefore, our findings suggest that spruces with quadrangular leaves and in clade-1
423 are expected to expand, whereas those with flattened leaves and in clade-2 and clade-3
424 are expected to retreat. This should be taken into account in the context of forming
425 strategies in response to future climate change.

426 **5 Summary and conclusions**

427 In summary, the influence of climate on the divergence of the morphology and
428 phylogeny of spruces is mediated by a number of biotic and abiotic factors, such as
429 geographical isolation, niche conservatism and ecological adaptation. A major finding
430 from this study is that temperature and precipitation parameters tend to be the main
431 driving factors for the primary divergence of spruce phylogeny and morphology,
432 respectively. Our hypotheses are largely verified by the findings of the present study.
433 However, exceptions to the overall pattern cannot be ignored. For example, although
434 most spruce species with quadrangular leaves tend to occur in drier habitats, Taiwan
435 spruce (*P. morrisonicola*) presents quadrangular leaves and is naturally distributed in
436 subtropical areas with abundant rainfall; thus, its present distribution is likely within a
437 refugium from the postglacial period (Tsukada, 1966; Xu et al., 1980). Further work
438 that considers all determinants is required to understand the forces driving ecological
439 divergence among spruce sister groups or species. In addition, our findings shed light
440 on the management issues with respect to spruce distributions under future climate
441 change.

442 **6 Data availability**

443 The relevant data are within the paper and its Supporting Information section.

444 **7 Author contributions**

445 GHW conceived and designed the experiments. All authors performed the experiment.
446 GHW and HL analyzed and interpreted the data, and wrote the paper. The authors
447 declare they have no conflict of interest.

448 **Supporting Information**

449 Additional supporting information may be found in the online version of this article:

450 **Appendix S1** Index of divergence (D) from the phylogeny-based and morphology-
451 based SEEVA evaluation of spruce species.

452 **Table S1** Index of divergence (*D*) from the phylogeny-based SEEVA evaluation of
453 spruce species worldwide.

454 **Table S2** Index of divergence (*D*) from the morphology-based SEEVA evaluation of spruce
455 species worldwide.

456 **9 Acknowledgements**

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573 **Table 1.** Factor analysis showing the eigenvalues, variance percentages, cumulative
574 percentages and correlations of 19 climatic variables with the five components.
575 Climatic variables in bold were selected for further analysis.

Climatic variables	Components				
	1	2	3	4	5
Eigenvalues	8.27	3.60	2.51	2.26	1.24
Variance %	43.52	18.93	13.21	11.89	6.51
Cumulative %	43.52	62.46	75.67	87.55	94.06
Annual Mean Temperature (AMT)	**0.803**	0.222	0.082	0.513	-0.152
Mean Diurnal Range (MDR)	-0.118	-0.155	-0.686	0.476	0.31
Isothermality (I)	0.687	0.283	-0.45	0.158	0.307
Temperature Seasonality (TS)	-0.928	-0.237	-0.099	0.204	-0.12
Max Temperature of Warmest Month (MTWM)	0.037	-0.155	-0.129	**0.968**	0.01
Min Temperature of Coldest Month (MTCM)	**0.931**	0.216	0.257	0.086	0.006
Annual Temperature Range (ATR)	**-0.854**	-0.267	-0.294	0.329	-0.001
Mean Temperature of Wettest Quarter (MTWeQ)	-0.123	0.091	-0.066	0.48	-0.788
Mean Temperature of Driest Quarter (MTDQ)	0.841	0.093	0.138	0.116	0.408
Mean Temperature of Warmest Quarter (MTWaQ)	0.14	0.02	0.04	0.918	-0.294
Mean Temperature of Coldest Quarter (MTCQ)	0.946	0.24	0.108	0.179	0.007
Annual Precipitation (AP)	0.306	**0.856**	0.365	-0.041	0.178
Precipitation of Wettest Month (PWM)	0.288	**0.942**	-0.006	-0.033	0.109
Precipitation of Driest Month (PDM)	0.147	0.255	**0.911**	0.008	0.087
Precipitation Seasonality (PS)	-0.109	0.255	-0.887	-0.006	-0.131
Precipitation of Wettest Quarter (PWeQ)	0.297	0.937	0.026	-0.038	0.134
Precipitation of Driest Quarter (PDQ)	0.175	0.302	0.894	-0.003	0.152
Precipitation of Warmest Quarter (PWaQ)	0.144	0.888	0.086	-0.057	-0.313
Precipitation of Coldest Quarter (PCQ)	0.323	0.402	0.418	-0.016	**0.652**

576

Table 2. Mean comparisons of the elevation and 8 climatic variables (mean±SD, abbreviations are the same as in Table 1) between sister groups at the first two split levels of both the phylogeny tree and the morphology tree. Mean±SD values marked with different letters indicate a significant difference at $P<0.05$, and the same letter indicates a non-significant difference ($P>0.05$).

	N	Elevation (m)	AMT (℃)	MTWM (℃)	MTCM (℃)	ATR (℃)	AP (mm)	PWM (mm)	PDM (mm)	PCQ (mm)
Phylogeny Nodes										
Sister Groups: node-2 (clade-1) vs. node-14 (clade-2 + clade-3)										
2	**1568**	**964±750** [a]	**3.2±4.2** [a]	**19.6±3.7** [a]	**-12.6±8.6** [a]	**32.1±9.5** [a]	**845.8±416.9** [a]	**117.1±52.3** [a]	**38.0±25.7** [a]	**158.9±124.2** [a]
14	**1820**	**1721±1150** [b]	**3.8±5.0** [b]	**21.8±3.9** [b]	**-13.9±8.8** [b]	**35.7±8.8** [b]	**910.7±727.6** [b]	**143.6±119.0** [b]	**26.9±27.8** [b]	**186.5±209.3** [b]
Sister Groups: node-15 (clade-2) vs. node-22 (clade-3)										
15	1100	**1176±906** [a]	**2.5±5.0** [a]	**22.5±3.6** [a]	**-16.6±8.2** [a]	**39.1±7.3** [a]	**784.1±442.6** [a]	**106.3±61.6** [a]	**35.7±27.7** [a]	190.7±180.0 [a]
22	720	**2554±971** [b]	**5.9±4.3** [b]	**20.6±4.0** [b]	**-9.9±8.1** [b]	**30.6±8.4** [b]	**1104.0±989.0** [b]	**200.8±157.0** [b]	**13.5±21.8** [b]	180.0±247.4 [a]
Sister Groups: node-3 vs. node-11 (two sister groups within clade-2)										
3	1502	**951±755** [a]	**3.0±4.2** [a]	**19.4±3.6** [a]	**-12.8±8.6** [a]	**32.2±9.7** [a]	**834.5±411.2** [a]	116.2±51.3 [a]	**37.4±25.8** [a]	**157.2±126.0** [a]
11	66	**1275±542** [b]	**7.1±2.8** [b]	**22.9±2.6** [b]	**-7.5±3.7** [b]	**30.4±2.8** [b]	**1101.8±464.7** [b]	137.8±70.0 [a]	**52.3±16.7** [b]	**196.3±63.3** [b]
Morphology Nodes										
Sister Groups: node-2 vs. node-25 (i.e., quadrangular leaf group vs. flattened leaf group)										
2	2857	**1191±915** [a]	**3.1±4.7** [a]	20.8±4.0 [a]	**-14.0±8.8** [a]	**34.8±9.7** [a]	**849.4±624.2** [a]	**120.0±95.2** [a]	**35.3±27.2** [a]	**163.8±146.4** [a]
25	531	**2337±1222** [b]	**5.8±3.7** [b]	20.7±3.7 [a]	**-9.3±6.6** [b]	**29.9±5.5** [b]	**1048.5±452.1** [b]	**192.2±67.9** [b]	**14.5±21.0** [b]	**226.8±279.7** [b]
Sister Groups: node-3 vs. node-24 (i.e., within quadrangular leaf group: seed scale closely arranged group vs. loosely arranged group)										
3	2530	**1059±850** [a]	**3.0±4.8** [a]	**20.5±3.9** [a]	**-14.3±9.2** [a]	34.8±10.2 [a]	**864.7±646.3** [a]	**121.6±97.8** [a]	**36.6±28.4** [a]	**155.8±135.1** [a]
24	327	**2219±729** [b]	**3.7±3.7** [b]	**22.8±4.0** [b]	**-12.1±4.8** [b]	34.8±4.2 [a]	**730.9±396.0** [b]	**107.7±70.6** [b]	**25.7±10.8** [b]	**225.9±204.9** [b]
Sister Groups: node-26 vs. node-29 (i.e., within flattened leaf group: seed scale closely arranged group vs. loosely arranged group)										
26	283	**2806±1301** [a]	**4.6±4.1** [a]	**19.0±3.3** [a]	**-12.4±7.3** [a]	**31.4±6.7** [a]	**996.1±564.2** [a]	190.1±77.4 [a]	15.1±23.7 [a]	**125.5±252.6** [a]
29	248	**1802±854** [b]	**7.2±2.5** [b]	**22.5±3.2** [b]	**-5.7±3.0** [b]	**28.2±2.9** [b]	**1108.4±261.7** [b]	194.6±55.3 [a]	13.8±17.4 [a]	**342.4±264.2** [b]
4	2118	**1124±890** [a]	3.0±4.9 [a]	**20.0±3.9** [a]	**-13.8±9.5** [a]	**33.8±10.5** [a]	853.8±682.2 [a]	**124.6±105.6** [a]	**33.3±26.2** [a]	**149.0±139.0** [a]
21	412	**724±487** [b]	3.2±4.3 [a]	**23.2±2.9** [b]	**-17.0±6.9** [b]	**40.1±6.0** [b]	921.0±412.3 [a]	**106.2±33.1** [b]	**53.2±33.0** [b]	**190.8±105.7** [b]

583 **Figure legends:**

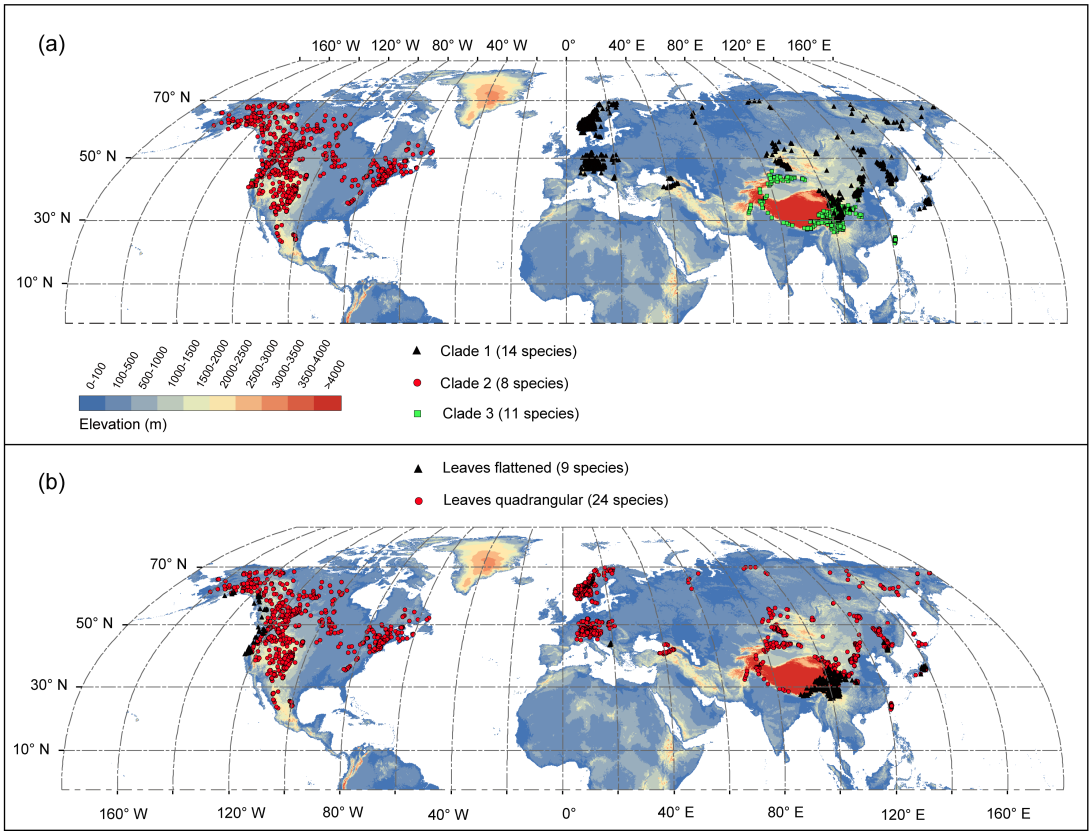
584 **Figure 1.** Sites were sampled across the entire range of spruces worldwide. Sites
585 marked with different symbols represent three phylogenetically distinct clades (a) and
586 two morphological groups (b). Elevation gradients are indicated by colored fields.

587 **Figure 2.** Divergence indices (scales range from 0-1) shown as histograms for elevation
588 and for the 8 climatic variables for each node of the phylogeny of *Picea* worldwide.
589 *Indicates a significant difference in ecological features after Bonferroni correction
590 (P<0.0016).

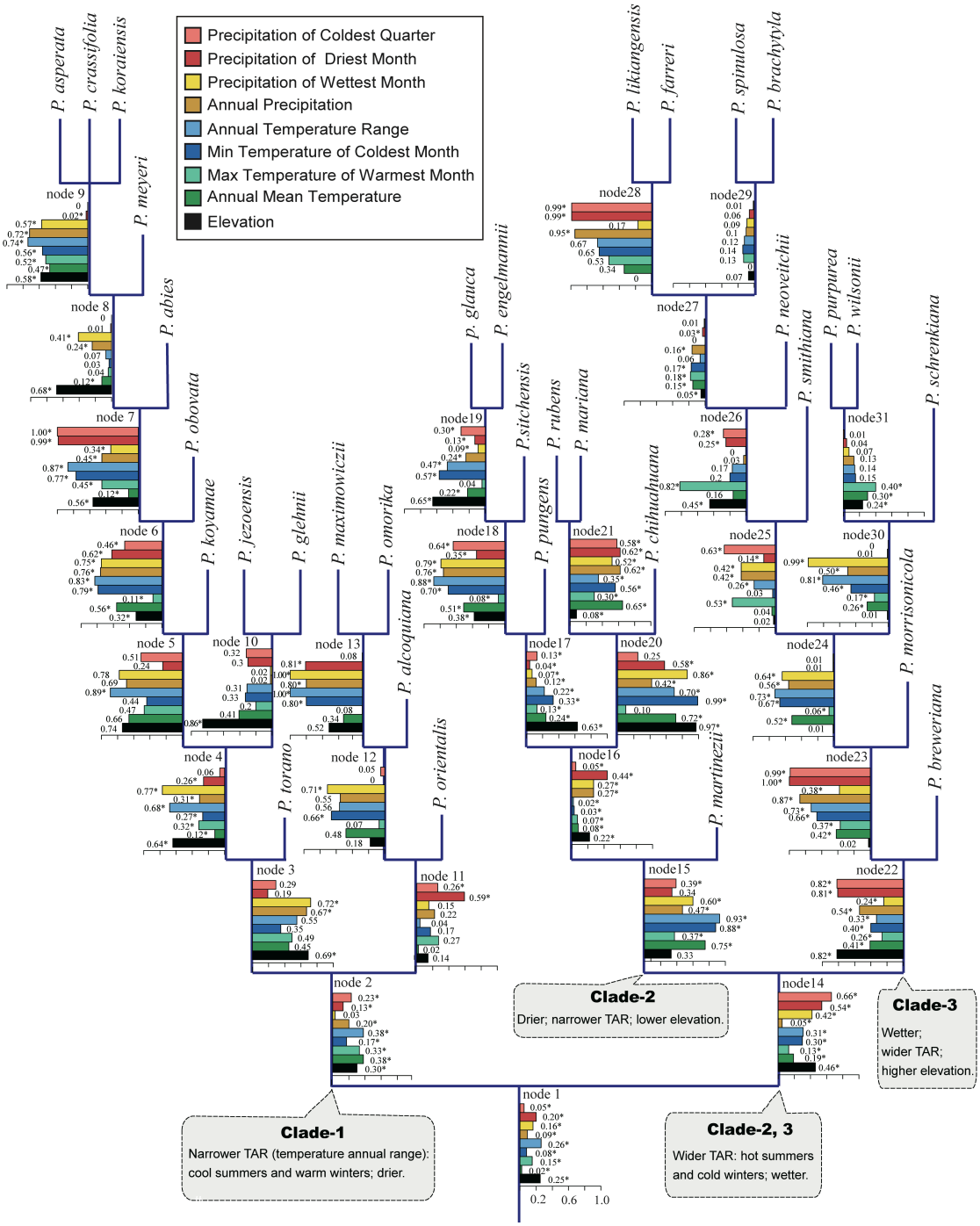
591 **Figure 3.** Divergence indices (scales range from 0-1) shown as histograms for elevation
592 and for the 8 climatic variables for each node of the morphology of *Picea* worldwide.
593 *Indicates a significant difference of ecological features after Bonferroni correction
594 (P<0.0016).

595 **Figure 4.** Box plots showing the index of divergence at each of the splitting levels in
596 the phylogenetic tree (a) and the morphological tree (b) of spruce species worldwide.
597 The central box in each box plot indicates the interquartile range and median, whereas
598 the whiskers show the 10th and 90th percentiles. Mean values marked with different
599 letters indicate a significant difference at P<0.01.

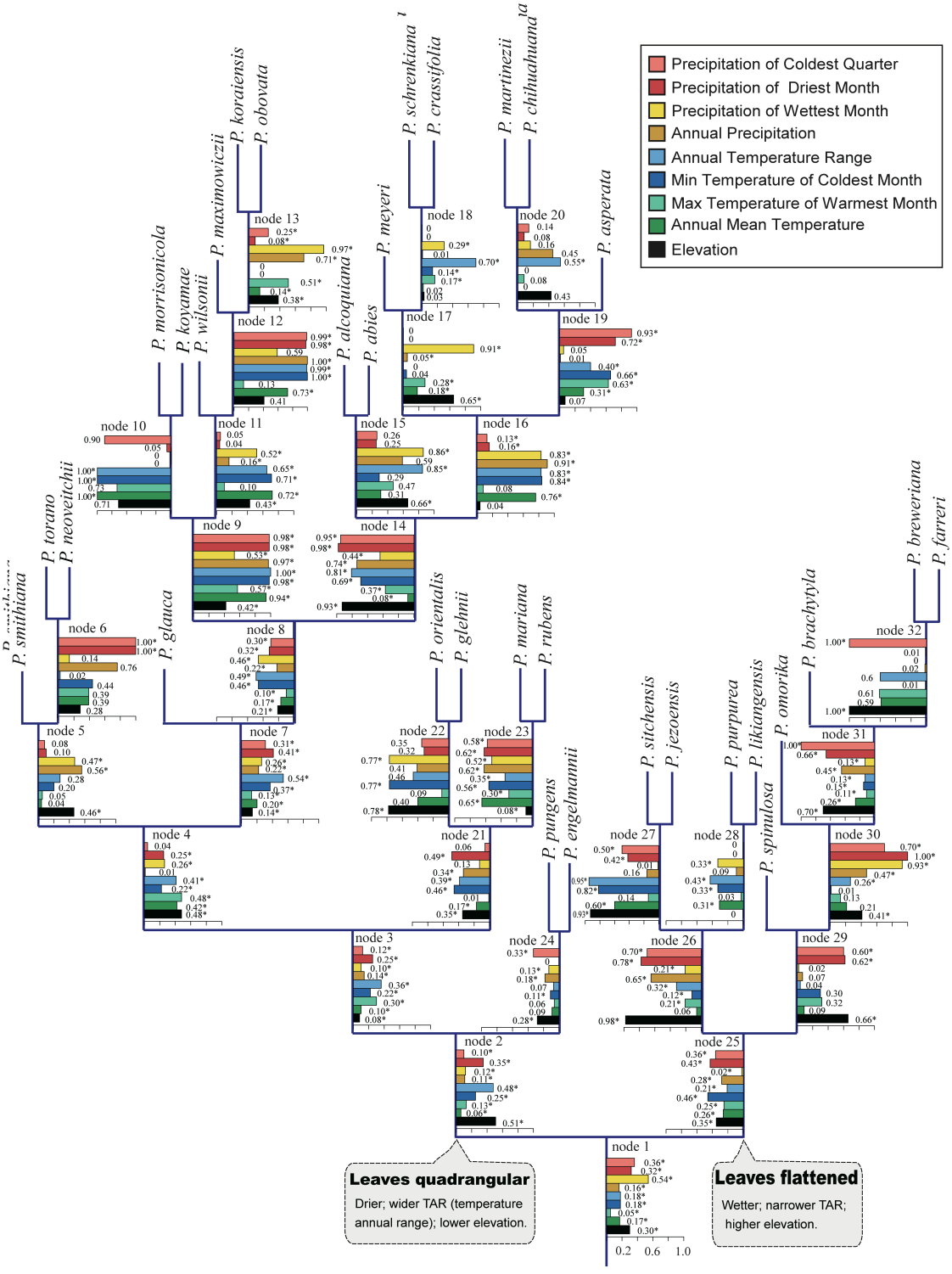
600 Figure 1


(a)
160° W
120° W
80° W
40° W
0°
40° E
80° E
120° E
160° E
70° N
50° N
30° N
10° N
0-100
100-500
500-1000
1000-1500
1500-2000
2000-2500
2500-3000
3000-3500
3500-4000
>4000
Elevation (m)
Clade 1 (14 species)
Clade 2 (8 species)
Clade 3 (11 species)
(b)
Leaves flattened (9 species)
Leaves quadrangular (24 species)
70° N
50° N
30° N
10° N
160° W
120° W
80° W
40° W
0°
40° E
80° E
120° E
160° E

601 Figure 2

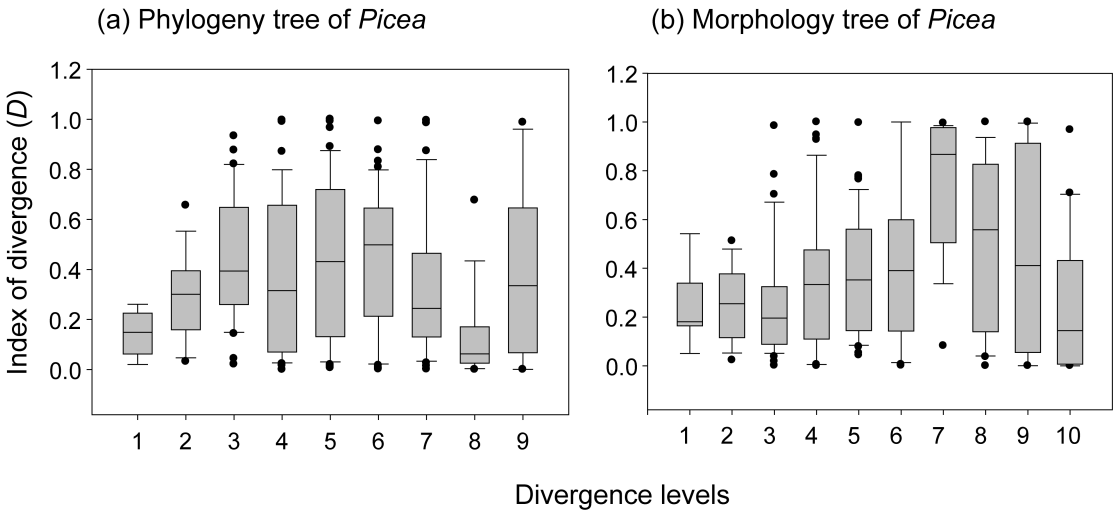

Precipitation of Coldest Quarter
Precipitation of Driest Month
Precipitation of Wettest Month
Annual Precipitation
Annual Temperature Range
Min Temperature of Coldest Month
Max Temperature of Warmest Month
Annual Mean Temperature
Elevation
P. asperata
P. crassifolia
P. koraiensis
P. likiangensis
P. farreri
P. spinulosa
P. brachytyla
P. meyeri
P. abies
P. obovata
P. koyamae
P. jezoensis
P. glehnii
P. maximowiczii
P. omorika
P. alcoquiana
P. glauca
P. engelmannii
P.sitchensis
P. rubens
P. mariana
P. chihuahuana
P. neoveitchii
P. smithiana
P. purpurea
P. wilsonii
P. schrenkiana
P. morrisonicola
P. breweriana
P. martinezii
P. orientalis
P. torano
node 1
node 2
node 3
node 4
node 5
node 6
node 7
node 8
node 9
node 10
node 11
node 12
node 13
node14
node15
node16
node17
node18
node19
node20
node21
node22
node23
node24
node25
node26
node27
node28
node29
node30
node31
Clade-1
Narrower TAR (temperature annual range): cool summers and warm winters; drier.
Clade-2
Drier; narrower TAR; lower elevation.
Clade-2, 3
Wider TAR: hot summers and cold winters; wetter.
Clade-3
Wetter; wider TAR; higher elevation.
0.2
0.6
1.0

602 Figure 3



603

604 Figure 4


(a) Phylogeny tree of *Picea*
(b) Morphology tree of *Picea*
Index of divergence (*D*)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9
1 2 3 4 5 6 7 8 9 10
Divergence levels