# Detecting climatically driven phylogenetic and morphological divergence among spruce (*Picea*) species 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. Spatial evolutionary and ecological vicariance analysis (SEEVA) 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 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, as well as 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 divergences of spruce phylogeny and morphology, respectively. Generally, the maximum *D* of the climatic variables was smaller in the basal nodes than in the remaining nodes. Notably, the primary divergence of morphology and phylogeny among the investigated spruces tended to be driven by different selective pressures. Given the climate scenario of severe and widespread drought over land areas in the next 30-90 years, our findings shed light on the prediction of spruce distribution under future climate change.

## Keywords

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

## 1 Introduction

Environmental conditions play important roles in speciation (Mayr, 1947; Darnell and Dillon, 1970; Wiens, 2004; Givnish, 2010; Schemske, 2010; Weber et al., 2017). However, quantitative investigations of environmental influences on the origin and divergence of species are less common than expected, especially in plants (Givnish, 2010; López-Reyes et al., 2015). 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; Luebert et al., 2017) 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

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

57 A species' ecological niche depends on both the species' adaptation to its present
58 habitat and the legacy of its ancestors (Wiens, 2004). Although species tend to retain
59 similar ecological niches as their immediate ancestors, which is generally called
60 phylogenetic niche conservatism (Münkemüller et al., 2015; Pyron et al., 2015),
61 natural selection of ecologically important traits is the key process that determines the
62 successful adaptation of incipient species (Peterson et al., 1999; Webb et al., 2002;
63 Wiens and Graham, 2005). In addition, speciation tends to occur in geographic
64 dimensions, whereas ecological differences evolve over time (Peterson et al., 1999).
65 Thus, there should be tradeoff between niche conservatism and ecological differences
66 among splits in the phylogeny of given taxa over evolutionary time scales. Spruces
67 likely originated in the early Tertiary or late Cretaceous era. The fossil spruce species
68 *Picea burtonii* Klymiuk et Stockey is regarded as the earliest fossil record for *Picea*

and dates to approximately 136 Ma (Klymiuk and Stockey, 2012). The ancestor of extant spruces dates to the Oligocene (Sigurgeirsson and Szmidt, 1993; LePage, 2001; Ran et al., 2006; Lockwood et al., 2013). The divergence times of extant spruces occurred over a long time scale, with a range of approximately 28 Ma to 1.8 Ma from the basal node to the terminal nodes (Lockwood et al., 2013). We hypothesize that there should be a relationship between the time since separation and the magnitude of ecological divergence or niche conservatism. Specifically, we expect to observe an increasing magnitude in terms of ecological divergence among sister groups from the basal nodes (older) to the terminal nodes (younger) on the evolutionary time scales because natural selection would favor species with high levels of ecological adaptation.

Although phylogenetically close species are likely to be similar in appearance to one another, differences in the rate of evolution may substantially obscure these similarities (Baum et al., 2005). In the genus *Picea*, none of the morphology-based classification schemes are congruent with or supported by the schemes derived from cpDNA-based phylogenies. Therefore, spruce species within a taxonomic section are not necessarily more similar in phylogenetic relatedness than those in different sections or subsections; this indicates that parallel evolution, i.e., the repeated appearance of similar characteristics that occur among distantly related species (Went, 1971; Hoekstra and Price, 2004; Schluter et al., 2004; Orr, 2005; Bailey et al., 2015), occurs in *Picea*. Therefore, we hypothesize that the divergence of morphology and phylogeny among the investigated spruce species may be subject to different selective

pressures under parallel evolution.

Evolutionary trees indicate historical relationships among organisms (Baum et al., 2005). This "tree-thinking" approach has been used in almost all branches of biology to detect relatedness among organisms (Baum and Offner, 2008) and to examine 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 ranging from cold-temperate to subtropical zones (Figure 1).

Thirty-four species are included in the genus *Picea* (Farjón, 2001). The global spruce checklist used in this study was primarily based on Farjón (2001) and refined

according to the Flora of China (Fu et al., 1999). Specifically, two species distributed in western China according to Farjón (1990), *Picea retroflexa* and *P. aurantiaca*, were treated as a synonym and as 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 geo-referenced 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 point 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 software QGIS (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

157 data and morphological data using statistical methods to investigate ecological
158 vicariance in speciation. The SEEVA compares the differences between each of the
159 climatic variables for each node. A divergence index (*D*) with (0, 1) scaling was
160 calculated for each climatic factor at each node. *D*=0 indicates no difference between
161 sister clades or groups, whereas *D*=1 indicates a maximum difference. Fisher's exact
162 test (Fisher, 1958), which generally provides a better *P*-value for tests with small
163 sample sizes, was performed to determine the significance of *D*. Because 31 and 32
164 independent tests were conducted for each of the climatic variables, a *P*-value less
165 than 0.0016 indicated a significant difference in the ecological features for splits at a
166 given node after performing a Bonferroni correction, i.e., $\alpha=0.05/(31 \text{ or } 32)\approx0.0016$.
167 Details on the calculations are available in Struwe et al. (2011). The SEEVA software
168 can be downloaded from http://seeva.heiberg.se.

169 A factor analysis was conducted to eliminate the redundant climatic variables, and a
170 principal component analysis (PCA) of the climatic variables was performed using the
171 SPSS statistical package (SPSS, Chicago, IL, USA). In addition, we ran the SEEVA
172 by taking all the 16 climate factors into account. To illustrate the results briefly and
173 clearly, we focused on how the mean value and extreme values of climate factors
174 could influence spruce divergence. The selected climatic variables must have both
175 higher divergence indices for the first split on the phylogenetic tree and the
176 morphological tree and relatively higher loading on the five component axes. As a
177 result, we mapped eight climate factors in the histograms, including four temperature
178 variables (annual mean temperature (AMT), minimum temperature of the coldest

179 month (MTCM), maximum temperature of the warmest month (MTWM) and
180 temperature annual range (TAR)) and four precipitation variables (annual
181 precipitation (AP), precipitation of the wettest month (PWM), precipitation of the
182 driest month (PDM) and precipitation of the coldest quarter (PCQ)). In addition,
183 elevation as a spatial variable was also used to detect the ecological vicariance among
184 sister groups because spruce is an elevation-sensitive taxon (Farjón, 1990; Taylor,
185 1993; Fu et al., 1999).

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

189 **3 Results**

190 **3.1 Variation in climatic variables**

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

201 precipitation of the coldest quarter, respectively.

202 **3.2 Ecological divergence among sister groups or species in the phylogeny of**
203 ***Picea***

204 Ecological divergence as indicated by the (0, 1) scaled index of *D* was significant
205 ($P$<0.0016, significance indicated as * where relevant) for 185 of the 279 comparisons
206 at 31 nodes in the phylogeny of *Picea* (see Table S1 in Appendix S1). The first split,
207 which yielded node-2 (clade-1) and node-14 (clade-2 and clade-3), was significant for
208 all 9 environmental variables. The annual temperature range ($D$=0.26*) showed
209 higher divergence, and it was followed by elevation ($D$=0.25*) and precipitation of
210 the driest month ($D$=0.20*). The spruce species in clade-1 tended to occur in climates
211 with a lower annual temperature range and lower precipitation compared with the
212 spruce species in node-14. The divergence within node-14 and between node-15
213 (clade-2) and node-22 (clade-3) was also significant for all 9 environmental variables.
214 The parameters precipitation of the coldest quarter, precipitation of the driest month
215 and precipitation of the wettest month had relatively high divergence ($D$=0.66* to
216 0.42*), elevation exhibited substantial divergence ($D$=0.46*), whereas the
217 temperature variables showed lower divergence ($D$=0.13* to 0.31*). Compared with
218 clade-3, clade-2 occurred in climates with lower precipitation levels and a higher
219 annual temperature range. Node-2 represented a split within clade-1 (the Eurasian
220 clade) between a subclade at a higher elevational zone (in the Caucasian area and
221 Japan) with a warmer and wetter climate and a subclade at a lower elevational zone
222 (esp. in the boreal area) with a cold and dry climate. The elevation and temperature

features showed relatively higher divergence ($D$=0.17* to 0.38*) compared with 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, indicated as * where relevant) for 196 of the 288 comparisons at 32 nodes in the morphological tree of *Picea* (see Table S2 in Appendix S1). Of the 32 nodes, we focused on three splits that represent several key morphological divergences 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 ($P$<0.0016, 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 ($P$<0.0016). Precipitation features ($D$=0.16*-0.54*), predominantly precipitation of the wettest month, showed much stronger divergence compared with that of 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 necessarily 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 phylogenetic 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

267 significant but relative low divergence (*D*=0.06*-0.25*). Compared with the results
268 for node-24 (loosely arranged seed scales), the species in node-3 (closely arranged
269 seed scales) tended to occur in lower elevational zones with higher precipitation of the
270 driest month and a wider variation of annual temperature range (Table 2). For the split
271 within node-25, both the minimum temperature of the coldest month (*D*=0.46*) and
272 precipitation of the driest month (*D*=0.43*) showed substantial divergence, with a
273 moderate divergence for elevation (*D*=0.35*). Compared with the results for node-26
274 (loosely arranged seed scales), the species in node-29 (closely arranged seed scales)
275 tended to occur in lower elevational zones with higher temperature and greater
276 precipitation in the coldest quarter (Table 2).

277 **3.4 Magnitude of ecological divergence and time since separation**

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

287 **4 Discussions**

288 **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.

This finding is likely associated with the incompatibility of the time scale between environmental data and ecological divergence because the environmental data extracted from the current locations tended to be more relevant to the divergence of younger nodes than older nodes. The low ecological divergence observed at the first split in both trees, on the one hand, should be an indicator of high ecological niche conservatism (Struwe et al., 2011); on the other hand, is likely related to the strong species interactions that obscure the splits. However, the higher divergence observed for the younger sister groups or sister species might suggest a strong selective effect of climate on extant spruce species derived from more recent splits and might also be associated with the fact that the smaller numbers of species of the sister groups within each node in the more recent splits have less-complicated trait compositions and

311 hence weak interactions. Our first hypothesis, i.e., an increasing magnitude in terms of
312 ecological divergence among sister groups from the basal nodes (older) to the
313 terminal nodes (younger) on evolutionary time scales is largely verified by the
314 findings of our study and those of a previous case study (Struwe et al., 2011).

315 Exceptions to the above-mentioned trend were observed for a few sister groups or
316 species in the phylogenetic tree. Specifically, within clade-3, significant ecological
317 divergence was not detected for the split (node-29 in Figure 2) between *P. spinulosa*
318 and *P. brachytyla*. These two sister species are distributed in the Circum-Tibetan
319 Plateau and their geographical ranges are adjacent. *P. spinulosa* is distributed in the
320 Mt. Himalaya region and has a narrow range (S Xizang, Bhutan, Nepal and Sikkim),
321 whereas *P. brachytyla* is distributed in the SE to E Tibetan Plateau and has a wide
322 range. These differences suggest that instead of ecological divergence, geographical
323 isolation caused by the deep valleys and high mountain peaks in this area, which act
324 as barriers to gene flow between species, might have played a major role in the
325 speciation of these two sister species (Li et al., 2010). In addition, we cannot rule out
326 the possibility that the selected climate parameters as well as their relatively coarse
327 resolution (about 1 km) do not adequately describe the climatic determinants of
328 spruce distributions.

329 In mountainous regions, 1 km of distance may cover an elevation interval of
330 hundreds of meters and therefore introduce several degrees of difference in
331 temperature, which may give rise to some uncertainties in the context of the detection
332 of some subtle variations such as within-species variation or variation among

elevation-sensitive species. In this case, as shown in Fig. 1 (a, b), instead of an elevation gradient, the geographical distributions both of the three phylogenetic clades and of the morphological groups (quadrangular leaves versus flattened leaves) are largely determined by horizontal gradients (latitude and longitude). Thus, although the resolution (1 km) of the climate data that we used in this study should be robust to interpret this large scale pattern, further studies 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 phylogenetic 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 necessarily 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

399 necessarily coincide with the phylogenetic relatedness of species (Went, 1971; Orr,
400 2005). Accordingly, spruce species with similar morphological characteristics but
401 distant phylogenetic relatedness may differ because of the tradeoff between niche
402 conservatism and ecological divergence. In addition, the composition of traits within a
403 species is also species specific. For example, the shape of the leaf cross section co-
404 varies along with the stomatal line position on the leaf surface, seed scale arrangement
405 and seed scale texture. However, evidence in support of the co-evolution between the
406 leaf cross section (quadrangular (Q) vs. flattened (F)) and seed scale arrangement
407 (closely (C) vs. loosely (L)) has not been observed. Trait combinations such as Q+C,
408 Q+L, F+C and F+L are found in 22, 2, 4 and 5 of the 33 species in *Picea,* respectively
409 (Farjón, 2001). Therefore, without providing additional details, a universal pattern of
410 ecological divergence cannot be predicted for the entire morphological tree of *Picea*.

411 **4.4 Divergence of morphology and phylogeny among spruce species is affected by**
412 **different selective pressures under parallel evolution**

413 Closely related species in a phylogenetic tree tend to be similar in appearance,
414 although this may not be so under parallel evolution (Hoekstra and Price, 2004; Baum
415 et al., 2005; Orr, 2005), and both cases can be observed in spruce. First, of the three
416 clades in the phylogenetic tree, most of the spruce species (19 of 22) in clade-1 and
417 clade-2 tended to have quadrangular leaves, whereas nearly half of the spruce species
418 (6 of 11) in clade-3 tended to have flattened leaves. In addition, two North American
419 species, *P. rubens* and *P. mariana*, are sister species in both constructed trees.
420 Accordingly, the morphological divergence and phylogenetic divergence of these

421 species are subject to the same selective pressures. Second, cases of parallel evolution
422 are quite obvious. For example, two Asian species, *P. purpurea* and *P. wilsonii*, are
423 sister species in the phylogenetic tree but are located in different sections of the
424 morphological tree; this scenario is also observed for another two North American
425 species, *P. glauca* and *P. engelmannii*. As a result, the morphological and
426 phylogenetic divergences for these species pairs are subject to different selective
427 pressures, providing a test of our second hypothesis. This finding suggests that the
428 divergence of morphology and phylogeny among the species in question may or may
429 not be subject to different selective pressures depending on the process of speciation.

430 **4.5 Significance and implications of the findings of this research in relation to**
431 **future climate change**

432 According to the 1997 UNPE standard climate zone classification (Middleton&
433 Thomas, 1997), 8 spruce species are in arid and semi-arid areas, 11 in dry sub-humid
434 areas, and 14 in humid areas. The scenario of global climate change predicts that there
435 would be severe and widespread droughts over land areas within the next 30-90 years,
436 resulting from decreased precipitation and/or increased evaporation, and the
437 significant increases in aridity already occur in many subtropical and adjacent humid
438 regions (Dai, 2012, Greve & Seneviratne, 2015). When overlapping the spruce
439 sampling points with the future Aridity Changes Map (data not shown), nearly all the
440 spruce species whose original distribution is in sub-humid and humid areas would be
441 subject to drought stress. Thus, our findings suggest that spruces with quadrangular
442 leaves and in clade-1 would be predicted to expand, while those with flattened leaves

443 and in clade-2 or clade-3 would be predicted to retreat. This should be taken into
444 account in the context of strategizing in response to future climate change.

445 **5 Summary and conclusions**

446 In summary, the influence of climate on the divergence of the morphology and
447 phylogeny of spruces is mediated by a number of biotic and abiotic factors, such as
448 geographical isolation, niche conservatism and ecological adaptation. A major finding
449 from this study is that temperature and precipitation parameters have tended to be the
450 main driving factors for the primary divergence of spruce phylogeny and morphology,
451 respectively. In addition, we observed an increasing magnitude in terms of ecological
452 divergence among sister groups from the basal nodes (older) to the terminal nodes
453 (younger) on the evolutionary time scales. Our hypotheses are therefore largely
454 verified by the findings of the present study. However, exceptions to the overall
455 pattern cannot be ignored. For example, although most spruce species with
456 quadrangular leaves tend to occur in drier habitats, Taiwan spruce (*P. morrisonicola*)
457 presents quadrangular leaves and is naturally distributed in subtropical areas with
458 abundant rainfall; thus, its present distribution is likely within a refugium from the
459 postglacial period (Tsukada, 1966; Xu et al., 1980). Further work that considers all of
460 the determinants is required to understand the forces driving ecological divergence
461 among spruce sister groups or species. In addition, our findings shed light on the
462 management issues with respect to spruce distribution under future climate change.

463 **6 Data availability**

464 The relevant data are within the paper and its Supporting Information files.

## 7 Author contributions

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

## 8 Supporting Information

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

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

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

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

## 9 Acknowledgements

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613

614 **Table 1.** Factor analysis showing the eigenvalues, variance percentages, cumulative
615 percentages and correlations of 19 climatic variables with the five components.
616 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 |
| **Temperature Annual Range (TAR)** | **-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** |

**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 phylogenetic tree and the morphological 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 (℃) | TAR (℃) | 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] |

622 **Figure legends:**

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

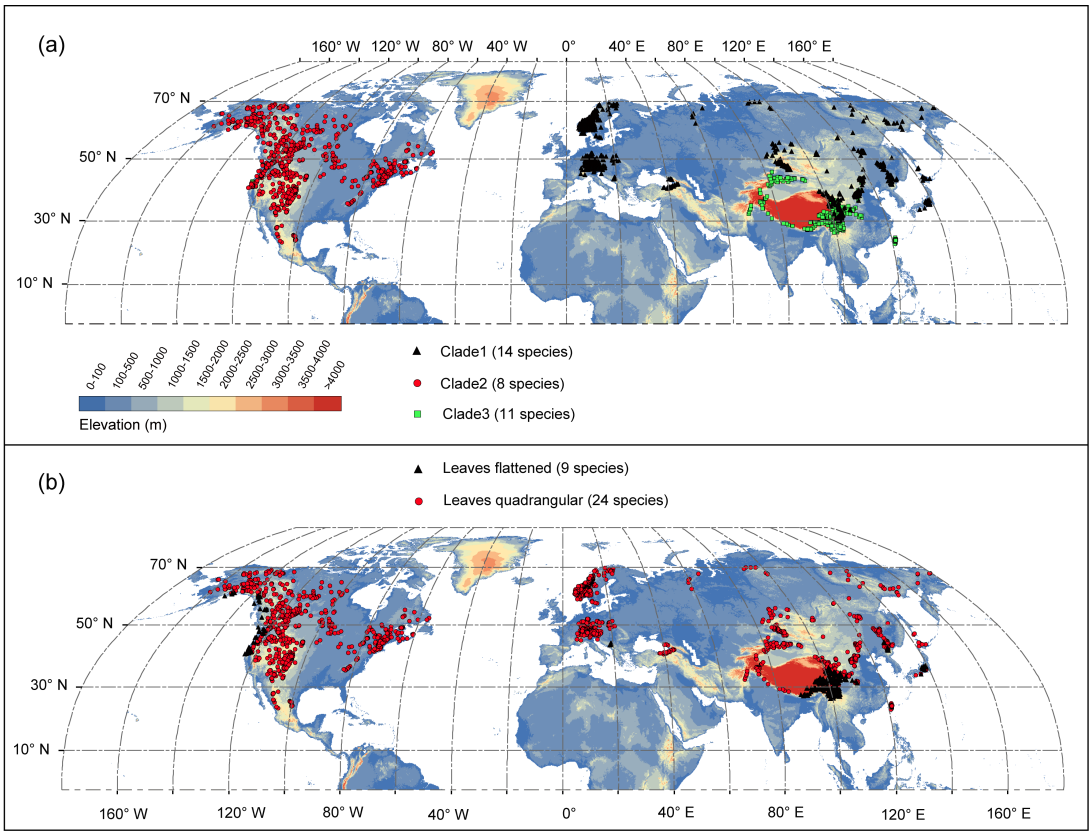
627 **Figure 2.** Divergence indices (scales range from 0-1) shown as histograms for
628 elevation and for the 8 climatic variables for each node of the phylogeny of *Picea*
629 worldwide. *Indicates a significant difference in ecological features after Bonferroni
630 correction ($P$<0.0016).

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

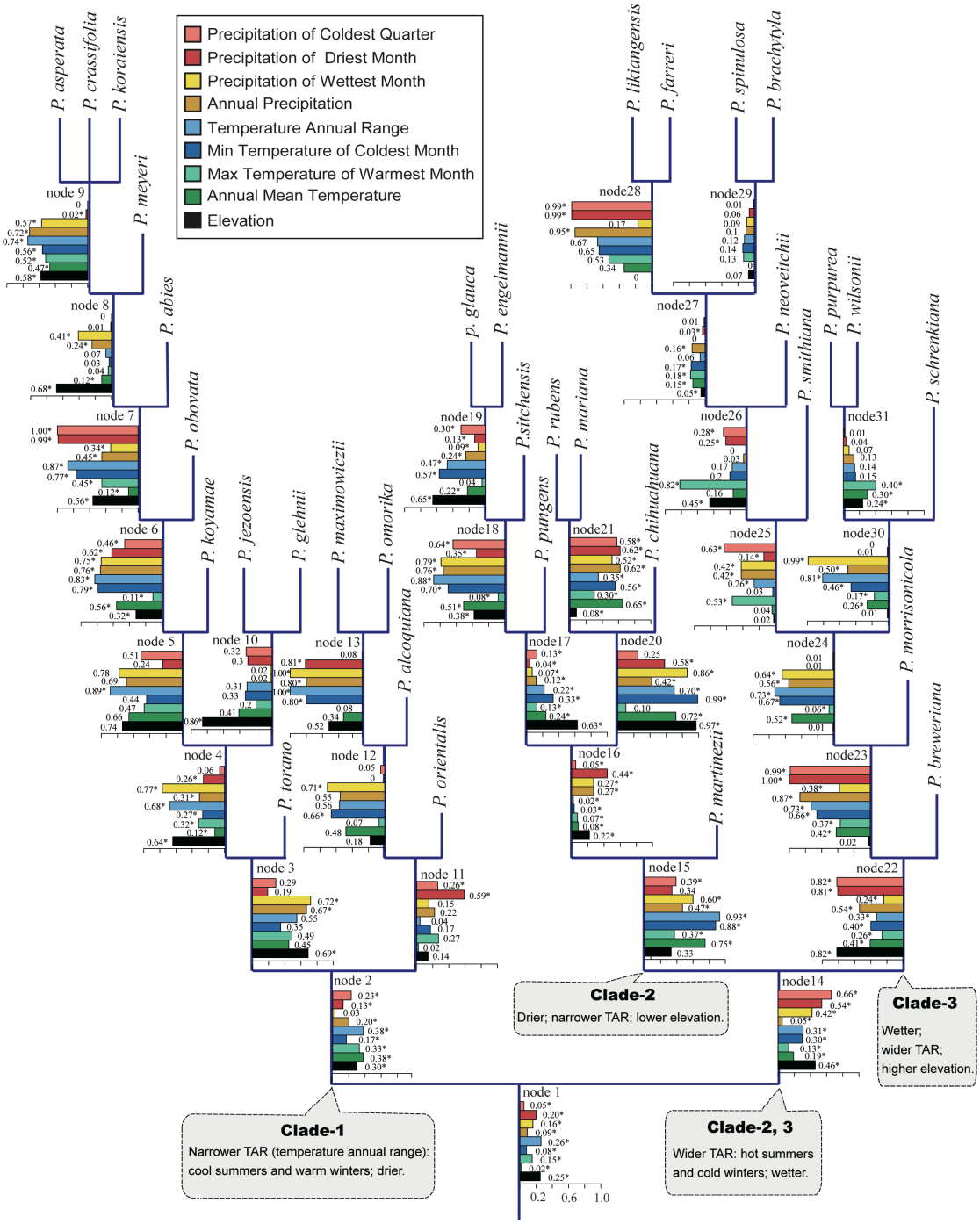
635 **Figure 4.** Box plots showing the index of divergence at each of the splitting levels in
636 the phylogenetic tree (a) and the morphological tree (b) of spruce species worldwide.
637 The central box in each box plot indicates the interquartile range and median, whereas
638 the whiskers show the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

640 Figure 1

641



642 Figure 2


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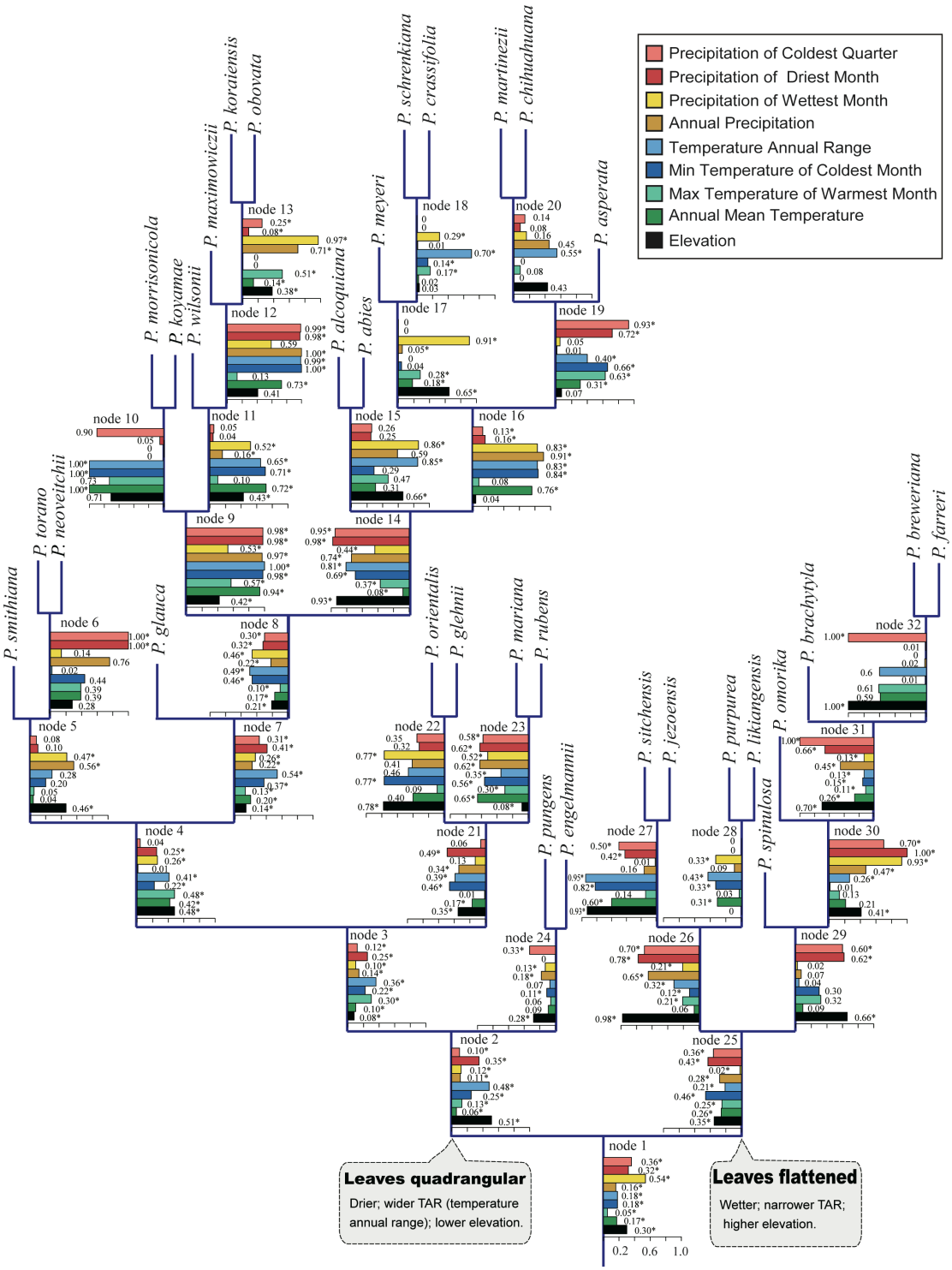
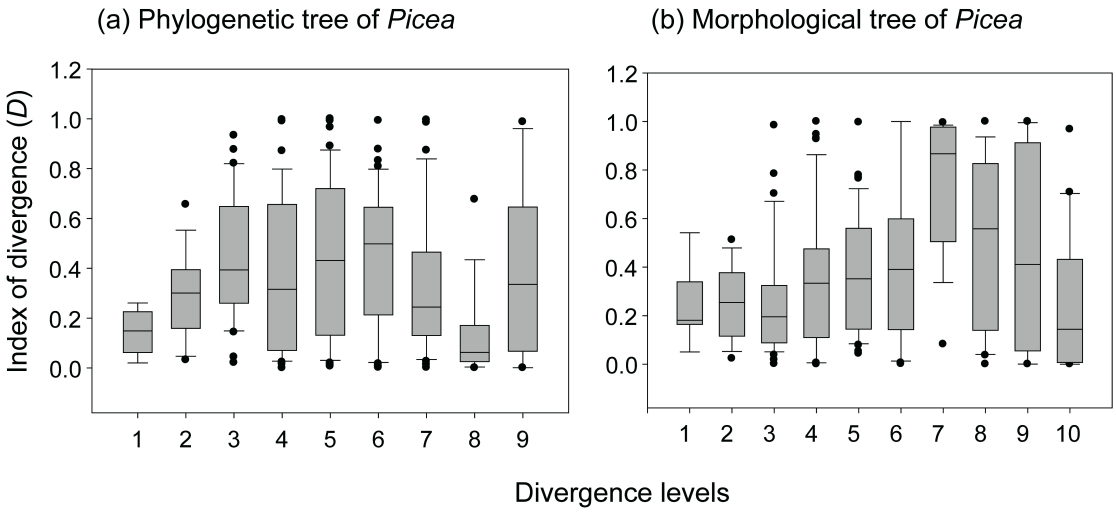
node21
node22
node23
node24
node25
node26
node27
node28
node29
node30
node31
Clade-1
Narrower TAR (temperature annual range): cool summers and warm winters; drier.
Clade-2
Drier; narrower TAR; lower elevation.
Clade-2, 3
Wider TAR: hot summers and cold winters; wetter.
Clade-3
Wetter; wider TAR; higher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

643 Figure 3

644



645 Figure 4


(a) Phylogenetic tree of *Picea*
(b) Morphological 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