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. 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 maximum *D* of the climatic variables was smaller in the basal nodes than in the remaining nodes. A major finding is that 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 in the next 30-90 years over land areas, our findings shed light on the prediction of spruce distribution under future climate change.

Keywords

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

1 Introduction

Environmental conditions play an important role 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

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

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

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

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

92 Evolutionary trees indicate historical relationships among organisms (Baum et al.,
93 2005). This “tree-thinking” approach has been used in almost all branches of biology
94 to detect relatedness among organisms (Baum and Offner, 2008) and to examine
95 ecological divergence between sister clades or species (Struwe et al., 2011). In this
96 study, tree-thinking methods were used to examine the ecological divergence among
97 spruce species worldwide by reconstructing a phylogenetic tree and a morphological
98 tree. A dataset of spruce species was compiled to test our hypothesis by answering the
99 following three questions: are the climatic variables extracted from the current spruce
100 locations correlated with the divergence among spruces? If so, is there a relationship
101 between the time since separation and the magnitude of ecological divergence? Lastly,
102 is the morphological and phylogenetic divergence among spruce species subject to
103 different selective pressures?

104 **2 Materials and Methods**

105 **2.1 Distribution data**

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

111 Thirty-four species are included in the genus *Picea* (Farjón, 2001). The global
112 spruce checklist used in this study was primarily based on Farjón (2001) but refined
113 according to the Flora of China (Fu et al., 1999). Specifically, because two species

114 distributed in western China according to Farjón (1990), *Picea retroflexa* and *P.*
115 *aurantiaca*, were treated as a synonym and a variety of *P. asperata,* respectively, in
116 the Flora of China. We followed the Chinese classification. Accordingly, the checklist
117 used for this study contained 33 spruce species.

118 Georeferenced data for the 33 spruce species was partially downloaded from the
119 Global Biodiversity Information Facility (GBIF), an international open data
120 infrastructure. Original data in the GBIF are derived from various sources, such as
121 natural history explorations (specimens or records) collected over the past 300 years,
122 current observations and automated monitoring programs (GBIF, 2015). We carefully
123 verified the original data downloaded from GBIF by excluding those data points with
124 geolocations outside of the natural distribution ranges (either horizontally or vertically
125 or both). As a result, 2397 point locations from the GBIF remained after the
126 verification, and they primarily represented spruce species in North America, Europe
127 and East Asia (Japan and Korea Peninsula). Additional data for the spruce species
128 from mainland China and Taiwan (991 locations for 16 species) were obtained from
129 geo-referenced herbarium collection records (490 sites) (Li et al., 2016) from the
130 herbarium of the Institute of Botany, Chinese Academy of Sciences; recent fieldwork
131 (370 sites, unpublished); and published sources (41 sites) (Tseng, 1991; Yang et al.,
132 2002). As a result, 3388 point locations for the 33 spruce species were available for
133 this analysis.

134 **2.2 Climatic variables**

135 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 data and morphological data using statistical methods to investigate

158 ecological vicariance in speciation. The SEEVA compares the differences between
159 each of the climatic variables for each node. A divergence index (*D*) with (0, 1)
160 scaling was calculated for each climatic factor at each node. *D*=0 indicates no
161 difference between sister clades or groups, whereas *D*=1 indicates a maximum
162 difference. Fisher's exact test (Fisher, 1958), which generally provides a better
163 *P*-value for tests with small sample sizes, was performed to determine the significance
164 of *D*. Because 31 and 32 independent tests were conducted for each of the climatic
165 variables, a *P*-value less than 0.0016 indicated a significant difference in the
166 ecological features for splits at a given node after performing a Bonferroni correction,
167 i.e., $\alpha=0.05/(31 \text{ or } 32)\approx0.0016$. Details on the calculations are available in Struwe et al.
168 (2011). The SEEVA software 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 mean value, extreme values of climate factors influence
174 spruce divergence. The selected climatic variables must have both higher divergence
175 indices for the first split on the phylogeny tree and the morphology tree, and relatively
176 higher loading on the five component axes. As a result, we mapped eight climate
177 factors in the histograms, including four temperature variables (annual mean
178 temperature (AMT), minimum temperature of the coldest month (MTCM), maximum
179 temperature of the warmest month (MTWM) and temperature annual range (TAR))

and four precipitation variables (annual precipitation (AP), precipitation of the wettest month (PWM), precipitation of the driest month (PDM) and precipitation of the coldest quarter (PCQ)). In addition, elevation as a spatial variable was also used to detect the ecological vicariance among sister groups because spruce is an elevation-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 the 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 variation 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 variation 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 variation 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

202 ***Picea***

203 Ecological divergence as indicated by the (0, 1) scaled index of *D* was significant
204 (P<0.0016, indicated as * where relevant) for 185 of the 279 comparisons at 31 nodes
205 in the phylogeny of *Picea* (see Table S1 in Appendix S1). The first split, which
206 yielded node-2 (clade-1) and node-14 (clade-2 and clade-3), was significant for all 9
207 environmental variables. The annual temperature range (*D*=0.26*) showed higher
208 divergence, and it was followed by elevation (*D*=0.25*) and precipitation of the driest
209 month (*D*=0.20*). The spruce species in clade-1 tended to occur in climates with a
210 lower annual temperature range and lower precipitation compared with the spruce
211 species in node-14. The divergence within node-14 and between node-15 (clade-2)
212 and node-22 (clade-3) was also significant for all 9 environmental variables. The
213 parameters precipitation of the coldest quarter, precipitation of the driest month and
214 precipitation of the wettest month had relatively high divergence (*D*=0.66* to 0.42*),
215 elevation exhibited substantial divergence (*D*=0.46*), whereas the temperature
216 variables showed lower divergence (*D*=0.13* to 0.31*). Compared with clade-3,
217 clade-2 occurred in climates with lower precipitation levels and a higher annual
218 temperature range. Node-2 represented a split within clade-1 (the Eurasian clade)
219 between a subclade at a higher elevational zone (in Caucasian area and Japan) with a
220 warmer and wetter climate and a subclade at a lower elevational zone (esp. in boreal
221 area) with a cold and dry climate. The elevation and temperature features showed
222 relatively higher divergence (*D*=0.17* to 0.38*) compared with the precipitation
223 variables (*D*=0.03* to 0.23*) (Figure 2, Table 2).

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

231 **3.3 Ecological divergence among sister groups or species in the morphology of**
232 ***Picea***

233 Ecological divergence was significant (P<0.0016, indicated as * where relevant) for
234 196 of the 288 comparisons at 32 nodes in the morphology tree of *Picea* (see Table S2
235 in Appendix S1). Of the 32 nodes, we focused on three splits that represent several
236 key morphological divergence in *Picea*. Specifically, the split of node-1 represents
237 divergence in the shape of the leaf cross section and the position of the stomatal line
238 on the leaf surface, whereas the split of node-2 and node-25 represents divergence in
239 the texture and seed scale arrangement. The remaining 29 splits, i.e., from node-3 to
240 node-24 and from node-26 to node-32, reflect divergence in the leaf size, seed cone
241 size, hairiness (pubescent vs. glabrous) and branchlet color, and these differences
242 were significant for approximately 65% of the comparisons (P<0.0016, Figure 3).

243 The first split of the morphology-defined topology tree (Figure 3) yielded node-2
244 (leaf quadrangular) and node-25 (leaf flattened) and was significant for all 9
245 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 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 Discussions

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

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

299 This finding is likely associated with the incompatibility of the time scale between
300 environmental data and ecological divergence because the environmental data
301 extracted from the current locations tended to be more relevant to the divergence of
302 younger nodes than older nodes. The low ecological divergence observed at the first
303 split in both trees, on the one hand, should be an indicator of high ecological niche
304 conservatism (Struwe et al., 2011); on the other hand, is likely related to the strong
305 species interactions that obscure the splits. However, the higher divergence observed
306 for the younger sister groups or sister species might suggest a strong selective effect
307 of climate on extant spruce species derived from more recent splits, and might also be
308 associated the fact that the fewer species of the sister groups within each node in the
309 more recent splits have relatively less complicated trait composition and hence weak
310 interactions. Our first hypothesis, i.e., an increasing magnitude in terms of ecological
311 divergence among sister groups from the basal nodes (older) to the terminal nodes

312 (younger) on the evolutionary time scales, is largely verified by the findings of our
313 study and those of a previous case study (Struwe et al., 2011).

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

328 In the mountainous regions, 1 km distance may cover an elevation interval of
329 hundreds of meters and therefore introduce several degrees of difference in
330 temperature, which may give rise to some uncertainties in the context of the detection
331 of some subtle variation such as within-species variation or among elevation-sensitive
332 species. In this case, as we can see from Fig.1 (a, b), instead of elevation gradient, the
333 geographical distribution of both the three phylogenetic clades and the morphological

groups (quadrangular leaves versus flattened leaves) is largely determined by horizontal gradients (latitude and longitude). Given this base, although the 1km-resolution of climate data we used in this study should be robust to interpret this large scale pattern, further works 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,

356 2000). In this process, old taxa became extinct or survived in refugia, whereas derived
357 taxa dispersed to new locations and underwent severe selection by climate (Hewitt,
358 2000; Hampe and Petit, 2010). Therefore, the formation of biogeographical plant
359 patterns is a product of interactions among these processes (Wolf et al., 2001).
360 In fact, considerable variations in geology and climate have occurred since spruce
361 originated in the late Oligocene. For example, the earliest spruce pollen fossil is from
362 the late Oligocene to the early Miocene in Asia and was found on the Tibetan Plateau
363 (Wu et al., 2007), and spruce pollen has frequently been found in sediments
364 originating from the late Pliocene and the Pleistocene in northern, eastern and
365 southwestern China (Xu et al., 1973; Xu et al., 1980; Shi, 1996) and Taiwan (Tsukada,
366 1966). A higher proportion of spruce pollen in specific sediments is generally
367 assumed to indicate a cold period, whereas a lower proportion of spruce pollen
368 indicates a warmer period (Xu et al., 1980). The proportion of spruce pollen in the
369 sediments varied substantially with the geological age of the sediments, suggesting
370 that spruce underwent frequent expansion and retreat during glacial cycles. In North
371 America, fossils of Brewer spruce (*P. breveriana*) have been observed in northeastern
372 Oregon in Miocene deposits that date to more than 15 Ma years ago; however, the
373 present distribution of Brewer spruce is different from the distribution of the fossil
374 locations, indicating that expansion and retreat occurred in the past (Waring et al.,
375 1975). It is difficult to match all the details of paleo-geological or paleo-climatic
376 events to the ecological divergence observed for specific nodes, although our findings
377 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 necessarily coincide with the phylogenetic relatedness of species (Went, 1971; Orr, 2005). Accordingly, spruce species with similar morphological characteristics but

400 distant phylogenetic relatedness may differ because of the tradeoff between niche
401 conservatism and ecological divergence. In addition, the composition of traits within a
402 species is also species specific. For example, the shape of the leaf cross section
403 co-varies along with the stomatal line position on the leaf surface, seed scale
404 arrangement and seed scale texture. However, evidence in support of the co-evolution
405 between the leaf cross section (quadrangular (Q) vs. flattened (F)) and seed scale
406 arrangement (closely (C) vs. loosely (L)) has not been observed. Trait combinations
407 such as Q+C, Q+L, F+C and F+L are found in 22, 2, 4 and 5 of the 33 species in
408 *Picea,* respectively (Farjón, 2001). Therefore, without providing additional details, a
409 universal pattern of ecological divergence cannot be predicted for the entire
410 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 to 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 shown that there
435 would have severe and widespread droughts in the next 30-90 years over land areas
436 resulting from either decreased precipitation and/or increased evaporation, and the
437 significant increases in aridity do occur in many subtropical and adjacent humid
438 regions (Dai, 2012, Greve & Seneviratne, 2015). When overlapping the spruce
439 sampling point to the future Aridity Changes Map (data not shown), nearly all the
440 spruce species whose original distribution in sub-humid and humid areas would
441 subject to drought stress. Given this, our findings suggest that spruces with
442 quadrangular leaves and in clade-1 are predicted to expand while those with flattened
443 leaves and in clade-2and clade-3 are predicted to retreat. This should be taken into

account in the context of strategy-making in response to future climate change.

5 Summary and conclusions

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

6 Data availability

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

7 Author contribution

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 conflict 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 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 (℃)	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]

Figure legends:

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

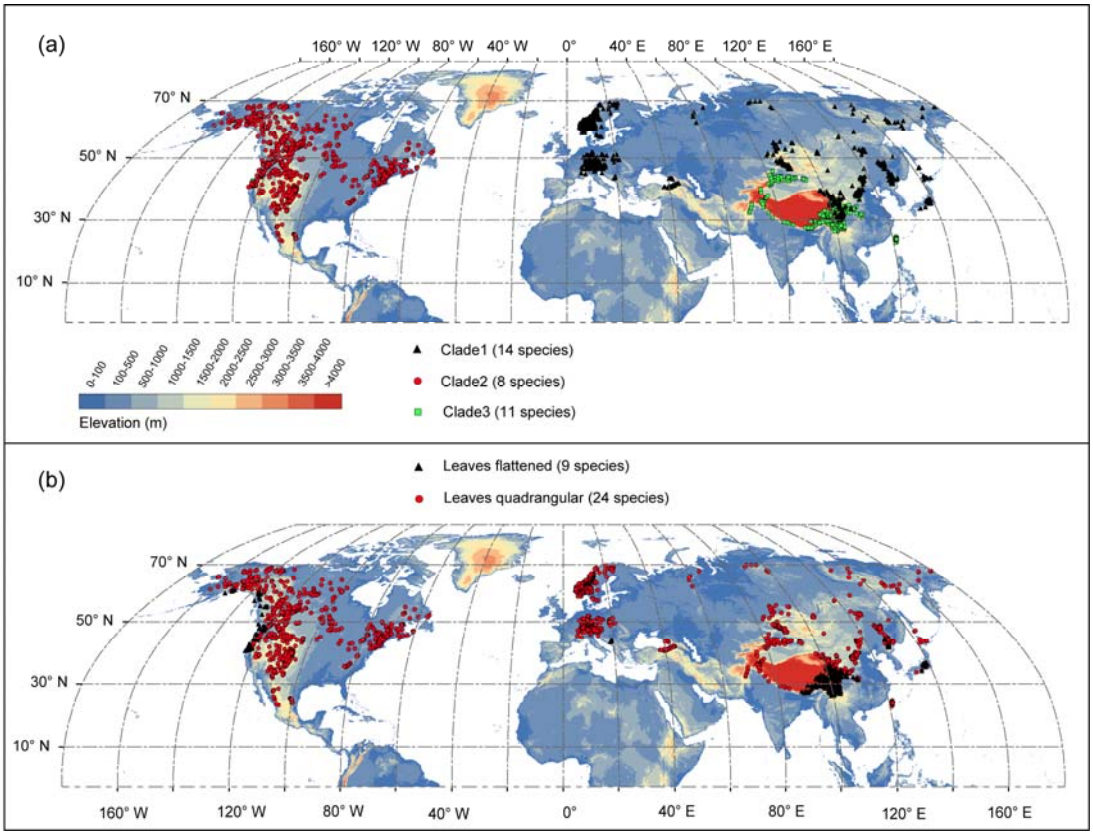
Figure 2. Divergence indices (scales range from 0-1) shown as histograms for elevation and for the 8 climatic variables for each node of the phylogeny of *Picea* worldwide. *Indicates a significant difference in ecological features after Bonferroni correction (P<0.0016).

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

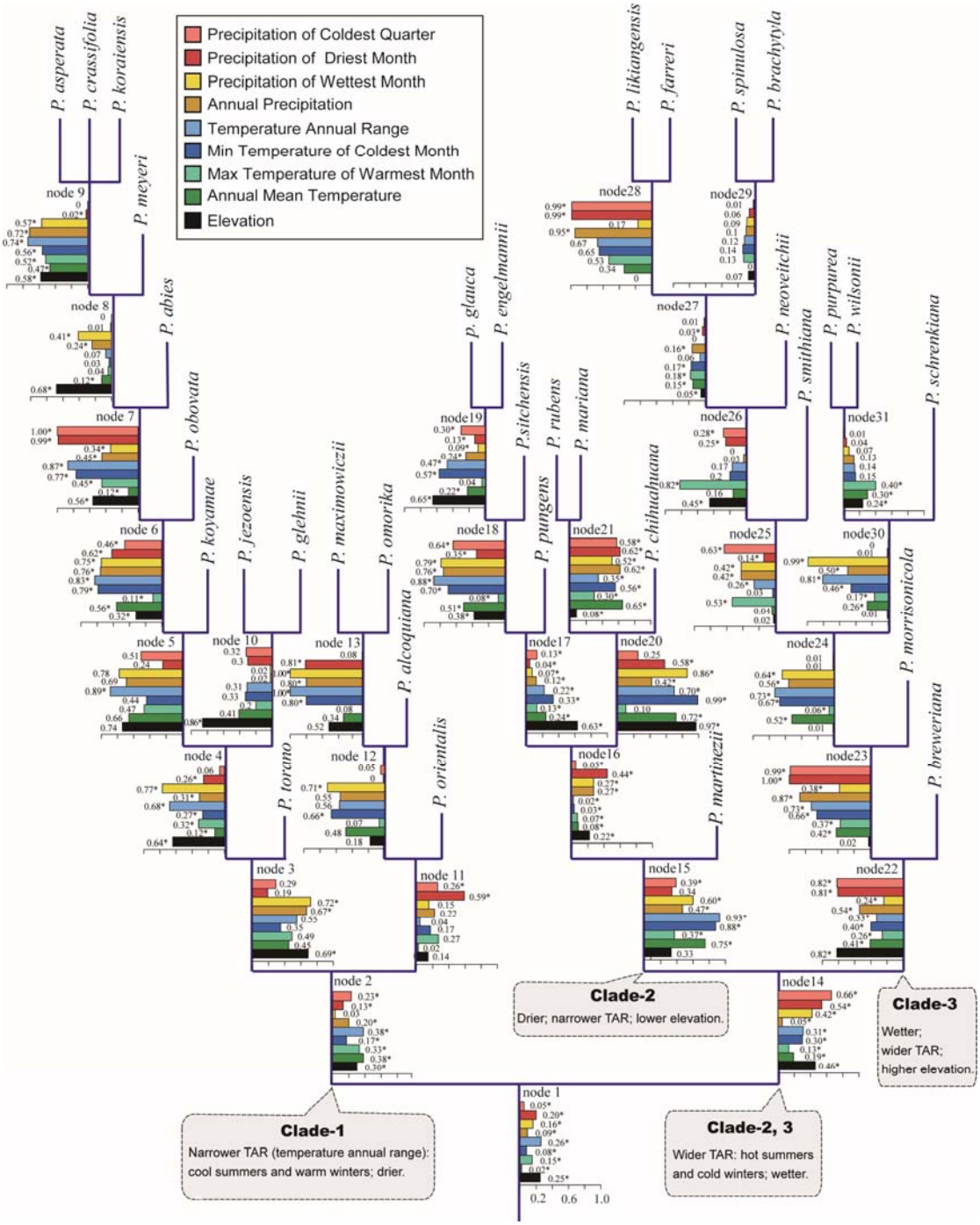
Figure 4. Box plots showing the index of divergence at each of the splitting levels in the phylogenetic tree (a) and the morphological tree (b) of spruce species worldwide. The central box in each box plot indicates the interquartile range and median, whereas the whiskers show the 10th and 90th percentiles. Mean values marked with different 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. 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. torano
P. orientalis
P. glauca
P. engelmannii
P.sitchensis
P. rubens
P. mariana
P. pungens
P. chihuahuana
P. martinezii
P. neoveitchii
P. smithiana
P. purpurea
P. wilsonii
P. schrenkiana
P. morrisonicola
P. breweriana
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
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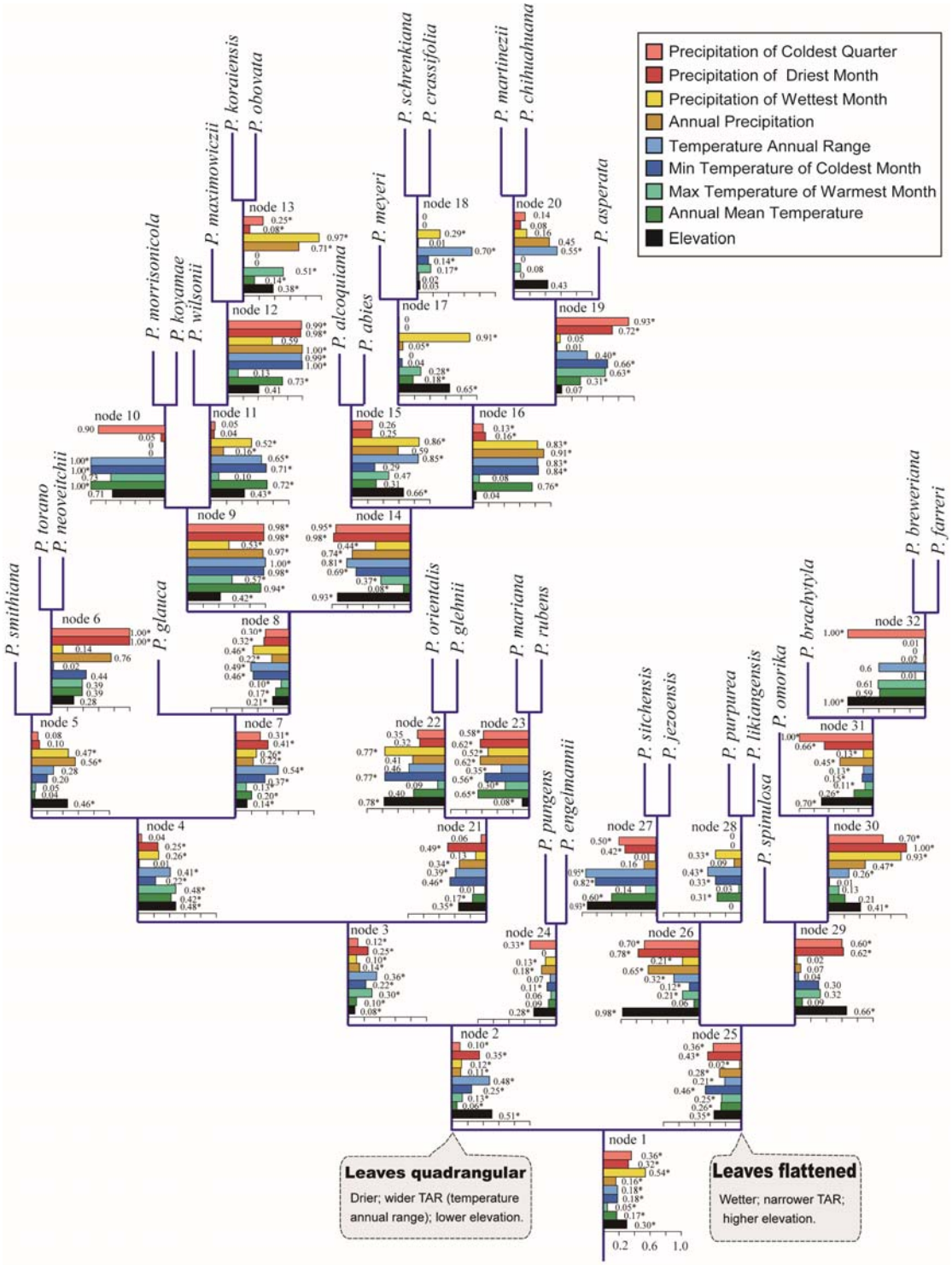
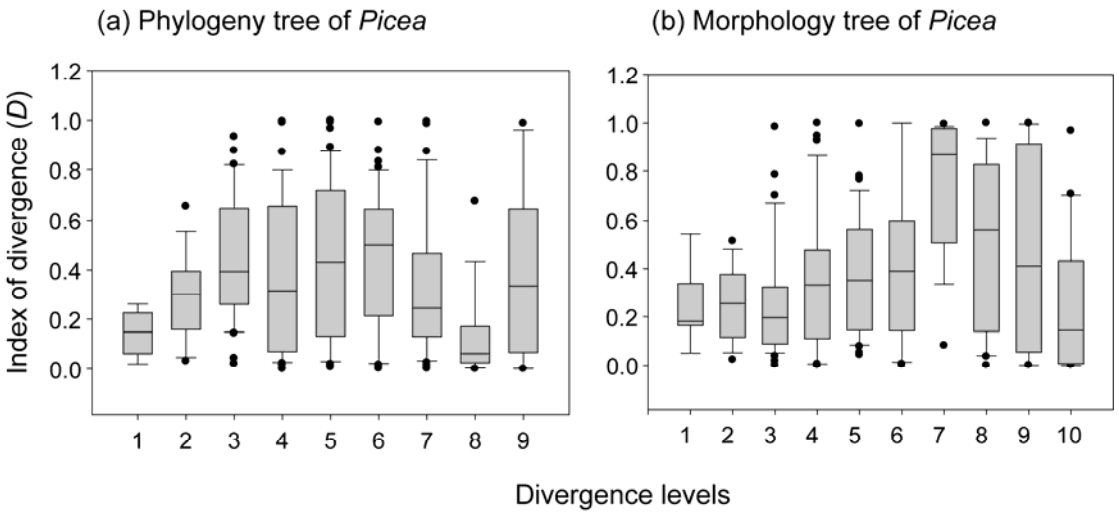
node30
node31
Clade-1
Narrower TAR (temperature annual range): cool summers and warm winters; drier.
Clade-2
Drier; narrower TAR; lower elevation.
Clade-2, 3
Wider TAR: hot summers and cold winters; wetter.
Clade-3
Wetter; wider TAR; higher elevation.
0.2
0.6
1.0

643 Figure 3

644



645 Figure 4


(a) 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