

Responses to Referee #1

Major Comment:

[Comment] I read the manuscript “Detecting climatically driven phylogenetic and morphological divergence among spruce species (*Picea*) worldwide” with delight. The manuscript explored the relationship between climate and the phylogenetic and morphological divergence of *Picea* species in the Northern Hemisphere, based on 3388 georeferenced distribution sites. Temperature and precipitation parameters were the main driving factors for the primary divergence of spruce phylogeny and morphology, respectively. The climatic data extracted from current spruce locations captured the ecological divergence among spruces. These results suggested that the primary divergence of morphology and phylogeny among the spruces tended to be driven by different selective pressures. The data and methods are appropriate for this study; the manuscript is well organized and presented. I found that the manuscript has a merit for publication in the journal *Biogeosciences*, pending on the authors can address my following concerns. My major concern is that if the climatic data used in this manuscript can represent the local climate of the distribution sites. The Worldclim dataset has been widely used in biogeographic studies. It can be used to surrogate the local climate in plain areas. However, it cannot represent the local climate in the mountainous regions because of the coarse resolution (about 1km). In the mountainous regions, 1 km distance may cover an elevational interval of hundreds of meters (and therefore introduce several degrees of difference in temperature). The authors need to discuss the caveat of using this dataset.

[Response] This is a good question. The coarse resolution (about 1km) of climate data from The Worldclim dataset would likely weaken the potential to interpret spruce distribution and divergence. Discussion of the caveat of using this dataset is absolutely needed and will be done when we get the chance to revise this MS. Thank you!

Nevertheless, we have the confidence that the climate data from The WorldClim dataset used in this study is suitable for interpreting the overall pattern, i.e., the first several splits that represent “the primary trigger” that led to the divergence of among spruce, which are the major findings of this study. As we can see from Fig.1 (a, b), instead of elevation gradient, the 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). Specifically, clade-1 is a Eurasian clade and clade-2 is a North American clade, while clade-3 is an Asian clade with only one North American species. As for as the morphological groups, spruces flattened leaves tend to occur in eastern Asia and the beach area of the northern America, while spruce with quadrangular leaves distribute in the rest part of the whole distribution range. Given this base, the 1km-resolution of climate data we used in this study should be robust to interpret this large scale pattern.

We confess this dataset may give rise to some uncertainties in the context of the detection of some subtle variation such as within-species variation or among elevation-sensitive species. In this case, although the splits at the terminal nodes are between species, that is to say that we don't have any within-species variation, the caveat with respect to the dataset must be discussed. 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.

Specific points:

[Comment 1] Line 66: “Nearly 34 species” should be “Thirty-four species”

[Response] We will make this change as suggested.

[Comment 2] Line 83: “niche conservatism” is not a process, but a pattern (result of the processes)

[Response] Agree! We will change the “process” to “pattern” in the revised manuscript.

[Comment 3] Line 130: “Between 34 and 35 species” is conflict to the “34 species” (line 66) Line 132 (and other areas): “flora of China” should be “Flora of China”

[Response] Thank you. We will make this change as suggested.

[Comment 4] Line 145, 148, 152: delete “approximately”

[Response] Thank you. We will make this change as suggested.

[Comment 5] Line 159-166: set abbreviations for the climatic variables (and use the abbreviations afterwards)

[Response] Thank you. We will make this change as suggested.

[Comment 6] Line 216-224: move to the “Materials and Methods” section

Reference

Harris I, Jones PD, Osborn TJ, Lister DH (2014) Updated high-resolution grids of monthly climatic observations - the CRU TS3.10 Dataset. *International Journal of Climatology*, **34**, 623-642.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965-1978.

Kriticos DJ, Webber BL, Leriche A, Ota N, Macadam I, Bathols J, Scott JK (2012) CliMond: global high-resolution historical and future scenario climate surfaces for bioclimatic modelling. *Methods in Ecology and Evolution*, **3**, 53-64.

Responses to Referee #2

Major Comment:

[Comment 1] Wang et al., analyzed the relations between current climate and ecological (phylogenetic and morphological) divergence among spruce species at a global scale. The topic is suitable for Biogeosciences, but I do not think it is suitable for this special issue “Ecosystem processes and functioning across current and future dryness gradients in arid and semi-arid lands”. The range of spruce (we could see in Figure 1) is not only limited in arid and semi-arid lands, but also covers a lot of other more wet regions. The main results of this paper are clear that phylogenetic and morphological divergence is driven by different climate variables, i.e., temperature for phylo and precipitation for morpho. But I have several questions/comments, which need carefully revised by the authors.

[Response] The major reason why we submitted this MS to the special issue “Ecosystem processes and functioning across current and future dryness gradients in arid and semi-arid lands” is that most spruce species are very important taxa in arid and semi-arid lands worldwide. Detecting climatically driven phylogenetic and morphological divergence among spruce species worldwide would deepen the understanding of ecosystem processes and functioning in arid and semi-arid lands.

To address this point, in this response, we extracted the Aridity Index (AI) for each point from the Global Aridity Index (Global-Aridity) and the Global Potential Evapo-Transpiration (Global-PET) Geospatial Database (<http://www.cgiar-csi.org/2010/04/134/>). According the 1997 UNPE standard (Middleton& Thomas, 1997) climate zone classification, 8 spruce species are in arid and Semi-Arid areas, 11 spruce species in Dry sub-humid areas, and 14 spruce species in humid areas. According to the scenario of global climate change, there would have severe and widespread droughts in the next 30-90 years over land areas resulting from either decreased precipitation and/or increased evaporation, and the significant increases in aridity do occur in many subtropical and adjacent humid regions [1, 2]. When overlapping the spruce sampling point to the future Aridity Changes Map (Fig. 1, 2 in this response), nearly all the spruce species whose original distribution in sub-humid and humid areas would subject to drought stress.

The Special issue “Ecosystem processes and functioning across current and future dryness gradients in arid and semi-arid lands” aims to provided platform for researches in plant species associations, plant distribution along environmental gradients, which is not only applicable for species distributed in arid and semi-arid areas, but also for the species subjected to aridity stress in future. Our findings would be helpful for management strategies and inform policy to climate change in future.

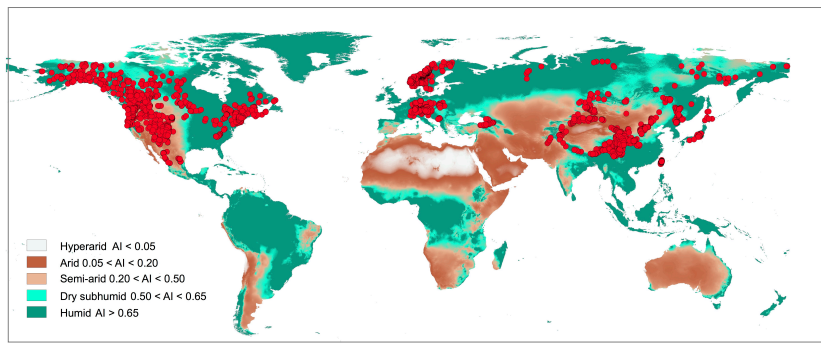


Fig. 1. The locations of sampling point in the study at different climate zone. The background image was the map of Global Aridity Index which obtained online (<http://www.cgiar-csi.org>) by the CGIAR-CSI with the support of the International Center for Tropical Agriculture (CIAT).

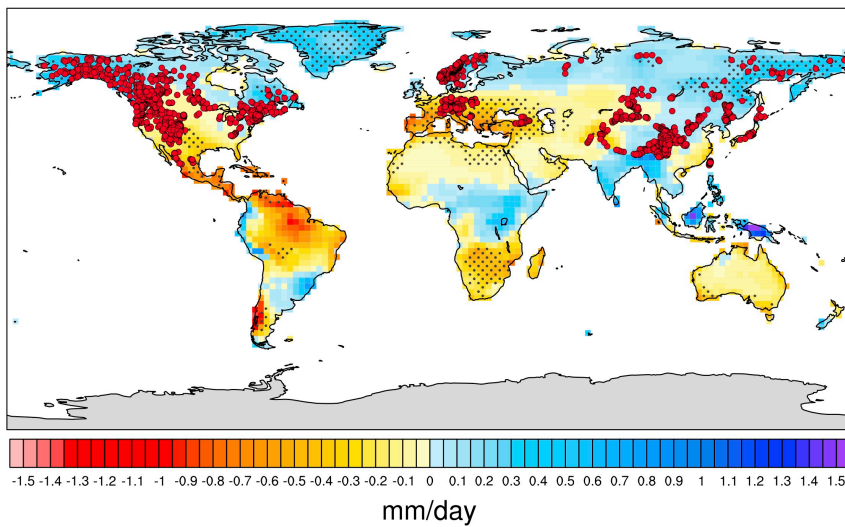


Fig. 2. The locations of sampling point in Aridity changes within the 21st century. The background image was the map of Changes in $P-Rn/\lambda$ comparing present-day (1980–2000) and future climate (2080–2100) following the RCP8.5 pathway.(P, precipitation; Rn, net radiation; λ , latent heat of vaporization).[1].

[Comment 2] Firstly, the abstract is not well written. There are too much information on methods and results. Usually, we first need some background, importance of the study, come up with the question, and what we do, what we found, and finally the importance of our findings. Furthermore, some information in the abstract are repeated, e.g., line 30-34 and line 40-41. Other minor problems in Abstract include: bioclimatic or cli- matic (should be consistent here and other parts of this paper); global and northern hemisphere are different; there are ecological divergence, phylo divergence,

morpho divergence and divergence, should be consistent or clearly defined; younger nodes are called remaining/terminal/end nodes/splits, should be consistent.

[Response] Thank you for your comments. We will make these changes as suggested.

[Comment 3] The use of current climate: The author also discussed this problem. As far as I know, there are not only current climate data in worldclim, but also paleoclimate. Although the paleoclimate there only date back to LGM, it still could reflect the climate situation for a longer time to some extent. I am wondering if this paleoclimate could be a better choice than current climate.

[Response] Due to expansion and retreat occurred in the past, the present distribution of spruce is different from the distribution of the fossil locations. Thus, paleoclimate data does not necessarily match the present distribution. The 3388 data points of the 33 spruce species were sampled on present locations. Current climate data should be more appropriate to interpret current distribution pattern of spruce species.

[Comment 4] The authors did PCA analysis and found that the first three axes could explain 75.67% of the variance, but the following analysis used 8 separate climate variables. I want to know why they choose these 8 variables, and not using the first three axes. Generally, 75% variance is OK. I guess the 4 temperature variables the author used are highly correlated, as well as the four precipitation variables. So I doubt the necessary to use so many climate variables. By the way, the numbers in the main text is not consistent with the numbers in table 1. For instance, the first axis explains 43.52% of the variance in Table 1, but 29.8% in the main text; other numbers are also wrong. In table 1, the first column, how did the authors choose the bold variables. I mean temperature seasonality is -0.928, and mean temperature of the coldest quarter is 0.946, higher than the AMT. The use of elevation is also questionable. The author at list did not discuss the effect of elevation in discussion.

[Response] We actually ran the SEEVA by taking all the 16 climate factors into account. To illustrate the results briefly and clearly, we need to reduce the redundant variables. We focused on how mean value, extreme values of climate factors influence spruce divergence. In addition, The climatic variables must have higher divergence indices for the first split on the phylogeny and morphology of *Picea*, and relatively higher loading on the five component axes. As a result, we mapped eight climate factors in the histograms on the phylogeny and morphology tree. Take an example, Min Temperature of Coldest Month and Mean Temperature of Coldest Quarter both have high loading on axis-1 of PCA: 0.931 vs. 0.946, but the former has higher divergence indices than the latter (0.0764 vs. 0.05524 in the phylogeny and 0.18 vs. 0.08 in the morphology). We therefore illustrated the results of the former variable.

Table-1 showed the eigenvalues, variance percentages, cumulative percentages and correlations of 19 bioclimatic factors but the rotated percentages were shown in the text. We will revise this inconsistency. Thanks.

Spruce is elevation-sensitive. We selected elevation as a variable because it can demonstrate a direct view with respect to spruce divergence, which would be helpful to understand how topography influence spruce divergence.

Specific points:

[Comment 1]: The results do not need to be divided into 6 parts, I think the last 4 parts could be merged into 1.

[Response] Agree. We will reorganize the text. We think 3.3, 3.4 and 3.5 should be merged into one section.

[Comment 2]: Some logic in the text is not reasonable. For instance, in line 87, information before “thus” and after “thus”, I don’t think they are well connected; line 178-189, the sequence of these parts is mess, line 188-189 should move to the front of the introduction of the SEEVA. The come up with several hypotheses in the introduction also feel not well connected with the text there. Anyway, the authors need to carefully check this throughout in the text.

[Response] Thanks. We will check these during the revision stage.

[Comment 3] Line 148, mainland China and Taiwan?

[Response] Agree. “mainland China and Taiwan” should be more formal. We will check these.

[Comment 4] Line 158-166, I am wondering if it’s necessary to list all the climate variables here.

[Response] Agree. A full list of climate variables has been shown in Table 1. We will check these during the revision stage.

[Comment 5] Line 349-350, how did the authors conclude like that? 6. Cannot or could not?

[Response] This paragraph highlighted the exceptions observed for a few sister groups or species in the phylogenetic tree to the overall pattern. We explained these exceptions as a result of geographical isolation and the limitation of the selected climate parameters that do not adequately describe the climatic determinants of spruce distributions.

Reference

Budantsev LY (1994) *The Fossil Flora of the Paleogene Climatic Optimum in North Eastern Asia*,

Springer Berlin Heidelberg.

Dai A (2012) Increasing drought under global warming in observations and models. *Nature Climate*

Change, **3**, 52-58.

Farjón A (2001) *World Checklist and Bibliography of Conifers (Second edn.)* England, Cambridge

University Press.

Giesecke T (2004) *The Holocene Spread of Spruce in Scandinavia*.

Greve P, Seneviratne SI (2015) Assessment of future changes in water availability and aridity.

Geophys Res Lett, **42**, 5493-5499.

- Hang S (2002) Evolution of Arctic-Tertiary flora in Himalayan-Hengduan mountains. *Acta Botanica Yunnanica*, **24**, 671-688.
- Kullman L (1995) New and firm evidence for Mid-Holocene appearance of *Picea abies* in the Scandes Mountains, Sweden. *Journal of Ecology*, **83**, 439-447.
- Liu JQ, Gao TG, Chen ZD, Lu AM (2002) Molecular phylogeny and biogeography of the Qinghai-Tibet Plateau endemic *Nannoglottis* (Asteraceae). *Molecular Phylogenetics & Evolution*, **23**, 307-325.
- Ran JH, Wei XX, Wang XQ (2006) Molecular phylogeny and biogeography of *Picea* (Pinaceae): implications for phylogeographical studies using cytoplasmic haplotypes. *Mol Phylogenet Evol*, **41**, 405-419.
- Spribille T, Chytrý M (2002) Vegetation surveys in the circumboreal coniferous forests: A review. *Folia Geobotanica*, **37**, 365-382.
- Struwe L, Smouse PE, Heiberg E, Haag S, Lathrop RG (2011) Spatial evolutionary and ecological vicariance analysis (SEEVA), a novel approach to biogeography and speciation research, with an example from Brazilian Gentianaceae. *Journal of Biogeography*, **38**, 1841-1854.
- Wu S, Yang YP, Fei Y (1995) On the flora of the alpine region in the Qinghai-Xizang (Tibet) Plateau, China. *Acta Botanica Yunnanica*.

A list of all relevant changes made in the manuscript

- We have simplified and specified abstract.
- We add a describe of climate zone of spruce species distributed in the method section and discussion section.
- We Added a statement of significance of the findings in relation to climate change to abstract, and added more discussions about significance and implications of the findings of this research in relation to future climate change.
- We have improved the English writing and sent the manuscript to a language service, made the paper more formal.

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

Guo-Hong Wang^{1*#}, He Li^{1#}, Hai-Wei Zhao^{1,2}, Wei-Kang Zhang¹

¹ State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

² University of the Chinese Academy of Sciences, Beijing 100049, China

*Address for correspondence: State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, No. 20 Nanxincun, Xiangshan, Beijing 100093, China

Tel: +86-010-6283-6585

Fax: +86-010-6259-0833

E-mail: ghwangaq@ibcas.ac.cn

#These authors contributed equally to this paper and should be regarded as co-first authors.

➤ **Running title:** Global ecological divergence of spruce

➤ **Number of words in the abstract:** 245

➤ **Number of words in the paper:** 6334

➤ **Number of references:** 49

➤ **Number of tables and figures:** 6

➤ **Supporting information:** 1

删除的内容: 332

删除的内容: 5996

删除的内容: 46

1 **Abstract**

2 This study aimed to elucidate the relationship between climate and the phylogenetic
3 and morphological divergence of spruces (*Picea*) worldwide. Climatic and
4 georeferenced data were collected from a total of 3388 sites distributed within the
5 global domain of spruce species. A phylogenetic tree and a morphological tree for the
6 global spruces were reconstructed based on DNA sequences and morphological
7 characteristics. The spatial evolutionary and ecological vicariance analysis (SEEVA)
8 method was used to detect the ecological divergence among spruces. A divergence
9 index (*D*) with (0, 1) scaling was calculated for each climatic factor at each node for
10 both trees. The results indicated that the annual mean values, extreme values and annual
11 range of the climatic variables were among the major determinants for spruce
12 divergence. The ecological divergence was significant ($P < 0.001$) for 185 of the 279
13 comparisons at 31 nodes in the phylogenetic tree, and for 196 of the 288 comparisons at
14 32 nodes in the morphological tree. Temperature parameters and precipitation
15 parameters tended to be the main driving factors for the primary divergence of spruce
16 phylogeny and morphology, respectively. Generally, the D_{max} of the climatic variables
17 was smaller in the basal nodes than in the remaining nodes. The primary divergence of
18 morphology and phylogeny among the investigated spruces tended to be driven by
19 different selective pressures. Given the future climate of severe and widespread drought
20 during the next 30-90 years over land areas, our findings shed light on the prediction of
21 spruce distributions under future climate change.

22 **Keywords**

删除的内容: R

删除的内容: .

删除的内容: A major finding is that t

删除的内容: scenario o

删除的内容: in

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

30 1 Introduction

31 Environmental conditions play an important role in speciation (Mayr, 1947; Darnell and
32 Dillon, 1970; Wiens, 2004; Givnish, 2010; Schemske, 2010). However, quantitative
33 investigations of environmental influences on the origin and divergence of species are
34 less common than expected, especially in plants (Givnish, 2010). For example, although
35 taxonomic and phylogenetic studies have explicitly addressed phylogenetic and
36 morphological divergence among spruces (Farjón, 1990; Sigurgeirsson and Szmidt,
37 1993; Fu et al., 1999; Ran et al., 2006; Li et al., 2010; Lockwood et al., 2013), ecological
38 differentiation among sister groups or species remains unknown. Ecological vicariance
39 differs from geographical vicariance (Wiley, 1988) and indicates the ecological
40 differentiation among sister groups or sister species within taxa, which provides
41 important information and ecological interpretations for the phylogenetic and
42 morphological divergence among taxa (Escudero et al., 2009; Struwe et al., 2011).

43 Spruce (*Picea* A. Dietrich) is an important component of boreal vegetation and
44 subalpine coniferous forests and has a wide geographical range that covers the Northern
45 Hemisphere and extends from the Eurasian continent to North America (Farjón, 2001;
46 Spribille and Chytry, 2002). Nearly thirty-four species are recognized in the genus
47 *Picea* worldwide (Farjón, 2001). Although taxonomic schemes of *Picea* based on
48 morphological characteristics differ slightly among authors, a consensus has been
49 reached for the criterion to determine the first several subdivisions (Liu, 1982; Farjón,
50 1990; Taylor, 1993; Fu et al., 1999). Accordingly, several sections within *Picea* have
51 been classified based on morphological similarity. For example, section *Picea* and

删除的内容: n

删除的内容: h

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

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

76 spruces occurred over a long time scale, with a range of approximately 28 Ma to 1.8
77 Ma from the basal nodes to the terminal nodes (Lockwood et al., 2013). We hypothesize
78 that there should be a relationship between the time since separation and the magnitude
79 of ecological divergence or niche conservatism. Specifically, we expect to observe an
80 increasing magnitude in terms of ecological divergence among sister groups from the
81 basal nodes (older) to the terminal nodes (younger) on the evolutionary time scales
82 because natural selection would favor species with high levels of ecological adaptation.

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

94 Evolutionary trees indicate historical relationships among organisms (Baum et al.,
95 2005). This “tree-thinking” approach has been used in almost all branches of biology
96 to detect relatedness among organisms (Baum and Offner, 2008) and to examine
97 ecological divergence between sister clades or species (Struwe et al., 2011). In this

删除的内容: necessarily

删除的内容: which indicateindicates

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

108 **2 Materials and Methods**

109 **2.1 Distribution data**

110 The sampling sites were selected from within the entire natural range of spruce species
111 in the Northern Hemisphere, extending over a wide geographical range (latitude: 22.8-
112 69.9°N; longitude: 53-165°W, 5-155°E; altitude: 103-4700 m a.s.l.) and exhibiting a
113 steep climatic gradient, varying from a cold-temperate zone to a subtropical zone
114 (Figure 1).

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

删除的内容: F

删除的内容: F

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

139 2.2 Climatic variables

140 A total of 19 climatic variables (Table 1) with a resolution of approximately 1 km² for
141 the 3388 locations were acquired and downloaded from WorldClim V. 1.4
142 (<http://www.worldclim.org>) (Hijmans et al., 2005). The values of each climate variable
143 at each site were extracted using the QGIS software package (<http://qgis.osgeo.org>),
144 and the final data were exported to an Excel worksheet for subsequent analysis.

145 2.3 Data analysis

删除的内容: -referenced

删除的内容: point

删除的内容: software

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

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

171 sample sizes, was performed to determine the significance of *D*. Because 31 and 32
172 independent tests were conducted for each of the climatic variables, a *P*-value less than
173 0.0016 indicated a significant difference in the ecological features for splits at a given
174 node after performing a Bonferroni correction, i.e., $\alpha=0.05/31$ or $32 \approx 0.0016$. Details on
175 the calculations are available in Struwe et al. (2011). The SEEVA software can be
176 downloaded from <http://seeva.heiberg.se>.

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

删除的内容: we ran the

删除的内容: all the

删除的内容: briefly and clearly

删除的内容: value,

删除的内容: must have both

删除的内容: ,

删除的内容: temperature

删除的内容: TAR

删除的内容: the

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

204 **3 Results**

205 **3.1 Variation in climatic variables**

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

217 **3.2 Ecological divergence among sister groups or species in the phylogeny of *Picea***

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

224 elevation ($D=0.25^*$) and precipitation of the driest month ($D=0.20^*$). The spruce
225 species in clade-1 tended to occur in climates with a lower annual temperature range
226 and lower precipitation than the spruce species in node-14. The divergence within node-
227 14 and between node-15 (clade-2) and node-22 (clade-3) was also significant for all 9
228 environmental variables. The parameters precipitation of the coldest quarter,
229 precipitation of the driest month and precipitation of the wettest month had relatively
230 high divergence ($D=0.66^*$ to 0.42^*), elevation exhibited substantial divergence
231 ($D=0.46^*$), whereas the temperature variables showed lower divergence ($D=0.13^*$ to
232 0.31^*). Compared with clade-3, clade-2 occurred in climates with lower precipitation
233 levels and a higher annual temperature range. Node-2 represented a split within clade-
234 1 (the Eurasian clade) between a subclade at a higher elevational zone (in Caucasian
235 area and Japan) with a warmer and wetter climate and a subclade at a lower elevational
236 zone (esp. in boreal area) with a cold and dry climate. The elevation and temperature
237 features showed relatively higher divergence ($D=0.17^*$ to 0.38^*) than the precipitation
238 variables ($D=0.03^*$ to 0.23^*) (Figure 2, Table 2).

删除的内容: compared with

删除的内容: compared with

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

248 **3.3 Ecological divergence among sister groups or species in the morphology of**

249 ***Picea***

250 Ecological divergence was significant ($P < 0.0016$) for 196 of the 288 comparisons at
251 32 nodes in the morphology tree of *Picea* (see Table S2 in Appendix S1). Of the 32
252 nodes, we focused on three splits that represent several key morphological divergence
253 in *Picea*. Specifically, the split of node-1 represents divergence in the shape of the leaf
254 cross section and the position of the stomatal line on the leaf surface, whereas the split
255 of node-2 and node-25 represents divergence in the texture and seed scale arrangement.
256 The remaining 29 splits, i.e., from node-3 to node-24 and from node-26 to node-32,
257 reflect divergence in the leaf size, seed cone size, hairiness (pubescent vs. glabrous) and
258 branchlet color, and these differences were significant for approximately 65% of the
259 comparisons (Figure 3).

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

删除的内容: compared with

删除的内容: that of

删除的内容: necessarily

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

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

295 **3.4 Magnitude of ecological divergence and time since separation**

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

305 **4 Discussion**

删除的内容: s

306 **4.1 Climatic data extracted from current spruce locations captures the ecological**
307 **divergence among spruces**

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

318 This finding is likely associated with the incompatibility of the time scale between
319 environmental data and ecological divergence because the environmental data extracted
320 from the current locations tended to be more relevant to the divergence of younger
321 nodes than older nodes. The low ecological divergence observed at the first split in both
322 trees should be an indicator of high ecological niche conservatism (Struwe et al., 2011);
323 however, it is likely related to the strong species interactions that obscure the splits. The
324 higher divergence observed for the younger sister groups or sister species might suggest
325 a strong selective effect of climate on extant spruce species derived from more recent
326 splits, and might also be associated the fact that the fewer species of the sister groups
327 within each node in the more recent splits have relatively less complicated trait
328 compositions and hence weak interactions. Our first hypothesis is largely verified by
329 the findings of our study and those of a previous case study (Struwe et al., 2011).

330 Exceptions to the above-mentioned trend were observed for a few sister groups or
331 species in the phylogenetic tree. Specifically, within clade-3, significant ecological
332 divergence was not detected for the split (node-29 in Figure 2) between *P. spinulosa*
333 and *P. brachytyla*. These two sister species are distributed in the Circum-Tibetan Plateau
334 and their geographical ranges are adjacent. *P. spinulosa* is distributed in the Mt.
335 Himalaya region and has a narrow range (S Xizang, Bhutan, Nepal and Sikkim),
336 whereas *P. brachytyla* is distributed in the SE to E Tibetan Plateau and has a wide range.
337 These differences suggest that instead of ecological divergence, geographical isolation
338 caused by the deep valleys and high mountain peaks in this area, which act as barriers
339 to gene flow between species, might have played a major role in the speciation of these

删除的内容: , on the one hand,

删除的内容: on the other hand,

删除的内容: However(Struwe et al., 2011). Thus, t

删除的内容: .

344 two sister species (Li et al., 2010). In addition, we cannot rule out the possibility that
 345 the selected climate parameters and their relatively coarse resolution (approximately 1
 346 km) do not adequately describe the climatic determinants of spruce distributions.
 347 In mountainous regions, a distance of 1 km may cover an elevation interval of
 348 hundreds of meters, introducing several degrees of difference in temperature, which
 349 may give rise to uncertainties in the detection of subtle variations, such as within-
 350 species variation, or among elevation-sensitive species. In this case, based on Fig. 1 (a,
 351 b), instead of the elevation gradient, the geographical distributions of the three
 352 phylogenetic clades and the morphological groups (quadangular leaves versus
 353 flattened leaves) are largely determined by horizontal gradients (latitude and longitude).
 354 Therefore, although the 1-km resolution climate data should be robust to interpret this
 355 large-scale pattern, further research that focus on the driving force underlying the
 356 variation of within-species or among elevation-sensitive species should use high-
 357 resolution climate data.

358 4.2 Temperature features tend to be the main driving factors of the primary 359 divergence of spruce phylogeny

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

- 删除的内容: s well as
- 删除的内容: about
- 删除的内容: -
- 删除的内容: the
- 删除的内容: distance
- 删除的内容: and therefore
- 删除的内容: e
- 删除的内容: some
- 删除的内容: context of the
- 删除的内容: some
- 删除的内容: as we can see from
- 删除的内容: Fig.1
- 删除的内容: both
- 删除的内容: is
- 删除的内容: Given this base
- 删除的内容: -
- 删除的内容: of
- 删除的内容: we used in this study
- 删除的内容:
- 删除的内容: work
- 删除的内容: s
- 删除的内容:

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

392 It is well established that the variations in the historical climate associated with the
393 advancement and retreat of ice sheets during the late Tertiary and Quaternary periods
394 played an important role in determining plant distributions (Walker, 1986; Hewitt,
395 2000). In this process, old taxa became extinct or survived in refugia, whereas derived
396 taxa dispersed to new locations and underwent severe selection by climate (Hewitt,
397 2000; Hampe and Petit, 2010). Therefore, the formation of biogeographical plant
398 patterns is a product of interactions among these processes (Wolf et al., 2001).

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

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

417 **4.3 Precipitation features tend to be the main driving factors of the primary** 418 **divergence of spruce morphology**

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

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

432 produce strong climatic signals; however, specific traits for different species do not
433 always exhibit the same response to specific environmental gradients (Wang et al., 2015;
434 Li et al., 2016). This inconsistency in response is likely due to parallel evolution
435 because morphological similarity among species does not always coincide with the
436 phylogenetic relatedness of species (Went, 1971; Orr, 2005). Accordingly, spruce
437 species with similar morphological characteristics but distant phylogenetic relatedness
438 may differ because of the tradeoff between niche conservatism and ecological
439 divergence. In addition, the composition of traits within a species is also species specific.
440 For example, the shape of the leaf cross section co-varies along with the stomatal line
441 position on the leaf surface, seed scale arrangement and seed scale texture. However,
442 evidence in support of the co-evolution between the leaf cross section (quadrangular
443 (Q) vs. flattened (F)) and seed scale arrangement (closely (C) vs. loosely (L)) has not
444 been observed. Trait combinations such as Q+C, Q+L, F+C and F+L are found in 22,
445 2, 4 and 5 of the 33 species in *Picea*, respectively (Farjón, 2001). Therefore, without
446 providing additional details, a universal pattern of ecological divergence cannot be
447 predicted for the entire morphological tree of *Picea*.

删除的内容: necessarily

删除的内容: necessarily

448 **4.4 Divergence of morphology and phylogeny among spruce species is affected by** 449 **different selective pressures under parallel evolution**

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

456 to have quadrangular leaves, whereas nearly half of the spruce species (6 of 11) in clade-
 457 3 tended to have flattened leaves. In addition, two North American species, *P. rubens*
 458 and *P. mariana*, are sister species in both constructed trees. Accordingly, the
 459 morphological divergence and phylogenetic divergence of these species are subject to
 460 the same selective pressures. Second, cases of parallel evolution are quite obvious. For
 461 example, two Asian species, *P. purpurea* and *P. wilsonii*, are sister species in the
 462 phylogenetic tree but are located in different sections of the morphological tree; this
 463 scenario is also observed for another two North American species, *P. glauca* and *P.*
 464 *engelmannii*. As a result, the morphological and phylogenetic divergences for these
 465 species pairs are subject to different selective pressures, which suggests that the
 466 divergence of morphology and phylogeny among the species in question may or may
 467 not be subject to different selective pressures depending on the process of speciation.

468 **4.5 Significance and implications of the findings of this research in relation to**
 469 **future climate change**

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

- 删除的内容: &
- 删除的内容: The scenario of global
- 删除的内容: there would have
- 删除的内容: in
- 删除的内容: over land areas resulting from
- 删除的内容: and/or
- 删除的内容: do
- 删除的内容: ,
- 删除的内容: &
- 删除的内容: A
- 删除的内容: C
- 删除的内容: s
- 删除的内容: M
- 删除的内容: of the

492 distribution encompasses sub-humid and humid areas is subject to drought stress.

删除的内容: in sss

493 Therefore, our findings suggest that spruces with quadrangular leaves and in clade-1

删除的内容: would

494 are expected to expand, whereas those with flattened leaves and in clade-2 and clade-3

删除的内容: Given this

495 are expected to retreat. This should be taken into account in the context of forming

删除的内容: predicted

496 strategies in response to future climate change.

删除的内容: while

删除的内容: predicted

删除的内容: y-making

497 5 Summary and conclusions

498 In summary, the influence of climate on the divergence of the morphology and

499 phylogeny of spruces is mediated by a number of biotic and abiotic factors, such as

500 geographical isolation, niche conservatism and ecological adaptation. A major finding

501 from this study is that temperature and precipitation parameters tend to be the main

删除的内容: ed

502 driving factors for the primary divergence of spruce phylogeny and morphology,

503 respectively. Our hypotheses are largely verified by the findings of the present study.

504 However, exceptions to the overall pattern cannot be ignored. For example, although

505 most spruce species with quadrangular leaves tend to occur in drier habitats, Taiwan

506 spruce (*P. morrisonicola*) presents quadrangular leaves and is naturally distributed in

507 subtropical areas with abundant rainfall; thus, its present distribution is likely within a

508 refugium from the postglacial period (Tsukada, 1966; Xu et al., 1980). Further work

509 that considers all determinants is required to understand the forces driving ecological

删除的内容: of the

510 divergence among spruce sister groups or species. In addition, our findings shed light

511 on the management issues with respect to spruce distributions under future climate

512 change.

513 6 Data availability

删除的内容: files

523 The relevant data are within the paper and its Supporting Information [section](#).

524 **7 Author contributions**

525 GHW conceived and designed the experiments. All authors performed the experiment.

526 GHW and HL analyzed and interpreted the data, and wrote the paper. The authors

527 declare they have no conflict of interest.

528 **Supporting Information**

删除的内容: 8

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

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

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

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

536 **9 Acknowledgements**

537 We thank Xing Bai, Lijiang Zhou, Miao Ma, Qinggui Wang, Hongchun Wang, Zhi Ma,

538 Ziyang Chen and Tiancai Chen for providing field assistance. This work was supported

539 by National Natural Science Foundation of China (41571045), the Chinese National

540 Basic Research Program (2014CB954201), and the National Natural Science

541 Foundation of China (30870398).

542 **References**

543 Baum, D. A., Smith, S. D., and Donovan, S. S.: Evolution. The tree-thinking challenge, *Science*,

544 310, 979-980, 10.1126/science.1117727, 2005.

547 Baum, D. A., and Offner, S.: Phylogenies & tree-thinking, *Am. Biol. Teach.*, 70, 222-229, 2008.

548 Dai, A.: Increasing drought under global warming in observations and models, *Nat. Clim. Change*,
549 3, 52-58, 2012.

550 Darnell, R. M., and Dillon, L. S.: Ecology and the origin of species. Introductory statement, *Am.*
551 *Zool.*, 10, 7-8, 1970.

552 Escudero, M., Valcarcel, V., Vargas, P., and Luceno, M.: Significance of ecological vicariance and
553 long-distance dispersal in the diversification of *Carex* sect. *Spirostachyae* (Cyperaceae),
554 *Am. J. Bot.*, 96, 2100-2114, 10.3732/ajb.0900134, 2009.

555 Farjón, A.: Pinaceae: Drawings and Descriptions of the Genera *Abies*, *Cedrus*, *Pseudolarix*,
556 *Keteleeria*, *Nothotsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix* and *Picea*, Cambridge
557 University Press, Konigstein, Germany, 1990.

558 Farjón, A.: World Checklist and Bibliography of Conifers, 2nd ed., Cambridge University Press,
559 Cambridge, UK, 2001.

560 Fisher, R. A.: Statistical Methods for Research Workers, 13th ed., Hafner Press, Hafner, NY, 1958.

561 Fu, L., Li, N., and Mill, R. R.: *Picea*, in: Flora of China, edited by: Wu, Z.-Y., and Raven, P. H.,
562 Science Press, Beijing, China, 25-32, 1999.

563 GBIF: Global Biodiversity Information Facility: <http://doi.org/10.15468/dl.mdqygv10>, last access:
564 Oct. 13, 2015.

565 Givnish, T. J.: Ecology of plant speciation, *Taxon*, 59, 1326-1366, 2010.

566 Greve, P. and Seneviratne, S. I.: Assessment of future changes in water availability and aridity,
567 *Geophys. Res. Lett.*, 42, 5493-5499, 2015.

568 Hampe, A., and Petit, R. J.: Cryptic forest refugia on the 'Roof of the World', *New Phytol.*, 185, 5-

删除的内容: Nature Climate

570 7, 10.2307/25609586, 2010.

571 Hewitt, G.: The genetic legacy of the Quaternary ice ages, *Nature*, 405, 907-913, 10.1038/35016000,
572 2000.

573 Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., and Jarvis, A.: Very high resolution
574 interpolated climate surfaces for global land areas, *Int. J. Climatol.*, 25, 1965-1978,
575 10.1002/joc.1276, 2005.

576 Hoekstra, H. E., and Price, T.: Parallel evolution is in the genes, *Science*, 303, 1779-1781, DOI
577 10.1126/science.1096413, 2004.

578 Klymiuk, A. A., and Stockey, R. A.: A lower Cretaceous (Valanginian) seed cone provides the
579 earliest fossil record for *Picea* (Pinaceae), *Am. J. Bot.*, 99, 1069-1082, 10.3732/ajb.1100568,
580 2012.

581 LePage, B. A.: New species of *Picea* A. Dietrich (Pinaceae) from the middle Eocene of Axel Heiberg
582 Island, Arctic Canada, *Bot. J. Linn. Soc.*, 135, 137-167, DOI 10.1111/j.1095-
583 8339.2001.tb01088.x, 2001.

584 Li, H., Wang, G., Zhang, Y., and Zhang, W.: Morphometric traits capture the climatically driven
585 species turnover of 10 spruce taxa across China, *Ecol. Evol.*, 6, 1203-1213,
586 10.1002/ece3.1971, 2016.

587 Li, Y., Stocks, M., Hemmila, S., Kallman, T., Zhu, H., Zhou, Y., Chen, J., Liu, J., and Lascoux, M.:
588 Demographic histories of four spruce (*Picea*) species of the Qinghai-Tibetan Plateau and
589 neighboring areas inferred from multiple nuclear loci, *Mol. Biol. Evol.*, 27, 1001-1014,
590 10.1093/molbev/msp301, 2010.

591 Liu, T.: A new proposal for the classification of the genus *Picea*, *Acta Phytotaxonomica et*

592 Geobotanica 33, 227-245, 1982.

593 Lockwood, J. D., Aleksic, J. M., Zou, J., Wang, J., Liu, J., and Renner, S. S.: A new phylogeny for
594 the genus *Picea* from plastid, mitochondrial, and nuclear sequences, *Mol. Phylogenet. Evol.*,
595 69, 717-727, 10.1016/j.ympev.2013.07.004, 2013.

596 Mayr, E.: Ecological factors in speciation, *Evolution*, 1, 263-288, Doi 10.2307/2405327, 1947.

597 ~~Middleton, N. and Thomas, D. S. G.: World atlas of desertification, *Geogr. J.*, 160, 210, 1997.~~

598 Orr, H. A.: The probability of parallel evolution, *Evolution*, 59, 216-220, 10.2307/3449009, 2005.

599 Peterson, A. T., Soberón, J., and Sanchez-Cordero, V. V.: Conservatism of ecological niches in
600 evolutionary time, *Science*, 285, 1265-1267, 1999.

601 Ran, J. H., Wei, X. X., and Wang, X. Q.: Molecular phylogeny and biogeography of *Picea* (Pinaceae):
602 Implications for phylogeographical studies using cytoplasmic haplotypes, *Mol. Phylogenet.*
603 *Evol.*, 41, 405-419, 10.1016/j.ympev.2006.05.039, 2006.

604 Schemske, D. W.: Adaptation and the origin of species, *Am. Nat.*, 176 Suppl 1, 4-25,
605 10.1086/657060, 2010.

606 Schluter, D., Clifford, E. A., Nemethy, M., and McKinnon, J. S.: Parallel evolution and inheritance
607 of quantitative traits, *Am. Nat.*, 163, 9-22, 10.1086/383621, 2004.

608 Shi, N.: Development of spruce and fir in north China during the Pliocene and the early Pleistocene:
609 palaeoclimatic implications, *Quaternary Sci.*, 16, 319-328, 1996.

610 Sigurgeirsson, A., and Szmidt, A. E.: Phylogenetic and biogeographic implications of chloroplast
611 DNA variation in *Picea*, *Nord. J. Bot.*, 13, 233-246, DOI 10.1111/j.1756-
612 1051.1993.tb00043.x, 1993.

613 Spribille, T., and Chytrý, M.: Vegetation surveys in the circumboreal coniferous forests: a review,

删除的内容: .

删除的内容: , and Programme, U. N. E.

删除的内容: The

删除的内容: aphical

删除的内容: Journal

619 Folia Geobot., 37, 365-382, Doi 10.1007/Bf02803253, 2002.

620 Struwe, L., Smouse, P. E., Heiberg, E., Haag, S., and Lathrop, R. G.: Spatial evolutionary and
621 ecological vicariance analysis (SEEVA), a novel approach to biogeography and speciation
622 research, with an example from Brazilian Gentianaceae, J. Biogeogr., 38, 1841-1854,
623 10.1111/j.1365-2699.2011.02532.x, 2011.

624 Taylor, R. J.: *Picea*, in: Flora of North America North of Mexico; volume 2: pteridophytes and
625 gymnosperms, edited by: Flora of North America Editorial Committee, Oxford University
626 Press, New York, 369-373, 1993.

627 Tseng, Y. S.: Studies on the vegetation ecology of Salihshianhsi watershed in central Taiwan II:
628 studies on the forest dynamics and population structure of Taiwan spruce forest, Master's
629 Thesis, Institute of Forestry, National Taiwan University, Taipei, 1991.

630 Tsukada, M.: Late pleistocene vegetation and climate in taiwan (formosa), Proc. Natl. Acad. Sci.
631 U.S.A., 55, 543-548, 10.2307/57266, 1966.

632 Walker, D.: Late Pleistocene early Holocene vegetational and climatic changes in Yunnan Province,
633 southwest China, J. Biogeogr., 13, 477-486, Doi 10.2307/2844968, 1986.

634 Wang, G. H., Liu, J. L., and Meng, T. T.: Leaf trait variation captures climate differences but differs
635 with species irrespective of functional group, J. Plant Ecol., 8, 61-69, 10.1093/jpe/rtu009,
636 2015.

637 Waring, R. H., Emmingham, W. H., and Running, S. W.: Environmental limits of an endemic spruce,
638 *Picea-breweriana*, Can. J. Bot., 53, 1599-1613, 1975.

639 Webb, C. O., Ackerly, D. D., McPeck, M. A., and Donoghue, M. J.: Phylogenies and community
640 ecology, Annu. Rev. Ecol. Syst., 33, 475-505, 10.1146/annurev.ecolsys.33.010802.150448,

删除的内容:

删除的内容: 369 – 373,

删除的内容: , NY

644 2002.

645 Went, F. W.: Parallel evolution, *Taxon*, 20, 197, 10.2307/1218877, 1971.

646 Wiens, J. J.: Speciation and ecology revisited: phylogenetic niche conservatism and the origin of
647 species, *Evolution*, 58, 193-197, 10.2307/3449309, 2004.

648 Wiens, J. J., and Graham, C. H.: Niche conservatism: integrating evolution, ecology, and
649 conservation biology, *Annu. Rev. Ecol. Evol. S.*, 36, 519-539,
650 10.1146/annurev.ecolsys.36.102803.095431, 2005.

651 Wiley, E. O.: Vicariance biogeography, *Annu. Rev. Ecol. Syst.*, 19, 513-542, 10.2307/2097164, 1988.

652 Wolf, P. G., Schneider, H., and Ranker, T. A.: Geographic distributions of homosporous ferns: does
653 dispersal obscure evidence of vicariance?, *J. Biogeogr.*, 28, 263-270, 10.2307/2656102,
654 2001.

655 Wu, Z., Wu, Z., Hu, D., Ye, P., and Zhou, C.: Geological evidences for the Tibetan Plateau uplifted
656 in late Oligocene, *Acta Geol. Sin.-Engl.*, 81, 577-587, 2007.

657 Xu, R., Tao, J. R., and Sun, X. J.: On the discovery of a *Quercus semicarpifolia* Bedin Mount Shisha
658 Pangma and its significance in botany and geology, *J. Integr. Plant Biol.*, 15, 103-119, 1973.

659 Xu, R., Kong, Z. C., and Du, N. Q.: Plant assemblages of *Picea* and *Abies* in the Pleistocene and
660 implications for Quaternary study *Quaternary Sci.*, 5, 48-56, 1980.

661 Yang, G. Z., Chen, Y. F., Zhao, W. C., Chen, X. Y., Wu, L. T., Zhao, G. R., and Lu, Z. F.: Plant
662 Resource Investigation in Nantzuhsien Creek Watershed in Yushan of National Park,
663 Yushan of National Park, Nantou, Taiwan, 2002.

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

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

- 删除的内容: Temperature
- 删除的内容: Annual
- 删除的内容: TAR
- 带格式表格

667

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

删除的内容:
 删除的内容:

删除的内容:
 删除的内容:

<i>N</i>	Elevation (m)	AMT (°C)	MTWM (°C)	MTCM (°C)	ΔTR (°C)	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 ^b
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 ^b	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

删除的内容: TAR

676

682 **Figure legends:**

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

删除的内容: .

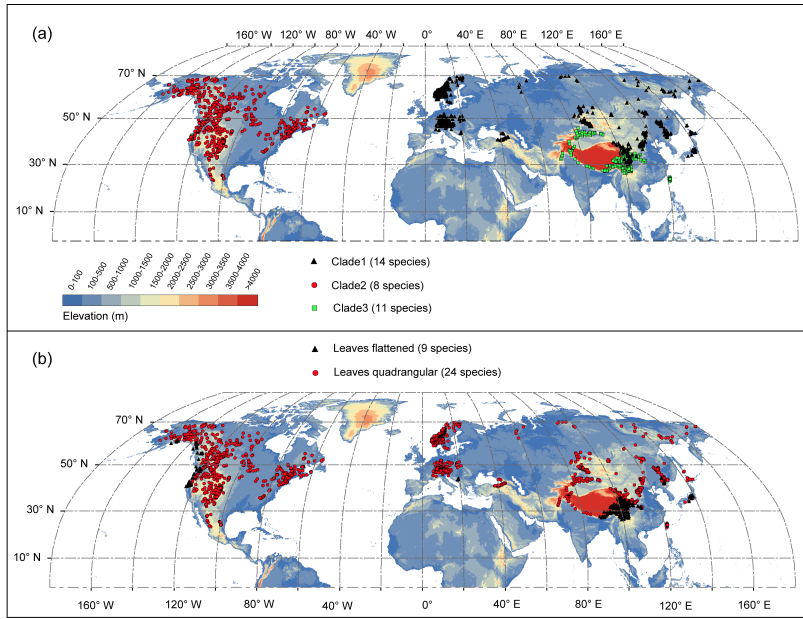
删除的内容: . respectively

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

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

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

701 Figure 1



702

批注 [E1]: Add a hyphen between 'Clade' .

批注 [E2]: Changed "temperature annual range" to "annual temperature range".

