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1 **New insights on stomata analysis of European conifers 65 years after the pioneering study of Werner**

2 **Trautmann (1953)**

3

4 *Walter Finsinger¹, Willy Tinner²*

5

6 ¹ ISEM, Univ Montpellier, CNRS, EPHE, IRD, 34095 Montpellier, France

7 ² Oeschger Centre for Climate Change Research and Institute of Plant Sciences, University of Bern, Bern, Switzerland

8

9 Corresponding author:

10 Walter Finsinger

11 Palaeoecology - Institute of Evolutionary Sciences (ISEM UMR 5554 CNRS)

12 University of Montpellier

13 2 place E. Bataillon - CC065 (Bat. 22)

14 F-34095 Montpellier Cedex 5

15 France

16

17 ORCID numbers:

18 Walter Finsinger: 0000-0002-8297-0574

19 Willy Tinner: 0000-0001-7352-0144

20

21 **Abstract**

1
2 22 Conifer-stomata analysis is an essential part of the palaeoecological toolbox because it allows determining the
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4 23 local presence of plant populations with a lower degree of uncertainty than pollen analysis. Although the
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6 24 European postglacial pool of conifer taxa is broad, stomata morphologies for only few taxa were investigated.
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8 25 Prior stomata morphology studies focused on taxa having wide distribution ranges in central and northern
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10 26 Europe, and stomata-morphologies for taxa occurring in southern European and Northern African mountain
11
12 27 regions have not been described, yet.

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14 28 Here, we present a qualitative assessment of stomata morphologies for 40 taxa from eight genera
15
16 29 (*Cupressus*, *Juniperus*, *Abies*, *Cedrus*, *Larix*, *Picea*, *Pinus*, and *Taxus*) that are present on the European
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18 30 continent and the southern borderlands of the Mediterranean Basin, thereby broadening substantially both the
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20 31 regional and taxonomical coverage of this now 65-years old technique. We found that visual identification of
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22 32 conifer stomata does not allow species-level identifications, supporting the notion of genus-specific stomata
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24 33 morphologies found in prior studies. For each genus we describe the stomata morphologies taking into account
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26 34 the varying shape of stomata features at different focusing levels. In addition, we provide stop-motion
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28 35 animations (publicly available at <http://dx.doi.org/10.6084/m9.figshare.7165261>) that may be useful tools for
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30 36 microscope analysts who want to acquaint oneself with conifer-stomata analysis.

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34 38 **Keywords:** stomata; conifers; vegetation history; Europe; reference-collection
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39 **Introduction**

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2 40 Natural ecosystems and biodiversity will be substantially affected by changes in climate and land-uses during the
3
4 41 ongoing century (Alcamo et al. 2007) and endemic plants in biodiversity hotspots appear particularly vulnerable
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6 42 to environmental changes (Malcolm et al. 2006). Organisms may be at risk if their habitat is fragmented or lost,
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8 43 and if they will be unable to rapidly adjust to new environmental conditions (Parmesan 2006). The risk of
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10 44 species loss may be particularly high for species having small or isolated populations such as those living on
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12 45 islands or in so-called ‘habitat islands’ on the continents (Whittaker and Fernandez-Palacios 2007).
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14 46 Among the species living in such ‘habitat islands’ are several conifer species. Due to their conservative traits
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16 47 (tough, long-lived needles; narrow tracheids enabling persistence in boreal regions), in Europe, Asia, and North
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18 48 America they are often restricted to high-latitudes, subalpine forests, arid regions, and on nutrient-poor or poorly
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20 49 drained soils, where they can escape or reduce the competitive effects of angiosperms (Coomes et al. 2005).
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22 50 The Mediterranean Basin hosts among the conifers a variety of endemic species (and subspecies) having
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24 51 currently small natural populations located in mountain regions (Fig. 1). Several of these species are deemed to
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26 52 be vulnerable, endangered, or critically endangered, i.e. species considered to be facing a high, very high, or
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28 53 extremely high risk of extinction in the wild (IUCN 2017). However, because these assessments rely only on
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30 54 recent population-size estimates, little is known concerning longer-term changes of range size and populations.
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32 55 Thus, for several of these endemic conifer species the historical legacies and the impacts of past environmental
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34 56 changes (climate, land-use, fire disturbance) are not well constrained over longer time spans.

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36 57 Past range shifts and population-size estimates are mostly inferred based on pollen records (e.g. Huntley
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38 58 and Birks 1983; Conedera et al. 2004; Giesecke et al. 2017; Brewer et al. 2017). However, particularly in
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40 59 mountain settings uncertainties related to pollen dispersal limit the possibility to infer the presence and history of
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42 60 parent trees around a study site (Herring et al. 2018). The palaeoecological toolbox offers, however, methods to
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44 61 prevail upon these limitations of pollen analysis: plant-macrofossil analysis (Birks and Birks 2000) and conifer-
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46 62 stomata analysis in pollen slides (Ammann et al. 2014) both can provide records at higher spatial resolution than
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48 63 pollen (Gervais and MacDonald 2001; Birks and Bjune 2010; Finsinger et al. 2017). However, conifer needles
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50 64 can be degraded after needle fall from parent trees and therefore be absent from plant-macrofossil records. By
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52 65 contrast, in such cases stomata can be found in pollen slides because their lignified components are more
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54 66 resistant than plant macrofossils to degradation in sedimentary archives, and chemicals used for pollen
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56 67 preparation do not degrade their lignified structures. Thus, pollen, plant macrofossils, and stomata provide

68 complementary evidences to unfold both altitudinal (Ammann et al. 2014; Vincze et al. 2017; Orbán et al. 2018),
69 latitudinal range shifts (Froyd 2005; Wagner et al. 2015), or range-size contractions (Tinner et al. 2013).

70 In his pioneering work, the European botanist Werner Trautmann (1953) investigated the morphologies
71 of stomata from central European trees and shrubs of six conifer genera (*Taxus baccata*, *Abies alba*, *Picea abies*,
72 *Larix decidua*, *Pinus* with 4 species, and *Juniperus* with 3 taxa; see Table 1) thereby laying the foundation of
73 sedimentary conifer-stomata analysis. He showed that stomata could be identified to genus level, an inference
74 later confirmed by Sweeney (2004), who focused on the six conifer species present in Scandinavia during
75 postglacial times (Table 1). Meanwhile, on the basis of Trautmann's pioneering work (Lang 1994) identification
76 keys of conifer stomata have been developed for species from North America (Hansen 1995; Lacourse et al.
77 2016), South America (Hansen et al. 2003), and China (Hu et al. 2016), thereby extending the spatial coverage
78 of conifer-stomata analysis to other continents and their floras.

79 While conifer-stomata analysis has increasingly been used and improved, the stomata morphologies of
80 southern European and Mediterranean endemic conifers have not been described, yet. Here, we portray and
81 compare the stomata morphologies for 40 conifer taxa that are native to the European continent and the southern
82 borderlands of the Mediterranean Basin (Table 1). The aim is to provide a first qualitative assessment of their
83 features that may be helpful to reconstruct past shifts in ranges and treelines in southern European and
84 Mediterranean mountain regions and might be extended later by quantitative analyses. Our main reason to stay
85 with qualitative analyses is that there is evidence that at continental scales some stomata measures (e.g. size)
86 may vary among populations (García Álvarez et al. 2009). Thus spatially highly resolved continental efforts
87 would be needed for quantitative analyses, which is out of scope for this study. To support our descriptions of
88 stomata morphologies, we devised a method to illustrate the stomata morphologies with stop-motion animations.
89 The animations offer stomata analysts the navigation across the different layers of the stomata structures, a
90 mandatory procedure to produce reliable stomata identifications (Trautmann 1953). Based on the microscope
91 analysis, we provide for each genus a qualitative description of features that we deem important to identify the
92 stomata to the lowest possible taxonomical level on the basis of qualitative criteria.

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95 **Materials & Methods**

96 *Collection of needles*

97 Conifer needles were collected from herbaria (the Herbarium Montpellier Université (MPU; Montpellier, France)
1 98 and the Royal Botanic Gardens Kew (KEW; London, UK)), and from living specimens growing at the Botanical
2
3 99 Gardens of the Universities of Montpellier (Jardin des Plantes; Montpellier, France), Vienna (Hortus Botanicus
4
5 100 Vindobonensis – HBV; Vienna, Austria), and Bern (BOGA; Bern, Switzerland), and from stands in Serbia and
6
7 101 Romania (see ESM_1 at (available at <http://dx.doi.org/10.6084/m9.figshare.7165261>). Species names written on
8
9 102 the nametags of collected specimens were crosschecked against the GBIF Backbone Taxonomy (GBIF
10
11 103 Secretariat 2017) and synonyms were replaced with the currently accepted species (or subspecies) names.
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15 16 105 *Laboratory treatment*

17
18 106 Needles of each specimen were first dried at 60°C overnight and thereafter cut with a razor blade along their
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20 107 major axis under a stereomicroscope to increase the likelihood that chemicals would digest the mesophyll and
21
22 108 detach both the cuticle from the underlying plant tissue and the stomata from the cuticle. Thereafter, needles of
23
24 109 each specimen were prepared using standard laboratory treatments used for pollen analysis (including hot KOH
25
26 110 10%, acetolysis, and a second hot KOH 10% treatment, but excluding HCl and HF treatments (following
27
28 111 MacDonald 2002), embedded in glycerine jelly, and mounted on slides. The number of needles prepared for
29
30 112 each specimen varies depending on the abundance of the material that was available.
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33 34 114 *Stop-motion animations*

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36 115 For each specimen, we took images of flat-lying stomata at 630x magnification (Leica HI PLAN 63x objective
37
38 116 with Koehler illumination set up) with a transmitted-light microscope (Leica DM 1000) equipped with a Leica
39
40 117 ICC50 HD 3.1 megapixels camera. Images were taken with an open aperture diaphragm to ensure high
41
42 118 resolution, small contrast, and small depth of view. At least 20 images were taken with the freeware Leica
43
44 119 Acquire v.3.4.1 software at increasing focussing depths. Each image features a scale bar (0.05 mm long) that
45
46 120 gives an estimate of stomata size. The images were z-stacked with the ImageJ v 1.50 software (Schneider et al.
47
48 121 2012), which provided stop-motion animations that may help the analyst to follow the stomata identification
49
50 122 procedure.
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53 54 124 *Terminology used*

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56 125 Although the terminology used to describe conifer-stomata morphology was extensively described in previous
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58 126 publications (e.g. Trautmann 1953; Hansen 1995; MacDonald 2002), we briefly summarise the main
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127 components of the stomata structure seen in equatorial view to provide the required vocabulary for stomata
128 identification (Figure 2).

129 Conifer stomata of species analysed in this study comprise two kidney-shaped guard cells joined
130 together at their ends, leaving an empty space between them (the stoma opening). The most distinctive guard cell
131 feature is the presence of unevenly thickened cell walls (Evert 2006). The cell-wall thickenings formed at the
132 junctions (the stems) extend from the stoma opening to the poles, which often terminate with polar hooks. The
133 cell walls facing towards the outer side of the needle (the ‘upper lamellae’) are more lignified than the cell walls
134 facing towards the inner side of the needle (the ‘lower lamellae’). The lignified cell wall bordering the stoma
135 opening (the ‘medial lamella’) is often thickened. Sometimes the upper and lower lamellae do not stay attached
136 together. Thus, it can happen that in pollen slides the stems are attached either to the lower lamellae or to the
137 upper lamellae.

140 **Results**

142 **Cupressaceae**

143 Genera: *Cupressus*, *Juniperus*

144 Species analysed: *Cupressus sempervirens* L., *Cupressus dupreziana* var. *atlantica* (Gaussen) Silba, *Cupressus*
145 *dupreziana* A. Camus, *Juniperus communis* L., *Juniperus communis* var. *saxatilis* Pall., *Juniperus drupacea*
146 Labill., *Juniperus oxycedrus* L., *Juniperus phoenicea* L., *Juniperus sabina* L., *Juniperus thurifera* L.

147 Stomata-type: *Cupressus*-type

148 The stomata from these taxa (Fig. 3) are characterised by relatively thick stems with arrow-tail-shaped poles.

149 Polar hooks are absent. The medial lamellae are generally thinner than the stems and distinct. When present, the
150 upper lamellae are distinct and almost as large as the lower lamellae. The pole-ward edges of both lamellae do
151 not reach to the poles, and the pole-ward medial border of the upper lamellae runs close to the stem.

155 **Pinaceae**

157 Genus: *Abies*

158 Species analysed: *Abies alba* Mill, *Abies borisii-regis* Mattf., *Abies cephalonica* Loudon, *Abies cilicica* (Antoine
159 & Kotschy) Carrière, *Abies nebrodensis* (Lojac.) Mattei, *Abies nordmanniana* Spach, *Abies nordmanniana* subsp.
160 *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen, *Abies numidica* de Lannoy ex Carrière, *Abies pinsapo*
161 Boiss., *Abies pinsapo* var *marocana* (Trab)

162 Stomata-type: *Abies*-type

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164 The stomata of the ten *Abies* taxa analysed (Fig. 4) have relatively thin stems with short and thin polar hooks,
165 which run perpendicular to the stem. The medial lamellae are almost as thin as the stem, sometimes indistinct
166 but generally more distinct than for *Larix* stomata. When present the upper lamellae are distinct, and are both
167 shorter and narrower than the lower lamellae. The pole-ward medial borders of the upper lamellae are not
168 parallel to the stem, giving the upper lamellae a butterfly-shaped form. In addition, the medial border of the
169 upper lamellae is generally slightly concave.

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172 Genus: *Cedrus*

173 Species analysed: *Cedrus atlantica* (Endl.) Manetti ex Carriere, *Cedrus libani* A. Rich.

174 Stomata-type: *Cedrus*-type

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176 The two *Cedrus* species have stomata characterised by relatively thick medial lamellae that delimit a circular
177 opening (Figs. 5a,c,e). The thickness of the stem is greatest on the upper side (Fig. 5b); towards the lower side of
178 the stoma the stem thins out near the stoma opening (Figs. 5a,e). Polar hooks are long and generally bent (Figs.
179 5a,b,c,e). The upper lamellae (Fig. 5d) are as wide as the lower lamellae, but are shorter. Similarly to *Abies*
180 stomata, the upper lamellae have a butterfly-shaped form (Figs. 5a,b,d).

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183 Genus: *Picea*

184 Species: *Picea abies* (L.) H. Karst, *Picea omorika* (Pancic) Purk, *Picea orientalis* (L.) Peterm.

185 Stomata-type: *Picea*-type

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187 The stomata of the three *Picea* species analysed are characterised by relatively thick and distinct medial lamellae
188 (Figs. 6a,c,f,g). The stem thickness varies along the stoma depth: on the lower side of the stoma the stem thins
189 out toward the stoma opening (Figs. 6a,c,f,g); on the upper side the stem has rather straight sides. The pole
190 hooks are short, bent, distinct, and are connected to the stem by a thin junction on the lower side of the stoma
191 (Fig. 6a,d,e). The upper lamellae are generally indistinct and are as wide and long as the lower lamellae. Their
192 pole-ward medial border runs parallel and close to the stem. Often the stomata bear a tooth at the polar edges of
193 the stoma opening.

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196 Genus: *Pinus*

197 Species: *Pinus brutia* Ten., *Pinus cembra* L., *Pinus halepensis* Mill., *Pinus heldreichii* H. Christ, *Pinus mugo*
198 Turra, *Pinus nigra* subsp. *laricio* (Poir.) Maire, *Pinus nigra* subsp. *salzmannii* (Dunal) Franco, *Pinus peuce*
199 Griseb., *Pinus pinaster* Aiton, *Pinus pinea* L., *Pinus sylvestris* L., *Pinus uncinata* Ram ex DC

200 Stomata-type: *Pinus*-type

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202 The stomata of these species bear thick and distinct medial lamellae and thick stems. The stems are notched
203 more or less sharply on the lower side of the stomata (Figs. 7c,d,e,f,g,h), and are curved on the upper side (Figs.
204 7b,c,d,f,i,j,n). Polar hooks are generally distinct, long, bent, and are directly attached to the stem. When present,
205 the upper lamellae are shorter and narrower than the lower lamellae.

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207 Genus: *Larix*

208 Species: *Larix decidua* Mill., *Larix sibirica* Ledeb.

209 Stomata-type: *Larix*-type

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211 The *Larix* stomata have relatively thin stems with long and thin polar hooks. The stem is slightly thinner on the
212 upper side (Fig. 8a) than on the lower side (Fig. 8b). The medial lamellae are very thin (Figs. 8a,c) and usually
213 much less distinct than in *Abies* stomata. When present the upper lamellae are indistinct, and are both shorter and
214 narrower than the lower lamellae (Figs. 8b,d). Similarly to *Abies* and *Cedrus*, the pole-ward medial borders of
215 the upper lamellae are not parallel to the stem. However, as opposed to *Abies*-type stomata, the medial border of
216 the upper lamellae is generally straight (Figs. 8b,d).

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2 218 **Taxaceae**

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4 219 Genus: *Taxus*

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6 220 Species: *Taxus baccata* L.

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8 221 Stomata-type: *Taxus*-type

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12 223 As opposed to stomata of the other genera, the stomatal complex of *Taxus baccata* includes subsidiary cells (Fig.

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14 224 8e) which are located above the upper lamellae. While these are a useful feature for the identification of *Taxus*

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16 225 *baccata* stomata, often stomata are found without the subsidiary cells (Fig. 8f). *Taxus baccata* stomata have

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18 226 relatively thick stems with short and thin polar hooks (Fig. 8f). The medial lamellae are very thin and usually

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20 227 indistinct. When present the upper lamellae are very distinct, and are both shorter and narrower than the lower

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22 228 lamellae, and have a butterfly-shaped form. Similarly to *Abies*-type stomata, the pole-ward medial border of the

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24 229 upper lamellae is generally curved.

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32 233 **Discussion**

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34 234 At the level of detail achieved in our study, the stomata of European species of the Cupressaceae family show

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36 235 similar features among genera. The stomata of the genera *Juniperus* and of *Cupressuss* seem to be

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38 236 indistinguishable with simple visual examination (Fig. 3). Moreover, the stomata of these taxa have

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40 237 morphological traits similar to those described by Trautmann (1953) for *Juniperus sabina* and can therefore be

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42 238 grouped within the *Juniperus*-type.

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44 239 By contrast, the stomata of European species of the Pinaceae family analysed in our study bear genus-

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46 240 specific features. This confirms earlier observations made by Trautmann (1953), who noted for instance that

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48 241 among species of *Pinus* sp. the stomata morphologies were not distinguishable with simple visual observations

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50 242 of stomata features. In keeping with this observation, Tonkov et al. (2018) recently grouped the stomata of *P.*

51
52 243 *mugo*, *P. sylvestris* and *P. peuce* as *Pinus* sp.. The notion of genus-specific stomata morphologies has been

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54 244 recently thoroughly tested and confirmed using statistical analysis of 10 geometrical measurements of

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56 245 morphological traits in a total of 315 stomata from 10 different genera of North American conifers (Lacourse et

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246 al. 2016). Similarly, Hu et al. (2016) and (Zhang et al. 2011) found on the basis of morphological traits that
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2 247 conifer species native to China could be identified to genus level.

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4 248 However, other investigations reported that measurements of geometrical features might be useful to
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6 249 separate stomata among and within genera. For instance, Yu (1997) found different width-to-length ratios for
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8 250 stomata of north American and Canadian *Thuja* and *Juniperus*, two genera belonging to the Cupressaceae family.
9
10 251 More recently García Álvarez et al. (2009, 2014) and Magyari et al. (2012) reported significant morphological
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12 252 differences among stomata from living populations of some European *Pinus* species on the basis of statistical
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14 253 analyses of morphological traits. Similarly, Hu et al. (2016) found species-specific differences of one
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16 254 morphological trait (the angle of attachment of the upper lamellae) among stomata of two *Abies* species native to
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18 255 China.

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20 256 In our study we refrained from numerical analyses of size measurements and morphological traits for
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22 257 two main reasons. Firstly, our main goal was to provide descriptions of stomata morphologies for the 40
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24 258 analysed taxa with a simple user-friendly tool that stomata analysts could refer to for stomata identification
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26 259 under standard transmitted-light microscopes at magnifications typically used for pollen analysis (e.g. x400 or
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28 260 x630). Classically, microscope analysts refer to dichotomous identification keys, static snapshot-type images of
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30 261 stomata, and schematic drawings to identify specimens. However, such tools only can illustrate a subset of the
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32 262 features of the overall morphology. Thus, for the reliable identification of stomata a good reference collection is
33
34 263 highly recommended (MacDonald 2002). The fact that some features change with focussing cell depth was
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36 264 already recognised by Trautmann (1953). For instance, he pointed out that the stem thickness and shape of *Pinus*
37
38 265 stomata varies along focus levels and therefore presented for each genus schematic drawings at two discrete
39
40 266 focusing depths when stomata cells are observed in equatorial view. In this respect our study went some steps
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42 267 further because the stop-motion animations (see ESM 2-40 at <http://dx.doi.org/10.6084/m9.figshare.7165261>)
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44 268 provide a higher number of images taken at different focussing depths, thereby allowing to virtually navigate
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46 269 across the different layers of the stomata in order to become acquainted with their 3-dimensional structure. The
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48 270 importance of the 3-dimensional structure of microfossils has also been acknowledged in prior studies. For
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50 271 instance, pollen atlases often illustrate a series of images of pollen grains taken at different focusing depths
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52 272 (Reille 1992; Beug 2004). While such stop-motion animations may not replace a reference collection, an
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54 273 animated sequence of images taken at different focus levels can be an effective tool to visualize the changing
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56 274 shapes of features that are visible under the microscope (Martin and Harvey 2017). Second, the morphological
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58 275 variability of stomata within species and the degree of morphological overlap among species may preclude such
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276 purely quantitative analysis (Lacourse et al. 2016). For instance, García Álvarez et al. (2009) reported for *P.*
1 277 *sylvestris* from Spain different stomata sizes (e.g. width, length) compared to the sizes measured by Sweeney
2 278 (2004) for Scandinavian specimens, indicating intraspecific variability of single morphological traits at
3 279 continental scales. We collected the majority of needles from specimens living in Botanical Gardens. Because
4 280 environmental conditions in Botanical Gardens represent (at best) only a fraction of the range of climatic
5 281 conditions and biotopes of wild populations across Europe or even Eurasia, size measurements might be
6 282 inaccurate. Conversely, because the overall stomata morphology is more stable at the species level (García
7 283 Álvarez et al. 2009), our qualitative descriptions should reflect the morphologies independently of intraspecific
8 284 variations. Moreover, our sampling size is rather small (only few needles from 1-2 specimens (Tab. 1)) and
9 285 larger sample sizes may be needed to define quantitatively the limits of taxonomic differentiation among
10 286 congeneric species (Lacourse et al. 2016).

21
22 287 In comparison to prior stomata-identification studies of European conifers (Trautmann 1953; Sweeney
23 288 2004) our study broadens substantially both, the regional and taxonomical coverage of this technique.
24 289 Trautmann (1953) focused only on central European and Alpine conifers, and Sweeney (2004) considered only
25 290 species native to Fennoscandia. Our assessment takes into account the larger variety of conifer species (and
26 291 subspecies) interspersed in southern European and northern African mountains. Overall, our descriptions largely
27 292 overlap with those of genus-level features that were detailed in prior studies, but some differences may be
28 293 highlighted. Trautmann (1953) noted that the pole-ward medial border of the upper lamellae is straight in *A. alba*
29 294 stomata and curved in *L. decidua* stomata. By contrast, we noted the opposite: a curved medial border in *Abies*-
30 295 type stomata (Fig. 4) and a straighter border in *Larix*-type stomata (Fig. 8). Our description of the pole-ward
31 296 medial border of the upper lamellae is however consistent with the description given by Sweeney (2004). Also,
32 297 for *Pinus* sp. stomata we noted the very frequent occurrence of notched stems, a feature that was illustrated in
33 298 Trautmann's schematic drawing but that received little attention in subsequent studies. Moreover, we describe
34 299 for the first time the stomata morphology of *Cedrus atlantica* and *Cedrus libani*, two species having small and
35 300 fragmented populations on the southern and eastern borderlands of the Mediterranean Basin. *Cedrus*-type
36 301 stomata seem absent from palaeoecological records within and around the current range of these two species
37 302 (Cheddadi et al. 2009; Hajar et al. 2010; Campbell et al. 2017). We cannot exclude the possibility that the
38 303 stomata of these species are less resistant to decay than the stomata of European conifers. However, *Cedrus*
39 304 stomata are resistant to chemical processing for pollen analyses (this study; Zhang et al. 2011). A plausible
40 305 explanation for the absence of *C. atlantica* and *C. libani* stomata in palaeoecological records is that their sparse
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306 tree cover may contribute to low abundance of needles being deposited. Moreover, the thin soil cover and
1 307 seasonal aridity in their native regions (Lamb et al. 1989; Hajar et al. 2010) may cause the decay of the needles
2 308 prior to their deposition in the lakes. The possibly faster decay in seasonally dry southern Europe may also
3 309 provide another plausible explanation for the absence of stomata in palaeoecological records from lowland sites
4 310 in southern Europe. This would fit with evidence for stomata findings in cooler and moister mountain regions
5 311 (e.g. Vescovi et al. 2010; Ammann et al. 2014; Tonkov et al. 2018) than in drier and warmer lowland sites.
6 312 While this may point out the limits of the method, we are confident that our descriptions be useful to take
7 313 advantage of this 65-years old technique that has great potential for inferring the local presence of conifer trees
8 314 and shrubs (Ammann et al. 2014).

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22 317 **Conclusions**

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24 318 In spite of the efforts made by palaeoecologists to improve the spatial coverage of pollen records in Europe
25 319 during the past decades, the long-term vegetation history and its relationship to past climate and land-use
26 320 changes and to changing disturbance regimes for a number of conifer species are still not well constrained.
27 321 Pollen production and dispersal may vary with habitat conditions (e.g. nutrients, competition, winds) so that
28 322 reconstructing the local presence of plant populations remains ambiguous if only based on presence of pollen
29 323 (false presence problem, Birks and Tinner 2016). Stomata are better indicators of local presence and together
30 324 with plant macrofossils may thus refine pollen-inferred reconstructions. Another limitation of pollen studies is
31 325 the low taxonomical resolution that limits identification to the genus (e.g. *Picea*, or *Abies*) or sometimes
32 326 subgenus level (e.g. *Pinus*; Moore et al. 1998; Beug 2004). While the descriptions of stomata morphologies
33 327 provided here do not allow species-level identifications, they support (albeit qualitatively) the notion of genus-
34 328 specific stomata morphologies of several conifer genera. Moreover, the novel stop-motion animations provided
35 329 by our work may be useful tools for palaeoecologists who want to acquaint oneself with stomata analysis. This
36 330 now 65-years old technique can substantially improve palaeoecological studies and its applications for
37 331 biodiversity conservation, and for assessments of climate change and anthropogenic impacts on vegetation.

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Data availability

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24 348 All stop-motion animations are publicly available at <http://dx.doi.org/10.6084/m9.figshare.7165261>.

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349 **Figure Captions**

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4 351 **Figure 1:** Maps illustrating the current distribution ranges of European conifer species and subspecies. Isolated
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6 352 populations are shown as point features (crosses, filled circles). The majority of distributions is based on
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8 353 shapefiles made available by Caudullo et al. (2017). However, the distribution of *Larix sibirica* is based on a
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10 354 shapefile developed by Malyshev (2008), the distributions of *Pinus peuce* and *Pinus heldreichii* are based on
11
12 355 shapefiles developed within the framework of the EUFORGEN project (Vendramin et al. 2008; Alexandrov and
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14 356 Andonovski 2011), and the distributions of *Juniperus drupacea*, *Cedrus atlantica*, and *Cedrus libani* are based
15
16 357 on Wazen and Fady (2016). Distributions of *Cupressus dupreziana* and *Picea orientalis* were manually digitised
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18 358 based on published maps (Kayacik 1955; Abdound et al. 2016). Maps were prepared within the R computing
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20 359 environment (R Core Team 2017).

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24 361 **Figure 2:** Sketched drawing of conifer stomata in equatorial view (modified after Trautmann, 1953). Shading
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26 362 indicates level of lignification.

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30 364 **Figure 3:** Snapshots of conifer stomata of (a) *Cupressus sempervirens* L., (b) *Cupressus dupreziana* A. Camus,
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32 365 (c) *Cupressus dupreziana* var. *atlantica* (Gaussen) Silba, (d) *Juniperus communis* L. (indistinct upper lamellae),
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34 366 and (e) *Juniperus communis* var. *saxatilis* Pall. (upper lamellae out of focus), (f) *Juniperus drupacea* Labill., (g)
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36 367 *Juniperus oxycedrus* L., (h) *Juniperus phoenicea* L., (i) *Juniperus sabina* L., (j) *Juniperus thurifera* L.. For the
37
38 368 stop-motion animations see ESM 2-11, respectively.

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42 370 **Figure 4:** Snapshots of conifer stomata (all with upper lamellae) of (a) *Abies alba* Mill, (g) *Abies borisii-regis*
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44 371 Mattf., (c) *Abies cephalonica* Loudon, (d) *Abies cilicica* (Antoine & Kotschy) Carrière, (e) *Abies nebrodensis*
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46 372 (Lojac.) Mattei, (f) *Abies nordmanniana* Spach, (g) *Abies nordmanniana* subsp. *equi-trojani* (Asch. & Sint. ex
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48 373 Boiss.) Coode & Cullen, (h) *Abies numidica* de Lannoy ex Carrière, and (i) *Abies pinsapo* Boiss. For the stop-
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50 374 motion animations see ESM 12-20, respectively.

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54 376 **Figure 5:** Snapshots of conifer stomata of (a-d) *Cedrus atlantica* (Endl.) Manetti ex Carrière, and (e) *Cedrus*
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56 377 *libani* A. Rich. For the stop-motion animations see ESM 21-22, respectively.

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379 **Figure 6:** Snapshots of conifer stomata of (a-d) *Picea abies* (L.) H. Karst, (e-f) *Picea omorika* (Pancic) Purk,
1 380 and (g-h) *Picea orientalis* (L.) Peterm. For the stop-motion animations see ESM 25-27, respectively.

3 381
5 382 **Figure 7:** Snapshots of conifer stomata of (a) *Pinus brutia* Ten., (b) *Pinus cembra* L., (c) *Pinus halepensis* Mill.,
7 383 (d) *Pinus heldreichii* H. Christ, (e) *Pinus mugo* Turra, (f) *Pinus nigra* subsp. *laricio* (Poir.) Maire, (g) *Pinus*
9 384 *nigra* subsp. *salzmannii* (Dunal) Franco, (h-i) *Pinus peuce* Griseb., (j) *Pinus pinaster* Aiton, (k) *Pinus pinea* L.,
11 385 (l) *Pinus sylvestris* L., (m-n) *Pinus uncinata* Ram ex DC. For the stop-motion animations see ESM 28-39,
13 386 respectively.

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17 388 **Figure 8:** Snapshots of conifer stomata of (a-b) *Larix sibirica* Ledeb, (c-d) *Larix decidua* Mill., and (e-f) *Taxus*
19 389 *baccata* L.. For the stop-motion animations see ESM 23, 24, and 40.

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60
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391 **References**

- 1
2 392 Abdound F, Griffiths A, Gardner MF (2016) *Cupressus dupreziana*, from the website, Threatened Conifers
3 393 of The World (<http://threatenedconifers.rbge.org.uk>). Last accessed 27/09/2018.
4
5 394 Alcamo J, Moreno JM, Nováky B, et al (2007) Europe. Climate Change 2007: Impacts, Adaptation and
6 395 Vulnerability. In: Contribution of Working Group II to the Fourth Assessment Report of the
7 396 Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, pp
8 397 541–580
9
10 398 Alexandrov AH, Andonovski V (2011) EUFORGEN Technical Guidelines for genetic conservation and use of
11 399 Macedonian pine (*Pinus peuce*). Bioversity International, Rome, Italy, p 6
12
13 400 Ammann B, Knaap WO van der, Lang G, et al (2014) The potential of stomata analysis in conifers to
14 401 estimate presence of conifer trees: examples from the Alps. *Veg Hist Archaeobotany* 23:249–264.
15 402 doi: 10.1007/s00334-014-0431-9
16
17 403 Beug H-J (2004) Leitfaden der Pollenbestimmung für Mitteleuropa und angrenzende Gebiete. Verlag Dr.
18 404 Friedrich Pfeil, München
19
20 405 Birks HH, Birks HJB (2000) Future uses of pollen analysis must include plant macrofossils. *J Biogeogr*
21 406 27:31–35
22
23 407 Birks HH, Bjune AE (2010) Can we detect a west Norwegian tree line from modern samples of plant
24 408 remains and pollen? Results from the DOORMAT project. *Veg Hist Archaeobotany* 19:325–340.
25 409 doi: 10.1007/s00334-010-0256-0
26
27 410 Birks HJB, Tinner W (2016) Past forests of Europe. In: San-Miguel-Ayanz J, de Rigo D, Caudullo G, et al.
28 411 (eds) European Atlas of Forest Tree Species. Publications Office of the European Union,
29 412 Luxembourg, pp 36–39
30
31 413 Brewer S, Giesecke T, Davis BAS, et al (2017) Late-glacial and Holocene European pollen data. *J Maps*
32 414 13:921–928. doi: 10.1080/17445647.2016.1197613
33
34 415 Campbell JFE, Fletcher WJ, Joannin S, et al (2017) Environmental Drivers of Holocene Forest Development
35 416 in the Middle Atlas, Morocco. *Front Ecol Evol* 5:. doi: 10.3389/fevo.2017.00113
36
37 417 Caudullo G, Welk E, San-Miguel-Ayanz J (2017) Chorological maps for the main European woody species.
38 418 Data Brief 12:662–666. doi: 10.1016/j.dib.2017.05.007
39
40 419 Cheddadi R, Fady B, Francois L, et al (2009) Putative glacial refugia of *Cedrus atlantica* deduced from
41 420 Quaternary pollen records and modern genetic diversity. *J Biogeogr* 36:1361–1371
42
43 421 Conedera M, Krebs P, Tinner W, et al (2004) The cultivation of *Castanea sativa* (Mill.) in Europe, from its
44 422 origin to its diffusion on a continental scale. *Veg Hist Archaeobotany* 13:161–179
45
46 423 Coomes DA, Allen RB, Bentley WA, et al (2005) The hare, the tortoise and the crocodile: the ecology of
47 424 angiosperm dominance, conifer persistence and fern filtering. *J Ecol* 93:918–935. doi:
48 425 10.1111/j.1365-2745.2005.01012.x
49
50 426 Evert RF (2006) Esau's Plant anatomy: meristems, cells, and tissues of the plant body: their structure,
51 427 function, and development, 3rd ed. Wiley-Interscience, Hoboken, N.J.
52
53 428 Finsinger W, Morales-Molino C, Gałka M, et al (2017) Holocene vegetation and fire dynamics at Crveni
54 429 Potok, a small mire in the Dinaric Alps (Tara National Park, Serbia). *Quat Sci Rev* 167:63–77. doi:
55 430 10.1016/j.quascirev.2017.04.032
56
57
58
59
60
61
62
63
64
65

- 431 Froyd CA (2005) Fossil stomata reveal early pine presence in Scotland: implications for postglacial
 1 432 colonization analyses. *Ecology* 86:579–586
 2
- 3 433 García Álvarez S, Morla Juaristi C, Paull R, García-Amorena I (2014) A taxonomic tool for identifying
 4 434 needle remains of south-western European *Pinus* species of the Late Quaternary. *Bot J Linn Soc*
 5 435 175:282–298
 6
- 7 436 García Álvarez S, Morla Juaristi C, Solana Gutiérrez J, García-Amorena I (2009) Taxonomic differences
 8 437 between *Pinus sylvestris* and *P. uncinata* revealed in the stomata and cuticle characters for use in
 9 438 the study of fossil material. *Rev Palaeobot Palynol* 155:61–68. doi:
 10 439 10.1016/j.revpalbo.2009.01.002
 11
- 12 440 GBIF Secretariat (2017) GBIF Backbone Taxonomy. Checklist dataset. <https://doi.org/10.15468/39omei>.
 13 441 Accessed 8 Jun 2018
 14
- 15 442 Gervais BR, MacDonald GM (2001) Modern pollen and stomate deposition in lake surface sediments from
 16 443 across the treeline on the Kola Peninsula, Russia. *Rev Palaeobot Palynol* 114:223–237. doi:
 17 444 10.1016/S0034-6667(00)00076-2
 18
- 19 445 Giesecke T, Brewer S, Finsinger W, et al (2017) Patterns and dynamics of European vegetation change
 20 446 over the last 15,000 years. *J Biogeogr* 44:1441–1456. doi: 10.1111/jbi.12974
 21
- 22 447 Hajar L, François L, Khater C, et al (2010) *Cedrus libani* (A. Rich) distribution in Lebanon: Past, present and
 23 448 future. *C R Biol* 333:622–630. doi: 10.1016/j.crvi.2010.05.003
 24
- 25 449 Hansen BC., Rodbell D., Seltzer G., et al (2003) Late-glacial and Holocene vegetational history from two
 26 450 sites in the western Cordillera of southwestern Ecuador. *Palaeogeogr Palaeoclimatol Palaeoecol*
 27 451 194:79–108. doi: 10.1016/S0031-0182(03)00272-4
 28 452
 29
- 30 452 Hansen BCS (1995) Conifer stomate analysis as a paleoecological tool: an example from the Hudson Bay
 31 453 Lowlands. *Can J Bot* 73:244–252
 32
- 33 454 Herring EM, Gavin DG, Dobrowski SZ, et al (2018) Ecological history of a long-lived conifer in a disjunct
 34 455 population. *J Ecol* 106:319–332. doi: 10.1111/1365-2745.12826
 35
- 36 456 Hu Y-Q, Mingram J, Stebich M, Li J-F (2016) A key for the identification of conifer stomata from N.E. China
 37 457 based on fluorescence microscopy. *Rev Palaeobot Palynol* 233:12–21. doi:
 38 458 10.1016/j.revpalbo.2016.06.005
 39
- 40 459 Huntley B, Birks HJB (1983) An atlas of past and present pollen maps for Europe: 0-13,000 years ago.
 41 460 Cambridge University Press, Cambridge
 42
- 43 461 IUCN (2017) The IUCN Red List of Threatened Species. Version 2017-3. <<http://www.iucnredlist.org>>
 44 462
 45 462 Kayacik H (1955) The distribution of *Picea orientalis* (L.) Carr. *Kew Bull* 10:481–490. doi:
 46 463 10.2307/4109240
 47 464
 48
- 49 464 Lacourse T, Beer KW, Hoffman EH (2016) Identification of conifer stomata in pollen samples from western
 50 465 North America. *Rev Palaeobot Palynol* 232:140–150. doi: 10.1016/j.revpalbo.2016.05.005
 51
- 52 466 Lamb HF, Eicher U, Switsur VR (1989) An 18,000-Year Record of Vegetation, Lake-Level and Climatic-
 53 467 Change from Tigalmamine, Middle Atlas, Morocco. *J Biogeogr* 16:65–74
 54
- 55 468 Lang G (1994) Quartäre Vegetationsgeschichte Europas. Methoden und Ergebnisse. G. Fischer, Jena
 56
- 57 469 MacDonald GM (2002) Conifer Stomata. In: *Tracking Environmental Change Using Lake Sediments*.
 58 470 Springer, Dordrecht, pp 33–47
 59
 60
 61
 62
 63
 64
 65

- 471 Malcolm JR, Canran L, Neilson RP, et al (2006) Global warming and extinctions of endemic species from
1 472 biodiversity hotspots. *Conserv Biol* 20:538–548
2
- 3 473 Malyshev LL (2008) AgroAtlas - Relatives - Range of distribution of Siberian Larch (*Larix sibirica*) . In:
4 474 Afonin AN, Greene SL, Dzyubenko NI, Frolov AN (eds) Interactive Agricultural Ecological Atlas of
5 475 Russia and Neighboring Countries. Economic Plants and their Diseases, Pests and Weeds [Online].
6 476 Available at: http://www.agroatlas.ru/en/content/related/Larix_sibirica/
7
- 8 477 Martin AC, Harvey WJ (2017) The Global Pollen Project: a new tool for pollen identification and the
9 478 dissemination of physical reference collections. *Methods Ecol Evol* 8:892–897. doi:
10 479 10.1111/2041-210X.12752
11
- 12 480 Moore PD, Webb JA, Collinson ME (1998) Pollen analysis. Blackwell Science Ltd, Oxford
13
- 14 481 Orbán I, Birks HH, Vincze I, et al (2018) Treeline and timberline dynamics on the northern and southern
15 482 slopes of the Retezat Mountains (Romania) during the late glacial and the Holocene. *Quat Int*
16 483 477:59–78. doi: 10.1016/j.quaint.2017.03.012
17
- 18 484 Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol*
19 485 *Syst* 37:637–669, doi:10.1146/annurev.ecolsys.37.091305.110100
20
- 21 486 R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical
22 487 Computing, Vienna, Austria
23
- 24 488 Reille M (1992) Pollen et spores d'Europe et d'Afrique du nord. Laboratoire de Botanique Historique et
25 489 Palynologie, Marseille
26
- 27 490 Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat*
28 491 *Methods* 9:671–675. doi: 10.1038/nmeth.2089
29
30
- 31 492 Sweeney CA (2004) A key for the identification of stomata of the native conifers of Scandinavia. *Rev*
32 493 *Palaeobot Palynol* 128:281–290. doi: 10.1016/S0034-6667(03)00138-6
33
- 34 494 Tinner W, Colombaroli D, Heiri O, et al (2013) The past ecology of *Abies alba* provides new perspectives
35 495 on future responses of silver fir forests to global warming. *Ecol Monogr* 83:419–439. doi:
36 496 10.1890/12-2231.1
37
- 38 497 Tonkov S, Possnert G, Bozilova E, et al (2018) On the Holocene vegetation history of the Central Rila
39 498 Mountains, Bulgaria: The palaeoecological record of peat bog Vodniza (2113 m). *Rev Palaeobot*
40 499 *Palynol* 250:16–26. doi: 10.1016/j.revpalbo.2017.12.006
41
- 42 500 Trautmann W (1953) Zur Unterscheidung fossiler Spaltöffnungen der mitteleuropäischen Coniferen. *Flora*
43 501 140:523–533. doi: 10.1016/S0367-1615(17)31952-3
44
- 45 502 Vendramin GG, Fineschi S, Fady B (2008) EUFORGEN Technical Guidelines for genetic conservation and
46 503 use for Bosnian pine (*Pinus heldreichii*). Bioversity International, Rome, Italy, p 6
47
- 48 504 Vescovi E, Ammann B, Ravazzi C, Tinner W (2010) A new Late-glacial and Holocene record of vegetation
49 505 and fire history from Lago del Greppo, northern Apennines, Italy. *Veg Hist Archaeobotany*
50 506 19:219–233. doi: 10.1007/s00334-010-0243-5
51
52
- 53 507 Vincze I, Orbán I, Birks HH, et al (2017) Holocene treeline and timberline changes in the South
54 508 Carpathians (Romania): Climatic and anthropogenic drivers on the southern slopes of the Retezat
55 509 Mountains. *The Holocene* 27:1613 –1630. doi: 10.1177/0959683617702227
56
- 57 510 Wagner S, Litt T, Sánchez-Goñi M-F, Petit RJ (2015) History of *Larix decidua* Mill. (European larch) since
58 511 130 ka. *Quat Sci Rev* 124:224–247. doi: 10.1016/j.quascirev.2015.07.002
59
60
61
62
63
64
65

512 Wazen N, Fady B (2016) Geographic distribution of 24 major tree species in the Mediterranean and their
1 513 genetic resources. Food and Agriculture Organization of the United Nations and Plan Bleu pour
2 514 l'Environnement et le Développement en Méditerranée, Rome, Italy
3
4 515 Whittaker RJ, Fernandez-Palacios JM (2007) Island Biogeography: Ecology, Evolution, and Conservation.
5 516 Oxford University Press
6
7 517 Yu Z (1997) Late Quaternary paleoecology of *Thuja* and *Juniperus* (Cupressaceae) at Crawford Lake,
8 518 Ontario, Canada: pollen, stomata and macrofossils. Rev Palaeobot Palynol 96:241–254
9
10 519 Zhang K, Zhao Y, Guo XL (2011) Conifer stomata analysis in paleoecological studies on the Loess Plateau:
11 520 An example from Tianchi Lake, Liupan Mountains. J Arid Environ 75:1209–1213. doi:
12 521 10.1016/j.jaridenv.2011.04.023
13
14 522
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16
17
18
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20
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23
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Figure 1a

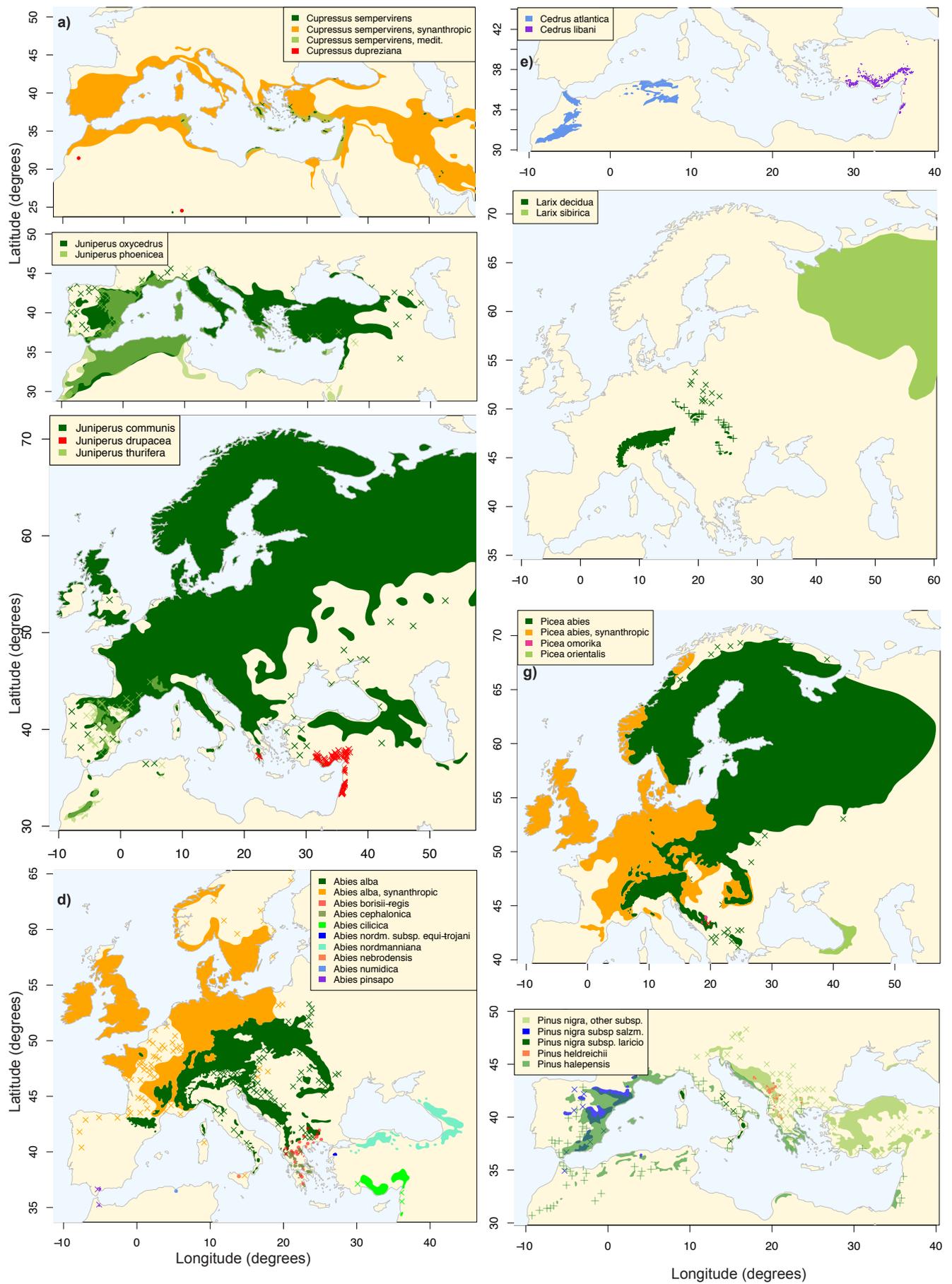


Figure 1b

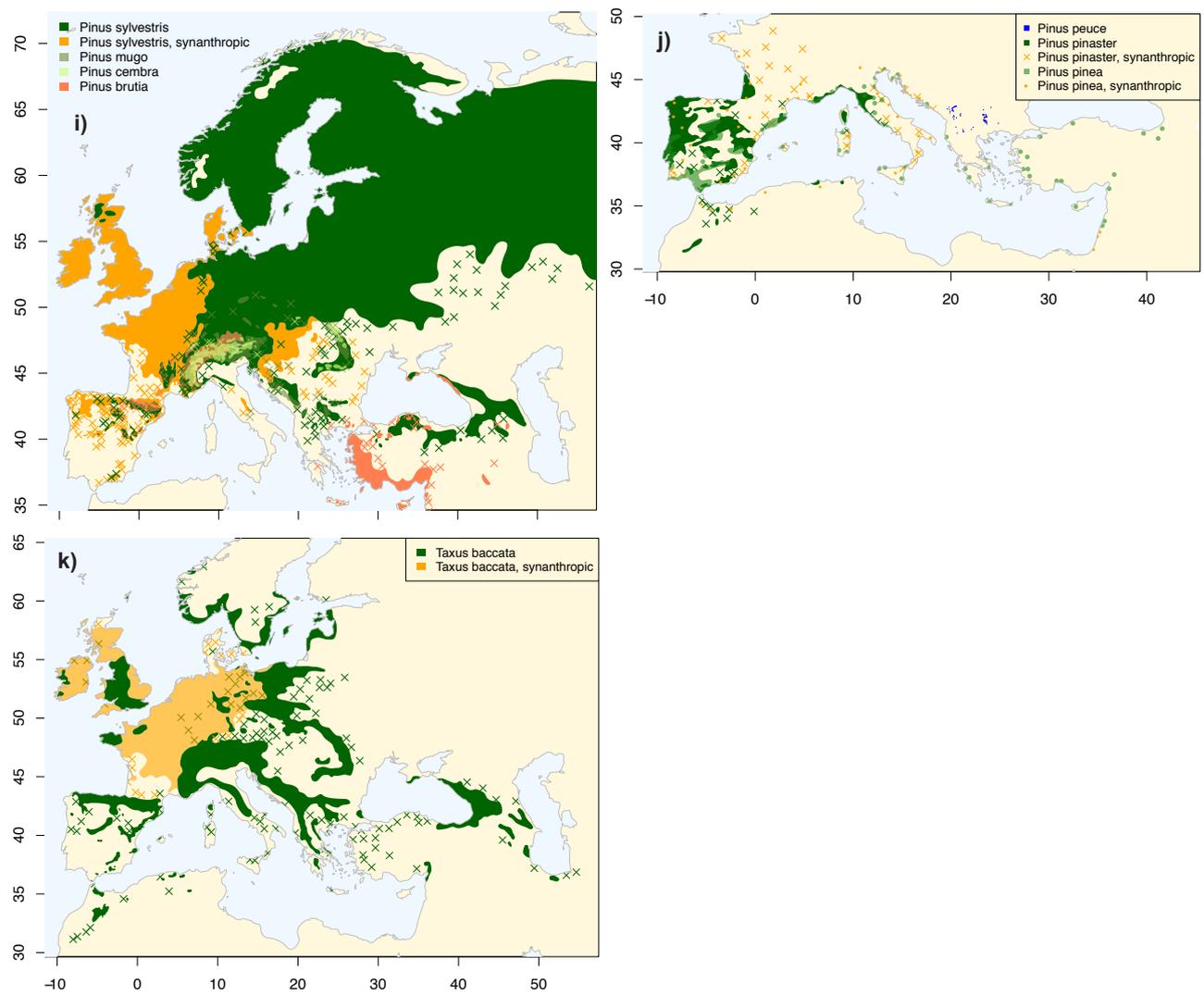


Figure 3

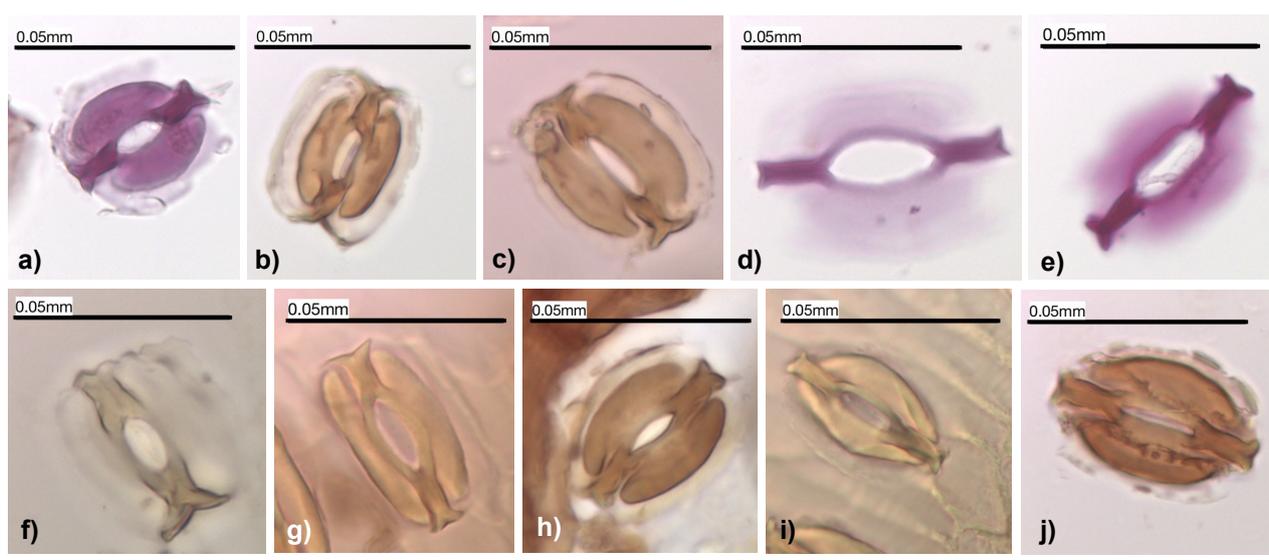


Figure 4

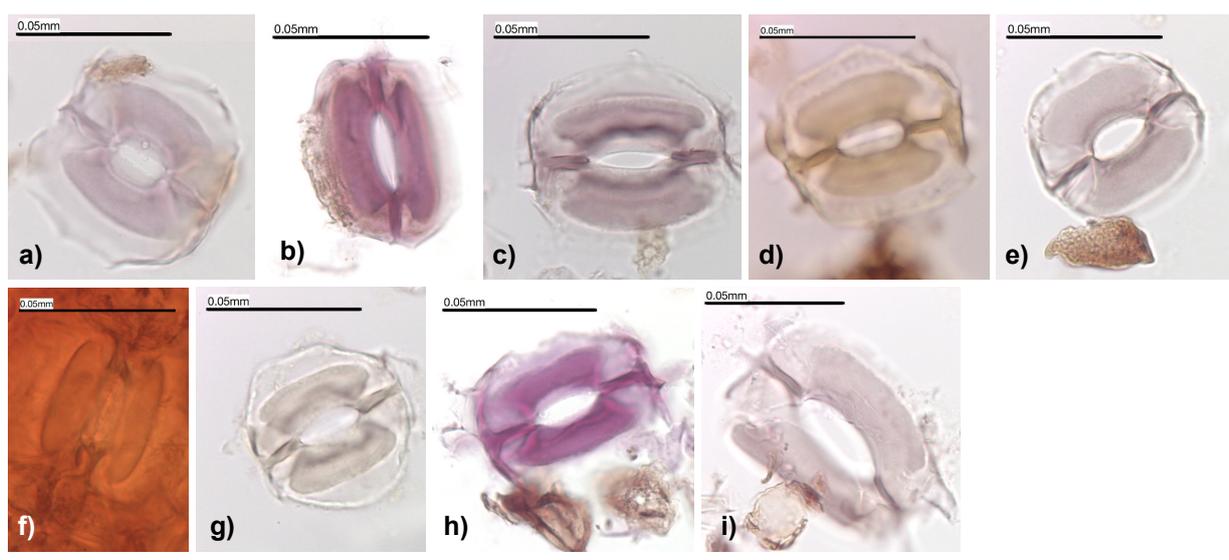


Figure 5

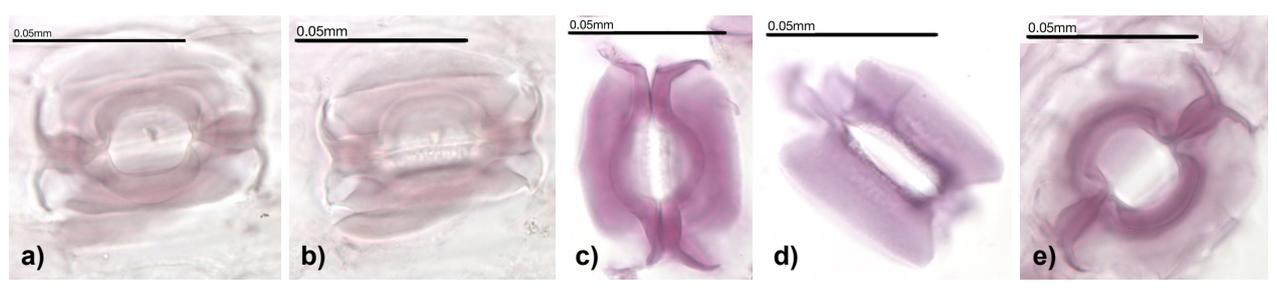


Figure 6

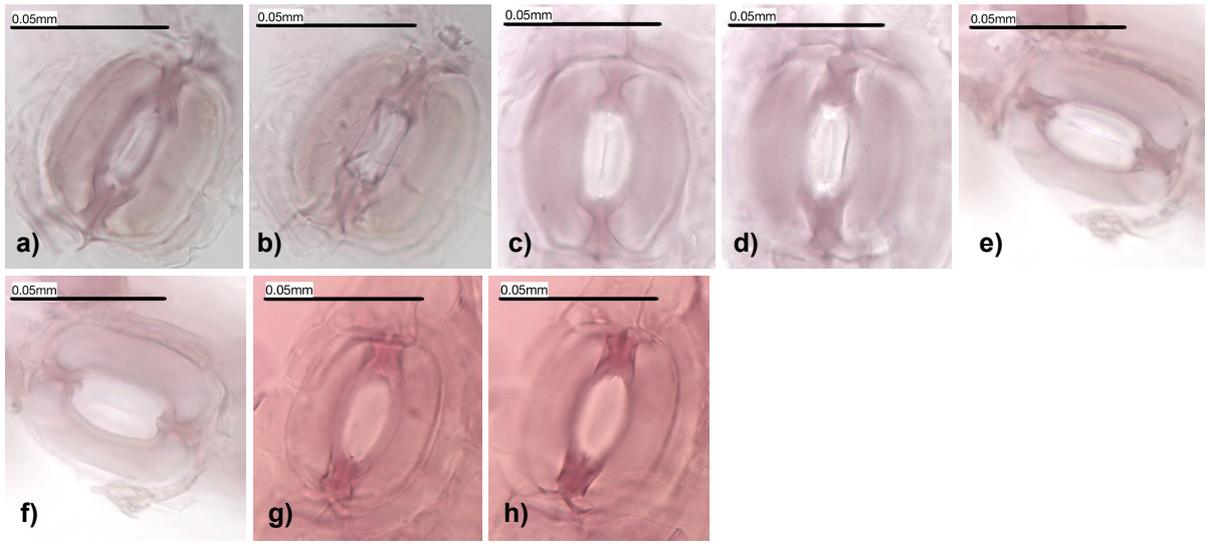


Figure 7

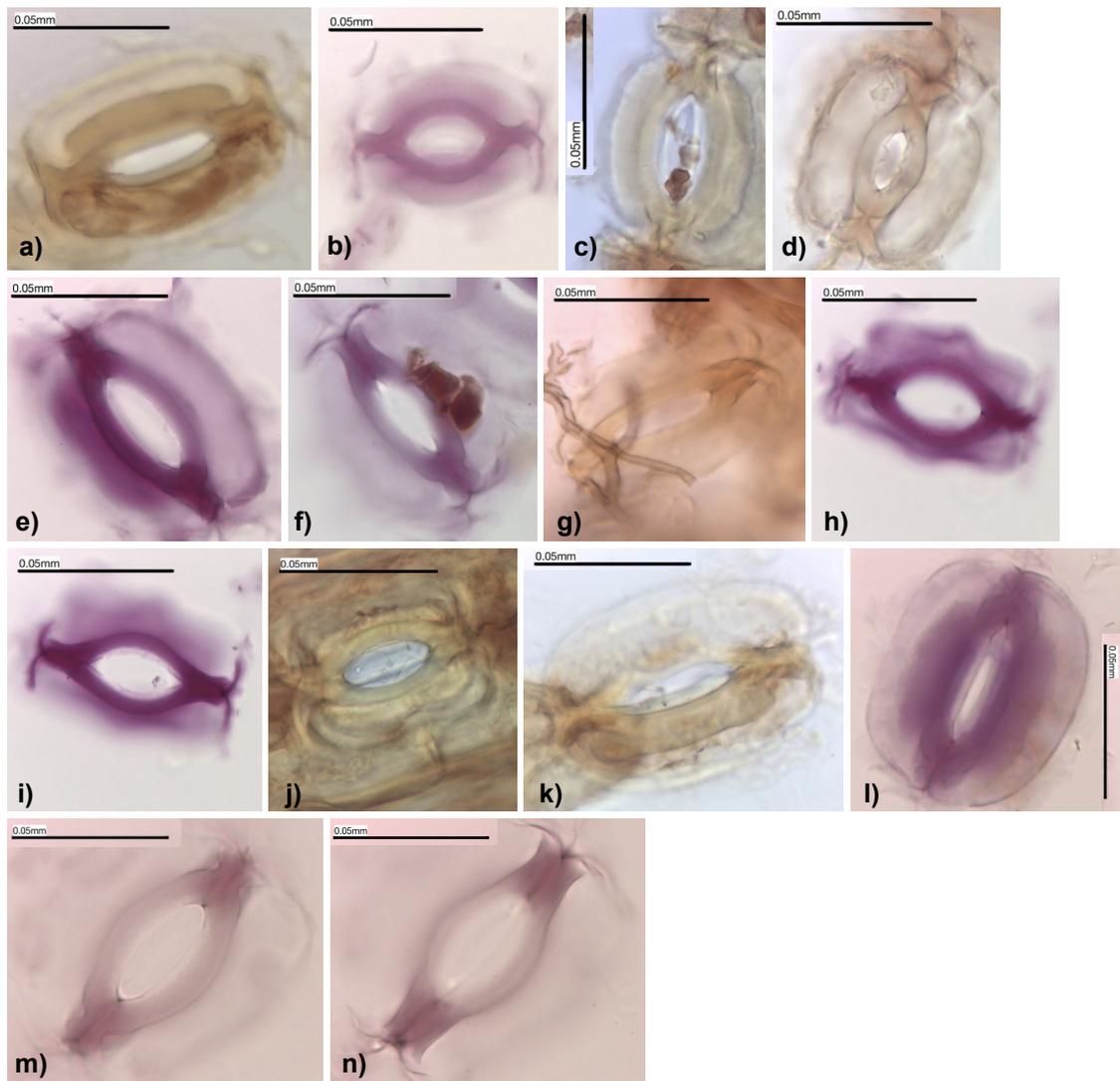


Figure 8

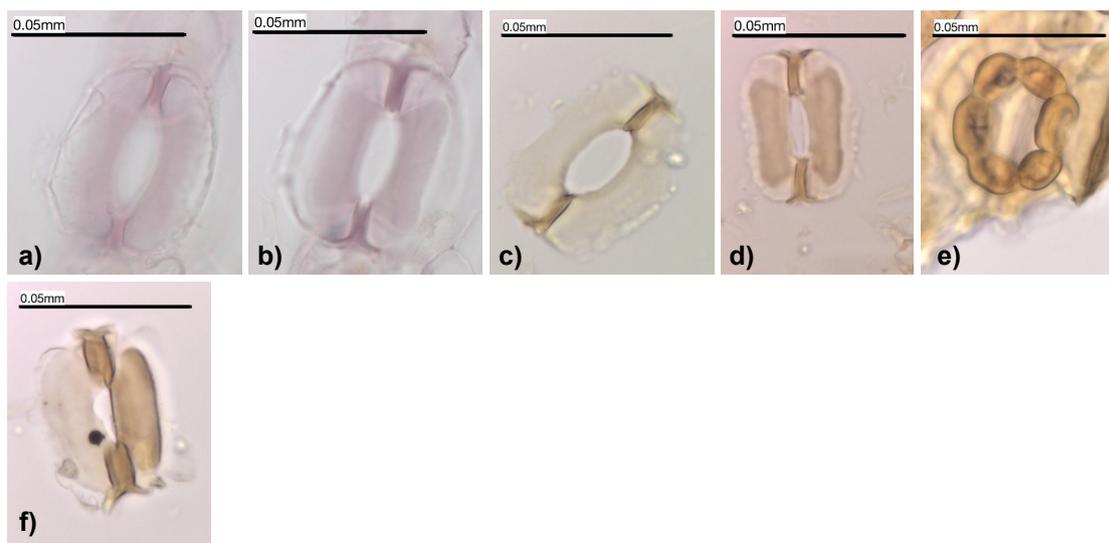


Table 1: List of species ordered by Family/Genus/Species name following the GBIF Backbone Taxonomy (GBIF Secretariat, 2017), and number of individuals whose stomata were analyzed in this study. Abbreviations of IUCN Red List categories: least concern (LC), near threatened (NT), vulnerable (VU), endangered (EN), critically endangered (CR), not evaluated (NE) (IUCN, 2017). * = needles collected from an isotype specimen, † = needles collected from a syntype.

Family	Genus	Species name	IUCN Red List Category	Stomata previously described	Number individuals in this study
Cupressaceae	<i>Cupressus</i>	<i>Cupressus sempervirens</i> L.	LC	/	1
		<i>Cupressus dupreziana</i> var. <i>atlantica</i> (Gaussen) Silba	CR	/	1
		<i>Cupressus dupreziana</i> A. Camus	EN	/	1
	<i>Juniperus</i>	<i>Juniperus communis</i> L.	LC	Trautmann (1953) Sweeney (2004)	1
		<i>Juniperus communis</i> var. <i>saxatilis</i> Pall.	LC	Trautmann (1953)	1
		<i>Juniperus drupacea</i> Labill.	LC	/	1
		<i>Juniperus oxycedrus</i> L.	LC	/	1
		<i>Juniperus phoenicea</i> L.	LC	/	1
		<i>Juniperus sabina</i> L.	LC	Trautmann (1953)	1
		<i>Juniperus thurifera</i> L.	LC	/	1
Pinaeaceae	<i>Abies</i>	<i>Abies alba</i> Mill	LC	Trautmann (1953) Sweeney (2004)	1
		<i>Abies borisii-regis</i> Mattf.	NE	/	1
		<i>Abies cephalonica</i> Loudon	LC	/	1
		<i>Abies cilicica</i> (Antoine & Kotschy) Carrière†	NT	/	1
		<i>Abies nebrodensis</i> (Lojac.) Mattei	CR	/	1
		<i>Abies nordmanniana</i> Spach	LC	/	1
		<i>Abies nordmanniana</i> subsp. <i>equi-trojani</i> (Asch. & Sint. ex Boiss.) Coode & Cullen*	EN	/	2
		<i>Abies numidica</i> de Lannoy ex Carrière	CR	/	1
		<i>Abies pinsapo</i> Boiss.	EN	/	1
		<i>Abies pinsapo</i> var. <i>marocana</i> (Trab)	EN	/	1
	<i>Cedrus</i>	<i>Cedrus atlantica</i> (Endl.) Manetti ex Carriere	EN	/	1
		<i>Cedrus libani</i> A. Rich.	VU	/	1
	<i>Larix</i>	<i>Larix decidua</i> Mill.	LC	Trautmann (1953)	1
<i>Larix sibirica</i> Ledeb.		LC	Sweeney (2004)	1	

<i>Picea</i>	<i>Picea abies</i> (L.) H. Karst	LC	Trautmann (1953) Sweeney (2004)	2
	<i>Picea omorika</i> (Pancic) Purk	EN	/	3
	<i>Picea orientalis</i> (L.) Peterm.	LC	/	1
<i>Pinus</i>	<i>Pinus brutia</i> Ten.	LC	/	1
	<i>Pinus cembra</i> L.	LC	Trautmann (1953)	2
	<i>Pinus halepensis</i> Mill.	LC	/	1
	<i>Pinus heldreichii</i> H. Christ	LC	/	2
	<i>Pinus mugo</i> Turra	LC	Trautmann (1953)	2
	<i>Pinus nigra</i> subsp. <i>laricio</i> (Poir.) Maire	LC	/	2
	<i>Pinus nigra</i> subsp. <i>salzmannii</i> (Dunal) Franco	LC	/	1
	<i>Pinus peuce</i> Griseb.	NT	/	1
	<i>Pinus pinaster</i> Aiton	LC	/	1
	<i>Pinus pinea</i> L.	LC	/	1
	<i>Pinus sylvestris</i> L.	LC	Trautmann (1953) Sweeney (2004)	1
	<i>Pinus uncinata</i> Ram ex DC	LC	/	1
	<i>Taxaceae</i>	<i>Taxus</i>	LC	Trautmann (1953) Sweeney (2004)