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Ruoyang Hu, Xuedong Li, Yong Hu, Runjie Zhang, Qiang Lv, Min Zhang, Xianyong Sheng, Feng Zhao, Zhijia Chen, Yuhan Ding, et al.

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3 **Adaptive evolution of the enigmatic *Takakia* now facing climate change**  
4 **in Tibet**

5

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## 76 **KEY WORDS**

77 abiotic stress; adaptive evolution; development; DNA repair; fast-evolving genome; gene expansion;  
78 global warming; molecular clock; UV radiation; palaeontology

79

## 80 **SUMMARY**

81 The most extreme environments are the most vulnerable to transformation under a rapidly changing  
82 climate. These ecosystems harbor some of the most specialized species, which will likely suffer the  
83 highest extinction rates. We document the steepest temperature increase (2010–2021) on record at  
84 altitudes of above 4,000 meters, triggering a decline of the relictual and highly adapted moss *Takakia*  
85 *lepidozoides*. Its *de novo* sequenced genome with 27,467 protein-coding genes includes unique  
86 adaptations to abiotic stresses and comprises the largest number of fast-evolving genes under  
87 positive selection. The uplift of the study site in the last 65 million years has resulted in life-  
88 threatening UV-B radiation and drastically reduced temperatures, and we detected several of the

89 molecular adaptations of *Takakia* to these environmental changes. Surprisingly, peculiar  
90 morphological features likely occurred earlier than 165 Mya. Following nearly 400 million years of  
91 evolution and resilience this species is now facing extinction.

92

## 93 **INTRODUCTION**

94 About 500 million years ago (Mya), when the ancestors of humans were tiny water-borne animals<sup>1</sup>,  
95 the Earth's rocky land masses were colonized by plants that evolved from freshwater algae. A series  
96 of biochemical and morphological innovations helped these first land plants to cope with and diversify  
97 in the new and harsher terrestrial environments, triggering a massive change in global carbon cycling  
98 through rock weathering and photosynthetic activity<sup>2,3</sup>. This drastically decreased atmospheric CO<sub>2</sub>,  
99 resulting in an ice age<sup>4</sup>, and elevated atmospheric O<sub>2</sub> concentration similar to modern levels, thereby  
100 facilitating the evolution of complex animals on land<sup>5,6</sup>. Stomata were one such critical innovations.  
101 These microscopic pores on plant surfaces, originated over 400 Mya, facilitating the greening of the  
102 land masses by permitting plant-atmosphere gas exchange and plant hydration<sup>7,8</sup>.

103 Starting 65 Mya, the India-Asian collision created the Himalayas with an average altitude  
104 exceeding 4,500 m<sup>9</sup>, resulting in one of the harshest environments on Earth<sup>10</sup>. A part of it, the Tibetan  
105 Plateau, is often referred to as "Roof of the World" where global warming drives upslope tree  
106 expansion and habitat loss of the endemic flora<sup>11</sup>. Due to their ecophysiological features and  
107 environmental sensitivity, mosses are considered as indicators of climate change<sup>12</sup>.

108 Mosses, together with liverworts and hornworts, compose the bryophytes, the sister lineage to  
109 vascular plants<sup>13</sup>. They likely share with the first land plants a gametophytic life style with freely  
110 moving sperm cells and a lack of roots, true vasculature, flowers and seeds<sup>14</sup>. Many bryophytes are  
111 pioneer plants<sup>15</sup> well adapted to harsh environments<sup>16,17</sup>. Several genome sequences of model  
112 bryophytes are available and have shed light on the early evolution of land plants<sup>18-22</sup>.

113 The genus *Takakia* comprises only two species: *T. lepidozoides* and *T. ceratophylla*, and its  
114 phylogenetic affinities to either algae, mosses or liverworts<sup>23-27</sup> was long ambiguous, until the  
115 discovery of their spore-bearing capsules that are similar to those of mosses<sup>25,28</sup>. *Takakia* is now  
116 resolved phylogenetically as the sister group to all other extant mosses<sup>29</sup>, from which it differs in leaf  
117 morphology and the mode of sporangial dehiscence, while their gametophyte resembles those of  
118 some liverworts<sup>23,25</sup>, as some biochemical features do. The possession of some algal features further  
119 suggests that *Takakia* may share some characteristics with the last common ancestor (LCA) of land

120 plants and provide critical insights into early land plant evolution.

121 Both species of *Takakia* co-occur only in the Tibetan Plateau, which may be viewed as the  
122 modern distribution center of *Takakia*<sup>28</sup>. In 2005, we found *T. lepidozoides* in the Gawalong glacier  
123 area above 4,000 meters elevation, well above the tree line (Figure 1A) where the endemic  
124 vegetation is undergoing drastic and profound changes due to local warming under climate change<sup>11</sup>,  
125 but the fate of specific species is unclear. To comprehensively explore the genetic and phenotypic  
126 characteristics of *T. lepidozoides* and correlate its adaptive evolution to environmental changes, we  
127 set up sample sites in a ~18 km<sup>2</sup> region, with real-time image and meteorological data recorders to  
128 characterize individual plant growth and reproductive development, and population dynamics in a  
129 climatic context. Further, we complemented the data of *Takakia* fossil and paleometeorology, and  
130 field observations with laboratory experiments on its physiology, its biochemistry and cell biology,  
131 and its genome bioinformatics, revealing adaptations to harsh environments, in particular, extreme  
132 tolerance to high UV radiation and freezing, resulted from the uplift of our experimental site started  
133 65 Mya.

134

## 135 **RESULTS**

### 136 **Global warming impacted population dynamics in the past decade**

137 We studied populations of *T. lepidozoides* in the southern part of the Tibetan Plateau, a top hotspot  
138 of global biodiversity conservation<sup>30</sup>. We established 68 sample sites (10×10 cm<sup>2</sup> each) in three  
139 areas at altitudinal intervals of about 100 m (Figures 1A–1C; Movie S1). The matrix properties of  
140 sample substrates comprised three types: soil, rock covered with a thin layer of soil and bare rock.  
141 The composition of mineral elements was analyzed from 20 soil samples (Data S1A).

142 The average annual temperature in the sampling area increased by 0.32 °C per decade from  
143 1961 to 2021 (Figure 1D). The snow-cover-free period for *Takakia* plants spans from June to October  
144 for 130 ± 11 days (Figure 1E; Table S1A), during which 60% of the annual average precipitation  
145 occurred, and air humidity and soil moisture were the highest (Data S1B). Between 2010 and 2021,  
146 the annual average temperature increased by 0.43 °C, which is the highest in documented history,  
147 and the Gawalong East Glacier retreated by 49 ± 3 m / year (Data S1C and S1D; Table S1B), above  
148 the average recorded for 82 glaciers in this area in 30 years (1970–2000)<sup>31</sup>.

149 Between 2010 and 2021, the average coverage of *T. lepidozoides* populations decreased by  
150 1.6% annually (Figure 1F), faster than that of four local common mosses (Figures S1A–S1D). By

151 contrast, the coverage of the liverwort *Herbertus sendtneri* increased by 2.27% / year (Figures S1E–  
152 S1G), that of the angiosperm *Diapensia wardii* by 0.88% / year (Figure S1H), and that of the  
153 lichenized fungus *Stereocaulon alpinum* by 0.47% / year (Figure S1M). We employed principal  
154 component analysis to further explore the major variation feature in populations and found that  
155 populations of the liverwort *Plagiochila recurvata* also increased in size (Data S1E; Table S1C).  
156 Based on Spearman rank correlation coefficient between changes in population size in these species  
157 and environmental factors, shifts in *T. lepidozoides* were significantly correlated to changes in  
158 maximal temperature during the growing season (Data S1E; Table S1D). The mineral content of the  
159 substrate supporting versus lacking *T. lepidozoides* (Data S1A) was significantly richer in N, Mg and  
160 Fe and poorer in P, S and Ca (Data S1A), suggesting that these macronutrients shape the local  
161 distribution of *T. lepidozoides*. We detected no significant differences between the dynamics of *T.*  
162 *lepidozoides* populations on different substrates, suggesting that only warming shapes the loss in  
163 coverage of this species (Data S1F; Table S1E).

164

#### 165 **Vegetative growth for stable network structures**

166 Spores of *T. lepidozoides* germinate to form thalloid protonemata, reminiscent of liverwort  
167 protonemata. These develop cylindrical rhizomes, which continuously branch or develop erect stems  
168 with deeply lobed leaves (phylloids) (Data S1G and S1H). Rhizoids are always lacking. In culture,  
169 erect stems grow  $1.52 \pm 0.03$  mm in 130 days (corresponding to the four months snow-free period  
170 in the field), compared to  $1.35 \pm 0.44$  mm in the field, while rhizomatous, leafless and stoloniform  
171 stems grow by  $2.87 \pm 0.02$  mm over the same period (Data S1H). The distance between two adjacent  
172 branches (rhizomes and erect stems) is  $1.73 \pm 1.56$  mm in the field and  $2.26 \pm 2.74$  mm in culture  
173 (Data S1H).

174 The branches do not originate from the leaf axil, unlike in angiosperms such as *Arabidopsis*  
175 *thaliana*. In contrast, it is similar to the randomness of lateral root-formation position in *A. thaliana*<sup>32</sup>.  
176 Branches originate from epidermal and subepidermal cell layers of the stem. While epidermal cells  
177 divide vertically, sub-epidermal cells divide periclinally and anticlinally, forming a dome, before  
178 gradually elongating to form a new branch (Figures 2A, 2B and S2; Movie S2). The cells in the middle  
179 part of stems are longer, with inclined walls between adjacent cells, and connect vertically at the  
180 intersection between the parental stem and the new branch (Figures 2A, 2B and S2). These potential  
181 vessel elements facilitate transport of macromolecules (Figure 2C). Thus, the connecting pattern is

182 similar to the connect model of vessel elements in roots (Figures 2D; Data S1I), but different from  
183 that in stems of vascular plants such as *Arabidopsis* (Figures 2E; Data S1I). New erect branches  
184 grow  $5.22 \pm 1.93$  mm (Data S1J) to achieve sexual maturity in ~3 years (Data S1J). Thus, individual  
185 rhizomes branch continuously, forming a population of clonal erect stems that ultimately decay at  
186 their base and maintain growth at the tip (Figures 2F and 2G; Data S1H). Erect stems and rhizomes  
187 continuously branch and grow in different directions, adjoined with surface mucilage hairs at cross-  
188 points (Data S1K), resulting in a network structure (Data S1L) that can withstand heavy snowstorms  
189 (Data S1M). Stems of *T. lepidozoides* show weaker negative geotropism (Data S1L, S1N) compared  
190 to other surveyed mosses (Data S1O), while positive phototropism is obvious (Data S1N).

191

## 192 **Reproductive strategy**

193 The sexual organs of *T. lepidozoides*, i.e., the male antheridia and female archegonia, could not be  
194 induced in culture. In wild populations, erect stems develop either antheridia or archegonia.  
195 Beginning in July, pale red antheridia are initiated and their number peaks in September (Data S1P).  
196 Stems carry  $9.13 \pm 2.07$  antheridia (Data S1G and S1P) or  $4.63 \pm 0.82$  archegonia at the stem tip  
197 (Data S1Q). One stem can produce antheridia continuously for ~3 years (Data S1P). Fertilization  
198 occurs in autumn, and zygotes or early embryos remain dormant under the snow at temperatures of  
199 -1 to -4 °C for about eight months. In mosses, embryos grow into unbranched sporophytes with  
200 terminal sporogenous capsules. In the field, light green *T. lepidozoides* sporophytes occur  
201 synchronously about ten days after snow melt in June. Following the rapid elongation of the capsule  
202 stalk (seta) to  $3.13 \pm 0.29$  mm within ~30 days, capsules expand and mature to become deep-brown  
203 in ~35 days (Data S1R and S1S; Movie S3). Generally, one stem supports one sporophyte, only  
204 occasionally two (Data S1R). At maturity capsules dehisce along a vertical spiral line to release  $19.5$   
205  $\pm 2.9$  thousand spores (Data S1S) in a radius of  $1.36 \pm 0.69$  cm (Data S1S). *T. lepidozoides* capsules  
206 lack stomata (Data S1T). In culture, spore germination rates reached 96%. In the field, we have not  
207 found new spore-derived populations since 2005. Of 52 samples, only 22 populations had  
208 reproductive organs: three with archegonia, nine with antheridia, and ten with mixed male and female  
209 stems. These populations did not differ significantly in their dynamics, revealing that *T. lepidozoides*  
210 mainly depends on clonal growth in the Tibetan Plateau.

211

## 212 **Tolerance to extreme UV-B radiation**

213 Exposure to solar UV-B radiation increases with altitudes and hence, at high elevations, mutagenic  
214 UV-B<sup>33</sup> is a potent stressor. We exposed *T. lepidozoides*, the model moss *Physcomitrium*  
215 (*Physcomitrella*) *patens*<sup>34</sup> and the model angiosperm *A. thaliana* to 144 kJ m<sup>-2</sup> UV-B, about ten times  
216 the intensity in our sample site, for two hours. Leaves of *A. thaliana* began wilting within 24 hours  
217 after treatment, and within 72 hours plants withered and died (Figure S3). Apical leaves of *P. patens*  
218 turned pale within 72 hours, and plants gradually died, whereas *T. lepidozoides* plants appeared to  
219 be unaffected (Figures 3A–3F). Cellular damage in *P. patens* included disordered chloroplast  
220 lamellae, expanded mitochondria, disintegrated nuclear membranes, agglomerated chromatin, and  
221 final cell collapse (Data S1U). By contrast, cells of *T. lepidozoides* exhibited only a slight  
222 condensation of heterochromatin but harbored significantly more oil bodies in response to UV-B  
223 (Data S1U).

224 These oil bodies were rich in neutral lipids and membrane lipids (Data S1V). The total lipid  
225 content was 1.91 µg / mg fresh weight (fw), including 61.61% neutral lipids, of which 53.41% are  
226 polyunsaturated fatty acids (PUFAs). In contrast, the total lipid content of *P. patens* was 1.01 µg /  
227 mg fw, including 19.36% neutral lipids, of which 36.29% are PUFAs (Data S1W). Thus, *T.*  
228 *lepidozoides* does not only contain much more overall lipids than *P. patens*, but especially more  
229 PUFAs, which are involved in tolerance<sup>35</sup> to various biotic and abiotic stresses. Upon UV-B treatment,  
230 the contents of diacylglycerol-*N,N,N*-trimethylhomoserine (DGTS), neutral lipids and PUFAs in  
231 neutral lipids increased significantly in *T. lepidozoides* (Figure 3G; Data S1X), compared to no DGTS  
232 or stagnation of neutral lipids in *P. patens* (Figure 3H; Data S1X). Monogalactoglycerolipid (MGDG)  
233 is the most abundant glycolipid in both mosses. Exposure to UV-B decreased its content slightly in  
234 *T. lepidozoides*, but significantly in *P. patens* (Data S1X). Phosphatidylcholine (PC), the main  
235 component of nonphotosynthetic membrane lipids, is the major phospholipid in both mosses. Upon  
236 radiation, the amount of PC remained stable in *T. lepidozoides* but decreased in *P. patens* (Data  
237 S1X).

238 After UV-B treatment, amounts of polyphenols and flavonoids doubled to tripled in *T.*  
239 *lepidozoides* to a fivefold equivalent of that in *P. patens* (Figures 3I and 3J; Table S1F), which was  
240 also supported by the result of non-targeted metabolic profiles (Data S1Y). Likewise, catalase activity  
241 increased while levels of the toxic malondialdehyde remained stable in *T. lepidozoides*, in contrast  
242 to *P. patens* (Figures 3K and 3L). While the maximum quantum efficiency of PSII photochemistry  
243 (Fv/Fm) was about 0.75 in both mosses, it decreased to 20% in *P. patens* and to 50–70% in *T.*

244 *lepidozoides* following UV-B treatment (Figure 3M). Likewise, the actual photochemical quantum  
245 efficiency (Fv/Fo) decreased sharply in *P. patens* but only slightly in *T. lepidozoides* (Figure 3N).  
246 The increased tolerance of *T. lepidozoides* to UV-B radiation was also visible on protein levels for  
247 both photosystems (Figures 3O and 3P). Taken together, these results revealed a strong defense  
248 response to UV-B in *T. lepidozoides* aimed at blocking radiation and at scavenging toxic ROS, and  
249 maintaining photosynthetic capacity by preventing lipid peroxidation and cell structure damage.

250

## 251 **Takakia Genome Features**

### 252 **Assembly, Annotation and Comparison**

253 The haploid genome of *T. lepidozoides* gametophytes that were initiated from a single spore was  
254 sequenced (Table S1G) and assembled. It comprises 325 Mbp, with 98.5% anchored to four  
255 chromosomes (Figures 4A and S4A), which coincides with our cytological observation (Figures S4B–  
256 S4E), and evidence from a Japanese isolate<sup>36</sup>. These chromosomes (1–4) are 96, 83, 77 and 64  
257 Mbp in size, respectively. The genome sequence represents 87% of the 375 Mbp estimated by flow  
258 cytometry (Data S2A; Table S1H) and 98% of the K-mer estimation of 331 Mbp (Data S2A), with a  
259 contig N50 length of 1.4 Mbp and a scaffold N50 length of 83 Mbp (Table S1I). The completeness of  
260 the genome estimated by BUSCO indicated that 80–97% of the conserved core gene sets are  
261 present (Data S2B). As in *P. patens*, we were unable to define specific centromeric regions. Gene  
262 annotation combined various strategies of *de novo*, homology, and transcriptome predictions. The  
263 final set contains 27,467 high confidence protein-coding gene models with mean gene length of  
264 3,301 bp, of which 85% have an assigned function (Table S1J). Further BUSCO analysis show that  
265 71–89% conserved core gene sets are present (Data S2B).

266 We found that over 52% of the *T. lepidozoides* genome (~172 Mbp) is composed of repetitive  
267 sequences. Long terminal repeat retrotransposons (LTRs) are the most abundant transposable  
268 elements (TEs), occupying more than 36% of the genome (Table S1K), similar to the 35.6% in the  
269 lycophyte *Selaginella moellendorffii*<sup>37</sup>, but different from the nearly 50% in *P. patens*<sup>38</sup> and the 22%  
270 in the liverwort *Marchantia polymorpha*<sup>19</sup>. Of the 119 Mbp LTRs in *T. lepidozoides*, 84% are from the  
271 gypsy superfamily and 5% are from the copia superfamily (Table S1K), different from the 48% and  
272 3.5%, respectively, in *P. patens*<sup>38</sup>. In *T. lepidozoides*, the median and mean age of copia elements  
273 is 0.85 Mya and 0.65 Mya, respectively, and of gypsy elements is 0.66 Mya and 0.47 Mya,  
274 respectively (Data S2B).

275 Moreover, we established the organellar genomes of *T. lepidozoides* with the plastome  
276 (NC\_005087.1) and chondriome (NC\_007945.1) of *P. patens* as seeds. The *T. lepidozoides*  
277 plastome is a circular molecule of 149,011 bp (Data S2C) and similar to the plastome of a Japanese  
278 isolate<sup>39</sup>. The *T. lepidozoides* chondriome is a circular molecule of 139,841 bp (Data S2C).

279

### 280 **Whole Genome Evolutionary History**

281 Intragenomic analysis detected 437 syntenic blocks containing 2,382 gene pairs with an average of  
282 5.45 gene pairs / block (Data S2D). Synonymous substitutions per site (Ks) age distributions showing  
283 a peak range from 0.7 to 1.1 (median 0.9) unveiled evidence in *T. lepidozoides* for an ancient  
284 diploidized state (Figures 4B; Data S2D). Based on an assumed rate of silent-site substitution of 9.4  
285 per silent site per billion years<sup>38</sup>, the diploidization occurred 58.51–37.23 Mya (median 47.87 Mya),  
286 during the period of the Tibetan Plateau uplifting<sup>9</sup>.

287 Regarding diploidization, including whole-genome duplication (WGD) and single-gene  
288 duplications, we identified 26,829 duplicated genes that were classified into five categories: 772  
289 WGD genes (2.88%), 452 tandem duplicated genes (TD, 1.68%), 1,172 proximal duplicated genes  
290 (PD, 4.37%), 6,005 transposed duplicated genes (TRD, 22.38%) and 18,428 dispersed duplicated  
291 genes (DSD, 68.69%). To identify the most recent and more ancient duplicated genes in *Takakia*,  
292 and how evolutionary forces inferred to affect them, we estimated the Ks values, nonsynonymous  
293 substitutions per site (Ka) values and Ka/Ks values for the gene pairs contained in each mode of  
294 gene duplication. We compared the Ks and Ka/Ks distribution among different modes of gene  
295 duplication. WGD genes showed a Ks peak ranging from 0.75–1.10 (median 0.90) and Ka/Ks peak  
296 ranging from 0.15–0.32 (median 0.23); TD genes showed a Ks peak ranging from 0.12–0.65 (median  
297 0.23) and Ka/Ks peak ranging from 0.18–0.52 (median 0.32); PD genes showed a Ks peak ranging  
298 from 0.06–0.46 (median 0.15) and Ka/Ks peak ranging from 0.20–0.63 (median 0.40); TRD genes  
299 showed a Ks peak ranging from 0.05–0.26 (median 0.12) and Ka/Ks peak ranging from 0.18–0.59  
300 (median 0.36); DSD genes showed a Ks peak ranging from 0.07–0.54 (median 0.18) and Ka/Ks  
301 peak ranging from 0.19–0.57 (median 0.33). Higher Ka/Ks ratios and smaller Ks values were found  
302 for PD gene pairs and TRD gene pairs than for other duplication modes (Figures 4C; Data S2E),  
303 suggesting a more rapid sequence divergence and a stronger positive selection on genes in these  
304 two modes than other duplication modes. These genes may have implications in stress responses  
305 involving hormones, such as jasmonic acid signalling (Data S2E). Most retained WGD genes

306 participated in post translational modifications of proteins, such as protein esterification and  
307 deacylation, which may be involved in stress responses<sup>40,41</sup> (Data S2E). According to the rate of  
308 silent-site substitution of 9.4 per silent site per billion years<sup>38</sup>, the WGD, TD, PD, TRD and DSD  
309 occurred 58.51–37.23 Mya (median 47.87 Mya), 34.57–6.38 Mya (median 12.23 Mya), 24.47–3.19  
310 Mya (median 7.98 Mya), 13.83–2.66 Mya (median 6.38 Mya) and 28.72–3.72 Mya (median 9.57 Mya)  
311 respectively, and most of duplications occurred during the Tibetan Plateau uplifting. Furthermore, 27  
312 singleton genes occur in *T. lepidozoides*, some of which may participate in DNA repair (Data S2E).

313

### 314 **Phylogenetic Position**

315 Inferences from 105 concatenated nuclear genes resolve *Takakia* as sister to all other mosses,  
316 including *Sphagnum*<sup>29</sup>, which was previously considered close to *Takakia*<sup>42</sup>, despite their  
317 conspicuous morphological dissimilarities. With the sequenced genome of *Takakia*, and genomes of  
318 two Charophyte species most closely related to land plants, we re-assessed the phylogenetic  
319 relationships of *Takakia* to other bryophytes based on an analysis of 818 nuclear genes from 56 plant  
320 species (Table S1L) and resolved *Takakia* as sister to other extant mosses, including *Sphagnum*  
321 (Data S2F), congruent with the outcome of our concatenated analysis and previous coalescent  
322 analyses<sup>29</sup>. Molecular clock analyses estimated that *Takakia* diverged from the lineage leading to  
323 other mosses ~389.92 Mya, ~38 million years before the separation of *Sphagnum* from other mosses,  
324 following the divergence of liverworts and mosses at 484.32 Mya (Figure 4D; Data S2F).

325

### 326 **Special Genes**

327 To gain further insights about how *Takakia* is related to mosses, and other bryophytes, we compared  
328 genes among those conserved orthogroups among bryophytes and also Charophytes. *Takakia*  
329 shares with one or more Charophytes 1,118 genes in 438 orthogroups (Data S2G) that are not found  
330 in *P. patens*, suggesting that these genes have been retained from the ancestor of land plants, but  
331 were lost in *P. patens*. In addition, *Takakia* shares with hornworts and liverworts 1,665 genes in 461  
332 orthogroups (Data S2G) that are not detected in *P. patens*, suggesting that they were present in the  
333 most recent ancestor to all bryophytes, and lost in a subsequent lineage giving rise to *P. patens*.  
334 Although the functions of many of these genes are not yet understood, it is possible that some of  
335 them are the basis for *Takakia* having some characteristics similar to liverworts and algae, but not to  
336 mosses.

337

## 338 **Genetic Foundation of Adaptive Features**

### 339 ***Positive Selection and Fast Evolution***

340 To gain insight into the evolutionary history of *Takakia*, we compiled a data set including orthologs  
341 from 12 plant taxa including one hornwort, four liverworts, five mosses and one eudicot. We  
342 employed maximum likelihood analysis using the branch-site model in PAML and detected positive  
343 selection signals in 83 out of 1,970 genes in *T. lepidozoides*. According to GO clustering, positively  
344 selected genes are enriched in the categories DNA repair, response to oxidative damage,  
345 photosynthesis and development (Table S1M). To identify fast-evolving genes, we estimated the  
346 global and local dN/dS (non-synonymous substitution rates / synonymous substitution rates) ratios,  
347 respectively. In total, 121 significantly fast-evolving genes were identified. Three functional  
348 annotation clusters ranked among the top 10 results: development, DNA repair and response to  
349 abiotic stress (Table S1N). In comparison with the liverwort *H. sendtneri* from the same environment,  
350 we identified 59 genes with convergent accelerated evolutionary rate (Table S1O) and 16 shared  
351 genes in all data sets (Table S1P). Their functions include metabolic process, energy generation,  
352 photosynthesis, oxidation-reduction, and defence response.

353 We calculated the dN/dS ratios for each lineage and compared *T. lepidozoides* to *A. thaliana*,  
354 *P. patens*, other mosses (excluding *T. lepidozoides*) and liverworts (Data S2H) and found that the  
355 upper boundary in *T. lepidozoides* was higher than of any other taxon group (Data S2H). This  
356 suggests that many fast-evolving genes occurred along the *Takakia* lineage. We further calculated  
357 the mean dN/dS ratios of GO categories for *T. lepidozoides*, *A. thaliana*, *P. patens*, and the LCA of  
358 all mosses (including *T. lepidozoides*) (Data S2H). Compared to *A. thaliana* and *P. patens*, we found  
359 a higher number of rapidly evolving GO categories in *T. lepidozoides* (342 in *T. lepidozoides* vs 78  
360 in *A. thaliana*, 208 in *T. lepidozoides* vs 124 in *P. patens*). Further, 56 GO categories evolved faster  
361 in the LCA of mosses than in *T. lepidozoides*. In contrast, 391 GO categories evolved faster in *T.*  
362 *lepidozoides* than in the LCA of all mosses. Among these are 31 GO categories that evolve faster  
363 in *T. lepidozoides* than in all other mosses (Table S1Q). These include response to desiccation,  
364 osmotic stress, heme biosynthesis, auxin metabolism and photosynthesis. Taken together, *T.*  
365 *lepidozoides* has the largest number of fast-evolving genes under positive selection on documented  
366 record.

367

## 368 **Transcription Associated Proteins**

369 Transcription associated proteins (TAPs) comprise transcription factors (TFs) and transcriptional  
370 regulators (TRs). Genome-wide, domain-based annotation of TAPs<sup>43</sup> identified 1,091 TAPs, of which  
371 427 are TFs and 664 TRs, which is more than that of the hornwort *A. angustus* (570) and liverwort  
372 *M. polymorpha* (682), but less than that of *P. patens* (1,638). However, the proportion of TAPs to  
373 protein-coding genes in *T. lepidozoides* (3.97%) is similar to *A. angustus* (3.89%) and *M. polymorpha*  
374 (3.53%) but less than in *P. patens* (4.97%) (Table S1R). In general, most TAP families in *T.*  
375 *lepidozoides* have a similarly low number in *A. angustus* and *M. polymorpha* than in *P. patens*. For  
376 example, the zf-HD family, which plays a key role in the control of growth and development<sup>44</sup>,  
377 comprises a single member in *T. lepidozoides*, *A. angustus* and *M. polymorpha*, but 12 in *P. patens*.  
378 Similarly, C2C2-CO-like TFs, which act between the circadian clock and genes controlling meristem  
379 identity<sup>45</sup>, are represented by two genes in *T. lepidozoides* and *A. angustus*, three in *M. polymorpha*,  
380 but 16 in *P. patens*. Interestingly, *T. lepidozoides* encodes no nucleic acid-binding cold shock domain  
381 protein (CSD) (Table S1R), which is an ancient and conserved family of proteins in various  
382 prokaryotes and eukaryotes<sup>46</sup>. In *Arabidopsis*, CSD proteins negatively regulate freezing tolerance<sup>47</sup>.

383 By contrast, with 438 genes, plant homeodomain zinc fingers (PHDs) are the most abundant  
384 TRs in *T. lepidozoides*, a diversity vastly outnumbering that in *A. angustus* (32), *M. polymorpha* (55)  
385 and *P. patens* (45) (Table S1R). PHDs are structurally conserved modules found in plants as  
386 epigenome readers that control gene expression through recruitment of multi-protein complexes of  
387 chromatin regulators, and play crucial roles in abiotic stress responses<sup>48</sup>. Among the 438 genes, 118  
388 are in small clusters of 2–5 genes in close proximity (fewer than 10 genes), with 164 of them lacking  
389 introns. Ks analysis showed these intronless genes have a peak ranging from 0.19–0.54 (median  
390 0.33), corresponding to their expansion 28.71–10.10 Mya (median 17.50 Mya), suggesting that at  
391 least some of them have experienced tandem duplications and retrotranspositions, respectively.

392 Mitochondrial transcription termination factors (mTERFs) are diversified in *T. lepidozoides* (18)  
393 compared to *A. angustus* (16), *M. polymorpha* (15) and *P. patens* (16) (Table S1R). mTERFs are  
394 localized in mitochondria or chloroplasts and involved in stimulating transcription and stabilizing post-  
395 transcriptionally processed mRNA, terminating transcription, promoting tRNA maturation, intron  
396 splicing and chloroplast ribosome assembly and translation<sup>49</sup>. They are important for stress  
397 responses and development<sup>50</sup>. The mTERFs in *T. lepidozoides* have a Ks peak range from 1.69–  
398 2.57 (median 2.07), suggesting that their diversity occurred 136.70–89.89 Mya (median 110.11 Mya).

399 Likewise, auxin/indole-3-acetic acid repressors (Aux/IAs) are diversified in *T. lepidozoides* (5)  
400 compared to *A. angustus* (2), *M. polymorpha* (3) and *P. patens* (4) (Table S1R). The diversity of  
401 Aux/IAs in *T. lepidozoides* occurred 218.62–142.02 Mya (median 218.09 Mya).

402

### 403 **Protein phosphorylation**

404 Protein phosphorylation is important for regulating protein activity and plays crucial roles in  
405 responses to environmental stresses<sup>51</sup>. In *Takakia*, 273 genes encode protein kinases, including 92  
406 that are in small dense clusters of 2–4 genes (Data S2I), suggesting that they were generated by  
407 tandem duplications. Moreover, 32 genes lack introns and are scattered throughout the genome,  
408 implying that some of them were acquired by retrotranspositions. According to Ks distribution, they  
409 diverged 145.07–84.76 Mya (median 107.26 Mya). However, 40 genes are younger than 30 Mya  
410 (Data S2I).

411

### 412 **PPR proteins and RNA-editing**

413 Nuclear-encoded pentatricopeptide (PPR) proteins localize to mitochondria and chloroplasts, control  
414 organellar gene expression, including RNA-editing<sup>52</sup>, and thus impact growth and development in  
415 response to stresses<sup>53,54</sup>. The *T. lepidozoides* genome encodes 1,926 PPRs, far more than that of  
416 *P. patens* (105) or *M. polymorpha* (69) (Figure 5A; Table S2A–S2H). An examination of their  
417 chromosomal positions revealed at least ~270 small clusters of two or more tandemly repeated  
418 genes (total 789 genes), and 271 of them have only 1–3 introns with a Ks value lower than 0.3,  
419 suggesting that they result from tandem duplications at about 15 Mya. About 429 PPR genes lack  
420 introns and are dispersed throughout the genome; 95% of them have a Ks value below 0.5 and 213  
421 of them have a Ks value below 0.3, suggesting that they expanded 30–15 Mya. Some of them might  
422 have experienced retro-transposon-mediated duplication (Data S2I). While in most bryophytes PPR  
423 proteins are equally distributed between plastids and mitochondria, the majority target mitochondria  
424 in *T. lepidozoides* (Figure 5B). RNA-editing occurs rarely in mosses (10 sites in *P. patens*). By  
425 contrast, we identified 912 editing sites in the *T. lepidozoides* organellar genomes, which is more  
426 than in *A. thaliana* (456) (Figure 5C; Table S2I and S2J). PPR-expression is induced by UV-B  
427 treatment (Figure 5D). Taken together, the expansion of PPR proteins and RNA-editing sites in *T.*  
428 *lepidozoides* may be linked to adaptation to extreme environments, such as exposure to elevated  
429 UV radiation (Figures 5E–5G).

430

### 431 **DNA repair**

432 UV-B radiation leads to pyrimidine dimers and (6-4) photoproducts, which can be excised and  
433 repaired in nuclear DNA by a group of proteins including ERCC1 and RAD2<sup>55</sup>. While *P. patens* and  
434 *A. thaliana* each encode one ERCC1 and one RAD2, *T. lepidozoioides* underwent a duplication of  
435 each these (Table S2K) about 5.57 Mya and 203.17 Mya, respectively. RAD51 is the eukaryotic  
436 homolog of bacterial RecA and a key factor in homologous recombination and DNA damage  
437 repair<sup>56,57</sup>. We identified five RAD51 genes in *T. lepidozoioides* compared to six in *P. patens* (Data  
438 S2J). In *T. lepidozoioides* a pair of RAD51 (Tle2c04609.1, Tle4c02755.1) arose at about 20.78 Mya,  
439 while paralogs in *P. patens* (Pp3c11\_22000V3.1, Pp3c7\_7920V3.1) separated at about 44.27 Mya.  
440 BRCA1 and BARD1 evolved from a common eukaryotic ancestor and are required for efficient DNA  
441 repair in humans and plants<sup>58</sup>. Like all other plants, except *P. patens* which lacks the gene, *T.*  
442 *lepidozoioides* encodes one BRCA1 (Table S2L). To trace its evolutionary history, we analysed the  
443 BRCA1 and BARD1 homologs in 11 bryophyte species with published genomes (two hornworts, one  
444 liverwort, eight mosses), and found that BRCA1 and BARD1 were lost in the ancestor to other  
445 mosses after *Takakia* separated from their LCA (Data S2K). Taken together, the genome of *T.*  
446 *lepidozoioides* is uniquely equipped with genes enhancing its ability to recover from DNA damage  
447 incurred by exposure to high UV radiations associated with its current habitat at high elevations in  
448 the Tibetan Plateau.

449

### 450 **Phenylalanine ammonia-lyases**

451 *T. lepidozoioides* is rich in phenolic compounds, including flavonoids (Figure 3J; Data S1Y), which are  
452 products involved in the phenylpropanoid pathway<sup>59</sup>. Phenylalanine ammonia-lyase (PAL) is the first  
453 and key enzyme in phenylpropanoid biosynthesis and is expressed in response to various stresses<sup>60</sup>.  
454 *T. lepidozoioides* encodes 20 PALs, more than *A. thaliana* (4), *M. polymorpha* (12) or *P. patens* (16).  
455 Sixteen of them cluster in a subclade, and nine of them are intronless. From these 20 PALs, 15  
456 expanded 50 Mya and the intronless PALs expanded 30 Mya, indicating a recent expansion via  
457 segmental or tandem duplications (Data S2L). In *Takakia*, PAL expression increases under UV-B  
458 stress and reaches the highest level during the recovery from UV-B exposure (Data S2L).

459

### 460 **Cytochromes P450**

461 CYP450s are widespread in eukaryotes. They catalyse various oxygenation / hydroxylation reactions  
462 that require molecular oxygen and depend on the electron transfer from NADPH via NADPH-P450  
463 reductase. CYP450s are involved in a variety of pathways producing primary and specialized  
464 metabolites<sup>61</sup>. In mosses, they are also important for the formation of the cuticle, a complex of  
465 phenol-enriched polymers on the epidermis that enables organogenesis and protects from  
466 dehydration<sup>62</sup>. We identified 71 CYP450s, close to the number in *P. patens* (80). Compared to *P.*  
467 *patens*, several clades of CYP450s such as CYP703A2 , CYP704 and CYP77, which are involved  
468 in fatty acid and hormone metabolism, are expanded in *T. lepidozoides* (Data S2M). This expansion,  
469 dated to 145.21–87.77 Mya (median 136.17 Mya), may explain why its fatty acid content is higher  
470 than that of *P. patens* under normal conditions and under UV stress.

471

## 472 **Genetic basis of phenotypes and development**

### 473 ***Leaf adaxial-abaxial polarity***

474 Most extant land plants have bifacial leaves with adaxial-abaxial (dorsiventral) polarity, which may  
475 have been different in the earliest land plants<sup>63</sup>. *T. lepidozoides* leaves have no such polarity (Data  
476 S1G). Polarity establishment in angiosperms is controlled by HD-ZIPIII TFs and KAN TFs<sup>64,65</sup>. While  
477 the former are present in *T. lepidozoides*, *P. patens* and *A. thaliana*, the latter are missing in *T.*  
478 *lepidozoides* (Table S2M), suggesting that the non-polar leaves resulted from the loss of KAN1  
479 (Figure 6A). The *Takakia* sp. fossil records dated to the Middle-Late Jurassic (165 Mya<sup>66</sup>) help us to  
480 trace the evolutionary history of morphological features in *T. lepidozoides*. The fossil plant shape  
481 (height  $12.48 \pm 2.46$  mm, stem diameter  $0.31 \pm 0.14$  mm; leaf length  $0.88 \pm 0.17$  mm, leaf diameter  
482  $0.04 \pm 0.01$  mm) is similar to that of extant *T. lepidozoides* (height  $11.14 \pm 2.90$  mm, stem diameter  
483  $0.26 \pm 0.08$  mm; leaf length  $0.75 \pm 0.16$  mm, leaf diameter  $0.04 \pm 0.01$  mm) in the experiment site of  
484 Tibet Plateau, and both of them hold similarly lobed terete leaves (Data S2N). Based on data of  
485 morphological characteristics of the fossil and extant *Takakia* plants, we suppose that *Takakia* might  
486 have lost adaxial-abaxial leaf polarity no later than 165 Mya and after the LCA of all mosses. To  
487 better understand the evolutionary history of KAN in mosses, we constructed a timetree using KAN  
488 homologs from 11 bryophyte species with two fossil calibrations and found that the KAN diversity  
489 within mosses occurred 396.15—240.84 Mya (median 314.86 Mya) (Data S2O), displaying these  
490 divergences of KANs did not disturb their function for maintaining the adaxial-abaxial leaf polarity  
491 among mosses. The ancestor of *Takakia* lost KAN likely between 389.92 Mya, when it separated

492 from the LCA of all mosses, and 165 Mya, resulting in the unique leaf morphology of *Takakia* among  
493 all mosses surveyed so far.

494 In addition, jasmonate signaling is important for leaf expansion<sup>63</sup>. From these factors, *T.*  
495 *lepidozoides* lacks MYC2 and JAZ1 (Data S2P; Table S2P). Another possibility is post-  
496 transcriptional regulation of gene expression via microRNAs (miRNAs), as *T. lepidozoides* has only  
497 one DICER1 among three DICER proteins (Data S2Q). In *P. patens*, knockout of *DCL1a* or *DCL1b*  
498 affects stem and leaf differentiation<sup>67</sup>. Together, these deficiencies may explain the deeply lobed leaf  
499 shape in *T. lepidozoides*, which is unique among mosses but found in some liverworts<sup>68</sup>.

500

### 501 **Rhizoids**

502 Rhizoids are haploid filamentous cells that facilitate interaction with the substrate, including water  
503 absorption and nutrient uptake<sup>69</sup>. They share similar regulatory developmental networks to those of  
504 root hairs of angiosperms<sup>70</sup>. Unlike other mosses, *T. lepidozoides* lacks rhizoids (Data S1G and  
505 S1H). The bHLH TFs RHD6 and RSL1 control root hair development in *A. thaliana* and rhizoid  
506 formation in *P. patens*<sup>70</sup>, while null mutants in *P. patens* and *M. polymorpha* lack rhizoids<sup>71,72</sup>. The  
507 Zygnematophyceae *Spirogloea muscicola* and *Mesotaenium endlicherianum*, the likely sister group  
508 to land plants that share the same subaerial/terrestrial habitat with the earliest-diverging bryophytes<sup>73</sup>,  
509 lack rhizoids and RHD6 homologs. At least one homolog of these TFs (RHD6 clade) occurs in each  
510 of the selected land plants, but none in *T. lepidozoides* (Figures 6B and S5A; Table S2N), suggesting  
511 that the loss of rhizoids resulted from the loss of these genes. The *Takakia* sp. fossil lack rhizoids  
512 (Data S2N), thus we suggest that *Takakia* lost rhizoids no later than the Middle-Late Jurassic after  
513 their ancestor separated from the LCA of all mosses. We constructed a timetree using RHD6  
514 homologs from 11 bryophyte species with two fossil calibrations and found that the RHD6 diversity  
515 within mosses occurred 326.81—163.40 Mya (median 246.95 Mya) (Data S2R), the lower boundary  
516 close to the date of fossil 165 Mya.

517

### 518 **Stomata**

519 Stomata are microscopic pores on plant surfaces that facilitate gas exchange, hydration and in *P.*  
520 *patens* the release of spores<sup>7</sup>. They were one of the first developmental innovations of land plants,  
521 at about 400 Mya<sup>2,74,75</sup>. However, *T. lepidozoides* lacks stomata (Data S1T). PpSCRM1 and PpSMF1,  
522 orthologs of bHLH TFs, regulate stomata development in *A. thaliana*, and control stomata formation

523 in *P. patens*, given that their individual knock-out resulted in the lack of stomata<sup>7</sup>. We identified two  
524 SCRM-homologs but no SMF-homologs in *T. lepidozoides* (Figures 6C, S5B–S5D; Table S2O),  
525 suggesting that the lack of stomata is due to a loss of SMF. The *Takakia* sp. fossil is uninformative  
526 for stomata. To better understand the evolutionary history of SMF in mosses, we constructed a  
527 timetree using SMF homologs from 11 bryophyte species with two fossil calibrations and found that  
528 the SMF diversity within mosses occurred 455.07—278.06 Mya (median 367.43 Mya) (Data S2S),  
529 the lower boundary earlier than the date of fossil 165 Mya.

530

### 531 **Tropisms**

532 Plant tropisms rely on auxin gradients<sup>76,77</sup>. In contrast to vascular plants, auxin concentrations in *T.*  
533 *lepidozoides* were highest at the top and lowest at the base of erect shoots, whereas the  
534 concentration remained constant along the rhizomes (Data S2T). The *T. lepidozoides* genome  
535 encodes three PIN proteins possibly related to auxin efflux (Data S2T), but only *TIPIN1*  
536 (Tle1c08012.1) has the classical domain structure. Ectopic expression of these genes in *A. thaliana*  
537 revealed that *TIPIN1* represses root hair growth greatly, *TIPIN2* (Tle4c04210.1) mildly, and *TIPIN3*  
538 (Tle1c06076.1) not at all (Data S2T). AtPIN1 antibodies detected TIPIN proteins on plasma  
539 membranes in cells from leaves and stems with no polar localization (Data S2U), which is different  
540 from leaf cells of *P. patens*<sup>78</sup> and root cells of *A. thaliana*<sup>76,77</sup>. The functional divergence of TIPIN1-3  
541 occurred 134.59–101.08 Mya. The unusual nonpolar localization of TIPIN1 in *Takakia* may account  
542 for its unusual weak negative geotropism, which facilitates the adaptive growth of populations on  
543 rugged and steep surfaces in the absence of rhizoids.

544

## 545 **DISCUSSION**

### 546 **Evolution of specific morphological traits predated the uplift of the Himalayas**

547 *Takakia* has been an enigmatic genus, as it combines a mixture of morphological traits of algae,  
548 liverworts and mosses. Our analysis of the *T. lepidozoides* genome combined with the fossil  
549 evidence suggests that its extant shape evolved earlier than 165 Mya, after their ancestor separated  
550 from the LCA of all other mosses, in which there were at least two periods of severe high  
551 temperatures according to paleometeorological data<sup>79</sup>. This predates the uplift of the Himalayas,  
552 which started 65 Mya. Based on fossils and phylogenomics data, it is believed that extant vascular  
553 plants and bryophytes originated from a more complex ancestral land plant during the Cambrian,

554 515–494 Mya<sup>80</sup>. Thus, we suggest that adaptive evolution of *Takakia* included the loss of stomata,  
555 rhizoids, and leaf polarity, rather than it representing an ancestral state.

556 The determination of leaf shape is controlled by complex molecular regulatory networks, which  
557 are involved in leaf origination, polarity fixation and blade expansion<sup>81,82</sup>. Our analysis of 11 bryophyte  
558 genomes revealed that *T. lepidozoides* encodes gene homologs related to leaf origination and blade  
559 expansion but lost the leaf polarity-determining TF KAN1 earlier than 165 Mya (Data S2O),  
560 suggesting that its ancestor had dorsiventral leaves, and that loss of polarity may be a special  
561 adaptive morphological trait. Likewise, all 11 bryophyte genomes, except *T. lepidozoides*, encode  
562 RHD6, which controls rhizoids development in bryophytes<sup>70</sup>, and diverged 326.81–163.40 Mya  
563 (median 246.95 Mya) (Data S2R). Therefore, we suggest that rhizoid loss is a consequence of the  
564 loss of RHD6 in the unique ancestor of *Takakia*. Fossils and phylogenomic data indicate that stomata  
565 were present in the LCA of land plants, prior to the divergence of bryophytes and tracheophytes, and  
566 subsequently lost multiple times during the diversification of bryophytes<sup>8</sup>. Our analysis of 11  
567 bryophyte genomes reveals that all of them, except *T. lepidozoides*, had genetic traces related to  
568 the TF SMF, which is associated with stomata development in embryophytes<sup>7</sup>, during  
569 455.07–278.06 Mya (median 367.43 Mya) (Data S2S). Therefore, we suggest that the loss of  
570 stomata resulted from the loss of SMF in *T. lepidozoides*, rather than this species never encoded  
571 SMF.

572 Instead of rhizoids that attach plants to the soil matrix, *T. lepidozoides* evolved a morphology  
573 unique among mosses. Plant tropism and branching are closely related to auxin signalling<sup>76,77</sup>.  
574 Aux/IAAs interact with ARFs leading to the repression of ARF-controlled genes, which are involved  
575 in tropisms, braching and root formation<sup>83-85</sup>. The timing history of Aux/IAA expansion (218.62–  
576 142.02 Mya), RHD6 loss (no later than 165 Mya) and PIN divergence (134.59–101.08 Mya) might  
577 reveal the reasons why *Takakia* plants display a weak negative geotropism, continued branching  
578 and randomness of branching positions. The expansion of Aux/IAA genes might have enhanced the  
579 function of Aux/IAA, strengthening the inhibitory effect of ARF-regulated gene expression, which could  
580 lead to weaker effects of auxin sigalling. The timing of RHD6 loss and Aux/IAA divergence may  
581 overlap and follow with the loss of polar PIN localization. Thus, there is no IAA distribution gradient  
582 (Data S2T) in *Takakia* in contrast to the situation in seed plants<sup>86</sup> and *P. patens*<sup>87</sup>. If such a causal  
583 relationship between the three gene families exists could be tested experimentally.

584

585 **The evolution of specific protective traits occurred during the uplift of the Himalayas**

586 While some morphological features characteristic of *Takakia* evolved before 65 Mya, the duplication  
587 and divergence of genes with implications in stress protection occurred mainly during the uplift of  
588 the Himalayas, namely between 50 Mya to present. This period is characterized by a sharp increase  
589 in harmful UV-B radiation and a sharp drop in temperature, both caused by the uplift of the region<sup>88</sup>.

590 The evolutionary deeply conserved RAD51 is a key factor in DNA repair and recombination in  
591 prokaryotes and eukaryotes<sup>56,57</sup>. Its enhanced expression under elevated UV-B levels results in  
592 increasing frequencies of homologous recombination to maintain genome stability in *A. thaliana* and  
593 potato plants<sup>33</sup>. In *Takakia*, two of the five RAD51 genes arose from duplications about 20.78 Mya  
594 when the experimental site was being uplifted to about 4,000 m elevation<sup>88</sup>, suggesting that this  
595 expansion enhances their protective effect on genome stability in responses to elevated UV-B  
596 radiation.

597 UV-B radiation leads to pyrimidine dimers and (6-4) photoproducts, which can be excised and  
598 repaired in nuclear DNA by a group of proteins including RAD2 and ERCC1<sup>55</sup>. Compared with one  
599 RAD2 and ERCC1 gene in all surveyed plants, two paralogs of each occurred in *Takakia* (Table S2K)  
600 from duplication dated to about 203.17 Mya and 5.57 Mya, respectively. While the driving force for  
601 RAD2 expansion remains enigmatic, the expansion of ERCC1 may have been driven by severe UV-  
602 B radiation at high altitude. BRCA1 and BARD1 evolved from a common eukaryotic ancestor and  
603 are required for efficient DNA repair in humans and plants<sup>58</sup>. While all surveyed moss genomes lack  
604 BRCA1 and BARD1, the *T. lepidozoides* genome encodes one BRCA1, suggesting that the common  
605 ancestor of the other mosses lost BRCA1 and BARD1 after their separation from the lineage that  
606 leads to *Takakia* (Data S2K).

607 One strategy of plants to resist UV-B damage is to produce abundant metabolites that effectively  
608 absorb radiation or eliminate ROS, such as accumulate flavones to increase UV-tolerance<sup>89</sup>, phenols  
609 and unsaturated fatty acids to enhance resistance to UV-B<sup>90</sup>. *T. lepidozoides* is rich in phenolic  
610 compounds, including flavonoids (Figure 3J; Data S1Y), which are products involved in the  
611 phenylpropanoid pathway<sup>59</sup>. PAL is the first and key enzyme in phenylpropanoid biosynthesis and is  
612 expressed in response to various stresses<sup>60</sup>. *T. lepidozoides* encodes 20 PALs, more than all  
613 surveyed plants, 15 of them expanded within 50 Mya and intronless PALs expanded 30 Mya,  
614 indicating a recent expansion via segmental or tandem duplications (Data S2L) likely in response to  
615 the changing environment during the uplifting of the Himalayas. Expression of these PALs increases

616 under UV-B stress and peaks during the recovery from this stress (Data S2L), resulting in the  
617 production of several flavonoids (Table S1F).

618 The uplift of the Himalayas has caused the annual average temperature in the area of our  
619 experimental site to drop from 27 °C to about 5 °C<sup>88</sup>, forcing *Takakia* plants to withstand freezing.  
620 CSD proteins are evolutionary deeply conserved in prokaryotes and eukaryotes<sup>46</sup>. In *Arabidopsis*,  
621 CSD proteins negatively regulate freezing tolerance as the null mutants showed higher freezing  
622 tolerance than WT when cold-acclimated. The increase in freezing tolerance was associated with  
623 up-regulation of CBF transcription factors and their downstream genes<sup>47</sup>. We constructed a timetree  
624 using CSD homologs from 11 bryophyte species with two fossil calibrations and found that the CSD  
625 diversity within mosses occurred 463.43—338.08 Mya (median 400.37 Mya) (Data S2V), the lower  
626 boundary earlier than the date of fossil 165 Mya. So we supposed the ancestor of *Takakia* may lost  
627 CSD after their ancestor separated from other mosses LCA, suggesting that CSD loss may help  
628 *Takakia* plants to withstand harsh conditions, such as being covered by snow for eight months at -  
629 4 °C.

630

### 631 **Limitations of the study**

632 Our decade long survey of the experimental site at 4 km altitude, together with physiological, cell  
633 biological, transcriptomic and genomic data comprehensively reveal the peculiar features of the so  
634 far enigmatic genus *Takakia*. A combination of molecular clock analyses and paleometeorological  
635 data provide evidence for molecular adaptations to the harsh environment during the uplift of the  
636 region that started 65 Mya. Nevertheless, we can not explain the current population decline in  
637 response to global warming. Remarkably, many of the morphological adaptations likely occurred  
638 earlier than 165 Mya Therefore, similar studies should be performed with relictual *Takakia*  
639 populations from different regions, such as Japan and North America. Such studies, together with  
640 our planned sequencing of more *Takakia* genomes from our 52 experimental quadrants will provide  
641 insights into genetic diversity and population dynamics. Subsequent selective-sweep and genome-  
642 wide association studies may lead to a better understanding of the causes underlying the rapid  
643 decline of this species in response to current global warming.

644

645 **Figure titles and legends**

646 **Figure 1. Geomorphologic and meteorological characteristics of the area in the Tibetan**  
647 **Plateau harboring *T. lepidozoides* populations.**

648 (A) The geomorphologic view of the survey area: exact location of experimental plots (red dots) in  
649 the area of ~18 km<sup>2</sup> near Gawalong East Glacier, Lat 29°45' N, Long 95°42' E, altitude 3,800–4,400  
650 meters, at Bomi County, Tibet, China, yellow dotted line marks the treeline position in survey area.  
651 (B) A population of *T. lepidozoides* growing on the surface of a cliff in area of Plot C (bar = 1 cm).  
652 (C) *Takakia* plants with capsules (red arrow) in one population of Plot B (bar = 5 mm). (D)  
653 Temperature change in the survey area from 1960 to 2021, with T° rising at a rate of 0.32°C per  
654 decade. (E) Temperature change per year in the sample plot from 2010 to 2021 (mean ± s.d.; n = 6  
655 investigated plots), the gray shadow area shows the period during which *Takakia* plants were  
656 covered by snow. (F) The coverage of *T. lepidozoides* population decreased continually at a rate of  
657 1.6% per year from 2010 to 2021. The vertical axis represents the relative coverage of *T.*  
658 *lepidozoides* in observation quadrants (n = 52), and the dotted line marks the relationship between  
659 the *T. lepidozoides* population size and year. The central mark of each box indicates the median,  
660 and the bottom and top edges of the box indicate the 25th and 75th percentiles respectively. The  
661 whiskers extend to the most extreme data points that are not considered as outliers.

662

663 **Figure 2. Branch origin and potential vessel elements connect in stems of *T. lepidozoides***  
664 **and in roots of *A. thaliana*.**

665 (A) The dome originates from cells of the epidermis (yellow 8 and white 6) and sub-epidermis (yellow  
666 7", 7', 7 and white 5) of parent stem and gradually elongates to form a branch, with potential vessel  
667 elements connected vertically (blue arrow), bar = 50 μm. (B) The parent stem and the branch  
668 comprise eight row cells, the vertical walls between epidermal cells (yellow arrows), the inclined walls  
669 between potential vessel elements in stem (white arrows) and at branch point (blue arrows), bar =  
670 50 μm. (C) Evans blue staining in the median longer cells in stem (arrow marks the branch point in  
671 the box of G), bar = 50 μm. (D) The connection of vessel elements between main root (white arrow)  
672 and lateral root (yellow arrows) in *Arabidopsis*, bar = 30 μm. (E) There is no direct connection of  
673 vessel elements between the stem (white arrows) and branch (yellow arrows) in *Arabidopsis*,  
674 between main stem (white arrows) and branch (yellow arrows) in *Arabidopsis*, bar = 500 μm. (F) The  
675 net structure population, green parts can maintain photosynthesis for 3 ~ 5 years, gradually aging

676 and death (blue arrow), and eventually become humus (white arrow), bar = 0.5 cm. (G) One part of  
677 an individual from F (bar = 2 mm), erect stems (red arrows), rhizomes (blue arrows).

678

679 **Figure 3. Physiological and biochemical properties in response to intense UV-B radiation in**  
680 **the two mosses *T. lepidozoides* and *P. patens*.**

681 Shoot phenotypes of *T. lepidozoides* (A–C) and *P. patens* (D–F) under normal conditions (A, D) 144  
682 kJ m<sup>-2</sup> UV-B treatment for 2 hours (B, E) and recovery (R) on the 3<sup>rd</sup> day (C, F) (bars = 1 mm). (G,  
683 H) Unsaturated fatty acid compositions under UV-B treatment and at different points of recovery in  
684 *T. lepidozoides* (G) and *P. patens* (H), red color represents MGDG, blue color represents PC, green  
685 color represents neutral lipids, purple color represents DGTS. (I) Polyphenol contents under UV-B  
686 treatment and recovery in *T. lepidozoides* (blue) and *P. patens* (red). (J) Flavonoid contents under  
687 UV-B treatment and recovery in *T. lepidozoides* (blue) and *P. patens* (red). (K) Active unit of catalase  
688 (CAT) under UV-B treatment and recovery in *T. lepidozoides* (blue) and *P. patens* (red). (L)  
689 Concentrations of malondialdehyde (MDA) under UV-B treatment and recovery in *T. lepidozoides*  
690 (blue) and *P. patens* (red). (M) The maximum quantum efficiency of PSII photochemistry (Fv/Fm)  
691 under UV-B treatment and recovery in *T. lepidozoides* (blue) and *P. patens* (red). (N) The actual  
692 photochemical quantum efficiency (Fv/Fo) under UV-B treatment and recovery in *T. lepidozoides*  
693 (blue) and *P. patens* (red). Western blot of photosystem components in *T. lepidozoides* (O) and *P.*  
694 *patens* (P) under normal condition, UV-B treatment, and recovery.

695

696 **Figure 4. Organization and characteristics of the *T. lepidozoides* genome.**

697 (A) Circos plot of the *T. lepidozoides* genome, extension from outer to inner showing the  
698 chromosomes with pentatricopeptide repeat regions (black band), GC contents in every 100 kb  
699 sliding window (purple histogram), transposable elements densities in every 100 kb sliding window  
700 (coral histogram), gene densities in every 100 kb sliding window (blue histogram), differential gene  
701 expression in UV radiations (solid line in red represents genes up-regulated, solid line in green  
702 represents genes down-regulated) and chromosomal syntenic relationships (solid lines with colors  
703 represent inter-chromosome collinearity, solid line in grey represents intra-chromosome collinearity).  
704 (B) WGD and divergence of *T. lepidozoides* indicated by Ks distribution. (C) The Ka/Ks ratio  
705 distributions of gene pairs derived from different modes of duplication. (D) *T. lepidozoides* time tree,  
706 numbers on the nodes represent divergence time, light purple bars show 95% confidence interval

707 length, nodes with black dots show fossil calibration points, the ordinal phylogeny structure with  
708 branch length shown on the top left, full phylogeny tree with branch length shown in Data S2F.

709

710 **Figure 5. PPR features in *T. lepidozooides*.**

711 (A) Classification of all PPRs in eight plant genomes based on structural motifs. (B) Word cloud of  
712 PPRs subcellular localization prediction shows that most of PPR proteins are targeted to  
713 mitochondria or chloroplasts. (C) RNA editing as confirmed by 30x sequence analysis, PPR proteins  
714 influence expression of mitochondria or chloroplasts transcripts by altering RNA sequence in *T.*  
715 *lepidozooides*. Most abundant RNA edit change is C to U nucleotides. (D) Expression levels of PPRs  
716 show significant differences in response to UV-B radiation and during recovery. (E) Gene ontology  
717 (GO) enrichment word cloud (biological process) of PPRs in response to UV-B radiation and during  
718 recovery. (F) GO enrichment word cloud (cellular component) of PPRs in response to UV-B radiation  
719 and during recovery. (G) GO enrichment word cloud (molecular function) of PPRs in response to UV-  
720 B radiation and during recovery.

721

722 **Figure 6. Genetic foundation of morphological features in *T. lepidozooides* versus in *P. patens*  
723 and *A. thaliana*.**

724 (A) Proteins regulating leaf (sporophytic) and phylloid (gametophytic) development. *T. lepidozooides*  
725 has terete leaves (phylloids) with no adaxial-abaxial polarity, probably due to the absence of the  
726 transcription factor KAN. Dashed lines with gene names mark the genes absent in the pathway,  
727 arrows indicate positive/promoting regulation, lines with perpendicular end bars indicate  
728 negative/repressing regulation, and crosses indicate that the pathway is blocked or absent. (B)  
729 Proteins regulating root hair (sporophytic) and rhizoid (gametophytic) development. *T. lepidozooides*  
730 has no rhizoids, probably due to the lack of the transcription factor RHD6. (C) Proteins regulating  
731 stomatal development. *T. lepidozooides* lacks stomata, probably due to the missing transcription  
732 factor SMF. In comparison to *A. thaliana*, *P. patens* lacks the transcription factor SCAP, probably  
733 resulting in less complex stomata. In both plants, stomata are restricted to the sporophyte; leaves in  
734 *A. thaliana* and spore capsules in *P. patens*.

735

736 **STAR Methods**

737 **RESOURCE AVAILABILITY**

738 **Lead contact**

739 Further information and requests for resources and reagents should be directed to and will be fulfilled  
740 by the lead contact, Yikun He (yhe@cnu.edu.cn).

741

742 **Materials availability**

743 This study did not generate new unique reagents. Clones of the *Takakia lepidozoides* plant that was  
744 sequenced are deposited at the International Moss Stock Center IMSC (<https://www.moss-stock-center.org/>) under accession number 41271.

746

747 **Data and code availability**

748 Whole-genome assembly and raw reads of *T. lepidozoides* have been deposited in the National  
749 Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov/>) under BioProject  
750 accession number PRJNA796444, the pseudomolecules genome with GenBank accession number  
751 JAKLZA000000000, the raw reads of DNA and RNA sequencing with Sequence Read Archive  
752 accession number SRP354614 (Illumina PCR-free reads SRR17573914—SRR17573930 and  
753 PacBio reads SRR17573931—SRR17573932). The genome assembly and raw data was also  
754 deposited in National Genomics Data Center (NGDC, <https://ngdc.cncb.ac.cn/?lang=en>) under  
755 BioProject accession number PRJCA014819, the genome with Genome Warehouse accession  
756 number GWHBRAK000000000 and the raw sequences data with Genome Sequence Archive  
757 accession number CRA009836. Metabolomics raw data of *T. lepidozoides* have been deposited in  
758 MetaboLights (<https://www.ebi.ac.uk/metabolights/>) under accession number MTBLS6999. The  
759 *Takakia* genome assembly sequences and gene annotations are available at  
760 <https://www.takakia.com>. This study does not report original code. All other data and materials are  
761 available from the corresponding authors upon reasonable request.

762

763 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

764 Natural samples of *T. lepidozoides* with mature capsules was collected from a shady, moist rock on  
765 Galongla Mountain, Bomi County, Tibet, China (29°45' N, 95°42' E) in October 2005 by Xuedong Li  
766 and Meizhi Wang. The *T. lepidozoides* tissue culture plantlets were grown in the greenhouses at

767 Beijing Key Laboratory of Plant Gene Resources and Biotechnology for Carbon Reduction and  
768 Environmental Improvement, College of Life Sciences, Capital Normal University, Beijing, China  
769 (39°56' N, 116°18' E) since 2005. The field study was carried out in Bomi County, Tibet, China (29°45'  
770 N, 95°42' E), altitude from 3,800 to 4,400 m since 2005.

771

## 772 **METHOD DETAILS**

### 773 **Field study**

774 The study site is located in Bomi County, Tibet, China (29°45' N, 95°42' E), altitude from 3,800 to  
775 4,400 m, each plot is 50 m × 50 m with 100 m interval of altitude. At each plot, three east-west  
776 transects of 10 m × 50 m with 10 m interval and three altitudinal gradient transects of 10 m × 50 m  
777 with 10 m interval were established. All 10 cm × 10 cm observation quadrants of *Takakia*  
778 *lepidozoides* were established randomly in the transect. The growing season in the site runs from  
779 late June to middle of October, quadrant records were collected twice at the beginning and end of  
780 the growing season each year. Real-time image recorders for quadrant and glacier were collected  
781 by outdoor wildlife cameras (TimelapseCams, Wingscapes). The meteorological data and sun  
782 radiation data were collected real-time by a microclimate station (MAWS830-FC, Hua Yun Instrument;  
783 L99, Hangzhou Luge Ltd.) and a radiant heat balance station (HYZY-SP, Hua Yun Instrument).  
784 Remote sensing observation data of Resources satellite three (Resources-3) and Gaofen-1 satellite  
785 (Gaofen-1) were collected from the National Geographical Information Monitoring Cloud Platform  
786 (<http://www.dsac.cn/>). The glacier ablation was calculated as

$$787 |distance| = \frac{\sqrt{(longitude_m - longitude_n)^2 + (latitude_m - latitude_n)^2}}{day_m - day_n} \times 365.$$

788 The Shannon-Wiener index was calculated as  $H' = \sum(P_i) (\ln P_i)$ , where  $P_i$  represented the  
789 coverage of species  $i$  in an observation quadrant. Spearman's rank correlation was used to analyze  
790 possible correlations among species, redundancy and canonical correspondence analysis to  
791 determine the effects of climatic variables on the population changes, where only commonly found  
792 species were considered. All multiple regressions and backward eliminations were performed by  
793 vegan package (version 2.5-7) (<https://CRAN.R-project.org/package=vegan>) in R. The potential  
794 distribution of *T. lepidozoides* was predicted using the ecological niche modeling software MaxEnt  
795 (version 3.4.1) ([https://biodiversityinformatics.amnh.org/open\\_source/maxent/](https://biodiversityinformatics.amnh.org/open_source/maxent/)) combined with data  
796 of the global climate from WorldClim (<https://www.worldclim.org/>). The 'Random test percentage' was  
797 set as 25, and 'Make pictures of predictions' and 'Do jackknife to measure variable importance' were

798 chosen, the remaining model values were set to default values in the initial model; ‘Random seed’  
799 was chosen, and 100 replicate models were run in the final model.

800 Soil samples were collected from four experimental plots; a 5-point cross sampling protocol was  
801 carried out in each plot. In brief, each plot was divided by a rectangle grid with a spacing distance of  
802 1 m, samples were retrieved from the top 5-cm surface soil in the four corner grids and the middle  
803 grid, then mixed by plot to measure the soil elemental compositions. Soil elemental compositions  
804 were analyzed using Inductively Coupled Plasma–Mass Spectrometry (iCAP TQ ICP–MS, Thermo  
805 Scientific), Inductively Coupled Plasma–Optical Emission Spectrometry (iCAP 7400 ICP–OES,  
806 Thermo Scientific) and X-ray fluorescence (ARL PERFORM’X, Thermo Scientific) to determine the  
807 content of macro- and micronutrients as well as toxic elements<sup>91,92</sup>.

808

### 809 **Plant materials**

810 Samples of *T. lepidozoides* with mature capsules were collected from a shady, moist rock on  
811 Galongla Mountain, Tibet, China. Healthy capsules were selected and surface-sterilized as follows:  
812 (1) five 3-min rinses with sterilized water, (2) five rounds of sterilization for 5 s with 75 % ethanol and  
813 washing for 1 min with sterilized water, (3) sterilization with 0.05 % HgCl<sub>2</sub> solution for 2 min and (4)  
814 five 1-min washes with sterilized water. The capsules were transferred into sterilized water to prepare  
815 a spore suspension, which was inoculated onto the surface of Beneke’s medium with some  
816 modification (200 mg L<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>, 100 mg L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 100 mg L<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 100 mg L<sup>-1</sup>  
817 CaCl<sub>2</sub>·2H<sub>2</sub>O, 15 mg L<sup>-1</sup> FeSO<sub>4</sub>·7H<sub>2</sub>O) containing 0.5 % (w/v) glucose (pH 5.8), and were cultivated  
818 under the following conditions: 14/10 °C day/night temperature, a 12-h/12-h light - dark photoperiod,  
819 illumination at 100 μmol photons m<sup>-2</sup> s<sup>-1</sup>, and approximately 85 % relative humidity (RH). Plants were  
820 transferred to the same medium and incubated under the same light and temperature conditions  
821 mentioned above every 2 weeks. The *Physcomitrella patens* wild-type “Gransden 2004” was grown  
822 on BCD medium (1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 1 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 45 μM FeSO<sub>4</sub>·6H<sub>2</sub>O, 10 mM KNO<sub>3</sub>, 0.22 μM  
823 CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.19 μM ZnSO<sub>4</sub>·7H<sub>2</sub>O, 10 μM H<sub>3</sub>BO<sub>3</sub>, 0.1 μM Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 2 μM MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.23  
824 μM CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.17 μM KI) containing 0.5 % (w/v) glucose (pH 6.5) with a light intensity of 100  
825 μmol m<sup>2</sup> s<sup>-1</sup> delivered in a 16-h/8-h light/dark cycle at 25 °C. Plants were transferred to the same  
826 medium and incubated under the same light and temperature conditions every 2 weeks, and the 2-  
827 weeks-old gametophytes were used as the material for future experiments, unless specifically  
828 mentioned. Seeds of *Arabidopsis thaliana* wild-type accession Col-0 lines were germinated on MS

829 medium (Sigma-Aldrich, M2909) containing 2 % (w/v) glucose (pH 6.0), and the plants were  
830 subsequently transferred to soil and grown in a growth chamber at 22 °C with 100  $\mu\text{mol photons m}^{-2}$   
831  $\text{s}^{-1}$ , a photoperiod of 16-h/8-h light/dark, and RH of 75%.

832

### 833 **UV-B treatment**

834 For UV-B irradiation the following plants were used: *A. thaliana* Col-0 25 days after germination in  
835 normal condition, plants of *T. lepidozoides* and *P. patens* grown for 24 h under 50  $\mu\text{mol photons m}^{-2}$   
836  $\text{s}^{-1}$  with the equivalent temperature and RH described above before starting the treatment. To all  
837 plants 20  $\text{W m}^{-2} \text{s}^{-1}$  UV-B was provided (2 x 312 nm XX-15B UV lamp, Spectronics) for 2 h. After UV-  
838 B irradiation, controls and UV-B-exposed samples were collected immediately, then plants were  
839 transferred to the normal condition, recover samples were collected at 6, 12, 24 hours and 3, 5, 7  
840 days after UV-B treatment.

841

### 842 **Cytological observations**

843 Samples for ultrastructural observation of cells were carried out according to Xiao et al.<sup>93</sup> with some  
844 modifications. Control and UV-B treated plants were fixed with 2 % glutaraldehyde in 0.1 M  
845 Sorensen's phosphate buffer for 24 h, dried under vacuum for 2 h, post-fixed in 1 % (w/v) osmium  
846 tetroxide for 2 h, and then dehydrated for 30 min in an ethanol to acetone gradient. The samples  
847 were then divided into two portions. One portion was embedded overnight in Spurr's low-viscosity  
848 resin. The embedded samples were dried at 40 °C for 2 days and then at 70 °C for 24 h. Sections  
849 were prepared with an ultramicrotome (EM UC6, Leica), stained with 1 % aqueous uranyl acetate  
850 and 0.1 % aqueous lead citrate for 30 min each, and examined with a transmission electron  
851 microscope (H7500, Hitachi). The other sample portion was air-dried overnight in a laminar flow  
852 hood, sputter-coated with gold-palladium for 90 s at 2.2 kV and examined with a scanning electron  
853 microscope (S4800, Hitachi).

854 The chromosome preparation was conducted according to a previous study<sup>94</sup>. Shoot tips of  
855 vigorously growing plants were harvested and fixed with Cannoy Solution (ethanol:acetic acid = 3:1)  
856 overnight. After washing with distilled water, the shoots were digested in a mixture of 2% cellulose  
857 (Sigma-Aldrich) and 1% pectinase (Sigma-Aldrich) at 37 °C for 90 min. After that, they were quickly  
858 rinsed in distilled water and fixed with the same fixation solution. The samples were spread on  
859 precooled slides and flame dried. Chromosomes were counterstained with 4',6-diamidino-2-

860 phenylindole (DAPI, Sigma-Aldrich) in an anti-fade solution (Vector Laboratories, H-1200-10).  
861 Images were captured under a Zeiss fluorescence microscope with a micro-charge-coupled camera  
862 (Imager A2, Zeiss).

863

#### 864 **Immunoblot of photosynthetic proteins**

865 Thylakoid membranes proteins were extracted as described<sup>95,96</sup>. Plants leaves were collected and  
866 ground under liquid nitrogen, then homogenized in an ice-cold isolation buffer containing 125 mM  
867 Tris-HCl pH 8.8, 1% (w/v) SDS, 10% (v/v) glycerol, 50 mM Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> with a chilled mortar and pestle  
868 and filtrated through two layers of cheesecloth. The filtrate was centrifuged at 5,000g for 10 min. The  
869 thylakoid pellets were washed with isolation buffer and recentrifuged. The thylakoid membranes  
870 were washed in 330 mM sorbitol and 50 mM BisTris-HCl, pH 7.0, and suspended in resuspension  
871 buffer (20% glycerol and 25 mM BisTris-HCl, pH 7.0) at 1.0 mg chlorophyll/mL. An equal volume of  
872 resuspension buffer containing 2% (w/v) DM was added to the thylakoid suspension in a dropwise  
873 manner. After incubation at 4°C for 5 min, insoluble material was removed by centrifugation at  
874 12,000g for 10 min. The supernatant was combined with one-tenth volume of 5% Serva blue G in  
875 100 mM BisTris-HCl, pH 7.0, 0.5 M 6-amino-n-caproic acid, and 30% (w/v) glycerol and applied to  
876 0.75-mm-thick 6 to 12% acrylamide gradient gels in a Hoefer Mighty Small vertical electrophoresis  
877 unit connected to a cooling circulator. For two-dimensional analysis, excised BN-PAGE lanes were  
878 soaked in SDS sample buffer and 5% β-mercaptoethanol for 30 min and layered onto 1-mm-thick  
879 15% SDS polyacrylamide gels containing 6 M urea. After SDS-PAGE, proteins were transferred to  
880 nitrocellulose membranes (Amersham Biosciences). The membranes were incubated with specific  
881 antibodies and visualized using the enhanced chemiluminescence method. The antibodies of  
882 AtCP47 and AtPsaD were from Liu et al.<sup>97</sup>.

883

#### 884 **Immunofluorescence labeling**

885 *T. lepidozoides* plants were fixed in FAA (3% w/v formaldehyde, 5% v/v acetic acid, 50% v/v ethanol)  
886 at room temperature overnight, embedded in STEEDMAN's wax after dehydration, and sectioned  
887 as described<sup>98</sup>. Rehydrated sections were then subjected to an antigen retrieval procedure<sup>99</sup>. Slides  
888 were first incubated in blocking buffer (2% nonfat milk in PBST) for 1 h, then with anti-AtPIN1  
889 antibody (PhytoAB, PHY1046S) at a dilution of 1:100 in blocking buffer with 2% normal goat serum  
890 for 5 h. Slides were washed with PBST buffer three times, each for 10 min, and then incubated for 2

891 h with an Alexa-fluor-488-conjugated anti-rabbit secondary antibody at a dilution of 1:200 (Invitrogen).  
892 After washing, the slides were mounted using ProLong Diamond Antifade solution (Life  
893 Technologies). A Leica DM5500 multifunctional microscope equipped with an Andor Zyla  
894 monochrome CCD and filter cubes for GFP and chlorophyll were used for fluorescence micrograph  
895 capture.

896

### 897 **Transgene constructs**

898 The *Arabidopsis EXPA7* (AT1G12560) promoter<sup>100</sup> and the 35S promoter were used to drive all PIN  
899 related transgenes. The binary vector *pCAMBIA1300-GFP-NOS* was used as the cloning vector.  
900 The *ProEXPA7:GFP-NOS* vector was generated by replacing the 35S promoter region with the  
901 genomic *EXPA7* promoter fragment by PCR from genomic DNA of *Arabidopsis* in the binary vector  
902 *pCAMBIA1300-GFP-NOS* using the primer sets in Table S2Q. The *Pro35S:AtPIN2* (AT5G57090),  
903 *ProEXPA7:AtPIN2*, *PpPINB* (Pp3c24\_2970V3.1), *TIPIN1* (Tle1c08012.1), *TIPIN2* (Tle4c04210.1),  
904 and *TIPIN3* (Tle1c06076.1)-*GFP* constructs were generated by connecting the *Pro35S* and  
905 *ProEXPA7* promoter to *PIN* coding regions by PCR from cDNA of corresponding species in the binary  
906 vector *pCAMBIA1300-GFP-NOS*. Plants were transformed with the floral dip method<sup>101</sup>. Root hairs  
907 of transgenic plants were observed as described<sup>102</sup>. For estimation of root hair length, digital  
908 photographs of roots were taken under a stereomicroscope (Zeiss, AXIO Zoom. V16) at 40x to 50x  
909 magnifications. The hairs in the hair maturation region were observed.

910

### 911 **Observation of branching origin**

912 To analyze the characteristics of the origin of the branches (stem and root) and the connection of the  
913 conducting elements at the cross-point between stems, the mPS-PI staining technique<sup>103</sup>, in which  
914 polysaccharides (especially cell walls) were specifically labeled with propidium iodide, with some  
915 modifications was used to reconstruct 3D images of plant organs. In brief, *T. lepidozoides* and *A.*  
916 *thaliana* organs were obtained and fixed in fixative (50% methanol and 10% acetic acid) at 4 °C  
917 overnight. After washing in ddH<sub>2</sub>O for three times, samples were incubated in 1% periodic acid at  
918 room temperature for 40 min. Next, samples were rinsed with ddH<sub>2</sub>O and incubated in Schiff's  
919 reagent (100 mM sodium metabisulphite and 0.15 N HCl) containing 100 mg/mL propidium iodide  
920 for 1 to 2 h. Finally, samples were transferred to glass bottom dishes and cleaned with a chloral  
921 hydrate solution (4 g chloral hydrate, 1 mL glycerol, and 2 mL water) for additional 1–2 d.

922 Fluorescence images were obtained using a Leica SP8 laser scanning confocal microscope, with  
923 the excitation wavelength at 561 nm and emission wavelength from 570 nm to 670 nm.

924

### 925 **Assay of macromolecule transport**

926 The Evans blue staining technique<sup>104</sup> with some modifications was used to monitor how  
927 macromolecules are transported in stems of *T. lepidozooides*. Plants from Tibet were collected and  
928 incubated in Evans blue staining buffer (3% w/v Evans Blue, 50 mM NaH<sub>2</sub>PO<sub>4</sub>) at room temperature  
929 for 3 min, followed by washing four times with deionized water. Digital photographs of stems were  
930 taken under a stereomicroscope (BA600-4, Motic).

931

### 932 **Measurement of free IAA**

933 IAA measurements were carried out according to Wang et al.<sup>105</sup> with some modifications. In brief,  
934 the tip, middle and base fragments were isolated separately from erects and rhizomes of *T.*  
935 *lepidozooides* plants under normal condition for 3 months subculture and collected, 200 mg (fresh  
936 weight) per sample were weighted, homogenized under liquid nitrogen, and extracted at -20 °C for  
937 24 h with 2 mL of cold methanol containing antioxidant and <sup>2</sup>H<sub>2</sub>-IAA (internal standard, CDN Isotopes).  
938 Purification was performed with Oasis MAX solid-phase extract cartridge (150 mg/6 cc; Waters).  
939 Measurement of free IAA was carried out using a UPLC-MS/MS system consisting of a UPLC system  
940 (ACQUITY UPLC; Waters) and a triple quadruple tandem mass spectrometer (Quattro Premier XE;  
941 Waters).

942

### 943 **Physiological experiments**

944 To estimate physiological changes under UV-B radiation, control and UV-B treated plants were  
945 collected and the contents of polyphenol (A143-1-1, plant total phenol test kit), catalase (A007-1-1,  
946 catalase assay kit), flavonoid (A142-1-1, plant flavonoids test kit) and malondialdehyde (A003-3-1,  
947 plant malondialdehyde assay kit) were measured using detection kits from the Nanjing Jiancheng  
948 Bioengineering Institute. Chlorophyll fluorescence was measured using a chlorophyll fluorometer  
949 (IMAGING-PAM M-series, Heinz Walz).

950 Two-dimension thin layer chromatography (2D-TLC) and gas chromatography/mass  
951 spectrometry (GC-MS) were employed to detect and identify total fatty acids<sup>106</sup>. In brief, plants were  
952 grinded in chlorohydrocarbon and a methyl alcohol mixture (1:2) at 4 °C, then mixed with 1M KCl to

953 a final volume ratio at 1:1:0.9, centrifuged for 10 min at 2,500 g. Subsequently, the organic phase  
954 was removed with N<sub>2</sub>. 20 × 20 cm × 250 μm glass Si gel plates (Silica Gel GF Uniplates, Sigma)  
955 were activated for 1 h at 100 °C and stored in a desiccator to increase reproducibility. Crude extract  
956 material of lipids applied to form a single small spot (2–4 mm diameter) in a corner 1 cm from either  
957 of two sides of a Si gel plate, then TLC plates were placed in a solvent chamber (CHCl<sub>3</sub>:CH<sub>3</sub>OH:H<sub>2</sub>O,  
958 65:25:4, v/v/v), and the extract spot was allowed to develop along the left-hand side of the plate.  
959 TLC plates were then removed from the solvent chamber immediately before the solvent front  
960 reached the top of the plates. These plates were allowed to air-dry and subsequently placed in  
961 another solvent chamber (CHCl<sub>3</sub>:CH<sub>3</sub>OH:NH<sub>3</sub>·H<sub>2</sub>O, 65:35:5, v/v/v) so that the developed lane of  
962 extract was placed horizontally just above the solvent. Each plate was allowed to develop until the  
963 solvent front reached 1 cm from the top of the plate and then removed from the chamber and air-  
964 dried. Sample spots were collected and mixed with 10 μg N-heptadecanoic acid, 5 μg butylated  
965 hydroxytoluene, 1.5 mL sulfuric acid: methyl ester (5:95), 85 °C for 1 h and then with 1 mL H<sub>2</sub>O, 2  
966 mL N-pentane, centrifuged for 10 min at 3,000 g. Subsequently, the water phase was removed. GC-  
967 MS (Trace GC 2000-Trace DSQ, Thermo Finnigan) was performed using a quartz capillary column.  
968 The heating program used an initial temperature of 50 °C, increasing at a rate of 10 °C/min to 150 °C  
969 and maintain 2 min, then increasing to 280 °C at a rate of 3 °C/min and maintain 10 min, then  
970 maintained at 250 °C until the running was completed. Individual compounds were identified by  
971 comparing the MS fragmentation patterns with those provided by the National Institute of Standards  
972 and Technology (<https://chemdata.nist.gov/>).

973 To classify the oil body components, the oil bodies were separated according to Zhang et al.  
974 (2010). In brief, plants were collected and washed three times with 50 ml 0.001% PBS Triton-X100;  
975 then washed with 50 ml buffer A (25 mm Tricine, pH 7.6, 250 mm sucrose, and 0.2 mm  
976 phenylmethylsulfonyl fluoride); homogenized using a polytron (Cole-Parmer® Labgen™ 125 and  
977 700 Tissue Homogenizers) in 10 ml buffer A, and centrifuged at 1,000 × g for 30 s; supernatant was  
978 homogenized again by nitrogen cavitation (Ashcroft Duralife Pressure Gauge) at 500 psi for 15 min  
979 on ice, and then centrifuged at 1,000 × g for 10 min; nine milliliters of this postnuclear supernatant  
980 was loaded into an SW40 tube, and 3 ml buffer B (20 mm HEPES, pH 7.4, 100 mm KCl, and 2 mm  
981 MgCl<sub>2</sub>) was overlaid on top. The tube was then centrifuged at 12,628 × g for 1 h at 4 °C. The oil body  
982 fraction was carefully collected from the top layer and washed three times with 200 μl buffer B. The  
983 oil body components were identified using 2D-TLC.

984

985 **Non-targeted metabolome profile**

986 Plants were collected and milled with 80% methyl alcohol and dried with N<sub>2</sub>. 25 mg powder per  
987 sample were solved with 50% acetonitrile. Chromatographic separation was performed on a Thermo  
988 Ultimate 3000 (Thermo Fisher Scientific) with 100 mm x 2.1 mm x 1.8 μm C18 column (ZORBAX  
989 SB-Aq, Agilent Technology). The chromatographic conditions were set as follows: flow rate of 0.3  
990 mL/min, column temperature of 40 °C, and injection volume of 4 μL. The mobile phase comprised  
991 water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), by using the following  
992 solvent gradient system: 0.0–6.0 min, 5–20% B; 6.0–10.0 min, 20–95% B; 10.0–11.0 min, 95–95%  
993 B; 11.0–11.5 min, 5% B; 11.5–14.5 min, 5% B.

994 All samples were run on a Q Exactive Orbitrap-mass spectrometer (Thermo Fisher Scientific)  
995 equipped with a heated electrospray ionization (ESI) interface (HESI-II). The QC sample was  
996 injected every six samples. Data with a mass range of m/z 70–1000 were acquired at positive and  
997 negative switching modes with data dependent MS/MS. The full scan and fragment spectra  
998 resolution were 70,000 FWHM and 17,500 FWHM, respectively. The ion source parameters were as  
999 follows: capillary temperature of 320 °C, vaporizer temperature of 350 °C, spray voltage of  
1000 3500/3000 V(+/-), sheath gas flow rate of 40 Arb, and the auxiliary gas flow rate of 5 Arb. The  
1001 acquisition mode of stepped NCE (normalized collision energy) was set to 15, 40 and 60, respectively.  
1002 Raw LC-MS data were processed by Compound Discoverer 3.2 (Thermo Fisher Scientific).  
1003 Compounds were identified by comparing the MS/MS spectra with those in mzCloud  
1004 (<https://www.mzcloud.org>) and ChemSpider (<http://www.chemspider.com/>).

1005

1006 **Genome and transcriptome sequencing**

1007 Total DNA for *de novo* genome sequencing was extracted from young gametophytes produced from  
1008 a single spore. For transcriptome sequencing we sampled tissues from *T. lepidozoides* including  
1009 gametophytes in different growth stages and under different growth conditions, including  
1010 protonemata, phylloids (leaves) at 7, 14, 21, 28, 35 and 42 days as well as phylloids under 144 kJ  
1011 m<sup>-2</sup>, 288 kJ m<sup>-2</sup>, 432 kJ m<sup>-2</sup>, 576 kJ m<sup>-2</sup>, 720 kJ m<sup>-2</sup> UV-B radiations and recovery in normal conditions  
1012 for 6 h.

1013 For PacBio sequencing, we prepared approximately 20-kb SMRT cell libraries. A total of 25.9  
1014 Gb data was sequenced on the PacBio system. All transcriptome libraries were sequenced using

1015 the Illumina platform. For the Hi-C sequencing and scaffolding, a Hi-C library was created from young  
1016 gametophytes of *T. lepidozoides* under normal conditions. In brief, the gametophytes were fixed with  
1017 formaldehyde and lysed, and the cross-linked DNA was then digested with Mbol overnight. Sticky  
1018 ends were biotinylated and proximity-ligated to form chimeric junctions. Chimeric fragments  
1019 representing the original cross-linked long-distance physical interactions were then processed into  
1020 paired-end sequencing libraries and 150-bp paired-end reads, which were sequenced on the Illumina  
1021 platform.

1022

### 1023 **Genome assembly**

1024 The contig-level assembly was performed on full PacBio long reads using the Canu package<sup>107</sup>  
1025 (version 2.0) (<https://github.com/marbl/canu>). We then polished the draft assembly using Arrow  
1026 (version 2.3.1) (<https://github.com/PacificBiosciences/GenomicConsensus>). To increase the  
1027 accuracy of the assembly, Illumina short reads were recruited for further polishing with the Pilon  
1028 program<sup>108</sup> (version 1.22) (<https://github.com/broadinstitute/pilon>). The paired-end reads from Hi-C  
1029 were uniquely mapped onto the draft assembly contigs. We used the 3D-DNA pipeline<sup>109</sup> (version  
1030 190716) (<https://github.com/aidenlab/3d-dna>) and Juicer<sup>110</sup> (version 1.11.08)  
1031 (<https://github.com/aidenlab/juicer>) to group them into scaffolds and chromosomes. Additionally, the  
1032 10-Kb and 20-Kb mate pair reads were used to connect sequence contigs into scaffolds using  
1033 SSPACE<sup>111</sup> (version 2.1.1) ([https://github.com/nsoranzo/sspace\\_basic](https://github.com/nsoranzo/sspace_basic)) with default parameters. To  
1034 minimize errors introduced in the scaffolding step, we only connected the sequence contigs  
1035 supported by linkage evidence from mate pair reads and HiC data. The SMRT reads were also  
1036 applied to fill the gap in scaffolds using PBJelly<sup>112</sup> (version 15.8.24)  
1037 (<https://sourceforge.net/projects/pb-jelly/>) with default parameters to generate longer sequence  
1038 contigs. The scaffolds clustering into 5 groups initially. As cytological evidence strongly supports 4  
1039 chromosomes in *T. lepidozoides*, 2 shortest pseudochromosomes were linked together after error  
1040 correction and manual inspection finally. The genome assembly quality was measured using  
1041 BUSCO<sup>113</sup> (version 4.0.6) (<https://busco.ezlab.org/>).

1042

### 1043 **Analysis of repetitive elements**

1044 Repetitive sequences and transposable elements were identified using a combination of *ab initio* and  
1045 homology-based methods. In brief, an *ab initio* repeat library for *T. lepidozoides* was predicted using

1046 LTR\_FINDER<sup>114</sup> (version 1.07) ([https://github.com/xzhub/LTR\\_Finder](https://github.com/xzhub/LTR_Finder)), RepeatModeler (version  
1047 1.0.11) (<http://www.repeatmasker.org/RepeatModeler/>) with default parameters. The library was  
1048 aligned to PGSB Repeat Element Database<sup>115</sup> (<http://pgsb.helmholtz-muenchen.de/plant/recat/>) to  
1049 classify the type of each repeat family. For identification of the repeats throughout the genome,  
1050 RepeatMasker (version 4.0.8) (<http://www.repeatmasker.org/>) was applied with both the *ab initio*  
1051 repeat databases and Repbase (<http://www.girinst.org/repbase>) using the WU-BLAST<sup>116</sup>  
1052 (<http://blast.wustl.edu/>) search engine. Overlapping transposable elements belonging to the same  
1053 repeat class were collated and combined. In addition, we annotated the tandem repeats using  
1054 Tandem Repeats Finder<sup>117</sup> (version 4.04) (<https://tandem.bu.edu/trf/trf.html>). Solo-LTR were  
1055 identified using LTRharvest<sup>118</sup> (version 1.6.1) ([http://genometools.org/tools/gt\\_ltrharvest.html](http://genometools.org/tools/gt_ltrharvest.html)). A  
1056 customized script was implemented to identify intact LTR/Copia and LTR/Gypsy retrotransposons.  
1057 ClustalW<sup>119</sup> (version 1.81) (<http://www.clustal.org/>) was applied to align 5' and 3' solo-LTRs to intact  
1058 LTR elements. The evolutionary distance of the two LTR sequences was estimated using the Kimura  
1059 two-parameter method embedded in BASEML in PAML<sup>120</sup> (version 4.9j)  
1060 (<http://abacus.gene.ucl.ac.uk/software/paml.html>). A substitution rate of  $1.3 \times 10^{-8}$  mutations per site  
1061 per year was used to convert evolutionary distance between 5' and 3' solo-LTRs to insertion age of  
1062 retrotransposons.

1063

#### 1064 **Gene prediction and functional annotation**

1065 Genscan<sup>121</sup> (version 1.0) (<http://genes.mit.edu/GENSCAN.html>) and Augustus<sup>122</sup> (version 3.3.3)  
1066 (<http://augustus.gobics.de/>) were used to carry out *de novo* predictions with gene model parameters  
1067 trained from *Chlamydomonas reinhardtii*, *Marchantia polymorpha*, *P. patens*, *Selaginella*  
1068 *moellendorffii* and *A. thaliana*. Furthermore, gene models were *de novo* predicted using MAKER<sup>123</sup>  
1069 (version 3.01.02) (<http://www.yandell-lab.org/software/maker.html>). We then evaluated the genes by  
1070 comparing MAKER results with the corresponding transcript evidence to select gene models that  
1071 were the most consistent. The gene prediction quality was measured using BUSCO<sup>113</sup> (version 4.0.6)  
1072 (<https://busco.ezlab.org/>). Protein-coding genes were retrieved and functionally annotated by blast  
1073 searches against databases including UniProtKB/Swiss-Prot and UniProtKB/TrEMBL<sup>124</sup>  
1074 (<https://www.uniprot.org/>). They were also subjected to Gene Ontology (GO) annotation and protein  
1075 family annotation by the InterProScan pipeline<sup>125</sup> (version 5.44-79.0) ([https://github.com/ebi-pf-](https://github.com/ebi-pf-team/interproscan)  
1076 [team/interproscan](https://github.com/ebi-pf-team/interproscan)). GO terms for each gene were assigned by blast searches against the GO

1077 database<sup>126</sup> (<http://geneontology.org/>).

1078

### 1079 **Organellar genomes**

1080 The organellar raw reads of *T. lepidozooides* were identified using BLASTn against the PacBio  
1081 subreads with plastome (NC\_005087.1) and chondriome (NC\_007945.1) of *P. patens* as references,  
1082 respectively. The organellar assembly were performed on raw reads using the Canu package<sup>107</sup>. The  
1083 organellar genome sequences were annotated as previously described<sup>127</sup>. Protein-coding genes,  
1084 rRNA genes, exact gene and exon/intron boundaries were annotated using GeSeq<sup>128</sup>  
1085 (<https://chlorobox.mpimp-golm.mpg.de/geseq.html>).

1086

### 1087 **Identification of genome synteny**

1088 We identified homologs using BLAST with default parameters, and scanned syntenic blocks  
1089 consisting of homologous genes within the *T. lepidozooides* genome using MCSan<sup>129</sup> (version 1.0.6)  
1090 (<https://github.com/tanghaibao/jcvi>).

1091 We identified WGD events in *T. lepidozooides* genome and two bryophytes (*M. polymorpha* and  
1092 *P. patens*) using the WGDI toolkit<sup>130</sup> (version 0.5.3) (<https://github.com/SunPengChuan/wgdi>). In  
1093 brief, delineation of paralogous gene families and one-to-one orthologs starts from all-versus-all  
1094 BLASTP similarity searches with “-evaluate 1e-5 -num\_alignments 10”. Collinear genes were first  
1095 identified with the parameter “-icl” of WGDI within each genome and between genomes, and the  
1096 collinear genes dot plots were used to count the syntenic ratios between different species to confirm  
1097 the polyploidy level of each species, then gene pairs located on the same chromosome or on the  
1098 same scaffold were removed, tandem gene pairs were also removed. Frequencies of synonymous  
1099 substitutions per synonymous site (Ks) between colinear genes were estimated using the Nei-  
1100 Gojobori approach as implemented in PAML<sup>120</sup>. The median Ks values of each block were selected  
1101 to perform the Ks peak fitting by WGDI with the parameter “-pf”. Ks peaks of *T. lepidozooides* vs. two  
1102 species (*M. polymorpha* and *P. patens*) syntenic orthologs allows calculating Ks per year (r) following  
1103  $r = Ks / (2 \times (\text{divergence time}))^{38}$ .

1104 We used DupGen\_finder<sup>131</sup> ([https://github.com/qiao-xin/DupGen\\_finder](https://github.com/qiao-xin/DupGen_finder)) with default  
1105 parameters to search for genome-wide duplications. First, all-versus-all local BLASTP was  
1106 performed using protein sequences with “-evaluate 1e-5 -num\_alignments 10”. Second, we identified  
1107 different modes of gene duplication as whole-genome duplicates (WGD), tandem duplicates (TD),

1108 proximal duplicates (less than 10 gene distance on the same chromosome: PD), transposed  
1109 duplicates (transposed gene duplications: TRD), or dispersed duplicates (other duplicates than WGD,  
1110 TD, PD and TRD: DSD). Third, the Ka (number of substitutions per nonsynonymous site), Ks, and  
1111 Ka/Ks values were estimated for gene pairs generated by different modes of duplication based on  
1112 the YN model in KaKs\_Calculator<sup>132</sup> (version 2.0, June 2009)  
1113 (<https://sourceforge.net/projects/kakscalculator2/>), after conversion of amino acid alignments into  
1114 the corresponding codon alignments with PAL2NAL<sup>133</sup> (version 14)  
1115 (<http://www.bork.embl.de/pal2nal/>).

1116

### 1117 **Phylogenomic reconstruction**

1118 To explore the phylogenetic position of *T. lepidozoides*, we constructed data sets from a total of 56  
1119 plant taxa (Table S1L), including nine genomes from Ensembl plants  
1120 (<http://plants.ensembl.org/index.html>) and JGI phytozome (<http://phytozome.jgi.doe.gov>), 20  
1121 transcriptomes from the onekp project  
1122 ([http://mirrors.iplantcollaborative.org/browse/iplant/home/shared/onekp\\_pilot](http://mirrors.iplantcollaborative.org/browse/iplant/home/shared/onekp_pilot)), 13 transcriptomes  
1123 from the NCBI sequence read archive (<https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?>), genes of  
1124 *Klebsormidium nitens* were obtained from the *K. nitens* genome project  
1125 ([http://www.plantmorphogenesis.bio.titech.ac.jp/~algae\\_genome\\_project/klebsormidium/index.html](http://www.plantmorphogenesis.bio.titech.ac.jp/~algae_genome_project/klebsormidium/index.html)  
1126 ), those of *Chara braunii* from ORCAE (<https://bioinformatics.psb.ugent.be/orcae/overview/Chbra>),  
1127 those of *Spirogloea muscicola* and *Mesotaenium endlicherianum* from Figshare  
1128 ([https://figshare.com/articles/dataset/Genomes\\_of\\_subaerial\\_Zygnematophyceae\\_provide\\_insights  
1129 \\_into\\_land\\_plant\\_evolution/9911876/1](https://figshare.com/articles/dataset/Genomes_of_subaerial_Zygnematophyceae_provide_insights_into_land_plant_evolution/9911876/1)), those from *Anthoceros agrestis* and *A. punctatus* from  
1130 University of Zurich (<https://www.hornworts.uzh.ch/en/download.html>), while the remaining data are  
1131 from our newly sequenced transcriptomes. Putative orthologous genes were identified using the best  
1132 reciprocal blast hits (BRBH) method. Eight genomes (*Anthoceros agrestis*, *Klebsormidium nitens*,  
1133 *Mesotaenium endlicherianum*, *Physcomitrella patens*, *Chara braunii*, *Marchantia polymorpha*,  
1134 *Penium margaritaceum*, *Takakia lepidozoides*) were selected for inferring low-copy orthologous  
1135 genes by OrthoMCL pipeline<sup>134</sup> (version 2.0.9) (<https://legacy.orthomcl.org/orthomcl.b5/>), then 818  
1136 single-copy orthologous genes (OGs) were selected and set as queries to search homologous  
1137 sequences from the 56 species (Table S1L) by HaMStR<sup>135</sup> (version 1.6.0)  
1138 (<https://github.com/BIONF/HaMStR>) with an e-value of  $1 \times 10^{-5}$ . Protein sequences of each OGs were

1139 aligned by MUSCLE<sup>136</sup> (version 3.8.31) (<http://www.drive5.com/muscle>) and poorly aligned regions  
1140 were trimmed (-automated1) using trimAl<sup>137</sup> (version 1.4) (<http://trimal.cgenomics.org>). Each OGs  
1141 were reconstructed by IQ-tree<sup>138</sup> (version 1.6.12) (<http://www.iqtree.org/>) with ultrafast bootstrap  
1142 parameter and suggested models, and then the 818 trees analyzed using ASTRAL<sup>139</sup> (version 5.7.1)  
1143 (<https://github.com/Smirarab/ASTRAL>) to infer species trees based on coalescent methods.

1144

### 1145 **Divergence time estimation**

1146 We used a total of nine fossil<sup>140-142</sup> calibrations for TreePL analysis (Table S2R). Considering the  
1147 arguments of the maximum age of five fossils, we set two calibrations: in set1, for the #3-7 fossils  
1148 only minimum ages were used for calibration, whereas in set2, minimum and maximum age  
1149 calibrations were applied to all fossils. The concatenation of the alignments of the 818 OGs was  
1150 used to calculate the branch length by IQ-tree, with the 818-gene coalescent tree as the constraint  
1151 tree. Divergence times were estimated using the penalized likelihood method<sup>143</sup> by treePL<sup>144</sup> (version  
1152 1.0) (<https://github.com/blackrim/treePL>). The cross-validation tested a set of smoothing parameters  
1153 and then an optimal smoothing value of 0.1 was selected.

1154

### 1155 **Testing positive selection and fast evolution**

1156 To gain insight into the evolutionary history of *T. lepidozooides*, we constructed a data set of orthologs  
1157 from 12 plant taxa (*Anthoceros angustus*, *Arabidopsis thaliana*, *Atrichum undulatum*, *Ctenidium*  
1158 *capillifolium*, *P. patens*, *Sphagnum palustre*, *T. lepidozooides*, *Conocephalum japonicum*,  
1159 *Haplomitrium mnioides*, *Herbertus sendtneri*, *Marchantia polymorpha* and *Selaginella moellendorffii*).  
1160 The CDS sequences corresponding to genomes of *P. patens*, *A. thaliana*, and *S. moellendorffii* were  
1161 obtained from the Ensembl plants web site (<http://plants.ensembl.org/index.html>), the *A. angustus*  
1162 transcriptome data from the NCBI sequence read archive ([https://sra-downloadb.be-](https://sra-downloadb.be-md.ncbi.nlm.nih.gov/sos2/sra-pub-run-15/SRR9662965/SRR9662965.1)  
1163 [md.ncbi.nlm.nih.gov/sos2/sra-pub-run-15/SRR9662965/SRR9662965.1](https://sra-downloadb.be-md.ncbi.nlm.nih.gov/sos2/sra-pub-run-15/SRR9662965/SRR9662965.1)), and the remaining data  
1164 are from our newly sequenced transcriptomes (Table S2S). Putative orthologous genes were  
1165 identified using the best reciprocal blast hits (BRBH) method. The homologous genes were first  
1166 detected by the TBLASTX program with an E-value cut-off of  $1 \times 10^{-5}$ . Well-annotated proteins of *A.*  
1167 *thaliana* were used as references to identify corresponding orthologs in the other 11 plants including  
1168 *T. lepidozooides*. Only orthologs with best-hit scores across all pairs of species were kept. To obtain  
1169 high-quality alignment and facilitate downstream analysis, all full orthologs data sets were aligned at

1170 the codon level. Finally, a total of 1970 putative orthologs were identified from 12 plants. We  
1171 employed a maximum likelihood approach<sup>145</sup> to detect positively selected genes (PSGs) along  
1172 specific lineages. The branch-site model implemented in the program CODEML in PAML<sup>120</sup> (version  
1173 4.9j) was used. The *T. lepidozoides* lineage was defined as foreground branch. For the foreground  
1174 branch we compared the selection model and the neutral model using a likelihood ratio test (LRT).  
1175 A Chi-square test was conducted for each gene to compute p-values. Multiple testing was corrected  
1176 by applying the false discovery rate (FDR) method. For a gene, if the selection model has a  
1177 significantly higher likelihood than the neutral model (FDR-adjusted p-value < 0.05), the gene on the  
1178 foreground branch may have experienced positive selection.

1179 To identify fast evolving genes (FSGs) in the *T. lepidozoides* lineage, we ran the one-ratio  
1180 branch model and two-ratio branch model with CODEML in PAML to estimate the global and local  
1181 dN/dS (non - synonymous substitution rates /synonymous substitution rates) ratio, respectively. The  
1182 one-ratio model assumes that all branches have been evolving at the same rate (null hypothesis),  
1183 and the two-ratio model allows the foreground branch to evolve under a different rate (alternative  
1184 hypothesis). *T. lepidozoides* was set to foreground. LRT was employed to compare the one-ratio  
1185 branch model to the two-ratio branch model. The LRT p-values were adjusted by FDR correction for  
1186 multiple testing. The abnormal dN/dS values (dN/dS < 0.001 or dN/dS >5) were excluded from our  
1187 analysis. If a gene had an FDR-adjusted p-value < 0.05 and also a higher dN/dS in the foreground  
1188 branch than in the background branch, it was considered as FSGs in the foreground branch. In  
1189 addition, we noticed that *H. sendtneri* also lived in the high-altitude area of the Tibetan Plateau. To  
1190 detect convergent accelerated evolution of genes between *T. lepidozoides* and *H. sendtneri*, we  
1191 defined both of them as foreground branches and repeated above-mentioned analysis. All detected  
1192 PSGs and FSGs were submitted to DAVID (<https://david.ncifcrf.gov/home.jsp>) for GO functional  
1193 enrichment analyses.

1194

### 1195 **Identification of transcription factors**

1196 For TFs and TRs, we used the HMMER search method. We downloaded the HMMER model of the  
1197 domain structure of each TF from the Pfam website (<https://pfam.xfam.org/>), referring to the TAPscan  
1198 TF database<sup>43</sup> (<https://plantcode.cup.uni-freiburg.de/tapscan/>). Preliminary candidates were  
1199 collected by searching the HMM profile for each species (<1 × 10<sup>-10</sup>). Then, we filtered genes that  
1200 did not match the SwissProt functional annotation (<1 × 10<sup>-5</sup>). Finally, we filtered genes containing

1201 an incorrect domain according to the domain rules of the TAPscan TF database. Most TFs/TRs were  
1202 confirmed by phylogenetic analysis.

1203

### 1204 **Gene family classification**

1205 We applied the standard OrthoMCL pipeline<sup>134</sup> (version 2.0.9)  
1206 (<https://legacy.orthomcl.org/orthomcl.b5/>) to identify orthologous gene families among *T.*  
1207 *lepidozoides*, *C. reinhardtii*, *K. nitens*, *C. braunii*, *M. polymorpha*, *P. patens*, *S. moellendorffii* and *A.*  
1208 *thaliana*. The longest protein from each gene was selected, and the proteins with a length less than  
1209 30 amino acids were removed. After this step, pairwise sequence similarities between all input  
1210 protein sequences were calculated using BLASTP with an E value cut-off of  $1 \times 10^{-5}$ . Markov  
1211 clustering (MCL) of the resulting similarity matrix was used to define the orthologue cluster structure  
1212 of the proteins, using an inflation value (-I) of 1.5 (OrthoMCL default). Gene families were aligned by  
1213 MAFFT<sup>146</sup> (version 7.453) (<https://mafft.cbrc.jp/alignment/software/>). Following alignment,  
1214 Gblocks<sup>147</sup> (version 0.91b) (<http://molevol.cmima.csic.es/castresana/Gblocks.html>) was used to  
1215 remove poorly aligned positions and divergent regions. RAxML<sup>148</sup> (version 8.2.12) ([https://cme.h-](https://cme.h-its.org/exelixis/web/software/raxml/)  
1216 [its.org/exelixis/web/software/raxml/](https://cme.h-its.org/exelixis/web/software/raxml/)) was used to construct the phylogenetic tree using the  
1217 PROTGAMMAIJTTF model and 1,000 bootstrap replicates. The divergence time was estimated  
1218 using the MCMCTree program in the PAML package<sup>120</sup>. CAFÉ (version 4.2.1)  
1219 (<https://hahnlab.github.io/CAFE/>) was used to estimate gene gain and loss.

1220

### 1221 **Fossil of *Takakia* sp.**

1222 The studied fossil, collected from the Middle-Late Jurassic Jiulongshan Formation at Daohugou (165  
1223 Mya<sup>66</sup>), Ningcheng County, Inner Mongolia, China. The fossil was identified as *Takakia* sp. by Yufei  
1224 Wang (Paleobotanist) and Yu Jia (Bryologist) from Institute of Botany, Chinese Academy of Sciences.  
1225 The fossil *Takakia*, No. CNU-PLA-NN2023056, is housed at the Key Lab of Insect Evolution and  
1226 Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China (CNUB;  
1227 Prof. Dong Ren, Curator). The photographs of fossil were taken with a Nikon SMZ 25 microscope  
1228 with an attached Nikon DS-Ri2 digital camera system.

1229

### 1230 **Evolution of specific morphological traits**

1231 To estimate the evolution of morphogenetic genes including stomata, rhizoids and leaf adaxial-

1232 abaxial polarity in *Takakia*, we carefully checked these gene homologs in 11 bryophytes (including  
1233 *T. lepidozoides*, two hornworts, i.e., *Anthoceros agrestis* and *Anthoceros angustus*, one liverwort,  
1234 i.e., *Marchantia polymorpha* and seven other mosses, i.e., *Calohypnum plumiforme*, *Ceratodon*  
1235 *purpureus*, *Entodon seductrix*, *Fontinalis antipyretica*, *Hypnum curvifolium*, *Physcomitrella patens*,  
1236 *Syntrichia caninervis*) (Table S2T) using BLASTP with identity cutoff 50%, e-value cutoff  $1 \times 10^{-20}$ .  
1237 Domains were checked in homolog candidates using HMMER. Maximum likelihood tree constructed  
1238 using RAxML with the PROTGAMMAJTT model based upon MAFFT alignments of proteins and  
1239 trimmed by trimAl. Tree is the consensus of 1,000 replicates. The divergence time was estimated  
1240 using the MCMCTree program in the PAML package<sup>120</sup>. We used two fossil<sup>140</sup> calibrations for  
1241 MCMCTree analysis (Table S2R), minimum age 405 Mya and maximum age 515.5 Mya calibrations  
1242 were applied.

1243

#### 1244 **Gene expression profiling**

1245 Poor quality or technical sequences in Illumina paired-end reads were removed using the  
1246 Trimmomatic preprocessing tool<sup>149</sup> (version 0.39)  
1247 (<http://www.usadellab.org/cms/?page=trimmomatic>). The qualified paired reads were then aligned  
1248 against the *T. lepidozoides* reference transcript sequences using Bowtie 2<sup>150</sup> (version 2.4.1)  
1249 (<http://bowtie-bio.sourceforge.net/bowtie2/index.shtml>) to find all alignments of a read with no more  
1250 than two mismatches. Subsequently, gene and isoform abundances were quantified from paired-end  
1251 RNA-seq data using the RSEM software package (version 1.3.3)  
1252 (<https://github.com/siddharthab/RSEM>). Differentially expressed transcripts or genes (DEGs) with  
1253 biological replicates were identified by running Bioconductor tools edgeR<sup>151</sup> (version 3.28.1)  
1254 (<https://bioconductor.org/packages/release/bioc/html/edgeR.html>), which implements a range of  
1255 statistical methods based on the negative binomial distributions. Orthologs of *T. lepidozoides* were  
1256 functionally annotated via the EggNOG database<sup>152</sup> (version 5.0) (<http://eggnog5.embl.de/>)  
1257 companion tool eggno-mapper<sup>153</sup> (version 2.0.1) (<http://eggnog-mapper.embl.de/>), with “-d euk --  
1258 tax\_scope 3208”. The inferred GO terms and Kyoto Encyclopedia of Genes and Genomes (KEGG)  
1259 pathways were used as the basis for DEG enrichment analyses performed with the R package  
1260 clusterProfiler<sup>154</sup> (version 3.14.3)  
1261 (<http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html>).

1262

1263 **QUANTIFICATION AND STATISTICAL ANALYSIS**

1264 The statistics of experiments can be found in the figure, table and/or data legends, including the  
1265 results represented as mean or median  $\pm$  SD, exact value of n, P values with significance levels. All  
1266 details of the statistics applied are provided alongside the respective analysis in the Method Details  
1267 section.

1268

1269 **SUPPLEMENTARY INFORMATION**

1270 **Figure S1.** Population changes from 2010 to 2021, related to Figure 1

1271 The coverage of commonly found species from 2010 to 2021. The vertical axis represents the  
1272 relative coverage of the species in observation quadrants (n = 52), and dotted line marks the  
1273 relationship between the species population size and the year. The central mark of each box  
1274 indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles  
1275 respectively, the whiskers extend to the most extreme data points not considered outliers). (**A – D**)  
1276 Most common mosses *Racomitrium himalayanum*, *Dicranum drummondii*, *Hypnum plumaforme*  
1277 and *Polytrichum juniperinum*, (**E – G**) common liverworts *Herbertus sendtneri*, *Plagiochila recurvata*  
1278 and *Wettsteinia rotundifolia*, (**H – L**) most common angiosperms *Diapensia wardii*, *Bergenia*  
1279 *purpurascens*, *Cassiope selaginoides*, *Saxifraga unguiculata* and *Pedicularis siphonantha*, (**M, N**)  
1280 lichens *Stereocaulon alpinum* and *Cladonia rangiferina*. (**O**) The Shannon-Wiener Index change  
1281 during 2010 to 2021.

1282

1283 **Figure S2.** The origin of branching in *T. lepidozoides*, observed with the mPS-PI staining technique,  
1284 related to Figure 2

1285 The sections (**A, B, C**) from three mutually perpendicular directions at the connection site (the cross  
1286 point of white dotted lines) between stem and branch, the numbers mark cells in the parent stem  
1287 (yellow) and the branch (white) respectively, both comprise eight rows of cells (1–8<sup>th</sup> row) in the  
1288 section (B), yellow arrows mark the vertical walls between epidermal cells, white arrows mark the  
1289 inclined walls between longer cells (potential vessel elements), bar = 50  $\mu$ m. The sections (**D, E, F**)  
1290 from three mutually perpendicular directions at the connection site (the cross point of white dotted  
1291 lines) between stem and branch, numbers mark cells in the parent stem (yellow) and the branch  
1292 (white) respectively, which shows the branch originated from cells of the epidermis (such as “yellow  
1293 8” and “white 6” marked cells) and sub-epidermis (such as “yellow 7”, 7’, 7 and “white 5” marked

1294 cells) of parent stem, bar = 50  $\mu\text{m}$ . Fluorescence images were obtained using a Leica SP8 laser  
1295 scanning confocal microscope, with the excitation wavelength at 561 nm and emission wavelength  
1296 from 570 nm to 670 nm.

1297  
1298 **Figure S3.** Effects of UV-B irradiation on *A. thaliana*, related to Figure 3

1299 Arabidopsis Col - 0 plants grown for 25 days under normal conditions were treated with or without  
1300 UV-B (A) Plants treated without or with 144  $\text{kJ m}^{-2}$  UV-B. (B) Plant recovery at 24 hours after UV-B  
1301 treatment. (C) Plant recovery at 48 hours after treatment. (D) Plant recovery at 72 hours after UV-B  
1302 treatment. Side length of the pots is 7.5 cm.

1303  
1304 **Figure S4.** Chromosomes in gametophyte cells of *T. lepidozoides*, related to Figure 4

1305 (A) Hi-C assembly heatmap shows four obvious chromosomes (blue boxes) composing the *T.*  
1306 *lepidozoides* genome. (B – E) Four chromosomes shown in a somatic prometaphase cell separated  
1307 from a stem tip, bar = 5  $\mu\text{m}$ .

1308  
1309 **Figure S5.** Genetic foundation of the lack of stomata and rhizoids, related to Figure 6

1310 Phylogenetic tree of genes relevant for the formation of rhizoids (A). Sequence alignments of SCRM  
1311 (B) and FAMA (C). Black boxes mark conserved domains. Phylogenetic tree of genes relevant for  
1312 the formation of stomata (D). Maximum likelihood tree using RAXML with the PROTGAMMAJTT  
1313 model based upon MAFFT alignments of proteins are the consensus of 1,000 replicates. Numbered  
1314 nodes show the percentage of bootstraps which support the node. Bootstraps with less than 50%  
1315 support are not shown. Orange font color represents *A. thaliana*, pink font color represents *T.*  
1316 *lepidozoides* and blue font color represents *P. patens*. Shade colors as shown on the right.

1317  
1318 **Table S1.** Field observation in survey areas, mass spectrum of metabolic compounds, sequencing  
1319 library, genome assembly and annotation, positive selection and fast evolution, with index in the first  
1320 sheet, related to STAR methods.

1321  
1322 **Table S2.** Genomic comparative analyses, key genes operating in morphological innovations and  
1323 environmental adaptations, with index in the first sheet, related to STAR methods.

1324

1325 **Data S1.** Field observation in survey areas, morphological characters, physiological and biochemical  
1326 properties of *T. lepidozoides*, related to STAR methods.

1327

1328 **Data S2.** Genome features, phylogenetic position, key genes operating in morphological innovations  
1329 and environmental adaptations, related to STAR methods.

1330

1331 **Movie S1.** Film of survey areas in the southern part of the Tibetan Plateau. Digital Earth data are  
1332 from Google Earth (<https://earth.google.com/>), remote sensing images captured by matrice 300 RTK  
1333 (DJI), related to Figure 1.

1334

1335 **Movie S2.** Film of a restructured stem. The raw images were converted to an avi file using Leica  
1336 LAS X (3.5.2) at 16 frames per second, related to Figure 2.

1337

1338 **Movie S3.** Time lapse photos of capsule development. Total 155 photos were taken at steps of 0.1  
1339 s (TimelapseCams, Wingscapes), related to Figure 1.

1340

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1362

### 1363 **AUTHOR CONTRIBUTIONS**

1364 Yikun He conceived the project; Yikun He and Ralf Reski designed the project; Yikun He, Ralf Reski  
1365 and Ruoyang Hu wrote the manuscript, supplementary notes, and methods; Xuedong Li, Runjie  
1366 Zhang, Ruoyang Hu, Xiaofeng Wu, Feng Zhao, Shuang Xing, Yong Hu, Lei Deng, Fuzhou Duan,  
1367 Wenji Zhao, Yu Jia and Meizhi Wang performed the field works; Ruoyang Hu, Yong Hu and Fang  
1368 Bao collected and sequenced the plant material; Yikun He and Xianyong Sheng performed three-  
1369 dimensional reconstruction of branching and rooting; Qiang Lv and Yuhan Ding performed transgene  
1370 experiments; Xiaofeng Wu, Ruoyang Hu, Runjie Zhang, Yikun He, Lihong Xiao and Kai Li performed  
1371 ultrastructural observations; Zhukuan Cheng and Hanli You observed chromosomes in somatic cells;  
1372 Min Zhang and Huan Yuan performed immunofluorescence labeling of AtPIN1; Zhijia Chen and  
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1384 Yong Zhang, Yang Liu and Shanshan Dong performed gene family clustering and comparative  
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1386 A. Rensing performed contamination analysis and annotated transcription associated proteins;

1387 Stefan A. Rensing supervised gene family clustering analysis; Yu Jia identified the fossil; Stefan A.  
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1391

## 1392 **DECLARATION OF INTERESTS**

1393 The authors declare no competing interests.

1394

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1841

## Key resources table

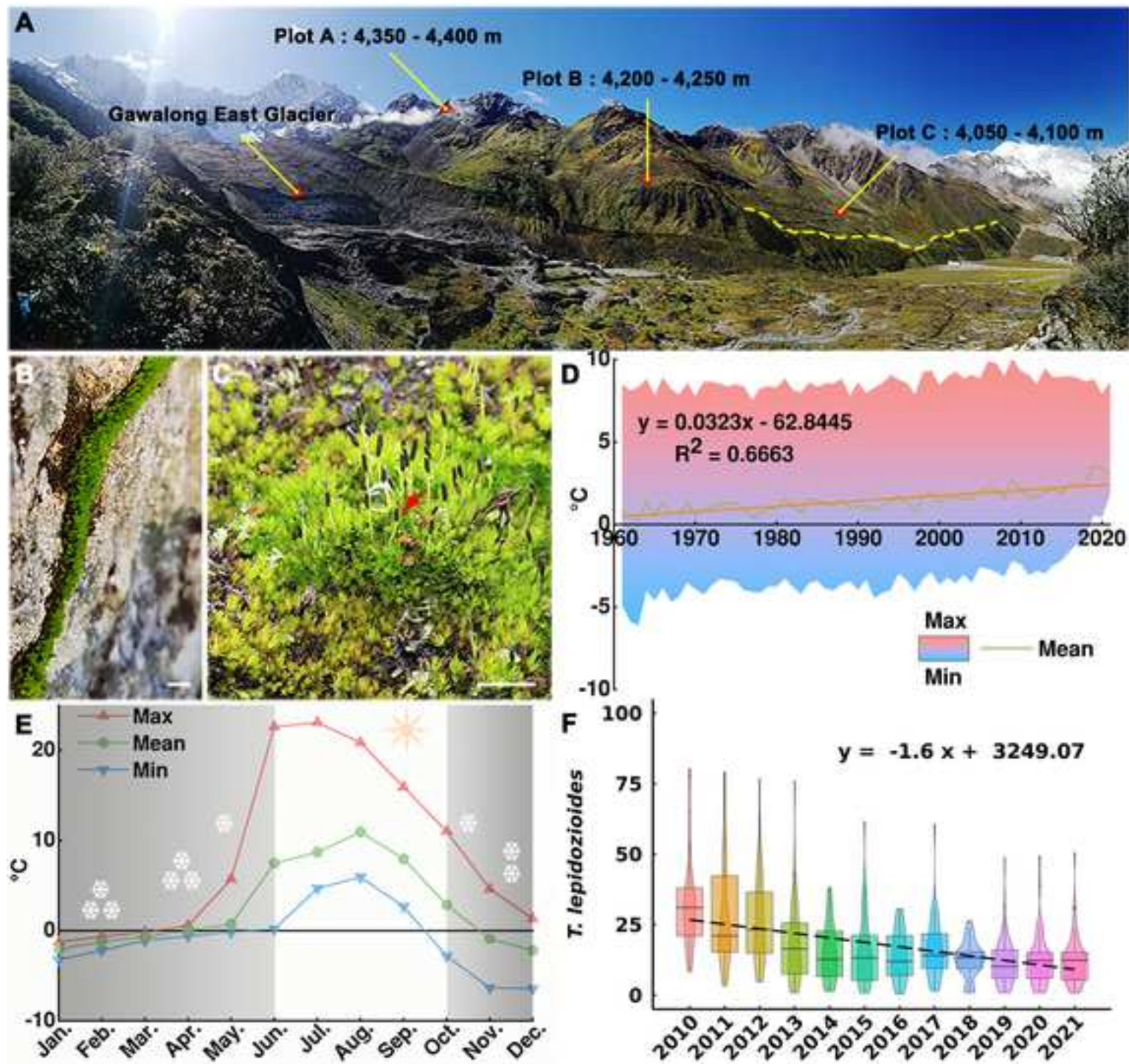
REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
AtCP47	97	N/A
AtPsaD	97	N/A
AtPIN1	PhytoAB	PHY1046S
<b>Bacterial and virus strains</b>		
<b>Biological samples</b>		
<i>Takakia lepidozoides</i>	This study	IMSC: 41271
<b>Chemicals, peptides, and recombinant proteins</b>		
<b>Critical commercial assays</b>		
<b>Deposited data</b>		
<i>Takakia lepidozoides</i> : Pacbio CCS, Hi-C, Illumina DNA and RNA sequencing data	This study	NCBI BioProject: PRJNA796444
<i>Takakia lepidozoides</i> : Pacbio CCS, Hi-C, Illumina DNA and RNA sequencing data	This study	NGDC BioProject: PRJCA014819
<i>Takakia lepidozoides</i> genome assembly and gene annotations	This study	<a href="https://www.takakia.com">https://www.takakia.com</a>
<i>Takakia lepidozoides</i> : Metabolomics raw data	This study	MetaboLights : MTBLS6999
<b>Experimental models: Cell lines</b>		

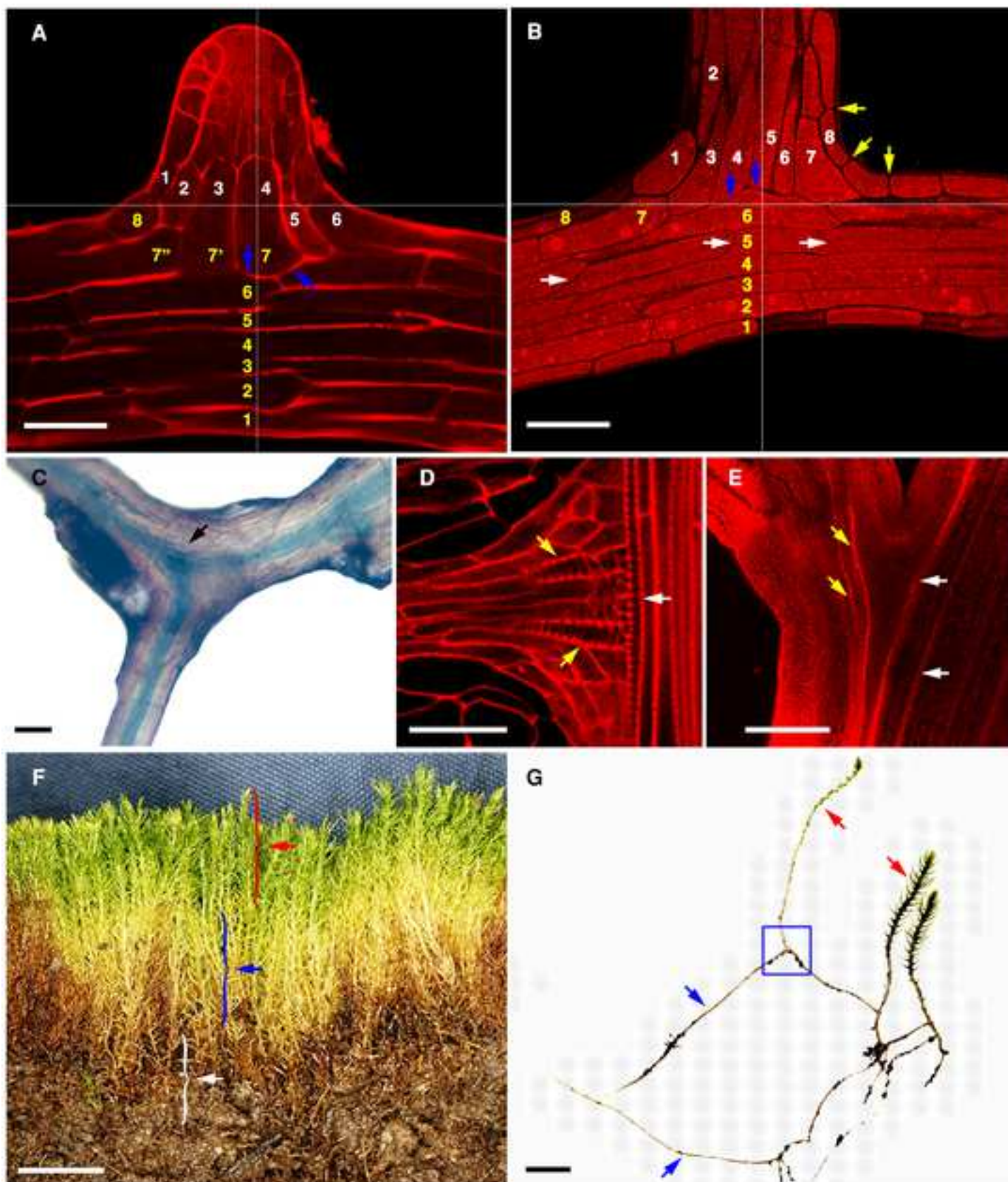
Experimental models: Organisms/strains		
Oligonucleotides		
Primers used for cloning and functional analysis of PIN (see Table S6 A)	This study	N/A
Recombinant DNA		
Software and algorithms		
Vegan version 2.5-7		<a href="https://CRAN.R-project.org/package=vegan">https://CRAN.R-project.org/package=vegan</a>
MaxEnt version 3.4.1		<a href="https://biodiversityinformatics.amnh.org/open_source/maxent/">https://biodiversityinformatics.amnh.org/open_source/maxent/</a>
Canu version 2.0	107	<a href="https://github.com/marbl/canu">https://github.com/marbl/canu</a>
Arrow version 2.3.1		<a href="https://github.com/PacificBiosciences/GenomicConsensus">https://github.com/PacificBiosciences/GenomicConsensus</a>
Pilon version 1.22	108	<a href="https://github.com/broadinstitute/pilon">https://github.com/broadinstitute/pilon</a>
3D-DNA version 190716	109	<a href="https://github.com/aidenlab/3d-dna">https://github.com/aidenlab/3d-dna</a>
Juicer version 1.11.08	110	<a href="https://github.com/aidenlab/juicer">https://github.com/aidenlab/juicer</a>
SSPACE version 2.1.1	111	<a href="https://github.com/nsoranzo/sspace_basic">https://github.com/nsoranzo/sspace_basic</a>
PBJelly version 15.8.24	112	<a href="https://sourceforge.net/projects/pb-jelly/">https://sourceforge.net/projects/pb-jelly/</a>
BUSCO version 4.0.6	113	<a href="https://busco.ezlab.org/">https://busco.ezlab.org/</a>
LTR_FINDER version 1.07	114	<a href="https://github.com/xzhu/LTR_Finder">https://github.com/xzhu/LTR_Finder</a>
RepeatModeler version 1.0.11		<a href="http://www.repeatmasker.org/RepeatModeler/">http://www.repeatmasker.org/RepeatModeler/</a>
RepeatMasker version 4.0.8		<a href="http://www.repeatmasker.org/">http://www.repeatmasker.org/</a>

WU-BLAST	116	<a href="http://blast.wustl.edu/">http://blast.wustl.edu/</a>
Tandem Repeats Finder version 4.04	117	<a href="https://tandem.bu.edu/trf/trf.html">https://tandem.bu.edu/trf/trf.html</a>
LTRharvest version 1.6.1	118	<a href="http://genometools.org/tools/gt_ltrharvest.html">http://genometools.org/tools/gt_ltrharvest.html</a>
ClustalW version 1.81	119	<a href="http://www.clustal.org/">http://www.clustal.org/</a>
PAML version 4.9j	120	<a href="http://abacus.gene.ucl.ac.uk/software/paml.html">http://abacus.gene.ucl.ac.uk/software/paml.html</a>
Genscan version 1.0	121	<a href="http://genes.mit.edu/GENSCAN.html">http://genes.mit.edu/GENSCAN.html</a>
Augustus version 3.3.3	122	<a href="http://augustus.gobics.de/">http://augustus.gobics.de/</a>
MAKER version 3.01.02	123	<a href="http://www.yandell-lab.org/software/maker.html">http://www.yandell-lab.org/software/maker.html</a>
InterProScan version 5.44-79.0	125	<a href="https://github.com/ebi-pf-team/interproscan">https://github.com/ebi-pf-team/interproscan</a>
GeSeq	128	<a href="https://chlorobox.mpi-golm.mpg.de/geseq.html">https://chlorobox.mpi-golm.mpg.de/geseq.html</a>
MCSscan version 1.06	129	<a href="https://github.com/tanghaibao/jcvi">https://github.com/tanghaibao/jcvi</a>
WGDI version 0.5.3	130	<a href="https://github.com/SunPengChuan/wgdi">https://github.com/SunPengChuan/wgdi</a>
DupGen_finder	131	<a href="https://github.com/qiao-xin/DupGen_finder">https://github.com/qiao-xin/DupGen_finder</a>
KaKs_Calculator version 2.0	132	<a href="https://sourceforge.net/projects/kakscalculator2/">https://sourceforge.net/projects/kakscalculator2/</a>
PAL2NAL version 14	133	<a href="http://www.bork.embl.de/pal2nal/">http://www.bork.embl.de/pal2nal/</a>
OrthoMCL version 2.0.9	134	<a href="https://legacy.orthomcl.org/orthomcl.b5/">https://legacy.orthomcl.org/orthomcl.b5/</a>
HaMStR version 1.6.0	135	<a href="https://github.com/BI-ONF/HaMStR">https://github.com/BI-ONF/HaMStR</a>
MUSCLE version 3.8.31	136	<a href="http://www.drive5.com/muscle">http://www.drive5.com/muscle</a>
trimAl version 1.4	137	<a href="http://trimal.cgenomics.org">http://trimal.cgenomics.org</a>
IQ-tree version 1.6.12	138	<a href="http://www.iqtree.org/">http://www.iqtree.org/</a>
ASTRAL version 5.7.1	139	<a href="https://github.com/smirarab/ASTRAL">https://github.com/smirarab/ASTRAL</a>
treePL version 1.0	144	<a href="https://github.com/blackrim/treePL">https://github.com/blackrim/treePL</a>
TAPscan	43	<a href="https://plantcode.uni-marburg.de/tapscan/">https://plantcode.uni-marburg.de/tapscan/</a>

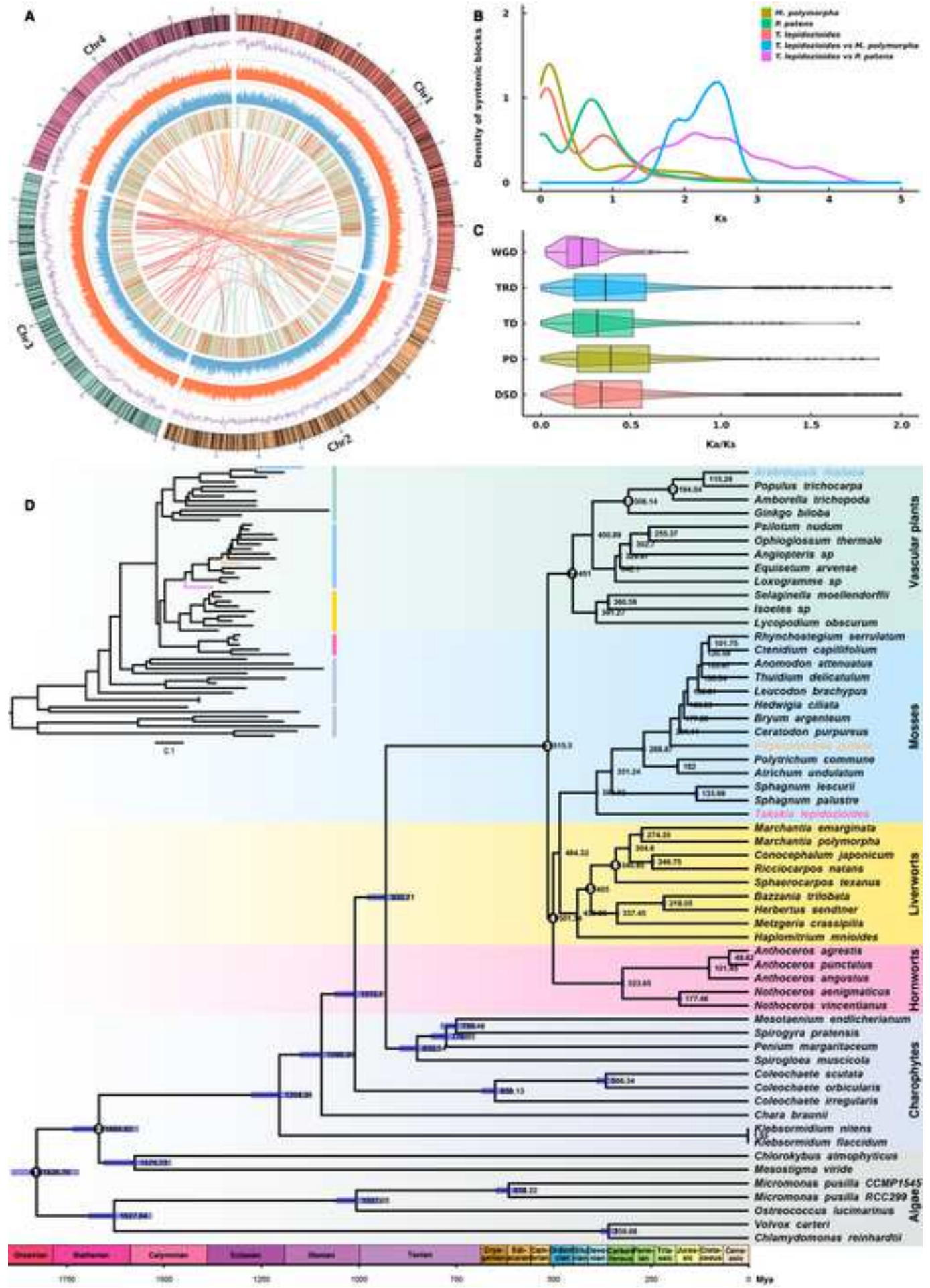
MAFFT version 7.453	146	<a href="https://mafft.cbrc.jp/alignment/software/">https://mafft.cbrc.jp/alignment/software/</a>
Gblocks version 0.91b	147	<a href="http://molevol.cmima.csic.es/castresana/Gblocks.html">http://molevol.cmima.csic.es/castresana/Gblocks.html</a>
RAxML version 8.2.12	148	<a href="https://cme.hits.org/exelixis/web/software/raxml/">https://cme.hits.org/exelixis/web/software/raxml/</a>
CAFÉ version 4.2.1		<a href="https://hahnlab.github.io/CAFE/">https://hahnlab.github.io/CAFE/</a>
Trimmomatic version 0.39	149	<a href="http://www.usadellab.org/cms/?page=trimmomatic">http://www.usadellab.org/cms/?page=trimmomatic</a>
Bowtie 2 version 2.4.1	150	<a href="http://bowtie-bio.sourceforge.net/bowtie2/index.shtml">http://bowtie-bio.sourceforge.net/bowtie2/index.shtml</a>
RSEM version 1.3.3		<a href="https://github.com/siddharthab/RSEM">https://github.com/siddharthab/RSEM</a>
edgeR version 3.28.1	151	<a href="https://bioconductor.org/packages/release/bioc/html/edgeR.html">https://bioconductor.org/packages/release/bioc/html/edgeR.html</a>
eggNOG-mapper version 2.0.1	153	<a href="http://eggNOG-mapper.embl.de/">http://eggNOG-mapper.embl.de/</a>
clusterProfiler version 3.14.3	154	<a href="http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html">http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html</a>
Other		
WorldClim version 1.4		<a href="https://www.worldclim.org/">https://www.worldclim.org/</a>
PGSB Repeat Element Database	115	<a href="http://pgsb.helmholtz-muenchen.de/plant/repeat/">http://pgsb.helmholtz-muenchen.de/plant/repeat/</a>
Repbase		<a href="http://www.girinst.org/rebase">http://www.girinst.org/rebase</a>
UniProtKB/Swiss-Prot and UniProtKB/TrEMBL	124	<a href="https://www.uniprot.org/">https://www.uniprot.org/</a>
Gene Ontology	126	<a href="http://geneontology.org/">http://geneontology.org/</a>
DAVID		<a href="https://david.ncifcrf.gov/home.jsp">https://david.ncifcrf.gov/home.jsp</a>
Pfam		<a href="https://pfam.xfam.org/">https://pfam.xfam.org/</a>
EggNOG version 5.0	152	<a href="http://eggNOG5.embl.de/">http://eggNOG5.embl.de/</a>
Plants used for phylogenetic analysis (see Table S4 A)	This study	N/A
Calibrations used in age estimation (see Table S6 B)	This study	N/A
Plants used for testing fast evolution and positive selection analysis (see Table S6 C)	This study	N/A
Plants used for estimating the time of morphogenetic genes lost (see Table S6 D)	This study	N/A

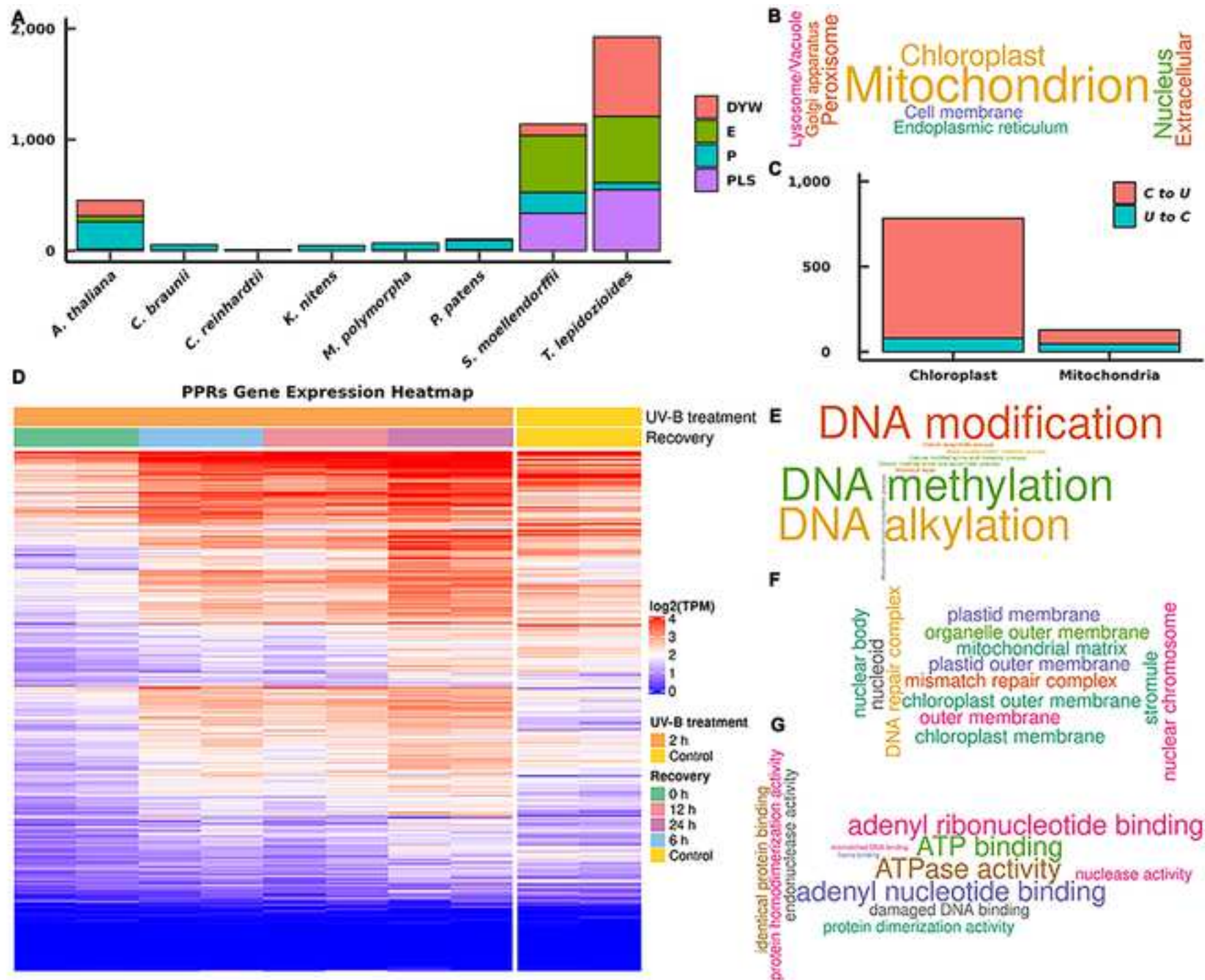


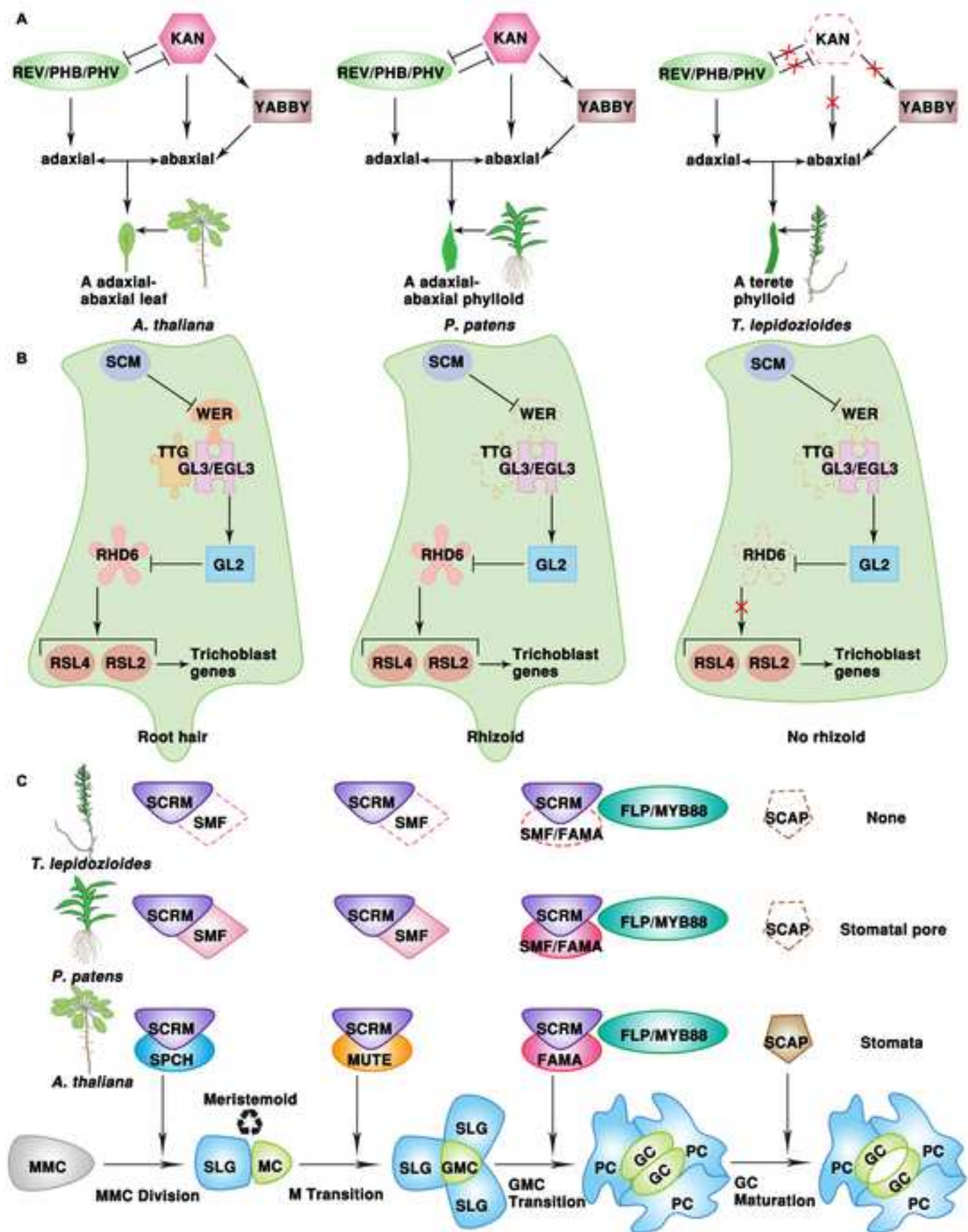






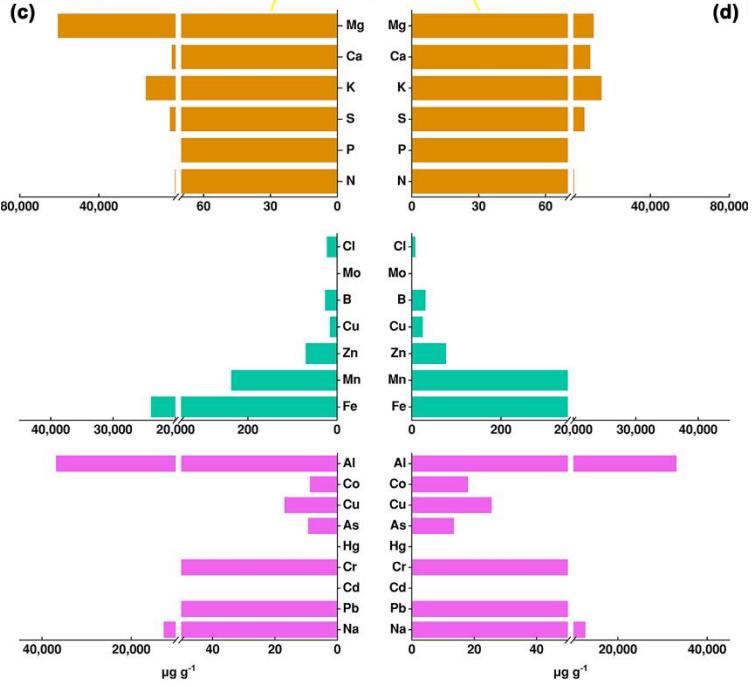
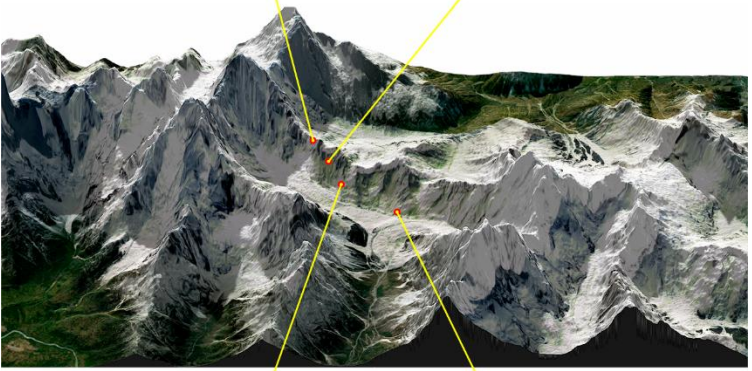
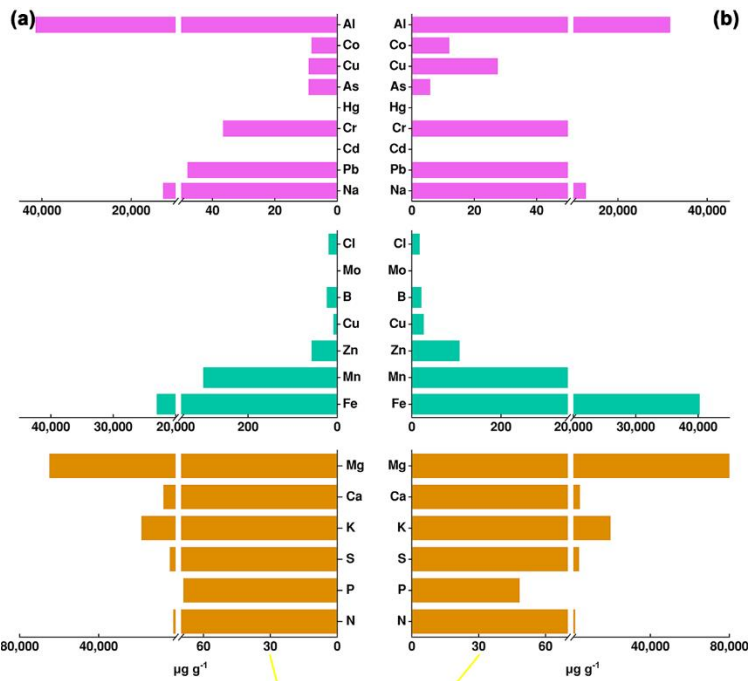






1 **Supplement data 1**

2

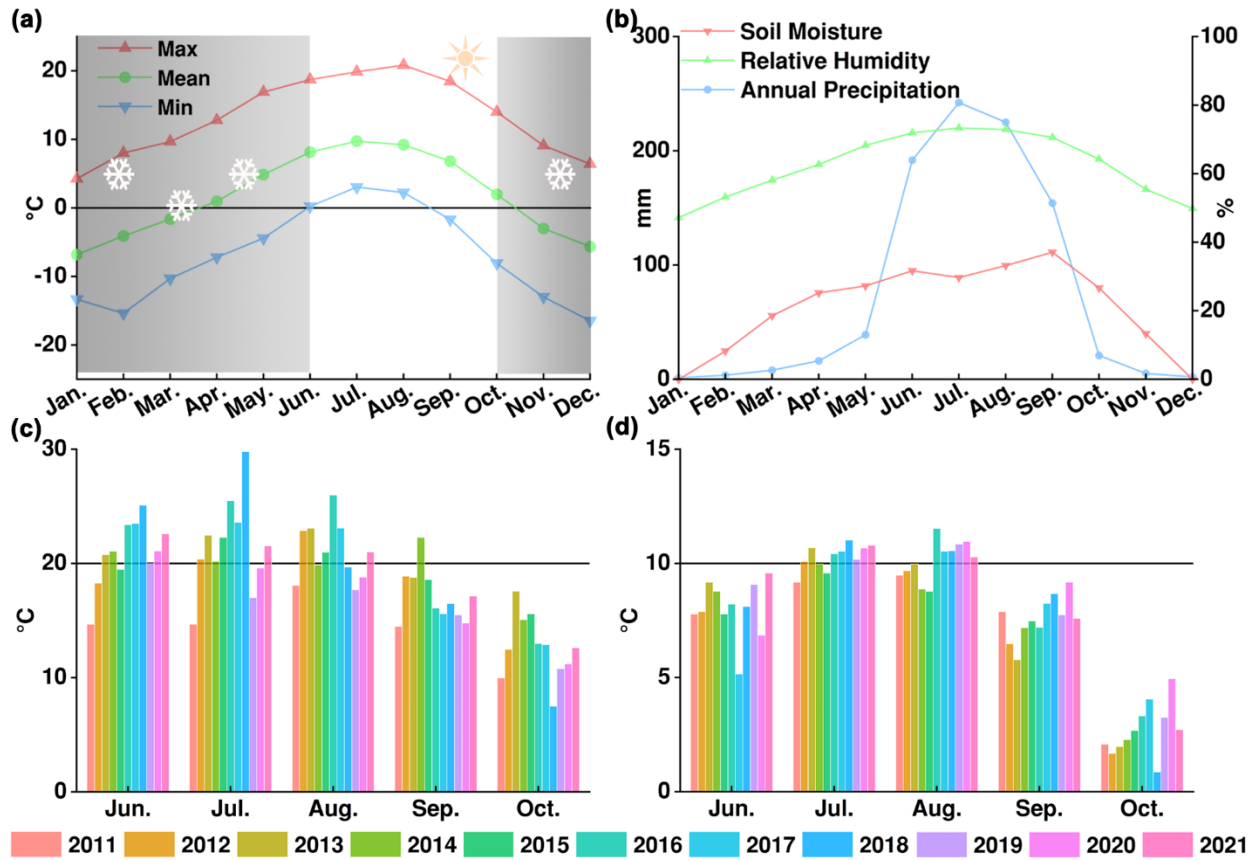


4

5 **Data S1 A. Soil mineral elements in the experimental area**

6 The soil mineral element components in plots with *T. lepidozooides* distribution (**a – c**) and  
7 without *T. lepidozooides* distribution (**d**). Red dots show the exact location of experiment plots in  
8 the area corresponding to Figure 1A from a different perspective. Pink color represents metal  
9 elements, green color represents microelements, and brown color represents macroelements.

10



11

12

13 **Data S1 B. Climate change from 2011 to 2021 in the experimental area**

14 The climate change in the survey area (plot B) from 2011 to 2021. (a) monthly temperature, (b)  
 15 monthly relative humidity, precipitation and soil moisture, (c) monthly maximum temperature  
 16 during growing season (June to October), (d) monthly average temperature during growing  
 17 season (June to October).

18

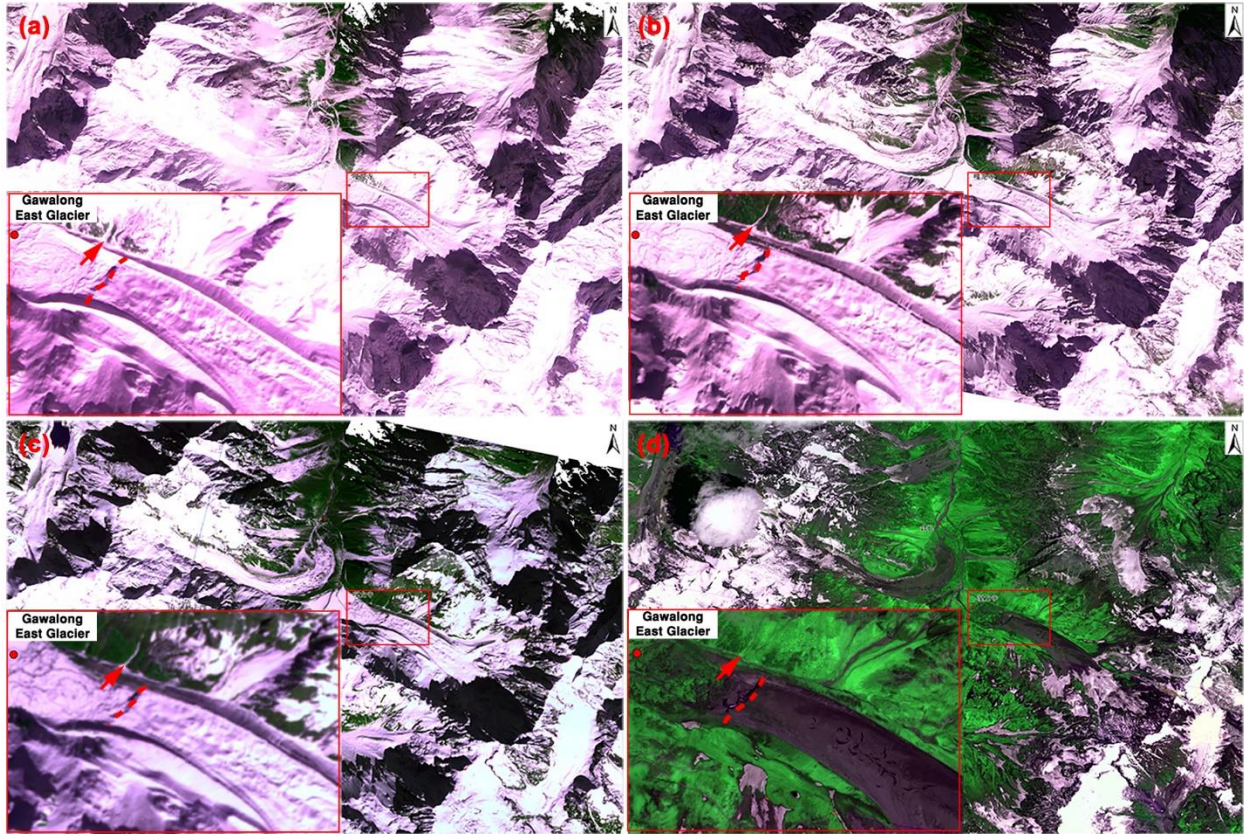


19  
20

21 **Data S1 C. Photographs of the edge of the Gawalong East Glacier**

22 Photographs of the edge of the glacier from 2011 to 2021. Years are indicated in red. The  
 23 yellow arrows show the mark of water flow (in a fixed position since 2005) near Gawalong East  
 24 Glacier. The yellow dotted lines show the edge of the glacier. The data of glacier retreat was  
 25 calculated from the measured distance between yellow arrow and dotted line and is compiled in  
 26 Table S1B.

27

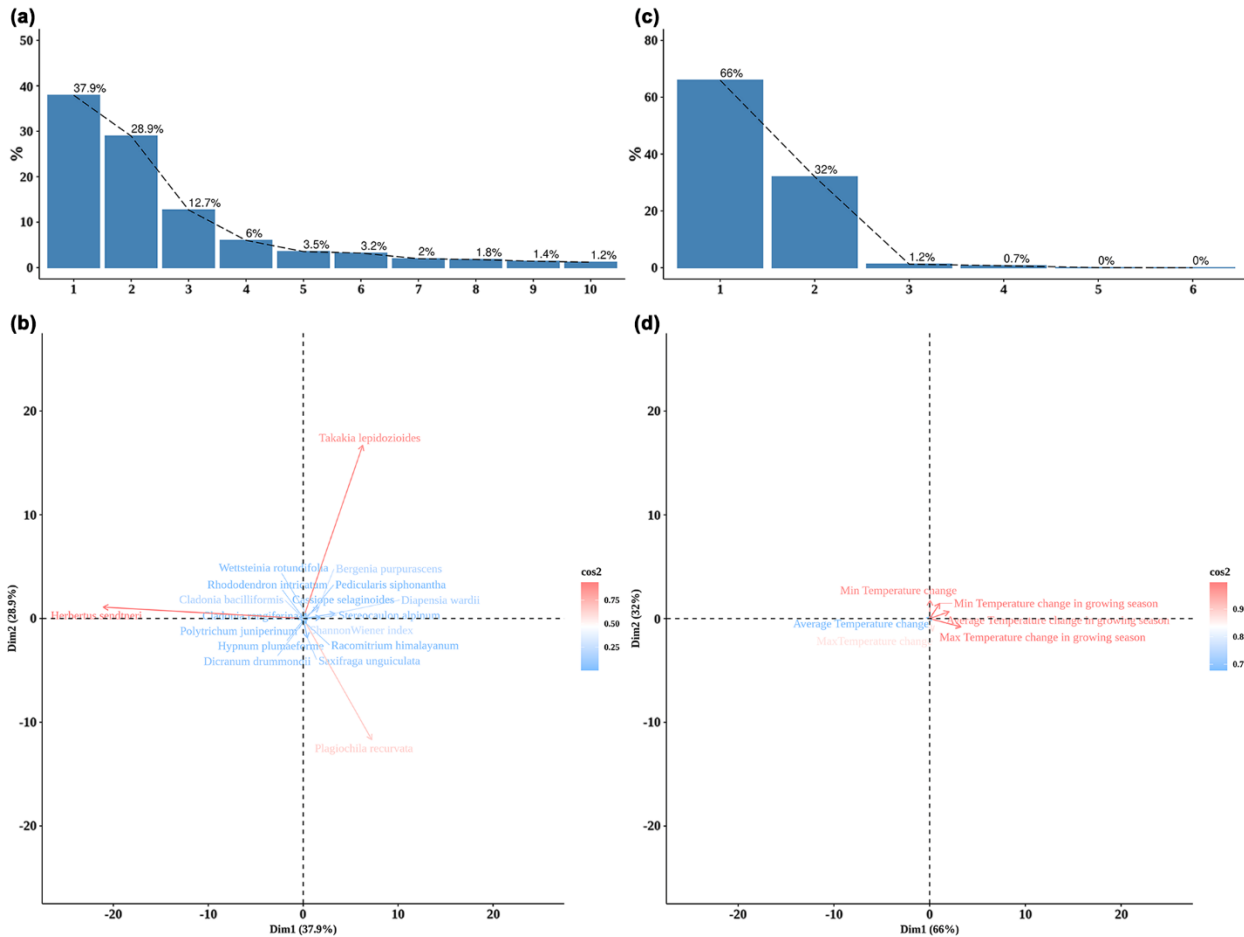


28  
29

30 **Data S1 D. Satellite images of the edge of the Gawalong East Glacier**

31 Satellite images of the glacier from Resources-3 (a, b) and Gaofen-1 (c, d). (a) Captured on Jan.  
 32 9<sup>th</sup>, 2013, (b) captured on Feb. 1<sup>st</sup>, 2014, (c) captured on Nov. 26<sup>th</sup>, 2013, (d) captured on Jul.  
 33 25<sup>th</sup>, 2015. Red boxes represent magnified views of the glacier. The red arrows show the mark  
 34 of water flow (in a fixed position since 2005) near Gawalong East Glacier. The red dotted lines  
 35 show the edge of the glacier. The space resolution ratio of both satellites is at 2 m, data from  
 36 the same satellite at different year was used to estimate glacier ablation. Detailed data of glacier  
 37 ablation estimation is compiled in Table S1B.

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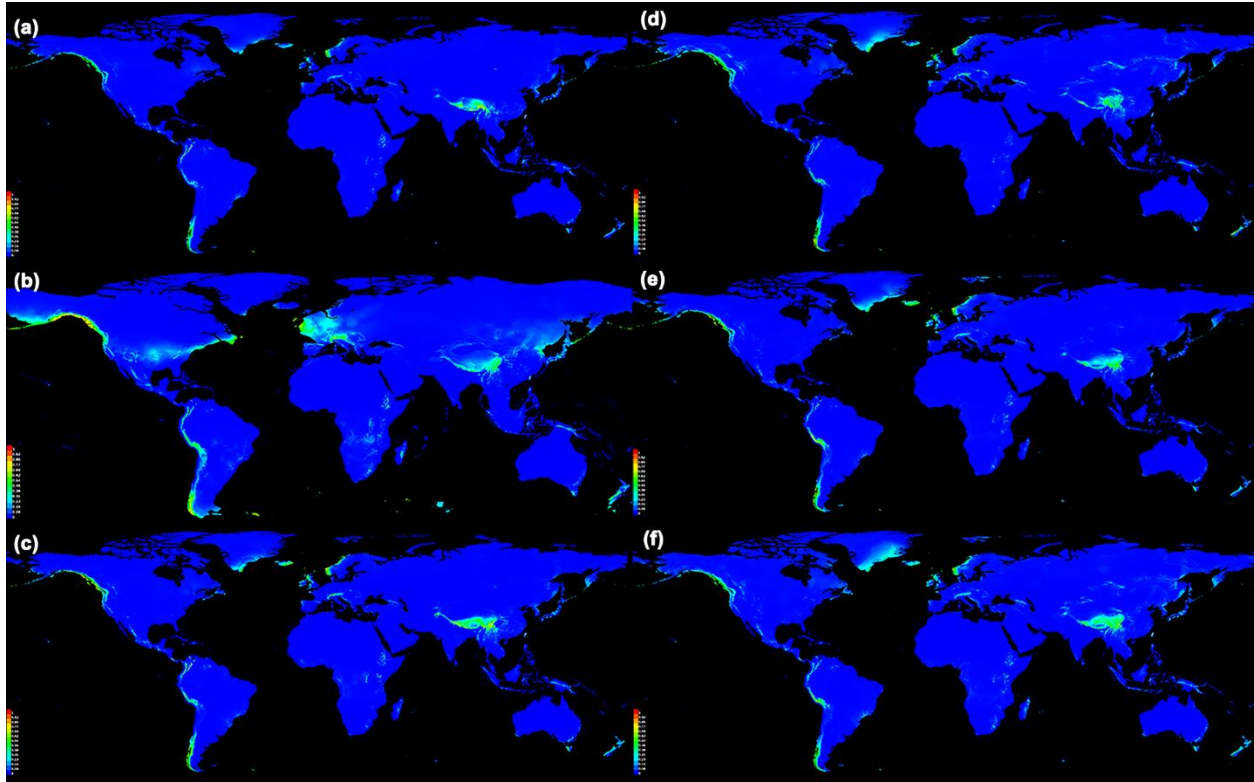


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40

#### 41 **Data S1 E. Correlation analysis of population changes and climatic changes**

42 Correlations among species population changes and climatic variables changes. (a) Principal  
 43 component analysis (PCA) of species population changes, depicted 3 principal component  
 44 contribution rate came up to 79.5 %. (b) Redundancy analysis (RDA) of species population  
 45 changes, depicted *T. lepidozoioides*, *H. sendtneri* and *P. recurvata* populations changed flexibly.  
 46 (c) PCA analysis of climatic variables, depicted 2 principal component contribution rate came up  
 47 to 98%. (d) Canonical correspondence analysis (CCA) of climatic variables, depicted  
 48 temperature in growing season significantly affected the populations. All multiple regressions  
 49 and backward eliminations were performed by vegan package ([https://CRAN.R-](https://CRAN.R-project.org/package=vegan)  
 50 [project.org/package=vegan](https://CRAN.R-project.org/package=vegan)) in R.

51

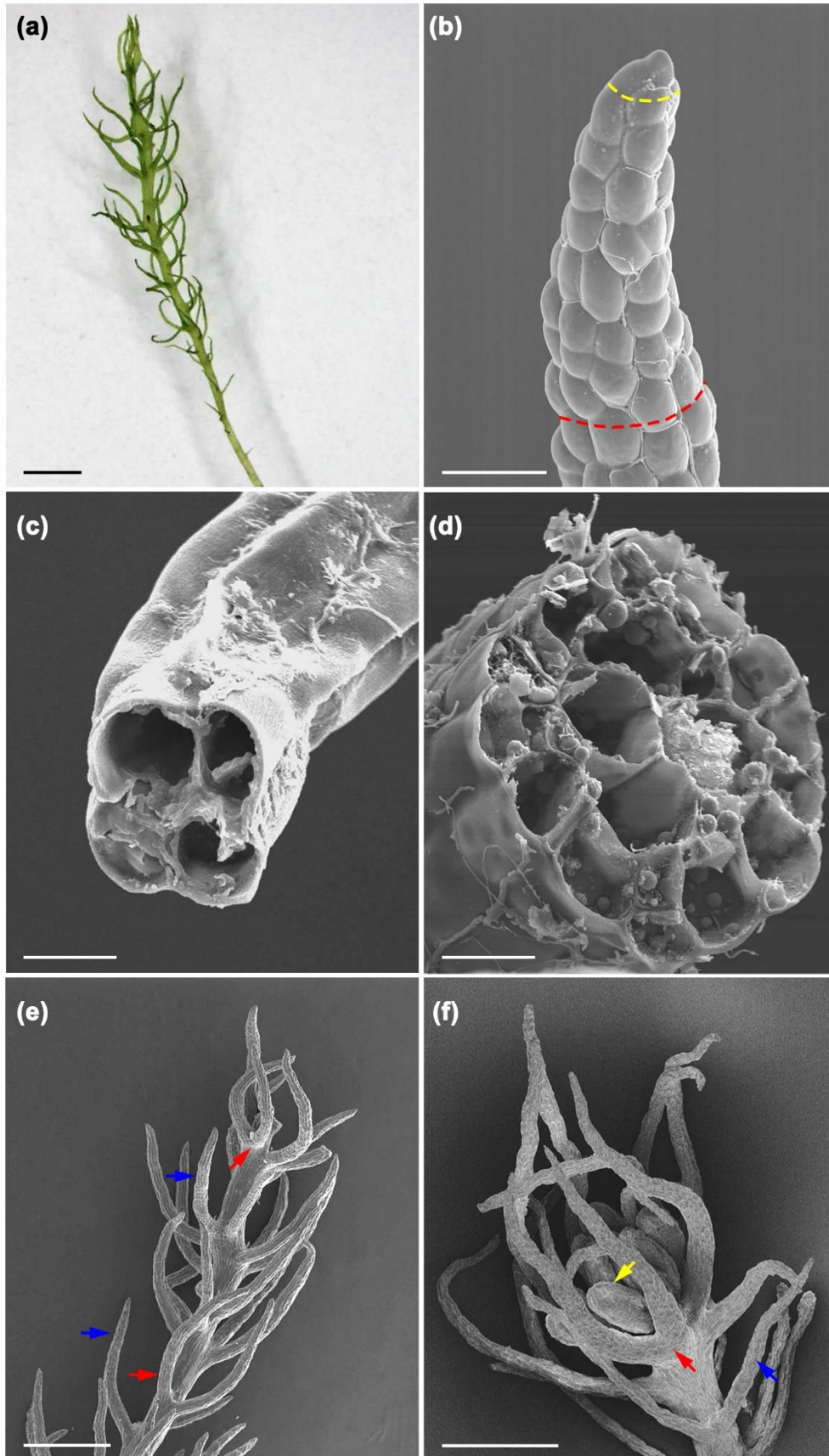


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53

54 **Data S1 F. Potential distribution of *T. lepidozoides* over time**

55 Potential distribution of *T. lepidozoides* (a) in the last interglaciation (~120,000 – 140,000 years ago), (b) in the last glacial maximum (about 22,000 years ago), (c) in the Holocene mega  
56 thermal (about 6,000 years ago), (d) 1970s to 2000s, (e) 2040s to 2060s, (f) 2060s to 2080s.  
57 Suitable conditions were classified according to Liu et al. [S1], regions with mean predicted  
58 value > 0.68 defined as suitable conditions, sites with value in 0.38 ~ 0.68 were taken as typical  
59 candidate distributions and predicted value lower than 0.38 were taken as pseudo absences.  
60 Colors indicate predicted probability that conditions are suitable, with warmer colors indicating  
61 high probability of suitable conditions for the species, green indicating conditions typical of those  
62 where the species is found, and lighter shades of blue indicating low predicted probability of  
63 suitable conditions.  
64

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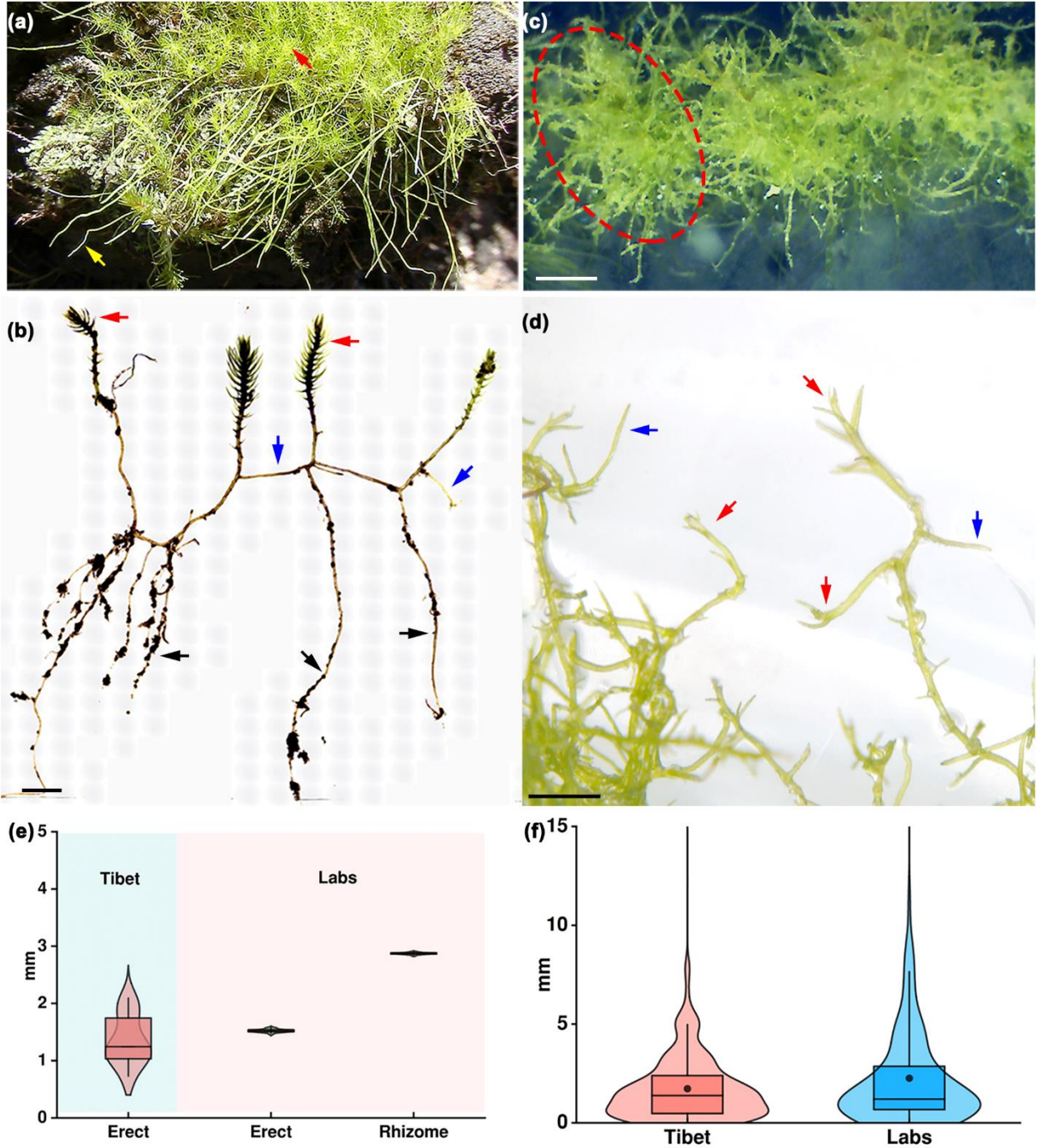


67

68 **Data S1 G. The leaf shape of *T. lepidozoides***

69 Plants are from the population of Plot B in the field. (a) An erect stem with finger-shaped leaves.  
70 (b – d) SEM images of a leaf (b), the transection (c) from the tip (yellow circle in b) and the  
71 transection (d) from below (red circle in b). (e – f) Arrows mark the single finger-shaped leaf  
72 (blue), finger-shaped leaf connected at the base (red) and antheridia (yellow arrow) located at  
73 the base of leaves on top of an erect stem. The picture in (a) was made using a stereo  
74 microscope (M125 C, Leica), pictures from (b) to (f) were made with a scanning electron  
75 microscope (S4800, Hitachi). Bar = 1 mm in (a), 50  $\mu\text{m}$  in (b), 20  $\mu\text{m}$  in (c) and (d), 500  $\mu\text{m}$  in  
76 (e) and (f).

77



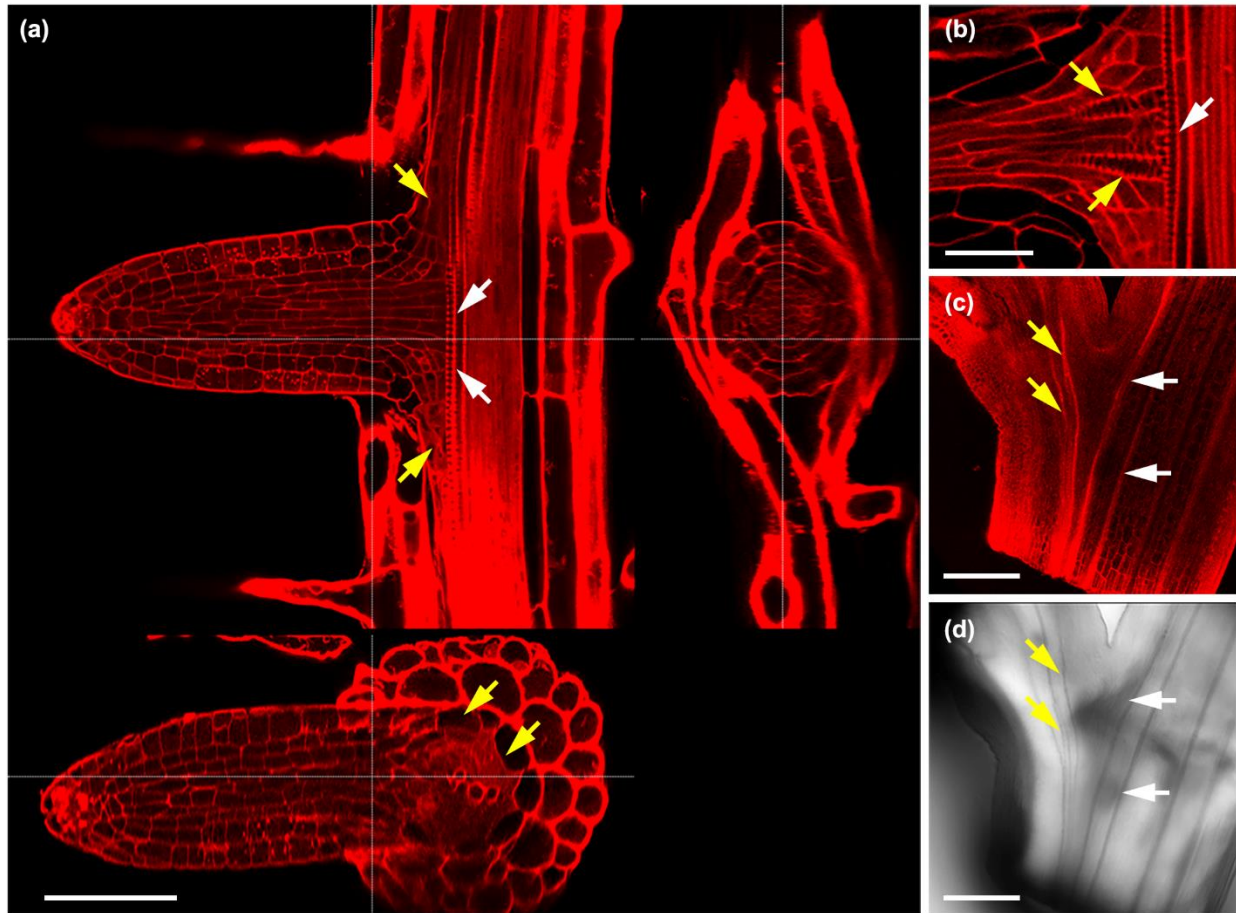
78  
79

80 **Data S1 H. Vegetative growth features of *T. lepidozoides***

81 (a) A natural population of *T. lepidozoides* in Plot B, red arrow marks erect stem, yellow arrow  
82 marks rhizome, photograph was made using EOS-1D X Mark II (Canon) with macro lens (MP-  
83 E65 mm, Canon). (b) One part of an individual plant isolated from the population of (a) (bar = 2  
84 mm), arrows show erect stems (red), rhizomes (blue) and aging/faded plant parts (black).

85 Observations were made using an optical microscope (BA600-4, Motic). (c) A population (circle)  
86 reproduced from a 3 – 4 mm fragment of erect stem or rhizome cultured on medium for 8  
87 months, bar = 2 mm, observation was made using a stereo microscope (Zeiss, AXIO Zoom.  
88 V16). (d) The branching pattern of plants in population of (c), red arrows mark erect stems, blue  
89 arrows mark rhizomes, bar = 1 mm, observation was made using an optical microscope (Zeiss,  
90 AXIO Zoom. V16). (e) The annual growth of stems and rhizomes in the field (n = 42, growth was  
91 measured with ImageJ from time-lapse photos) and laboratory (n = 72, measured with ImageJ  
92 from microscope). (f) The distance between two adjacent branches in the field (n = 174,  
93 measured with ImageJ) and laboratory (n = 135, measured with ImageJ). The central mark of  
94 each box indicates the median, and the bottom and top edges of the box indicate the 25th and  
95 75th percentiles respectively, the black dots in each box indicates the mean values.

96

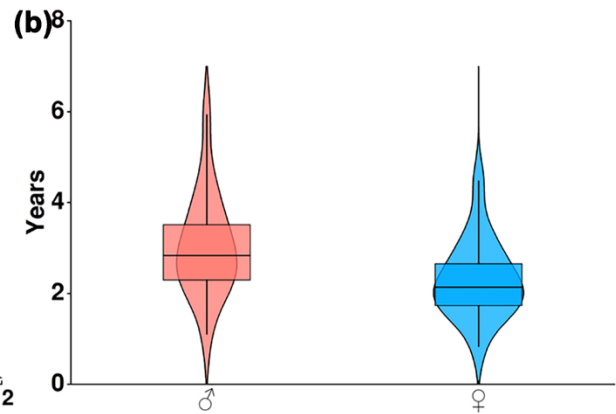
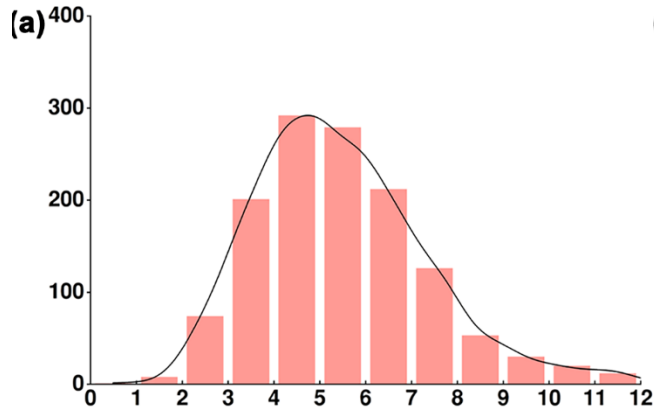


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98

99 **Data S1 I. The connection mode of vessel elements in vascular bundles between lateral-**  
100 **main roots and branches in *A. thaliana*, observed with the mPS-PI staining technique**

101 (a) The sections from three mutually perpendicular directions at the connection site (the cross  
102 point of white dotted lines) between main root and lateral root, yellow arrows show pericycle  
103 cells, white arrows show vessel elements in vascular bundles of main root, bar = 100 μm. (b)  
104 Arrows show vessel elements in main root (white) and lateral root (yellow), bar = 30 μm. (c, d)  
105 Arrows show vessel elements in main stem (white) and branch (yellow), PI stain-fluorescent  
106 image (c) and bright-field image (d) for the same sample, bar = 500 μm. Fluorescence images  
107 were obtained using a Leica SP8 laser scanning confocal microscope, with the excitation  
108 wavelength at 561 nm and emission wavelength from 570 nm to 670 nm.

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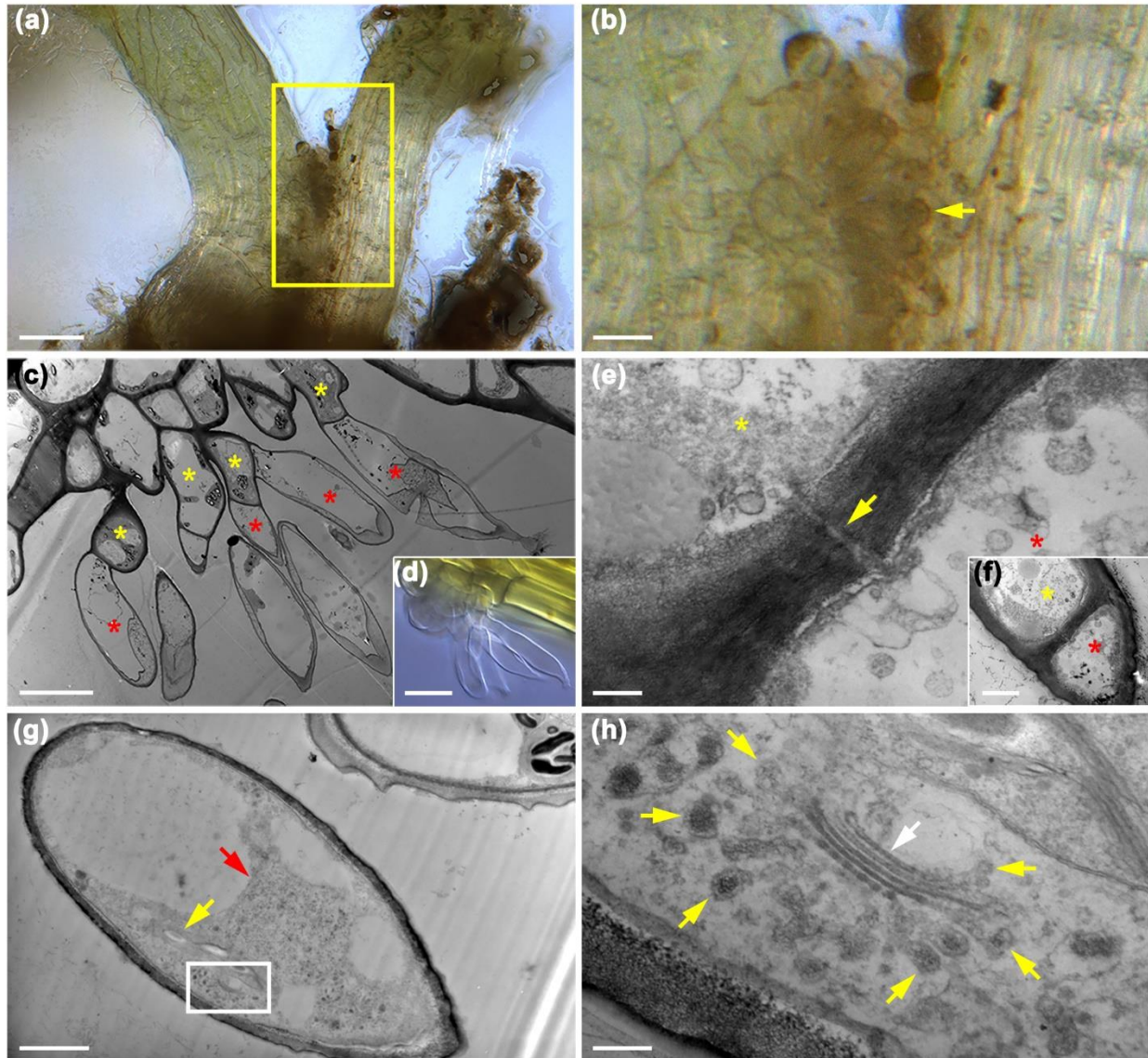
110

111

112 **Data S1 J. Sexual reproduction features of *T. lepidozooides***

113 (a) Distribution blot of the height of erects in the fields. Horizontal axis represents the height of  
 114 erects in mm, vertical axis represents the frequency of occurrence (n = 1,315). (b) The timing  
 115 that erect stems began sexual reproduction. Male plants achieve sexual maturity in  $3.06 \pm 1.14$   
 116 years (median 2.83), while female plants achieve sexual maturity in  $2.31 \pm 0.86$  years (median  
 117 2.14) years of vegetative growth (n = 17). The central mark of each box indicates the median,  
 118 and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively.

119



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122 **Data S1 K. The mucilage hairs on the surfaces of stems in *T. lepidozoides***

123 (a) Plants collected from field populations. Mucilage hairs at the cross point between branches,  
 124 bar = 100  $\mu\text{m}$ . (b) Magnified views of boxed areas in (a), yellow arrow marks mucilage hairs with  
 125 humus, bar = 20  $\mu\text{m}$ . (c) TEM picture of mucilage hairs showing that mucilage hairs are  
 126 multicellular. Yellow stars mark the basal cells, red stars mark the tip cells, bar = 10  $\mu\text{m}$ . (d)  
 127 Mucilage hairs originate from a stem in cultured populations shown at right corner in (c), bar =  
 128 20  $\mu\text{m}$ . (e) The basal cell (yellow star) and tip cell (red star) are connected by plasmodesmata  
 129 (yellow arrow), magnified from (f), bar = 200 nm. (f) A mucilage hair with basal cell (yellow star)  
 130 and tip cell (red star), bar = 1  $\mu\text{m}$ . (g) A tip cell of mucilage hairs contains a nucleus (red arrow),

131 chloroplast (yellow arrow) and Golgi apparatus (white box), bar = 20  $\mu\text{m}$ . (h) The Golgi  
132 apparatus (white arrow) with secretory vesicles (yellow arrows), magnified from the box of (g),  
133 bar = 200 nm. (a) and (d) were made using an optical microscope (BA600-4, Motic), (b) was  
134 made using a stereo microscope (M125 C, Leica), (c), (e) – (h) were made with a transmission  
135 electron microscope (H7500, Hitachi).

136

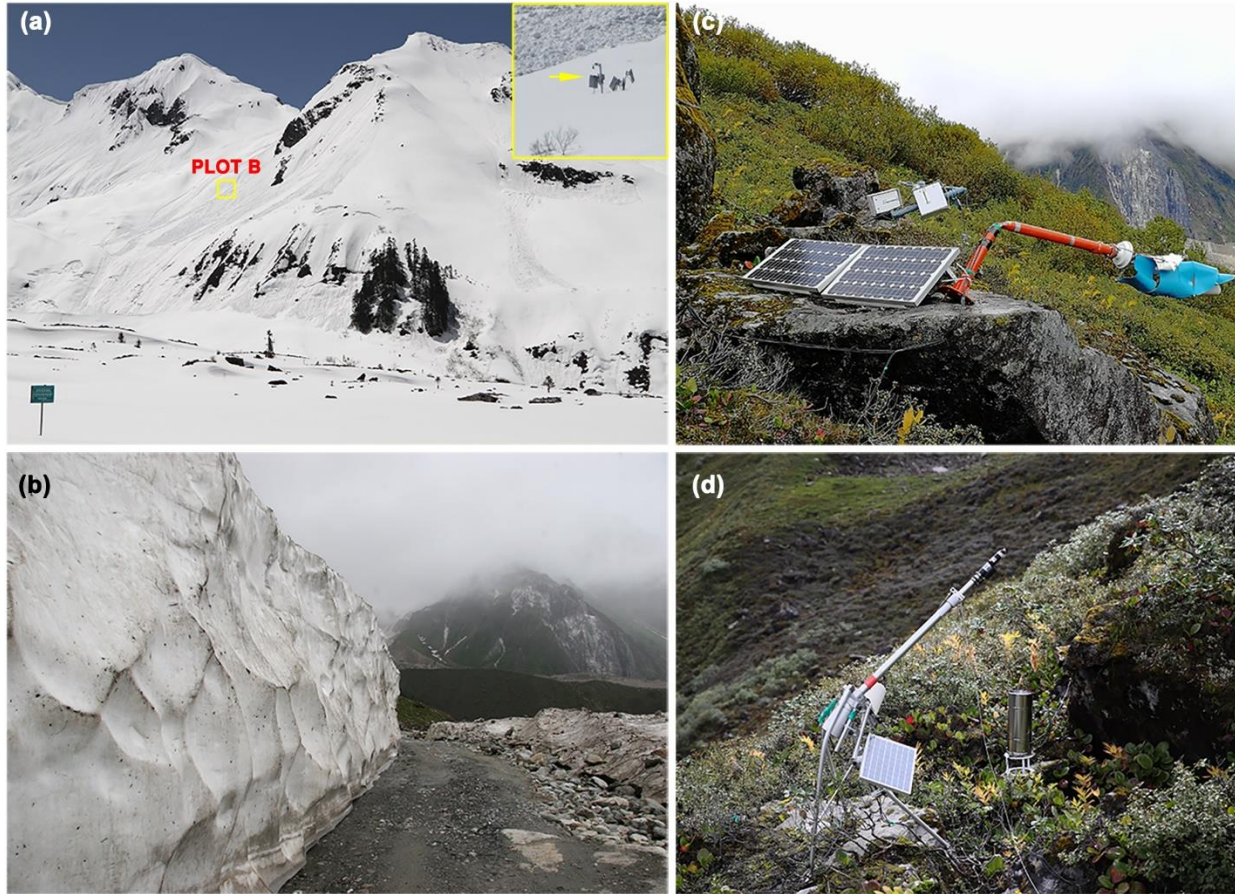


138

139 **Data S1 L. *T. lepidozoides* populations in the field**

140 (a) A population hanging on the top edge of a cave. (b) Magnified views of boxed areas in (a),  
141 gametophytes show weak negative geotropism at the apex (yellow arrow), red arrow marks the  
142 direction of gravity, and white arrow marks the sunlight direction for most of a day. (c) A  
143 population on a steep rock surface. (d) Magnified views of boxed areas in (c), sporophyte shows  
144 weak negative geotropism at the apex (yellow arrow), red arrow marks the direction of gravity,  
145 and white arrow marks the sunlight direction for most of a day. Photographs were made using  
146 EOS-1D X Mark II (Canon) with macro lens (MP-E65 mm, Canon).

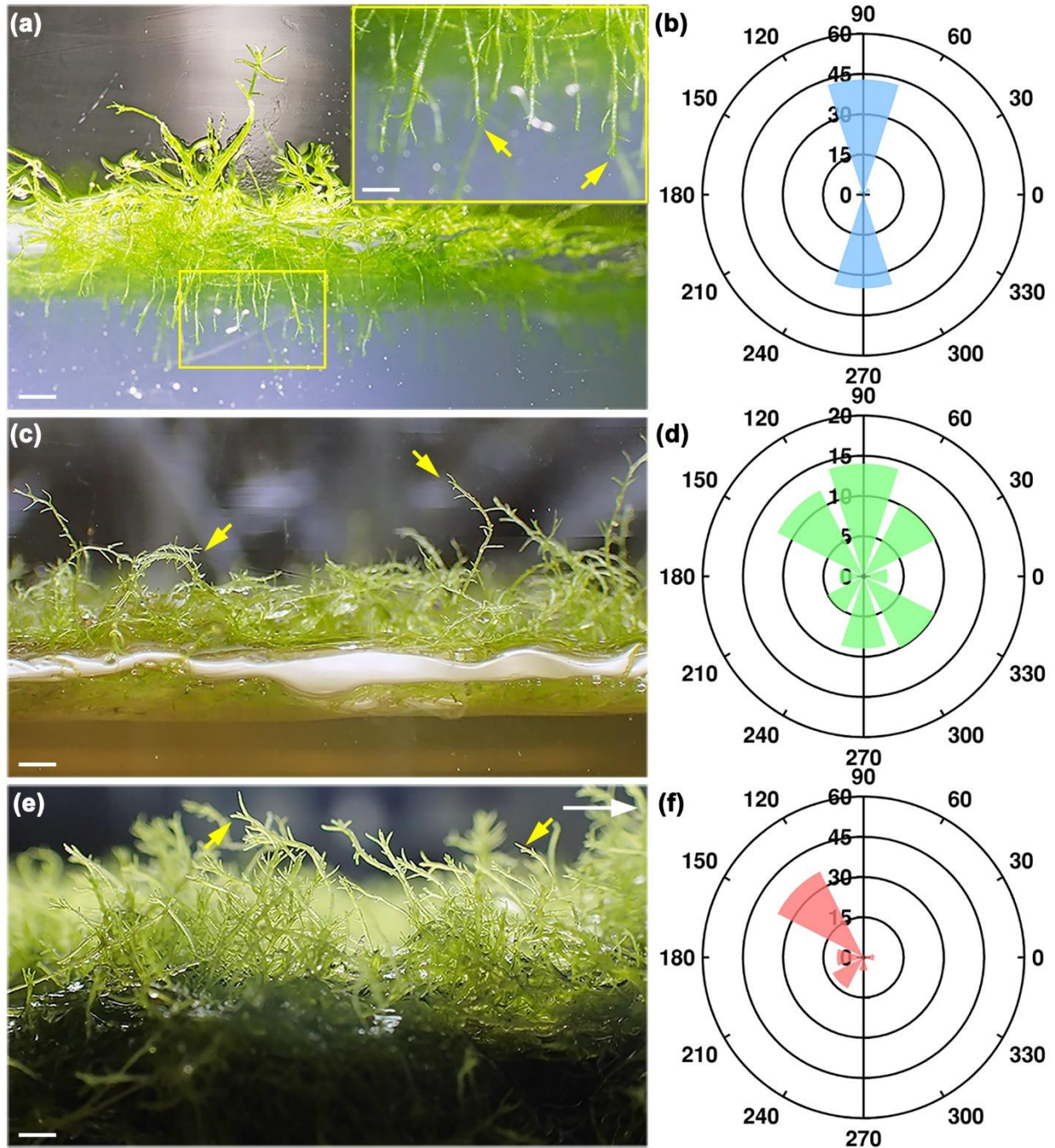
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150 **Data S1 M Heavy snowstorm during winter in the experiment sites**

151 Heavy snowstorm during winter (a, b) and meteorological instrument was broken (c, d) by the  
 152 heavy snow in the experiment plots. (a) The picture was captured by an unmanned aerial  
 153 vehicle (Matrice 300 RTK, DJI) on 5<sup>th</sup> May, 2020, yellow box marks meteorological instrument in  
 154 plot B and magnified at top right (yellow arrow). (b) The picture was captured on 20<sup>th</sup> June, 2008  
 155 in plot B. (c – d) The pictures were captured on 20<sup>th</sup> August, 2019 in plot B.  
 156

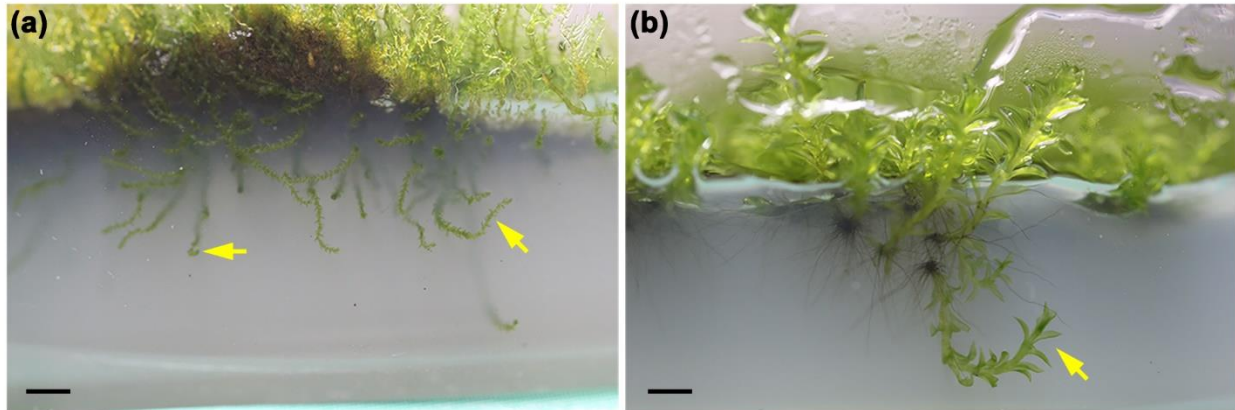


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159 **Data S1 N. The tropism features of *T. lepidozoides* in culture**

160 Plants were grown under normal (a, b), continuous dark (c, d) and continuous unilateral light (e,  
161 f) for 8 months. (a) Bar = 2 mm, box magnified at top right with bar = 1 mm, the apex lacks  
162 obvious negative geotropism (yellow arrow). (b) The response was quantified by counting the  
163 number of tips (n = 70) in all 30° sectors. (c) Bar = 2 mm, the apex lacks obvious tropism

164 (yellow arrow). The response was quantified by counting the number of tips ( $n = 66$ ) in all  $30^\circ$   
165 sectors (**d**). (**e**) Bar = 2 mm, white arrow marks the direction of continuous unilateral light, the  
166 apex clearly shows phototropism (yellow arrow). The response was quantified by counting the  
167 number of tips ( $n = 60$ ) in all  $30^\circ$  sectors (**f**). Horizontal axis represents sector area, the vertical  
168 axis represents the count number.  
169



170  
171

172 **Data S1 O Negative geotropism of the mosses *Bryum argenteum* and *Physcomitrium***  
173 ***patens* in culture**

174 Negative geotropism of (a) *Bryum argenteum* (bar = 1 mm) and (b) *Physcomitrium patens* (bar  
175 = 1 mm). *B. argenteum* and *P. patens* shoots firstly grow downward (typical geotropism) without  
176 spaces suitable for them to grow upward because the spaces were occupied by other shoots.  
177 When the surrounding space becomes available, they return to grow upward (yellow arrow) with  
178 apparent negative geotropism. *B. argenteum* was grown on half strength MS medium with a  
179 light intensity of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  delivered in a 16-h/8-h light/dark cycle at 22 °C for 8 weeks; *P.*  
180 *patens* was grown on BCD medium with a light intensity of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  delivered in a 16-  
181 h/8-h light/dark cycle at 25 °C for 8 weeks. Observations were made using a stereo microscope  
182 (M125 C, Leica).

183

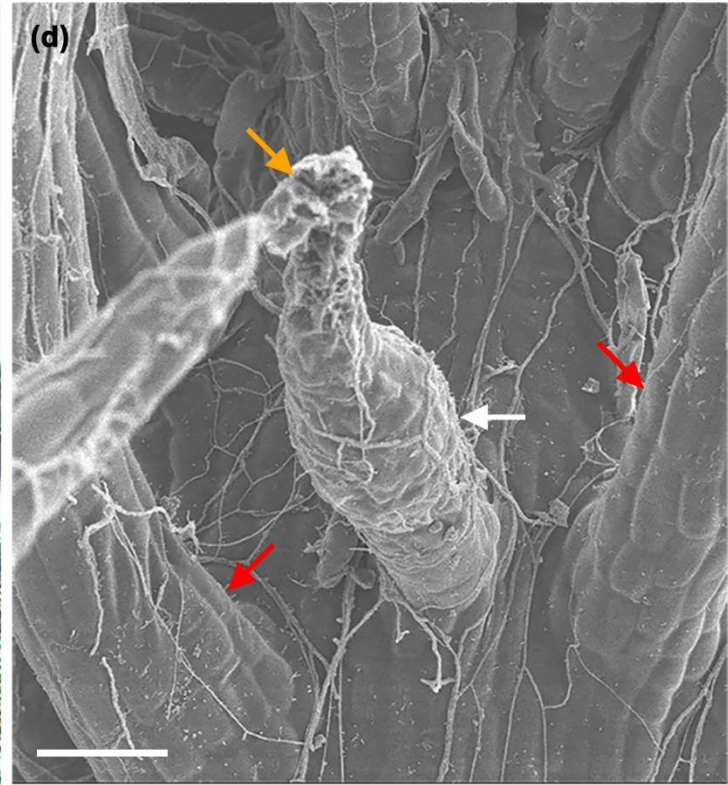
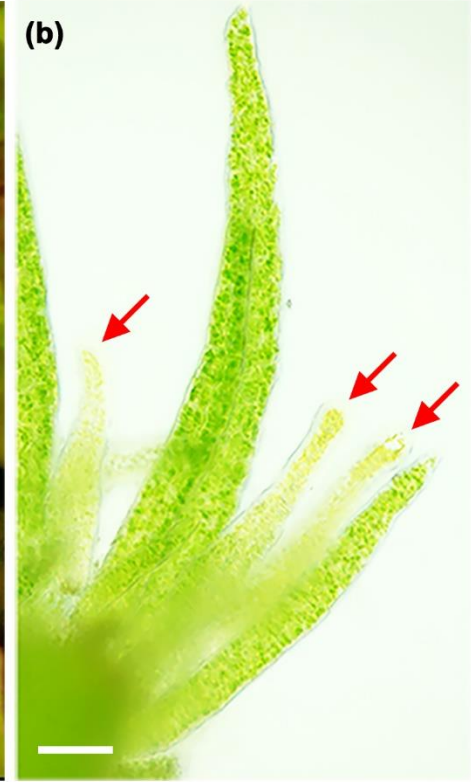
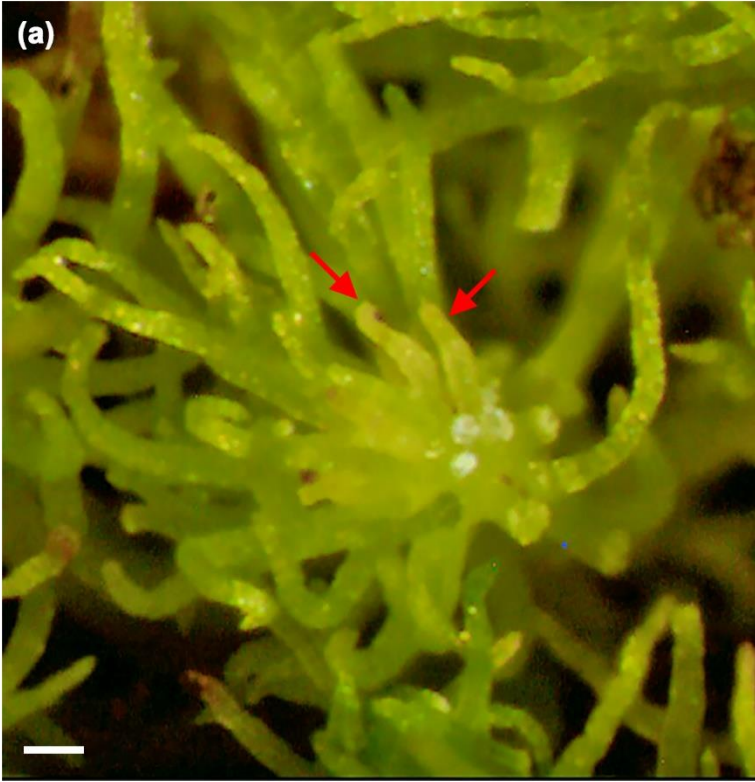


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186 **Data S1 P. Antheridia development in *T. lepidozoides***

187 Development of antheridia in the field. (a) Young antheridia in early July (bar = 2 mm). (b)  
188 Mature antheridia in late September, bar = 2 mm. (c) A male erect stem with antheridia (arrow).  
189 Red antheridia are a product of the current year and pale green antheridia are a product of the

190 previous year, bar = 200  $\mu\text{m}$ . **(d)** Magnified view of mature antheridia, bar = 200  $\mu\text{m}$ . **(e)**  
191 Magnified view of mature and dehisced antheridia. White arrow marks a dehisced antheridium,  
192 bar = 200  $\mu\text{m}$ . (a) and (b) were made using EOS-1D X Mark II (Canon) with macro lens (MP-  
193 E65 mm, Canon), (c) was made with an optical microscope (BA600-4, Motic), (d) and (e) was  
194 made using a stereo microscope (M125 C, Leica).  
195

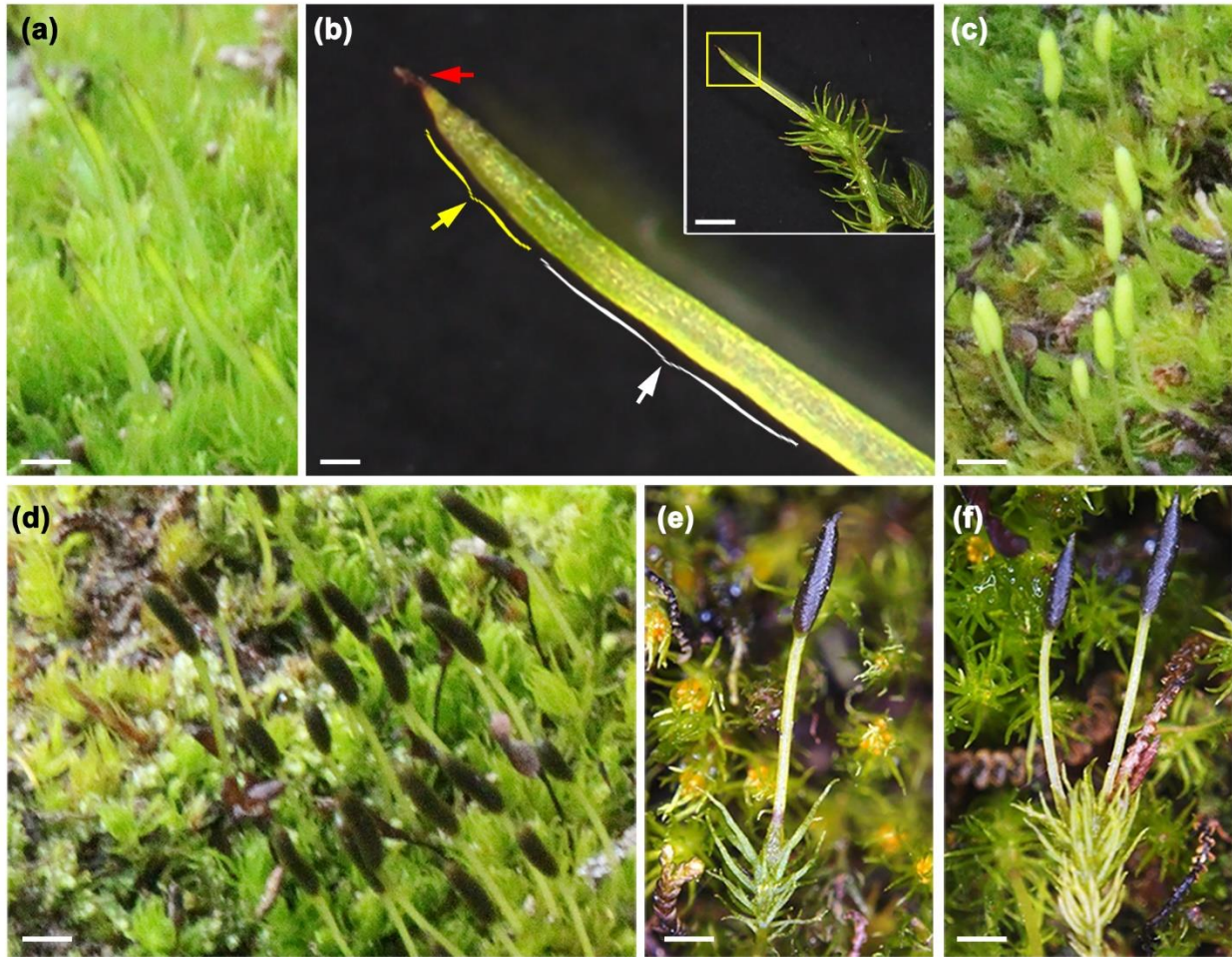


196  
197

198 **Data S1 Q. Archegonia development in *T. lepidozoides***

199 Development of archegonia in the field. **(a)** Young archegonia (red arrows) at the base of leaves  
200 on top of the erect stem, bar = 100  $\mu\text{m}$ . **(b)** A female erect stem with archegonia (red arrows) at  
201 the base of leaves, bar = 100  $\mu\text{m}$ . **(c)** A mature archegonium with an open canal at the top  
202 (orange arrow) and immature archegonia (red arrows), bar = 50  $\mu\text{m}$ . **(d)** SEM micrograph of a  
203 mature archegonium (white arrow) with an opening (orange arrow). Red arrows mark terete  
204 phylloids, bar = 50  $\mu\text{m}$ . (a) was made with a stereo microscope (M125 C, Leica), (b) and (c)  
205 were made with an optical microscope (BA600-4, Motic), (d) was made with a scanning electron  
206 microscope (S4800, Hitachi).

207

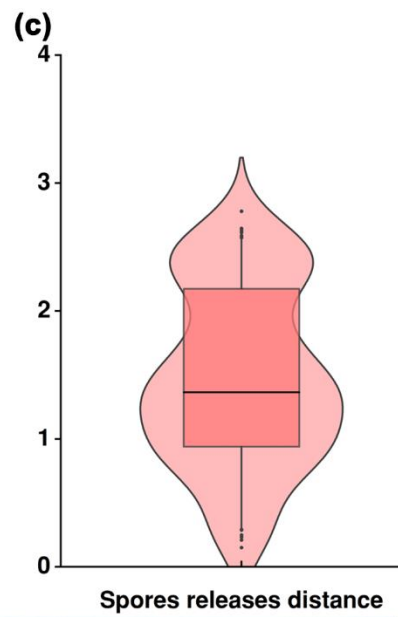
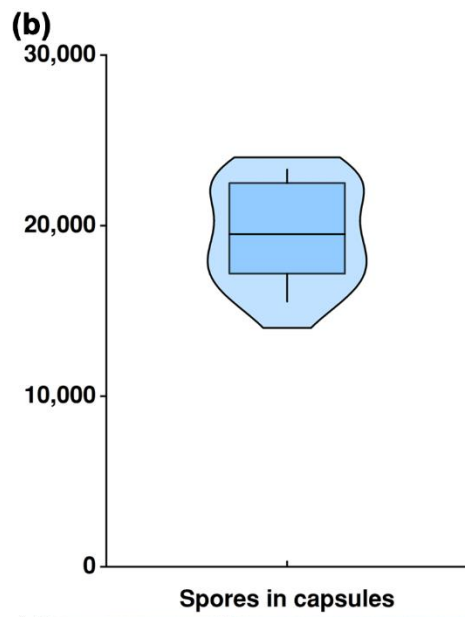


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210 **Data S1 R. Sporophyte development in *T. lepidozoides***

211 Development of sporophytes in the field. (a) Young sporophytes with unexpanded capsules in  
 212 early July, bar = 1 mm. (b) A young sporophyte developing (magnified from top right yellow box,  
 213 bar = 200  $\mu$ m) from a fertilized archegonium with the calyptra (red arrow) on the apiculus of the  
 214 unexpanded capsule (yellow arrow), white arrow marks seta, bar = 50  $\mu$ m. (c) Sporophytes with  
 215 capsule expansion in late July, bar = 1 mm. (d) Sporophytes with mature capsules in mid-  
 216 October, bar = 2 mm. (e) Single erect stem with one mature capsule, bar = 1mm. (f) Single  
 217 erect stem with two mature capsules, bar = 1mm.

218

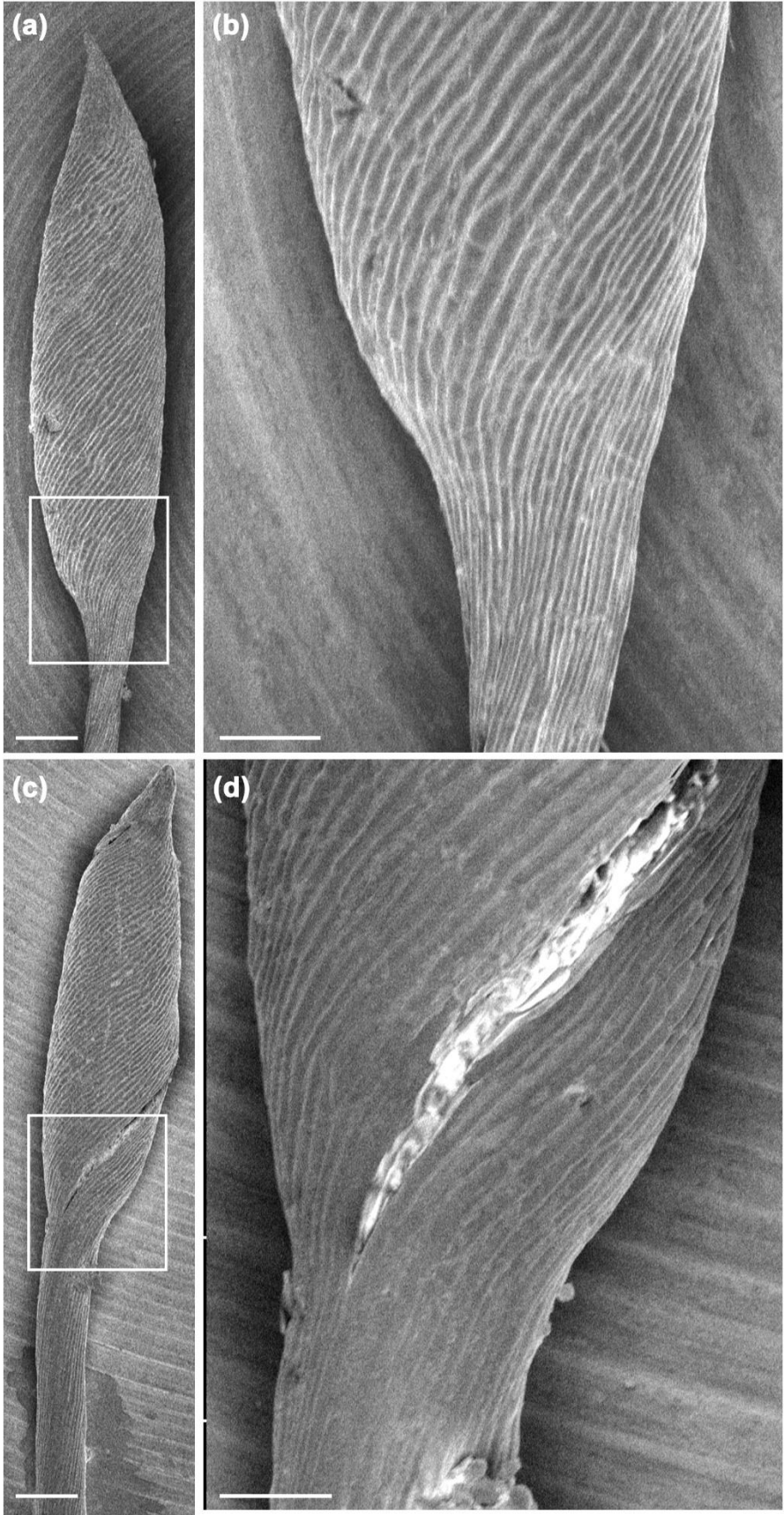


220

221 **Data S1 S. Capsule features of *T. lepidozooides***

222 (a) Analysis of sporophyte growth and development. Growth was measured with ImageJ from  
223 time-lapse photos in every 10 days in 2014 (n = 40). The central mark of each box indicates the  
224 median, and the bottom and top edges of the box indicate the 25th and 75th percentiles,  
225 respectively. (b) Spore numbers in one capsule (n = 8). (c) Spore dispersion distance (in cm)  
226 under laboratory conditions in (d), all radius data were from 4 capsules with 30 individual  
227 measurements in each. The central mark of each box indicates the median, and the bottom and  
228 top edges of the box indicate the 25th and 75th percentiles, respectively. (d) Experimental  
229 setting for (c). Erect stem with mature capsule collected from the field and inserted into 1 cm<sup>3</sup>  
230 agar in a petri dish. The dish was placed in a chamber with silica gel to mimic dehiscence. The  
231 distance from spore to erect bottom were measured with ImageJ, photograph was made using a  
232 stereo microscope (M125 C, Leica), bar = 5 mm.

233

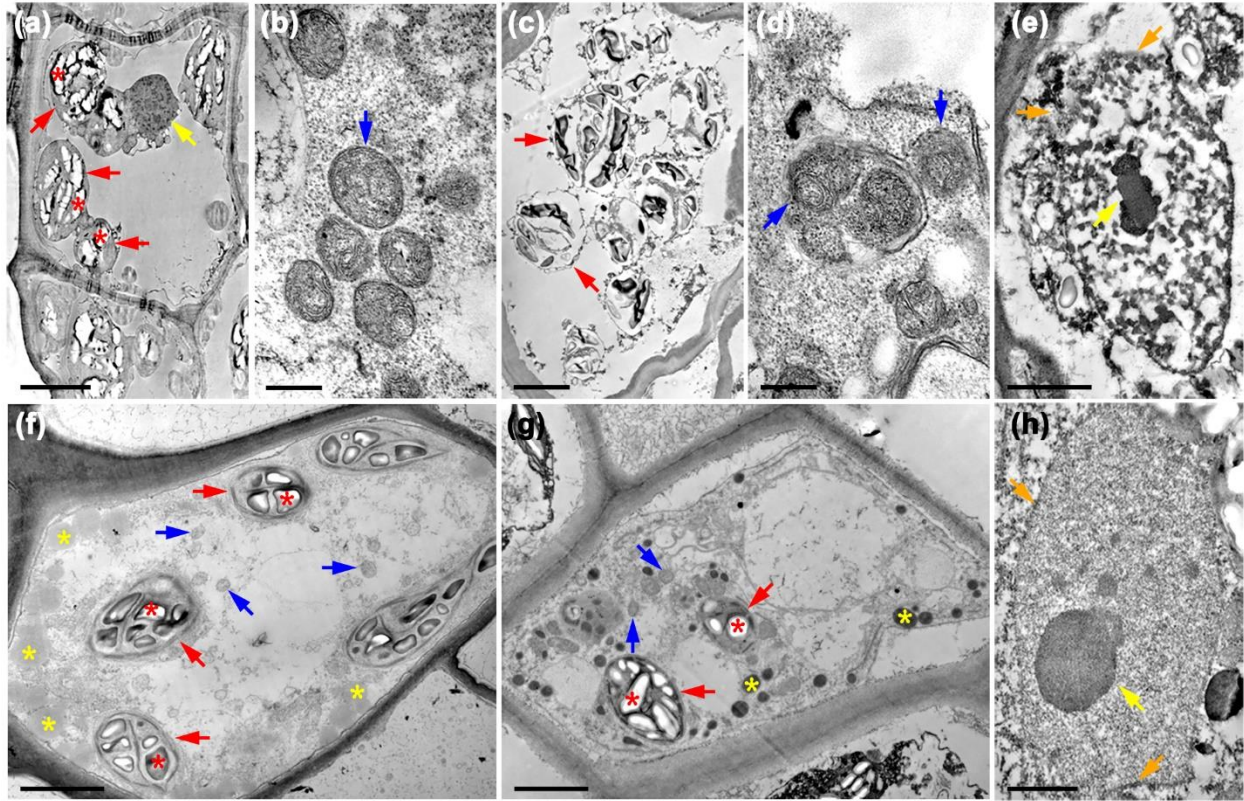


235

236 **Data S1 T. SEM of *T. lepidozoides* capsules**

237 SEM micrographs of *T. lepidozoides* capsules showing that the sporophyte lacks stomata at the  
238 capsule base, unlike other moss sporophytes such as *P. patens* [S2]. Young (**a, b**) and mature  
239 capsule with spiral split (**c, d**). (b) and (d) are magnified views of boxed areas in (a) and (c)  
240 respectively. (a) – (c) bars = 200  $\mu\text{m}$ , (d) bar = 100  $\mu\text{m}$ . Observations were made with a  
241 scanning electron microscope (S4800, Hitachi).

242



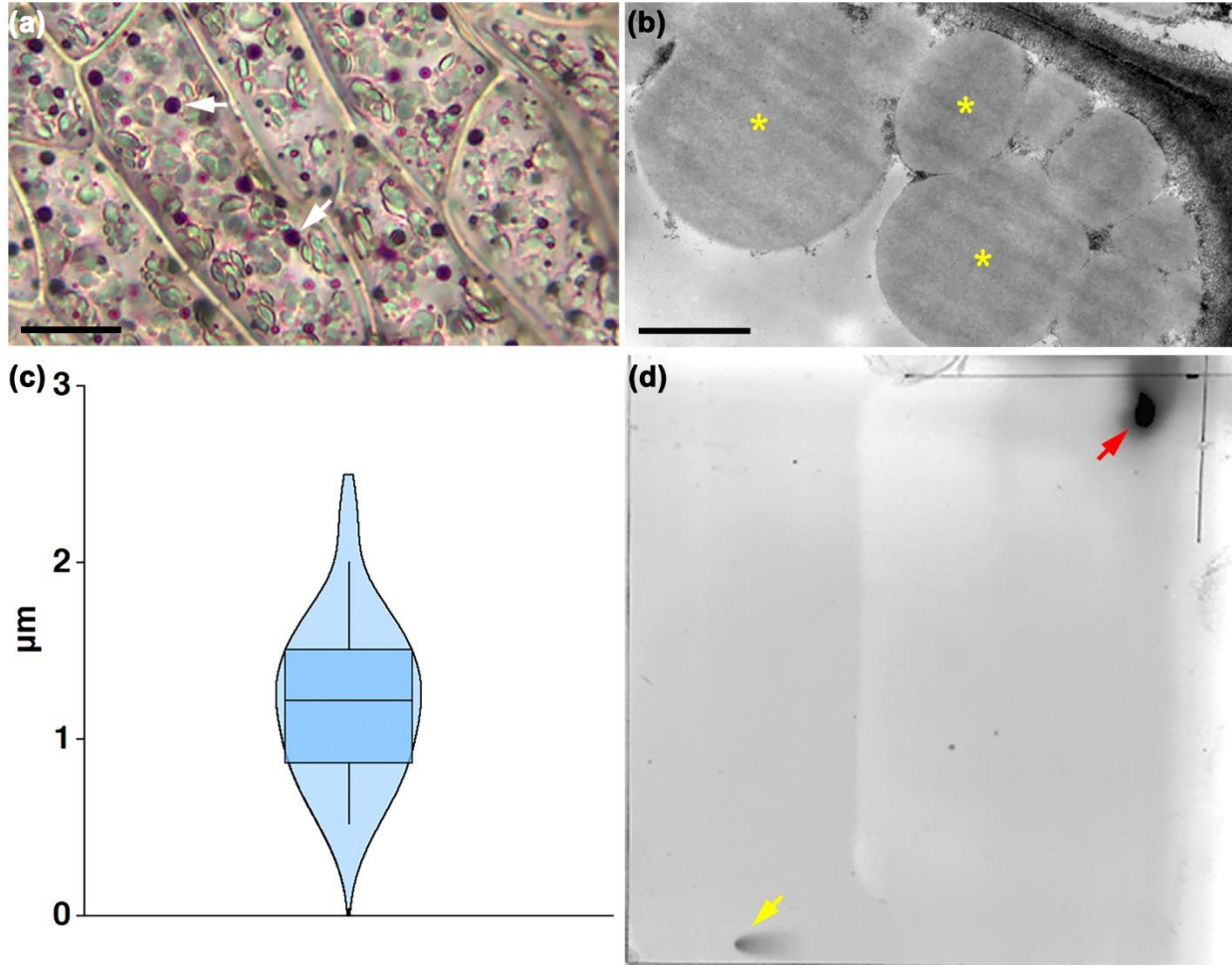
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244

245 **Data S1 U. TEM of cells before and after UV-B radiation in *P. patens* and *T. lepidozoides***

246 TEM micrographs of *P. patens* (a – e) and *T. lepidozoides* (f – h) before and after UV-B  
 247 radiation and recovery. (a, b) Chloroplasts (red arrows) with starch grains (stars) and  
 248 mitochondria (blue arrow) in the cell under normal condition. (c – e) Collapsed chloroplasts (red  
 249 arrows), expanded mitochondria (blue arrows) and a collapsed nucleus with disintegrated  
 250 nuclear membranes (orange arrows) and nucleolus (yellow arrow) surrounded by agglomerated  
 251 chromatin in cells recovering for 48 to 72 hours after UV-B radiation. (f) Chloroplasts (red  
 252 arrows) with starch grains (red stars), mitochondria (blue arrows) and oil bodies (yellow stars) in  
 253 the cell under normal condition. (g, h) Chloroplasts (red arrows) with starch grains (red stars),  
 254 mitochondria (blue arrows), oil bodies (yellow stars) and a nucleus with intact nuclear  
 255 membrane (orange arrows) and nucleolus (yellow arrow) surrounded by a slight condensed  
 256 chromatin in cells recovering for 48 to 72 hours after UV-B radiation. Observations were made  
 257 with a transmission electron microscope (H7500, Hitachi), bar = 5  $\mu\text{m}$  in (a) and (c), 3  $\mu\text{m}$  in (g),  
 258 2  $\mu\text{m}$  in (e) and (f), 1  $\mu\text{m}$  in (h), 400 nm in (b) and (d).

259

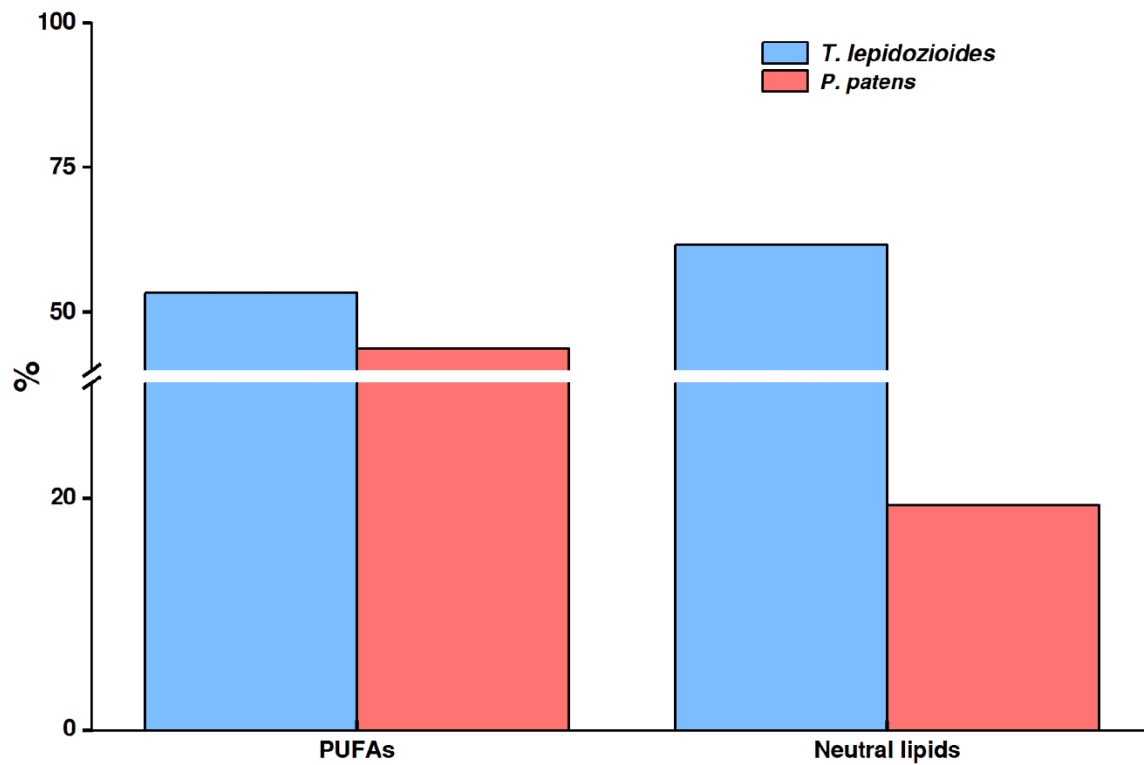


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262 **Data S1 V. The features of oil bodies in *T. lepidozooides***

263 (a) Oil bodies in living cells of *T. lepidozooides* stems after Sudan black staining, white arrows  
 264 mark oil bodies, bar = 200  $\mu\text{m}$ . (b) TEM micrograph of oil bodies, yellow stars mark oil bodies,  
 265 bar = 1  $\mu\text{m}$ . Observations were made with a transmission electron microscope (H7500,  
 266 Hitachi). (c) Radius of oil bodies in living cells (about 1.22  $\mu\text{m}$ ) of stems (n = 20). The central  
 267 mark of each box indicates the median, and the bottom and top edges of the box indicate the  
 268 25th and 75th percentiles respectively. (d) 2D-TLC to identify oil body components. Red arrow  
 269 marks nonpolar lipids, yellow arrow marks phosphatidylserine, which is the main content of  
 270 plasma membranes [S3].

271



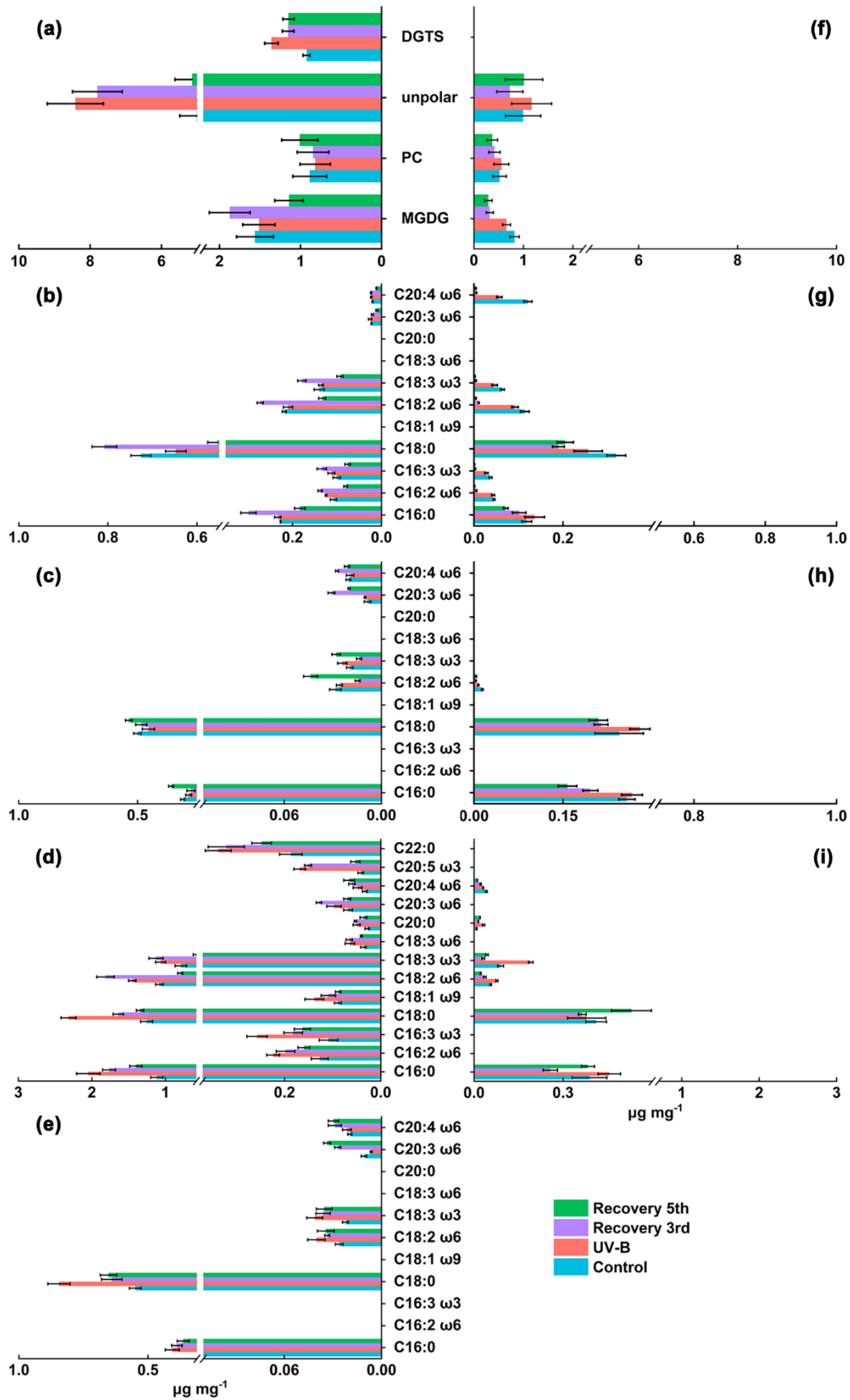
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273

274 **Data S1 W. The lipid contents of *T. lepidozoides* and *P. patens***

275 *T. lepidozoides* and *P. patens* plants were collected, PUFAs and neutral lipids were qualitatively  
 276 and quantitatively analyzed by 2D-TLC and GC-MS. PUFAs: polyunsaturated fatty acids.

277



279

280 **Data S1 X. Lipid composition in *T. lepidozoides* and *P. patens* before and after UV-B**  
281 **radiation**

282 Lipid changes before and after UV-B radiation in *T. lepidozoides* (a – e) and *P. patens* (f – i). *T.*  
283 *lepidozoides* and *P. patens* gametophytes grown under normal condition for two weeks, UV-B  
284 treatment, qualitative and quantitative analysis of lipids. (a, f) Total lipids. (b, g)  
285 monogalactoglycerolipid (MGDG). (c, h) phosphatidylcholine (PC). (d, i) Nonpolar lipids. (e)  
286 diacylglycerol-N,N,N-trimethylhomoserine (DGTS) in *T. lepidozoides*. There were no detectable  
287 DGTS in *P. patens*. Blue color represents plants under normal conditions (control), red color  
288 represents plants under 144 kJ m<sup>-2</sup> UV-B treatment for 2 hours (UV-B), violet represents plants  
289 recovery on the 3<sup>rd</sup> day after UV-B, and green represents plants recovery on the 5<sup>th</sup> day after  
290 UV-B.

291



293

294 **Data S1 Y. Non-targeted metabolic profiles of *T. lepidozoides* and *P. patens***

295 The metabolic profiles differed significantly between *T. lepidozoides* and *P. patens* during UV-B  
296 radiation and recovery. In total, 85 metabolites were detected and clustered into 3 major clades.  
297 Pathway analysis of clade 1 identified 3 pathways that were enriched to flavone and flavonol  
298 biosynthesis, flavonoid biosynthesis, and purine metabolism. Clade 2 pathways were mainly  
299 enriched in purine metabolism, pyrimidine metabolism, tyrosine metabolism. (a) Negative ion  
300 mass spectrum of *P. patens* and *T. lepidozoides*. (b) Positive ion mass spectrum of *P. patens*  
301 and *T. lepidozoides*. (c) Heatmap clusters of in total 85 metabolites. Detailed data of the 85  
302 metabolites is compiled in Table S6. (d) Pathway enrichment of clade 1 in (c). (e) Pathway  
303 enrichment of clade 2 in (c). The horizontal axis “pathway impact” is the cumulative percentage  
304 from the matched metabolite nodes.

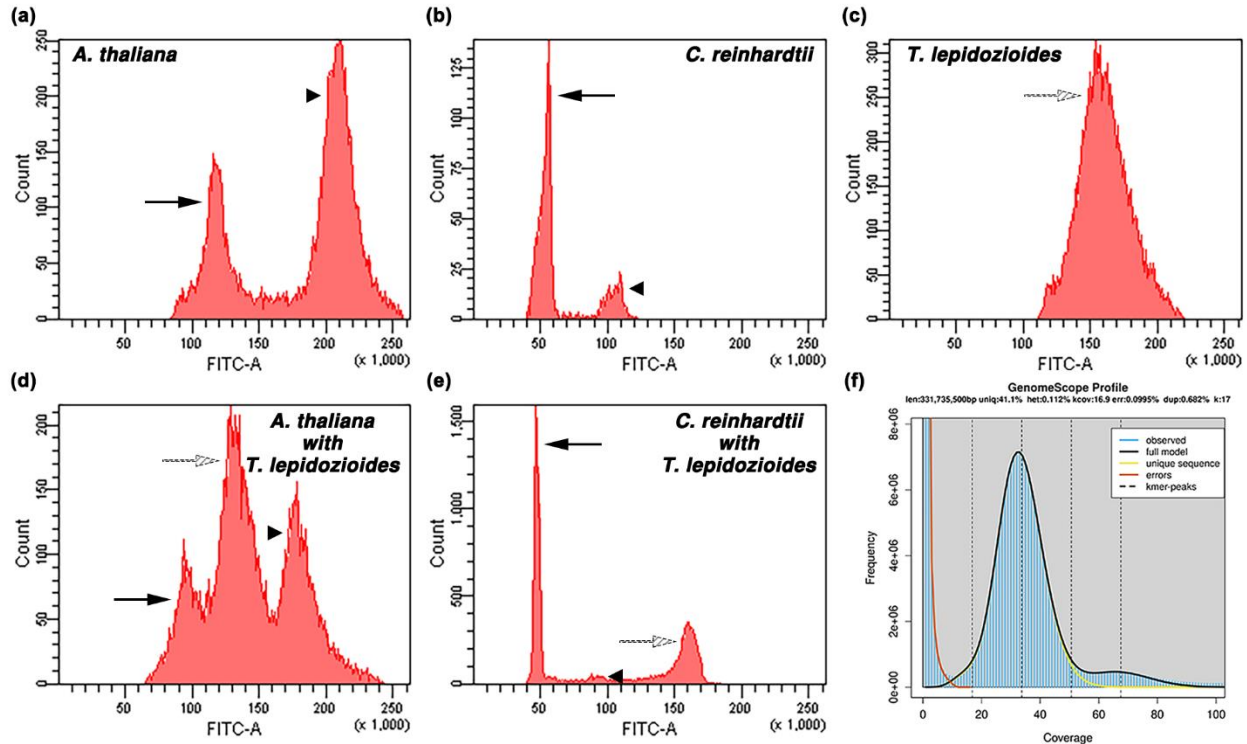
305

306 **SUPPLEMENTAL REFERENCES**

- 307 S1. Liu, C.R., Newell, G., and White, M. (2016). On the selection of thresholds for predicting  
308 species occurrence with presence-only data. *Ecol. Evol.* 6, 337–348.  
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- 310 S2. Chater, C.C., Caine, R.S., Tomek, M., Wallace, S., Kamisugi, Y., Cuming, A.C., Lang,  
311 D., MacAlister, C.A., Casson, S., Bergmann, D.C., et al. (2016). Origin and function of  
312 stomata in the moss *Physcomitrella patens*. *Nat. Plants* 2, 16179, 16179.  
313 10.1038/nplants.2016.179.
- 314 S3. Leventis, P.A., and Grinstein, S. (2010). The distribution and function of  
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317 10.1146/annurev.biophys.093008.131234.  
318

1 **Supplement data 2**

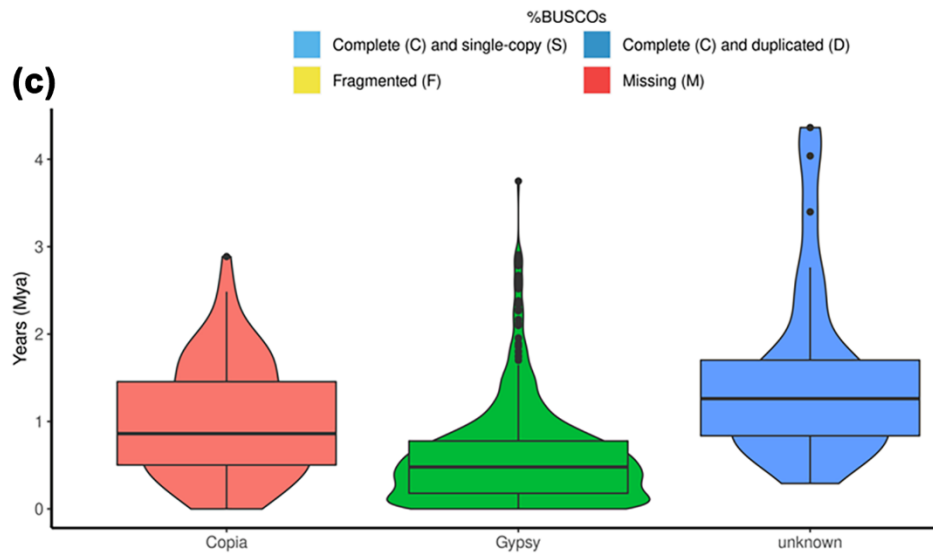
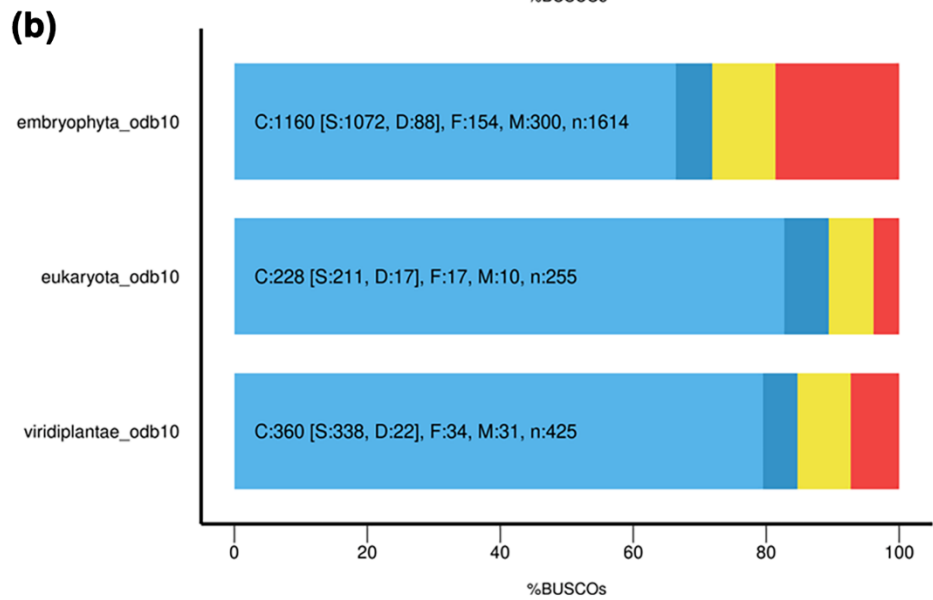
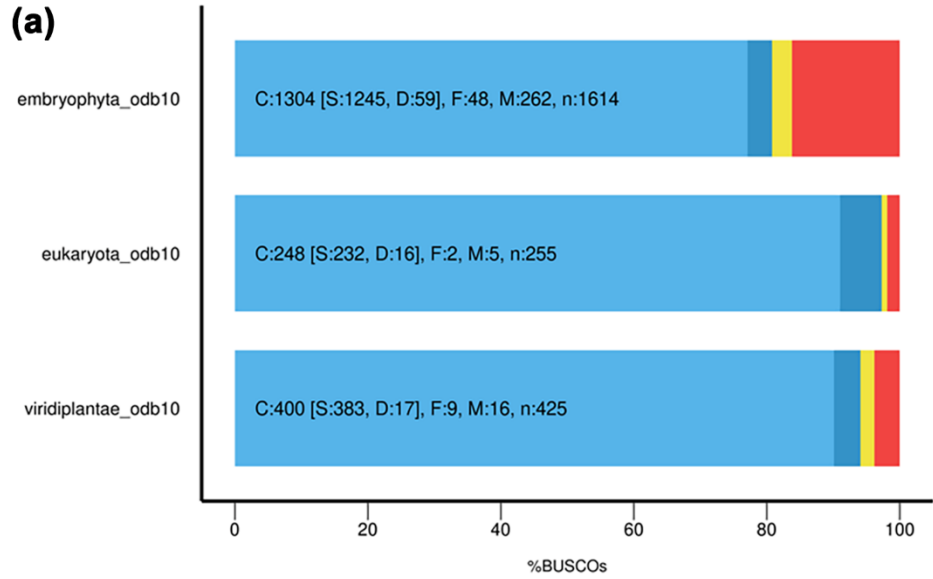
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## 5 Data S2 A. Genome size estimations of *T. lepidozooides*

6 (a – e) Estimate of *T. lepidozooides* genome size based on flow cytometry (FCM). FCM was carried  
 7 out according to Reski et al. [S1] in a cell sorter (FACSaria, BD) with SYTOX Green stain. (a)  
 8 Histogram of fluorescence signals of *A. thaliana*, two major peaks at around 100 (arrow) and 200  
 9 (triangle) with ploidy levels at 2C and 4C, respectively. (b) Histogram of fluorescence signals of  
 10 *C. reinhardtii*, two major peaks at around 50 (arrow) and 100 (triangle) with ploidy levels at 1C  
 11 and 2C, respectively. (c) Histogram of fluorescence signals of *T. lepidozooides*, only one peak at  
 12 around 150 (dotted arrow). (d) Histogram of fluorescence signals of *A. thaliana* and *T.*  
 13 *lepidozooides* nucleus mixture, the signals of *T. lepidozooides* (dotted arrow) near 150 between  
 14 two major peaks of *A. thaliana* (arrow and triangle), the estimated DNA contents with a ratio of  
 15 1.39:1 between *T. lepidozooides* and *A. thaliana* (2C). (e) Histogram of fluorescence signals of *C.*  
 16 *reinhardtii* and *T. lepidozooides* nucleus mixture, the signals of *T. lepidozooides* (dotted arrow) near  
 17 150, and two major peaks of *C. reinhardtii* at around 50 (arrow) and 100 (triangle), the estimated  
 18 DNA contents with a ratio of 3.42:1 between *T. lepidozooides* and *C. reinhardtii* (1C). (f) Estimate  
 19 of *T. lepidozooides* genome size based on sequencing. Total 20 Gbp Illumina paired-end reads  
 20 were used in estimation according to Marcais et al. [S2] and Vurture et al. [S3]. The estimated  
 21 genome size of *T. lepidozooides* is 331 Mbp.



23

24 **Data S2 B. Genome features of *T. lepidozooides***

25 BUSCO assessment of assembly (a) and annotation (b). The latest all eukaryotic organism gene  
26 set eukaryote odb10, all plants gene set viridiplantae odb10 and all land plants gene set  
27 embryophyta odb10, were used as the reference, respectively, and Arabidopsis was used in the  
28 -sp option, 97.3% in eukaryota, 94.1% in viridiplantae and 80.8% in embryophyte gene sets are  
29 present in the final assembly. The annotation proportions were 89.4%, 84.7% and 71.9%,  
30 respectively. (c) Insertion time of *T. lepidozooides* LTR retrotransposons. The central mark of each  
31 box indicates the median, and the bottom and top edges of the box indicate the 25th and 75th  
32 percentiles, respectively. The whiskers extend to the most extreme data points not considered  
33 outliers. The median age of *copia* is 0.85 Mya, the median age of *gypsy* is 0.47 Mya, and most  
34 LTRs in *T. lepidozooides* (unknown) have an insertion age of 1 ~ 2 Mya.

35

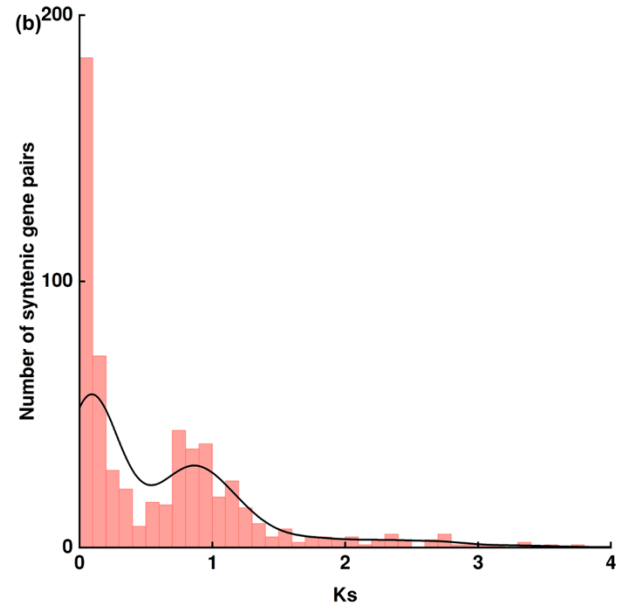
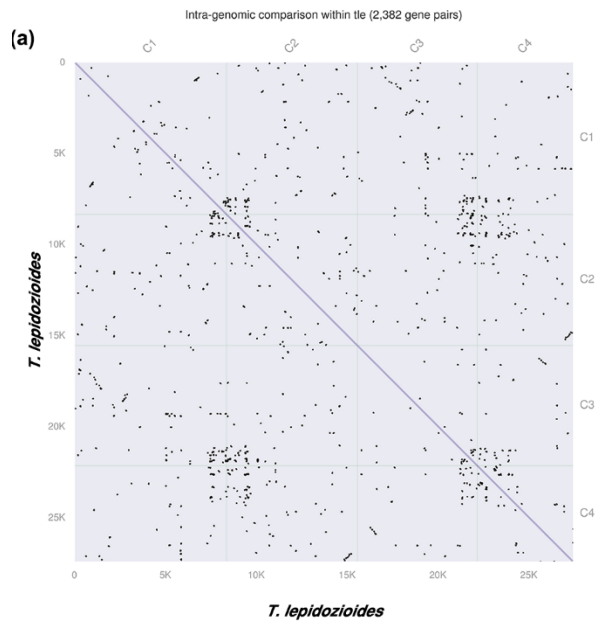


37

38 **Data S2 C. Organellar genomes of *T. lepidozoides***

39 (a) The complete circular *T. lepidozoides* chloroplast genome comprises 149 Kb in four parts:  
40 long single copy section (LSC), inverted repeat A (IRA), small single copy section (SSC), and  
41 inverted repeat B (IRB). The chloroplast genome encodes 129 genes, including 37 tRNAs. (b)  
42 The complete circular *T. lepidozoides* mitochondrial genome comprises 139 Kb. It encodes 70  
43 genes, including 24 tRNAs. The figure was generated using OGDRAW program from  
44 CHLOROBX (<https://chlorobox.mpimp-golm.mpg.de/index.html>). Color-coded boxes indicate  
45 the genes in the genome.

46



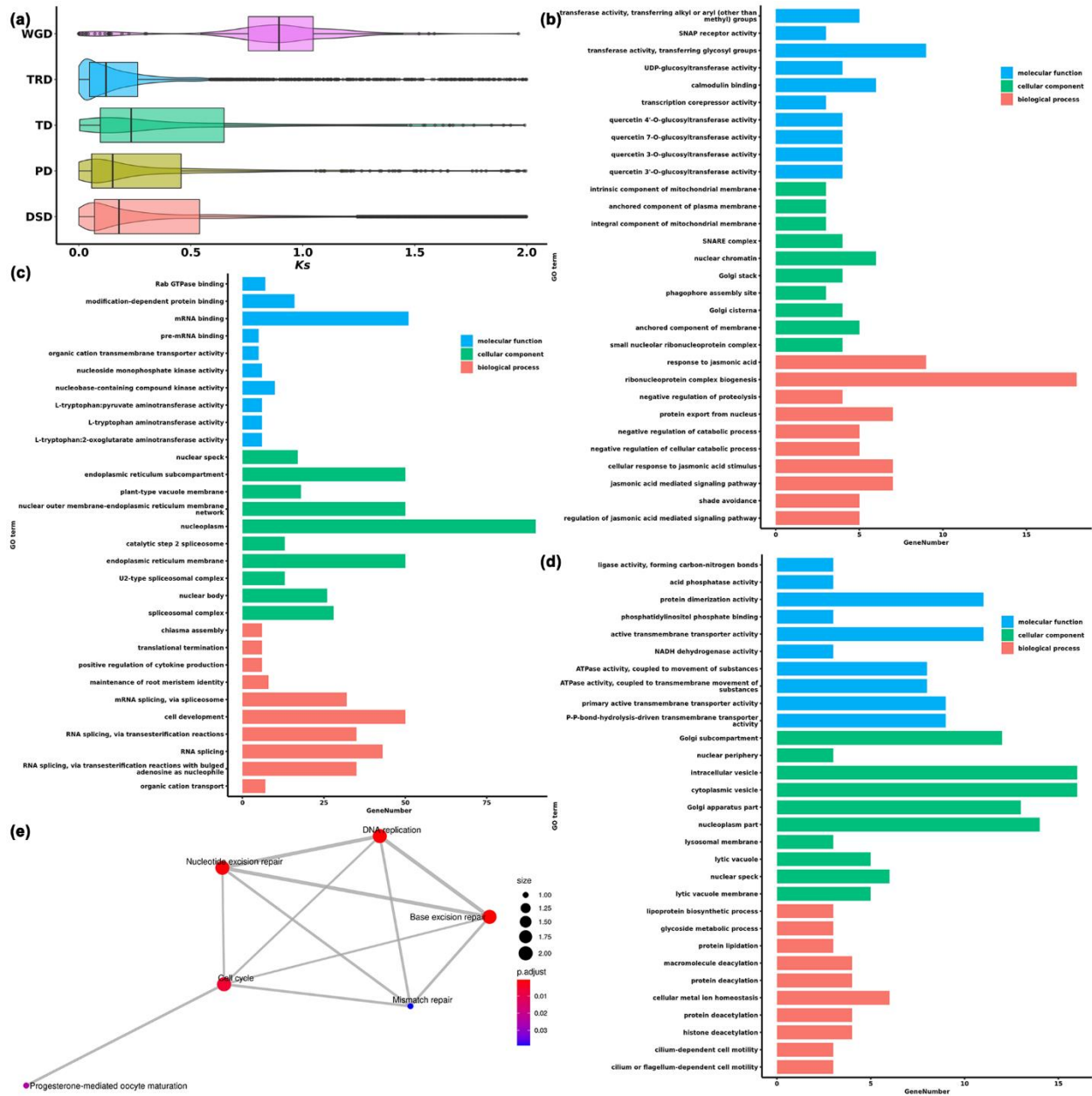
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48

49 **Data S2 D. Genome characters of *T. lepidozooides***

50 (a) Synteny blocks of *T. lepidozooides* genome. (b) Ks values distribution reveal diploidy events  
 51 in *T. lepidozooides*.

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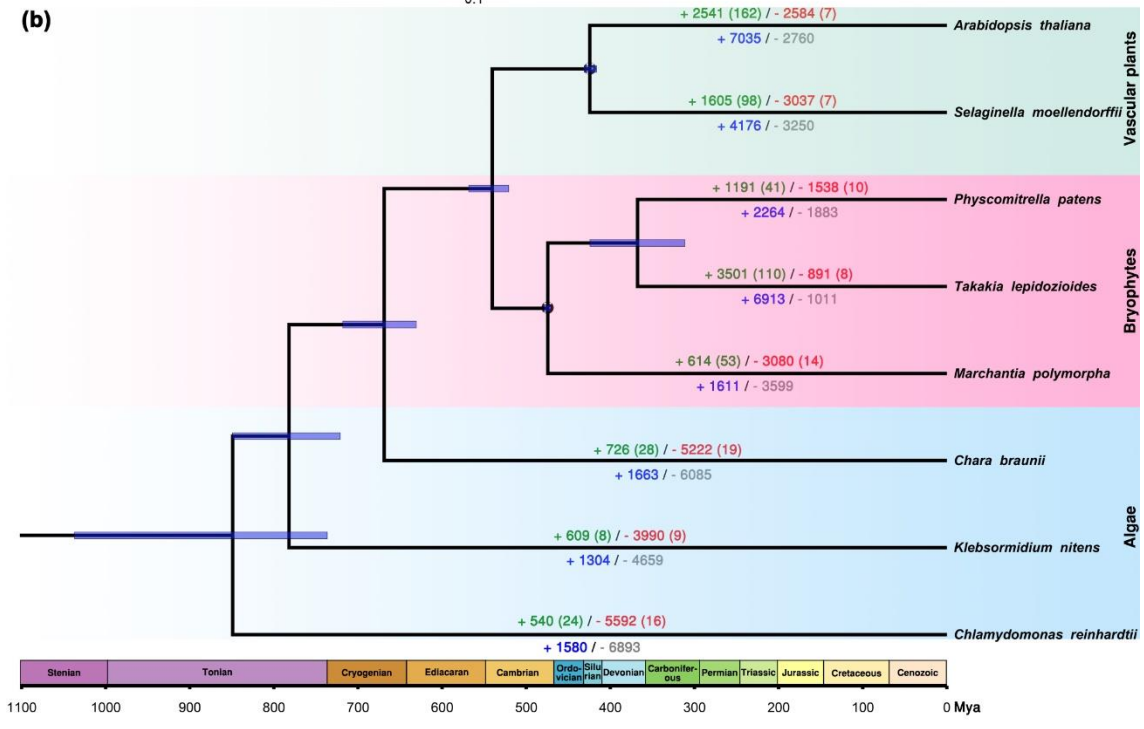
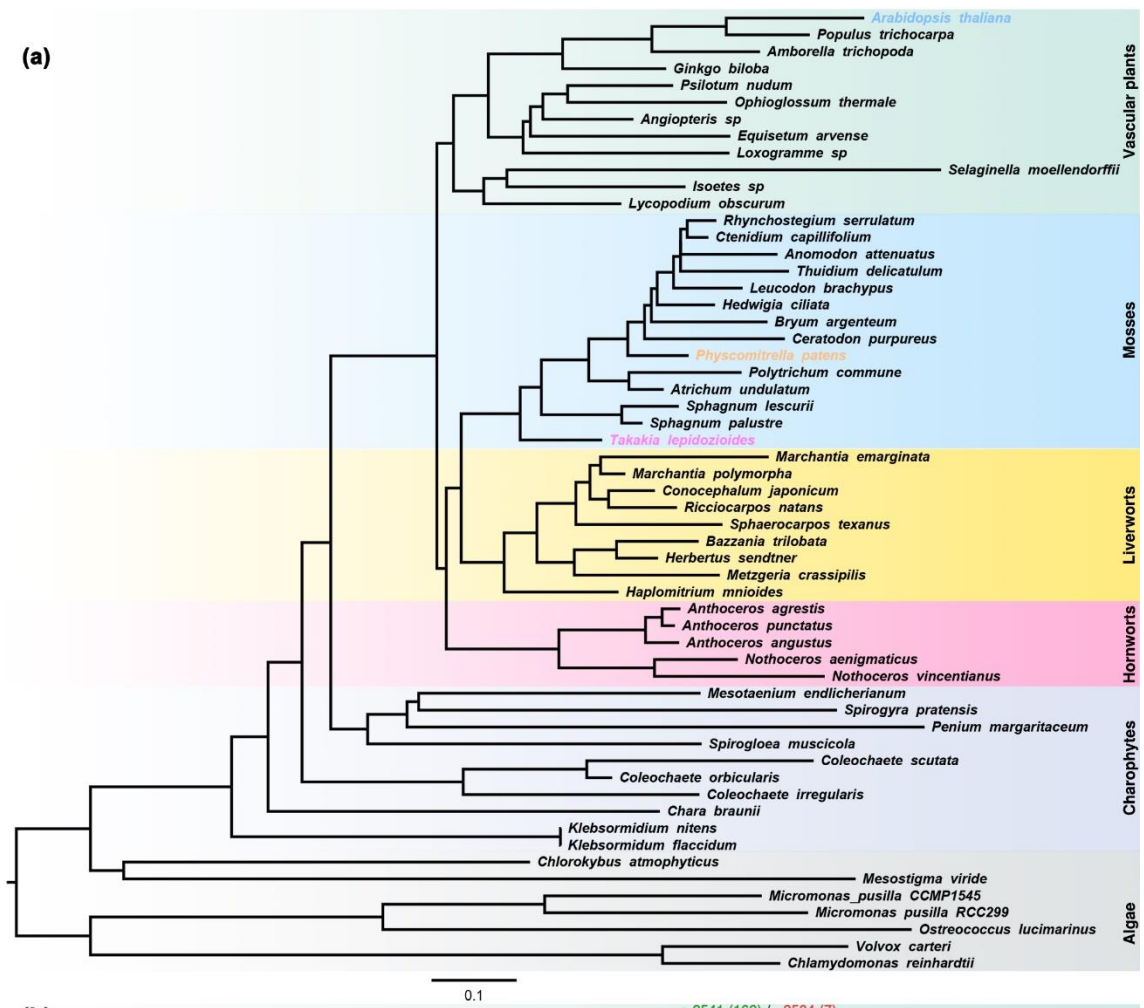
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## 55 Data S2 E. Evolution and function of duplicated genes in *T. lepidozoides*

56 (a) Ks distribution of the five modes of gene duplications. WGD: whole-genome duplication, TD:  
 57 tandem duplication, PD: proximal duplication, TRD: transposed duplication, DSD: dispersed  
 58 duplication. In the boxplot representations, points are outliers; the centre line is the median; the  
 59 lower and upper hinges correspond to the first and third quartiles (25th and 75th percentiles);  
 60 whiskers extend to the minimum (left whiskers) and maximum (right whiskers) estimates located  
 61 within the 1.5x interquartile range (IQR) of the first and third quartiles, respectively. (b) Gene

62 ontology (GO) enrichment of PD in *T. lepidozoides* (c) GO enrichment of TRD in *T. lepidozoides*.  
63 (d) GO enrichment of WGD in *T. lepidozoides*. (e) Kyoto Encyclopedia of Genes and Genomes  
64 (KEGG) enrichment of singleton genes in *T. lepidozoides*.  
65



67

68 **Data S2 F. Phylogenetic position and divergence time of *T. lepidozoides***

69 (a) Phylogenetic tree of 56 plant species based on 818 low-copy orthologs amino acid sequence.

70 The tree of 56 plant species inferred from five plant clades reveals *T. lepidozoides* as the sister  
71 group to all other mosses, rather than to liverworts or to the LCA of mosses and liverworts. (b) *T.*

72 *lepidozoides* time tree with gene family expansion/contraction analysis compared to

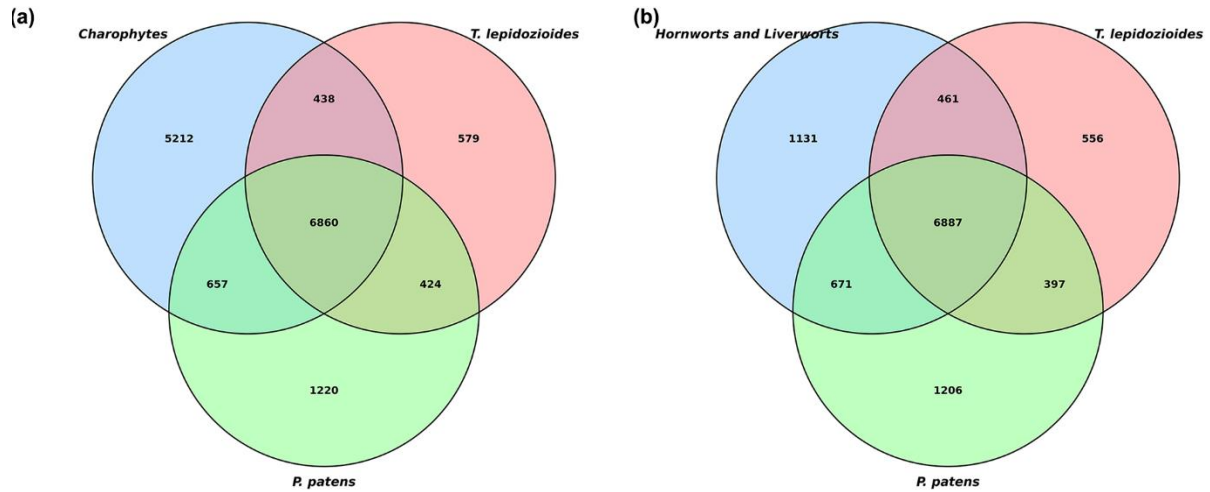
73 representatives of seven other plant lineages, coloured numbers above branches show gene

74 family expansions (+) and contractions (-), coloured numbers under branches show gene gains

75 (+) and losses (-), light purple bars show 95% confidence interval lengths, black dots on nodes

76 mark fossil calibration points.

77



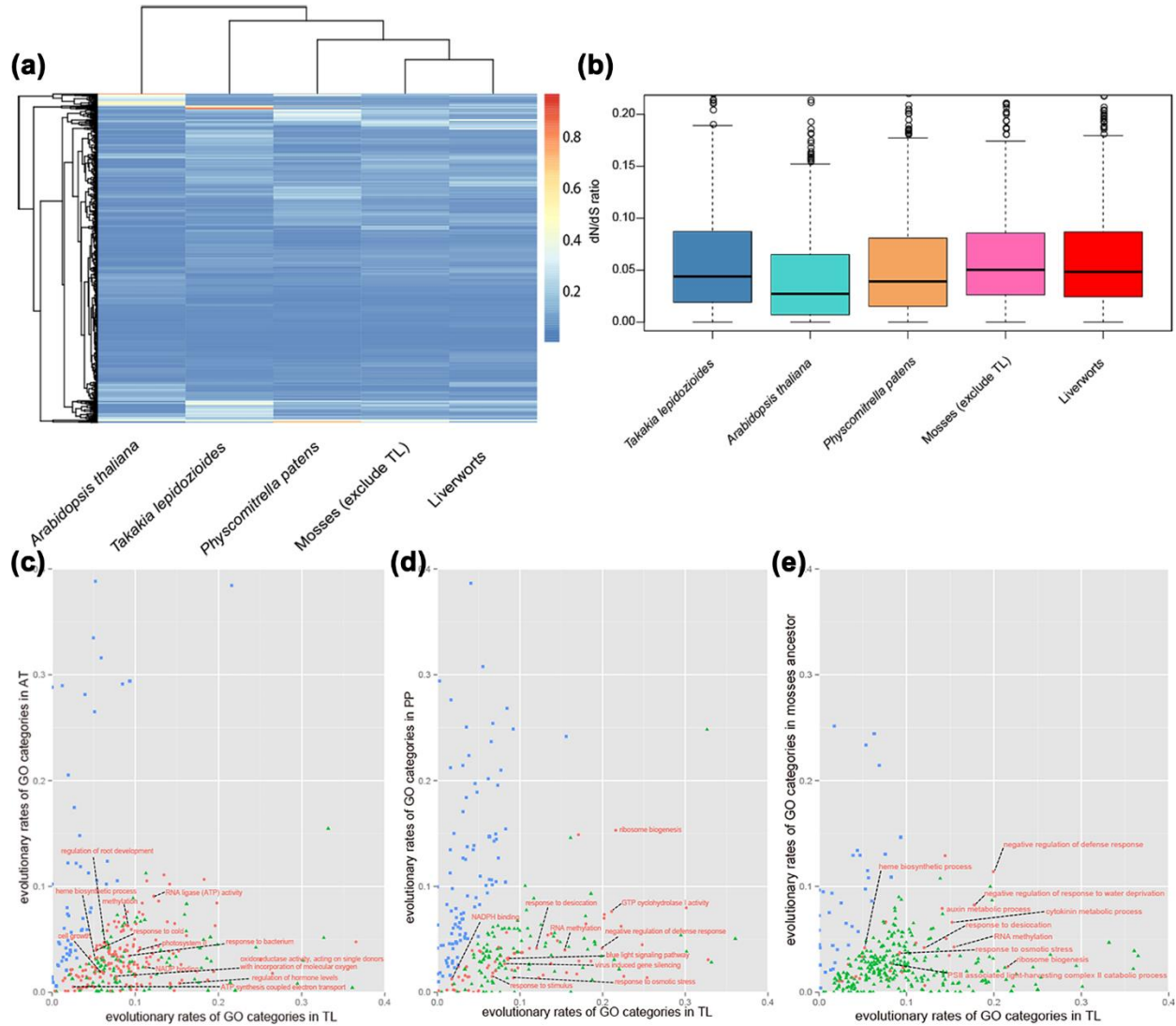
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80 **Data S2 G. Gene families characters of *T. lepidozooides***

81 **(a)** Gene families of *T. lepidozooides*, *P. patens* and Charophytes (the union of *K. nitens*, *C. braunii*,  
 82 *M. endlicherianum* and *S. muscicola* sets). **(b)** Gene families of *T. lepidozooides*, *P. patens* and  
 83 hornworts and liverworts (the union of *A. angustus* and *M. polymorpha* sets).

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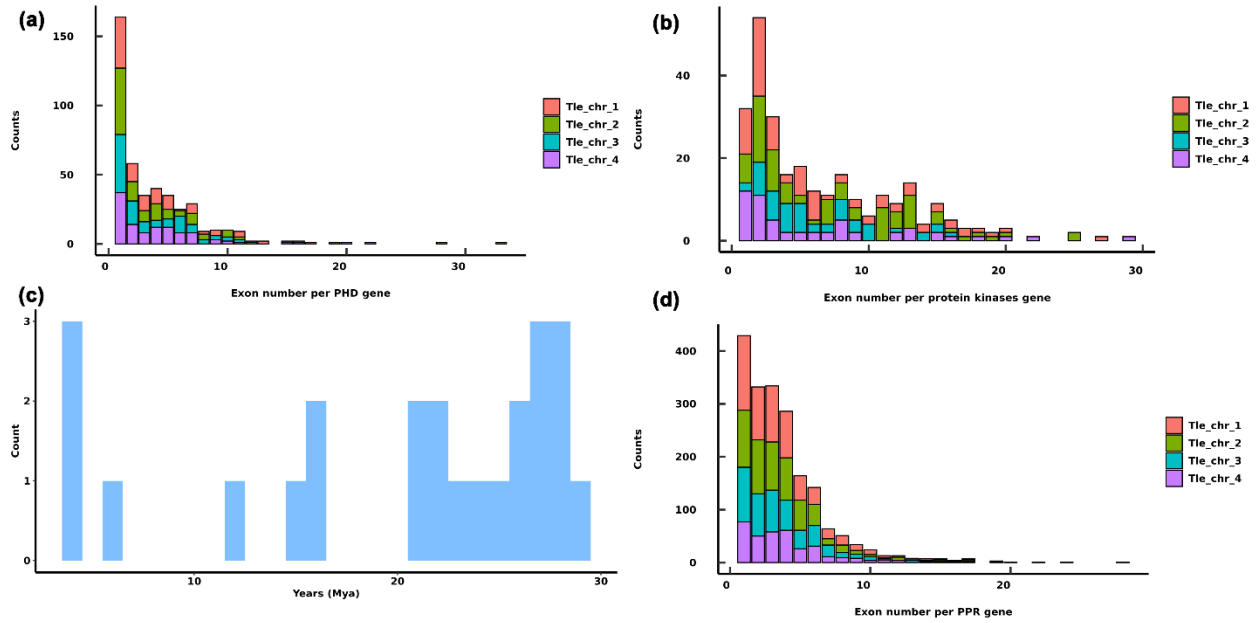


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87 **Data S2 H. Positive selection and fast evolution**

88 (a) Heatmap showing dN/dS ratio for individual genes in *A. thaliana*, *T. lepidozooides*, *P. patens*,  
 89 all mosses except *T. lepidozooides*, and all liverworts. Each horizontal line represents a gene. (b)  
 90 Boxplot showing dN/dS ratios for individual genes in *T. lepidozooides*, *A. thaliana*, *P. patens*, all  
 91 mosses except *T. lepidozooides*, and all liverworts. The upper boundary of the evolution rate range  
 92 scale in *T. lepidozooides* is higher than that of other taxon groups surveyed. (c) GO annotation of  
 93 significantly fast evolving genes in *T. lepidozooides* and *A. thaliana*. Blue squares represent GO  
 94 categories with significantly higher mean dN/dS in *A. thaliana*, red points represent fast evolving  
 95 GO categories specific to *T. lepidozooides*, green triangles represent fast evolving GO categories  
 96 shared by all mosses; TL, *T. lepidozooides*; AT, *A. thaliana*. (d) GO annotation of significantly fast

97 evolving genes in *T. lepidozoides* and *P. patens*. Blue squares represent GO categories with  
98 significantly higher mean dN/dS in *P. patens*, red points represent fast evolving GO categories  
99 specific to *T. lepidozoides*, green triangles represent fast evolving GO categories shared by all  
100 mosses; TL, *T. lepidozoides*; PP, *P. patens*. (e) GO categories of rapidly evolving genes in *T.*  
101 *lepidozoides* and the common ancestor of all mosses including *T. lepidozoides*. Green triangles  
102 represent 56 GO categories with significantly higher mean dN/dS in the common ancestor of all  
103 mosses, blue squares represent 32 fast evolving GO categories specific to *T. lepidozoides*, red  
104 points represent 359 fast evolving GO categories shared by all mosses including *T. lepidozoides*.  
105

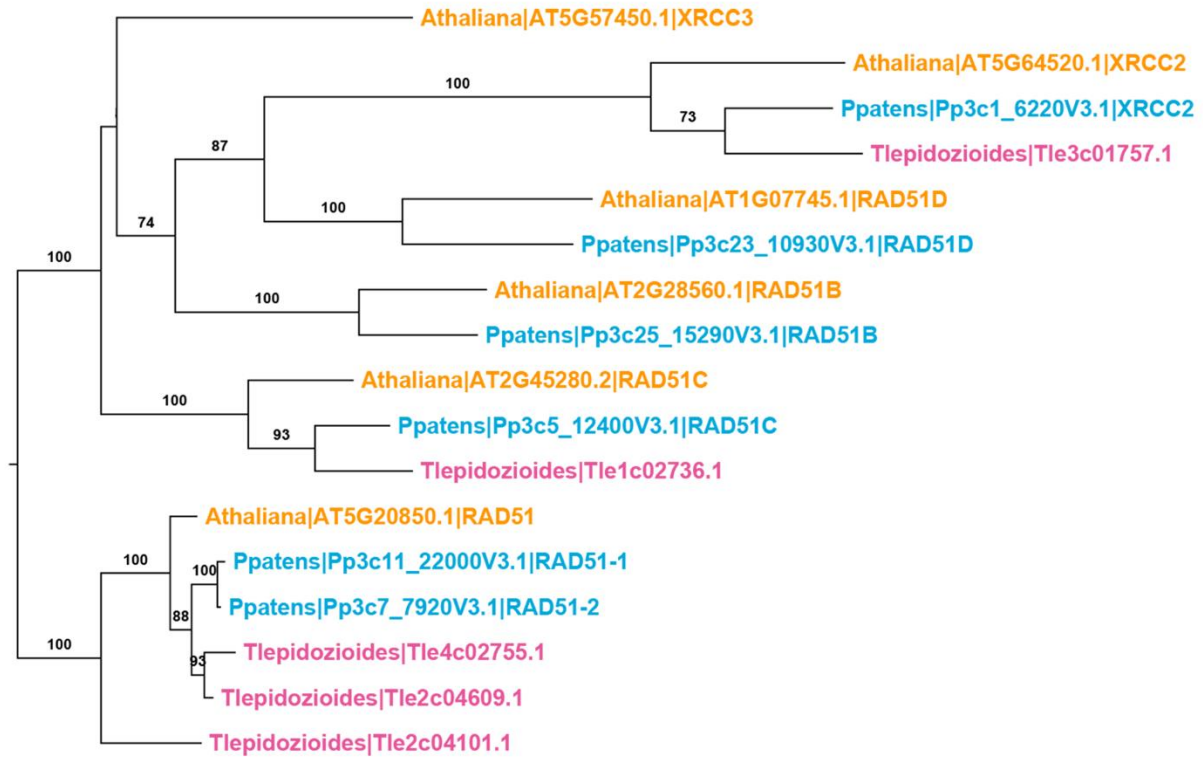


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108 **Data S2 I. Statistics in PHDs, protein kinases and PPRs of *T. lepidozoides***

109 (a) Statistics of exon numbers in each PHD. (b) Statistics of exon numbers in each protein kinase.  
110 (c) Numbers of protein kinases which are younger than 30 Mya. (d) Statistics of exon numbers in  
111 each PPR.

112

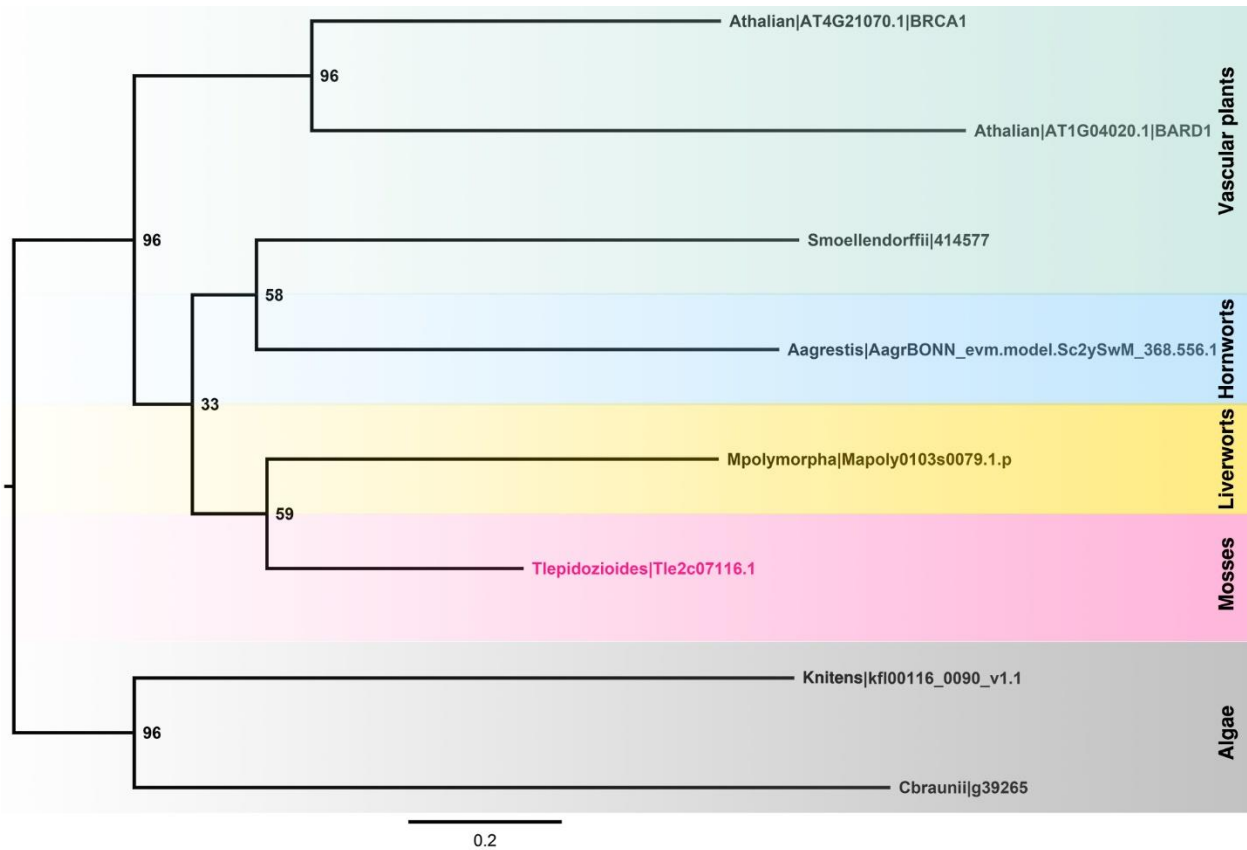


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115 **Data S2 J. Phylogenetic trees of RAD51**

116 Maximum likelihood trees using RAxML with the PROTGAMMAJTT model based upon MAFFT  
 117 alignments of proteins are the consensus of 1,000 replicates. Numbered nodes show the  
 118 percentage of bootstraps which support the node, bootstraps with less than 50% support are not  
 119 shown. Orange font color represents *A. thaliana*, pink font color represents *T. lepidozioides* and  
 120 blue font color represents *P. patens*. Shade colors as shown on the right.

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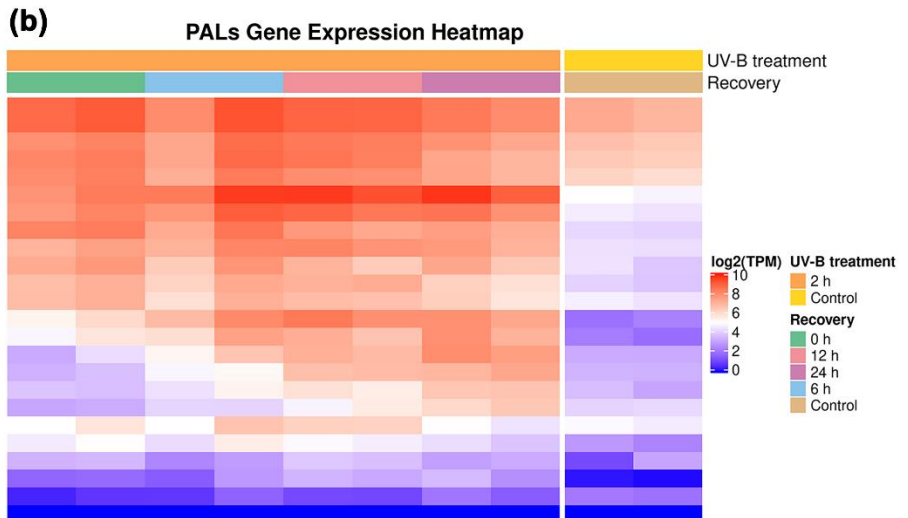
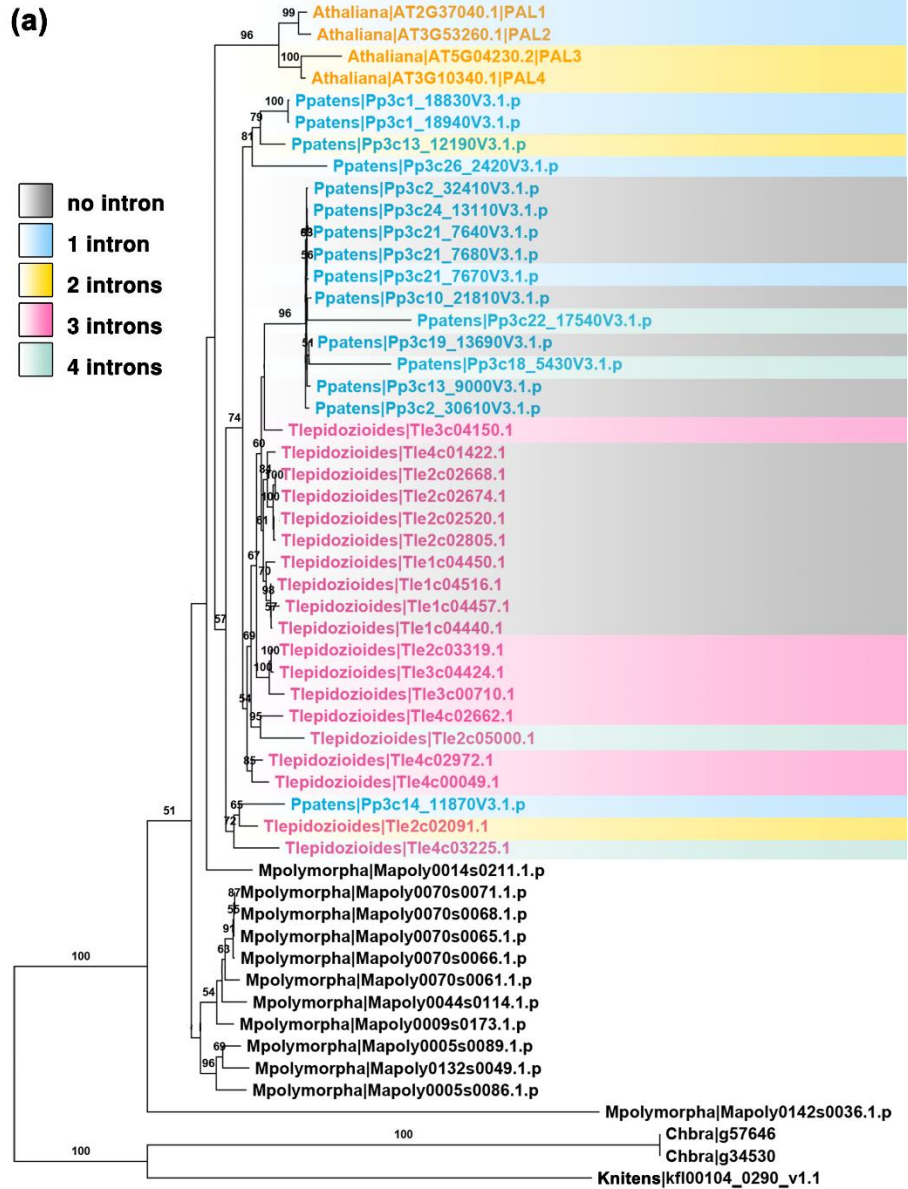


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124 **Data S2 K. Phylogenetic tree of BRCA1-homologs in bryophytes**

125 BRCA1 (AT4G21070) were found using blastp with identity cutoff 50%, e-value cutoff  $1 \times 10^{-20}$ .  
 126 Maximum likelihood tree using RAxML with the PROTGAMMAJTT model based upon MAFFT  
 127 alignments of proteins and trimmed by trimAl. Tree is the consensus of 1,000 replicates.  
 128 Numbered nodes show the percentage of bootstraps which supported the node.

129

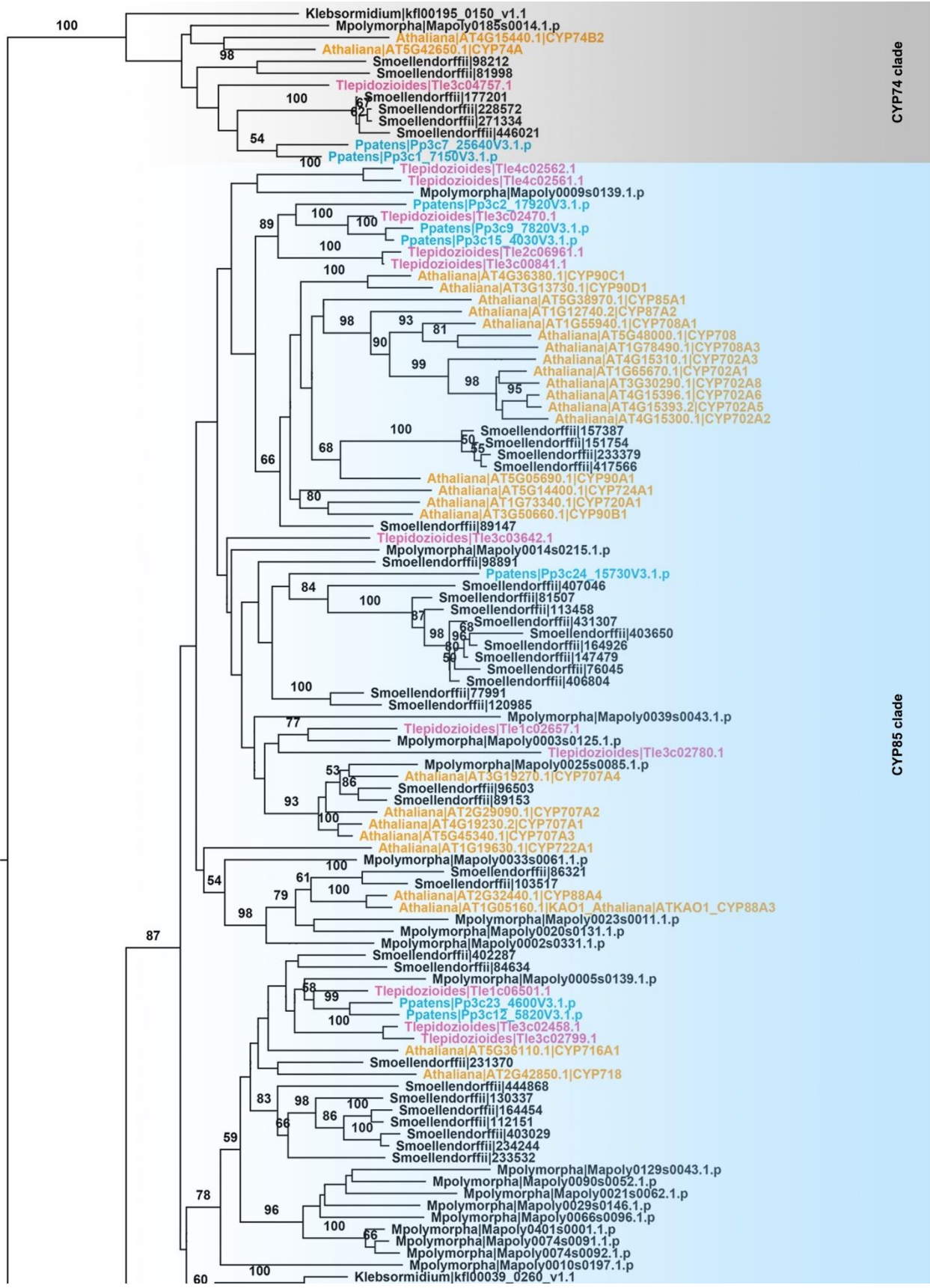


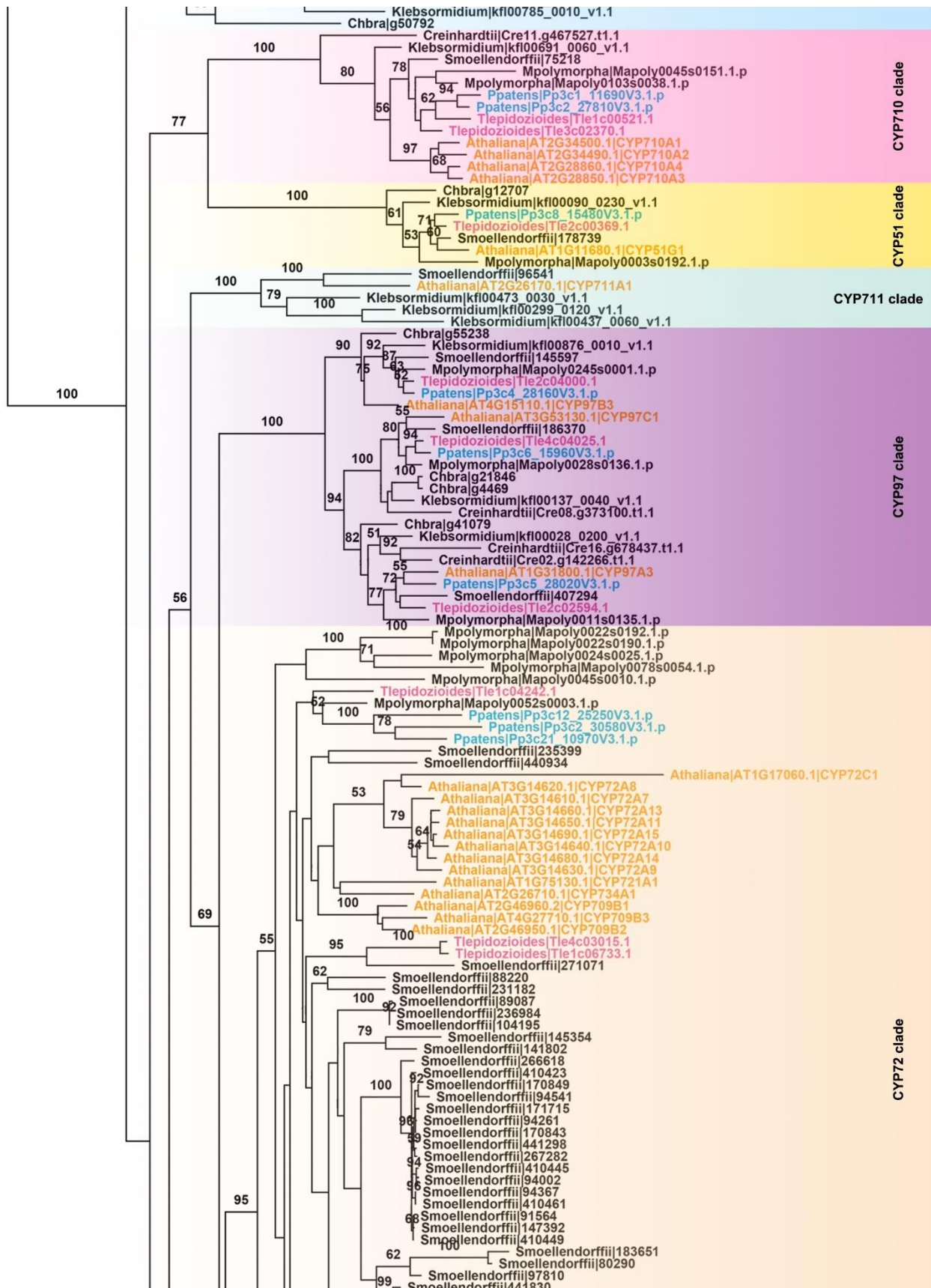
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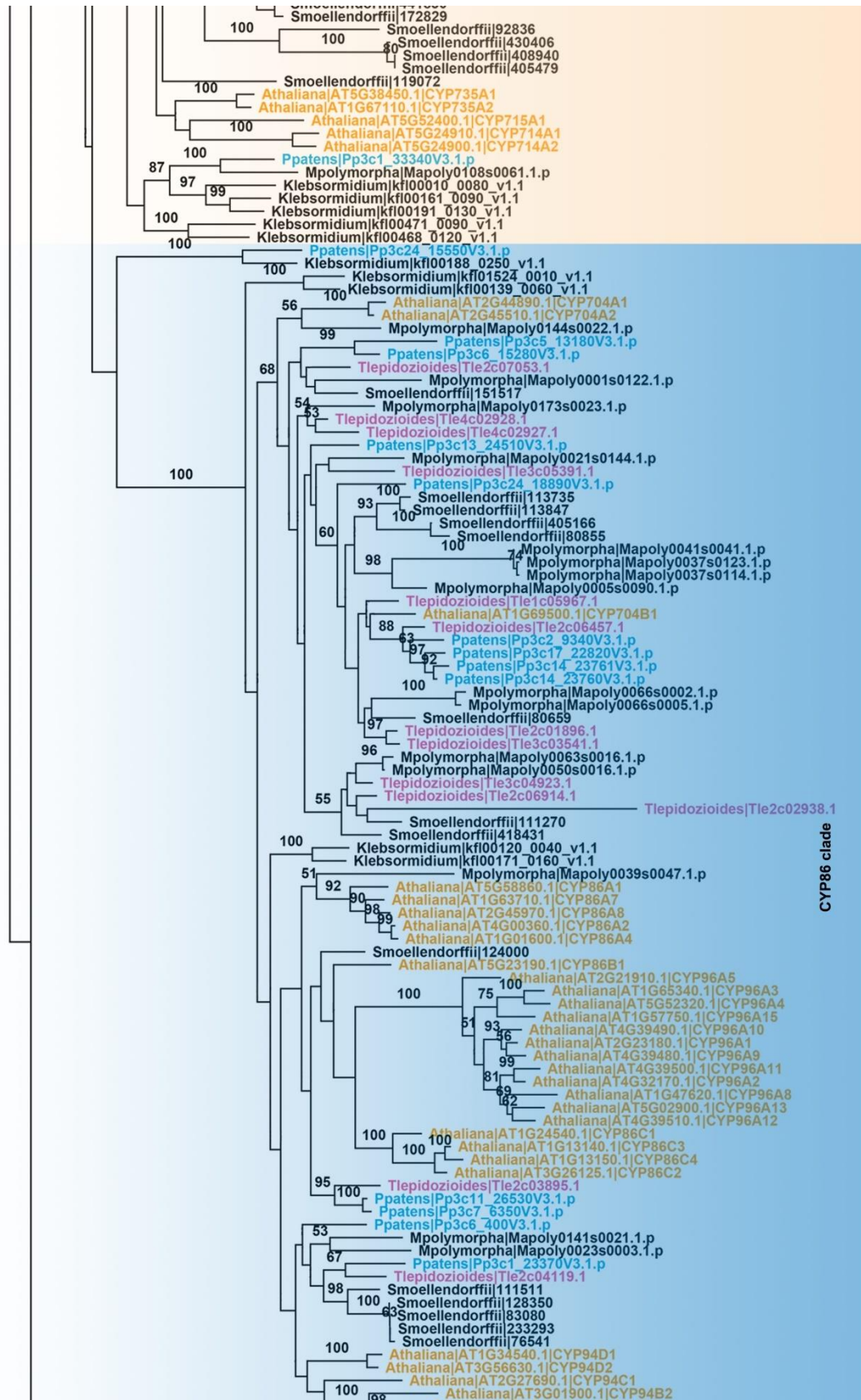
132 **Data S2 L. PAL features in *T. lepidozooides***

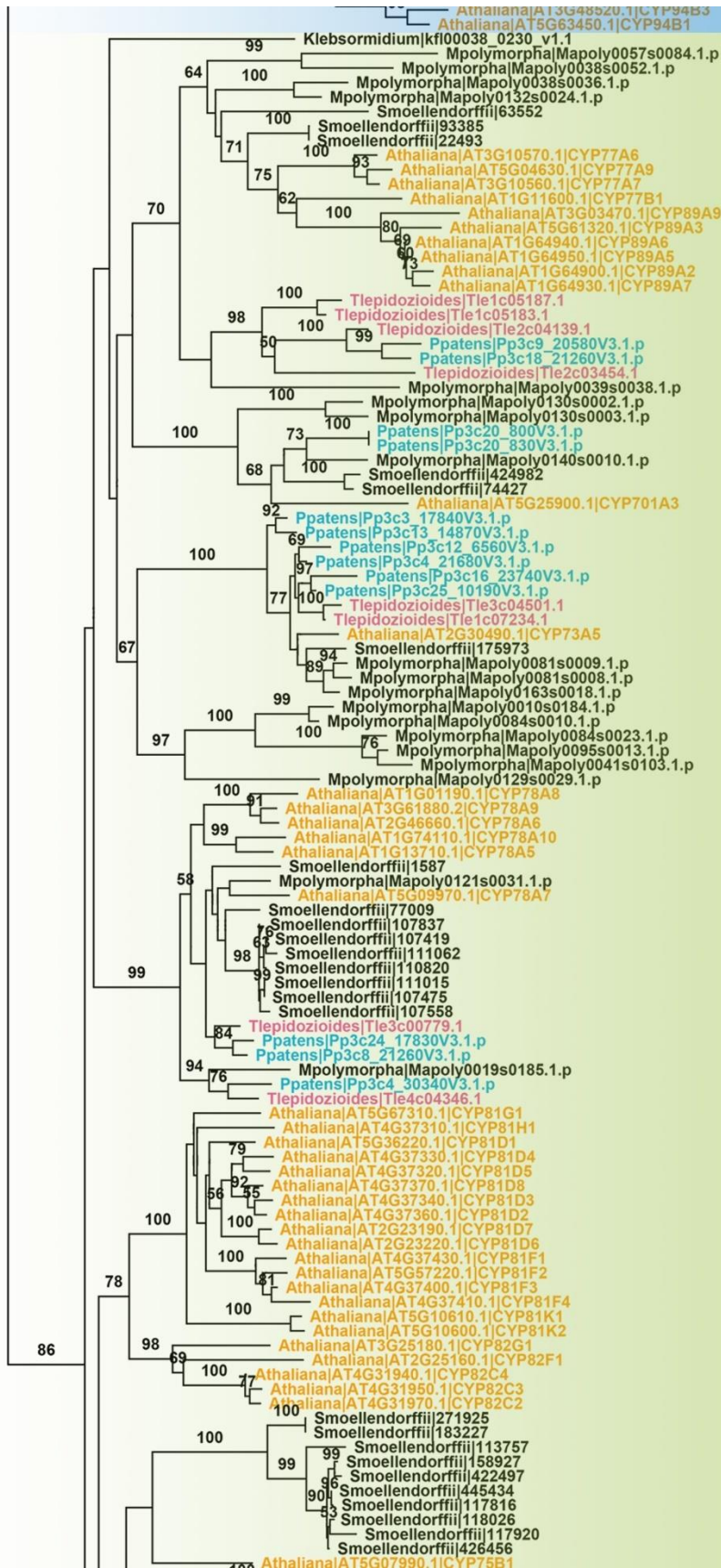
133 (a) Phylogenetic tree of PAL genes. Maximum likelihood tree using RAxML with the  
134 PROTGAMMAJTT model based upon MAFFT alignments of proteins and trimmed by trimAl. Tree  
135 is the consensus of 1,000 replicates. Numbered nodes show the percentage of bootstraps which  
136 support the node, bootstraps with less than 50% support are not shown. Orange font color  
137 represents *A. thaliana*, pink font color represents *T. lepidozooides* and blue font color represents  
138 *P. patens*. Shade colors as shown on the top left. (b) Expression levels of PALs in response to  
139 UV-B radiation and during recovery.

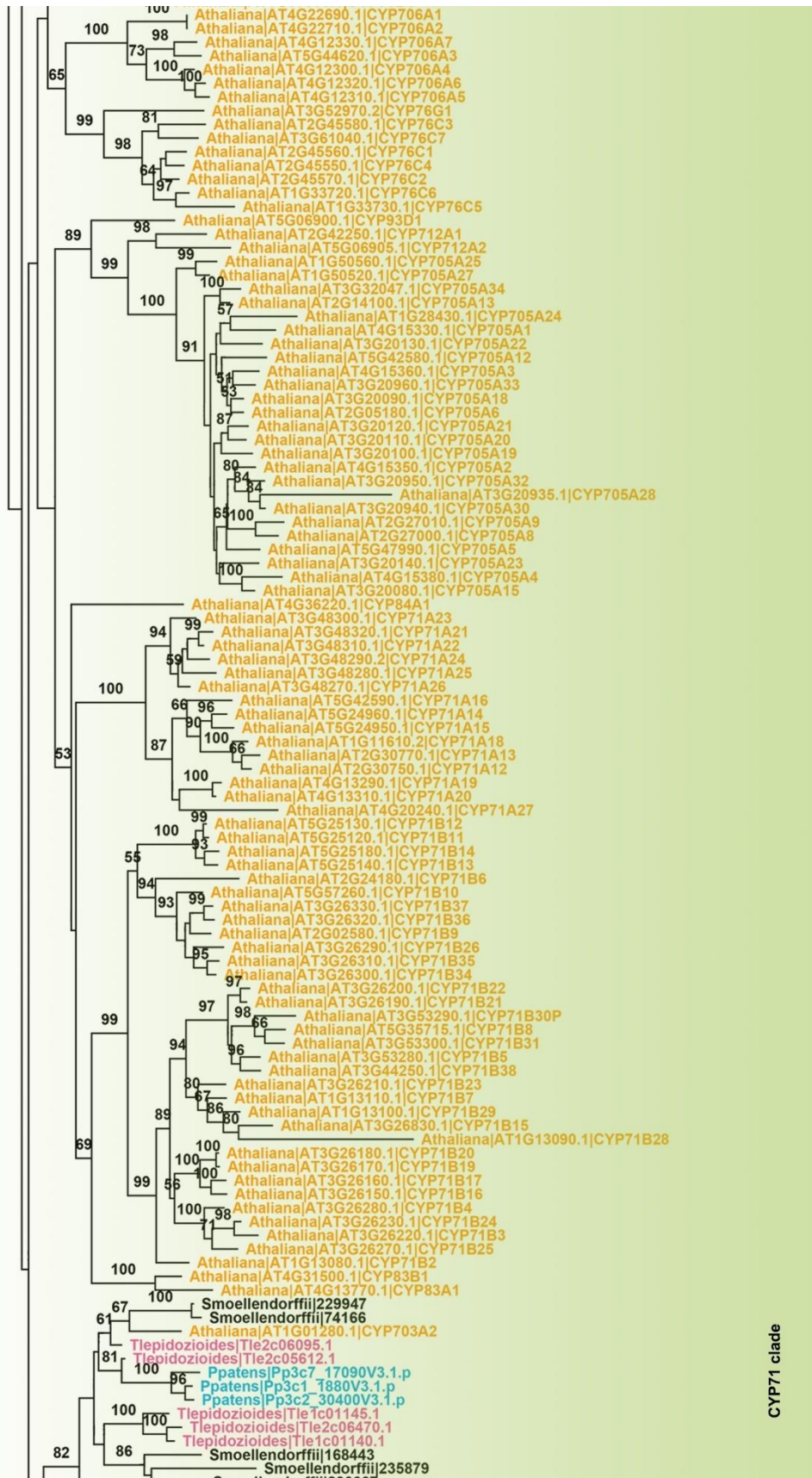
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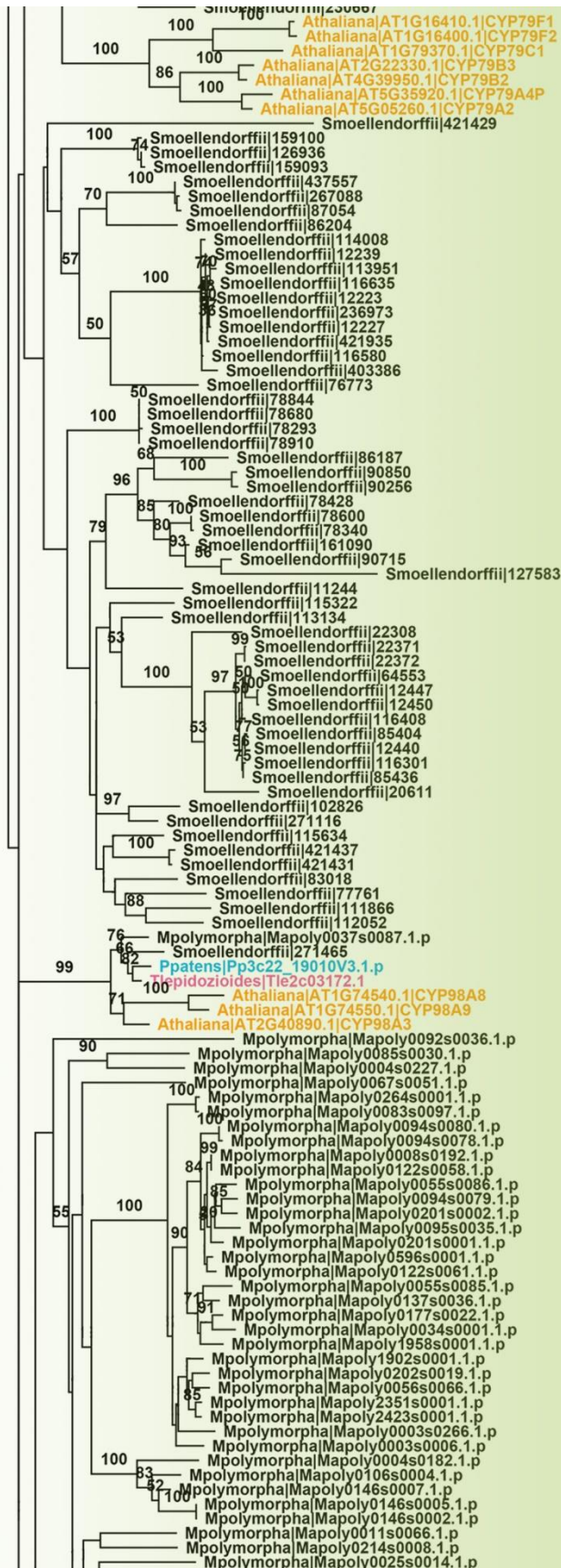


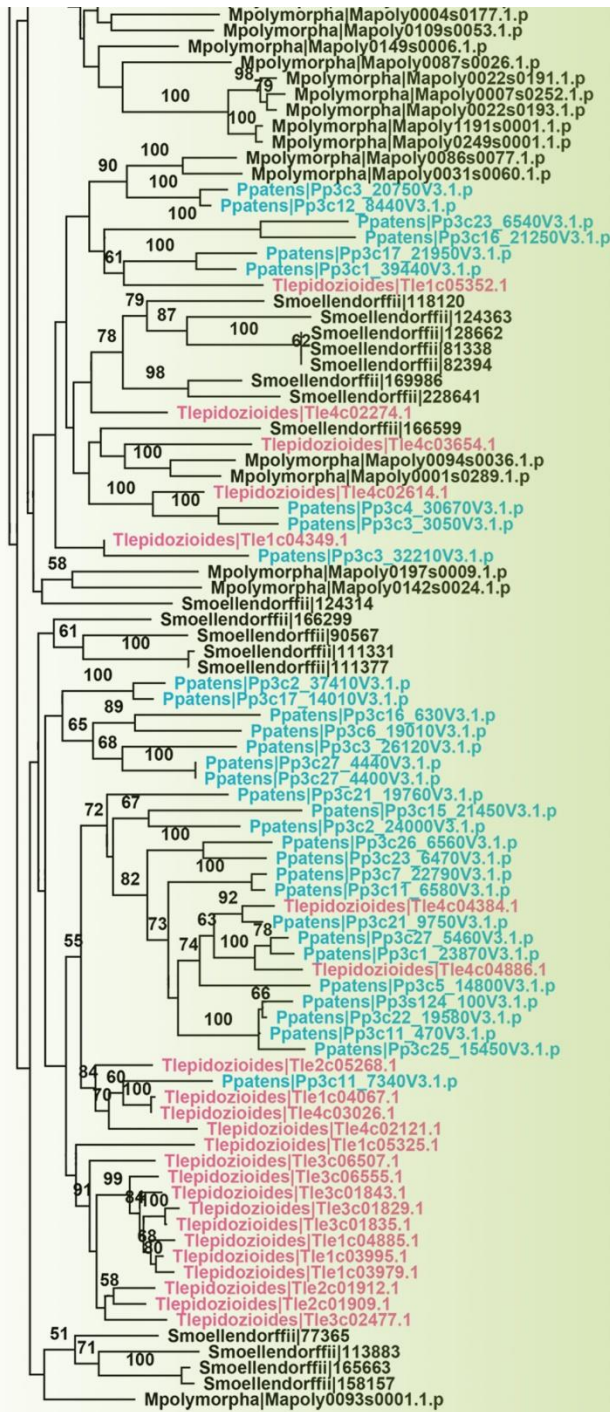












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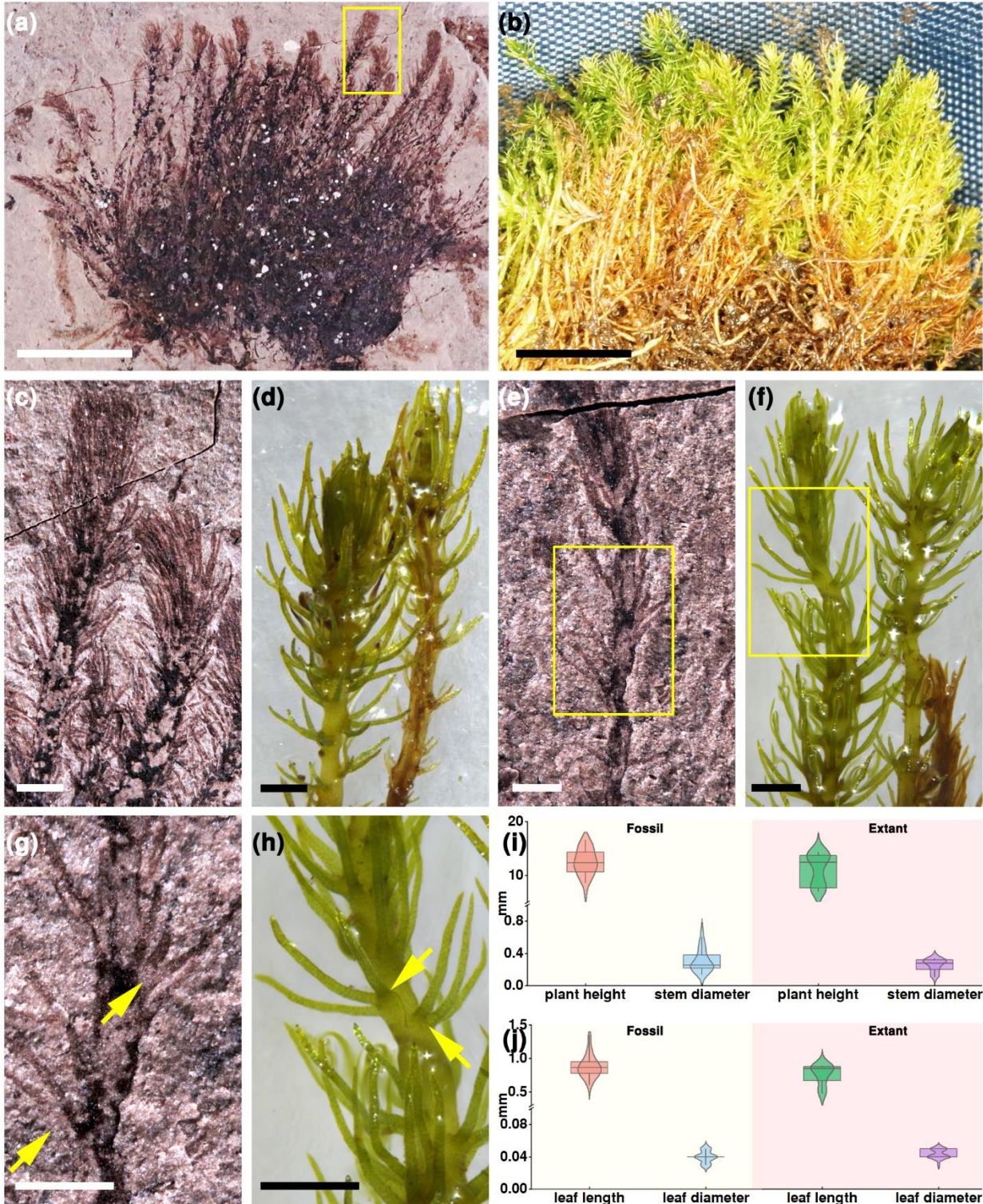
149 **Data S2 M. Phylogenetic tree of CYP450s**

150 Maximum likelihood tree using RAxML with the PROTGAMMAJTT model based upon MAFFT

151 alignments of proteins and trimmed by trimAl. Tree is the consensus of 1,000 replicates.

152 Numbered nodes show the percentage of bootstraps which supported the node, bootstraps with

153 less than 50% support are not shown. Orange font color represents *A. thaliana*, pink font color  
154 represents *T. lepidozoides* and blue font color represents *P. patens*. Shade colors as shown on  
155 the right.  
156

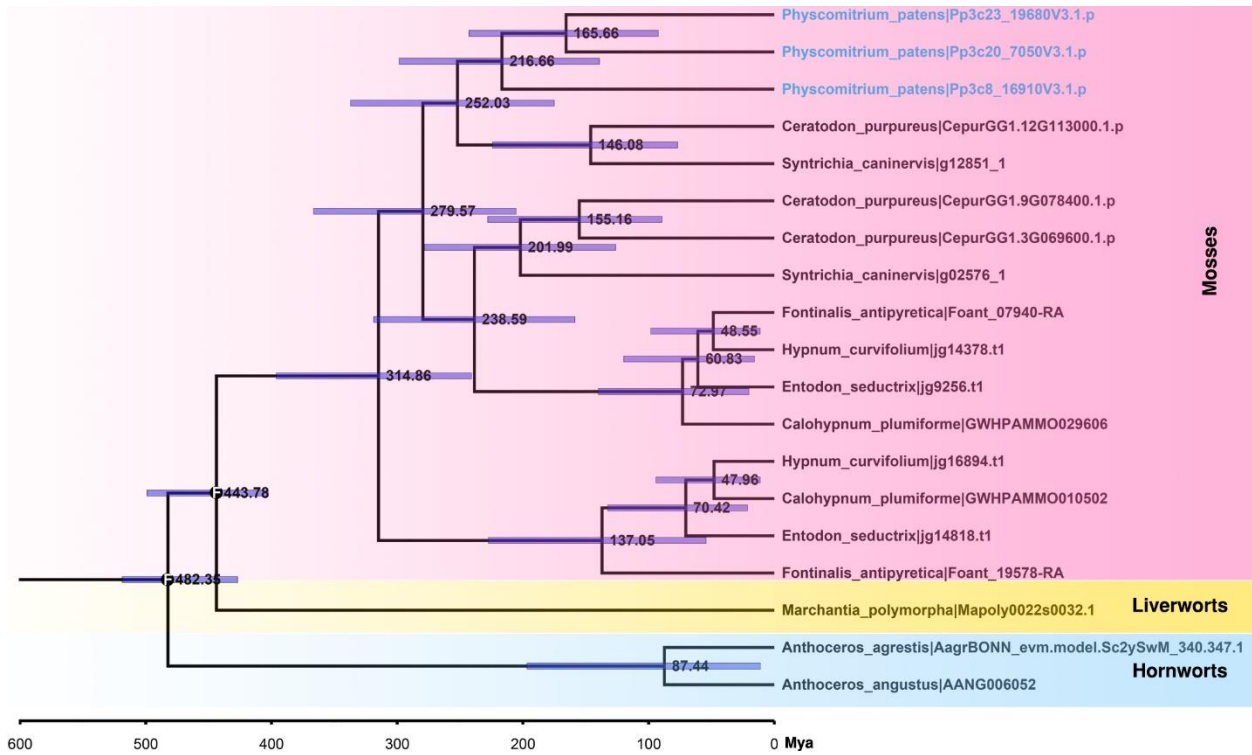


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159 **Data S2 N. Plant morphological characteristics of *Takakia* sp. fossil and *T. lepidozoides***

160 (a) A population of fossil *Takakia* sp. from the Middle–Late Jurassic Daohugou Biota, China, bar  
161 = 1 cm. (b) A natural population of *T. lepidozoides* from the experiment site of Tibetan Plateau,  
162 bar = 1 cm. (c) Magnified views of boxed areas in (a), bar = 500  $\mu\text{m}$ . (d) Extant plants of *T.*  
163 *lepidozoides* in the experiment site of Tibetan Plateau, bar = 500  $\mu\text{m}$ . (e) Details of fossil *Takakia*  
164 sp. fossil plant with lobed terete leaves, bar = 500  $\mu\text{m}$ . (f) Details of *T. lepidozoides* plant with  
165 lobed terete leaves in the experiment site of Tibetan Plateau, bar = 500  $\mu\text{m}$ . (g) Magnified views  
166 of boxed areas in (e), a yellow arrow marks a lobed terete leaf, bar = 500  $\mu\text{m}$ . (h) Magnified views  
167 of boxed areas in (f), a yellow arrow marks a lobed terete leaf, bar = 500  $\mu\text{m}$ . (i) The plant type,  
168 height  $12.48 \pm 2.46$  mm, stem diameter  $0.31 \pm 0.14$  mm in fossil ( $n = 30$ ); height  $11.14 \pm 2.90$  mm,  
169 stem diameter  $0.26 \pm 0.08$  mm in extant plants ( $n = 25$ ). (j) The leaf type, leaf length  $0.88 \pm 0.17$   
170 mm, leaf diameter  $0.04 \pm 0.01$  mm in fossil ( $n = 30$ ); leaf length  $0.75 \pm 0.16$  mm, leaf diameter  
171  $0.04 \pm 0.01$  mm in extant plants ( $n=25$ ).

172

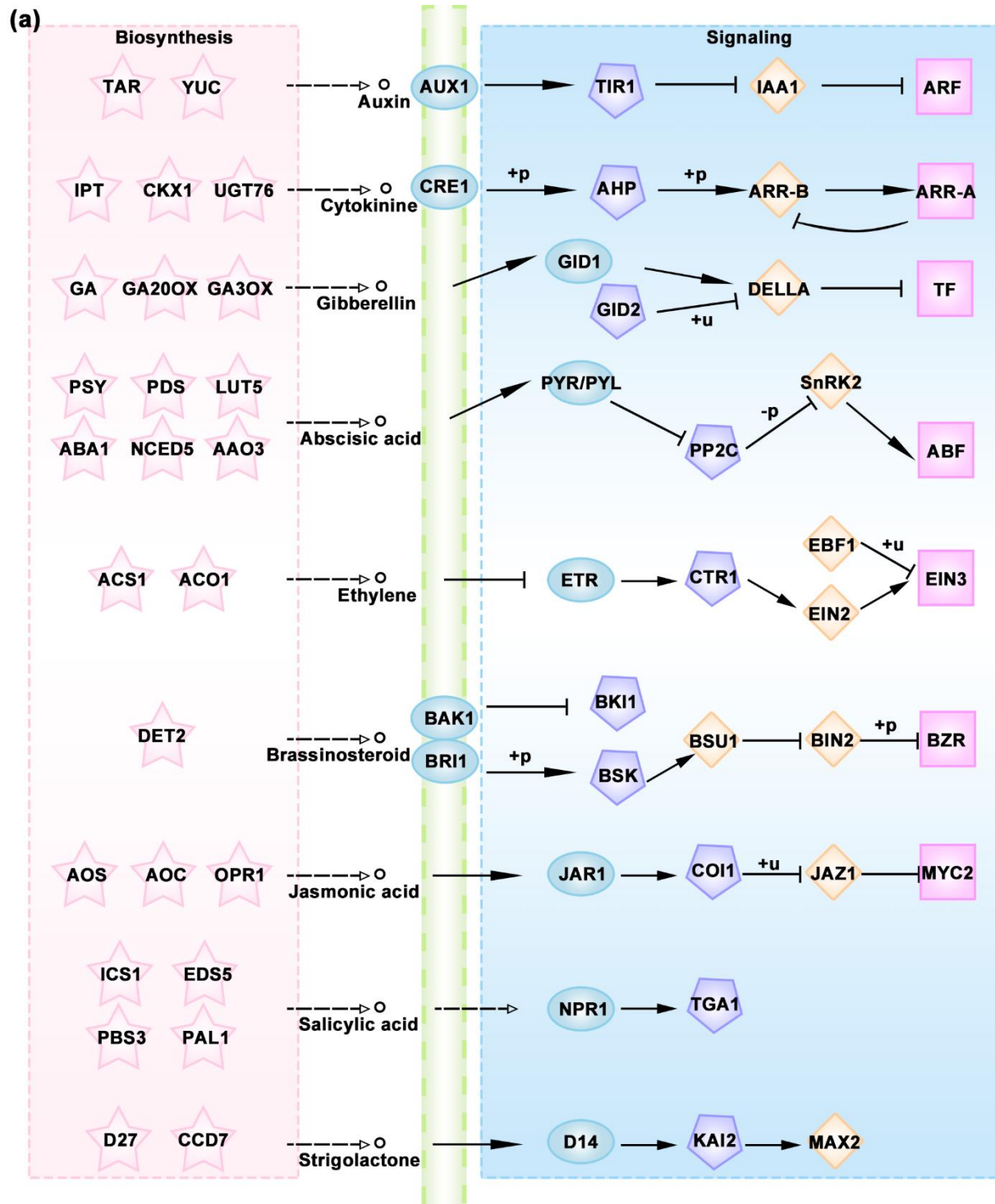


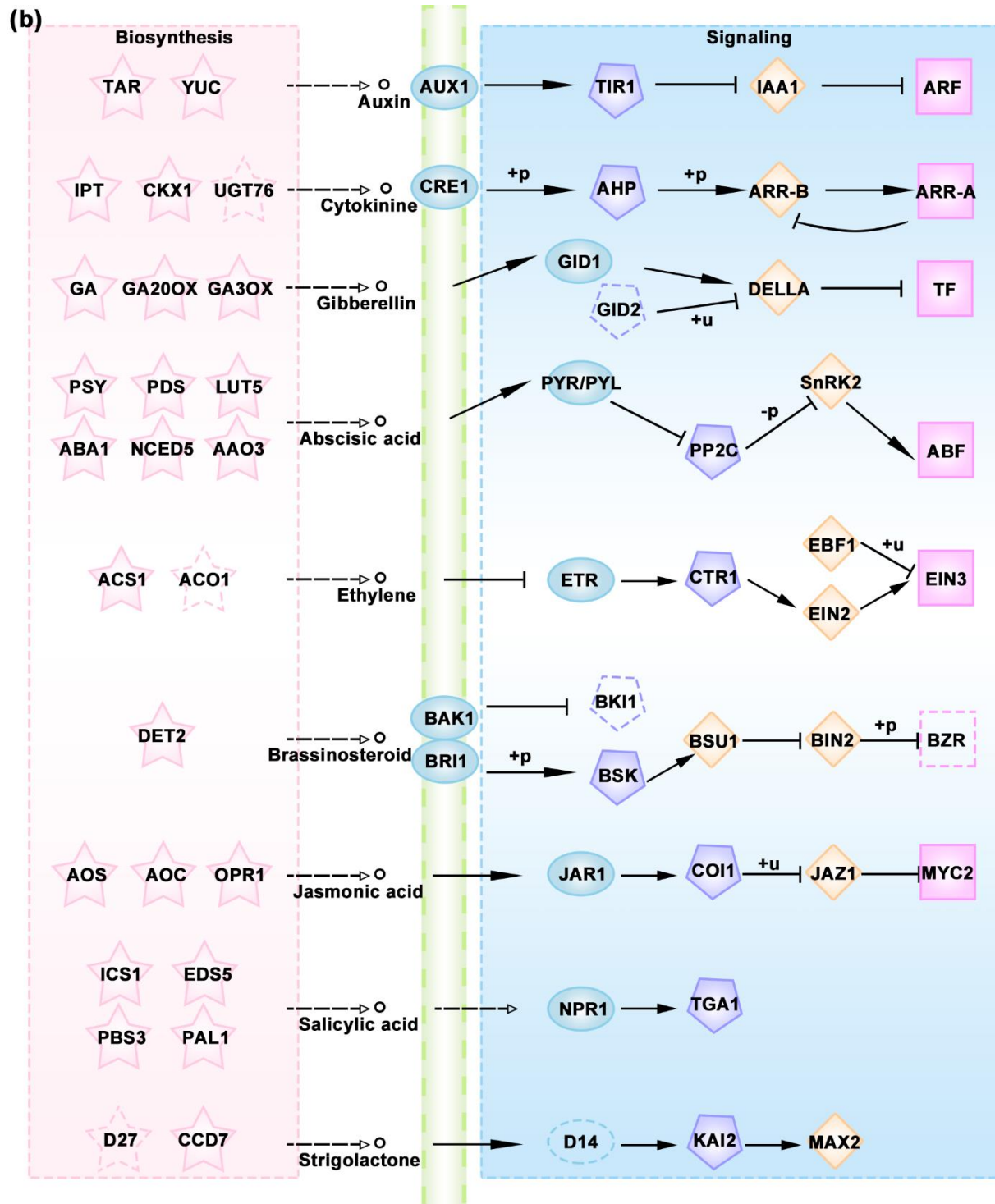
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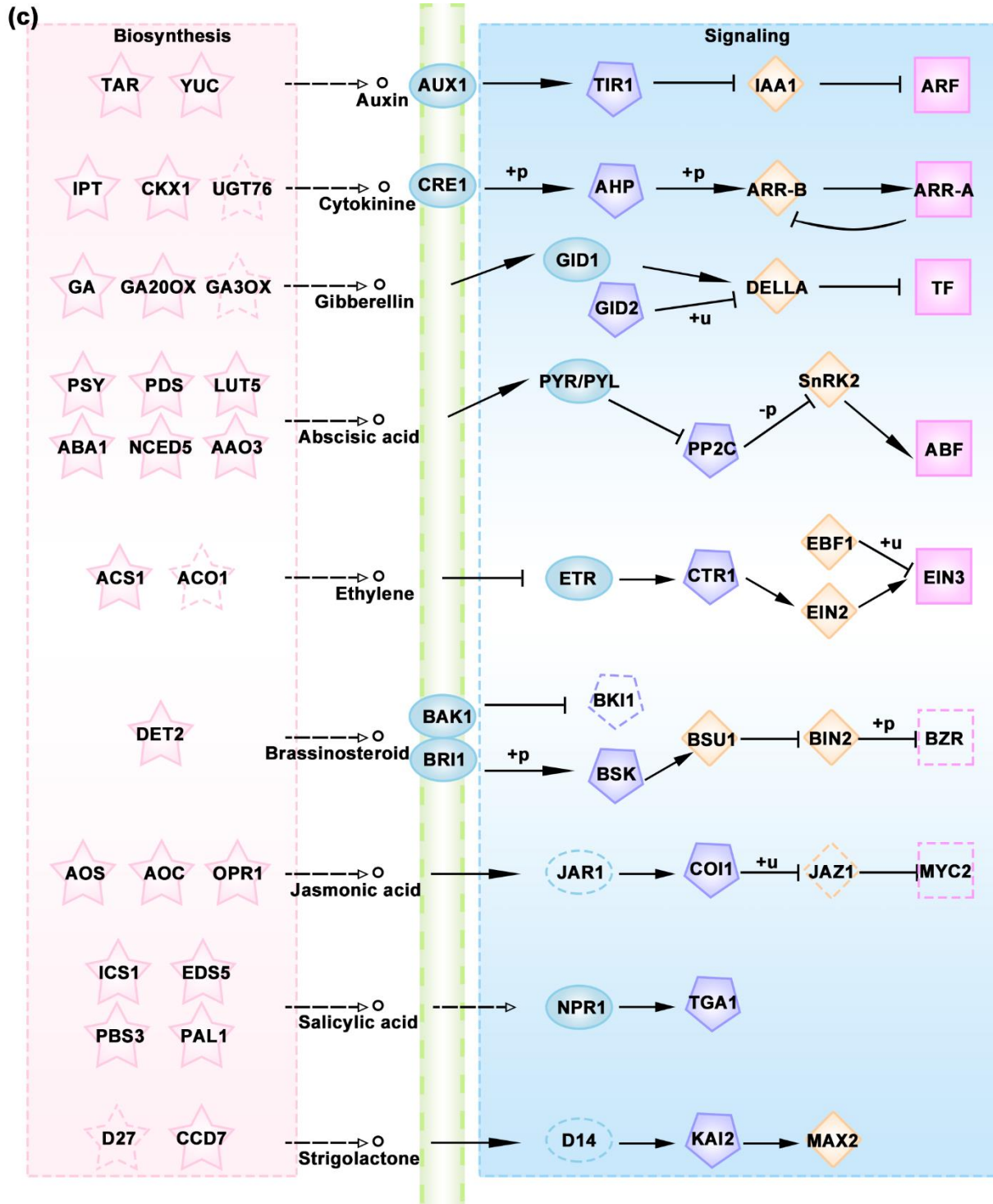
175 **Data S2 O. Phylogenetic timetree of KAN-homologs in bryophytes**

176 KAN (AT5G16560) were found using blastp with identity cutoff 50%, e-value cutoff  $1 \times 10^{-20}$ .  
 177 Maximum likelihood tree using RAXML with the PROTGAMMAJTT model based upon MAFFT  
 178 alignments of proteins and trimmed by trimAl. Tree is the consensus of 1,000 replicates. Numbers  
 179 on nodes represent divergence time, light purple bars show 95% confidence interval lengths,  
 180 nodes with black dots show fossil calibration points.

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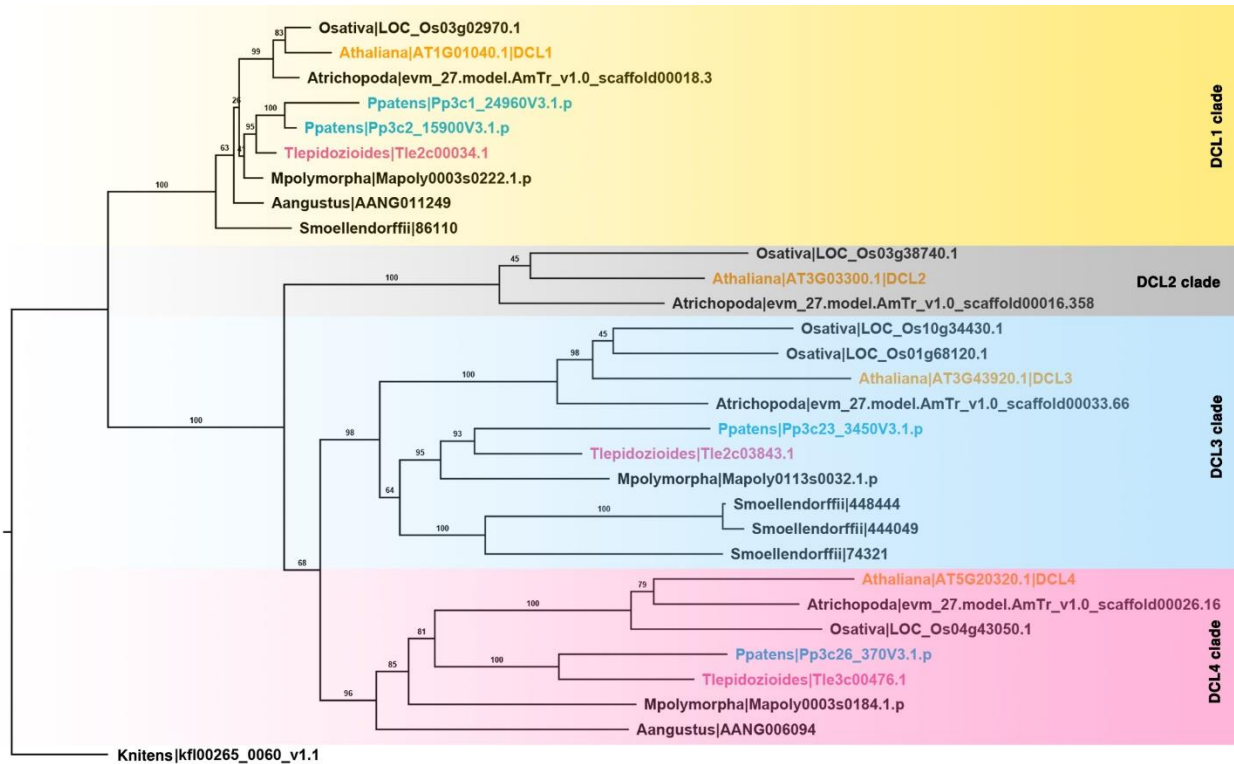
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186 **Data S2 P. Comparison of hormone pathway genes in *A. thaliana*, *P. patens* and *T.***  
187 ***lepidozoides***

188 Plant hormones pathways in *A. thaliana* (**a**), *P. patens* (**b**) and *T. lepidozoides* (**c**). Dashed lines  
189 with gene name mark the genes absent in the pathway, arrows indicate positive/promoting  
190 regulation, and lines with perpendicular end bars indicate negative/repressing regulation. *T.*  
191 *lepidozoides* lacks MYC2 and JAZ1, which may be involved in leaf expansion.

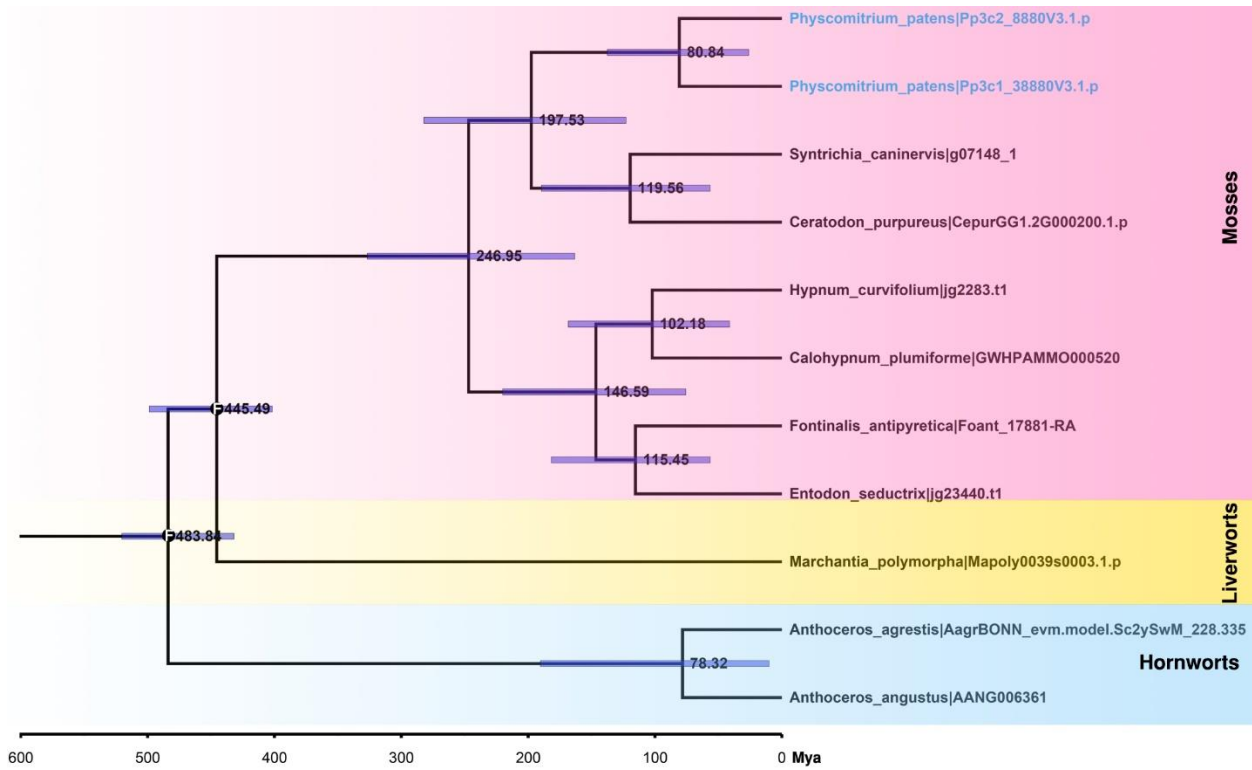
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195 **Data S2 Q. Phylogenetic trees of Dicer proteins**

196 BLASTP was performed using Dicer of *A. thaliana* sequences to find candidate Dicer proteins.  
 197 Putative Dicer proteins were selected with “ $E < 1 \times 10^{-20}$ , identity > 30%, coverage > 70%”.  
 198 Maximum likelihood trees using RAxML with the PROTGAMMAJTT model based upon MAFFT  
 199 alignments of proteins are the consensus of 1,000 replicates. Numbered nodes show the  
 200 percentage of bootstraps which support the node, bootstraps with less than 50% support are not  
 201 shown. The four clades of DICER-like (DCL) plant proteins are color-coded. Orange font color  
 202 represents *A. thaliana*, pink font color represents *T. lepidozoides* and blue font color represents  
 203 *P. patens*. Shade colors as shown on the right.  
 204



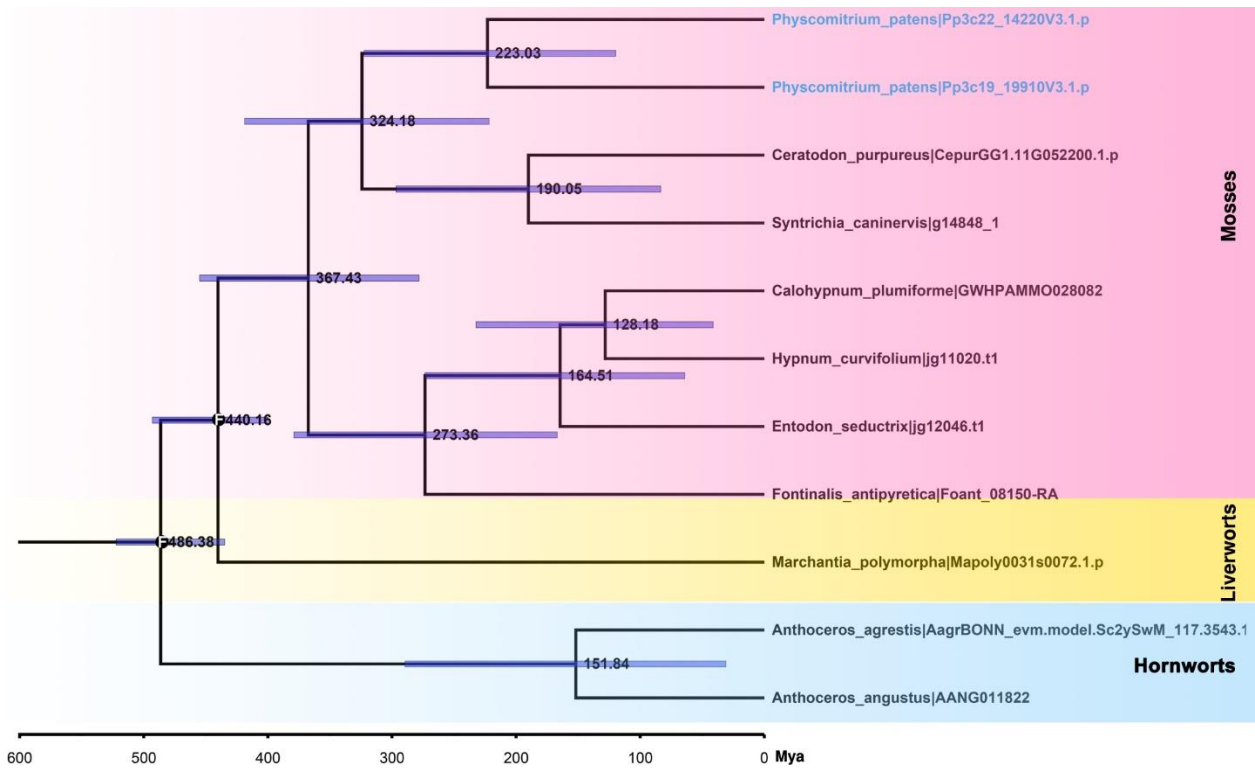
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207 **Data S2 R. Phylogenetic timetree of RHD6-homologs in bryophytes**

208 RHD6 (AT1G66470) homologs were found using blastp with identity cutoff 50%, e-value cutoff  
 209  $1 \times 10^{-20}$ . Maximum likelihood tree using RAxML with the PROTGAMMAJTT model based upon  
 210 MAFFT alignments of proteins and trimmed by trimAl. Tree is the consensus of 1,000 replicates.  
 211 Numbers on nodes represent divergence time, light purple bar shows 95% confidence interval  
 212 lengths, nodes with black dot show fossil calibration points.

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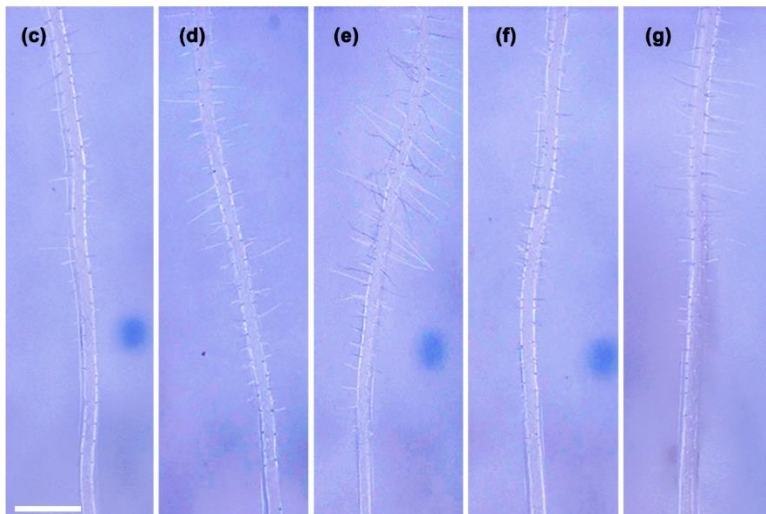
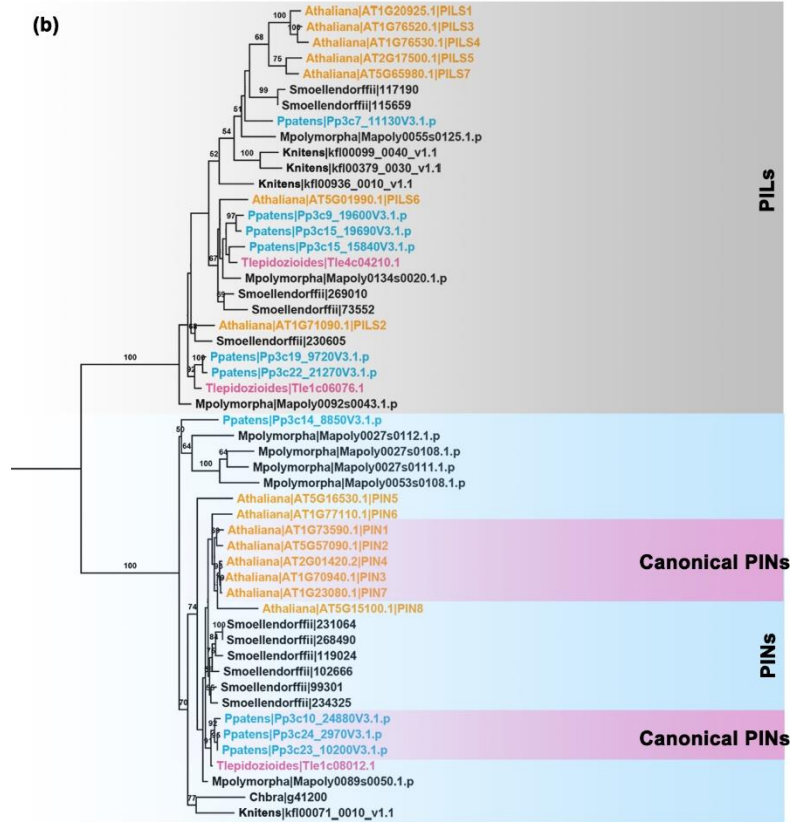
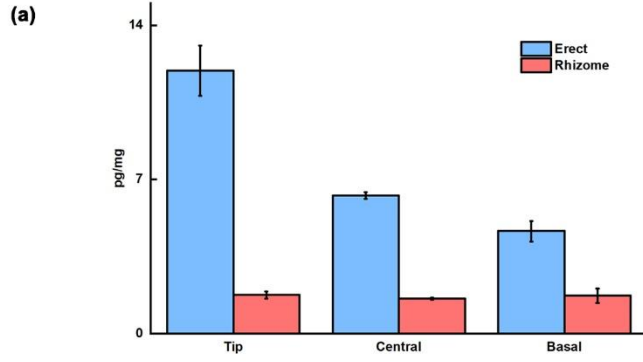


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## 216 Data S2 S. Phylogenetic tree of SMF-homologs in bryophytes

217 FAMA (AT3G24140) homologs were found using blastp with identity cutoff 30%, e-value cutoff  
 218  $1 \times 10^{-20}$ . Then HMMER used to check bHLH domain in FAMA-homolog candidates. Maximum  
 219 likelihood tree using RAxML with the PROTGAMMAJTT model based upon MAFFT alignments  
 220 of proteins and trimmed by trimAl. Tree is the consensus of 1,000 replicates. Numbers on nodes  
 221 represent divergence time, light purple bar show 95% confidence interval length, nodes with black  
 222 dot show fossil calibration points.

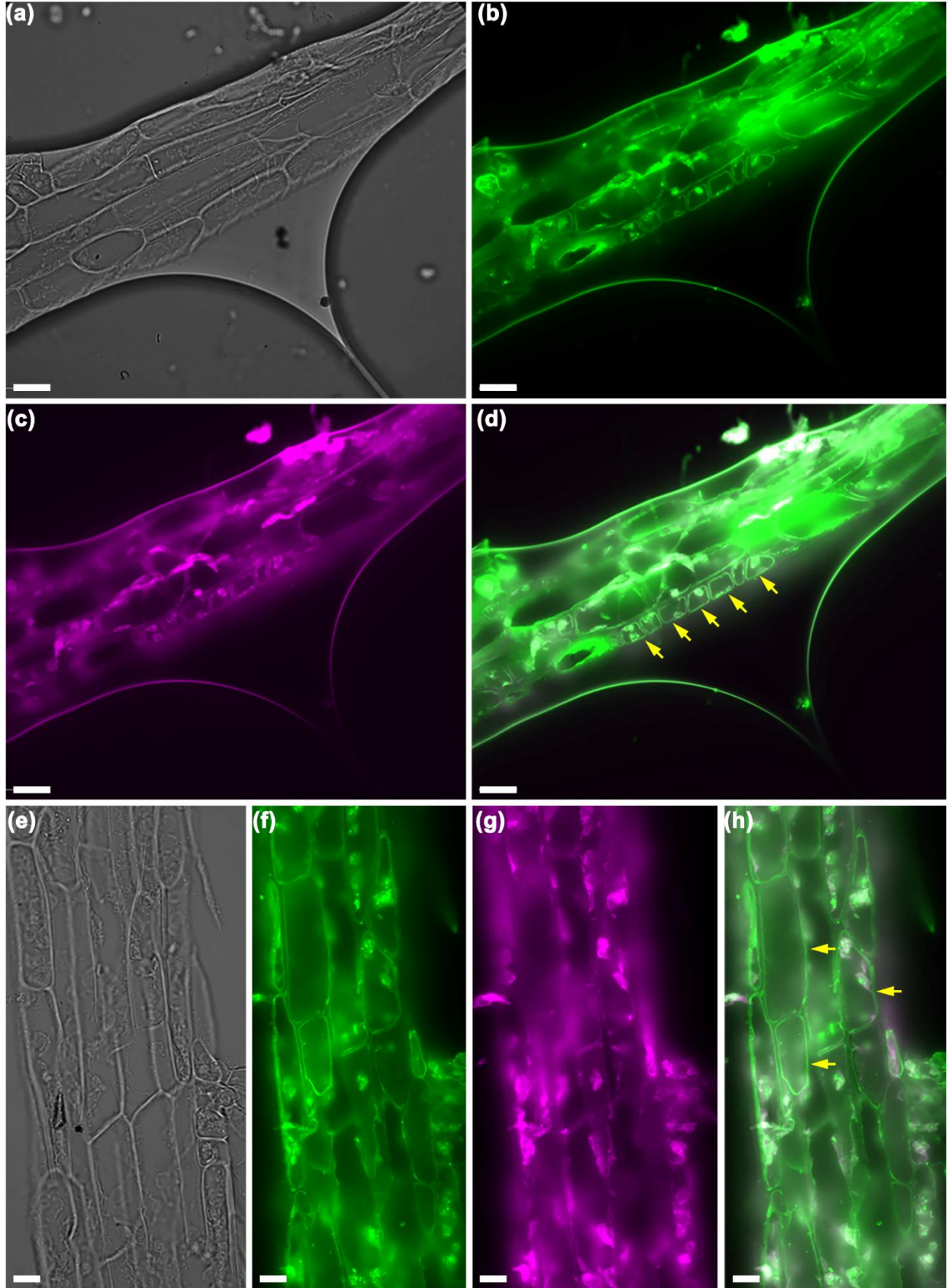
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226 **Data S2 T. IAA content and PINs**

227 (a) Gradient of IAA content from top to base in *T. lepidozoides* stems (mean  $\pm$  s.d.; n = 5). Auxin  
228 concentrations were highest at the top and lowest at the stem base, and there was no detectable  
229 gradient in rhizomes. (b) Phylogenetic tree of PINs. Maximum likelihood tree using RAxML with  
230 the PROTGAMMAJTT model based upon MAFFT alignments of proteins are the consensus of  
231 1,000 replicates. Numbered nodes show the percentage of bootstraps which support the node.  
232 Orange font color represents *A. thaliana*, pink font color represents *T. lepidozoides* and blue font  
233 color represents *P. patens*. Shade colors as shown on the right. (c – g) The root phenotypes of  
234 *TIPIN1-3* transgenic *A. thaliana* plants, bar = 200  $\mu$ m. (c) TIPIN1, (d) TIPIN2, (e) TIPIN3, (f)  
235 PpPINB, (g) AtPIN2.  
236

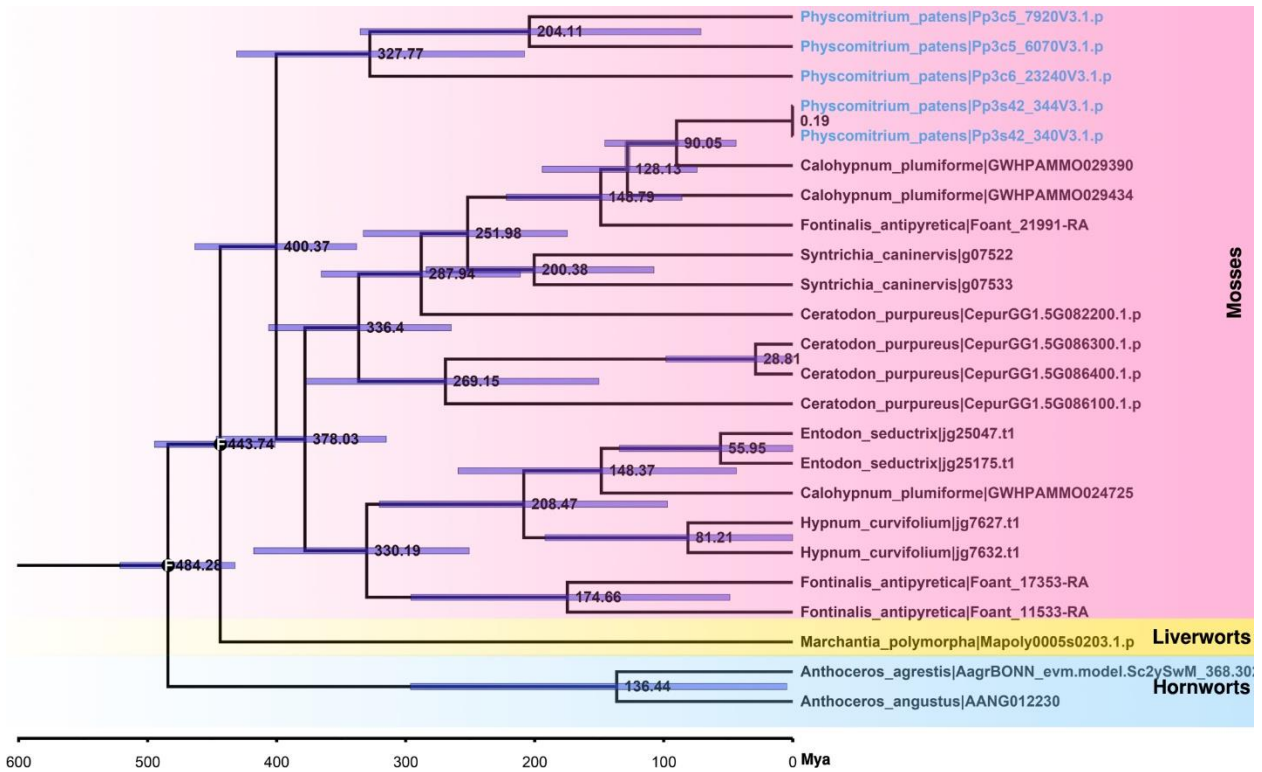


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239 **Data S2 U. Immune-localization of anti-AtPIN antibodies in cells of *T. lepidozoides***

240 The positive signal (arrows) of anti-AtPIN antibodies immune-localization in leaf cells (**a – d**) and  
241 erect-stem cells (**e – h**). Images of bright-field microscopy of leaf cells (**a**) and erect-stem cells  
242 (**e**). The immunofluorescence micrographs of the localization of anti-AtPIN1 antibodies for leaf  
243 cells (**b**) and erect-stem cells (**f**). The chlorophyll signals for leaf cells (**c**) and erect-stem cells (**g**).  
244 The merged views of antibody signals and chlorophyll signals for leaf cells (**d**) and erect-stem  
245 cells (**h**). Yellow arrows mark the plasma membrane. Bar = 20  $\mu\text{m}$ .

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249 **Data S2 V. Phylogenetic tree of CSD-homologs in bryophytes**

250 CSD domains were found using HMMER. Maximum likelihood tree using RAXML with the  
 251 PROTGAMMAJTT model based upon MAFFT alignments of proteins and trimmed by trimAl. Tree  
 252 is the consensus of 1,000 replicates. Numbers on the nodes represent divergence time, light  
 253 purple bars show 95% confidence interval length, nodes with black dots show fossil calibration  
 254 points.

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256 **SUPPLEMENTAL REFERENCES**

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