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1 **1.36 million years of Mediterranean forest refugium dynamics in response to glacial-interglacial**
2 **cycle strength**

3 Short title: Glacial-interglacial dynamics in a forest refugium

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28 **Summary**

29 The sediment record from Lake Ohrid (Southwestern Balkans) represents the longest continuous lake
30 archive in Europe extending back to 1.36 million years (Ma). We reconstruct the vegetation history based
31 on pollen analysis of the DEEP core to reveal changes in vegetation cover and forest diversity during
32 glacial-interglacial (G-IG) cycles and early basin development. The earliest lake phase saw a significantly
33 different composition rich in relict tree taxa and few herbs. Subsequent establishment of a permanent
34 steppic herb association around 1.2 Ma implies a threshold response to changes in moisture availability
35 and temperature and gradual adjustment of the basin morphology.

36 A change in the character of G-IG cycles during the Early-Middle Pleistocene Transition, is reflected in
37 the record by reorganization of the vegetation from obliquity to eccentricity-paced cycles. Based on a
38 quantitative analysis of tree taxa richness, the first large-scale decline in tree diversity occurred around
39 0.94 Ma. Subsequent variations in tree richness were largely driven by the amplitude and duration of G-IG
40 cycles. Significant tree richness declines occurred in periods with abundant dry herb associations, pointing

41 to aridity affecting tree population survival. Assessment of long-term legacy effects between global
42 climate and regional vegetation change reveals a significant influence of cool interglacial conditions on
43 subsequent glacial vegetation composition and diversity. This effect is contrary to observations at high
44 latitudes, where glacial intensity is known to control subsequent interglacial vegetation, and the evidence
45 demonstrates that the Lake Ohrid catchment functioned as a refugium for both thermophilous and
46 temperate tree species.

47 **Significance statement**

48 Forest conservation and restoration are important means to counter threats caused by habitat fragmentation
49 and global change. Diverse and resilient forests can only be maintained and developed if we understand
50 their sensitivity to past climate change. The sedimentary record of the oldest extant lake in Europe, Lake
51 Ohrid (North Macedonia, Albania), shows both the survival and extinction of tree species during glacial
52 and interglacial stages (G-IG) of the Quaternary. Pollen analysis reveals that the area was an effective
53 refuge for tree populations through G-IG periods. The lake body locally buffered the climate, allowing
54 repeated recovery of tree populations that were close to disappearance. However, extended periods of
55 aridity during G-IG intensification after 0.94 Ma caused many tree taxa to disappear.

56

57

58 Introduction

59 Identification and protection of past forest refugia, supporting a relict population, has gained interest in
60 light of projected forest responses to anthropogenic climate change (1-4). Understanding the past and
61 present composition of Mediterranean forest refugia is central to the study of long-term survival of tree
62 taxa and the systematic relation between forest dynamics and climate (5). The Quaternary vegetation
63 history of Europe, studied for over a century, is characterized by successive loss of tree species (6-8).
64 Species loss was originally explained by the—repeated—migration across E-W oriented mountain chains
65 during glacial-interglacial (G-IG) cycles (9). Later views gave more importance to the survival of tree
66 populations during warm and arid stages in southern refugia (10, 11). Tree survival likely depends on
67 persistence of suitable climate and tolerable levels of climate variability, as well as niche differentiation
68 and population size at the refugium (12), although the precise relation between—regional—extinctions,
69 climate variability and local edaphic factors is not well known (13). Mediterranean mountain regions are
70 considered to serve as forest refugia over multiple glacial cycles and frequently coincide with present-day
71 biodiversity hotspots (14). Across the Mediterranean, increases in aridity and fire occurrence have
72 impacted past vegetation communities (15-18). Comprehensive review of available Quaternary
73 Mediterranean records indicates that Early (2.58-0.77 Ma) and Middle Pleistocene (0.77-0.129 Ma) tree
74 diversity was higher compared to the present (13, 19-21). Particularly drought intolerant, thermophilic
75 taxa were more abundant and diverse (8) but with strong spatial and temporal variations in tree diversity
76 across the region. Long-term relationships between refugia function and environmental change over
77 multiple G-IG cycles are hard to quantify due to the rarity of long, uninterrupted records.

78 The Early-Middle Pleistocene Transition (EMPT) between 1.4 Ma and 0.4 Ma (22), is of particular
79 importance for understanding the relation between past climate change, vegetation dynamics and
80 biodiversity in the Mediterranean region. The EMPT is characterized by a gradual transition of G-IG cycle
81 duration from obliquity (41-kyr) to eccentricity (100-kyr) scale with increasing amplitude of each G-IG
82 cycle (e.g. 23, 24). The EMPT was accompanied by long-term cooling of the deep and surface ocean and
83 was likely caused by atmospheric CO₂ decline and ice-sheet feedbacks (25-30). In Europe, the EMPT is
84 associated with pronounced vegetation changes and local extinction and isolation of small tree populations
85 (31).

86 Here we document vegetation history for the last 1.36 Ma of the Lake Ohrid (LO) catchment, located at
87 the Albanian/North Macedonian border at 693 m above sea level (m asl, Fig. 1), the longest continuous
88 sedimentary lake record in Europe (32, 33). The chronology of the DEEP core (ICDP site 5045-1;
89 41°02'57'' N, 20°42'54'' E, Fig. 1) is based on tuning of biogeochemical proxy data to orbital parameters
90 with independent tephrostratigraphic and paleomagnetic age control (32, 33). The Balkan Peninsula has
91 long been considered an important glacial forest refugium for presently widespread taxa such as *Abies*,
92 *Picea*, *Carpinus*, *Corylus*, *Fagus Ostrya*, *Quercus*, *Tilia*, and *Ulmus* (7, 34-36). More than 60% of the
93 Balkans is currently located >1,000 m asl (36), providing steep latitudinal and elevational gradients to
94 support refugia under both cold and warm conditions. Today, the LO catchment is dominated by (semi-)
95 deciduous oak (*Quercus*) and hornbeam (*Carpinus/Ostrya*) forests. Above 1,250 m mixed mesophyllous
96 forest with montane elements occurs (*Fagus* and at higher elevation *Abies*), which above 1,800 m
97 elevation develops into sub-alpine grassland with *Juniperus* shrubs (see ref. 37 for site details). Isolated
98 populations of *Pinus peuce* and *P. nigra* currently grow in the area (37-40).

99 Previous analysis of pollen composition of the last 500 kyr at the DEEP site revealed that the LO has been
100 an important refugium. Arboreal pollen (AP) is deposited continuously and changes in abundance on
101 multi-millennial timescales were associated with G-IG cycles, whereas millennial-scale variability is
102 tightly coupled to Mediterranean sea-surface temperature variations (37, 41-45). Subsequent studies
103 confirm the refugial character of the site recording Early Pleistocene (1.365–1.165 Ma) high relict tree
104 diversity and abundance—and significant hydrological changes, including an increase in lake size and
105 depth (38). Here we present the first continuous palynological record from LO with millennial resolution
106 (~2 kyr) back to 1.36 Ma to assess the systematic relationships between tree pollen abundance, forest
107 diversity, and glacial-interglacial (G-IG) climate variability.

108 Our objective is to: 1) infer the impact of past climate variability on local vegetation across the EMPT, 2)
109 estimate tree species diversity in the catchment, and 3) examine how the amplitude and duration of
110 preceding glacial/interglacial intervals affected the vegetation development and plant species diversity in
111 this refugial area.

112 **Results and Discussion**

113 *Vegetation responses to basin development and glacial-interglacial dynamics*

114 The DEEP pollen record was divided into pollen superzones that reflect G-IG cycles (labelled OD-1 to
115 OD-40; Table S1, Fig. S1), following the approach of Sadori et al. (37, 41). These G-IG variations are
116 characterized by alternations between arboreal pollen (AP %) and herbs (non-arboreal pollen; NAP %)
117 (Fig. 2), superimposed on a long-term trend toward more herbs and shrubs (Figs. S3 and S4).

118 The pollen data indicate two phases of major vegetation reorganizations (Fig. 2). Prior to the first
119 transition at 1.16 Ma (OD-31/32), trees were abundant during the majority of glacial cycles (only 4
120 samples have AP <40% before 1.16 Ma), which is in contrast to subsequent glacial stages with frequent
121 AP values of 20-30 %. Similarly, interglacial herb percentages rose across the 1.16 Ma transition (typical
122 NAP <5% increased to 15-20 %), after which steppic herb associations (*Poaceae*, *Artemisia*,
123 *Amaranthaceae* and *Asteroidae*) and semi-arid shrubs zones established (mainly *Cupressaceae* and
124 *Hippophæ*, Fig. 2ab). Forest composition during the early lake phase (1.36-1.16 Ma) was characterized by
125 abundant *Quercus cerris*-type, *Cedrus*, *Tsuga*, *Carya* and *Picea*, relatively low *Pinus*, and above-average
126 levels of *Corylus*, *Fagus*, *Liquidambar* and *Pterocarya* (Figs. S1, S3, Table S1). Palynological, biomarker,
127 diatom, and geochemical data suggest a gradual expansion and deepening of the lake during the first 100
128 kyr of its existence (1.365–1.265 Ma), which resulted in the inundation of extensive wetlands surrounding
129 the lake (38, 46). The low herb abundances during interglacials prior to 1.16 Ma suggest largely closed
130 forest cover with no extensive herb associations in the catchment. Following the Early Pleistocene (OD-
131 39–OD-33; MIS 42–36), when the Ohrid catchment was rather forested and the soil composition remained
132 stable (38), an increase in herb percentages during the OD-31 (MIS 34) records an opening of the
133 landscape, considering that extensive wetland areas were inundated with the expansion and deepening of
134 the lake during these early G-IG cycles (38). This rapid increase in *Artemisia* and *Amaranthaceae*
135 percentages in OD-31 was most likely associated with a threshold response to glacial conditions since
136 preceding glacials did not cause similarly strong vegetation shifts. This initial appearance of a steppic herb
137 association after 1.16 Ma is consistent with independent evidence of a trend to more aridity in the
138 Mediterranean region (21). At the same time, the Greek Tenaghi Philippon (TP, Figs. 1 and 2) pollen
139 record shows a less pronounced shift to more herbs, suggesting an additional local-scale driver of

140 intensified aridity at LO. Fossil diatom analysis (38, 46) place the shift to steppe vegetation in a period of
141 lake deepening. Graben formation due to NE-SW geologic extension since the Pliocene likely drove the
142 lake deepening—and was accompanied by regional uplift (47). This uplift could explain part of the herb
143 increase at LO by increasing the elevational vegetation gradient in the region and strengthening the
144 aridity-driven reduction in forest cover, but this scenario requires further study.

145 The second transition at 0.94 Ma (OD-24/25) shows an increase in G-IG vegetation variability,
146 characterized by reduced mesophyllous tree abundances during glacial periods (<10% compared to ~20%
147 prior to the transition). High G-IG contrasts in vegetation prevailed after 0.94 Ma, except for OD-14 (MIS
148 14), which was a weakly developed glacial period (30). *Artemisia* abundance, indicative of cool-arid
149 conditions in montane settings (48), increased in post-0.94 Ma glacials, reaching ~30-40% during the most
150 recent glacial stages (MIS 6 and 2-4; Fig. S1) (37, 41). Semi-arid shrubs (Fig. 2) were more abundant in
151 herb-dominated samples with relatively low Poaceae abundance. *Abies* became more abundant post-0.94
152 Ma, reaching notable maxima at about 0.5 Ma (OD-13) and 0.2 Ma (OD-6). *Cedrus* abundance declined
153 with each G-IG cycle after 0.94 Ma (Fig. S1). *Quercus cerris*-type abundance was higher in many, but not
154 all interglacials following the transition, relative to the 1.16-0.94 Ma period. Montane tree abundances
155 were highly variable and, following the definition of Tzedakis et al. (49), occur principally during
156 relatively cool (phases of) interglacials, such as in OD-6 (MIS 5a/c)—and OD-13 (MIS 13) (Fig. 2).
157 Mediterranean tree taxa, commonly under-represented in pollen data (21), were relatively low in
158 abundance throughout the sequence, but reached values of ~10% in zones OD-6, 10, 14, 15 and 21. Most
159 of these phases were concurrent with insolation maxima (precession minima, Fig. S4).

160 The full record extends the observed relationship between AP/NAP changes (37) and global and
161 Mediterranean stacks of benthic marine oxygen isotopes (24, 50) back to 1.36 Ma. The insolation
162 threshold for deglaciation increased during the EMPT (30), leading to eccentricity-paced glaciations (25,
163 26). The increased contrast in abundance and longer duration of forest and steppic periods are a clear
164 expression of the EMPT (Figs. 2 and S6). MIS 24-22 (~0.94-0.86 Ma) is considered the first prolonged
165 eccentricity-paced glacial cycle (28), and was associated with a prolonged phase of high herb abundance
166 (NAP 30-90%) and an unprecedentedly long (>50 ky) period of low (<30%) mesophyllous taxa at LO
167 (zones OD-24 to OD-22). Although glacial vegetation composition and G-IG amplitude increases are
168 evident, changes in the interglacial vegetation composition following the EMPT are less obvious (Figs. S1
169 and S3). A key characteristic of the LO record is the clear precession-scale variability in mesophyllous
170 tree abundance, particularly for deciduous oaks, which is driven by regional atmospheric teleconnections
171 that resulted in phases of high winter rainfall during precession minima (33, 50) (Fig. S6). The vegetation
172 response to this forcing is particularly clear post-EMPT in interglacial periods MIS 5, 7, 9 and 13 and
173 contrasts with the low amplitude precessional forcing and the corresponding low mesophyllous variability
174 in MIS 11 and 19 (Figs. 2 and S4). High precession amplitude during glacials (e.g. MIS 8 and 6) did not
175 lead to a similar expansion of mesophyllous forest, suggesting that during glacials the precession-forced
176 humidity increases were not enough to alter vegetation. Although a vegetation response to changes in
177 precession is evident in the entire sequence (33; Fig. S6), cross-correlation results show that phase
178 relations between individual tree taxa vary over time (Fig. S6), reflecting a change in vegetation patterns
179 as a result of intensification of G-IG cycles. Prior to 0.94 Ma, the *Abies* maxima lagged deciduous oaks by
180 >12 kyr and, together with NAP and *Pinus*, primarily displayed low-amplitude variation characteristic of
181 obliquity-forced G-IG cycles. In contrast, the timing of *Abies* became closer to the deciduous oak maxima
182 (mean offset of 6 kyr) between 0.94 Ma-present (Fig. S6) consistent with a more pronounced vegetation

183 change from forest to steppe characteristic of stronger, eccentricity-paced G-IC cycles. Similarly, *Pinus*
184 abundance gradually varied across the EMPT with high values during glacial maxima and antiphase
185 relation to deciduous oak before 0.94 Ma, to mostly interglacial abundance maxima in the last 0.5 Myr.
186 Together with *Abies*, *Pinus* varied on precession timescales in this later phase with a 6 kyr offset relative
187 to deciduous oaks (Fig. S6). A commonly observed increase of deciduous oaks prior to late successional
188 taxa, such as *Carpinus* (5), was not seen at LO, which likely reflects its refugium character and relative
189 lack of vegetation change within interglacials.

190 ***Relict tree abundance and regional trends***

191 The Ohrid DEEP record shows high abundance and diversity of tree taxa (Fig. 3) that are currently extinct
192 in mainland Europe, or occur as relict populations on Mediterranean islands, in western Asia, and/or North
193 Africa (8, 31). The assemblages also include extant taxa that were locally rare or absent at LO during
194 much of the Pleistocene, such as *Fagus* and *Juglans*. Between 1.36 - 1.16 Ma, primarily *Cathaya*, *Cedrus*,
195 *Tsuga*, *Eucommia*, *Carya*, *Fagus*, *Liquidambar*, *Pterocarya* and *Zelkova* were recorded (OD 40-32). From
196 1.16 Ma onwards, *Castanea*, *Buxus*, *Celtis*, Hamamelidaceae, *Taxodium*-type and *Sequoia*-type
197 established in the catchment, while *Cedrus* and *Fagus* decreased in abundance. In contrast to most other
198 taxa, *Fagus* had a disjunct distribution of abundance before 0.92 Ma, followed by isolated occurrences,
199 and strong recovery during the last 100 kyr. A few other taxa show phases of recovery; *Cedrus* was
200 present until 0.8 Ma, subsequently between 0.67 - 0.43 Ma, and between 0.13 - 0.03 Ma. *Pterocarya* was
201 nearly absent between 0.72 - 0.53 Ma and, *Juglans*, *Castanea* and *Buxus* were absent between 0.48 - 0.33
202 Ma, Fig. 3) after which they recovered. Most other relicts and rare taxa had a—near—continuous presence
203 until ~1.0-0.8 Ma (e.g. *Carya*, *Liquidambar*, *Pterocarya*, *Taxodium*-type, *Tsuga*, *Zelkova*), after which
204 they occurred in low frequencies during—short phases of—some interglacials and finally disappear.

205 Scattered occurrences might represent declining local populations, periods of vegetative growth with little
206 or no production of pollen (e.g. 51), and/or stands at greater distance such as from the Lake Prespa
207 catchment (Fig. 1) that today has a slightly different forest composition (39, 40). Long-distance transport
208 (52) or reworking might be additional causes for isolated pollen occurrences in single samples (42). No
209 long-term decrease or systematic offset in count sums explains the major phases of taxa decline (Table
210 S1), and brief intervals of low abundances occur in subsequent samples, which supports the reliable
211 detection of small tree populations in the analysis. The tephra horizons in the DEEP core are sourced to
212 Italian volcanic provinces despite numerous potential eastern (Aegean, Anatolian) volcanic sources,
213 consistent with dominant westerly winds (33, 42, 53). This information argues against a long-term shift in
214 prevailing wind direction to explain the overall pollen abundance patterns. Sediment and diatom analysis
215 and available chronostratigraphic information also exclude significant reworking of pollen grains (33, 54),
216 but cannot be entirely ruled out.

217 In the context of regional patterns of Quaternary relict tree taxa (13), notable observations in the LO
218 record include the near-continuous presence of *Taxodium*-type pollen until 1.0 Ma and between 0.92-0.85
219 Ma. Other continuous records of *Taxodium*-type only ranged until ~1.5 Ma, although scattered
220 occurrences are known from central Italy and Greece until ~0.5 Ma (13). Likely the shallow early lake
221 phase offered good habitat to some taxa of Taxioideae and other wetland trees such as *Liquidambar* and
222 *Carya* (although *Carya* is not exclusively a wetland tree). The last occurrences (outside current relict
223 populations) of *Cathaya* (0.85 Ma), *Cedrus* (0.03 Ma, discontinuous) and *Zelkova* (0.07 Ma) are most
224 closely comparable to available Italian records (13), while the *Eucommia* presence after 1.1 Ma at LO is

225 only otherwise known from the Tenaghi Phillipon record in NE Greece (55-58) and, curiously, NW
226 Europe (59). The Tenaghi Phillipon record so far is the only continuous European pollen record of
227 comparable length, dated to 1.35 Ma (60). The last occurrences of *Carya* (0.25 Ma), *Pterocarya* (0.4 Ma),
228 and *Liquidambar* and *Tsuga* (0.7 Ma) at LO and Tenaghi Phillipon occur at the same time, and *Carya* is
229 also known from MIS 9 in central Italy (51). In high-resolution analyses of selected intervals from LO,
230 scattered grains of *Tsuga* and *Parrotia* have been reported until 0.4 Ma (MIS 11), which significantly
231 post-dates known occurrences of relict taxa in southern Europe and might represent long-distance
232 transport or reworking (42).

233 ***Richness variations and intensification of glacial-interglacial variability***

234 The palynological richness of tree taxa, estimated by species accumulation curves (Fig. 3), shows
235 relatively high values (> 25 taxa) during the entire record in line with the suggested refugial character of
236 the site and the surrounding region (37, 42, 43, 45). Compared to the base of the record, tree richness
237 before 0.94 Ma was high (>40 taxa) and relatively stable, while the total relict tree abundance was high
238 (>30% in interglacials) and co-varied with G-IG cycles as defined by the marine benthic $\delta^{18}\text{O}$ stack (24).
239 High-resolution rarefaction analysis (61), previously performed on both tree and herb pollen for the Early
240 Pleistocene dataset from LO (1.365–1.165 Ma), showed a gradual decline in total palynological richness
241 within the first 100 kyr of lake existence followed by a stabilization for the rest of the interval (38).
242 During the early development phase of LO, the palynological richness was possibly affected by declining
243 landscape openness due to lake level rise and receding wet meadows. However, the early decline is
244 mirrored in our present analysis, which considers tree pollen only and uses the cumulative richness of
245 consecutive samples to detect rare pollen occurrences (see Methods).

246 The tree richness record responds to the amplification and lengthening of G-IG cycles during the EMPT in
247 several steps. Relict tree relative abundance (principally *Cedrus*) decreased sharply at 1.16 Ma related to
248 the establishment of the permanent herb association from OD-31, while tree richness increased and
249 became more variable (Fig. 3). Regional tectonic uplift (47) likely contributed to a stronger elevational
250 gradient with increased vegetation differentiation and, as is observed for modern large lake basins (62,
251 63), potential development of a locally milder microclimate for diverse tree populations to persist despite
252 the greater regional aridity. The first prominent decline in tree richness occurred at 0.94 Ma with the first
253 prominent eccentricity-paced G-IG cycle, and most relict taxa show a discontinuity in their temporal range
254 at that time (Fig. 3). Tree richness recovered over the next two G-IG cycles, followed by renewed decline
255 between 0.7 and 0.5 Ma during the end of EMPT. Richness remained relatively stable and mostly well
256 below 40 taxa until 0.35 Ma, and subsequently recovered to 40-50 taxa (MIS 9-7), coincident with lower
257 amplitude G-IG variability in benthic $\delta^{18}\text{O}$ (24). Tree richness after 0.2 Ma (MIS 6-1) returned to
258 moderately variable, intermediate values of mostly <40 taxa (Fig. 3).

259 Regional tree taxa loss is evident during MIS 22 (NE Spain and S France), MIS 16 (Greece and Aegean,
260 NE Spain and S France), MIS 20-14 (Northern Italy), and MIS 12 (central/southern Italy) (13). Although
261 these changes have not been quantified as richness estimates and represent composite records from
262 multiple basins, the general pattern of taxa loss seen at LO during the EMPT is supported. Comparable to
263 the LO record, Tenaghi Phillipon shows maximum tree diversity prior to MIS 24-22, and a clear extended
264 minimum after MIS 16, with no major extinctions during MIS 12 (60). A further similarity between these
265 two records is low interglacial abundance of *Corylus* and *Fagus*. These similarities in the decline of tree
266 diversity suggest that the signals at LO and Tenaghi Phillipon are part of a regional trend.

267 The LO record shows that even during glacial periods, when forest vegetation belts were compressed and
268 herb vegetation expanded, tree diversity remained high except in the most extensive glaciations, in support
269 of the refugial characteristics of the catchment. Equally, pronounced interglacials (MIS 5e, 11c, 31) had
270 relatively little impact on tree richness. Although there is no one-to-one relation between tree richness and
271 the amplitude or duration of G-IG cycles, long-term richness declines did not occur during interglacials
272 (declines during MIS 4-3b, 7a-6d, 9c, 12c, 16a, 19b, 24). Strikingly, long-term (>20 kyr) recoveries also
273 occurred exclusively during either glacial or interstadial conditions, not interglacials (MIS 9b, 10b/a, 18d-
274 a, and 21d-a, 30/29 transition), with the notable exception of MIS 35. The tree richness declines were
275 associated with aridity-adapted herb associations (e.g. OD-2, -4, -16 and -24 with high *Artemisia*,
276 *Amaranthaceae*, Fig. S3), suggesting drought severity as an important driver in addition to G-IG
277 temperature change. Conversely, glacials with no significant tree richness decline (e.g. OD-7, -11, -20)
278 generally have higher amounts of Poaceae and Cyperaceae, indicative of greater —soil— moisture. This
279 pattern in moisture variability is also observed in stable isotope-based water balance reconstructions from
280 LO back to 0.6 Ma (64). Svenning (8) recognized that greater drought tolerance favored the relatively late
281 disappearance of *Tsuga*, *Eucommia*, *Carya* and *Pterocarya* in Europe which is consistent with the early
282 disappearance of more drought sensitive *Taxodium*-type we observe. *Tsuga* disappearance in the Italian
283 Peninsula has been explained by drought (16) and associated wildfires (18). The strong tree richness
284 recovery at LO during MIS 9-7 coincides with less extreme G-IG cycles. The marine benthic $\delta^{18}\text{O}$
285 composite (24) and regional pollen evidence suggest that MIS 7 was a cool and moist interglacial (49, 65),
286 and ice buildup during the preceding glacial was not extensive (30).

287 The exact roles of temperature and moisture availability in diversity decline are difficult to quantify due to
288 lack of continuous independent records. Mediterranean sea-surface temperature variations show variations
289 of up to 10 °C across G-IG cycles (66) suggestive of a dominant temperature control of vegetation
290 composition on multi-millennial timescales. Long climate model hindcasts (Fig. 2, ref. 33) regressed
291 against AP% confirm this pattern (modelled annual surface air temperature [SAT], $r^2=0.40$, >0.0001 ; total
292 annual precipitation [TAP], $r^2=0.10$, >0.0001) but additionally show that moisture variability explains
293 more variance in the AP% than temperature at precessional timescales (SAT, $r^2=0.07$, >0.0001 ; TAP,
294 $r^2=0.18$, >0.0001), likely due to strong convective precipitation during enhanced insolation (33, 50).
295 Modern pollen data (67) show that deciduous *Quercus* pollen (>10%) occur at minimum mean annual
296 temperatures of >5 °C. Since deciduous oaks have been continuously present in the LO catchment for the
297 last 1.36 Myr (Fig. S1), this sets a lower limit for glacial temperatures for the entire record. Although
298 temperature variation has been a primary control of G-IG vegetation patterns, the enhanced variability on
299 precessional time scales in the latter half of the record (Fig. S6) was likely controlled by moisture
300 availability, which therefore also was an important factor in the gradual decline in tree richness.

301 **Legacy effects on vegetation composition and richness**

302 The mean composition of the LO record, reflected by the first axis of a Detrended Correspondence
303 Analysis (DCA, Figs S3 and S4), shows a strong negative correlation with maximum marine benthic $\delta^{18}\text{O}$
304 stack values (24) ($r=-0.84$, Fig. S5), which represents minimum global temperature and/or maximum
305 global ice volume reached during each correlative pollen zone. Evidence of a potential legacy effect from
306 previous glacial periods on subsequent interglacial vegetation composition, such as described for the high-
307 latitude paleolake El'gygytgyn (68), is limited at LO ($r=-0.49$, Fig. S5), and likely reflects the refugial
308 character of the region. In contrast, the maximum interglacial marine benthic $\delta^{18}\text{O}$ stack values (24) have a

309 relatively strong negative correlation with the composition of the subsequent glacial pollen assemblage
310 ($r=-0.71$, Fig. S5), indicating increased persistence of relative cool-adapted vegetation caused by—short
311 phases of—cool conditions during interglacials. This relation might represent a ‘cold refugium’ behavior
312 whereby relatively cool interglacial conditions promote the survival and subsequent expansion of cold-
313 adapted vegetation elements during glacials. For tree richness, both the concurrent glacial ($r=-0.55$) and
314 preceding maximum interglacial benthic $\delta^{18}\text{O}$ stack values have the most pronounced negative correlation
315 ($r=-0.59$, Fig S5). This comparison suggests that maximum cooling in both glacials and interglacials is
316 important for tree population survival and, consequently, long-term forest diversity.

317 In summary, the shift to eccentricity-paced G-IG cycles during the EMPT appears responsible for the
318 long-term decline in temperate tree species at Lake Ohrid, whereas diversity was able to recover during
319 low-amplitude G-IG cycles. The changed dynamics also affected the phase relation of montane taxa with
320 respect to maximum insolation forcing. Especially the diversity signal varied between G-IG cycles of
321 varying intensity and duration. Differences in G-IG temperature contrasts, moisture availability and
322 frequency of polar air outbreaks (69), were key factors in the vegetation history at LO. Precession forcing
323 controlled organic matter production and deciduous oak abundance at LO through variation in the length
324 and severity of the dry season (33), which likely also affected the long-term species survival on G-IG time
325 scales. Unique characteristics of the LO basin, especially its topography and the thermal buffer capacity of
326 the large lake resulted in relatively high glacial AP% and a more effective forest refugium compared to the
327 Drama Basin area (TP site in eastern Greece), which was more vulnerable to polar air outbreaks (70). The
328 long LO record reflects global-scale environmental patterns and therefore is of great importance to further
329 quantify the relation of forest diversity and variations in climate. The recorded moisture sensitivity sheds
330 new light on Mediterranean forest development in glacial and interglacial periods, and can help determine
331 the resilience of future forests to 21st century climate change (4) and long-term species survival (2).

332

333 **Methods**

334 *Palynology processing and analysis*

335 Quantitative pollen analysis of the Ohrid DEEP site down to the base of the permanent lake phase was
336 carried out at 64-cm resolution (~1.9 kyr). A total of 697 sediment samples have been processed and
337 analyzed for pollen and spores. Palynological processing followed standard procedures outlined by Faegri
338 et al. (71), consisting of treatment of 1.0–1.5 g dry sediment with cold HCl (37 %), cold HF (40 %) and
339 hot NaOH (10 %) to dissolve carbonates, silicates, and humic acids, respectively. Residues were mounted
340 in glycerin for analysis by transmitted light microscopy. Pollen identification and counting approach is
341 described in detail in Sadori et al. (37, 41). Reported percent abundances are based on the total terrestrial
342 pollen sum excl. *Pinus* due to overrepresentation and potential long-distance transport of this taxon. The
343 pollen percentage sum is based on a mean of 533 (including *Pinus*) and 253 grains/sample (excluding
344 *Pinus*). The reported deciduous oak abundances (Fig. S1, S6) are represented by the combined percentages
345 of *Quercus robur* and *Q. cerris*-types *sensu* Beug et al. (72). Deciduous oak abundances are commonly
346 used as indicators for mid-elevation, relatively humid forest across the Mediterranean region (e.g. 21, 73,
347 74). Assemblage change over time was summarized through detrended correspondence analysis (DCA)
348 incorporated in PAST v. 3.21 (75) on all upland tree and herb taxa >1% (Fig. S3 and S4). Centroid values
349 provide a mean axis score for each pollen zone (Fig. S3).

350 *Tree richness estimates*

351 Palynological detection of rare taxa, and therefore richness estimates, is generally considered problematic
352 as sample counts are commonly around 300 grains, and samples span different length of time, often have
353 poor pollen preservation and overrepresentation of few pollen taxa (13). Richness counts rarefied to a
354 common count sum (61) partly resolves these issues but information is lost in the process since the sample
355 richness is scaled back to the sample with the lowest counts sum, which limits the signal. Our approach
356 uses species accumulation curves (SAC) (76), which effectively bin sets of samples within a window to
357 amplify the total pollen sum for the taxa richness estimate. Subsequently, the window is shifted up one
358 sample and richness is recalculated, a process which is repeated along the entire record. We limited the
359 analysis to tree taxa only since forest dynamics is our principal interest. Also, the majority of tree taxa in
360 this region are wind pollinated, producing abundant pollen. Pollen types are well described (72) and
361 misidentification is therefore minimal. Uncertain identifications were binned into larger taxonomical units
362 (into Hamamelidaceae, *Alnus*, *Tsuga* and *Picea* types), prior to the diversity analysis. The curve of
363 cumulative tree richness levels off as more samples are added (77). The exact shape of the SAC depends
364 on species evenness (76) and therefore on vegetation structure. To address these effects, overlapping
365 SACs of up to 25 samples were constructed, based on all available pollen counts, to determine the
366 appropriate sample window size at which the richness reaches an approximately stable value. The applied
367 method pools the frequency of species relative to sample size (Kindt's exact accumulator, function
368 'specaccum' in the R-package vegan 2.5-5; ref. 78). Based on the overlapping SACs (Fig S2), minimum,
369 optimal and maximum window sizes were selected of, respectively, 10, 15, and 20 samples (Fig. 3).
370 Larger window sizes increase the total taxa count marginally (see methods Fig. S2), but also result in loss
371 of resolution. We therefore consider the window size of 15 as the optimal reflection of richness in both
372 resolution and confidence between the pollen zones.

373 Tree pollen richness values for each of the window sizes were subsequently calculated based on the
374 'collector' method that adds successive samples as they occur for a 1-step sliding window of variable size
375 along the entire sequence. Corresponding ages are based on the midpoint of the respective window size.
376 Our approach effectively estimates richness based on a large cumulative count sum of over 5000 grains
377 sample⁻¹ and ensures a comparison between forest elements only. In addition, minimal sample-specific
378 bias due to the high cumulative sums lead to optimal representation of rare taxa occurrences at pollen zone
379 resolution (77). To enable comparison of the SAC-based richness estimates, we show rarefaction analysis
380 in Fig. S4, computed with the 'rarefy' function in the R-package vegan 2.5-5 (78). To limit the effect of
381 low pollen counts in glacial samples on the rarefaction results we have omitted samples with <150 tree
382 pollen from the analysis. The expected number of taxa, $E(T_{150})$, and associated 95% confidence interval
383 are therefore based on the common value of 150 in 533 samples (Fig. S4). The rarefaction analysis
384 highlight the high-resolution changes while the SAC is more robust and reflective of long-term change
385 since the taxa count reach saturation (Fig. S2). The pollen source area and resulting richness is influenced
386 by the lake size (77), which, due to lake-level variations, could have varied in the early part of the record
387 (38).

388 The reconstructed tree taxa richness represents the Ohrid catchment per pollen zone at a taxonomical
389 resolution typical for pollen analysis, i.e., at genus level. All tree taxa recorded as pollen in the uppermost
390 sediments are present in the current LO catchment area (GBIF data October 2020). For instance, sparse
391 grains of *Picea*, *Betula* and *Tilia* pollen are mirrored by the limited distribution of these species in the

392 present vegetation (37), while significant *Pinus*, *Q. robur*-type, *Q. cerris*-type, *Q. ilex*-type, *Carpinus*,
393 *Ostrya* and *Abies* pollen abundances reflect local tree populations in the catchment today. This adds
394 confidence to down core records reflecting actual presence of taxa in the catchment, although the exact
395 timing of (dis)appearance of specific species should be confirmed by macrofossil analysis of near-shore
396 cores, the general pattern provides important insights into the effectiveness of the LO catchment refugium
397 function.

398 ***Time series analysis***

399 Time series analyses for *Abies*, *Pinus* (Fig. S6) follow the approach reported for deciduous oaks and total
400 AP in Wagner et al. (33). Data series were resampled at regular intervals (linear interpolation) and
401 submitted to continuous wavelet transform (CWT, Morlet window) incorporated in PAST v. 3.21 software
402 (75). In addition, phase relations were calculated using cross-correlation (79) for three time periods; 0-500
403 ka, 500-940 ka and 940-1320 ka, representing the periods approximately prior, during and following the
404 EMPT. We limited the analysis to selected abundant tree taxa (*Abies*, *Pinus*, *Carpinus*, Cupressaceae
405 [excluding *Taxodium*-type] and sclerophyllous oaks) and NAP, for which significant phase relations are
406 determined relative to maximum deciduous oak abundance (*Q. cerris*-type and *Q. robur*-type) in each
407 period. Negative numbers indicate a mean lead with respect to deciduous oak abundance, and positive a
408 lag.

409 **Legacy effects**

410 Analysis of subsequent G-IG cycles at the Lake El'gygytyn pollen record from NE Siberia revealed an
411 obvious legacy effect of glacial intensity on vegetation composition in the ensuing interglacial due to the
412 enhanced permafrost and distance of forest refugia (68). To test for possible legacy at LO we regressed
413 zone-averaged pollen composition data and tree richness values against climate proxy data. Following
414 Herzschuh et al. (68), we use the LR04 global benthic $\delta^{18}\text{O}$ stack (24) as a measure of glacial intensity.
415 We regressed minimum, maximum and mean $\delta^{18}\text{O}$ of contemporaneous and preceding stages (based on 2
416 kyr interpolated values from LR04) against the pollen-zone average values from the first DCA axis,
417 representing the principal compositional changes in the vegetation proxy record (Fig. 2).

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604

605 **Figure captions**

606 **Fig 1:** (A) Location of Lake Ohrid (LO) and Tenaghi Phillipon (TP) on the Balkan Peninsula. (B) Local
607 setting around LO, bathymetry (80) and DEEP coring site (adapted from ref. 32).

608 **Fig 2:** (A) **Pollen diagram** of ecological groups (%) against chronology of Wagner *et al.* (33): montane
609 trees (*Abies*, *Betula*, *Cedrus*, *Fagus*, *Ilex*, *Picea*, *Taxus*, *Tsuga*); mesophyllous trees (*Acer*, *Buxus*,
610 *Carpinus betulus*, *Carya*, *Castanea*, *Celtis*, *Corylus*, *Fraxinus excelsior/oxycarpa*, *Hedera*,
611 *Ostrya/Carpinus orientalis*, *Pterocarya*, *Quercus cerris*-type, *Quercus robur*-type, *Tilia*, *Ulmus*, *Zelkova*);
612 Sclerophyllous trees (*Arbutus*, *Cistus*, *Fraxinus ornus*, *Olea*, *Phillyrea*, *Pistacia*, *Quercus ilex*-type,
613 *Rhamnus*); wetland trees (*Alnus*, *Liquidambar*, *Platanus*, *Populus*, *Salix*, *Tamarix*, *Taxodium*-type); semi-
614 arid shrubs (*Ephedra*, Ericaceae, *Hippophaë*, Cupressaceae [mostly *Juniperus*-type]). (B) Tenaghi
615 Philippon (TP, location see Fig. 1): AP percentages excluding *Pinus*, *Betula* and *Juniperus*-type (55-58,
616 60). (C) LOVECLIM model timeseries of annual precipitation and (D) mean surface air temperature for
617 the Lake Ohrid grid cell (33). (E) MEDSTACK planktonic $\delta^{18}\text{O}$ data (81). (F) Stacked benthic $\delta^{18}\text{O}$ data
618 for ODP sites 967 and 968 from the eastern Mediterranean (82). (G) LR04 $\delta^{18}\text{O}$ global benthic stack (24).

619 **Fig 3: relicts and richness** (A) Range and relative abundance of relict tree taxa in the DEEP site of Lake
620 Ohrid together with (B) total % AP (filled blue) and % total relict taxa (filled grey, excl. *Fagus*). Bars
621 represent % abundances for relict and rare tree taxa and are mirrored to enhance visibility. (C) Tree taxa
622 richness of the DEEP site is based on taxa accumulation curves of tree pollen along a moving window.
623 Single data points represent total richness from species accumulation curves (see methods) with a sliding
624 window of size 10 (red dotted line), 15 (black line), or 20 (green dashed line). All LO data is plotted
625 against the chronology of Wagner *et al.* (33). (D) LR04 $\delta^{18}\text{O}$ global benthic stack (24).





