

1.36 million years of Mediterranean forest refugium dynamics in response to glacial–interglacial cycle strength

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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
- 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
- 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
- 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
- aridity during G-IG intensification after 0.94 Ma caused many tree taxa to disappear.

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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
- during glacial-interglacial (G-IG) cycles (9). Later views gave more importance to the survival of tree
 populations during warm and arid stages in southern refugia (10, 11). Tree survival likely depends on
- populations during warm and and suges in southern relagia (10, 11). The survival mery depends on
 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
- considered to serve as forest refugia over multiple glacial cycles and frequently coincide with present-day
- 51 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
- 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
- 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
- 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 (\sim 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
- 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

samples have AP <40% before 1.16 Ma), which is in contrast to subsequent glacial stages with frequent

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 Asteroideae) and semi-arid shrubs zones established (mainly Cupressaceae and
- 124 *Hippophaë*, Fig. 2ab). Forest composition during the early lake phase (1.36-1.16 Ma) was characterized by
- 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,
- 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
- 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
- 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
- the lake during these early G-IG cycles (38). This rapid increase in *Artemisia* and Amaranthaceae
- 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
- 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, characterized by reduced mesophyllous tree abundances during glacial periods (<10% compared to ~20% 146 147 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 \sim 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). Ouercus 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

- 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
- 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 Mediterranean stacks of benthic marine oxygen isotopes (24, 50) back to 1.36 Ma. The insolation 161 162 threshold for deglaciation increased during the EMPT (30), leading to eccentricity-paced glaciations (25, 26). The increased contrast in abundance and longer duration of forest and steppic periods are a clear 163 164 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 (NAP 30-90%) and an unprecedentedly long (>50 ky) period of low (<30%) mesophyllous taxa at LO 166 167 (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 relations between individual tree taxa vary over time (Fig. S6), reflecting a change in vegetation patterns 178 179 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*
- abundance gradually varied across the EMPT with high values during glacial maxima and antiphase
- 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

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
- taxa, *Fagus* had a disjunct distribution of abundance before 0.92 Ma, followed by isolated occurrences,
- and strong recovery during the last 100 kyr. A few other taxa show phases of recovery; *Cedrus* was
- present until 0.8 Ma, subsequently between 0.67 0.43 Ma, and between 0.13 0.03 Ma. *Pterocarya* was
- 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
- they occurred in low frequencies during—short phases of—some interglacials and finally disappear.

Scattered occurrences might represent declining local populations, periods of vegetative growth with little
 or no production of pollen (e.g. 51), and/or stands at greater distance such as from the Lake Prespa
 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
- long-term decrease or systematic offset in count sums explains the major phases of taxa decline (Table
 S1), and brief intervals of low abundances occur in subsequent samples, which supports the reliable
- 210 ST), and offer intervals of low abundances occur in subsequent samples, which supports the renable 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,
- consistent with dominant westerly winds (33, 42, 53). This information argues against a long-term shift in
- prevailing wind direction to explain the overall pollen abundance patterns. Sediment and diatom analysis
- and available chronostratigraphic information also exclude significant reworking of pollen grains (33, 54),
- but cannot be entirely ruled out.
- 217 In the context of regional patterns of Quaternary relict tree taxa (13), notable observations in the LO
- 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
- occurrences are known from central Italy and Greece until ~0.5 Ma (13). Likely the shallow early lake
- 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
- populations) of *Cathava* (0.85 Ma), *Cedrus* (0.03 Ma, discontinuous) and *Zelkova* (0.07 Ma) are most
- closely comparable to available Italian records (13), while the *Eucommia* presence after 1.1 Ma at LO is

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

233 Richness variations and intensification of glacial-interglacial variability

- The palynological richness of tree taxa, estimated by species accumulation curves (Fig. 3), shows
- relatively high values (> 25 taxa) during the entire record in line with the suggested refugial character of
- the site and the surrounding region (37, 42, 43, 45). Compared to the base of the record, tree richness
- 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 are defined by the marine benthic δ^{18} O stack (24).
- 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
- 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
- several steps. Relict tree relative abundance (principally *Cedrus*) decreased sharply at 1.16 Ma related to
- the establishment of the permanent herb association from OD-31, while tree richness increased and
- 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,
- 63), potential development of a locally milder microclimate for diverse tree populations to persist despite
- the greater regional aridity. The first prominent decline in tree richness occurred at 0.94 Ma with the first prominent eccentricity-paced G-IG cycle, and most relict taxa show a discontinuity in their temporal range
- at that time (Fig. 3). Tree richness recovered over the next two G-IG cycles, followed by renewed decline
- between 0.7 and 0.5 Ma during the end of EMPT. Richness remained relatively stable and mostly well
- below 40 taxa until 0.35 Ma, and subsequently recovered to 40-50 taxa (MIS 9-7), coincident with lower
- amplitude G-IG variability in benthic δ^{18} 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
- 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
- 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
- two records is low interglacial abundance of *Corylus* and *Fagus*. These similarities in the decline of tree
- 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

- herb vegetation expanded, tree diversity remained high except in the most extensive glaciations, in support
- of the refugial characteristics of the catchment. Equally, pronounced interglacials (MIS 5e, 11c, 31) had
- relatively little impact on tree richness. Although there is no one-to-one relation between tree richness and
- the amplitude or duration of G-IG cycles, long-term richness declines did not occur during interglacials
 (declines during MIS 4-3b, 7a-6d, 9c, 12c, 16a, 19b, 24). Strikingly, long-term (>20 kyr) recoveries also
- occurred exclusively during either glacial or interstadial conditions, not interglacials (MIS 9b, 10b/a, 18d-
- a, and 21d-a, 30/29 transition), with the notable exception of MIS 35. The tree richness declines were
- associated with aridity-adapted herb associations (e.g. OD-2, -4, -16 and -24 with high Artemisia,
- Amaranthaceae, Fig. S3), suggesting drought severity as an important driver in addition to G-IG
- 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
- LO back to 0.6 Ma (64). Svenning (8) recognized that greater drought tolerance favored the relatively late
- disappearance of *Tsuga*, *Eucommia*, *Carya* and *Pterocarya* in Europe which is consistent with the early
- disappearance of more drought sensitive *Taxodium*-type we observe. *Tsuga* disappearance in the Italian
- Peninsula has been explained by drought (16) and associated wildfires (18). The strong tree richness
- recovery at LO during MIS 9-7 coincides with less extreme G-IG cycles. The marine benthic δ^{18} O
- composite (24) and regional pollen evidence suggest that MIS 7 was a cool and moist interglacial (49, 65),
- 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
- 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
- against AP% confirm this pattern (modelled annual surface air temperature [SAT], $r^2=0.40$, >0.0001; total
- annual precipitation [TAP], $r^2=0.10$, >0.0001) but additionally show that moisture variability explains more variance in the AP% than temperature at precessional timescales (SAT, $r^2=0.07$, >0.0001; TAP,
- r²=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
- 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
- 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 δ^{18} O
- 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 δ^{18} O stack values (24) have a

- 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-
- adapted vegetation elements during glacials. For tree richness, both the concurrent glacial (r=-0.55) and preceding maximum interglacial benthic δ^{18} O stack values have the most pronounced negative correlation
- preceding maximum interglacial benthic δ^{18} O stack values have the most pronounced negative correlation (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
- 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
- and severity of the dry season (33), which likely also affected the long-term species survival on G-IG time
- scales. Unique characteristics of the LO basin, especially its topography and the thermal buffer capacity of
- 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
- 526 long LO record reflects global-scale environmental patients and therefore is of great importance to further
- quantify the relation of forest diversity and variations in climate. The recorded moisture sensitivity sheds new light on Mediterranean forest development in glacial and interglacial periods, and can help determine
- the resilience of future forests to 21^{st} 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

carried out at 64-cm resolution (~1.9 kyr). A total of 697 sediment samples have been processed and

analyzed for pollen and spores. Palynological processing followed standard procedures outlined by Faegri

- et al. (71), consisting of treatment of 1.0–1.5 g dry sediment with cold HCl (37 %), cold HF (40 %) and
- hot NaOH (10 %) to dissolve carbonates, silicates, and humic acids, respectively. Residues were mounted
- in glycerin for analysis by transmitted light microscopy. Pollen identification and counting approach is

described in detail in Sadori et al. (37, 41). Reported percent abundances are based on the total terrestrial
 pollen sum excl. *Pinus* due to overrepresentation and potential long-distance transport of this taxon. The

- pollen percentage sum is based on a mean of 533 (including *Pinus*) and 253 grains/sample (excluding
- *Pinus*). The reported deciduous oak abundances (Fig. S1, S6) are represented by the combined percentages
- of *Quercus robur* and *Q. cerris*-types *sensu* Beug et al. (72). Deciduous oak abundances are commonly
- 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)
- 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 uses species accumulation curves (SAC) (76), which effectively bin sets of samples within a window to 356 357 amplify the total pollen sum for the taxa richness estimate. Subsequently, the window is shifted up one sample and richness is recalculated, a process which is repeated along the entire record. We limited the 358 analysis to tree taxa only since forest dynamics is our principal interest. Also, the majority of tree taxa in 359 360 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 cumulative tree richness levels off as more samples are added (77). The exact shape of the SAC depends 363 364 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 appropriate sample window size at which the richness reaches an approximately stable value. The applied 366 367 method pools the frequency of species relative to sample size (Kindt's exact accumulator, function 'specaccum' in the R-package vegan 2.5-5; ref. 78). Based on the overlapping SACs (Fig S2), minimum, 368 369 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 of resolution. We therefore consider the window size of 15 as the optimal reflection of richness in both 371 372 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. Our approach effectively estimates richness based on a large cumulative count sum of over 5000 grains 376 377 sample⁻¹ and ensures a comparison between forest elements only. In addition, minimal sample-specific bias due to the high cumulative sums lead to optimal representation of rare taxa occurrences at pollen zone 378 resolution (77). To enable comparison of the SAC-based richness estimates, we show rarefaction analysis 379 in Fig. S4, computed with the 'rarefy' function in the R-package vegan 2.5-5 (78). To limit the effect of 380 low pollen counts in glacial samples on the rarefaction results we have omitted samples with <150 tree 381 382 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 since the taxa count reach saturation (Fig. S2). The pollen source area and resulting richness is influenced 385 386 by the lake size (77), which, due to lake-level variations, could have varied in the early part of the record (38). 387

388 The reconstructed tree taxa richness represents the Ohrid catchment per pollen zone at a taxonomical

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
- timing of (dis)appearance of specific species should be confirmed by macrofossil analysis of near-shore
- 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
- determined relative to maximum deciduous oak abundance (*Q. cerris*-type and *Q. robur*-type) in each
 period. Negative numbers indicate a mean lead with respect to deciduous oak abundance, and positive a
- 407 period. Negative numbers indicate a mean read 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'gygytgyn 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
- 213 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 δ^{18} O stack (24) as a measure of glacial intensity.
- 415 We regressed minimum, maximum and mean δ^{18} 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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605 Figure captions

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

- **Fig 2: (A) Pollen diagram** of ecological groups (%) against chronology of Wagner et al. (33): montane
- 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 δ^{18} O data (81). (F) Stacked benthic δ^{18} O data
- for ODP sites 967 and 968 from the eastern Mediterranean (82). (G) LR04 δ^{18} 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
- 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
- against the chronology of Wagner et al. (33). (**D**) LR04 δ^{18} O global benthic stack (24).





