ALL TOGETHER NOW: AN INTERNATIONAL PALYNOLOGICAL TEAM DOCUMENTS VEGETATION AND CLIMATE CHANGES DURING THE LAST 500 KYR AT LAKE OHRID (SE EUROPE)

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ABSTRACT: Lake Ohrid (Balkan peninsula) is the oldest European extant lake and one of the deepest and largest. Such a unique, terrestrial natural archive is especially relevant for both paleoenvironmental and paleoclimatic reconstructions but also for genetic studies. In the frame of the International Continental Scientific Drilling Program (ICDP), a deep drilling campaign was carried out within the scope of the Scientific Collaboration on Past Speciation Conditions in Lake Ohrid (SCOPSCO) project in 2013. Here, we present the summary of palynological analyses carried out in the upper 200 m of the overall 569 m long DEEP site sediment succession from the central part of the lake. These studies, performed by an international palynological team, document the main floristic, vegetation and climate changes during the last ca 500 kyr, at a millennial-scale resolution (~1.6 kyr). The continuous sediment infill permitted to trace multiple non-forested/forested phases as a response to Glacial/Interglacial cycles as well as to sub-Milankovitch climate changes. The pollen record, corresponding with marine isotope stages MIS 13 to MIS 1, points to a progressive change from cooler and wetter to warmer and drier interglacials. New palynological studies are underway to reconstruct vegetational and climatic conditions over older intervals as well as to obtain high resolution data for some key intervals such as MIS 5-6, MIS 11-12, MIS 35-42. The complete record of changes in flora composition and vegetation during both glacials and interglacials will furnish indispensable insights for understanding the role of refugia, ecosystem resilience and maintenance of terrestrial biodiversity in the Mediterranean area.

Keywords: Lake Ohrid, pollen, flora, vegetation, climate, Pleistocene, Balkan peninsula

1. INTRODUZIONE

In 2013, within the frame of the International Continental Scientific Drilling Program (ICDP), a deep drilling campaign was carried out as part of the Scientific Collaboration on Past Speciation Conditions in Lake Ohrid (SCOPSCO) project, on the Balkan peninsula (Fig. 1). Several sites in Lake Ohrid (LO in the following), with the deepest one having a depth of some 568 m, were drilled to address several scientific aims (Wagner et al., 2014). Most important is to define both the depositional context and the range of past natural variability and magnitude of climate changes within a coherent chronologic frame.

Previous molecular clock analyses of DNA on endemic genera (e.g. *Dina*, Hirudinea, Erpobdellidae) that have evolved within the lake suggested, by estimating the onset of intralacustrine diversification, a time frame of approximately 2-3 Ma (Trajanovski et al., 2010). However recent evidence indicates an age of 1.2-1.9 Ma for the origin of LO (Wagner et al., 2014; Lindhorst et al., 2015). The occurrence of freshwater endemic species (including both flora and fauna taxa) was also a prime motivation for the study of present conditions at LO in order to observe their response to the increasing anthropogenic pressure in the lake. Based on field surveys, monitoring data, published records, expert interviews, conservation concerns and associated major threats were traced (e.g. Kostoski et al., 2010).

Palynology, together with a large amount of geological and stratigraphical investigations (Franke et al., 2016) was applied to the study of the LO sedimentary succession since 2014, especially to provide evidence on the history of changes of both flora composition and vegetation structure. Both items are indispensable for climate deductions as well as to assess the distribution

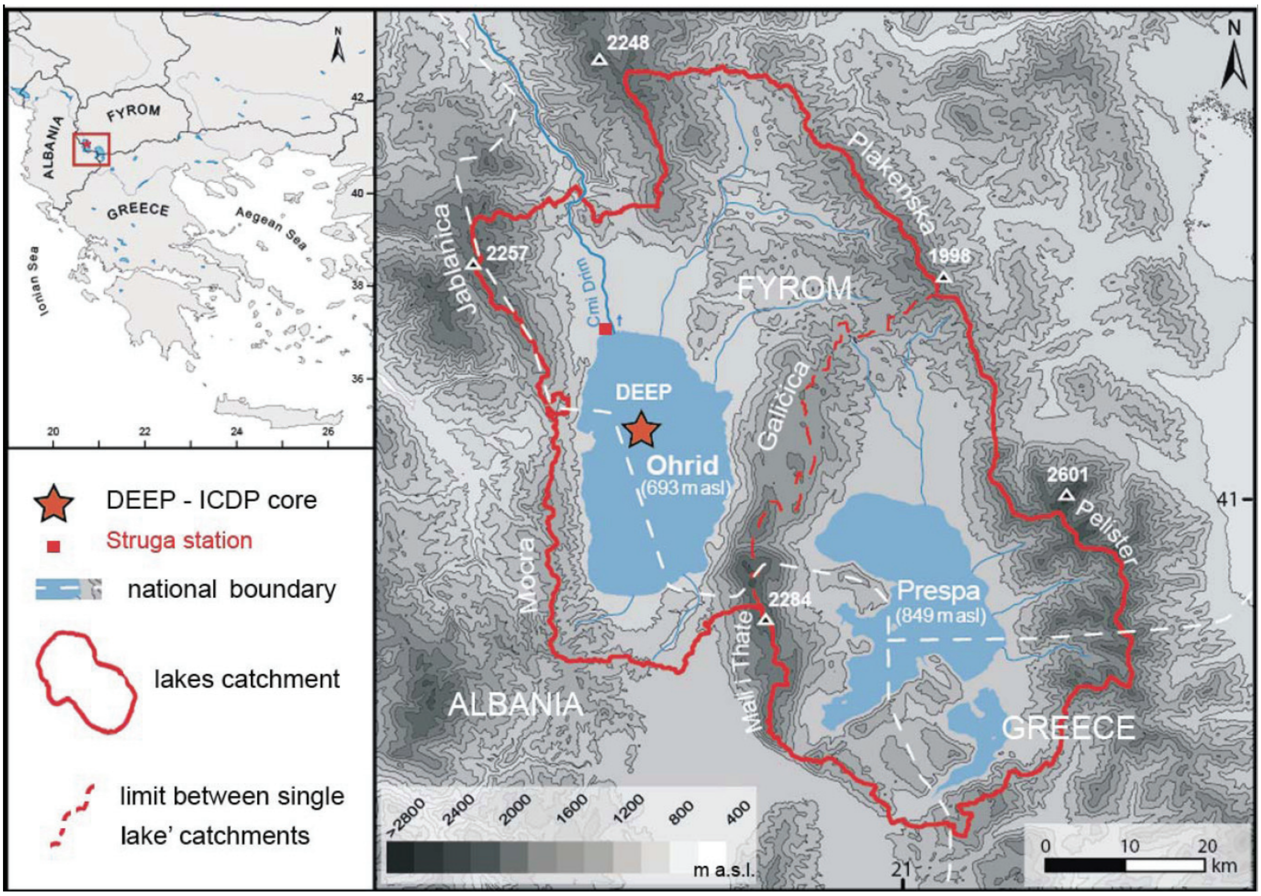

Fig. 1 - Location map of Lake Ohrid (modified from Panagiotopoulos, 2013).

of plant refugia in southern Balkans, i.e. one of the three Mediterranean refuge areas so far identified along with southern Iberian Peninsula and southern Italy. Such evidence is especially relevant to get a better understanding of the role played by (glacial) refugia in vegetation dynamics during the Pleistocene in Europe (e.g. Médail & Diadema, 2009; Fletcher et al., 2013; Tzedakis et al., 2013 and references therein). Here we summarize and discuss the first results of palynological studies, carried out by an international team of palynologists, from the upper part of the DEEP site sediment succession and covering the last 500 kyr (Francke et al., 2016; Sadori et al., 2016). The palynology work schedule for the year 2016 is also presented.

2. GENERAL SETTING

Lake Orhid (40°54′-41°10′ N and 20°38′-20°48′ E; Fig. 1) is located at the border between Albania and the Former Yugoslav Republic of Macedonia (FYROM), at an altitude of 693 m above sea level (a.s.l.). The ICDP deep drilling campaign took place using the Deep Lake Drilling System (DLDS) operated by the Drilling, Observation and Sampling of the Earths Continental Crust (DOSECC) consortium, in spring 2013. More than 2100 m of sediments were recovered from four different drill sites (Figs 2, 3); onsite core processing comprised analyses of core catcher material and magnetic susceptibility measurements. All cores were shipped to the University of Cologne for in progress and future analyses (Figs 4, 5; i.e. lithological, sedimentological, and biogeochemical). The main drill site in the central part of the lake (Fig. 1; 41°02’57’’ N, 20°42’54’’ E) permitted to recover 1526 m of core in total (DEEP site 5045-1) in six overlapping holes down to a maximum composite depth of 569 m. For more details see Francke et al. (2016) and references therein.

2.1. Present features

Lake Ohrid, 30.3 km long and 15.6 km wide, covers an area of ca 360 km^2 (Fig. 1); its maximum water depth is 293 m. LO is mainly fed by groundwater from karstic sources in the relatively small natural catchment area of 1042 km^2, which was artificially enlarged to 1487 km^2 in 1962 (Matzinger et al., 2006). However the occurrence of several surface springs and possibly also some subaquatic inflows from the nearby Lake Prespa (10 km to the east, at 849 m a.s.l., i.e. around 150 m above LO from which is separated by the Galičica mountain range) are responsible for a larger effective size of the catchment (Stankovic, 1960; Matzinger et al., 2006; Fig. 1). The surface outflow of LO is the river Crni Drim in the northern part of the lake, which accounts for 63% of the water loss, with the remaining 37% accounted for by

Fig. 2 - The barge at the deep site at lake Ohrid (photo courtesy S. Schorr).

Fig. 3 - The HBI boat/Core handing at Lake Ohrid on April 2013. On board researchers of Cologne university: B. Wagner, N. Leicher and F. Wild. (photo courtesy S. Schorr)

Fig. 4 - Cores in the refeer at the University of Cologne (a, b), (photo courtesy S. Schorr)

Fig. 5 - Core description and processing at the University of Cologne. At work N. Leicher and colleagues. (photo courtesy S. Schorr).

evaporation (Watzin et al., 2002). The current lake state is oligotrophic, due to the large water volume and the low nutrient availability (Wagner et al., 2010). The thermopluviometric diagram of the Struga meteorological station shows the main climate features for the close area located North of LO, at an altitude of 694 m (Figs. 1, 6). The average annual temperature is here 11.3 °C. The annual precipitation amounts to 878 mm. July is the warmest and driest month, with an average temperature of 20.5 °C and 42 mm of rain. Most of the precipitation falls in November, averaging 114 mm. January is the coldest month, with temperatures averaging 2.0 °C. This climate is considered to be Cfb (temperate rainy climate, warm summers) according to the Köppen-Geiger climate classification (e.g. Kottek et al., 2006). The catchment vegetation (see Fig. 7a and b for some panoramic views) is distributed mainly in altitudinal belts, from the lake level (700 m) to the top of mountains (>2200 m), with mixed deciduous oak forest, beech forest at lake level followed at higher elevation by mesophilous/montane species; mixed forests at the upper limit of the forested area and alpine pasturelands and grasslands are found over the timberline, currently at around 1900 m (Matevski et al., 2011). The presence of glaciers is documented on top of the Galičica Mountains during glacial periods (Ribolini et al., 2011).

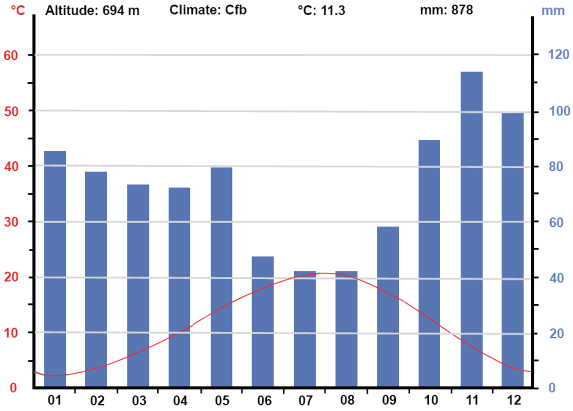

Fig. 6 - The thermopluviometric diagram of Struga metereological station (Fig. 1) at the north side of Lake Ohrid (data from http://en.climate-data.org/location/29778/).

Fig. 7 - View of the present vegetation around Lake Ohrid. (a) Saint John at Kaneo; (b) The rocky southeastern shore of the lake.

2.2. Geological and stratigraphical framework

The LO morphostructure with high mountains to the west ("Mokra Mountain" Chain, up to 2156 m) and east ("Galičica Mountain" Chain, up to 2254 m) (Fig. 1) is mainly the result of a pull-apart like opening of the basin during the late phases of the Alpine orogeny (Aliaj et al., 2001; Hoffmann et al., 2010). The oldest bedrock (Devonian) is present in the northeastern part of the basin whereas Triassic carbonates and siliciclastics occur in the southeast, east, and northwest (e.g., Wagner et al., 2009; Hoffmann et al., 2010; Vogel et al., 2010). Jurassic and Cretaceous metamorphic and magmatic rocks crop out in the west (Hoffmann et al., 2010). Quaternary lacustrine and fluvial deposits cover the plains to the north and to the south (Hoffmann et al., 2010; Vogel et al., 2010) overlaying Pliocene continental mudstones and claystones. The tectonic activity in the area is attested, until present day, in the lateral parts of LO (Reicherter et al., 2011; Lindhorst et al., 2012; Wagner et al., 2012) by several earthquakes (NEIC database, USGS) and mass wasting deposits (Lindhorst et al., 2016).

2.3. Summary of main project results

Most of the data collected by various investigators, during the 2 years following the core-drilling period since 2013, have been focused on lithology, sedimentology, tephrostratigraphy, and (bio-) geochemistry (see for all details Wagner et al., 2014, Francke et al., 2016 and references therein, Leicher et al., 2016). Here we summarize those results from studies carried out in cores from the central part of the lake giving, in the next paragraph, special emphasis to the palynological contribution (Sadori et al., 2016) concerning the upper 200 m of the overall 569 m long DEEP site sediment succession.

1. The DEEP site sediment succession (in Francke et al., 2016) consists of hemipelagic sediments including several tephra layers and rare mass wasting deposits (< 5 cm). They were organized, on the basis of the initial core description and the calcite content, into three main lithotypes (1-3). Lithotypes 1 and 2 deposits comprise calcareous and slightly calcareous silty clay respectively, whereas lithotype 3 deposits consist of clastic, silty clayey material.
2. The tectono-sedimentary structure of the basin as reconstructed by Lindhorst et al. (2015) includes three main seismic units overlying the acoustic basement associated with fluvial deposits and lacustrine sediments. The seismic facies analysis revealed a prominent cyclic pattern associated with Glacial/Interglacial cycles with a mean sedimentation rate of 0.41 mm yr^{-1} for the last 430 kyrs.
3. According to the age model based on radiometric ages of eleven tephra layers (1st order tie points) and

on tuning of biogeochemical proxy data to orbital parameters (2^{nd} order tie points; Laskar et al., 2004), the upper portion of the sedimentary succession, 247.8 m thick, covers the last 637 kyr (Francke et al., 2016, Leicher et al., 2016).

4. According to the isotopes ($\delta^{18}O$ and $\delta^{13}C$) from the upper ca 247.5 m of sediments, LO experienced a period of general stability between MIS 15 to 13. Wetter climate conditions characterized both MIS 11 and MIS 9. The successive interglacials were marked by progressively drier conditions (Lacey et al., 2016).

3. THE PALYNOLOGICAL CONTRIBUTION

The first SCOPSCO palynological meeting was held at the *Dipartimento di Biologia Ambientale* in Rome (Italy) on 8 and 9 January 2014. It led to the composition of an international palynological team with 12 European scientists from 5 countries under the supervision and coordination of Laura Sadori (University of Rome). In order to provide coherent flora and vegetation data, indispensable for both successive quantification analyses to infer regional paleoclimatic signals and to ensure comparability of results among the different laboratories, common protocols for both chemical-physical and microscope analyses were discussed and established. Moreover cross-check analyses on selected pollen samples by more than one analyst were adopted.

3.1. Protocol for laboratory analyses

Each sample, previously described for its main visual lithological features was weighed and 0.5 to 1.5 g of dry sediment were chemically treated. In order to estimate the pollen concentration, two tablets containing a known amount of *Lycopodium* spores (Stockmarr, 1971) were added in this first step to ensure that all palynomorphs will be treated similarly. The different chemical attacks (see below) were each time followed by both washing of sediment with distilled water and decantation. HCl (37%) was used to remove the carbonate fraction of the sediment as well as to dissolve the *Lycopodium* tablets. HF (40%) was used to remove silicates and hot NaOH (10%) to disperse organic matter and remove humic acids. At the end some drops of glycerol were added to the residue.

For the present work 306 sediment samples, at 64 cm interval down to the depth of 197.55 m of the DEEP core have been processed.

3.2. Protocol for optical microscope analysis

The list of pollen flora taxa, the counts, diagrams, bibliographic and photographic documentation are available to all components of the palynological team in a shared cloud folder. Here several general articles on both modern and ancient pollen taxa are also available. Special emphasis was given to selected taxa in order to facilitate their morphological identification and taxonomy. The difficulty and even the impossibility to always precisely discriminate, at the optical microscope, the different deciduous, semi-deciduous and evergreen components of the genus *Quercus* has long been discussed as they obviously produce some limitations for the ecological and climatic reconstructions. Beug (2004), Chester & Raine (2001), Reille (1992, 1995, 1998) and Smit (1973) documentation and criteria were carefully evaluated. Finally we decided to apply an informal subdivision, which possibly represents, at present, the best compromise for analyses at the optical microscope despite it was originally defined by electron microscope analyses (Smit, 1973). Accordingly, oak pollen has been divided in three main types: *Quercus robur* type (including all deciduous oaks), *Quercus ilex* type (including all the evergreen oaks minus *Q. suber*) and *Quercus cerris* type (including semi-deciduous oaks plus *Q. suber*). *Juniperus* type was established to include pollen grains of *Cupressus*, *Juniperus* and *Taxus*.

Further efforts are being made to collect materials in view of the ongoing studies on the oldest intervals in the sedimentary core, especially pictures of pollen grains belonging to taxa having a good spread in the Mediterranean area during Pliocene and Early Pleistocene and now showing a disjunct geographical distribution. For the interval under examination, special attention was paid to trace the occurrence and abundance of some taxa such as *Carya*, *Cathaya, Cedrus*, Hamamelidaceae, *Liquidambar*, *Pterocarya*, *Tsuga, Zelkova*.

3.3. The time schedule

The project unfolds over three main steps. The two first allow the study of the entire sedimentary succession at low resolution (from the top down to 200 m, i. e. the present study and from 200 m to the bottom core, respectively), in relatively short time. According to the age model by Francke et al. (2016) the mean resolution between two samples from the upper portion is ~1600 years. Being the first step just accomplished (Sadori et al., 2016), the study of the interval between 200 m and the bottom core has started (Step 2). At the same time higher resolution analyses (Step 3) are also in progress in the sedimentary portions corresponding to MISS 5-6, 10-12, 35-42.

3.4. Palynological results and discussion

Palynological analyses pointed out a rich palynoflora including 175 taxa (153 terrestrial plants including 10 freshwater plants). Quantitative data high enough to trace different pollen diagrams were possible in 296 of the 306 available samples (see, figs 2, 3 and table 1 in Sadori et al., 2016). Samples with final counts less than 80 pollen grains (in addition to *Pinus*) were excluded. Mean pollen counts of 824 terrestrial pollen grains have been achieved. Pollen concentration is quite variable, ranging from ca. 4000 to ca. 910,000 pollen grains g^{-1}. Pollen grains do no exhibit major mechanisms of degradation but bisaccate pollen, especially *Pinus*, is often fragmented. Dinoflagellate cysts are sporadically present. Significant reworking phenomena were absent, as also indicated by the lithology, except of very few, thin mass movement deposits (Francke et al., 2016). Pollen assemblages in the upper 200 m are characterized by a general good richness, which however fluctuates in its values in dependence of both climate (being lower during glacials and higher during interglacials) and time interval (e.g. richness in NAP notably increases in the younger intervals). The main floristic features are summarized below. Trees are mainly represented by *Pinus* pollen. Among other Pinaceae taxa, *Abies* and *Picea* are present throughout; *Tsuga* is scattered and *Cedrus* as well as *Cathaya* are absent. Deciduous broadleaved trees are represented mainly by *Quercus* spp. followed by *Carpinus betulus*, *Ostrya*/*Carpinus orientalis*, *Ulmus*, *Zelkova*, *Acer*, *Tilia* and *Juglans*. Among the only sporadically present taxa we number *Celtis* and *Carya*. Among Mediterranean trees, *Quercus ilex* type is dominant with *Phillyrea, Pistacia,* plus the pioneer shrub *Hippophaë*. *Betula* and especially *Fagus* occur in a discontinuous way. Herbaceous/non arboreal taxa are particularly represented by pollen of Asteraceae, especially *Artemisia*, Poaceae and Amaranthaceae. Moreover Cyperaceae, Caryophyllaceae, Ranunculaceae, *Helianthemum*, Plantaginaceae. Polygonaceae, Rubiaceae, Euphorbiaceae, Dipsacaceae, Gentianaceae, Rosaceae and Caprifoliaceae also occur but discontinuously and at low frequencies. Pteridophyta are generally well represented. Algal taxa are principally represented by *Pediastrum* and *Botryococcus*, though discontinuously.

The analysis of changes in pollen concentration and percentages of both single taxa and arboreal pollen sum (AP) versus non-arboreal pollen sum (NAP), is summarized in Fig. 8. In the text *Pinus* percentages refer to a pollen sum including all AP (plus *Pinus*) and NAP; all the other taxa were calculated with respect to the pollen sum of AP (but *Pinus* excluded) and NAP.

A prevalent contraposition between non arboreal pollen (including Poaceae, Amaranthaceae, *Artemisia*) and woodland (including *Abies*, *Picea*, *Quercus robur* type, *Quercus cerris* type) taxa is well evident throughout the sedimentary succession (Fig. 8). Such pattern reflects the vegetational response to the succession of Glacial/Interglacial cycles between MIS 13 and MIS 1 (Francke et al., 2016). Moreover, minor expansions of AP during glacials as well as forest opening during interglacials pointed out interstadials and stadials, respectively.

Glacials at LO

At LO (Fig. 8), pollen zones OD-12, OD-11, OD-9 and OD-7 roughly corresponding to older glacials MIS 12, MIS 10 and MIS 8 exhibit Poaceae as the most abundant NAP taxon, followed sometimes by Cyperaceae (MIS 12) or Amaranthaceae (MIS 10), and subsequently by Asteroideae, Cichorioideae, *Artemisia* and *Hippophaë* (particularly abundant during MIS 10); *Pinus* as a whole, shows a large occurrence in both concentration and percentage values, exhibiting striking relatively millennial-scale variations in addition to the glacial -interglacial cycles. It fluctuates between 28% and 98%. Montane coniferous taxa show significant fluctuations too as expressed by *Picea* (range 0%- 67%) and *Abies* (range 0%-63%). MIS 8 is marked with respect to MIS 12 and 10 by a peculiar increase of both *Juniperus* and *Artemisia.* The younger glacials are marked by successive distinct patterns in the abundances of some taxa. From MIS 6 onwards a progressive decrease of *Pinus*, as well as of *Abies* and *Picea*, parallels a notable increase of *Artemisia* followed by Poaceae and Amaranthaceae. A relevant increase of *Juniperus* (up to 55%) marks the top of MIS 6.

The establishment of harsh climate conditions possibly promoted various environmental changes; it is a fact that montane taxa, after MIS 6, never reach previous values. The successive, younger glacials are especially characterized by the dominance of *Artemisia* followed by Poaceae and Amaranthaceae; *Pinus* still well represented in percentages, shows a remarkable decrease in concentrations since MIS 6. *Abies* shows a slight percentage increase just after the onset of MIS 5 whereas *Picea* occurs sporadically and in low values.

Overall total pollen concentration during glacials is lower than in interglacials; probably the larger expansion of open environments (i.e. herbaceous taxa) or even bare soils, which favored enhanced phases of erosion and transport of clastic material in the basin may have diluted the pollen content. Such depositional processes are expressed by the rather exclusive occurrence of lithotype 3 sediments, except lithotype 1 in MIS 8 (Francke et al., 2016).

Interglacials at LO

The climate signature in the depositional environment is well expressed by the dominance of a peculiar and diverse pattern with respect to that dominant during glacials. In fact during interglacials lithotypes 1 and 2 sediments prevail with rare occurrences of lithotype 3 sediments. A progressive overall shift from cooler and wetter to warmer and drier interglacials was pointed out.

As a whole the strong chronological frame for LO enables precise terrestrial-marine correlations using the comparison with the main coeval regional sites (e.g. Follieri et al., 1989; Okuda et al., 2001; Tzedakis et al., 2004; 2006; Brauer et al., 2007; Roucoux et al. 2008, 2011; Litt et al., 2014; Pross et al., 2015) and synchronization of single Marine Isotope Stages (Zanchetta et al., 2016). A striking feature of LO respect to the other long terrestrial pollen records of Europe and Near East, is the relatively continuous presence of taxa from all major vegetation belts despite the significant Glacial/Interglacial variability throughout the profile.

In particular, similar patterns of vegetation development during the last Glacial/Interglacial cycles are testified by previous palynological studies (Panagiotopoulos et al., 2014) on the adjacent Lake Prespa (Fig. 1) for the last 92 ka. Similar forested phases, dominated by deciduous trees indicative of higher temperatures and moisture availability, developed during both MIS 5 and MIS 1. Open landscapes during lower temperatures and moisture availability phases were prevalent during MIS 4, 2, and also 3 despite the significant presence of temperate trees. With respect to Prespa, the longer LO pollen record provides a unique documentation of the history of flora composition and vegetation structure here updated to the MIS 13 p.p. According to the summary AP vs NAP diagram (Fig. 8) an overall progressive enlargement of the open vegetal formations is quite evident since MIS 13, with a major rise to dominance of steppe formations with *Artemisia* (Fig. 8) occurring from the MIS 7/MIS 6 transition onwards. The quite long glacial MIS 6 is especially marked by a sig-

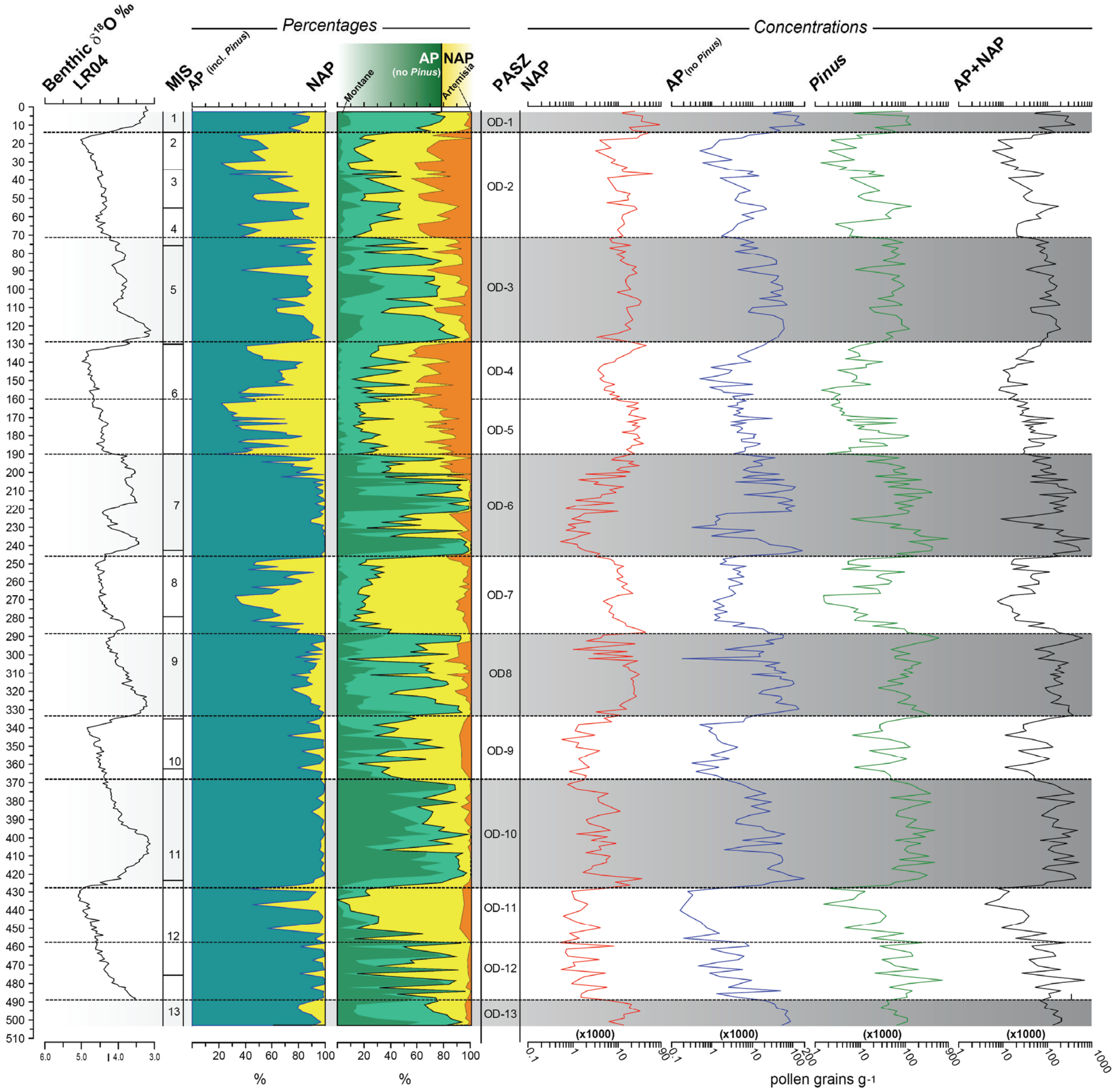

Fig. 8 - Summary pollen data of Lake Ohrid (FYROM), DEEP core against chronology (Francke et al., 2016). From the left: marine records, LR04 δ^{18}O benthic stack (Lisiecki and Raymo, 2005); terrestrial records, percentages summary pollen diagrams, and selected concentration pollen curves. AP: Arboreal Plants; NAP: Non Arboreal Plants; MIS: Marine Isotope Stages; PASZ: Pollen Assemblage SuperZones.

nificant increase for *Artemisia,* which reaches values up to ca 47%. Ongoing pollen-based climate reconstructions will be able to establish the magnitude of changes in temperatures but especially in the regime of precipitations. The persistent dry conditions during MIS 6 probably enhanced intense erosive processes. After this significant paleoenvironmental and paleoclimate change, probably the major of the last 500 kyr, we observe a general reduction of the altitudinal coniferous taxa (*Abies* and *Picea*) particularly evident during interglacials. Possibly a progressive and generalized decrease in the humidity values plays a major role in the reorganization of vegetation assemblages. Such a prominent event is traceable for *Picea* (see fig. 2 in Sadori et al., 2016) which became subordinated with respect to *Abies* since MIS 6. The knowledge of both the stratigraphic distribution and the past change in the abundances of previous taxa is really helpful to assess their current spatial distribution under the effects of different ecological processes (e.g., competition, survival, biological diversity) within forest communities as well as to choose correct preservation and conservation strategies.

4. CONCLUSION

LO is a unique continental site including at least the last 1.2 Ma history of changes on the Balkan peninsula, a key area of the Mediterranean. The productive collaboration among the different components of the international palynological team provided effective implementation of the SCOPSCO project being a long and continuous pollen record for the last 500 kyr produced in a short period of time for the standard time requested by palynological analyses. The achievement of this first step (Step 1) as well as the ongoing collaboration aimed at the completion of the entire record by the end of 2016 (Step 2) and to the implementation of high resolution studies in key intervals (Step 3) are truly relevant, the full pollen record permitting to trace by the analysis over multiples Glacial/Interglacial cycles

i. the climate variability since 1.2 Ma when subtropical ecosystems disappeared from the central Mediterranean area,
ii. the history of taxa migration and extinctions under the effects of regional to global events
iii. the role of refugia areas over time.

The palynological evidence along with the geological, micropaleontological and stratigraphical data, now well documented by a large number of studies, provide indispensable elements of knowledge for the reconstruction of the late Quaternary paleoclimatic changes in the eastern Mediterranean area within a strong chronologic frame. Such an integrated approach has paramount importance as it permits to trace a comprehensive paleoenvironmental history of LO which is needed to both its appropriate present management and in the context of predicting its future.

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