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Supplementary Materials for

Deep drilling reveals massive shifts in evolutionary dynamics after formation of ancient ecosystem

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/40/eabb2943/DC1)

Data S1 to S3

Supplementary Materials

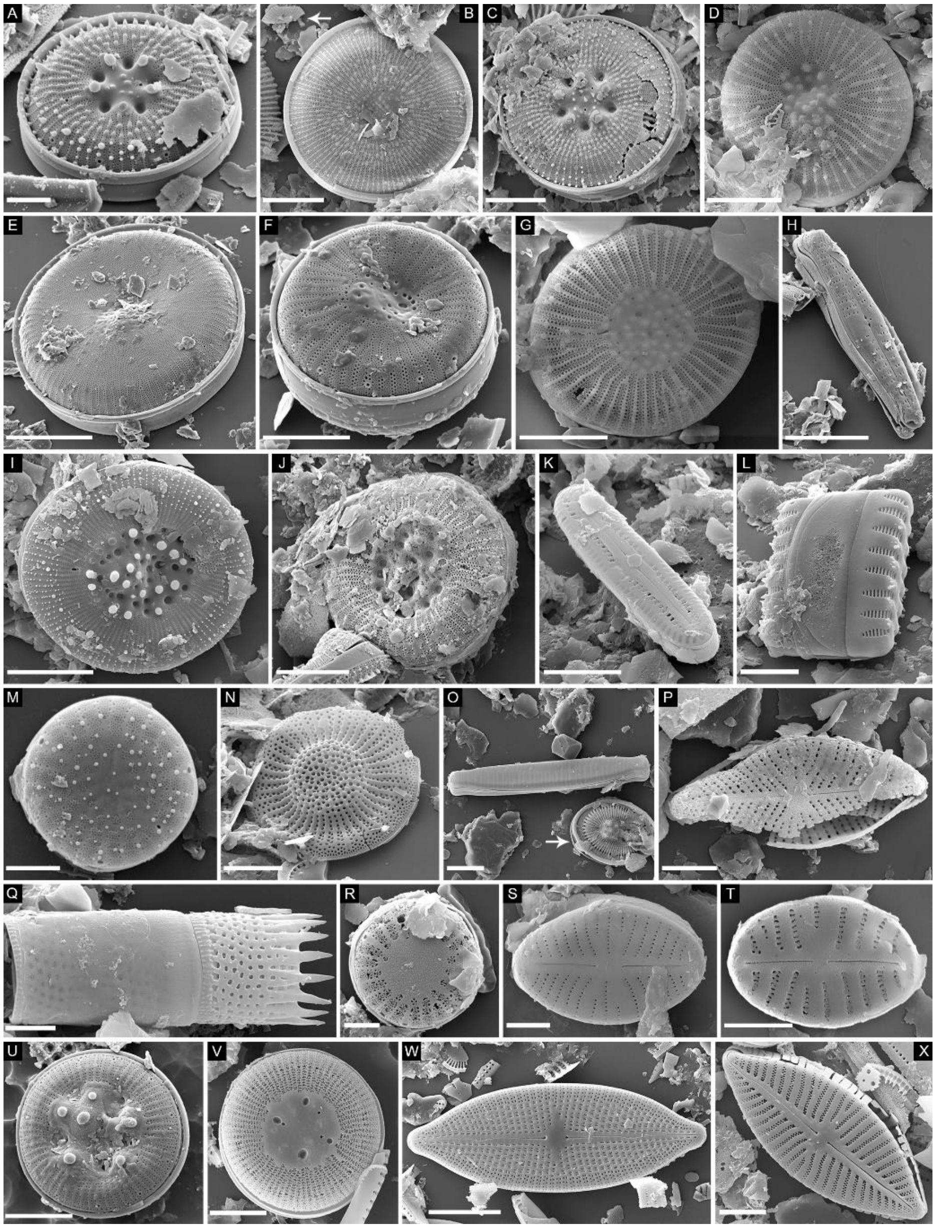

Fig. S1. Representative scanning electron micrographs showing the preservation of planktonic and benthic diatoms throughout the Lake Ohrid DEEP-5045-1 sediment record. (**A**) *Pantocsekiella ocellata* var. 2 in 'pristine' state. (**B**) *Cyclotella fottii* in 'pristine' state; note the white arrowed *Pantocsekiella ocellata* in progressively dissolved and broken state. (**C**) *Pantocsekiella ocellata* var. 2 in weakly dissolved and broken state. (**D**) *Cyclotella fottii* in moderately dissolved state. (**E**) *Cyclotella fottii* in 'pristine' state. (**F**) *Cyclotella fottii* in 'pristine' state. (**G**) *Cyclotella fottii* in moderately dissolved state. (**H**) *Gomphonema mihoi* in weakly dissolved state. (**I**) *Cyclotella cavitata* in 'pristine' state. (**J**) *Cyclotella cavitata* in progressively dissolved state. (**K**) *Fallacia* sp. nov. in 'pristine' state. (**L**) *Staurosirella pinnata* in moderately dissolved state. (**M**) *Cribrionella ohridana* in 'pristine' state. (**N**) *Stephanodiscus transylvanicus* in dissolved state. (**O**) *Diatoma ehrenbergii* in 'pristine' state; note the white arrowed *Cyclotella cavitata* in a progressively dissolved state. (**P**) *Placoneis balcanica* in progressively dissolved state. (**Q**) *Aulacoseira subarctica* in 'pristine' state. (**R**) *Cribrionella ohridana* in progressively dissolved state. (**S**) *Sellaphora* sp. in 'pristine' state. (**T**) *Sellaphora* sp. in dissolved state. (**U**) *Pantocsekiella* sp. nov. 2 in 'pristine' state. (**V**) *Pantocsekiella* sp. nov. 1 in progressively dissolved state. (**W**) *Aneumastus* sp. nov. 2 in 'pristine' state; (**X**). *Paraplaconeis prespanensis* in progressively dissolved stage. (**A**–**D**). Interglacial stage (MIS-5), from ~0.07– 0.13 Ma. (**E**–**H**). Glacial stage (MIS-6), from ~0.13–0.18 Ma. (**I**–**L**). Interglacial stage (MIS-15), from ~0.57–0.62 Ma. (**M**–**P**). Glacial stage (MIS-16), from ~0.62–0.66 Ma. (**Q**–**T**). Glacial stage (MIS-38), from ~1.24–1.29 Ma. (**U**–**X**). Interglacial stage (MIS-41), from ~1.29–1.36 Ma. Note the morphological variability in *Cyclotella fottii* during the MIS 6/5 glacial-interglacial cycle. Scale bars (**B**, **E**, **H**–**J**, **O**, and **W**) = 10 μm; (**C**, **D**, **F**, **G**, **K**, **L**, **N**, **P**, **U**, **V**, and **X**) = 5 μm; (**A**, **Q**, **S**, and **T**) = 2 μm; Figs (**M** and **R**) = 1 μm.

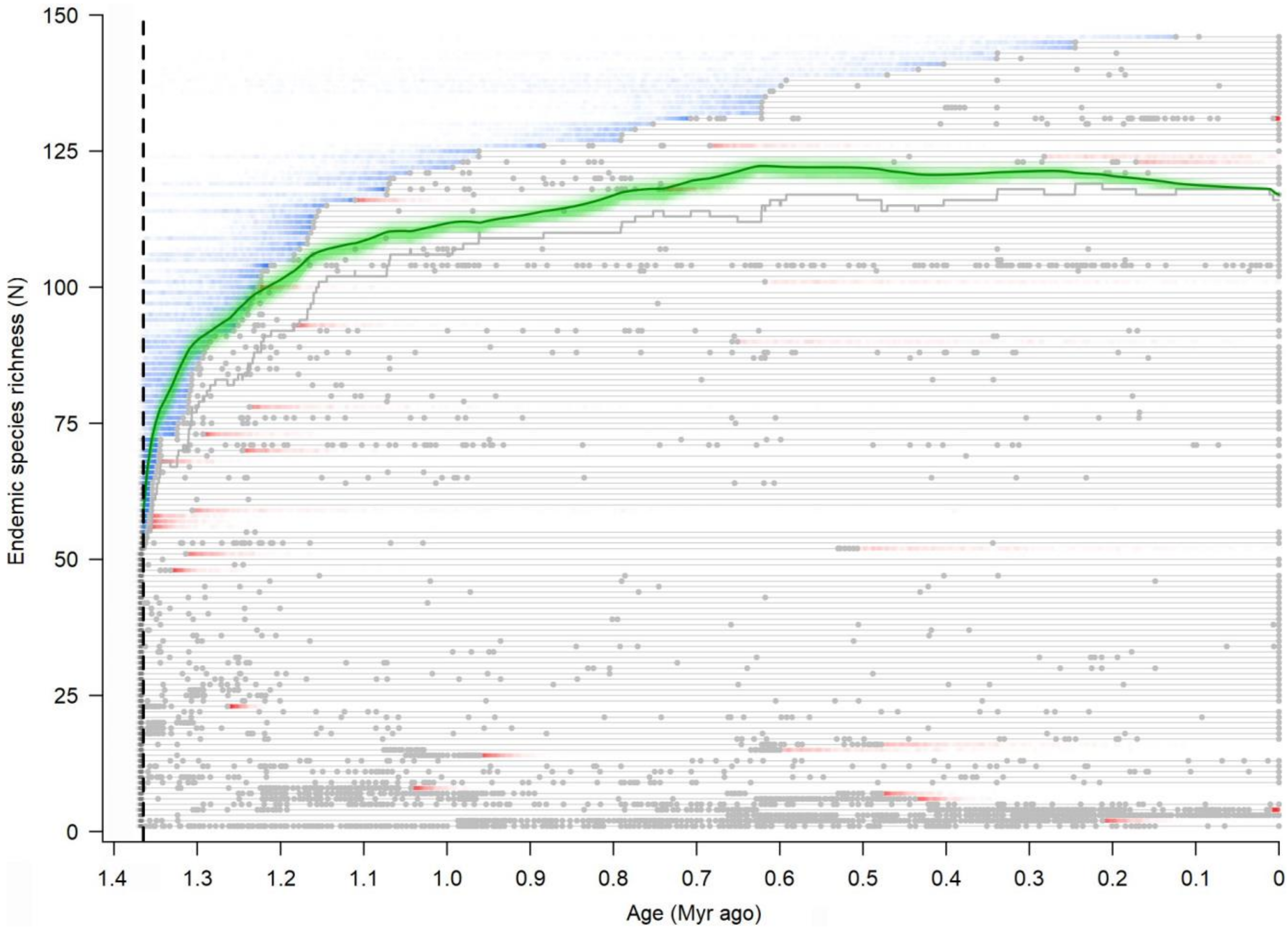

Fig. S2. Lake Ohrid species accumulation curves for endemic diatom species. Species are ordered according to time of appearance (speciation). Black dashed line, time of lake formation 1.36 Myr ago; grey dots, fossil counts; blue dots, speciation events (incl. preservation bias); red dots, extinction events (incl. preservation bias); dark grey line, uncorrected species accumulation curve; green line, species accumulation curve corrected for preservation bias; green shading, 95% credible interval.

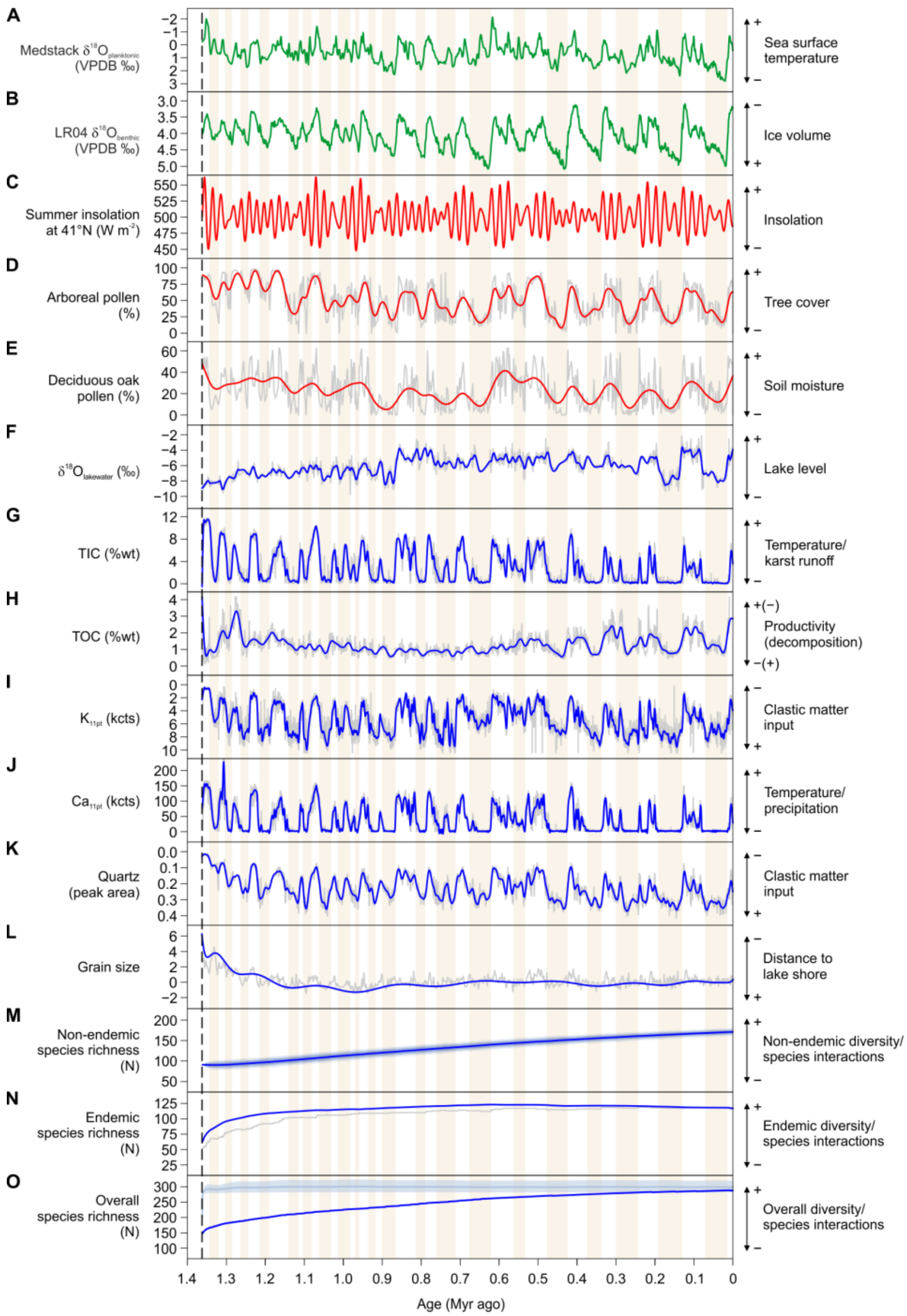

Fig. S3. Lake Ohrid environmental and climate indicator time series and diatom species richness curves. Raw data are shown in grey, processed data in colour (for details see Materials and Methods). Global, regional and local indicators are shown as green, red and blue curves, respectively. Black dashed line, time of lake formation; brown bars, glacial periods. Data for panels **A, B, D, E** and **G–K** were published previously. (**A**) Medstack $\delta^{18}O$ planktonic isotope ratios in parts per thousand relative to VPDB (*33*); (**B**) LR04 benthic $\delta^{18}O$ stack isotope ratios in parts per thousand relative to VPDB (*34*); (**C**) Northern Hemisphere summer insolation at the latitude of Lake Ohrid (41° N) (*35*); (**D**) Percentages of arboreal pollen excluding *Pinus* pollen at Lake Ohrid (*13*); (**E**) Percentage of pollen from deciduous oaks at Lake Ohrid (*13*); (**F**) Lake Ohrid $\delta^{18}O_{lakewater}$ based on calcite and siderite $\delta^{18}O$ information; (**G**) Lake Ohrid total inorganic carbon (TIC) concentrations (*13*); (**H**) Lake Ohrid total organic carbon (TOC) concentrations (*13*); (**I**) Lake Ohrid potassium (K) counts from XRF scanning (*13*); (**J**) Lake Ohrid calcium (Ca) counts from XRF scanning (*13*); (**K**) Lake Ohrid relative sedimentary quartz content (*13*); (L) Lake Ohrid grain size information (summarized by ordination); (**M**) Lake Ohrid species accumulation curve for non-endemic diatom species corrected for preservation bias; (**N**) Lake Ohrid species accumulation curves for endemic diatom species. Blue accumulation curve, data corrected for preservation bias; (**O**) Lake Ohrid species accumulation curve for endemic and non-endemic diatom species combined. The diatom community has not yet reached a dynamic equilibrium. Maximum standing diversity in the dataset studied is 288 species; estimated equilibrium diversity varies as a function of lake size (shaded blue area). **M, N, F, L, N**, Data are the same as in Fig. 2.

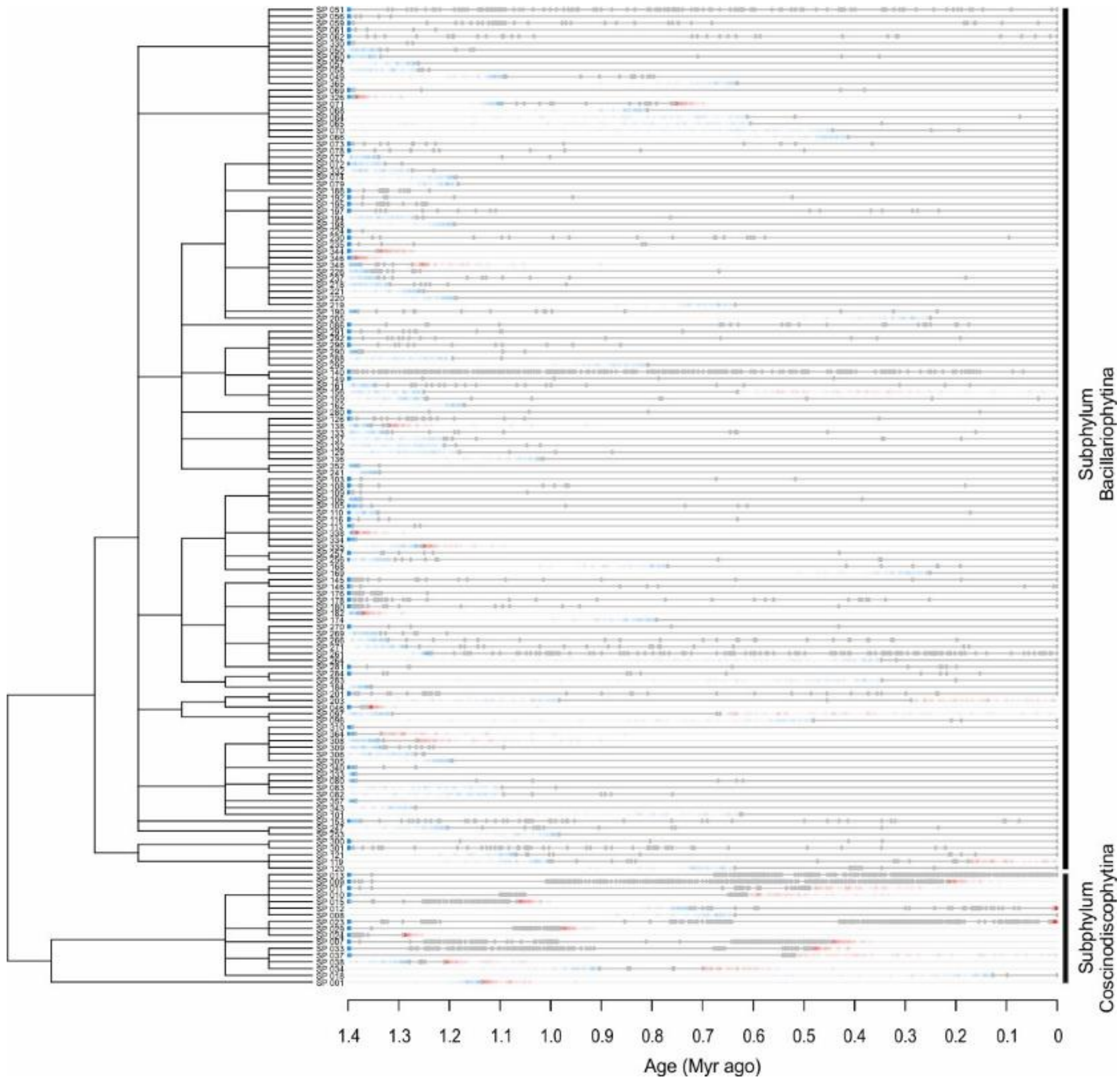

Fig. S4. Lake Ohrid diatom systematic relationships and speciation and extinction events of endemic species. Systematic tree is based on difference between taxonomic ranks that equal a branch length of 1 unit (for details see Materials and Methods). For species codes see Data S1. Species are ordered according to systematic relationships at the considered taxonomic levels (*52*). Grey dots, fossil counts; blue dots, speciation events (incl. preservation bias); red dots, extinction events (incl. preservation bias). There is a significant signal of selective extinction (D = 0.37, $p_{D<1} = 0.00$, $p_{D>0} = 0.13$), as can be seen, for example, in the planktonic subphylum Coscinodiscophytina, in which most members go extinct over time.

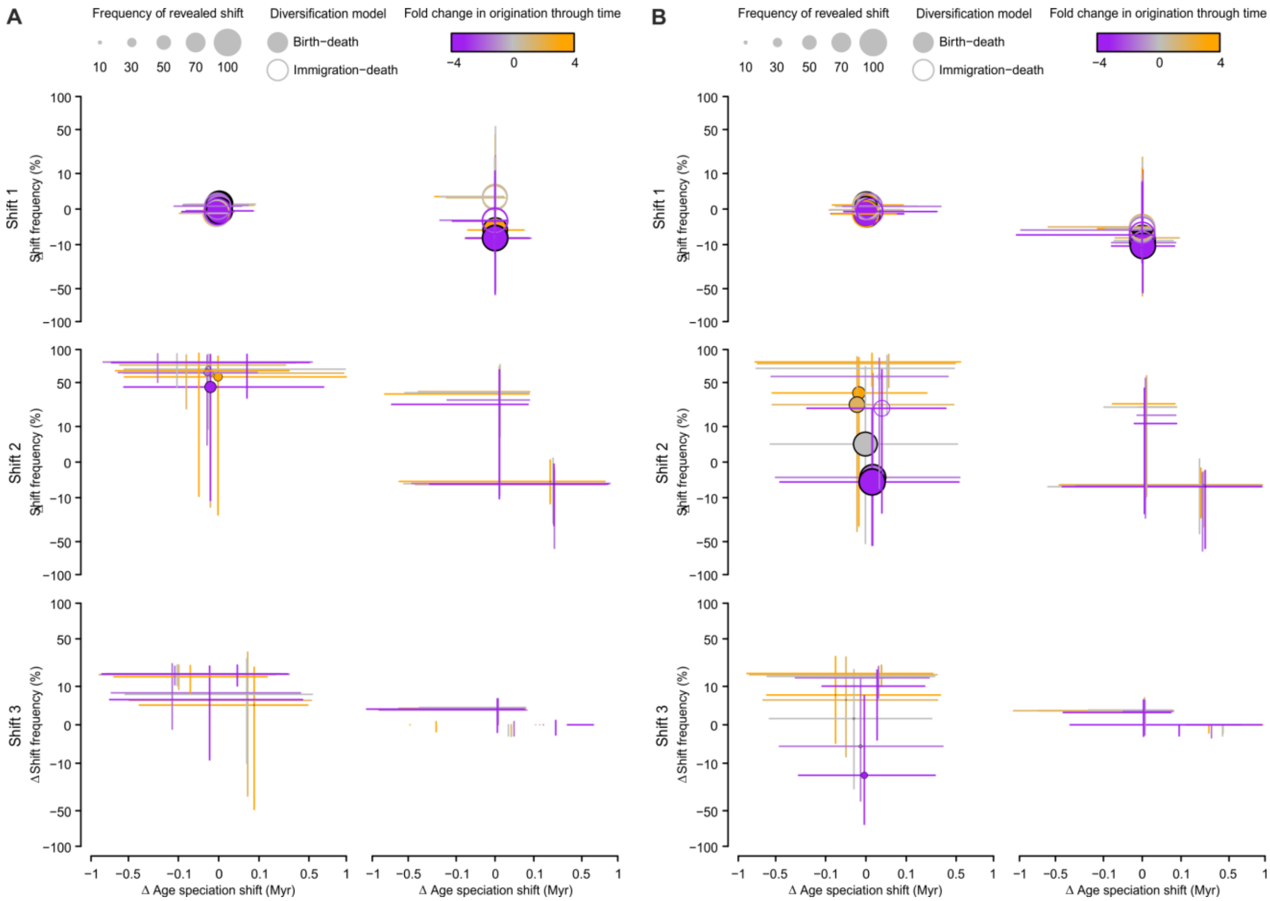

Fig. S5. Robustness of inferred ages of rate shifts and their frequency in being sampled during the Bayesian inference against the effect of missing < 25% extant species. (A) Simulated first appearance times of species by a phylogenetic Yule model representing exclusively in-situ cladogenesis and **(B)** probabilistic sampling of first appearance times with in- and decreasing rates and probabilities, respectively (color gradient). Rate shifts were inferred with birth-death and colonization-death models in PyRate (closed and open circles, respectively). We summarized the prevalence of inferred shifts for the complete sampling (circle size), how the frequency of sampled shifts changes (y-axis; with positive values indicating more frequently sampled shifts in the incomplete data) and the effect on shift age (x-axis; with positive values indicating a bias towards older shifts under incomplete sampling). Circles are placed at the median across the 100 simulations and lines display the 95% variation interval. The results show that a complete taxon sampling would not affect the age of the oldest shifts (shift 1) for the speciation and extinction rates, and even a 4 fold decrease in the speciation rate through time (i.e., missing taxa are old) would only reduce the frequency of sampling the extinction shift during Bayesian inference by 10%. The identified younger, less pronounced drop in the speciation rate (shift 2) could be 0.1 Ma younger under a complete taxon sampling and would be sampled less frequently when the missing taxa are young. Small circles indicate that an additional 2nd shift in the extinction rate or 3rd shift in the speciation rate could not be detected by a simulated complete taxon sampling.

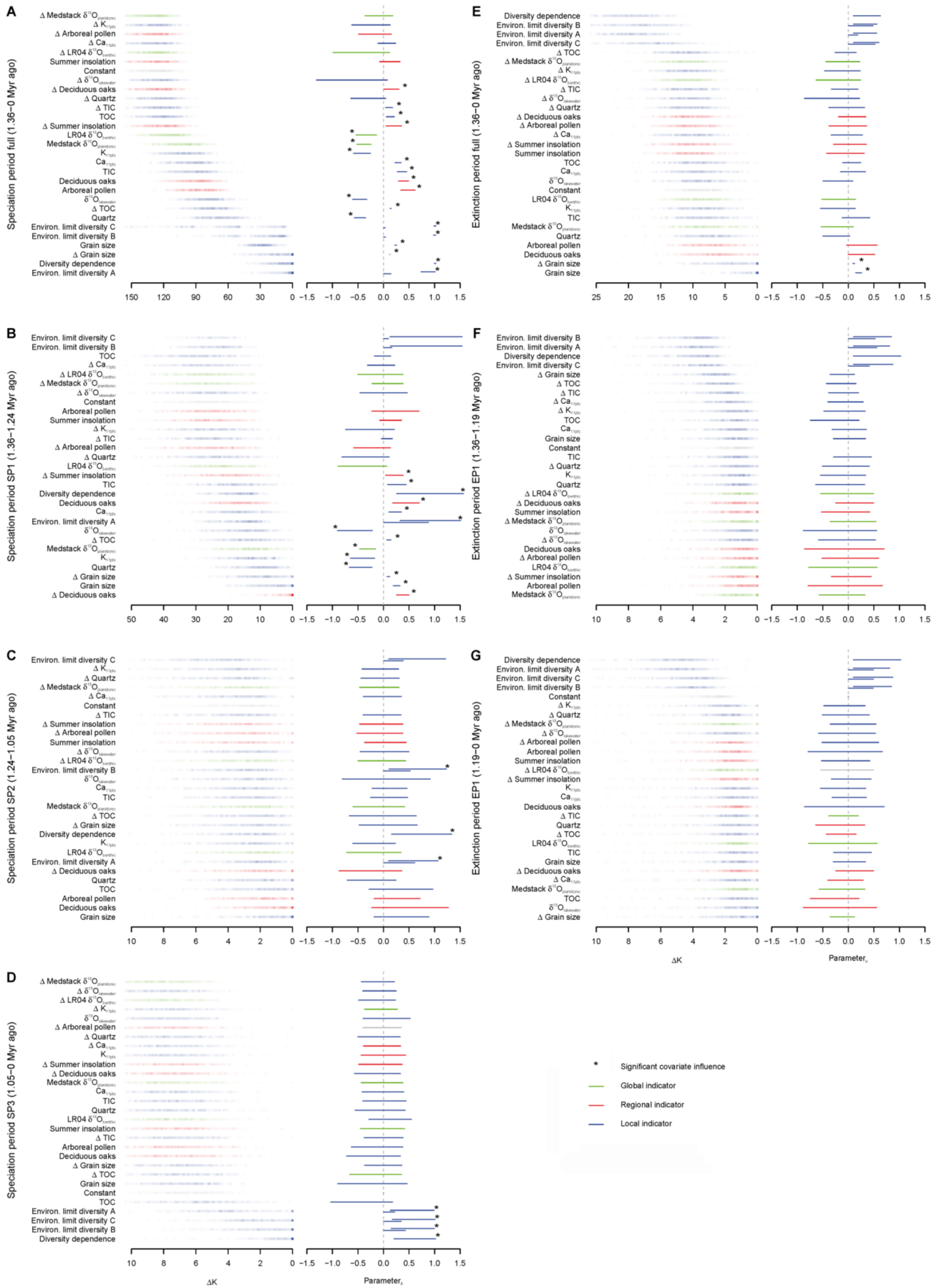

Fig. S6. Comparison of relative model support and correlation strength of speciation and extinction rates with climate, environmental and diversity indicators. Plots summarize the results of the diversification analyses of covariate influence over the entire period of 1.36 Myr **(A, E)** and within the three speciation **(B–D**) and two extinction rate periods **(F, G)**. For environmental and climate indicators, both the influence of the change of the parameter per 1,000 years (Δ) and that of the total value were tested. Left panels of the plots show the ranking of covariates according to the difference in the Bayes Factor K to the best-fit model within 300 replicated datasets (dots), thereby integrating the uncertainty in speciation and extinction times. Lines in the right panels quantify the uncertainty in model parameters for the correlation between rates and covariates across all replicates through 95% HPD intervals. The model parameter shown for the diversity dependent model is the reciprocal of the carrying capacity, with values below 1 indicating an unattained environmentally-defined ecological limit. Models for an environmentally-defined ecological limit include a second line for the parameter Arrhenius-z, which shows the exponent for converting the environmental covariate to a carrying capacity. For all other models, the only parameter displayed is the linear correlation strength γ of rates and covariates. Covariates with model parameters significantly different from zero, i.e., which do not intersect the grey dashed line, are marked with an asterisk. Covariates shown to the left of the dashed line (–) are negatively correlated, to the right of the dashed line (+) are positively correlated. Environ. limit diversity A, B, C = environmentally-dependent limits to diversity based on grain size information, $\delta^{18}O_{lakewater}$ values and quartz content, respectively.

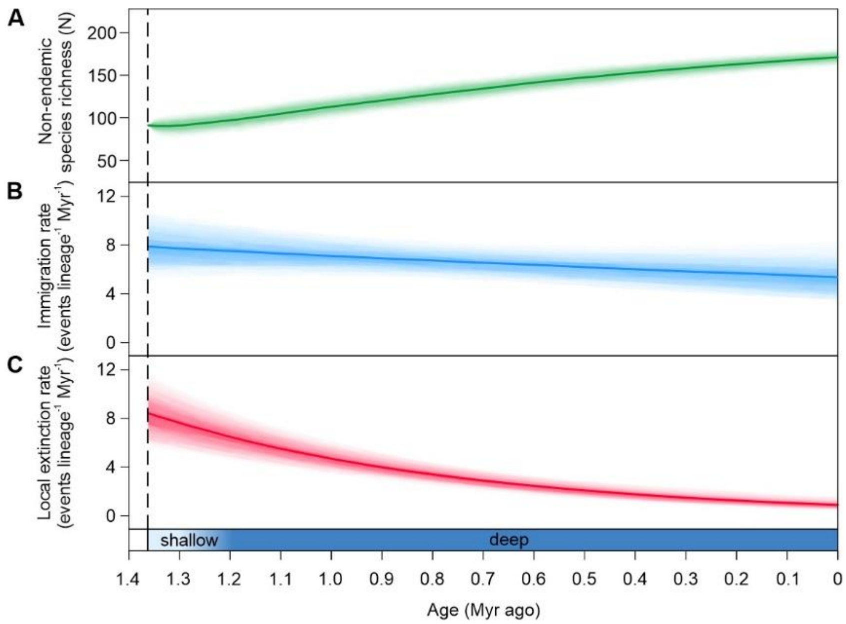

Fig. S7. Species accumulation curve and immigration and local extinction rates for non-endemic diatom species in Lake Ohrid spanning the past 1.36 Myr. Black dashed line, time of lake formation; blue bar at bottom of figure, lake phases (shallow and deep) (*17*). **(A)** Species accumulation curve for non-endemic diatom species corrected for preservation bias (data are the same as in Fig. S3M); **(B)** Per-lineage immigration rate and 95% confidence interval (blue shading); **(C)** Per-lineage local extinction rate and 95% confidence interval (red shading).

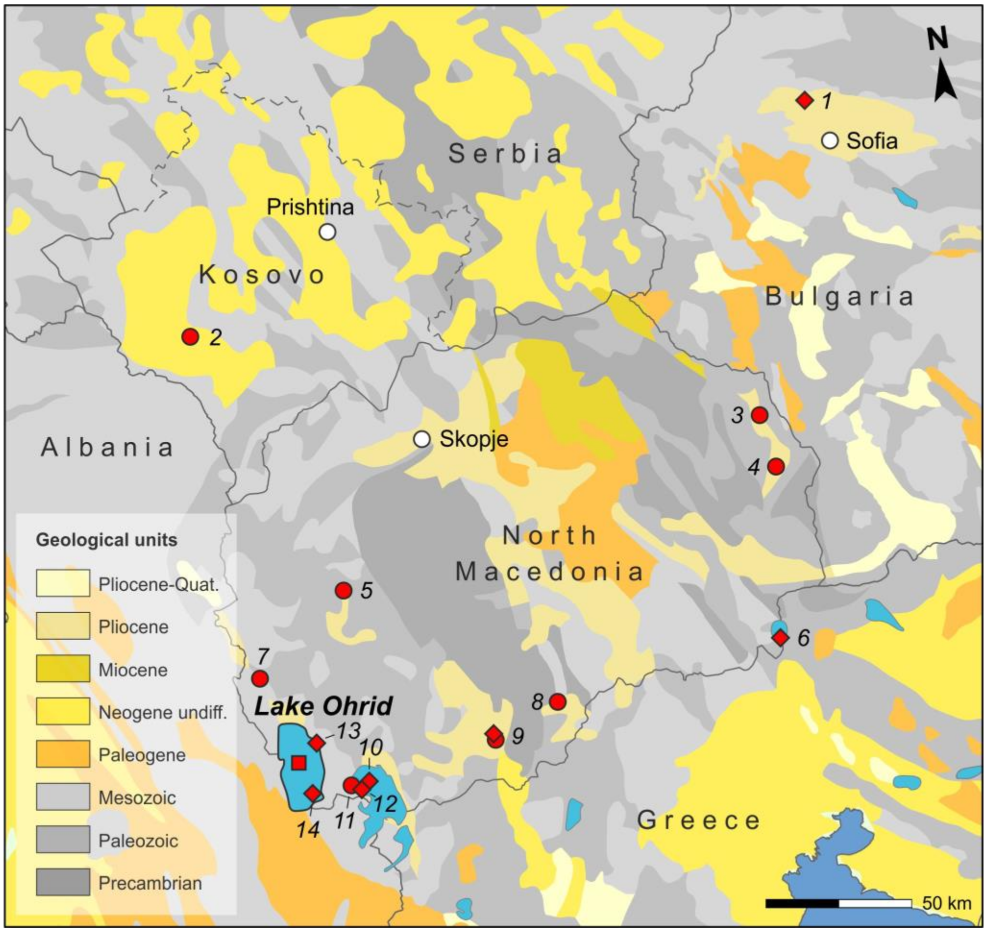

Fig. S8. Geological map of the southern Balkans showing the location of Late Miocene–Pleistocene diatomites and lake successions that were used to determine what species are endemic to Lake Ohrid. Major cities are indicated with white circles, outcrops with red circles, the Ohrid DEEP core site with a red square, other coring locations with red diamonds. The geological map is simplified from the International Geological Map of Europe and Adjacent Areas (IGME 5000). Stratigraphic details were taken from the literature (*65*). 1, core C-14 taken near Katina (Sofia Basin, Novi Iskar Formation); 2, outcrop near Dakovica (Metohia Basin, Metohia Formation); 3, outcrop near Star Istevnik (Delchevo-Pehchevo Basin, Pantcharevo Formation); 4, outcrop near Brestovi (Berovo Basin, Pantcharevo Formation); 5, outcrop near Ovcha reka (Kichevo Basin, unnamed Pliocene formation); 6, Lake Dojran core Co1260 (12.5 kyr); 7, outcrop near Crn Drim (Piskupshtina Basin, Piskupshtina Formation); 8, outcrops near Vitolishte and Manastir (Mariovo Basin, Nerezi-Vitachevo formations); 9, outcrop "Suvodol mine" and core B-466 near Vranjevci (Pelagonia Basin, Pelagonian Formation); 10, Lake Prespa core Co1215 (90.0 kyr); 11, outcrop between Zarina and Leskovec (Prespa Basin, unnamed Pliocene formation); 12, Lake Prespa core Co1204 (48.0 kyr; 13, Lake Ohrid core Co1202 (135 kyr); 14, Lake Ohrid core Lz1120 (40 kyr).

Data S1. (separate file)
Diatom counts and taxonomic, life style, endemicity and voucher information for the Lake Ohrid DEEP site.

Data S2. (separate file)
Grain size data for the Lake Ohrid DEEP site.

Data S3. (separate file)
$\delta^{18}O_{lakewater}$ data for Lake Ohrid Co1262 and DEEP sites.

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