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*Article*

# Diversity and Spatial Distribution of Extant Freshwater Ostracodes (Crustacea) in Ancient Lake Ohrid (Macedonia/Albania)

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**Abstract:** We carried out an intensive sampling survey in ancient Lake Ohrid (Macedonia/Albania), covering all seasons, to determine total species number, relative species abundances and spatial distribution of Ostracoda. We identified 32 living species that belong to seven families (Candonidae, Ilyocyprididae, Cyprididae, Leptocytheridae, Limnocytheridae, Cytherideidae, and Darwinulidae) and 15 genera (*Candona*, *Fabaeformiscandona*, *Candonopsis*, *Cypria*, *Cyclocypris*, *Ilyocypris*, *Eucypris*, *Prionocypris*, *Bradleystrandesia*, *Herpetocypris*, *Dolerocypris*, *Amnicythere*, *Paralimnocythere*, *Cytherissa*, and *Darwinula*). Six additional species were identified from empty carapaces and valves. Dominant families in Lake Ohrid were Candonidae and Limnocytheridae, representing 53% and 16% of all species, respectively. Prevalence of species flocks in these two families confirms the "young" ancient status of the lake. *Amnicythere* displays a preference for oligo-haline to meso-haline waters, but some species are found in saline environments, which suggests Lake Ohrid has a marine history. Recent studies, however, indicate fluvial/glaciofluvial deposition at the onset of Lake Ohrid sedimentation. *Candona* is the most diverse genus in Lake Ohrid, represented by 12 living species. *Paralimnocythere* is represented by five living species and all other genera are represented by one or two species. Reports of *Candona bimucronata*, *Ilyocypris bradyi, Eucypris virens*, *Eucypris* sp., *Prionocypris zenkeri*, *Bradleystrandesia reticulate*, *Herpetocypris* sp. 2, and *Dolerocypris sinensis* are firsts for this lake. Living ostracodes were collected at the maximum water depth (280 m) in the lake (*Candona hadzistei*, *C. marginatoides*, *C. media*, *C. ovalis*, *C. vidua*, *Fabaeformiscandona krstici*, *Cypria lacustris*, *C. obliqua* and *Amnicythere karamani*). *Cypria lacustris* was overall the most abundant species and *Cypria obliqua* displayed the highest abundance at 280 m water depth. Principal environmental variables that influence ostracode distributions in Lake Ohrid are water depth and conductivity. In general, species richness, diversity and evenness were greater in waters <60 m deep, with highest values often found in the littoral zone, at depths <30 m. Candonids, however, displayed highest diversity in the sublittoral (30–50 m) and profundal (50–280 m) zones. The most frequent species encountered are taxa endemic to the lake (14 living species), which have a wide depth range (≤280 m), and display higher abundance with greater water depth. Non-endemic species were rare, limited to water depths <50 m, and were found mainly in the north part of the lake where anthropogenic pressure is high. Several cosmopolitan species were encountered for the first time, which suggests that these widespread species are new arrivals that may replace endemics as human impacts increase.

**Keywords:** Balkan Ostracoda; biodiversity; endemism; water depth; anthropogenic impact

## 1. Introduction

Ancient lakes possess long continental archives of past climate and environmental changes and are hotspots of biodiversity and speciation dynamics for endemic taxa [1–5]. The Balkan region is a biogeographically diverse area characterized by ancient lakes. Besides well-known Lake Ohrid, Lakes Prespa, Skutari, Pamvotis, and Dojran are also ancient lakes [6–9]. Lake Ohrid hosts a highly diverse endemic freshwater fauna and flora [10]. Its geologic age and mode of formation remain controversial [11]. Lake Ohrid was selected for drilling by the International Continental Scientific Drilling Program (ICDP) because of its high potential as a paleoenvironmental and paleoclimatic archive [12]. Lake Ohrid possesses >220 endemic faunal species [13], most belonging to the Amphipoda, Gastropoda, Isopoda, Ostracoda, Porifera, and Tricladida [10]. Stankovič [14], Salemaa [15], Frogley and Preece [7], and Albrecht and Wilke [10] suggested that 33 (63%) of the 52 described ostracode species from Lake Ohrid and its catchment are endemic. Petkovski (2005, unpubl. data) listed 41 species for Lake Ohrid proper, excluding the catchment. Early works on Ostracoda focused on taxonomy [16–26] and there is little information on species ecological preferences, distribution within the lake, life cycles, or habitats. Mikulić and Pljakić [27] published the only paper that presents the spatial distribution of one genus, *Candona,* in Lake Ohrid. Greatest *Candona* diversity occurred between 20 m and 50 m water depth.

Ostracodes are microcrustaceans, generally ≤3 mm long, and are one of the few microfossil groups preserved in the last two interglacial sediment sequences from the lake [13,28]. Belmecheri *et al*. [13]

and Wagner *et al*. [28] used ostracode fossil assemblages in sediment cores to infer past climate and environmental changes. Belmecheri *et al*. [13] emphasized that most ostracode data came from the littoral zone and that, in general, the lake was under-sampled and taxonomic revision was needed. We carried out four field campaigns in Lake Ohrid to identify species that occur in the lake today [29,30]. In a previous study [30], the relationship between ostracode species in Lake Ohrid and environmental variables was explored. The main objective was to gather autecological information for all species and determine species temperature tolerances, a prerequisite for paleoenvironmental reconstructions [30]. In this study, we investigated the spatial distribution of each taxon and ostracode diversity and species richness in the lake. This information will improve the potential for using Ostracoda as bioindicators of climate and environment, and will help to interpret fossil species assemblages from sediment cores to be recovered by the Lake Ohrid Drilling Project.

*Study Region*

Lake Ohrid lies along the border between Macedonia and Albania, at 695 m a.s.l. The maximum water depth (293 m) is found in the southeast part of the basin. The lake has a surface area of 360 $km^2$ and a volume of 50.7 $km^3$ [31,32]. The steep-sided basin is located in a tectonic graben system of Pliocene to Pleistocene age [11] and is surrounded by mountains that reach ~1500 m a.s.l. to the west ("Mokra" mountain chain) and >1750 m a.s.l to the east ("Galičica" mountain chain) [28]. The Mokra is composed of serpentine (peridotites) overlain by Triassic limestone, whereas the Galičica consists mainly of Triassic limestone [14]. The timing of lake formation is still unclear and a subject of debate. Stankovič [14] suggested a 2–3 Ma age for the tectonic formation of the basin, whereas Spirkovski *et al*. [33] proposed an age of 4–10 Ma. Recent studies by Wagner *et al*. [34] and Lindhorst *et al.* [31] suggested a younger age (1.9 Ma).

An equally important question relates to the origin of the lake's water. Albrecht and Wilke [10] offered four hypotheses for the origin of Lake Ohrid. Hypothesis 1 ("Mesohellenic Trough" hypothesis) suggests that the Mesohellenic Trough was a narrow basin that extended from northern Greece northwest to the Korca Basin during the late Eocene and the middle Miocene and that modern Lake Ohrid filled by connection to the Aegean Basin. Hypothesis 2 ("Tethys" hypothesis) suggests that Lake Ohrid was part of a system of lacustrine basins that separated from the Tethys during the early and middle Pliocene. A connection between the Ohrid Basin and the Pannonian Basin via a series of freshwater lakes during the middle to late Miocene is postulated in hypothesis 3 ("Lake Pannon" hypothesis). Hypothesis 4 ("*De novo*" hypothesis) proposes that Lake Ohrid formed *de novo* in a dry *polje*, from springs or rivers. Albrecht and Wilke [10] regard the *de novo* scenario as the most tenable. Stankovič [14] notes that the fauna and flora of Lake Ohrid belong to freshwaters and that there is no evidence for marine or brackish immigrants in Lake Ohrid.

The Lake Ohrid catchment is influenced by Mediterranean climate, and less so by continental climate [35]. For the period 1961–1990, minimum air temperature was −5.7 °C, maximum air temperature was 31.5 °C, and average annual air temperature was 11.1 °C. Ice cover never formed [36]. Maximum precipitation occurs in December and March, and the late summer is dry [15]. Mean annual precipitation averages about 750 mm [28].

Lake Ohrid is an oligomictic lake that presents complete overturn roughly every seventh winter. The top 150–200 m of the water column display the usual seasonal stratification of typical deep temperate lakes. Deeper water temperatures show a small depth gradient between −0.02 and 0.15 °C per 100 m. Stratification of the water column below 150 m is controlled by salinity [37].

Numerous karstic springs contribute approximately 53% to the net hydrologic input, with the balance equally divided between river inflows and direct precipitation [10]. Spring inflows influence ostracode species distribution and ecology because they supply the cool, oxygen-rich water favored by all endemic forms in Lake Ohrid [37,38].

Vegetation of the Ohrid region is of the sub-Mediterranean type and belongs to the *Quercetalia pubescentis* order, especially in the zone of *Quercus cerris* forests. *Quercetum macedonicae* EM prov., *Pinetum pallasianae macedonicum* EM, and *Carpinetum orientalis macedonicum* associations occupy the lower belt of the Ohrid Basin (700–1000 m a.s.l.). The entire Ohrid region has, however, been largely deforested, giving rise to another characteristic vegetation consisting of small trees and shrubs, such as *Pistacia terebinthus* and *Asparagus tenuifolius* [14].

It has been suggested that the subaqueous springs create a high number of ecological niches and spring water has been important in the evolution of the Lake Ohrid ecosystem [32,38]. Furthermore, small-scale differences in sediment composition may be responsible for the patchiness in sub-structuring of benthic communities. This heterogeneity may also be linked to evolutionary processes. Grain-size distribution, metal concentrations, and concentrations of particular mineral phases are closely tied to catchment geology and transport processes, and show strong spatial variability in lake surface sediments [39].

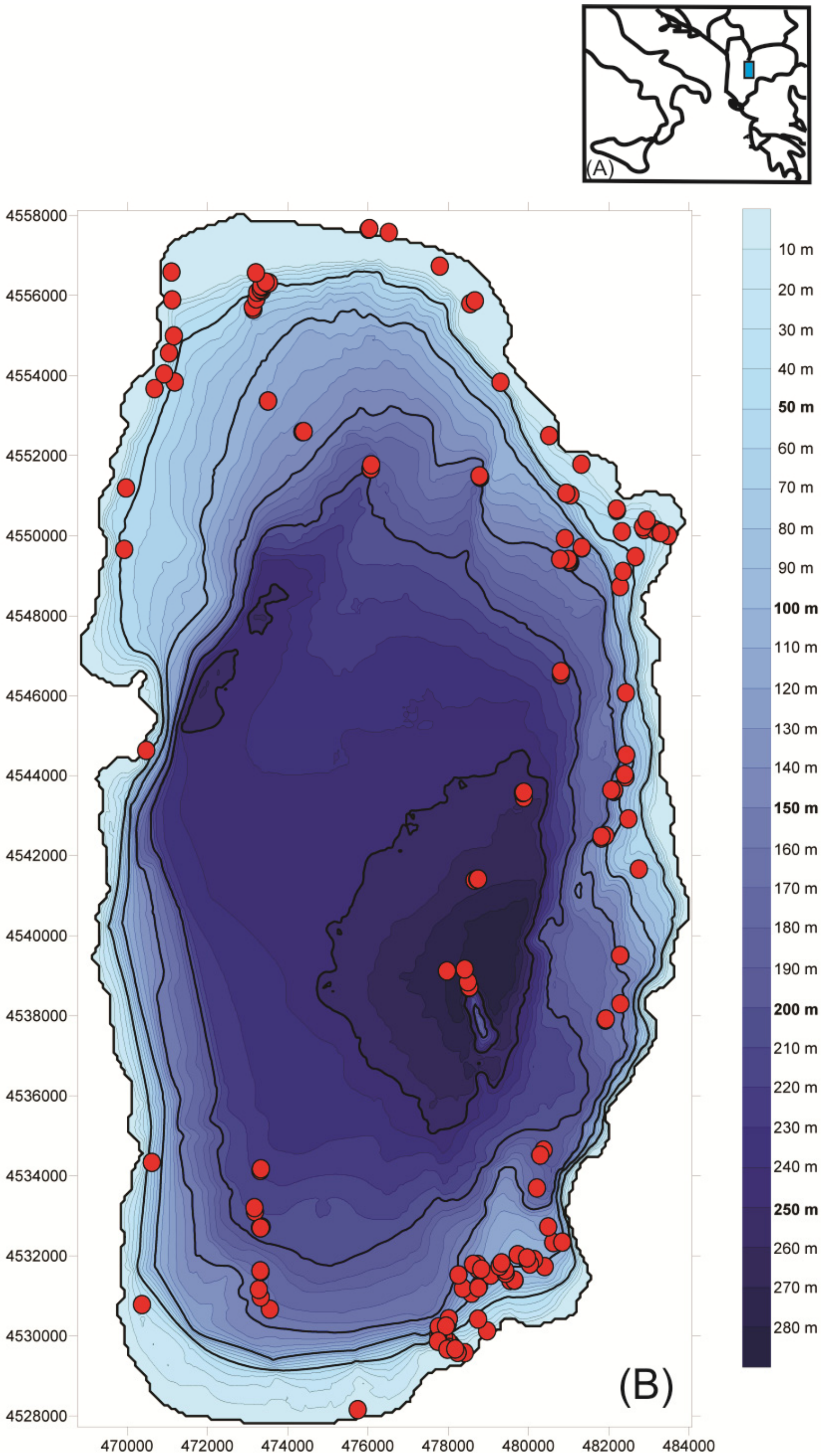
Little information is available about the biodiversity of higher aquatic plants [10], but the macrophyte diversity in the lake is apparently relatively low. Four major zones (belts) of macrophyte vegetation are found: *Cladophora*, *Phragmites*, *Potamogeton* and *Chara* [40]. The *Chara* belt is the best studied zone and extends into the lower littoral, to about 20 m depth [10].

## 2. Sampling Survey

We carried out four field campaigns covering all seasons (March/April 2009, August/September 2009, February 2010, and June 2010) [30], during which we collected a total of 187 surface sediment samples from the littoral zone to 280 m depth (Figure 1). Sampling efforts focused on water depths to 235 m, but additional samples were recovered from 270 m to 280 m. Physical and chemical variables and sediment information for each sampling site are found at [41].

In areas of the lake where sediment is coarse, samples were retrieved with an exhauster, spoon net, UWITEC gravity corer, Ekman grab, dredge or sediment-suction device. The sediment suction device consisted of a metal pipe to which a 5-m-long flexible hose was attached. Sediment was drawn into a 125-µm-mesh sieve, using a manual membrane pump. In areas where sediment is finer, samples were collected with a gravity multi-corer (three cores, each with a diameter of 11 cm). The uppermost 2 cm of each core (190 $cm^3$) was washed through a 125-µm-mesh sieve immediately after collection. All sieve residues were preserved in 95–99% ethanol.

**Figure 1.** Inset map showing (**A**) the location of Lake Ohrid (rectangle) on the border of Macedonia/Albania and (**B**) a bathymetric map of the lake, displaying the sediment sampling sites.




In the laboratory, ostracodes were picked from the residues with fine brushes, using a stereo microscope (Leica MZ 7.5) with a magnification up to 50×. Valves and soft bodies were separated and soft parts were dissected and mounted in Hydro-Matrix$^{TM}$ (Micro Tech Lab, Graz, Austria). The taxonomic works of Klie [16–19], Holmes [20], Meisch [42], Mikulić [21], Namiotko *et al.* [43], Petkovski [22–26] and Petkovski *et al.* [44] were used to identify species. Digital images of valves were taken with a light microscope (DM5000B; camera type Leica DFC320, Leica Microsystems GmbH, Wetzlar, Germany). Scanning Electron Microscope (SEM) pictures of ostracode valves and carapaces were taken at the Zoological Museum of the University of Hamburg, with a Scanning electron microscope LEO 1525 (Carl Zeiss Inc., Jena, Germany) and at the University of Cologne, with a CamScan-CS-44 (CamScan, Cranberry Township, PA, USA).

A total of 187 samples were included in numerical analyses, of which 27 samples contained no ostracodes. Furthermore, juvenile ostracodes that could not be identified to species level, were omitted from interpretation. Ostracode relative abundances are found at [41].

Species richness (number of species in a sample, S) is indicated for all samples, whereas the Shannon diversity index (H) [45] and the index of evenness (E) [46] were calculated only for samples taken with the gravity multicorer. Adult counts, *i.e.*, valves, empty carapaces and individuals with soft parts were included in analyses. Sample abundances per unit volume (190 $cm^{-3}$) were only determined for the multi-corer samples. Species richness (S), diversity (H) and evenness (E) were modeled as a function of the spatial coordinates using a non-parametric, locally weighted regression (loess) with a span of 0.5 [47]. Additionally, abundance of species present in 10 or more sites was generalized as a function of collection depth and conductivity through non-parametric smoothing regressions [48]. All statistical analysis was performed in R [49].

## 3. Results and Discussion

Reported numbers of ostracode species in Lake Ohrid differ among authors, suggesting the need for a taxonomic review. Early taxonomic work began in the 1930s and is compiled in Table 1. It is difficult, however, to ascertain the number of specimens collected and analyzed in those early studies. Holmes [20] suspected that his species list was incomplete because spring and summer taxa might have already disappeared by the time he visited the lake between 1 August and 10 September 1935. Furthermore, he collected specimens from a restricted area of the lake. Our study is the first to sample during all seasons of the year.

### *3.1. Ostracode Diversity*

We collected living specimens of 32 species belonging to seven families (Candonidae, Ilyocyprididae, Cyprididae, Leptocytheridae, Limnocytheridae, Cytherideidae, and Darwinulidae) (Box 1 and Figures 2–5) [30]. Six additional species were identified from only hard parts: *Candona expansa*, *C. holmesi*, *C. marginata*, *C. triangulata*, *Cypria ophtalmica*, and *Paralimnocythere umbonata*. These species were excluded from consideration because valves and empty carapaces may have been transported to the sampling locations and, therefore, may not represent living species at the sites. Fifty-three percent of the ostracode species belong to Candonidae and 16% to Limnocytheridae. Only one species belonging to Cytherideidae (*Cytherissa lacustris*) was found, which is typical for ancient lakes. Other *Cytherissa* species are endemic to Lake Baikal [50]. Our results from the younger, though ancient Lake Ohrid agree with Frogley *et al.* [51], in that they reported that "relatively younger" ancient lakes (0.75–8 Ma old) typically display high faunal diversity, with species flocks of

Limnocytheridae and Candonidae being the most common groups. Such "younger" ancient lakes, with the same dominant subfamilies found in Lake Ohrid, include among others, Lakes Malawi, Biwa and Titicaca. Older lakes such Tanganyika (9–12 Ma) and Baikal (25–35 Ma) are truly ancient both geologically and biologically and most species flocks belong to Cytherideidae and Candonidae.

**Box 1.** Taxonomic ranking of ostracode species collected from Lake Ohrid. Species marked with asterisk (*) were identified using valves or empty carapaces, species endemic to the lake are marked with an open circle (°) (modified from [30]).

Class Ostracoda Latreille, 1806
Order Podocopida Sars, 1866
Suborder Podocopina Sars, 1866
Infraorder Cypridocopina Jones, 1901
Superfamily Cypridoidea Baird, 1845
Family Candonidae Kaufmann, 1900
Subfamily Candoninae Kaufmann, 1900
Genus *Candona* s. str. Baird, 1845
*Candona bimucronata* Klie, 1937
*°*Candona expansa* Mikulić, 1961
°*Candona goricensis* Mikulić, 1961
°*Candona hadzistei* Petkovski *et al.*, 2002
°*Candona hartmanni* Petkovski, 1969
*°*Candona holmesi* Petkovski, 1960
°*Candona litoralis* Mikulić, 1961
°*Candona margaritana* Mikulić, 1961
*°*Candona marginata* Klie, 1942
*Candona marginatoides* Petkovski, 1960
°*Candona media* Klie, 1939
°*Candona ohrida* Holmes, 1937
°*Candona ovalis* Mikulić, 1961
°*Candona trapeziformis* Klie, 1939
*°*Candona triangulata* Klie, 1939
°*Candona vidua* Klie, 1942
Genus *Fabaeformiscandona* Krstić, 1972
°*Fabaeformiscandona krstici* (Petkovski, 1969)
Genus *Candonopsis* Vávra, 1891
*Candonopsis kingsleii* (Brady & Robertson, 1870)
Subfamily Cyclocypridinae Kaufmann, 1900
Genus *Cypria* Zenker, 1854
*Cypria lacustris* Sars, 1890
*Cypria obliqua* Klie, 1939
**Cypria ophtalmica* (Jurine, 1820)
Genus *Cyclocypris* Brady & Norman, 1889
*Cyclocypris ovum* (Jurine, 1820)
Family Ilyocyprididae Kaufmann, 1900
Subfamily Ilyocypridinae Kaufmann, 1900
Genus *Ilyocypris* Brady & Norman, 1889
*Ilyocypris bradyi* Sars, 1890

**Box 1.** *Cont.*

Family Cyprididae Baird, 1845
Subfamily Eucypridinae Bronshtein, 1947
Genus *Eucypris* Vávra, 1891
*Eucypris virens* (Jurine, 1820)
*Eucypris* sp.
Genus *Prionocypris* Brady & Norman, 1896
*Prionocypris zenkeri* (Chyzer & Toth, 1858)
Subfamily Cypricercinae McKenzie, 1971
Genus *Bradleystrandesia* Broodbakker, 1983
*Bradleystrandesia reticulata* (Zaddach, 1844)
Subfamily Herpetocypridinae Kaufmann, 1900
Genus *Herpetocypris* Brady & Norman, 1889
*Herpetocypris* sp.
*Herpetocypris* sp.2
Subfamily Dolerocypridinae Triebel, 1961
Genus *Dolerocypris* Kaufmann, 1900
*Dolerocypris sinensis* (Sars, 1903)
Superfamily Cytheroidea Baird, 1850
Family Leptocytheridae Hanai, 1957
Subfamily Leptocytherinae Hanai, 1957
Genus *Amnicythere* Devoto, 1965
*Amnicythere karamani* (Klie, 1939)
Family Limnocytherinae Klie, 1938
Subfamily Limnocytherinae Klie, 1938
Genus *Paralimnocythere* Carbonnel, 1965
*Paralimnocythere alata* (Klie, 1939)
*Paralimnocythere georgevitschi* (Petkovski, 1960)
*Paralimnocythere karamani* (Petkovski, 1960)
*Paralimnocythere ochridense* (Klie, 1934)
*Paralimnocythere slavei* Petkovski, 1969
**Paralimnocythere umbonata* (Klie, 1939)
Family Cytherideidae Sars, 1925
Genus *Cytherissa* Sars, 1925
*Cytherissa lacustris* (Sars, 1863)
Infraorder Darwinulocopina Sohn, 1988
Superfamily Darwinuloidea Brady & Norman, 1889
Family Darwinuloidae Brady & Norman, 1889
Genus *Darwinula* Brady & Robertson, 1885
*Darwinula stevensoni* (Brady & Robertson, 1870)

The 32 ostracode species found alive belong to 15 genera (Box 1 and Figures 2–5). The most diverse genus in the lake was *Candona*, with 12 species identified from live individuals and four species from valves and empty carapaces (*C. expansa*, *C. holmesi*, *C. marginata* and *C. triangulata*). Besides the genus *Candona*, the subfamily Candoninae was represented by the genera *Candonopsis* (*C. kingsleii*) and *Fabaeformiscandona* (*F. krstici*). The second most diverse genus was *Paralimnocythere*. We found five living species (*P. alata*, *P. georgevitschi*, *P. karamani*, *P. ochridense*, and *P. slavei*), but only shells of *P. umbonata*. The genus *Cypria* included two living species (*C. lacustris* and *C. obliqua*) and one species identified from valves and empty carapaces

(*C. ophtalmica*). All other genera were represented by one or two species. A distinctive feature of the ostracode fauna in this lake is the occurrence of the species *Amnicythere karamani*. Species of the genus *Amnicythere* normally live in oligo-haline to meso-haline waters and marine environments [52], but some occur in springs, rivers and lakes [50]. Although the *de novo* hypothesis has been suggested as the most probable explanation for the lake's origin, the presence of *Amnicythere* suggests a marine origin for the lake [29]. Recent studies, however, indicate fluvial/glaciofluvial sedimentation at the onset of Lake Ohrid [34], suggesting other reasons for the presence of this species in the lake. According to Namiotko *et al*. [43], this species probably derived from Lake Pannon species that colonized lakes in southern Europe through a stepping-stone process that enabled adaptation to the freshwater environment.

**Figure 2.** Pictures of ostracodes from Lake Ohrid: *Fabaeformiscandona krstici* (**1**) Left valve (LV), external view, male; *F. krstici* (**2**) Right valve (RV), internal view, male; *Candona hadzistei* (**3**) RV, external view, male; *C. hadzistei* (**4**) RV, internal view, female; *Candona margaritana* (**5**) RV, internal view, female; *C. margaritana* (**6**) LV, internal view, female; *C. marginata* (**7**) RV, internal view, female; *Candona goricensis* (**8**) RV, external view, female; *Ilyocypris bradyi* (**9**) LV, external view, female; *I. bradyi* (**10**) RV, internal view, female; *Darwinula stevensoni* (**11**) LV, external view, female; *D. stevensoni* (**12**) RV, internal view, female; *Cytherissa lacustris* (**13**) RV, external view, female; *C. lacustris* (**14**) LV, internal view, female; *Candona goricensis* (**15**) LV, internal view, female; *Herpetocypris* sp. 2 (**16**) LV, external view, female. Arrows point to anterior (modified from [30]).

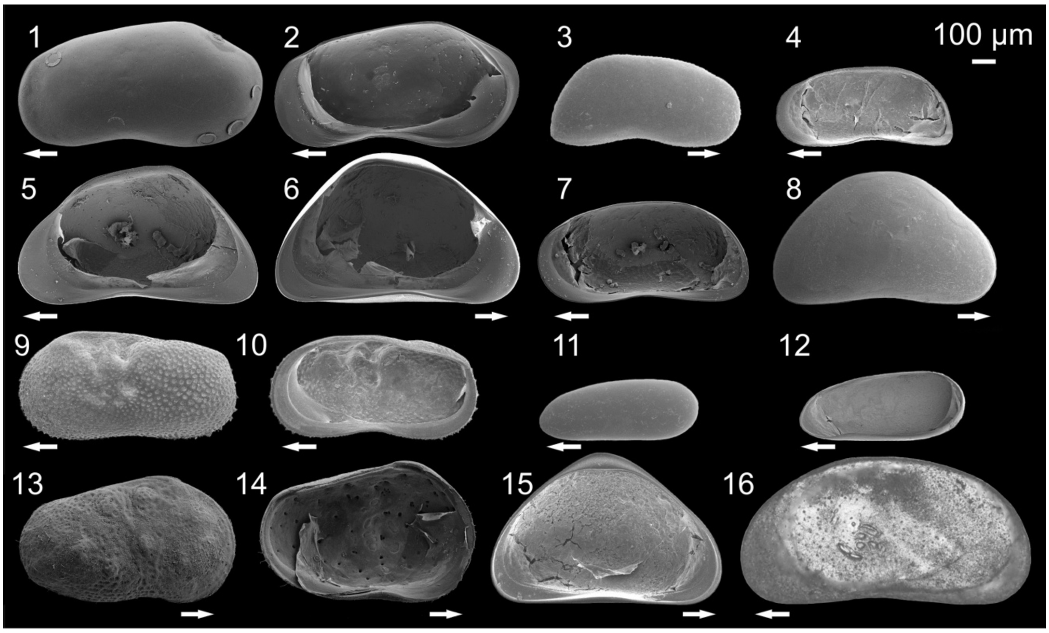


**Figure 3.** Pictures of ostracodes from Lake Ohrid: *Candona triangulata* (**1**) Left valve (LV), external view, female; *C. triangulata* (**2**) Right valve (RV), internal view, female; *Candona litoralis* (**3**) RV, external view, male; *Candona media* (**4**) LV, external view, female; *C. media* (**5**) RV, internal view, female; *Candona trapeziformis* (**6**) RV, internal view, male; *C.trapeziformis* (**7**) RV, internal view, female; *Candona vidua* (**8**) LV, external view, female; *C. vidua* (**9**) RV, internal view, female; *Candona ovalis* (**10**) LV, external view, female; *C. ovalis* (**11**) LV, internal view, female; *Candonopsis kingsleii* (**12**) RV, external view, male; *C. kingsleii* (**13**) LV, internal view, male; *Candona hartmanni* (**14**) RV, internal view, male; *C. hartmanni* (**15**) RV, internal view, female; *Candona ohrida* (**16**) RV, external view, male; *C. ohrida* (**17**) LV, internal view, male; *Candona holmesi* (**18**) RV, internal view, female. Arrows point to anterior part of valves (modified from [30]).

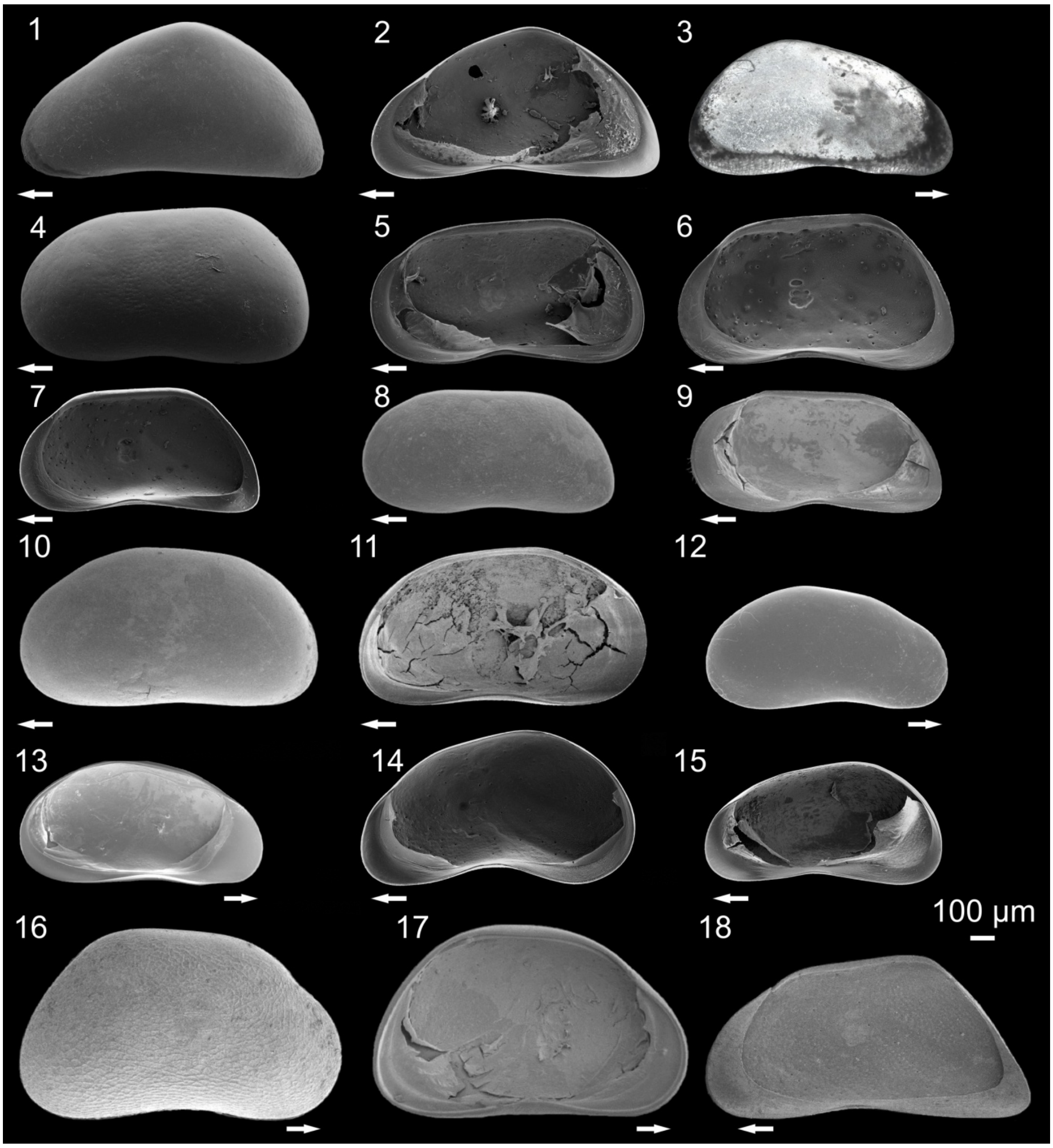
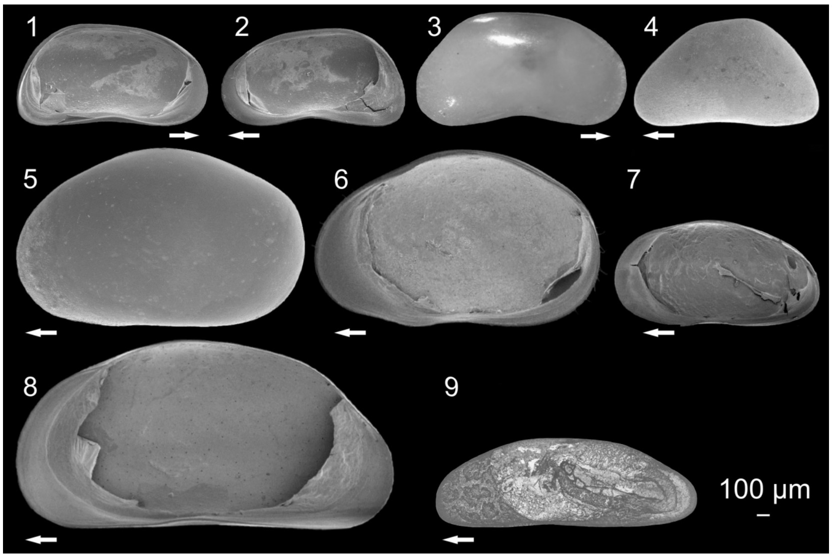


**Figure 4.** Pictures of ostracodes from Lake Ohrid: *Candona marginatoides* (**1**) Left valve (LV), internal view, female; *C. marginatoides* (**2**) Right valve (RV), internal view, female; *Candona bimucronata* (**3**) RV, external view, female; *Candona expansa* (**4**) LV, external view, female; *Eucypris virens* (**5**) LV, external view, female; *E. virens* (**6**) RV, internal view, female; *Bradleystrandesia reticulata* (**7**) RV, internal view, female; *Eucypris* sp. (**8**) RV, internal view, female; *Dolerocypris sinensis* (**9**) LV, external view, female. Arrows point to anterior part of valves (modified from [30]).




It was previously uncertain how many ostracode species inhabit the lake and surrounding waters and earlier studies reported different species numbers. In a literature review, Martens [53] reported 38 species from 13 genera in Lake Ohrid, but did not provide a species list. Martens and Schön [54] mentioned that the lake possesses ~50 species. A total of 52 species, in the lake and its surrounding wetlands, is found in Frogley *et al.* [51] and Griffiths and Frogley [55]. According to Frogley *et al.* [51], endemic ostracodes include three species of *Amnicythere* (*Leptocythere*), four of *Paralimnocythere*, and 25 candonids. Petkovski (2005, unpubl. data) reduced the list of species (excluding the catchment area) to a total of 41, because some species were described several times under different names (Table 1). Such repeated description of some species under different names may be one reason we still miss some previously described ostracode species. It is also possible that some species have disappeared from the lake during the last few decades. We found eight living species that had never been reported in the lake: *Candona bimucronata*, *Ilyocypris bradyi*, *Eucypris virens*, *Eucypris* sp., *Prionocypris zenkeri*, *Bradleystrandesia reticulata*, *Herpetocypris* sp. 2, and *Dolerocypris sinensis*. *Candona bimucronata,* however, was found by Petkovski *et al.* [44] in a small rheocrene stream northeast of the City of Struga at Lake Ohrid, and *D. sinensis* was encountered in a puddle in a meadow near Struga [24]. *Ilyocypris bradyi* and *P. zenkeri* were reported by Holmes from nearby wetlands [20].

**Figure 5.** Pictures of ostracodes from Lake Ohrid: *Cyclocypris ovum* (**1**) Right valve (RV), internal view, female; *Cypria obliqua* (**2**) Left valve (LV), external view, female; *C. obliqua* (**3**) RV, internal view, female; *C. lacustris* (**4**) RV, internal view, female; *Cypria ophtalmica* (**5**) LV, internal view, isolated valve; *Amnicythere karamani* (**6**) LV, external view, female; *A. karamani* (**7**) RV, internal view, female; *Paralimnocythere ochridense* (**8**) LV, external view, male; *P. ochridense* (**9**) RV, internal view, female; *Paralimnocythere karamani* (**10**) LV, external view, female; *P.karamani* (**11**) RV, internal view, female; *Paralimnocythere alata* (**12**) LV, external view, female; *Paralimnocythere slavei* (**13**) LV, external view, female; *P. slavei* (**14**) LV, external view, male; *Paralimnocythere georgevitschi* (**15**) LV, external view, female; *Paralimnocythere umbonata* (**16**) LV, external view, female; *P. umbonata* (**17**) RV, internal view, female; *Prionocypris zenkeri* (**16**) LV, external view, juvenile. Arrows point to anterior part of valves (modified from [30]).

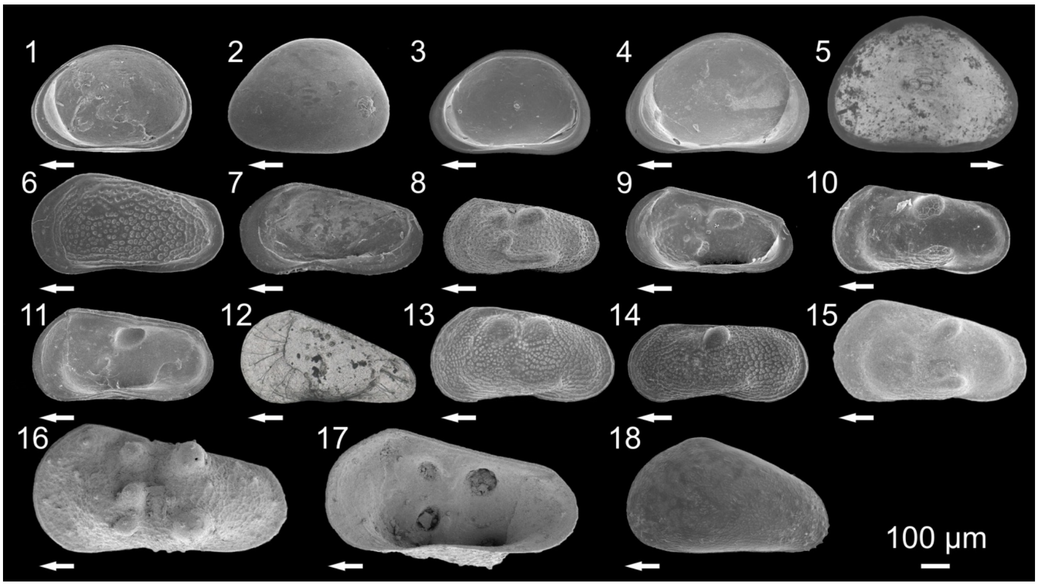



**Table 1.** Checklist of ostracode species from Lake Ohrid, including synonymy. Comparison with the ostracode fauna reported in this paper and by [30] (+living species, * valves or empty carapaces), species reported previously in the lake from other authors, and Petkovski (2005, unpubl. data).

| Ostracode Species in Lake Ohrid | This Paper | Other Authors | Petkovski (2005, Unpubl. Data) |
|---|---|---|---|
| *Amnicythere angulata* | | (Klie, 1939b) | + |
| *Amnicythere karamani* | + | (Klie, 1939b) | + |
| *Amnicythere prespensis* | | (Petkovski & Keyser, 1992) | + |
| *Amnicythere proboscidea* | | (Klie, 1939b) | + |
| *Bradleystrandesia reticulata* | + | | |
| *Candona acricauda* | | Mikulić, 1961 | synonym of *Candona holmesi* |
| *Candona alta* | | Klie, 1939a | *Candona neglecta* (review needed) |
| *Candona bimucronata* | + | | |
| *Candona candida* | | Holmes, 1937 | |
| *Candona compressa* | | Klie, 1942 | *Pseudocandona compressa* |

**Table 1.** *Cont.*

| Ostracode Species in Lake Ohrid | This Paper | Other Authors | Petkovski (2005, Unpubl. Data) |
|---|---|---|---|
| *Candona cristatella* | | Klie, 1939a | synonym of *Candona ohrida* |
| *Candona depressa* | | Klie, 1939a | + |
| *Candona expansa* | * | Mikulić,1961 | + |
| *Candona fabaeformis* | | Klie, 1939a | *Eucandona* (*Fabaeformiscandona*) *krstici* |
| *Candona formosa* | | Mikulić,1961 | + |
| *Candona goricensis* | + | Mikulić,1961 | + |
| *Candona hadzistei* | + | Petkovski *et al*. 2002 | + |
| *Candona hartmanni* | + | Petkovski, 1969a | + |
| *Candona holmesi* | * | Petkovski, 1960a | + |
| *Candona jordeae* | | Petkovski *et al*. 2002 | + |
| *Candona litoralis* | + | Mikulić,1961 | + |
| *Candona lucida* | | Petkovski, 1969a | renamed in *Candona jordeae* |
| *Candona lychnitis* | | Petkovski, 1969a | + |
| *Candona macedonica* | | Mikulić,1961 | + |
| *Candona margaritana* | + | Mikulić,1961 | |
| *Candona marginata* | * | Klie, 1942 | + |
| *Candona marginatoides* | + | Petkovski, 1960b | + |
| *Candona media* | + | Klie, 1939a | *Candona neglecta* (review needed) |
| *Candona neglecta* | | Holmes, 1937 | + |
| *Candona ohrida* | + | Holmes, 1937 | + |
| *Candona ovalis* | + | Mikulić,1961 | + |
| *Candona parvula* | | Mikulić,1961 | renamed in *Candona hadzistei* |
| *Candona* sp. | | Petkovski, 2005 (unpub. data) | + |
| *Candona sublitoralis* | | Mikulić,1961 | synonym of *Candona ohrida* |
| *Candona trapeziformis* | + | Klie, 1939a | + |
| *Candona triangulata* | * | Klie, 1939a | + |
| *Candona vidua* | + | Klie, 1942 | + |
| *Candonopsis kingsleii* | + | Klie, 1942 | + |
| *Cyclocypris ovum* | + | Klie, 1939a | + |
| *Cypria lacustris* | + | Petkovski, 1960b | + |
| *Cypria obliqua* | + | Klie, 1939a | + |
| *Cypria ophtalmica* | * | Holmes, 1937 | |
| *Cytherissa lacustris* | + | Klie, 1942 | + |
| *Darwinula stevensoni* | + | Holmes, 1937 | + |
| *Dolerocypris sinensis* | + | | |
| *Eucypris virens* | + | | |
| *Eucypris* sp. | + | | |
| *Fabaeformiscandona krstici* | + | Petkovski, 1969a | + as *Eucandona* (*Fabaeformiscandona*) *krstici* |
| *Herpetocypris* sp. 2 | + | | |
| *Ilyocypris bradyi* | + | | |
| *Paralimnocythere alta* | + | Klie, 1939b | + |
| *Paralimnocythere georgevitschi* | + | (Petkovski, 1960c) | + |

**Table 1.** *Cont.*

| Ostracode Species in Lake Ohrid | This Paper | Other Authors | Petkovski (2005, Unpubl. Data) |
|---|---|---|---|
| *Paralimnocythere karamani* | + | (Petkovski, 1960c) | + |
| *Paralimnocythere ochridense* | + | (Klie, 1934) | + |
| *Paralimnocythere slavei* | + | (Petkovski, 1969b) | + |
| *Paralimnocythere umbonata* | * | (Klie, 1939b) | + |
| *Physocypria* sp. (*Physocypria kraepelini*?) | | Holmes, 1937 | *Physocypria kliei*? (review needed) |
| *Prionocypris zenkeri* | + | | |
| *Pseudocandona compressa* | | | + |
| *Pseudocandona elongata* | | Holmes, 1937 | + |
| *Pseudocandona slavei* | | Petkovski, 1969a | + |

### *3.2. Spatial Distribution, Species Richness and Diversity of Ostracodes*

Lorenschat and Schwalb [30] studied the ecological preferences of ostracodes in Lake Ohrid and indicated that water depth and conductivity (salinity) are the two main environmental variables that determine their distribution. We therefore focused on these two variables, even though we were aware that ostracode distribution may be influenced by other variables. Many of the species were widely distributed in the lake (Figures 6–8). Endemic species had a broad water-depth range and non-endemic species occurred mainly at shallow depths.

**Figure 6.** Depth distributions of living ostracodes in Lake Ohrid.

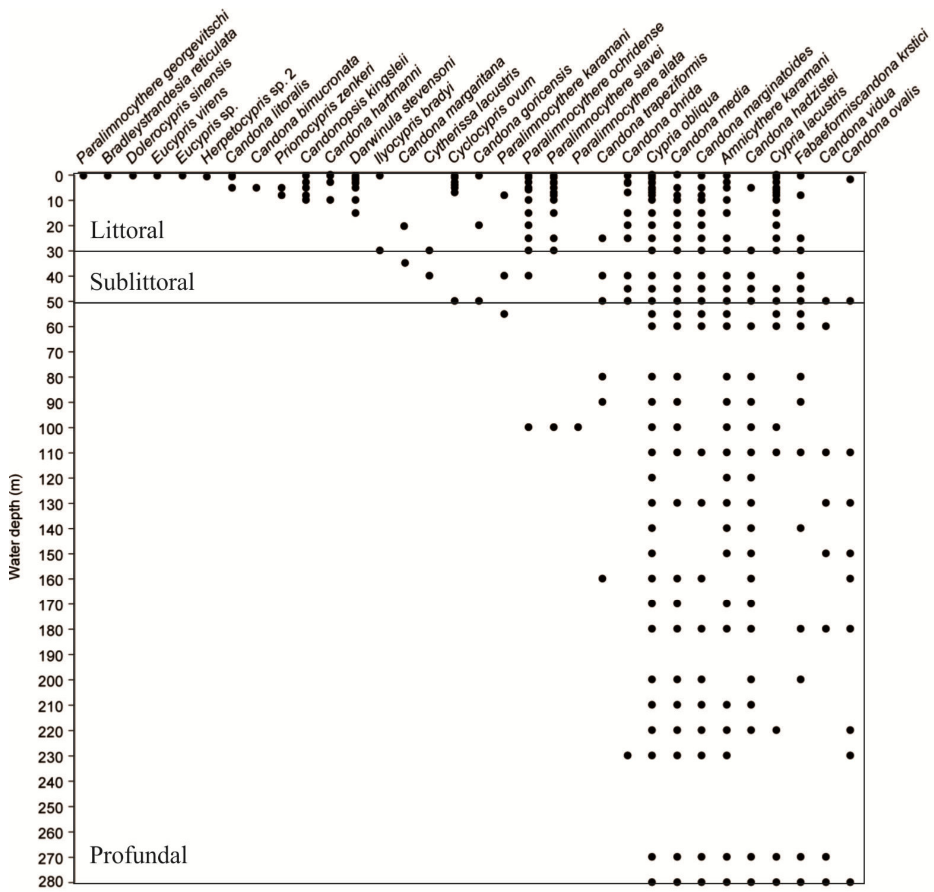


**Figure 7.** 3D scatter plot of species relative abundance (%) as a function of depth (m) and water conductivity (µS $cm^{-1}$) reported for species collection sites. Vertical lines and spheres indicate the species relative abundances. Blue surface shows modeled abundance through smooth regression. Only species present in ≥10 sites are shown.

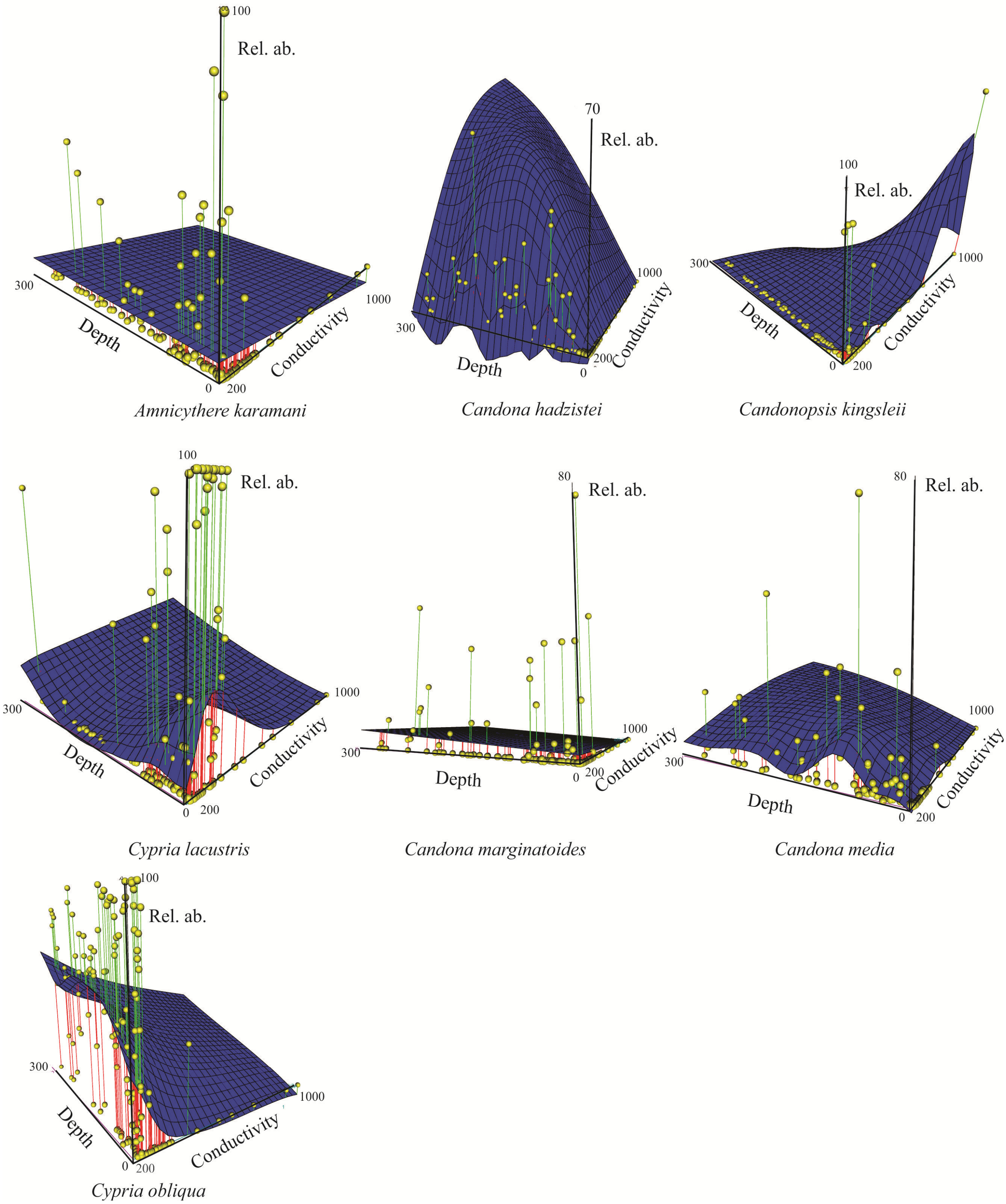
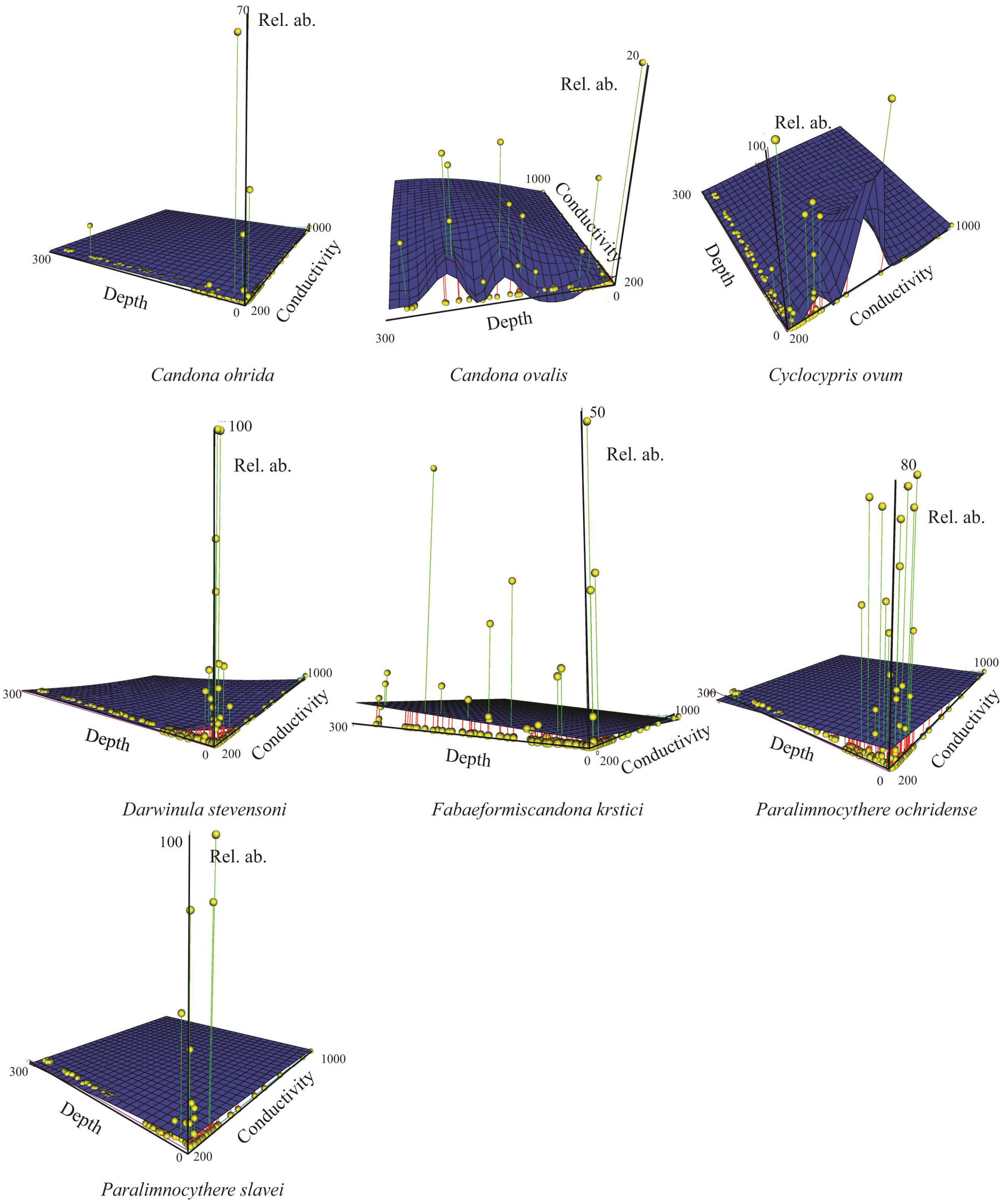


**Figure 8.** 3D scatter plot of species relative abundance (%) as a function of depth (m) and water conductivity (μS cm$^{-1}$) reported for species collection sites. Vertical lines and spheres indicate the species relative abundances. Blue surface shows modeled abundance through smooth regression. Only species present in ≥10 sites are shown.



3.2.1. Spatial Distribution

Species of *Paralimnocythere* generally have highest abundances in the north part of the lake and *P. ochridense* showed the highest abundance of all species from this genus (777 individuals 190 $cm^{-3}$). Only the two endemic species, *Paralimnocythere alata* and *P. georgevitschi*, are restricted to the north part of the lake, and five non-endemic species were also found only in the north part of the lake. *Dolerocypris sinensis*, *Eucypris virens*, *Eucypris* sp., and *Bradleystrandesia reticulata* occurred in the northeast part of the lake, which is affected by agricultural land use. *Candona hartmanni* was found by Petkovski [26] along the muddy northeast shore of the lake. *Prionocypris zenkeri* inhabited only the northwest part of the lake, near the springs of Kališta, and *Candona bimucronata* colonized exclusively the Bay of Ohrid. *Cytherissa lacustris* occurred only in the area influenced by the springs of Sveti Naum and *Herpetocypris* sp. 2 was found in the littoral area near the street in the village of Peštani. Empty carapaces of *Candona expansa* were found only in the northern part of the lake and valves and empty carapaces of *Candona holmesi* were recovered at 14 sampling locations distributed around the entire lake. Valves and empty carapaces of *Candona marginata* and *Cypria ophtalmica* were found in only one sample from the eastern shore. Dead individuals of *Candona triangulata* occurred in three samples from the eastern shore and remains of *Paralimnocythere umbonata* were found in one sample from the western shore of the lake. In the southern part of the lake, *P. ochridense* showed high abundance in one sample taken in the area of Sveti Naum (516 individuals 190 $cm^{-3}$). Otherwise, abundances for other species ranged between 1 and 9 individuals/190 $cm^3$ in the southern part of the basin. Species richness and the diversity and evenness indexes were higher in the northern and eastern parts of the lake (Figures 9–11).

**Figure 9.** Spatially modeled species richness (S) in Lake Ohrid.

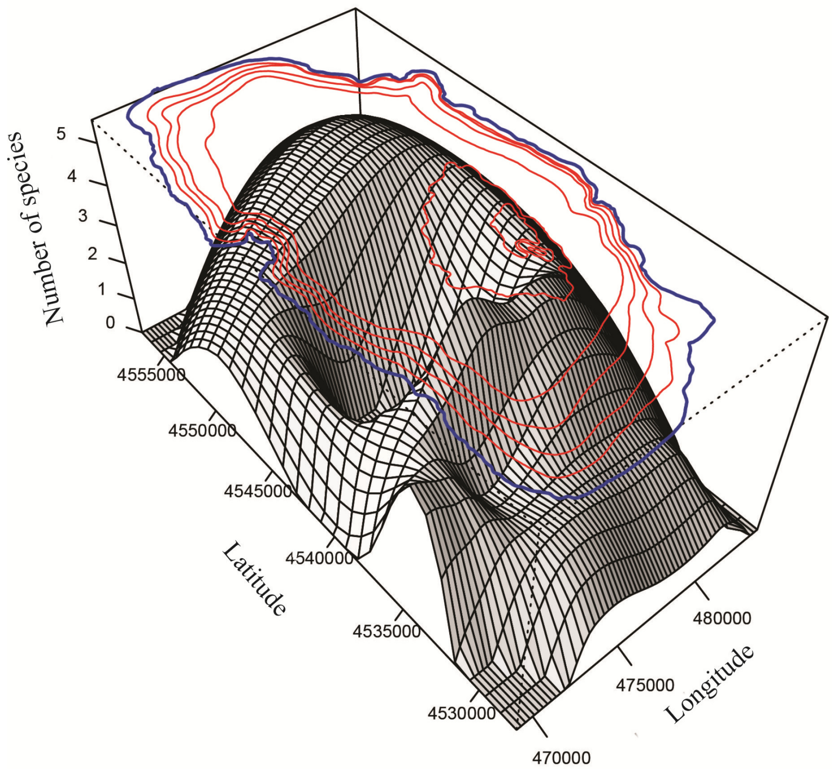


**Figure 10.** Spatially modeled species diversity (H) in Lake Ohrid.

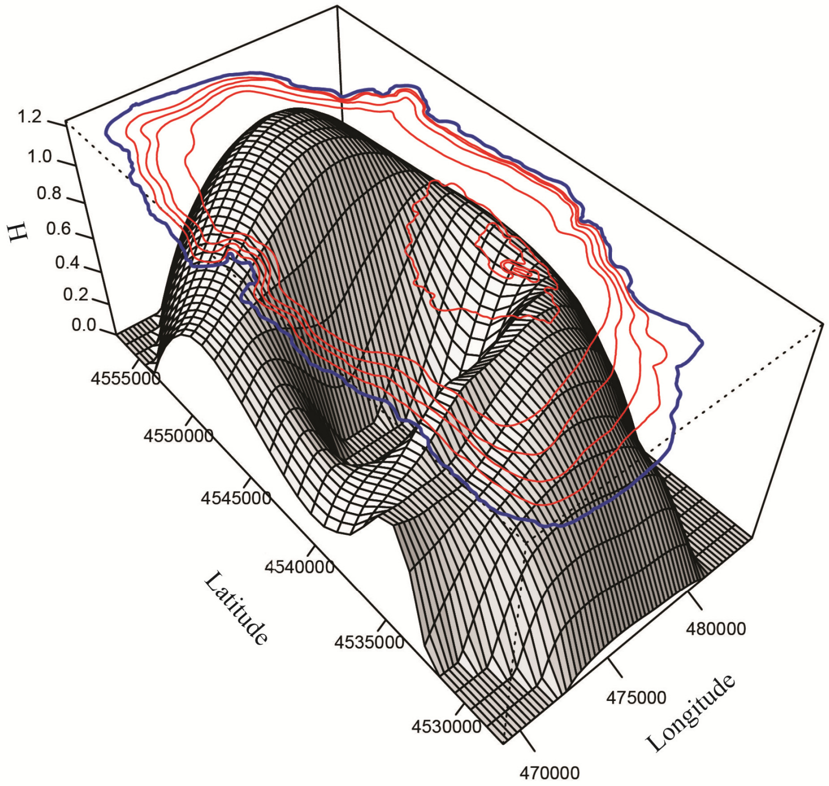
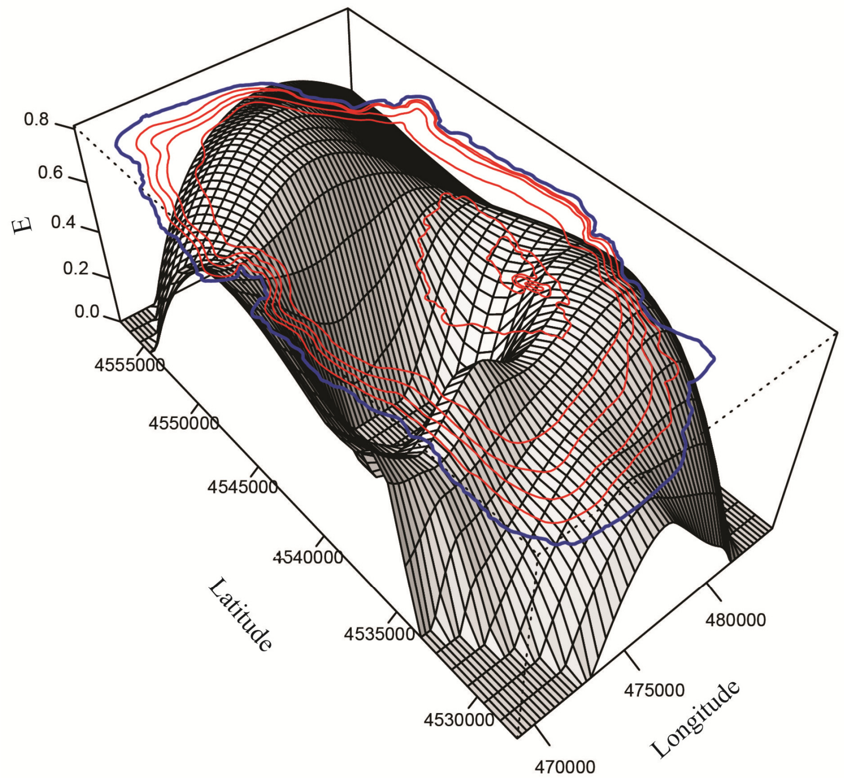



**Figure 11.** Spatially modeled species evenness (E) in Lake Ohrid.



3.2.2. Vertical Distribution

Figures 7 and 8 display 3D plots of the relative abundance, water depth and conductivity data corresponding to the species collection sites. Our studies are the first in Lake Ohrid that sampled down to 280 m water depth [29,30]. We found that some species were restricted to littoral, sublittoral or profundal zones, whereas other species had wider distributions (littoral-sublittoral zones or littoral-profundal zones, Figures 6–8).

3.2.2.1. Littoral Zone (≤30 m)

Three species of *Candona* (*C. litoralis*, *C. bimucronata* and *C. hartmanni*) occurred only in the littoral zone (Figure 6), which we define as water depths to 30 m because we found submerged aquatic vegetation (*Chara* and *Potamogeton*) down to this depth during all sampling campaigns. Besides the three species of *Candona*, *P. georgevitschi*, *C. litoralis*, *C. bimucronata*, and *C. hartmanni*, and nine other species inhabited only the littoral zone (Figure 6). The "real littoral *Candona* species" *C. litoralis* was found by Mikulić [21] down to 20 m water depth, and *C. bimucronata* was reported by Griffiths and Brancelj [56] from 13 m to 15 m in Lake Bohinj (Slovenia). Highest abundance of all Ohrid species was displayed by *C. lacustris*: 2468 individuals 190 $cm^{-3}$ at 9 m water depth and 1910 individuals 190 $cm^{-3}$ at 0.3 m (Figure 7). Highest abundances of this species were reported mainly in waters with low conductivities. *Paralimnocythere georgevitschi* was found only at 0.3 m water depth. *Paralimnocythere ochridense* showed the highest abundance of all species from this genus (777 individuals 190 $cm^{-3}$) at 30 m water depth and in collection sites with low conductivity (Figure 8), and also displayed high abundance in a sample from the area of Sveti Naum taken at 7 m depth (516 individuals 190 $cm^{-3}$). Klie found this species in the littoral of Lake Ohrid [16] and empty carapaces down to 10 m [17]. Petkovski [25] reported *P. slavei* from the littoral zone. *Ilyocypris bradyi* appeared near the springs of Sveti Naum at 30 m water depth (14 and 9 individuals 190 $cm^{-3}$) and at 0.5 m (1 individual 190 $cm^{-3}$). Meisch [42] suggested that individuals of *I. bradyi* found in lakes are usually discharged from nearby springs. *Darwinula stevensoni* occurred in water depths between 0.2 m and 15 m, where conductivities were low (Figures 6 and 8) and highest abundance was at 0.4 m water depth (30 individuals 190 $cm^{-3}$). Holmes *et al.* [20] found *D. stevensoni* in water depths from 7 m to 10 m. According to Meisch [42] this species lives at depths of 0–12 m, with a maximum abundance at 6 m. We confirmed that *Candonopsis kingsleii* prefers the littoral zone of lakes [42]. It was collected at 0.3 and 10.2 m water depth (Figure 6), with a maximum abundance (202 individuals 190 $cm^{-3}$) at 3 m depth. It seems to tolerate water conductivities up to 1000 µS $cm^{-1}$ (Figure 7). Klie [19] reported *C. kingsleii* in Lake Ohrid, but the location and water depth were not indicated. *Prionocypris zenkeri* was found in two samples from 5 m and 8.7 m water depth near the northwest shore, but abundances were very low (1 and 2 individuals 190 $cm^{-3}$) (Figure 6). This area near the village of Kališta is affected by subaquatic and surface springs. This species is known to occur in waters connected to springs and the interstitial habitat of streams, and occasionally enters lakes from nearby streams [42]. *Eucypris virens* was found at 0.3 and 0.5 m water depth in the northeastern part of the lake, as were *D. sinensis* (0.3 m depth), *Eucypris* sp. (0.3 m depth) and *B. reticulata* (0.5 m depth) (Figure 6). None of these species had been reported in the lake before. *Eucypris virens*, *D. sinensis* and *B. reticulata* are species that colonize temporary waters in open fields [42] and it cannot be ruled out that these species immigrated in waters from the northeast part of the Lake Ohrid watershed, which is affected by agricultural land use. We found *Herpetocypris* sp. 2 in one sample from 0.7 m depth. In general, highest species richness was found in the littoral zone (Figure 9), especially in the Bay of Ohrid and near the springs of Kališta. High species richness, diversity and evenness characterize the littoral zone, especially in the northern and eastern parts of the lake (Figures 9–11).

3.2.2.2. Sublittoral (30–50 m)

Few species are restricted to the sublittoral zone. *Candona margaritana* was found down to 35 m and *C. goricensis* down to 50 m. *Candona margaritana* colonized water depths ranging from 20 m to 35 m, but Mikulić [21] found it between 20 m to 100 m. *Cytherissa lacustris* was present in our samples from 30 and 40 m water depth and maximum abundance occurred at 30 m (94 individuals 190 $cm^{-3}$) (Figure 6). Klie [57] reported that it occurs in the littoral zone of lakes, but prefers deeper water, and according to Meisch [42], it inhabits the sublittoral and profundal zones of cold, deep lakes and the littoral and sublittoral zones of high-altitude, alpine lakes. Similar to the littoral zone, high species richness, diversity and evenness were reported for the sublittoral zone, especially at 30 m and 50 m water depth.

3.2.2.3. Profundal (50–280 m)

*Candona trapeziformis* was found at a maximum depth of 163 m and *C. ohrida*, which seems to prefer low conductivities, at a maximum depth of 232 m (Figure 8). All other species of *Candona* and the genera *Fabaeformiscandona*, *Cypria* and *Amnicythere* occurred down to the maximum depth of 280 m. Comparison of our data to those from previous sampling campaigns is difficult because sampling during the earlier projects was restricted to water depths between the littoral and 160 m. One deep sample, from a water depth of 240 m, was analyzed by Klie [19]. *Candona vidua* occurred in our samples from 50 m to 280 m. Klie [19] encountered it at water depths of 40, 160, and 240 m and Mikulić and Pljakić [27] found it between 20 m and 100 m. Ostracode abundances in our study were low at 280 m water depth. The most frequent species was *C. obliqua* (95 individuals 190 $cm^{-3}$), followed by *C. lacustris* (57, 18 and 7 individuals 190 $cm^{-3}$ in three samples), which prefers conductivities <500 $\mu S\ cm^{-1}$ (Figure 7). All other species at 280 m water depth showed low abundances (1–4 individuals 190 $cm^{-3}$). Samples collected near Sveti Naum displayed high species richness. Below 50 m water depth, species richness, diversity and evenness are relatively low, although there are peaks at 110, 135, 200, 220 and 280 m water depth (Figures 9–11).

3.2.2.4. Littoral-Sublittoral Taxa (≤50 m)

We found *C. goricensis* in the littoral and sublittoral zone (0.5, 20, and 50 m), whereas Mikulić [21] found this species only between 20 m and 50 m water depth. *Candona media* was found at 40 and 100 m water depth by Klie [18] and between 20 m and 100 m by Mikulić and Pljakić [27]. This species shows a broad water-depth distribution and a preference for low conductivities (Figure 7). *Candona ovalis* was discovered by Mikulić [21] only between 20 m and 100 m water depth, and our results suggest that it prefers waters with low conductivities. *Paralimnocythere karamani* inhabited water depths to only 54 m. This species and *P. georgevitschi* (a littoral species) were reported from Lake Ohrid by Petkovski [23], but sample depths were not reported. *Cyclocypris ovum* appeared in water depths from 0.3 m to 50 m (Figure 6), with a maximum abundance at 5.5 m (394 individuals 190 $cm^{-3}$). Preferred conductivity is <700 µS $cm^{-1}$. Klie [18] found this species in the lake down to 10 m water depth, and according to Meisch [42] it is most common in the littoral zone of lakes, but also occurs down to water depths of 70 m [57]. As indicated, the littoral and sublittoral zones are characterized by high species richness, diversity and evenness (Figures 9–11).

3.2.2.5. Wide Depth-Range Taxa

The genera *Candona*, *Fabaeformiscandona*, *Cypria* and *Amnicythere* showed the greatest water-depth ranges (Figure 6). *Candona trapeziformis* occurred in our samples from 25 m to 163 m water depth. Klie [18] indicated only "great depth" for locations where it was encountered and Mikulić and Pljakić [27] found this species between 20 m and 100 m. *Candona ohrida* was present between 0.3 m and 232 m water depth. Highest abundances were seen in shallow waters and indicated a preference for water with low conductivity (Figure 8). Holmes [20] collected *C. ohrida* in a shallow pool on a marsh near Lake Ohrid and Mikulić [21] reported it (as *C. sublitoralis*, Table 1) between 20 m and 50 m. We found four species of *Candona* that inhabited the lake from the littoral zone down to 280 m water depth: *C. hadzistei*, *C. marginatoides*, *C. media*, and *C. ovalis*. These species also showed a preference for low conductivities (Figure 7). Mikulić [21] reported *C. hadzistei* (as *C. parvula*, Table 1) in the littoral, sublittoral, and profundal zones. Depth preferences for *C. marginatoides* were not reported in previous studies. *F. krstici* was collected by us from 0.2 m down to 280 m water depth (Figures 6 and 8), whereas Petkovski [26] reported *F. krstici* from the transition between the littoral and the sublittoral zone. We found *C. lacustris* and *C. obliqua* from 0.1 m down to 280 m depth. *Cypria lacustris* was reported by Petkovski [22] in deeper parts of the lake and *C. obliqua* by Klie [17] at 40, 100, and 120 m water depth. *Amnicythere karamani* inhabited water depths from 0.4 down to 280 m (Figures 6 and 7) and Klie [17] collected this species only at 40 m. This species was found in waters with high conductivities (Figure 7). Most species of *Paralimnocythere* occurred down to a maximum water depth of 100 m (Figure 6), but *P. alata* appeared only in one sample taken from 100 m. Klie [17] found this species at 120 m water depth, but also at 40 m. We discovered maximum abundances of *P. ochridense* and *P. slavei* in the littoral zone, but we also found individuals down to water depths of 100 m (Figures 6, 8). Generally, the diversity of candonids was higher in the sublittoral and profundal zones and lower in the littoral zone. This partially confirmed the results of Mikulić and Pljakić [27] who also found highest candonid diversity in the sublittoral zone.

### *3.3. Biogeography and Anthropogenic Impact on Ostracode Distribution*

The endemic ostracode species in Lake Ohrid are highlighted in Box 1. Fourteen of 32 collected species displayed well-preserved soft parts, and five were identified only from hard parts. Martens [53] reported 38 species of which 25 species (66%) were endemic, and mentioned that two thirds of the Ohrid endemics belong to the family Candonidae. Our findings indicate 44% of the Ohrid ostracodes are endemic and 79% of the endemics (11 species) belong to the Candonidae. *Candona marginatoides*, *C. obliqua*, *A. karamani* and *P. ochridense* occur in Lakes Ohrid and Prespa [22]. *Darwinula stevensoni* is the only truly cosmopolitan species in the lake. *Candonopsis kingsleii*, *Cyclocypris ovum*, *I. bradyi*, *E. virens*, *B. reticulata*, and *C. lacustris* are found in the Holarctic, *D. sinensis* in the Palaearctic (Eurasia), and *P. zenkeri* in Europe and Asia Minor (Turkey) [42]. *Paralimnocythere karamani* is known from Lakes Ohrid and Prespa and was also found in a spring and a river in the Skadar Valley, Montenegro [58]. *Candona bimucronata* inhabits waters in the montane, subalpine and high alpine areas of Macedonia and Montenegro and it is also common in the mountains of Bosnia, Kosovo, and Slovenia [55,56]. *Cypria lacustris* was described from areas including Iran, Bulgaria, Montenegro, Serbia, Croatia and Slovenia [22]. Meisch [42] mentioned that *C. lacustris* and *C. ophtalmica* appear to be extreme forms of a single species that is variable in autecology, carapace shape, structure of the penis, male clasping organs and all genital processes of the female. We propose that *C. lacustris* and *C. ophtalmica* are two different species because the genital processes of the female specimens differ; *C. ophtalmica* has two processes and *C. lacustris* has only one. But because the differences between processes show variations, it will be necessary to carry out DNA analysis to determine if this is one or two taxa. *Cypria ophtalmica* is a nearly cosmopolitan species, absent only from Australia.

Our findings regarding the diversity of endemic species contradict the results of Martens [1]. He reported that endemic diversity is higher in the littoral zone than in the sublittoral or profundal zones of ancient lakes. We found cosmopolitan species and those distributed in the Holarctic or the Palaearctic in <50 m water depth, whereas species endemic to the lake occurred mostly in deeper water. Few lakes in the world possess living ostracodes at great water depths, which highlights the importance of this finding. Live specimens were also reported at water depths >40 m in Lake Petén Itzá, Guatemala [59]. Pérez *et al*. [60,61] also showed that water depth and conductivity are the factors that most influence species distribution in water bodies on the neotropical Yucatán Peninsula.

Ostracodes in Lake Ohrid show a distribution pattern similar to that for Gastropoda [32], in that the most abundant snail species are endemic and non-endemic species are relatively rare. Endemic snail species displayed a broad water-depth range, whereas non-endemic species were largely restricted to shallow waters, down to only ~5 m. Furthermore, most of the widespread species, which were previously unknown from the lake, occur mainly at the north end where the two largest Macedonian cities are located (Ohrid and Struga), and where much land is in agriculture. This may be evidence for increasing anthropogenic pressure on the lake, because cosmopolitan species often replace rare endemic taxa [62]. Kostoski *et al*. [63] also pointed out that anthropogenic pressure threatens endemic species. Pieri *et al*. [64] found that wastewater discharge in the Ledra River Basin (NE Italy) systematically causes replacement of local, rare ostracode species by common species. Because all the cosmopolitan species found in this survey were not reported in previous studies, we suspect that recent increases in anthropogenic pressures, combined with the wider tolerance ranges of cosmopolitan species, is leading to replacement of endemic species by widespread taxa in the littoral zone. Endemic ostracode species were relatively evenly distributed in the lake and did not show preferences for areas influenced by spring inflows, as mentioned by Matzinger *et al.* [37] and Matter *et al.* [38].

## 4. Conclusions

Lake Ohrid is characterized by high ostracode species richness. The most speciose genus in the lake is *Candona* (12 living species) and the second most prominent genus is *Paralimnocythere* (five living species). The "young" ancient lake status was confirmed by the prevalence of species flocks of Limnocytherinae and Candonidae. *Cypria lacustris* displayed the highest abundances (2468 individuals 190 $cm^{-3}$) among encountered species. Species abundances at 280 m water depth were relatively low, with highest values of 95 individuals 190 $cm^{-3}$ (*C. obliqua*). Ostracode species living at 280 m water depth include *C. obliqua, C. hadzistei*, *C. marginatoides*, *C. media*, *C. ovalis*, *C. vidua*, *F. krstici*, *C. lacustris*, and *A. karamani*. Highest species richness was detected at 5 m water depth (15 species). Lower species richness in deep waters may reflect extreme environmental conditions (e.g., low oxygen content), and higher richness in the littoral zone may be a consequence of high $O_2$ and diverse microhabitats. The water-depth distribution of ostracodes in the lake is probably a consequence of multiple interacting factors including oxygen concentration, water temperature, bottom substrate and food availability. In addition, ostracodes adapt to the environment by being mobile. Eight species were detected in the lake for the first time: *C. bimucronata*, *I. bradyi*, *E. virens*, *Eucypris* sp., *P. zenkeri*, *B. reticulata*, *Herpetocypris* sp. 2, and *D. sinensis*. These species and all the other cosmopolitan species found in the lake are restricted to water depths <50 m. However, given the increasing anthropogenic pressure on Lake Ohrid and the large tolerance ranges of some cosmopolitan species, we expect that endemic ostracodes will be progressively replaced by widespread species. The presence of *A. karamani* may be important for providing insights into the origin of Lake Ohrid. Although *Amnicythere* species usually inhabit oligo-haline to meso-haline waters, some species are marine, which may suggest a marine origin for the lake. Recent studies, however, indicate fluvial/glaciofluvial sedimentation at the onset of Lake Ohrid.

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## Author Contributions

This study was designed and conducted by Antje Schwalb and Julia Lorenschat. Julia Lorenschat organized and carried out the field trips, laboratory analysis (identification and counting), statistical analysis and manuscript writing. Antje Schwalb provided funding for the development of this study (DFG project) and made important revisions to the submitted manuscript. Liseth Pérez was the corresponding author and was in charge of the manuscript final corrections suggested by multiple reviewers. Alexander Correa-Metrio was responsible for all statistical analyses and graphs, and Ullrich von Bramann provided the lake bathymetric data. Mark Brenner contributed with manuscript editing and language improvement. All authors read and approved the final manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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