

This discussion paper is/has been under review for the journal Biogeosciences (BG).
Please refer to the corresponding final paper in BG if available.

Testing the spatial and temporal framework of speciation in an ancient lake species flock: the leech genus *Dina* (Hirudinea: Erpobdellidae) in Lake Ohrid

S. Trajanovski¹, C. Albrecht², K. Schreiber², R. Schultheiß², T. Stadler³,
M. Benke², and T. Wilke²

¹Hydrobiological Institute Ohrid, Naum Ohridski 50, 6000 Ohrid, Republic of Macedonia

²Department of Animal Ecology & Systematics, Justus Liebig University,
Heinrich-Buff-Ring 26-32 IFZ, 35392 Giessen, Germany

³Institute of Integrative Biology, Swiss Federal Institute of Technology, Universitätsstrasse 16,
8092 Zürich, Switzerland

Received: 21 May 2010 – Accepted: 7 June 2010 – Published: 1 July 2010

Correspondence to: T. Wilke (tom.wilke@allzool.bio.uni-giessen.de)

Published by Copernicus Publications on behalf of the European Geosciences Union.

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

Ancient Lake Ohrid on the Balkan Peninsula is considered to be the oldest ancient lake in Europe with a suggested Plio-Pleistocene age. Its exact geological age, however, remains unknown. Therefore, molecular clock data of Lake Ohrid biota may serve as an independent constraint of available geological data, and may thus also help to refine age estimates. Such evolutionary data may also help unravel potential biotic and abiotic factors that promote speciation events.

Here, mitochondrial sequencing data of one of the largest groups of endemic taxa in Lake Ohrid, the leech genus *Dina*, is used to test whether it represents an ancient lake species flock, to study the role of horizontal and vertical barriers in Lake Ohrid for evolutionary events, to estimate the onset of intralacustrine diversification in this group based on molecular clock analyses, and to compare this data with data from other endemic species for providing an approximate time frame for the origin of Lake Ohrid.

Based on the criteria speciosity, monophyly and endemism, it can be concluded that Lake Ohrid *Dina*, indeed, represents an ancient lake species flock. Lineage sorting of its species, however, does not seem to be complete. Analyses of population structures of *Dina* spp. in the Ohrid watershed indicate a horizontal zonation of haplotypes from spring and lake populations, corroborating the role of lake-side springs, particularly the southern feeder springs, for evolutionary processes in endemic Ohrid taxa. Vertical differentiation of lake taxa, however, appears to be limited, though differences between populations from the littoral and the profundal are apparent. Molecular clock analyses indicate that the most recent common ancestor of extant species of this flock is approximately 1.99 ± 0.83 Ma old, whereas the split of the Lake Ohrid *Dina* flock from a potential sister taxon outside the lake is estimated at 8.30 ± 3.60 Ma. Comparisons with other groups of endemic Ohrid species indicated that in all cases, intralacustrine diversification started ≤ 2 Ma ago. Thus, this estimate may provide information on a minimum age for the origin of Lake Ohrid. Maximum ages are less consistent and generally less reliable. But cautiously, a maximum age of 3 Ma is suggested. Interestingly, this time

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



frame of approximately 2–3 Ma for the origin of Lake Ohrid, generated based solely on evolutionary data, well fits the time frame most often used in the literature by geologists. Future studies must show whether this concurrence holds true.

1 Introduction

Analyses of major diversification events in ancient lakes, that is, lakes that have continuously existed for a hundred thousand or even million years, play a major role in studies of speciation and radiation patterns (e.g., Brooks, 1950; Martens et al., 1994; Martens, 1997; Rossiter and Kawanabe, 2000). From a temporal point of view, ancient lakes constitute prime systems for studying evolutionary events over geological time scales. Due to their prolonged existence, for example 35 million years (Ma) for Lake Baikal, they have accumulated thick sedimentary layers, which often provide an excellent paleoenvironmental record (e.g., Cohen, 2003). Moreover, since these lake basins typically constitute relatively closed systems, they are ideal for documenting the effects of environmental events on endemic lake faunas. Examples for such events acting on local and regional scales include lake-level fluctuations (e.g., Cohen, 1994; Cohen et al., 2007; Schultheiß et al., 2009), salinity changes (Cohen, 2003), and volcanic eruptions (Degens et al., 1973; Wagner et al., 2008; Sulpizio et al., 2010). On a global scale, tectonic and oceanic events, climate change, anoxia, and meteoric impact are among the most influential physical disturbances that are thought to have triggered biotic changes (e.g., Benton, 2009).

The deep drilling programs in Lake Baikal, for example, have revealed cyclicities of 20 000, 40 000, and 100 000 years in physical parameters, all of which are related to the three Milankovich orbital parameters (e.g., Kashiwa et al., 1999). In fact, continental deep drilling programs have been conducted in several ancient lakes, opening a new era of exploration of lake histories (e.g., Scholz et al., 2007).

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Comparisons of drilling core and other geological data with dated molecular phylogenies of extant endemic species, however, often indicate severe discrepancies between old lakes with a long limnological history on the one hand and young endemic faunal elements on the other. This is because catastrophic environmental events may have repeatedly wiped out or at least depleted ancient lakes faunas. Examples include Lake Malawi (e.g., Sturmbauer et al., 2001; Schultheiß et al., 2009; Genner et al., 2010), Lake Kivu (e.g., Degens et al., 1973), and Lake Titicaca (e.g., Mourguiart and Montenegro, 2002). Even for the oldest lake in the world, Lake Baikal, researchers have shown that endemic faunal elements are often one order of magnitude younger than the lake itself (e.g., Sherbakov, 1999; Wilke, 2004). One of the few ancient lakes for which no such discrepancy has been reported so far, is the European ancient Lake Ohrid situated on the Balkan Peninsula (for a review of the limnology and biodiversity of Lake Ohrid see Albrecht and Wilke, 2008). Several ancient lake species flocks, that is, relatively species-rich monophyletic groups of endemic species (Greenwood, 1984), have been reported for Lake Ohrid that very likely evolved within the lake and that are up to 2 Ma old. At the same time, many researchers suggest a lake age of 2–3 Ma (see Discussion for details). The problem, however, is that the latter estimate is largely based on preliminary geological data that is about 100 years old (i.e., Cvijić, 1911; also see the Discussion for details). Therefore, these estimates have to be treated with caution pending further investigation. In fact, evolutionary data on Lake Ohrid biota may serve as an independent constraint to available geological data, and may thus help refining age estimates.

The second dimension of speciation events in Lake Ohrid besides time – the spatial one – might also be closely linked to geological and environmental events such as lake-level fluctuations and major earthquakes. Within the Ohrid watershed, different spatial scales of endemism have been proposed by Albrecht and Wilke (2008):

- species endemic to Lake Ohrid and its adjacent springs,
- species endemic to the surrounding springs,

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- species endemic to the lake proper, and
- species endemic to parts of the lake separated by horizontal and vertical barriers.

Previous studies of selected species flocks in Lake Ohrid (e.g., Albrecht et al., 2006; Wilke et al., 2007; Wysocka et al., 2008) have provided first insights into patterns of allopatric and parapatric speciation in Lake Ohrid. Two of the most interesting findings are that i) adjacent springs of Lake Ohrid (particularly the southern feeder springs around Sv. Naum on the Macedonian side and Zagorican/Tushemisht on the Albanian side) harbour endemic species, some of them being regarded as sister species to lake taxa, and ii) there are distinct vertical zones within the lake presumably promoting either parapatric speciation along geographical/ecological gradients or even “micro-allopatric” speciation due to bathymetrical barriers (reviewed in Albrecht and Wilke, 2008).

A comprehensive picture of patterns and processes of speciation in Lake Ohrid, however, is still missing and the role of vertical and horizontal differentiation is still subject to controversial discussions. This is partly due to the facts that i) only a limited number of species flocks have been studied so far within a rigorous phylogenetic framework, ii) most flocks are relatively small, typically comprising only a handful of species, and iii) there are only very few groups that inhabit all key habitats in Lake Ohrid ranging from the littoral to the deepest parts of the profundal.

A group that fulfils the latter two criteria is the leeches (Hirudinea) of Lake Ohrid (Sket and Šapkarev, 1992). According to these authors, Lake Ohrid and its surrounding springs are inhabited by 26 species (12 of them being endemic), which belong to 5 families: Piscicolidae (2); Glossiphoniidae (9); Hirudinidae (1), Haemopidae (1), and Erpobdellidae (13). Sket (1989) first drew attention to a potentially endemic radiation in Lake Ohrid hirudinids, describing the intralacustrine speciation within the genus *Dina* (family Erpobdellidae). The putative species flock is characterized by a high degree of anatomical similarities among its members. However, body shape variation is considerable, making determination a difficult task (Sket, 1989). Interestingly, there are pronounced seasonal changes in the distribution of *Dina* spp. and preliminary data

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



suggests both a vertical and horizontal separation of taxa within the lake (e.g., Sket, 1989).

Phylogenetic relationships of worldwide Hirudinea have recently been investigated (e.g., Trontelj et al., 1996; Siddall and Burrenson, 1998; Apakupakul et al., 1999; Pfeiffer et al., 2004, 2005; Utevsky and Trontelj, 2004; Phillips and Siddall, 2009). Some studies also included the Erpobdellidae (Trontelj and Sket, 2000; Sket et al., 2001; Siddall, 2002; Borda and Siddall, 2004). However, no similar analyses exist as yet for the putative *Dina* flock in Lake Ohrid. This is very unfortunate because this flock is not only one of the very few presumed hirudinid ancient lake species flocks in the world, it also includes a sufficient number of species for testing patterns and processes of speciation in Lake Ohrid.

Here we used DNA sequences from the mitochondrial cytochrome *c* oxidase subunit I (COI) gene from Lake Ohrid *Dina* spp. and related taxa in order to:

1. test whether *Dina* spp., do indeed, represent an ancient lake species flock,
2. study the role of horizontal and vertical barriers in Lake Ohrid for speciation in this group,
3. estimate the onset of intralacustrine diversification in this group based on molecular clock analyses, and
4. compare this data with data from other endemic species groups to provide an approximate time frame for the origin of Lake Ohrid solely based on evolutionary data.

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



2 Materials and methods

2.1 Specimens studied

Specimens of nominal *Dina* spp. were collected from the Lake Ohrid area (lakes Ohrid, Prespa, and Mikri Prespa, including adjacent springs) between 2004 and 2009 (Fig. 1). Where possible, we included topotypical materials (Table 1). In addition, we obtained specimens of the type species of the genus *Dina*, *D. lineata*, from Germany as well as topotypes of *D. lineata lacustris* from a small glacial lake some 34 km north of Lake Ohrid. For comparison, we also studied nominal *Dina* species from Greece and Spain as well as representatives of the closely related genus *Erpobdella*. Specimens of *Glossiphonia complanata* and *Batrachobdella algira* (both family Glossiphoniidae) were used for outgroup comparison. Genetic information of additional taxa (including *Erpobdella* spp., *Trocheta* spp., and *Mooreobdella melanostoma*) were obtained from GenBank (Fig. 2).

Most of the specimens, especially from the littoral, were collected using a rectangular dredge (frame dimension 400×200 mm) with a mesh size of 0.5 mm. A van Veen grab (225 cm²) was utilized for collecting samples from the profundal of Lake Ohrid (depths 120, 130, 180, and 200 m). Additionally, traps were prepared in Lake Ohrid using plastic bottles filled with fish bait. Specimens from springs were gathered by hand picking from stones. If possible, taxa were identified in the field by ST with a field stereomicroscope based on the morphological (e.g., size, colour, suckers) and anatomical (e.g., atrium, cornea, testes) characteristics suggested by Šapkarev (1975, 1990), Lukin (1976), Sket (1981), Sket and Šapkarev (1986), and Nesemann and Neubert (1999). After identification, specimens were preserved in 80% ethanol for DNA work.

2.2 DNA isolation and sequencing

The method described in Wilke et al. (2006) was used for isolating DNA from individual leeches. The primers for amplifying a fragment of the COI gene with a target length

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



of 658 base pairs (excluding 51 bp primer sequence) were LCO1490 and HCO2198 as described by Folmer et al. (1994). It should be noted that COI is the mitochondrial marker most often used in annelid molecular studies (Halanych and Janosik, 2006). Sequences (forward and reverse) were determined using the LI-COR (Lincoln, NE) DNA sequencer Long ReadIR 4200 and the Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ). The protein-coding COI sequences were unambiguously aligned in BioEdit 7.0.4.1 (Hall, 1999).

The first base pairs behind the 3' end of each primer were difficult to read. We therefore uniformly cut off these parts, leaving a 592 bp-long completely overlapping fragment for the COI gene. The 109 newly created sequences (GenBank accession numbers HM246525–HM246633) were used for a hierarchical set of phylogenetic and phylogeographical analyses, together with 12 sequences obtained from GenBank (for GenBank accession numbers see Fig. 2).

2.3 Phylogenetic and phylogeographical analyses

2.3.1 Phylogenetic analyses

A family-level phylogenetic analysis was performed using a dataset of 47 sequences from representatives of *Dina* spp., *Erpobdella* spp., *Trocheta* spp., and *Mooreobdella* sp., with *Glossiphonia complanata* and *Batracobdella algira* as outgroup taxa. The purpose of this analysis was to infer the position of *Dina* spp. from the Ohrid watershed within the family Erpobdellidae and to test whether they represent an ancient lake species flock sensu Greenwood (1984) and Schön and Martens (2004). Based on preliminary phylogenetic analyses, we included only 16 taxa from Lake Ohrid in this analysis in order to avoid a possible shift of the performance of the model selection, and thus the actual phylogenetic analysis, towards intraspecific relationships. These 16 taxa comprise 7 nominal species as well as 9 undetermined haplotypes.

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



is, whether a strict molecular clock can be assumed (recall that the larger family-level dataset did not show signs of saturation). As model-choice criterion, we used the Bayes factor (see Ronquist and Deans, 2010, for a review). The Bayes factor B is the ratio of the marginal likelihoods of two given models M_0 and M_1 . If the models differ by a factor of $2 \ln B > 10$, then model M_0 is strongly favoured (Kass and Raftery, 1995). The marginal likelihood can be approximated by the harmonic mean of the likelihood values from an MCMC sample. According to Hipsley et al. (2009), an advantage of the Bayes factor compared to likelihood ratio tests is that it automatically penalizes complex models, thus guarding against overfitting.

With harmonic means of $-\ln=2091.07$ and $-\ln=2095.27$ for the clock and no-clock models and a Bayes factor of -8.4 , the more complex model (i.e., the one without clock assumption) was not favoured. The less parameter-rich strict-clock model therefore appears to be appropriate.

In the absence of a *Dina*-specific substitution rate, we then estimated divergence times from all clock-enforced trees of both Bayesian runs (except burn-in), utilizing the trait specific COI Protostomia molecular clock rate of $1.24\% \pm 0.22\%$ Ma for the HKY model suggested by Wilke et al. (2009). This rate has been shown to be robust among invertebrate taxa with similar biological and life-history characteristics (i.e., dioecious subtropical or tropical taxa with a generation time of ~ 1 year and a body size of approximately 2–50 mm). All these criteria are met by *Dina* spp. from the Ohrid area.

Finally, we calculated 95% confidence intervals for our time estimates including both the error of the phylogenetic analyses (in terms of branch-length variation of individual trees) and the error of the clock rate (see above) by utilizing a propagation of uncertainty approach (for details see Wilke et al., 2009; also see Wilke et al., 2010).

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



It should be noted that we did not correct our clock estimates for ancestral polymorphism since the universal Protostomia COI clock applied here is also not corrected (see Wilke et al., 2009). As the Protostomia clock, however, is based on phylogenetic events that are, on average, 3 Ma old, there might be a small bias towards the overestimation of divergence times for events younger than 3 Ma and towards underestimation for events older than that.

2.3.3 Network analysis

For phylogeographical analyses (both network analyses and tests for genetic structure) of *Dina* spp. within the Ohrid watershed, we used sequences from a total of 87 specimens (from hereon called the *Dina* dataset). First, a statistical parsimony haplotype network was constructed utilizing the program TCS 1.21 (Clement et al., 2000), with the connection limit set to 95% and gaps being treated as fifth state. Then, we ran a second analysis, reducing the connection limit to 90% in an attempt to infer connections among sub-networks.

2.3.4 Test for genetic structure

We used the *Dina* dataset to perform an exact test as suggested by Raymond and Rousset (1995) and implemented in Arlequin 3.5.1.2 (Excoffier et al., 2005) to study possible genetic differentiations among taxa inhabiting different habitats (i.e., springs, littoral, sublittoral, profundal) and the role of horizontal and vertical barriers in Lake Ohrid. We feel that this test is appropriate as lineage sorting does not seem to be complete in Lake Ohrid *Dina* spp. (see Results for details). The assumed null hypothesis of the test statistics is a random distribution of different haplotypes among groups (i.e., panmixia). The significance of pairwise differentiation of specimens from different habitats was evaluated by running the Markov chain for 1 000 000 steps.

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



3 Results

3.1 Phylogenetic analyses

The family-level phylogenetic analysis utilizing 47 sequences from representatives of *Dina* spp., *Erpobdella* spp., *Trocheta* spp. and *Mooreobdella* sp. resulted in the majority-rule consensus tree shown in Fig. 2. In this tree, basal relationships are poorly resolved, resulting in a large polytomy of several non-Ohrid taxa. All clades harbouring specimens of *Dina* spp., however, are extremely well resolved, all with Bayesian posterior probabilities (BPP) of 1.00. Accordingly, nominal *Dina* spp. are not monophyletic but cluster in four different clades. The first “*Dina*” clade comprises specimens of *D. latestriata* from Lake Mikri Prespa (topotypes) and Lake Prespa as well as a closely related taxon (here called *D. cf. latestriata*) from the Southern Balkan Lake Trichonis. The second “*Dina*” clade consists of the two specimens of *D. punctata* studied from Spain. The third clade is made up of specimens of the type species of *Dina*, *D. lineata*, comprising representatives of nominal *D. l. lineata* from Germany and topotypes of *D. l. lacustris* from a small glacial lake north of Lake Ohrid. The fourth and largest *Dina* clade in our study contains all and only *Dina* specimens from Lake Ohrid (incl. feeder springs). Thus, Lake Ohrid *Dina* forms a monophyletic group. This fourth clade is sister to the *Dina* clade comprising the type species of the genus. The sister-group relationship is supported by a BPP of 1.00. The *Dina* clade from Lake Ohrid shows a very shallow phylogenetic structure and nominal taxa are, in part, not well resolved.

For this and subsequent analyses, we labelled specimens that resembled a known morphospecies but which could not be assigned unambiguously with the epithet *conferre* (cf.). Morphologically and genetically distinct specimens that could not be assigned to previously described species were referred to as “sp.” (also see Table 1).

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



3.2 Molecular clock analyses

The molecular clock analyses (Fig. 2) indicate that the monophyletic group of endemic *Dina* species from the Lake Ohrid watershed (hence called Lake Ohrid *Dina* flock) is relatively young. The most recent common ancestor (MRCA) of extant species of this flock is estimated to be 1.99 Ma old with a 95% confidence interval of ± 0.83 Ma.

This estimate is based on an average node depth of 0.0246 ± 0.0093 and a clock rate of $0.0124 \pm 0.0022 \text{ Ma}^{-1}$ for the HKY model (see Materials and methods section).

The split of the Lake Ohrid *Dina* flock from its presumed sister taxon outside the lake (i.e., *Dina lineata*) is estimated to have occurred 8.30 Ma ago with a confidence interval of ± 3.60 Ma (average node depth 0.129 ± 0.041).

3.3 Network analyses

The network analysis applying a 95% connection limit resulted in four distinct sub-networks (Fig. 3). The first and by far the largest sub-network consists of all specimens belonging to *D. cf. kuzmani*/cf. *eturpschem*, *D. cf. profunda*, *D. cf. svilesta*, *D. krilata*, *D. lepinja*, *D. lyhnida*, *D. ohridana* as well as four undetermined haplotypes (*Dina* spp.). The second sub-network comprises eleven undetermined specimens with a total of seven haplotypes, whereas sub-networks three and four comprise only one undetermined haplotype. If the connection limit is lowered to 90%, all sub-networks became interconnected (see grey lines in Fig. 2).

On the one hand, a number of specimens could not be unambiguously assigned to nominal species and substitutional differences among species are generally low. On the other hand, specimens of most morphologically distinct taxa share haplotypes or cluster together. This observed pattern, together with a number of reticulations seen in the networks, indicates that lineage sorting in Lake Ohrid *Dina* spp. is still not fully complete.

The assignment of character states for horizontal (springs) and vertical (littoral, sublittoral, profundal) distribution to haplotypes shows that haplotypes are frequently

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



shared by specimens collected from different vertical zones such as littoral/sublittoral as well as sublittoral/profundal. There are, however, no haplotypes shared by specimens from all three vertical zones and there are no clusters of specimens from the littoral and profundal. As for the horizontal distribution (i.e., springs vs. lake proper), all spring haplotypes are highly distinct from lake haplotypes except for the most common haplotype in our dataset, which is shared by a total of 14 specimens from springs and the lake itself.

3.4 Test for genetic structure

The exact test for pairwise sample differences based on haplotype frequencies did not reject the global null hypothesis of panmixia of population from different vertical zones (Table 2). However, there are significant pairwise differences between haplotypes from springs and all lake habitats.

4 Discussion

4.1 Phylogenetic position of the Lake Ohrid *Dina* flock

In Europe, the Erpobdellidae is the predominant leech family with three species-rich nominal genera, *Erpobdella* Blainville, 1918, *Trocheta* Dutrochet, 1817, and *Dina* Blanchard, 1892. The latter genus comprises at least 24 species and subspecies (Nesemann and Neubert, 1999; Grosser et al., 2007; Grosser and Eiseler, 2008).

Systematics and phylogeny of the family, however, are still discussed controversially. Siddall (2002), for example, has argued that the morphological and anatomical characters used to define genera of Erpobdellidae were not reliable. He also found, based on molecular data, that the genus *Dina* is not monophyletic. Therefore, he formally synonymised the genera *Dina*, *Mooreobdella*, *Nephelopsis*, and *Trocheta* under the genus *Erpobdella*. Whereas some researchers followed this suggestion (e.g., Borda

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



and Siddall, 2004; Oceguera-Figueroa et al., 2005), other workers argued that there are morphological characters that can be used to distinguish at least some of the above genera (e.g., Hovingh, 2004) and continued to treat *Dina* and *Trocheta* as valid (e.g., Pfeiffer et al., 2005; Grosser et al., 2007; Grosser and Eiseler, 2008).

Whereas we did find European *Dina* to be paraphyletic in our dataset as previously suggested by Siddall (2002), we also found *Dina* specimens from Lake Ohrid to form a monophyletic clade with the type species of *Dina*, *D. lineata*, as its sister taxon. Moreover, *Dina* spp. from Lake Ohrid could be unambiguously distinguished from *Erpobdella*, sympatrically occurring in the lake (see Fig. 1). Pending a comprehensive revision of the family Erpobdellidae, we therefore prefer to retain the genus name *Dina* for the clade comprising the endemic Lake Ohrid taxa and the type species of the genus.

Our phylogenetic analysis not only showed the Ohrid *Dina* species to be monophyletic, it also revealed a number of genetically (and morphologically) distinct taxa that may represent new species. Sket (1989) and Sket and Šapkarev (1992) had already suspected the existence of at least two undescribed species. The occurrence of cryptic species is not unusual in leeches as demonstrated recently for the medicinal leeches (Trontelj and Utevsky, 2005; Siddall et al., 2007). Overall, the total number of species within the Lake Ohrid *Dina* flock remains unknown, largely because of a conflict between morphological and molecular data (which, in turn, could be due to incomplete lineage sorting). Thus future studies based on detailed anatomical analyses and fast-evolving nuclear markers would have to address this problem.

Interestingly, we also found one taxon, "*Dina*" *latestriata*, which had been described from Lake Mikri Prespa, not to belong to the Ohrid group (Fig. 2), corroborating the faunal distinctness of Lake Ohrid. Such distinctness has been suggested before based on biogeographical and phylogenetical studies in diverse taxa (e.g., Albrecht and Wilke, 2008; Hauswald et al., 2008; Schultheiß et al., 2008; Albrecht et al., 2009; Wilke et al., 2010).

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



As to the question whether the Ohrid group, indeed, represents an ancient lake species flock, the conservative species flock definition by Greenwood (1984) is based on the following criteria: speciosity, monophyly and endemcity. All these criteria are met by the *Dina* species in Lake Ohrid. For this reasoning, we fully support Sket (1989) in that the endemic species of Lake Ohrid represent an ancient lake species flock.

It should be noted that *D. lineata*, the presumably widespread type species, is also mentioned in the literature for Lake Ohrid (e.g., Sket and Šapkarev, 1992), though the researchers state that they are not certain as to the species identification because only juvenile specimens were studied. Our sampling indicates that *D. lineata* occurs in the vicinity of Lake Ohrid, but we were unable to find it in the actual lake.

4.2 Horizontal and vertical zonation of *Dina* spp. in Lake Ohrid

The relatively high rate of intralacustrine speciation within the Lake Ohrid *Dina* flock raises questions about whether there are distinct horizontal and vertical barriers or gradients in the lake that may have promoted evolutionary events. The role of such barriers has been discussed in the literature for more than 50 years (e.g., Hadžišče, 1956; Hubendick, 1960; Radoman, 1985). Two of the most prominently considered barriers in the Ohrid watershed are the horizontal differentiation of species occurring in springs and the lake proper and the vertical differentiation of taxa (species and sub-species) occurring above and below the *Chara* belt (i.e., an almost continuous belt of *Chara* algae in the lower littoral, impeding migration of benthic invertebrates; for details see Albrecht and Wilke, 2008).

Whereas several cases of horizontal zonations between springs and the lake proper have been reported (e.g., Radoman, 1983; Albrecht et al., 2006, 2008; Albrecht and Wilke, 2008; Wysocka et al., 2008; also see Haufe et al., 2010), strong vertical barriers or gradients within the lake remain mostly hypothetical (e.g., Hadžišče, 1956; Hubendick, 1960; Radoman, 1985; Albrecht and Wilke, 2008; but see Wysocka et al., 2008). Part of the problem is that, as mentioned in the Introduction, there are only very

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



few groups that inhabit all vertical zones of Lake Ohrid, ranging from the littoral to the profundal.

Our findings for the horizontal zonation of haplotypes from spring and lake populations corroborate the role of lake-side springs, particularly of the southern feeder springs, for evolutionary processes in endemic Lake Ohrid taxa. This, in turn, raises questions as to the role of isolation of lake and spring populations for allopatric/parapatric speciation, and why these springs, which probably were repeatedly inundated by the lake during evolutionary times (for lake-level fluctuations see Lezine et al., 2010; Lindhorst et al., 2010), continue to harbor highly distinct haplotypes. Some researchers have even raised the question as to whether the feeder springs of Lake Ohrid are possibly older than the actual lake, and whether these spring populations may be the ancestors of the respective lake faunas (e.g., Albrecht et al., 2006; Albrecht and Wilke, 2008). While we see no good evidence for a common spring origin of lake taxa in our dataset, we would like to raise awareness about some of the old haplotypes or groups of haplotypes in spring populations of *Dina* spp. and advocate future detailed population genetic studies of these highly interesting populations.

Regarding a vertical differentiation of lake taxa, population differences among the vertical zones studied appear to be small (Table 2). Nonetheless, some structure is obvious from the vertical (littoral, sublittoral, profundal) distribution of haplotypes plotted in Fig. 3. Accordingly, specimens from the littoral and profundal never share haplotypes, whereas specimens from adjacent depth zones do. However, haplotypes from profundal specimens are much less distinct than haplotypes from spring specimens (see above). Therefore, we have some doubts whether these specimens from greater depths have significantly contributed to the *Dina*-diversity seen in Lake Ohrid today, and we see no evidence that these specimens hold a reservoir function within Lake Ohrid (i.e., that they enabled the survival of relict lineages during massive environmental changes such as strong lake level fluctuations).

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



4.3 Evolutionary evidence and the time frame for the origin of Lake Ohrid

As stated in the Introduction, the exact age of Lake Ohrid is unknown (for a review of available data see Albrecht and Wilke, 2008). Whereas there is newer geological data for the time frame for the formation of the Ohrid Graben (i.e., 5–8 Ma; Dumurdzanov et al., 2004), most information about the limnological age of the actual lake is directly or indirectly based on geological studies of Cvijić (1911: 716), who wrote that “the Ohrid valley probably originated during the Pliocene”. Later, Stanković (1960) referred to Cvijić (1911) and suggested that the Ohrid Basin was formed at the end of the Pliocene. Other researchers (e.g., Spirkovski et al., 2001) even considered an age of Lake Ohrid of up to 10 Ma, but without stating on which evidence this information is based. There is, however, new data for the limnological age of Lake Ohrid from Lindhorst et al. (2010). Based on sediment thickness and estimated sedimentation rates, the authors suggest a minimum age of 1.1 Ma (Fig. 4).

Given the conflicting geological information on the age of Lake Ohrid, molecular clock estimates of major evolutionary events in Lake Ohrid species flocks may help to refine these dates by establishing lower and upper constraints.

The upper constraint could come from the onset of speciation in those species flocks. If we assume that speciation occurred intralacustrine, the first divergence event in the respective flock would constitute the minimum age of Lake Ohrid because at this time, the lake must have already existed. In contrast, if we calculate the divergence time of the MRCA of lake taxa with the presumed sister taxon from outside the lake then we would receive a potential maximum age for the lake. Of course, to do so, we would have to assume that the divergence event happened outside the lake and that the lake did not already exist at that time. Whereas data on, for example, the population structure and ecology of the taxa involved may help to provide some evidence for that, these assumptions appear to be difficult to test. Therefore, the lower constraint suggested here should be treated with caution. Moreover, missing taxa (either not sampled or already extinct) may also affect the clock estimations. However, in this case, the test

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



statistic suggested here is highly conservative. If, for example, a particular species flock is not completely sampled, then this could lead to an underestimation of divergence times and therefore of the minimum age of Lake Ohrid, but never to an overestimation (for details of potential sampling biases in molecular clock estimations see Fig. 5 in Wilke et al., 2009). In contrast, if the actual sister species of a particular species flock is not sampled but only a more distantly related taxon instead, then this could lead to an overestimation (but not underestimation) of divergence times and therefore the maximum age of Lake Ohrid (see Wilke, 2004 and Wilke et al., 2009 for details).

The molecular clock analyses conducted here for Lake Ohrid *Dina* (Fig. 2) indicate that the MRCA of extant species of this flock is approximately 1.99 Ma old with a confidence interval of ± 0.83 Ma. The age of the split of the Ohrid *Dina* flock from its sister taxon outside the lake (i.e., *Dina lineata*) is estimated at 8.30 ± 3.60 Ma. The upper limit shows good concordance with the minimum ages for other endemic taxa available in the literature (Fig. 4) ranging from approximately 1.3 Ma for the limpet genus *Acroloxus* in Lake Ohrid (Albrecht and Wilke, 2006) to some 2 Ma for the endemic *Salmo trutta* complex (Sušnik et al., 2006).

Considerably less data is available for the lower constraint, which also shows a higher variability. Estimates for the split of endemic Ohrid taxa to taxa outside the lake range from approximately 2.85 ± 1.29 Ma for the pyrgulinid gastropod species flock (Wilke et al., 2007) to 8.30 ± 3.60 Ma calculated here for the *Dina* flock.

Overall, the data summarized in Fig. 4 provides good support that the minimum age of Lake Ohrid is some 2 Ma. For the reasons outlined above, support for the maximum age estimation is weaker and approximately 3 Ma is a rough estimate. Interestingly, this time frame of approximately 2–3 Ma for the origin of Lake Ohrid, generated solely based on evolutionary data, almost exactly fits the time frame most often used in the literature by geologists. Future studies conducted by geologists and evolutionary biologist will tell whether this concurrence holds true.

Acknowledgements. The work was supported by German Research Foundation grants Wi1901/8-1, AL 1076/3-1, and MAK 17/1/06 to TW, CA, and ST, respectively. We thank Jonce A. Šapkarev for sharing his expert knowledge on *Dina* spp. Uwe Jueg provided valuable out-group samples. Silvia Nachtigall is gratefully acknowledged for helping with the molecular lab work.

References

- Albrecht, C., Hauffe, T., Schreiber, K., and Wilke, T.: Mollusc biodiversity and endemism in the putative ancient Lake Trichonis (Greece), *Malacologia*, 51, 357–375, 2009.
- Albrecht, C. and Wilke, T.: Testing the “old lakes – old taxa assumption”: freshwater limpets (Gastropoda: Acroloxidae) in the European ancient lakes, *Berl. Paläobiol. Abh.*, 9, 7, 2006.
- Albrecht, C., Trajanovski, S., Kuhn, K., Streit, B., and Wilke, T.: Rapid evolution of an ancient lake species flock: freshwater limpets (Gastropoda: Ancyliidae) in the Balkan Lake Ohrid, *Org. Divers. Evol.*, 6, 294–307, 2006.
- Albrecht, C. and Wilke, T.: Lake Ohrid: biodiversity and evolution, *Hydrobiologia*, 615, 103–140, 2008.
- Albrecht, C., Wolff, C., Glöer, P., and Wilke, T.: Concurrent evolution of ancient sister lakes and sister species: the freshwater gastropod genus *Radix* in lakes Ohrid and Prespa, *Hydrobiologia*, 615, 157–167, 2008.
- Apakupakul, K., Siddall, M. E., and Burreson, E. M.: Higher-level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences, *Mol. Phylogenet. Evol.*, 12, 350–359, 1999.
- Benton, M. J.: The Red Queen and the Court Jester: species diversity and the role of biotic and abiotic factors through time, *Science*, 323(5915), 728–732, 2009.
- Borda, E. and Siddall, M. E.: Arhynchobdellida (Annelida: Oligochaeta: Hirudinidae): phylogenetic relationships and evolution, *Mol. Phylogenet. Evol.*, 30, 213–225, 2004.
- Brooks, J. L.: Speciation in ancient lakes, *Q. Rev. Biol.*, 25, 30–60, 131–176, 1950.
- Clement, M., Posada, D., and Crandall, K. A.: TCS: a computer program to estimate gene genealogies, *Mol. Ecol.*, 9, 1657–1659, 2000.

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Cohen, A.: Extinction in ancient lakes: biodiversity crises and conservation 40 yr after J. L. Brooks, in: Speciation in Ancient Lakes, edited by: Martens, K., Gooderis, B., and Coulter, G., Arch. Hydrobiol. – Ergebnisse der Limnologie, 44, 451–479, 1994.

Cohen, A.: Paleolimnology. The history and evolution of lake systems, Oxford University Press, New York, 2003.

Cohen, A. S., Stone, J. R., Beuning, K. R. M., Park, L. E., Reinthal, P. N., Dettman, D., Scholz, C. A., Johnson, T. C., King, J. W., Talbot, M. R., Brown, E. T., and Ivory, S. J.: Ecological consequences of early Late Pleistocene megadroughts in tropical Africa, P. Natl. Acad. Sci. USA, 104, 16422–16427, 2007.

Cvijić, J.: Fundamentals of geography and geology of Macedonia and Old Serbia, Book III, Serbian Academy of Sciences, Special Edition, Beograd (in Serbian), 1911.

Degens, E. T., von Herzen, R. P., Wong, M. K., Deuser, W. G., and Jannasch, H. W.: Lake Kivu: structure, chemistry and biology of an East African rift lake, Geol. Rundsch., 62, 245–277, 1973.

Drummond, A. J. and Rambaut, A.: BEAST: bayesian evolutionary analysis by sampling trees, BMC Evol. Biol., 7, 214, doi:10.1186/1471-2148-7-214, 2007.

Dumurdzanov, N., Serofimovski, T., and Burchfield, B. C.: Evolution of the neogene-pleistocene basins of Macedonia, Geol. Soc. Am. Digital Map Chart Series 1, 1–20, 2004.

Excoffier, L., Laval, G., and Schneider, S.: Arlequin (version 3.0): an integrated software package for population genetics data analysis, Evol. Bioinform., 1, 47–50, 2005.

Folmer, O., Black, M., Hoeh, W., Lutz, R. A., and Vrijenhoek, R. C.: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates, Mol. Mar. Biol. Biotech., 3, 294–299, 1994.

Genner, M. J., Knight, M. E., Haesler, M. P., and Turner, G. F.: Establishment and expansion of Lake Malawi rock fish populations after a dramatic Late Pleistocene lake level rise, Mol. Ecol., 19, 170–182, 2010.

Greenwood, P. H.: What is a species flock? in: Evolution of fish species flocks, edited by: Echelle, A. A. and Kornfield, I., Orono Press, Maine, 13–19, 1984.

Grosser, C. and Eiseler, B.: Beschreibung von *Dina pseudotrocheta* sp. nov. (Hirudinea: Erpobdellidae) aus dem deutsch-belgisches Grenzgebiet, Lauterbornia, 65, 27–41, 2008.

Grosser, C., Moritz, G., and Pešić, V.: *Dina minuoculata* sp. nov. (Hirudinea: Erpobdellidae) – eine neue Egelart aus Montenegro, Lauterbornia, 59, 7–18, 2007.

- Hadžišće, S.: III. Beitrag zur Kenntnis der Gastropodenfauna des Ohridsees, Beschreibung der bis jetzt unbekannten Schnecken und Beispiele der Speciation bei den Gastropoden des Ohridsees, Recl. Trav. Station Hydrobiol. Ohrid, 4, 57–107, 1956.
- Halanych, K. M. and Janosik, A. M.: A review of molecular markers used for Annelid phylogenetics, Integr. Comp. Biol., 46, 533–543, 2006.
- Hall, T. A.: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucl. Acids S., 41, 95–98, 1999.
- Hauffe, T., Albrecht, C., Schreiber, K., Trajanovski, S., and Wilke, T.: Spatially explicit analyses of gastropod compositions in ancient Lake Ohrid reveal a hierarchical distribution of endemic taxa and distinct biodiversity hotspots, Biogeosciences Discuss., submitted, 2010.
- Hauswald, A.-K., Albrecht, C., and Wilke, T.: Testing two contrasting evolutionary patterns in ancient lakes: species flock vs. species scatter in valvatinid gastropods of Lake Ohrid, Hydrobiologia, 615, 169–179, 2008.
- Hipsley, C. A., Himmelmann, L., Metzler, D., and Müller, J.: Integration of Bayesian molecular clock methods and fossil-based soft bounds reveals early Cenozoic origin of African lacertid lizards, BMC Evol. Biol., 9, 151, 2009.
- Hovingh, P.: *Erpobdella (Dina) parva* complex (Annelida: Hirudinea: Arhynchobdellida: Erpobdellidae): additional description of *Erpobdella parva*, *E. dubia*, and *E. lahontana* and taxonomic revision, Hydrobiologia, 517, 89–105, 2004.
- Hubendick, B.: The Ancyliidae of Lake Ochrid and their bearing on intralacustrine speciation, P. Zool. Soc. Lond., 133, 497–529, 1960.
- Kass, R. E. and Raftery, A. E.: Bayes factors, J. Am. Stat. Assoc, 90, 773–795, 1995.
- Kashiwaya, K., Ryugo, M., Horii, M., Sakai, H., Nakamura, T., and Kawai, T.: Climatolimnological signals during the past 260 000 yr in physical properties of bottom sediments from Lake Baikal, J. Paleolimnol., 21, 143–150, 1999.
- Lézine, A.-M., von Grafenstein, U., Andersen, N., Belmecheri, S., Bordon, A., Caron, B., Cazet, J.-P., Erlenkeuser, H., Fouache, E., Grenier, C., Huntsman-Mapila, P., Hureau-Mazaudier, D., Manelli, D., Mazaud, A., Robert, C., Sulpizio, R., Tiercelin, J.-J., Zanchetta, G., and Zeqollari, Z.: Lake Ohrid, Albania, provides an exceptional multi-proxy record of environmental changes during the last glacial–interglacial cycle, Palaeogeogr. Palaeoclimatol. Palaeoecol., 287, 116–127, 2010.

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Lindhorst, K., Vogel, H., Krastel, S., Wagner, B., Hilgers, A., Zander, A., Schwenk, T., Wessels, M., and Daut, G.: Stratigraphic analysis of lake level fluctuations in Lake Ohrid: an integration of high resolution hydro-acoustic data and sediment cores, *Biogeosciences Discuss.*, 7, 3651–3689, doi:10.5194/bgd-7-3651-2010, 2010.
- 5 Lukin, E. I.: Fauna SSSR. Pijavki, T. 1. Izd. Nauka, Leningrad, 1976.
- Martens, K.: Speciation in ancient lakes (review), *Trends Ecol. Evol.*, 12, 177–182, 1997.
- Martens, K., Coulter, G., and Goddeeris, B.: Speciation in ancient lakes – 40 yr after Brooks, in: *Speciation in Ancient Lakes*, edited by: Martens, K., Gooderis, B., and Coulter, G., *Arch. Hydrobiol.*, 44, 75–96, 1994.
- 10 Mourguiart, P. and Montenegro, M. E.: Climate changes in the Lake Titicaca area: evidence from ostracod ecology, *Geophys. Monograph.*, 131, 151–165, 2002.
- Nesemann, H. and Neubert, E.: Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea, *Süßwasserfauna von Mitteleuropa IX*, Spektrum Verlag, Heidelberg, 1999.
- Nylander, J. A. A.: MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, 2004.
- 15 Ocegüera-Figueroa, A., León-Règagnon, V., and Siddall M. E.: Phylogeny and revision of Erpobdelliformes (Annelida, Arhynchobdellida) from Mexico based on nuclear and mitochondrial gene sequences, *Rev. Mex. Biodiv.*, 76, 191–198, 2005.
- Pfeiffer, I., Brenig, B., and Kutschera, U.: The occurrence of an Australian leech species (genus *Helobdella*) in German freshwater habitats as revealed by mitochondria DNA sequences, *Mol. Phylogenet. Evol.*, 33, 214–219, 2004.
- 20 Pfeiffer, I., Brenig, B., and Kutschera, U.: Molecular phylogeny of selected predaceous leeches with reference to the evolution of body size and terrestriality, *Theor. Biosci.*, 124, 55–64, 2005.
- 25 Phillips, A. J. and Siddall, M. E.: Poly-paraphyly of Hirudinidae: many lineages of medicinal leeches, *BMC Evol. Biol.*, 9, 246, doi:10.1186/1471-2148-9-246, 2009.
- Radoman, P.: Hydrobioidea, a superfamily of Prosobranchia (Gastropoda), I. Systematics, Serbian Academy of Sciences and Arts, Belgrade, 1–256, 1983.
- Radoman, P.: Hydrobioidea, a superfamily of Prosobranchia, Faculty of Science – Department of Biology, Beograd, 1985.
- 30 Raymond, M. and Rousset, F.: An exact test for population differentiation, *Evolution*, 49, 1280–1283, 1995.

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Ronquist, F. and Deans, A. R.: Bayesian phylogenetics and its influence on insect systematics, *Annu. Rev. Entomol.*, 55, 189–206, 2010.
- Ronquist, F. and Huelsenbeck, J. P.: MrBayes 3: Bayesian phylogenetic inference under mixed models, *Bioinformatics*, 19, 1572–1574, 2003.
- 5 Rossiter, A. and Kawanabe, H.: Ancient lakes: biodiversity, ecology and evolution, *Advances in ecological research*, Vol. 31, Academic Press, London New York, 1–624, 2000.
- Šapkarev, J. A.: Sistematika i rasprostranjenje pijavica (Hirudinea) Makedonije (Taxonomy and distribution of leeches (Hirudinea) from Macedonia), *Biosistematika*, 1, 87–99, 1975.
- Šapkarev, J.: New leeches (Hirudinea) of the ancient Lake Ohrid, *Fragmenta Balcanica*, 14, 155–162, 1990.
- 10 Scholz, C. A., Johnson, T. C., Cohen, A. S., King, J. W., Peck, J. A., Overpeck, J. T., Talbot, M. R., Brown, E. T., Kalindekafe, L., Amoako, P. Y. O., Lyons, R. P., Shanahan, T. M., Castañeda, I. S., Heil, C. W., Forman, S. L., McHargue, L. R., Beuning, K. R., Gomez, J., and Pierson, J.: East African megadroughts between 135 and 75 thousand years ago and bearing on early-modern human origins, *P. Natl. Acad. Sci. USA*, 104(42), 16416–16421, 2007.
- Schön, I. and Martens, K.: Adaptive, pre-adaptive and non-adaptive components of radiations in ancient lakes, a review, *Org. Div. Evol.*, 4, 137–156, 2004.
- Schultheiß, R., Albrecht, C., Bößneck, U., and Wilke, T.: The neglected side of speciation in ancient lakes: phylogeography of an inconspicuous mollusc taxon in lakes Ohrid and Prespa, *Hydrobiologia*, 615, 141–156, 2008.
- 20 Schultheiß, R., Van Bocxlaer, B., Wilke, T., and Albrecht, C.: Old fossils-young species: on the evolutionary history of an endemic gastropod assemblage in Lake Malawi, *P. Roy. Soc. Lond. B Bio.*, 276, 2837–2846, 2009.
- 25 Sherbakov, D. Y.: Molecular phylogenetic studies on the origin of biodiversity in Lake Baikal, *Trends Ecol. Evol.*, 14, 92–95, 1999.
- Siddall, M. E.: Phylogeny of the leech family Erpobdellidae (Hirudinida: Oligochaeta), *Invertebr. Syst.*, 16, 1–6, 2002.
- Siddall, M. E. and Bureson, E. M.: Phylogeny of leeches (Hirudinea) based on mitochondrial cytochrome c oxidase subunit I, *Mol. Phylogenet. Evol.*, 9, 156–162, 1998.
- 30 Siddall, M. E., Trontelj, P., Utevsky, S. Y., Nkamany, M., and MacDonald, K. S.: Diverse molecular data demonstrate that commercially available medicinal leeches are not *Hirudo medicinalis*, *P. Roy. Soc. Lond. B Bio.*, 274, 1481–1487, 2007.

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Sket, B.: Rhynchobdellid leeches (Hirudinea, Rhynchobdellae) in the relic Ohrid Lake region, Biol. Vestnik, 29, 67–90, 1981.
- Sket, B.: Intralacustrine speciation in the genus *Dina* (Hirudinea, Erpobdellidae) in Lake Ohrid (Yugoslavia), Hydrobiologia, 18, 4–58, 1989.
- 5 Sket, B., Dovč, P., Jalžič, B., Kerovec, M., Kučinić, M., and Trontelj, P.: A cave leech (Hirudinea, Erpobdellidae) from Croatia with unique morphological features, Zool. Scr., 30, 223–229, 2001.
- Sket, B. and Šapkarev, J.: *Dina lepinja* sp. n. (Hirudinea, Erpobdellidae), a new endemic leech from the ancient Lake Ohridsko Ezero, Biol. Vestnik, 34, 89–92, 1986.
- 10 Sket, B. and Šapkarev, J.: Distribution of Hirudinea (Annelida) in the ancient Ohrid Lake region, Arch. Hydrobiol., 124, 225–237, 1992.
- Spirkovski, Z., Avramovski, O., and Kodzoman, A.: Watershed management in the Lake Ohrid region of Albania and Macedonia, Lake Reserv. Manage., 6, 237–242, 2001.
- Stanković, S.: The Balkan Lake Ohrid and its Living World. Monographiae Biologicae Vol. IX. Uitgeverij Dr. W. Junk, Den Haag, 1960.
- 15 Sturmbauer, C., Baric, S., Salzburger, W., Ruber, L., and Verheyen, E.: Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes, Mol. Biol. Evol., 18, 144–154, 2001.
- Sulpizio, R., Zanchetta, G., D'Orazio, M., Vogel, H., and Wagner, B.: Tephrostratigraphy and tephrochronology of lakes Ohrid and Prespa, Balkans, Biogeosciences Discuss., 7, 3931–3967, doi:10.5194/bgd-7-3931-2010, 2010.
- 20 Sušnik, S., Knizhin, I., Snoj, A., and Weiss, S.: Genetic and morphological characterization of a Lake Ohrid endemic, *Salmo (Acantholingua) ohridanus* with a comparison to sympatric *Salmo trutta*, J. Fish Biol., 68, Supplement A, 2–23, 2006.
- 25 Trontelj, P. and Sket, B.: Molecular re-assessment of some phylogenetic, taxonomic and biogeographic relationships between the leech genera *Dina* and *Trocheta* (Hirudinea: Erpobdellidae), Hydrobiologia, 438, 227–235, 2000.
- Trontelj, P., Sket, B., Dovč, P., and Steinbrück, G.: Phylogenetic relationships in European erpobdellid leeches (Hirudinea: Erpobdellidae) inferred from restriction-site data of the 18S ribosomal gene and ITS2 region, J. Zool. Syst. Evol. Res., 34, 85–93, 1996.
- 30 Trontelj, P. and Utevsky, Y. S.: Celebrity with a neglected taxonomy: molecular systematics of the medicinal leech (genus *Hirudo*), Mol. Phylogenet. Evol., 34, 616–624, 2005.

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Utevsky, Y. S. and Trontelj, P.: Phylogenetic relationships of fish leeches (Hirudinea, Piscicolidae) based on mitochondrial DNA sequences and morphological data, *Zool. Scr.*, 33, 375–385, 2004.
- Wagner, B., Reicherter, K., Daut, G., Wessels, M., Matzinger, A., Schwalb, A., Spirkovski, Z., and Sanxha, M.: The potential of Lake Ohrid for long-term palaeoenvironmental reconstructions, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 259, 341–356, 2008.
- Wilke, T.: How dependable is a non-local molecular clock? A reply to Hausdorf et al. (2003), *Mol. Phylogenet. Evol.*, 30, 835–840, 2004.
- Wilke, T., Albrecht, C., Anistratenko, V. V., Sahin, S. K., and Yildirim, M. Z.: Testing biogeographical hypotheses in space and time: faunal relationships of the putative ancient lake Egirdir in Asia Minor, *J. Biogeogr.*, 34, 1807–1821, 2007.
- Wilke, T., Davis, G. M., Qiu, D., and Spear, R. C.: Extreme mitochondrial sequence diversity in the intermediate schistosomiasis host *Oncomelania hupensis robertsoni*: another case of ancestral polymorphism?, *Malacologia*, 48, 143–157, 2006.
- Wilke, T., Schultheiß, R., and Albrecht, C.: As time goes by: a simple fool's guide to molecular clock approaches in invertebrates, from the symposium “Molluscs as models in evolutionary biology: from local speciation to global radiation” presented at the World Congress of Malacology, held from 15 to 20 Jul 2007 in Antwerp, Belgium, *Am. Malacol. Bull.*, 27, 25–45, 2009.
- Wilke, T., Schultheiß, R., Albrecht, C., Bornmann, N., Trajanovski, S., and Kevrekidis, T.: Native *Dreissena* freshwater mussels in the Balkans: in and out of ancient lakes, *Biogeosciences Discuss.*, 7, 4425–4461, doi:10.5194/bgd-7-4425-2010, 2010.
- Wysocka, A., Kostoski, G., Kilikowska, A., Wróbel, B., and Sell, J.: *Proasellus* (Crustacea, Isopoda) species group endemic to the Balkan Lake Ohrid: a case of ecological diversification?, *Fund. Appl. Limnol.*, 172, 301–313, 2008.
- Xia, X., Xie, Z., Salemi, M., Chen, L., and Wang, Y.: An index of substitution saturation and its application, *Mol. Phylogenet. Evol.*, 26, 1–7, 2003.

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 1. Specimens of Hirudinea studied, including locality information, locality codes according to Fig. 1, and DNA voucher numbers. Nomenclature follows Nesemann and Neubert (1999). For GenBank accession numbers and information on sequences obtained from GenBank see text.

Species	Locality (locality code)	Geographical coordinates	DNA voucher #
<i>Dina l. lineata</i> (O. F. Müller, 1774)	Germany, Mecklenburg-Western Pomerania, Parchim district, small pool	53.445° N, 11.910° E	5990, 6800, 6801
<i>Dina l. lacustris</i> Sket, 1968	Macedonia, Ligemii Bogovines (type locality)	41.95069° N, 20.79578° E	9114, 9115, 9213
<i>Dina lepinja</i> Sket & Šapkarev, 1986	Macedonia, Lake Ohrid (O14)	41.0931° N, 20.7953° E – 41.0931° N, 20.7891° E	5943, 5952, 5996, 6305
	Macedonia, Lake Ohrid (O12)	41.1028° N, 20.8019° E – 41.1028° N, 20.7967° E	5954, 6001
	Macedonia, Lake Ohrid (O16)	41.08783° N, 20.79277° E	6911
	Macedonia, Lake Ohrid (O21)	40.93950° N, 20.77754° E	6920
<i>Dina cf. svilesta</i> Sket, 1989	Macedonia, Lake Ohrid (O18)	41.0563° N, 20.7919° E – 41.0563° N, 20.7982° E	3949
	Macedonia, Lake Ohrid (O17)	41.05635° N, 20.79671° E	6922
<i>Dina lyhnida</i> Šapkarev, 1990	Macedonia, Lake Ohrid (O4)	41.1031° N, 20.7302° E	5956, 6004, 6309, 6799
	Macedonia, Lake Ohrid (O5)	41.105° N, 20.744° E	5946, 5961
	Macedonia, Lake Ohrid (O6)	41.105° N, 20.757° E	6308, 5962
	Macedonia, Lake Ohrid (O14)	41.0931° N, 20.7953° E – 41.0931° N, 20.7891° E	6377
	Macedonia, Lake Ohrid (O16)	41.08783° N, 20.79277° E	6912
	Macedonia, Lake Ohrid (O21)	40.93950° N, 20.77754° E	6918
	Macedonia, Lake Ohrid (O3)	41.107° N, 20.649° E	3937
	Macedonia, Lake Ohrid (O13)	41.1005° N, 20.8003° E – 41.1006° N, 20.7943° E	6378
<i>Dina krilata</i> Sket, 1989	Macedonia, Lake Ohrid (O1)	41.143° N, 20.687° E	3947, 3948
	Macedonia, Lake Ohrid (O12)	41.1028° N, 20.8019° E – 41.1028° N, 20.7967° E	5937, 5950, 6307, 5998
	Macedonia, Lake Ohrid (O16)	41.08783° N, 20.79277° E	6909
	Macedonia, Lake Ohrid (O17)	41.05635° N, 20.79671° E	6916

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 1. Continued.

Species	Locality (locality code)	Geographical coordinates	DNA voucher #
<i>Dina cf. kuzmani</i> Šapkarev, 1990/ <i>Dina cf. eturpshem</i> Sket, 1989	Albania, Lake Ohrid (O23)	40,91579° N, 20,65533° E	11 375, 11 376
	Albania, Lake Ohrid (O22)	40,93412° N, 20,6478° E	11379
	Macedonia, Lake Ohrid (O14)	41.0931° N, 20.7953° E – 41.0931° N, 20.7891° E	5955, 6304, 5994
	Macedonia, Lake Ohrid (O11)	41.1065° N, 20.7966° E – 41.1100° N, 20.8026° E	3939
	Macedonia, Lake Ohrid (O16)	41.08783° N, 20.79277° E	6913
	Macedonia, Lake Ohrid (O11)	41.1065° N, 20.7966° E – 41.1100° N, 20.8026° E	3940, 3945
	Macedonia, Lake Ohrid (O12)	41.1028° N, 20.8019° E – 41.1028° N, 20.7967° E	5999, 6372
	Macedonia, Lake Ohrid (O18)	41.0563° N, 20.7919° E – 41.0563° N, 20.7982° E	3955
	Macedonia, Lake Ohrid (O17)	41.05635° N, 20.79671° E	6914
	Macedonia, Lake Ohrid (O16)	41.08783° N, 20.79277°	6910
<i>Dina cf. profunda</i> Šapkarev, 1990	Macedonia, Lake Ohrid (O8)	41.1030° N, 20.7578° E	5945 , 5959
	Macedonia, Lake Ohrid (O7)	41.1037° N, 20.7447° E	6798
	Macedonia, Lake Ohrid, (O18)	41.0563° N, 20.7919° E – 41.0563° N, 20.7982° E	3950
<i>Dina ohridana</i> Sket, 1968	Albania, Lake Ohrid (O20)	40.97531° N, 20.64453° E	6297, 6299
	Macedonia, Lake Ohrid (O12)	41.1028° N, 20.8019° E – 41.1028° N, 20.7967° E	5960, 6002
	Macedonia, Lake Ohrid (O21)	40.93950° N, 20.77754° E	6919
	Macedonia, Lake Ohrid (O14)	41.0931° N, 20.7953° E – 41.0931° N, 20.7891° E	5997
	Macedonia, Lake Ohrid (O17)	41.05635° N, 20.79671° E	6915
	Macedonia, Lake Ohrid (O19)	41.01262° N, 20.80513° E	6917
	Macedonia, Lake Ohrid (O2)	41.12643° N, 20.64184° E	6298
	Macedonia, Lake Ohrid (O2)	41.0931° N, 20.7953° E – 41.0931° N, 20.7891° E	6793
	Macedonia, spring lake of Sv. Naum (S3)	40.91444° N, 20.74226° E	11 363, 11 364, 11 367, 11 368

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 1. Continued.

Species	Locality (locality code)	Geographical coordinates	DNA voucher #
<i>Dina</i> sp.	Macedonia, spring near old camping site of Sv. Naum (type locality) (S4)	40.91208° N, 20.74213° E	11 381, 11 382, 11 383
	Macedonia, spring lake near main springs of Sv. Naum (S5)	40.91019° N, 20.74793° E	11 373, 11 374
	Macedonia, Ohrid, Studenčišta spring (S2)	41.102° N, 20.814° E	5991
	Macedonia, Lake Ohrid (O1)	41.143° N, 20.687° E	3935
	Macedonia, Lake Ohrid (O15)	41.093° N, 20.793° E	3951
	Macedonia, Lake Ohrid (O9)	41.11084° N, 20.78839° E	6295
	Macedonia, Lake Ohrid (O10)	41.11082° N, 20.78838° E	6296
	Macedonia, Lake Ohrid (O12)	41.1028° N, 20.8019° E – 41.1028° N, 20.7967° E	6000, 6795
	Macedonia, Lake Ohrid (O3)	41.107° N, 20.649° E	3944
	Macedonia, spring Sv. Naum (S3)	40.91444° N, 20.74226° E	11 365, 11 366, 11 369, 11 370, 11 372
<i>Dina latestriata</i> Neubert & Neseman, 1995	Albania, Lake Ohrid (O23)	40.91579° N, 20.65533° E	11 377, 11 378
	Albania, Lake Ohrid (O22)	40.93412° N, 20.6478° E	11 380
	Albania, spring complex at the beginning of the village Zagorican (S6)	40.88973° N, 20.71312° E	11 361, 11 362
	Albania, Lake Prespa (P2)	40.76848° N, 20.92642° E	6300
	Greece, Lake Trichonis	38.58893° N, 21.46703° E	6363
	Macedonia, Lake Prespa (P1)	40.86655° N, 20.98989° E	6924
<i>Dina punctata</i> Johansson, 1927	Greece, Lake Mikri Prespa (type locality) (MP1)	40.74215° N, 21.10763° E	9206
	Spain, Valencia, Province Castellon, Municipality Algimia de Almonacid, Fuente Donace	39.91800° N, 0.43760° W	9106, 9107

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

I◀

▶I

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 1. Continued.

Species	Locality (locality code)	Geographical coordinates	DNA voucher #
<i>Erpobdella monostrata</i> (Lindenfeld & Pietruszynski, 1890)	Germany, Mecklenburg-Western Pomerania, Ludwigslust district, Lake Neustädter See	53.39054° N, 11.55809° E	9096
<i>Erpobdella nigricollis</i> (Brandes, 1900)	Germany, Mecklenburg-Western Pomerania, Ludwigslust district, Lake Neustädter See	53.39054° N, 11.55809° E	9100
<i>Erpobdella octoculata</i> (Linnaeus, 1758)	Macedonia, Lake Ohrid (O14)	41.0931° N, 20.7953° E – 41.0931° N, 20.7891° E	5995
<i>Erpobdella</i> sp.	Macedonia, Lake Prespa (P1)	40.86655° N, 20.98989° E	6923
	Bejbunar spring (S1)	41.110° N, 20.818° E	5933
<i>Erpobdella testacea</i> (Savigny, 1822)	Germany, Mecklenburg-Western Pomerania, Ludwigslust district, Lake Neustädter See	53.39054° N, 11.55809° E	9098
<i>Erpobdella vilnensis</i> (Liskiewicz, 1925)	Germany, Mecklenburg-Western Pomerania, Ludwigslust district, forest pool	53.43° N, 11.41° E	5989
	Germany, Saxony-Anhalt, W of Osterwieck, River Ilse	51.967° N, 10.710° E	6805
<i>Batrachobdella algira</i> (Moquin-Tandon, 1846)	Spain, Andalusia, Province Huelva, Municipality Cortelazor, outflow of spring Fuene Lavadora	37.95559° N, 4.04210° W	9205
<i>Glossiphonia complanata</i> (Linnaeus, 1758)	Germany, Mecklenburg-Western Pomerania, creek	53.81848° N, 10.92799° E	9200

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 2. Results of the exact test of significant pairwise genetic differences (level 0.05) between *Dina* spp. from different habitats of the Ohrid watershed.

	Springs	Littoral	Sublittoral	Profundal
Springs				
Littoral	+			
Sublittoral	+	–		
Profundal	+	–	–	

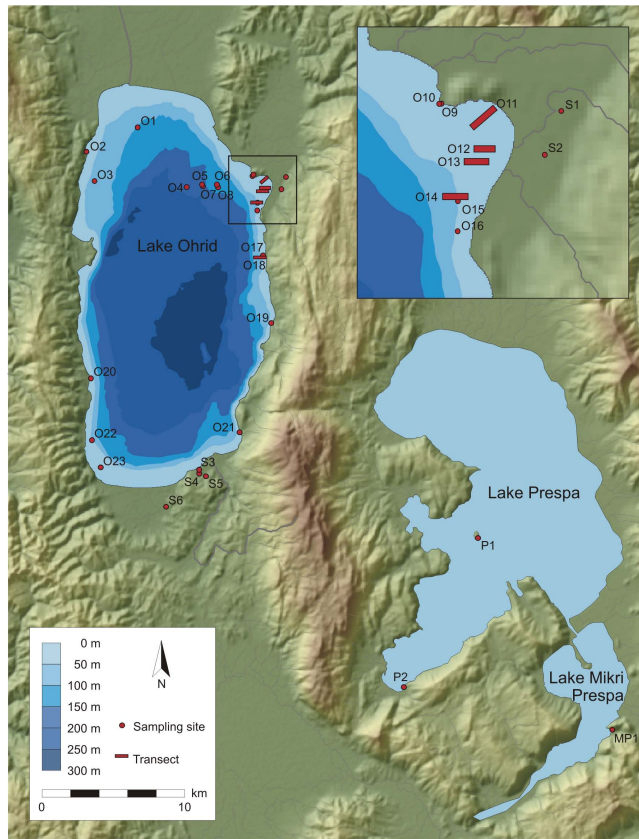


Fig. 1. Map of the Balkan lakes Ohrid, Prespa, and Mikri Prespa showing collecting sites for specimens of the family Erpobdellidae. The insert in the upper right corner shows an enlargement of the Ohrid Bay together with the respective collecting sites. Locality details are given in Table 1. Base map adapted from Mountain High Maps[®], Copyright[©] 1993, Digital Wisdom[®], Inc.

BGD

7, 5011–5045, 2010

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

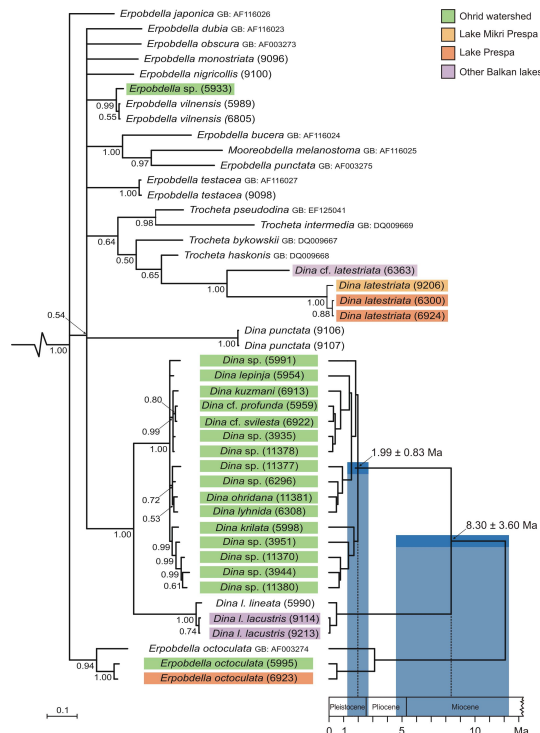


Fig. 2. Bayesian phylogeny of selected species of Erpobdellidae inferred from the mitochondrial COI gene (left). The outgroup taxa *Glossiphonia complanata* and *Batracobdella algira* were removed a posteriori. Bayesian posterior probabilities are given next to the nodes. The scale bar indicates the number of substitutions per site. A strict clock Bayesian tree of selected taxa from the Lake Ohrid *Dina* flock is shown on the right. The 95% confidence intervals for the onset of intralacustrine diversification and for the split with taxa from outside the lake are indicated by blue bars. Specimens from the Balkans are marked by coloured bars. Number in parenthesis behind taxon names are the respective DNA voucher numbers (see Table 1 for details). Accession numbers are provided for sequences obtained from GenBank.

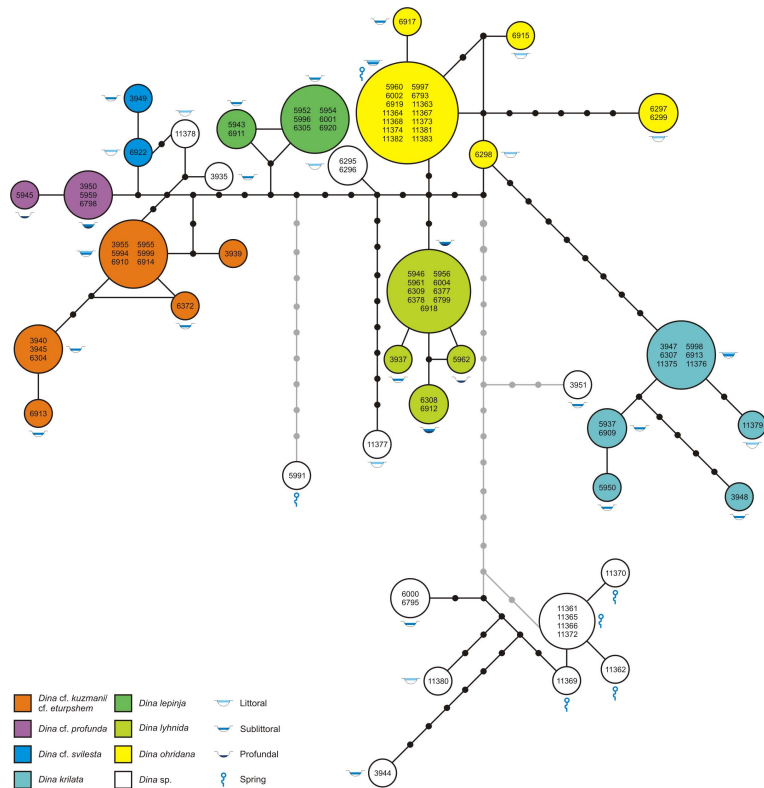


Fig. 3. Statistical parsimony haplotype network of specimens of the Lake Ohrid *Dina* flock based on the mitochondrial COI gene (connection limit 95%). Haplotypes are colour-coded according to taxa (DNA voucher numbers are provided inside the circles; see Table 1 for details). Areas of circles representing the haplotypes found are proportional to the number of specimens sharing the respective haplotype. Missing haplotypes are indicated by black dots. The grey lines show the connections among sub-networks when reducing the TCS connection limit to 90%. Major habitat types are indicated by symbols.

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



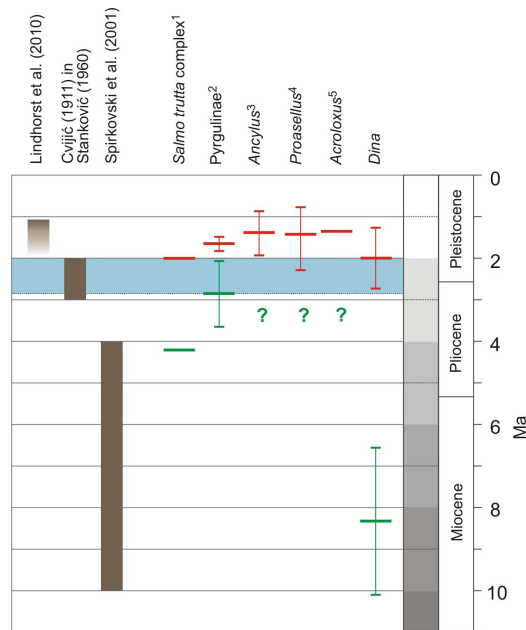


Fig. 4. Comparisons of geological and evolutionary estimates for the age of Lake Ohrid. Brown bars indicate geological estimates (note that the estimate of Lindhorst et al., 2010 represents a minimum age). The narrow red bars indicate the onset of intralacustrine diversification in groups of endemic Lake Ohrid taxa together with their standard deviations (not CI). The narrow green bars indicate the average timing and standard deviations of the split with their respective sister groups outside the lake. These estimates set the minimum and a potential maximum time frame for the origin of Lake Ohrid faunas (blue band) based on evolutionary data. See text for details.

¹ Sušnik et al., 2006.

² Wilke et al., 2007, 2009. Note that the authors did not include all species of the Pyrgulinae species flock in their analysis. Therefore, the onset of intralacustrine diversification in this group is very likely underestimated.

³ Albrecht et al., 2006. Note that the authors regarded an endemic taxon from the feeder springs of Lake Ohrid as a non-Ohrid taxon. For reasons of consistency, this taxon is here considered to be part of the Ohrid group.

⁴ Wysocka et al., 2008. Note that the authors calculated the age for the older of two *Proasellus* groups at 2.83 Ma. For their tree estimations, they used the GTR+I+G substitution model, but utilized the K2P model for subsequent molecular dating. Here we have recalculated the divergence time based on the GTR+I+G rate suggested by Wilke et al., 2009.

⁵ Albrecht and Wilke, 2006.

The *Dina* species flock in Lake Ohrid

S. Trajanovski et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

