RESEARCH ARTICLE

Microscopic eukaryotes living in a dying lake (Lake Koronia, Greece)

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Abstract

The morphological and phylogenetic diversity of the microscopic eukaryotes of the Lake Koronia water column was investigated during a mass kill of birds and fish in August–September 2004. The dominant morphospecies corresponded to the known toxin-producing species *Prymnesium parvum*, followed by *Amoebidium* sp., a taxon belonging to the group of parasitic *Mesomycetozoea*, and the common chlorophyte *Pediastrum boryanum*. *Prymnesium* exhibited heteromorphic life-cycle stages (flagellate and nonmotile coccoid cells). Phylogenetic analysis with 18S rRNA gene suggested that these heteromorphic stages belonged to the *Platychrysis–Prymnesium* monophyletic group. The most abundant phylotype was almost identical to *P. boryanum*. The fungal phylotypes were related to the *Chytridiomycota*, and the ciliate-like ones were closely related to *Enchelys polynucleata* and *Pattersoniella vitiphila*. Two phylotypes representing novel members belonging to the *Jakobida* and the *Apicomplexa* were also found. The microscopic eukaryotes of Lake Koronia include several organisms that are related to parasitic life modes.

Introduction

Recently, there has been a considerable increase in the number of phylogenetic studies based on SSU rRNA molecular analyses, aiming to uncover the microeukaryotic diversity of various ecosystems and thus reveal the degree of the ecological and evolutionary significance of this diversity (López-Garcia *et al.*, 2001; Massana *et al.*, 2004; Takishita *et al.*, 2007; Lefèvre *et al.*, 2008). The use of PCR-based techniques on environmental samples, especially from extreme environments such as anoxic (Dawson & Pace, 2002; Stoeck & Epstein, 2003; Stoeck *et al.*, 2003b; Behnke *et al.*, 2006), hydrothermal (Edgcomb *et al.*, 2002; López-Garcia *et al.*, 2003), deep-sea hypersaline (Edgcomb *et al.*, 2009) or oil-polluted systems (Dalby *et al.*, 2008), has often unveiled novel clades unrelated to any known eukaryotes, as well as primitive branches placed in already established groups. To the best of our knowledge, only a few studies have dealt with microeukaryotic molecular diversity in eutrophic lakes (Lefranc *et al.*, 2005; Lepère *et al.*, 2007; Marshall *et al.*, 2008), while there is no study of microeukaryotic molecular diversity published for hypertrophic or heavily degraded lakes. Most of the microeukaryotes found in eutrophic lakes belonged to the functional groups of autotrophs, parasites, toxin producers, saprophytes, strict phagotrophs or mixotrophs. Taxa such as *Fungi*, *Ciliophora*, *Cryptophytes* and *Chlorophytes* are commonly found, suggesting a widespread prevalence of these microeukaryotes in eutrophic lakes (Marshall *et al.*, 2008).

Light microscopy methods alone are laborious, often even unsuccessful in identifying relatively small organisms (generally $< 20\,\mu m$) or groups of microeukaryotes with no distinctive morphological characteristics (Caron *et al.*, 2004; Stoeck *et al.*, 2006; Yan *et al.*, 2007). In addition, most of these species cannot be presently maintained in cultures (Stoeck *et al.*, 2006). Molecular analyses can facilitate these drawbacks by revealing the extensive diversity of known and especially novel groups in natural samples (Caron *et al.*, 2004). However, a few problems arise from the use of molecular approaches as well. For example, in many studies, there is a biased estimation of the full microeukaryotic diversity, due to limitations of PCR-based approaches (Qiu *et al.*, 2001) and the use of a single set of primers (Caron *et al.*, 2004). Furthermore, some of the molecular methods involve highly technical procedures, which may be suitable for the initial detection of the organisms, but provides no

information about their ecology. Therefore, a combination of rRNA and microscopy methodologies is required in order to acquire a more detailed description of the microeukaryotic diversity of environmental samples (Stoeck *et al.*, 2003a).

In the present study, the genetic diversity of the microeukaryotic community in a shallow, hypertrophic, heavily polluted and modified lake (Lake Koronia, Greece) was investigated using 18S rRNA gene analysis, during a mass kill of birds and fish in August–September 2004. The results of the 18S rRNA gene analysis were compared with the community composition based on microscopic analysis. The aims of this study were to supplement the existing knowledge regarding the diversity of microscopic eukaryotes in Lake Koronia with molecular analysis and to focus on the detection of potentially parasitic and toxin-producing taxa, which have deleterious effects on the food web components of the lake and pose a hazard to wildlife and public health.

Materials and methods

Lake Koronia is located in northern Greece (40°40′58″N, 23°09′33″E) at 75 m above sea level. It is a shallow, hypertrophic, highly polluted and heavily modified lake, in the final stages before death. It has undergone a massive decrease in lake volume over the past 20 years, with dramatic decreases in surface area and maximum depth due to anthropogenic interference (i.e. overpumping for irrigation purposes and industrial use of lake water), significant changes in salinity (from freshwater to > 10 PSU; M. Moustaka-Gouni, unpublished data) and collapse of its food web (M. Moustaka-Gouni *et al.*, in preparation). In the 1970s, the lake's surface area was 46.2 km^2, with a maximum depth of 8 m, while in 1995 the surface area was 30 km^2 and the maximum depth was 1 m. Finally, in the summer of 2002, the lake dried up completely. Early in 2003, water started accumulating again in the lake and to date the maximum depth is < 1 m (Moustaka-Gouni *et al.*, 2004).

Water samples for microscopic analysis were collected on 25 August and 11 September 2004 at the deepest point of the lake (< 1 m). Sampling details, processing of the samples, and *in situ* physical–chemical prevailing parameters of the samples are described elsewhere (Moustaka-Gouni *et al.*, 2004; Michaloudi *et al.*, 2009). For each sampling date, at least 10 replicates of live and preserved samples were examined in 2-mL sedimentation chambers using an inverted microscope with phase contrast (Nikon SE 2000). Microscopic eukaryotes were identified using taxonomic keys and the works by Carter (1937), Green *et al.* (1982), Gayral & Fresnel (1983) and Larsen (1999). Microeukaryote counts (cells, colonies and coenobia) were performed using the Utermöhl method (Utermöhl, 1958).

Water samples for DNA extraction were transported to the laboratory in 1.5-L airtight plastic bottles and placed in insulated boxes in the dark. Within 2 h of collection, 100 mL of water was filtered through Whatman GF/C filters and these were stored at -20 °C immediately after filtration until further analysis. The use of an isopore membrane filter was not feasible as it became clogged after 10–20 mL of water.

DNA was extracted using the UltraClean Soil DNA isolation kit (MoBio Laboratories) according to the manufacturer's protocol after slicing the filters with a sterile scalpel. For PCR amplification, 0.5 μL of the DNA template was used. The 18S rRNA gene was amplified using the EukA (5′-AACCTGGTTGATCCTGCCAGT-3′) (Medlin *et al.*, 1988) and Euk1633rE (5′-GGGCGGTGTGTACAARGRG-3′) (Dawson & Pace, 2002) primers. PCR included an initial denaturation step at 95 °C for 2 min, which was followed by 40 cycles consisting of denaturation at 95 °C for 40 s, annealing at 50 °C for 40 s and elongation at 72 °C for 2 min and 15 s; a final 7-min elongation step at 72 °C was included. Each PCR from the two samples was repeated with different cycle numbers (between 20 and 35). The lowest number of cycles that gave a positive signal (i.e. 26 cycles for both samples) was then used for cloning and sequencing in order to eliminate some of the major PCR innate limitations (von Wintzigerode *et al.*, 1997; Spiegelman *et al.*, 2005) and to avoid differential representation of 18S rRNA genes with low and high copy numbers.

PCR products from the minimum number of cycles were visualized on a 1% agarose gel under UV light, purified with the Montage Purification Kit (Millipore Inc.) and cloned using the TOPO XL PCR cloning kit (Invitrogen Co.) with electrocompetent cells according to the manufacturer's specifications. For each clone library, a maximum of 49 clones containing insert of *c.* 1600 bp were grown in liquid Luria–Bertani medium with kanamycin and their plasmids were purified using the Nucleospin Plasmid QuickPure kit (Macherey-Nagel GmbH and Co. KG, Germany) for DNA sequencing.

Sequence data were obtained by capillary electrophoresis (Macrogen Inc., Seoul, Korea) using the BigDye Terminator kit (Applied Biosystems Inc.) with the primers M13F (5′-GTAAAACGACGGCCAG-3′), M13R (5′-CAGGAAACAGCTATGAC-3′) and 1179rE (5′-CCCGTGTTGAGTCAAATT-3′). Each sequence read was *c.* 950 bp. For each individual clone, forward and reverse reads were assembled, and then the assembled sequences were checked for chimeras using the CHIMERA-CHECK function of the Ribosomal Database Project II (Maidak *et al.*, 2001).

For detection of the closest relatives, all sequences were compared with the BLAST function (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence data were compiled using the MEGA4 software (Tamura *et al.*, 2007) and aligned with

the sequences obtained from the GenBank (http://www.ncbi.nlm.nih.gov) databases, using the CLUSTALX aligning utility. Phylogenetic analyses were performed using minimum evolution and parsimony methods implemented in MEGA4 (Tamura *et al.*, 2007). Heuristic searches under minimum evolution criteria used 1000 random-addition replicates per data set, each followed by tree bisection–reconnection topological rearrangements. The topology of the tree was based on neighbour-joining according to Jukes–Cantor. Bootstrapping under parsimony criteria was performed with 1000 replicates. Sequences of unique phylotypes found in this study have GenBank accession numbers FJ157330–FJ157340. Library clone coverage was calculated using the formula $[1-(n_1/N)]$ (Good, 1953), where n_1 is the number of operational taxonomic units (OTU) represented by only one clone and N is the total number of clones examined in each library.

Formaldehyde-preserved samples were analysed by scanning electron microscopy (SEM). An aliquot of each sample (5 mL) was filtered through 1.2-μm pore size (13 mm diameter) Millipore Isopore membrane filters for qualitative observation. The filters were left to air dry and were then placed on individual aluminium stubs using a carbon tape. The stubs were placed in a Hummer VI-A gold coater and coated with a thin film (about 15 nm) of gold. Analysis was conducted using a Carl Zeiss Leo 1450VP scanning electron microscope fitted with a tungsten filament.

Results

A total of 11 and 13 morphospecies of microeukaryotes were identified with microscopic observation of the samples collected on 25 August and 11 September 2004, respectively (Table 1). The dominant morphospecies corresponded to the known toxin-producing species *Prymnesium parvum*, which reached high cell numbers on 11 September 2004 ($> 200 \times 10^6$ cells L^{-1}). This species exhibited a heteromorphic life cycle comprising of free-swimming flagellated cells and nonmotile coccoid cells (Fig. 1a–c). High numbers on September 2004 (20×10^6 cells L^{-1}) were also observed for the protist *Amoebidium* sp. (Fig. 1d–f). Other morphospecies that were observed were attributed to groups of *Chlorophyta* (Fig. 1g and h) and *Ciliophora*, as well as *Euglenophyta*, *Bacillariophyta* and *Cryptophyta* (Table 1).

According to Good's C estimator for the library clone coverage, an asymptotic curve > 0.80 was reached in samples from both dates (Fig. 2). A total of 24 and 49 eukaryotic rRNA inserts in clone plasmids were sequenced from 25 August and 11 September 2004, respectively, and 11 representative phylotypes were detected based on a $\geq 98\%$ similarity cut-off limit (Table 2, Fig. 3). The two most abundant phylotypes occurred in both sampling dates, while five and four unique phylotypes occurred on 25 August and 11 September 2004, respectively.

On 25 August 2004, the 18S rRNA gene clone library was dominated by *Chlorophyta* phylotypes (kor-250804-16 and kor-250804-18) (Table 2). Phylotype kor-250804-16 alone represented 58.4% of the total abundance and was closely related to *Pediastrum boryanum* var. *cornutum*. The second most abundant group was the *Haptophyta* (20.8%), with phylotype kor-250804-5 closely related ($> 98\%$) to *Platychrysis* sp. The rest of the phylotypes found belonged to the taxa of *Ciliophora*, *Fungi*, *Mesomycetozoea* and *Jakobida* (20.8%).

On 11 September 2004, the 18S rRNA gene clone library was dominated by the phylotype kor-250804-16 as well (81.7%), followed by kor-250804-5 (6.1%) and kor-1109040-24 (6.1%), fungus-related phylotypes. The rest of the detected phylotypes were related to *Apicomplexa*, *Chytridiomycota* and *Ciliophora* (6.1%).

In total, for the two sampling dates, the dominant taxonomic affiliations of the phylotypes were *Chlorophyta* (75.3%), *Haptophyta* (11.0%), *Fungi* (6.8%), *Apicomplexa* (1.4%), *Ciliophora* (2.7%), *Mesomycetozoea* (1.4%) and *Jakobida* (1.4%).

Discussion

We investigated the diversity of microscopic eukaryotes in the hypertrophic, heavily polluted and modified Lake Koronia, Greece, by combining morphological and phylogenetic analyses, in August–September 2004. Both approaches revealed a total of 21 different taxa. According to Good's estimator, our 18S rRNA gene clone libraries were large enough to have revealed $> 80\%$ of the existing microeukaryotic diversity (Kemp & Aller, 2004). There are two reasons for the low phylogenetic species richness we found, which are related to: (a) the use of a single pair of PCR primers, while it has been suggested for microeukaryotes in extreme environments that with the use of multiple-primer pairs, PCR reveals a greater amount of phylotypes (Stoeck *et al.*, 2006), and (b) the use of GF/C filter, which excludes the picoeukaryotes.

The microeukaryotic diversity of the lake during the period of the study was low based on the apparent richness of OTUs (phylotypes detected/total clones sequenced) (15.1%). Only a few studies of the microeukaryotic molecular diversity in eutrophic freshwaters have been published, all revealing a much larger diversity. For example, in a temperate eutrophic drinking water reservoir, Marshall *et al.* (2008) found the apparent richness of OTUs to be 56% while in a temperate eutrophic lake, Lefranc *et al.* (2005) found it to be 25.5%.

Table 1. Occurring morphospecies identified in water samples from Lake Koronia, Greece

Morphospecies	25 August 2004	11 September 2004
Mesomycetozoea		
Amoebidium sp.	+	+
Chlorophyta		
Monoraphidium mirabile (W. & G.S. West) Pankow	+	−
Oocystis sp.	+	+
Pediastrum boryanum (Turpin) Meneghini	+	+
Planktosphaeria gelatinosa G.M. Smith	+	−
Scenedesmus ecornis (Ehrenberg) Chodat	−	+
Scenedesmus subsicatus Chodat	+	+
Schroederia setigera (Schröder) Lemmermann	−	+
Tetraëdron minimum (A. Braun) Hansgirg	+	+
Euglenophyta		
Euglena sp.	−	+
Phacus sp.	+	+
Bacillariophyta		
Nitzschia closterium (Ehrenberg) W. Smith	+	+
Nitzschia paleacea Grunow	+	+
Cryptophyta		
Cryptomonas sp.	−	+
Haptophyta		
Prymnesium parvum N. Carter	+	+

+ and −, present and absent morphospecies, respectively.

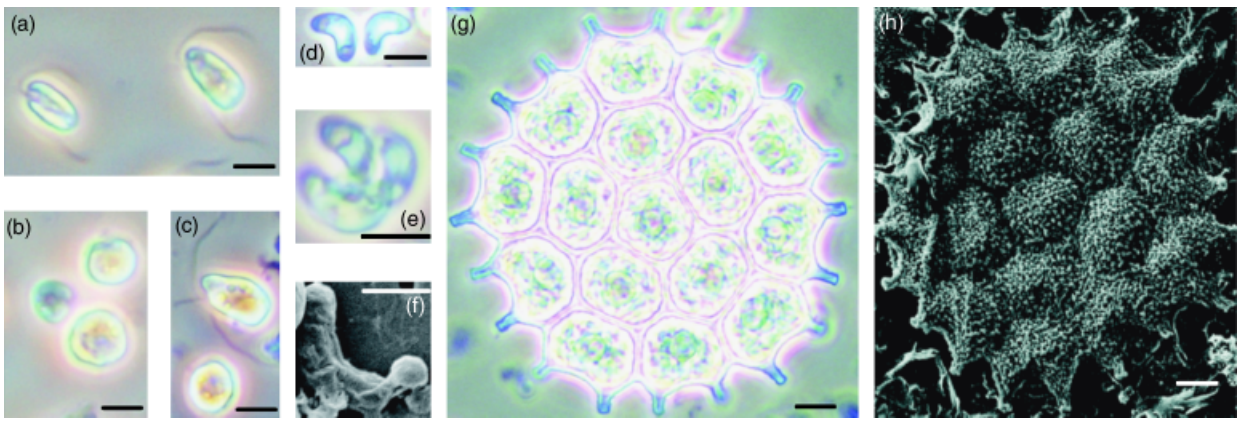

Fig. 1. Dominant microscopic eukaryotes of Lake Koronia, Greece. Light micrographs (phase contrast) of live cells (except for Fig. 1f and 1h, SEM micrographs of preserved samples). (a) Swimming flagellates of the Haptophyte *Prymnesium parvum* with sub-equal flagella and non-coiling haptonema, (b) Non-motile coccoid cells of *P. parvum*, (c) Flagellate cells of *P. parvum* settled to the bottom of the sedimentation chamber; one has stopped swimming and exhibited a round shape, (D - F). Different sides of the Mesomycetozoea *Amoebidium* sp. and (G, H). The chlorophyte *Pediastrum boryanum*. Scale bar: 5μm.

Phylotype (kor-250804-16) dominated the eukaryotic microbial community on both dates and was closely related to the chlorophyte *P. boryanum* as shown by both microscopic observation and molecular analysis. In the clone libraries, it constituted 58.4% and 81.7% of the clones on 25 August and 11 September 2004, respectively, whereas its abundance was estimated to be $< 10\%$ of the total eukaryote abundance with microscopic counts. This could be attributed to the fact that each *Pediastrum* individual is in fact a coenobium comprised of at least 16 cells in our samples; thus, each microscopic 'individual' is expected to have multiple 18S rRNA gene copies. Another chlorophyte-like phylotype (kor-250804-18) occurred as a singleton, but was only 93% similar to an uncultivated member of this phylum originating from soil.

The second most dominant phylotype (kor-250804-5) in both of the clone libraries was a *P. parvum*-like species, which reached a very high population density in the lake in

September 2004, among the highest ever recorded for *P. parvum* (Moustaka-Gouni *et al.*, 2004). The water samples contained actively swimming flagellates (Fig. 1a) and nonmotile coccoid cells (Fig. 1b). After several hours of microscopic observation of water samples, the flagellate cells stopped swimming and exhibited a round shape (Fig. 1c). The flagellate cells microscopically corresponded to the species *P. parvum*, in accordance with the presence of a short, noncoiled haptonema, their swimming trait cell and their shape and size (Carter, 1937; Green *et al.*, 1982). SEM examination did not reveal scales in preserved samples probably as a result of the presence of high concentrations of dissolved and particulate matter in the samples during fixation. Coccoid cells (a nonmotile stage of the organism), several of which had collapsed flagella, were similar to the closely related, but rarely recorded species of *Platychrysis* (Carter, 1937). *Platychrysis* is distinguished morphologically from *Prymnesium* by the occurrence of the dominant neustonic amoeboid cell stage (Gayral & Fresnel, 1983). Flagellated cells of *Platychrysis* resemble those of *P. parvum* (Edvardsen & Medlin, 2007).

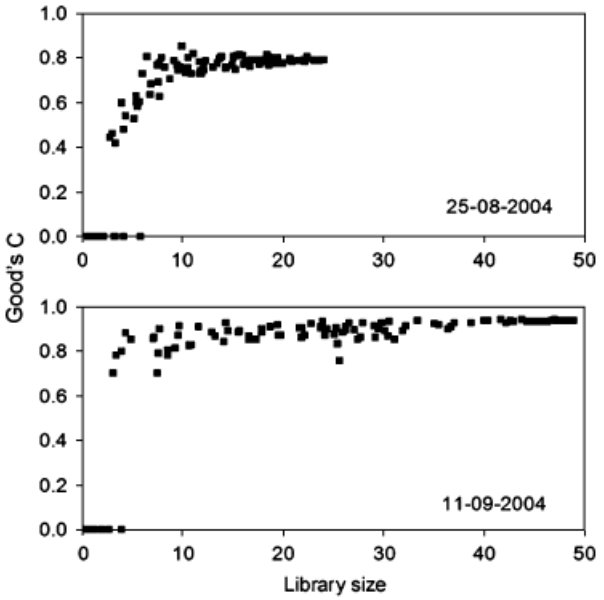

Fig. 2. Clone library coverage based on Good's C estimator of the eukaryotic 18S rDNA clone libraries from Lake Koronia. The ratio of observed phylotypes: predicted phylotypes (S_{Chao1}) was 0.405 and 0.668 at 25 August and 11 September 2004, respectively.

The close relationship between *Platychrysis* and *Prymnesium* has also been demonstrated by molecular phylogeny. Members of *Platychrysis* fall within the *Prymnesium* clade in the 18S rRNA gene phylogenetic tree of haptophytes and should be transferred to this genus (Edvardsen & Medlin, 2007). In accordance to this suggestion, only one phylotype in our study (kor-250804-5) fell in a *Platychrysis–Prymnesium* clade (Fig. 3). Based on our morphological and phylogenetic analysis, and the ecological niche of the species (Michaloudi *et al.*, 2009), this heteromorphic haptophyte was assigned to *P. parvum*.

Prymnesium parvum is considered a candidate species forming 'ecosystem disruptive blooms', EDAB species (i.e. dense monospecific blooms that cause detrimental alternation of ecosystem function) (Sunda *et al.*, 2006). This organism can survive in a wide range of salinities and it blooms in coastal and brackish inland waters worldwide (Edvardsen & Paasche, 1998). In some cases, its blooms coincide with massive deaths of fish (Lindholm *et al.*, 1999). This has also been the case in Lake Koronia during the study period (Moustaka-Gouni *et al.*, 2004; Michaloudi *et al.*, 2009).

One of the three fungi-like phylotypes found (kor-110904-17) was related to the *Chytridiomycota*, certain members of which occur primarily as phytoplankton parasites in aquatic systems (Ibelings *et al.*, 2004; Lefèvre *et al.*, 2007). The parasitic life cycle of chytrids can be an important factor controlling seasonal succession of phytoplankton, as

Table 2. Occurring 18S rRNA gene phylotypes in the water column of Lake Koronia, Greece

	No. of similar clones (> 98%)			
Phylotypes	25 August 2004	11 September 2004	Putative affiliation	Closest relative (% similarity) [GenBank accession no.]
kor-250804-16	14	40	*Chlorophyta*	*Pediastrum boryanum* (99%) [AY663035]
kor-250804-5	5	3	*Haptophyceae*	*Platychrysis* sp. (98%) [AB183611]
kor-110904-24	0	3	*Fungi*	Clone WIM48 (93%) [AM114806]
kor-110904-15	0	1	*Apicomplexa*	Clone Elev-18S-1206 (81%) [EF024716]
kor-110904-17	0	1	*Chytridiomycota*	Clone PFG9SP2005 (93%) [EU162638]
kor-110904-3	0	1	*Ciliophora*	*Enchelys polynucleata* (96%) [DQ411861]
kor-250804-11	1	0	*Ciliophora*	*Pattersoniella vitiphila* (99%) [AJ310495]
kor-250804-14	1	0	*Fungi*	Clone CCW24 (92%) [AY230211]
kor-250804-18	1	0	*Chlorophyta*	Clone WIM12 (93%) [AM114821]
kor-250804-27	1	0	*Mesomycetozoea*	Clone DSGM-66 (90%) [AB275066]
kor-250804-8	1	0	*Jakobida*	*Jakoba libera* (84%) [AF411288]

infection of one species may favour the development of other algal species (reviewed in Ibelings *et al.*, 2004), which in turn can alter the succession. As chytrids can become epidemic under both winter and summer conditions, namely under the combination of low temperature and low irradiance and high temperature and high irradiance (Bruning, 1991), their activity in late summer in Lake Koronia could also involve parasitism on the prevailing abundant phytoplankton biomass. Although *Phlyctidium scenedesmi* has been reported as a specific parasite for *P. boryanum* (Masters, 1971), which is the most abundant algal phylotype and abundant morphospecies in our study, the closest known relatives of the Lake Koronia's chytrid-like phylotype are *Chytriomyces* spp., the most frequently occurring and abundant species in freshwater systems (James *et al.*, 2006). The other two clones (kor-250804-14 and kor-250804-24) could not be affiliated to any known fungal taxon and were related to fungal phylotypes retrieved from complex soil and hypoxic marine habitats.

One phylotype (kor-250804-27) was positioned within the class of *Mesomycetozoea* (Fig. 3), a monophyletic group, which consists mainly of obligate or facultative parasites of various animals (Mendoza *et al.*, 2002), and it showed low

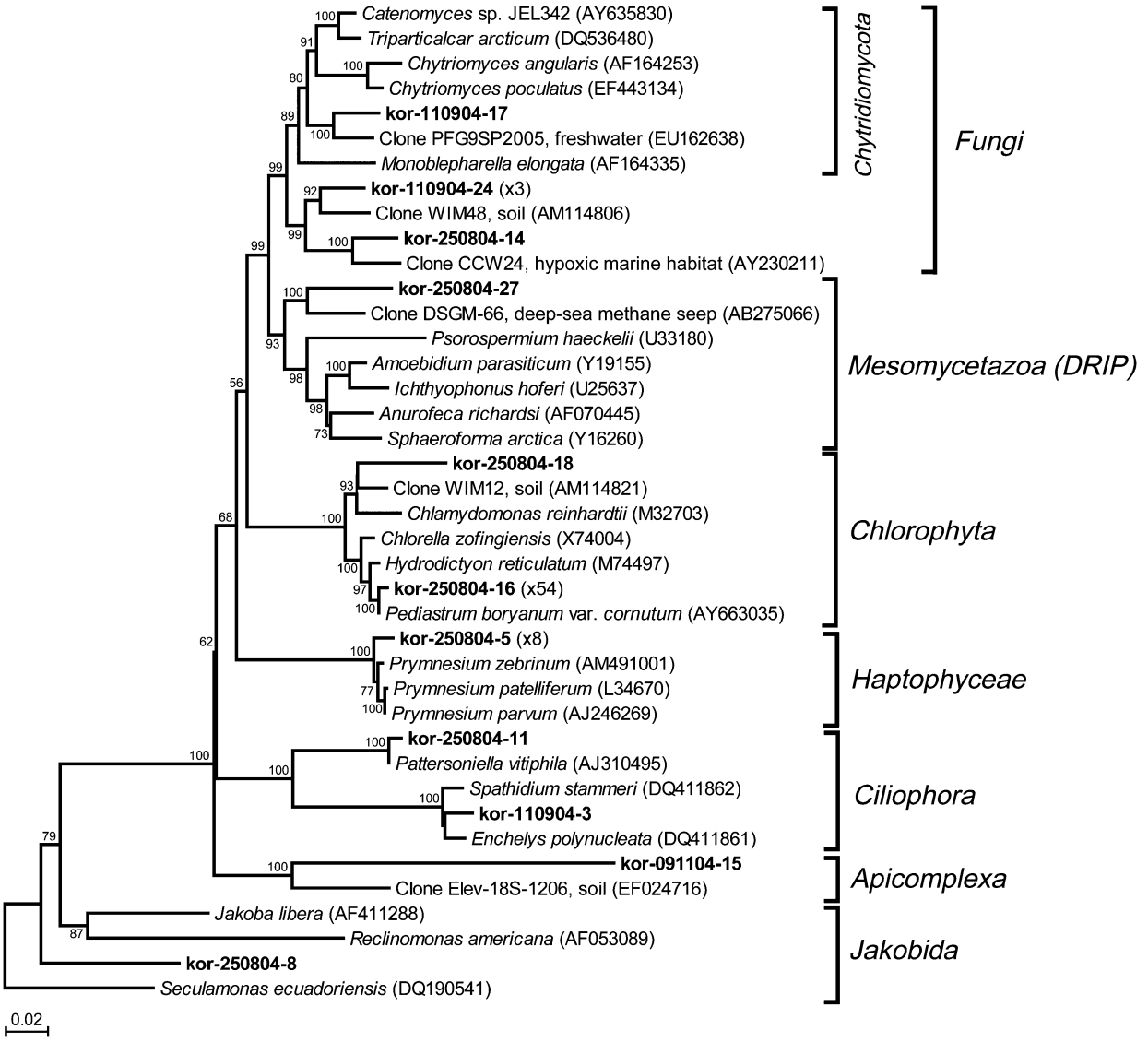

Fig. 3. Phylogenetic tree of relationships of 18S rDNA (*c.* 1600 bp) of the representative eukaryotic clones found in the water column of Lake Koronia, Greece, based on the neighbour-joining method as determined by distance Jukes-Cantor analysis. One thousand bootstrap analyses (distance) were conducted, and percentages greater than 50% are indicated at the nodes. Groups (bold letters) that have ≥98% similar nucleic acid sequences are represented by a single sequence, with the number of clones out of the total in parentheses. The numbers in brackets are GenBank accession numbers. Scale bar represents 2% estimated distance.

similarity (< 90%) to *Amoebidium parasiticum*. Phylogenetic studies suggest that the *Mesomycetozoea* clade diverged near the animal–fungal dichotomy (Ragan *et al*., 1996), suggesting that such unicellular protists were the predecessors of multicellular protoanimals, from which the major metazoan lineages emerged (Lang *et al*., 2002). The members of Amoebidiales have only been reported to be arthropod, crustacean and insect larvae parasites (Benny & O'Donnell, 2000; White *et al*., 2006), but *A. parasiticum* is considered an apparent nonpathogenic microorganism (Mendoza *et al*., 2002). Our microscopic observation revealed a morphospecies that shared some morphological similarities to the species *A. parasiticum* and *Amoebidium appalchense*. In particular, we observed mainly motile lunate-shaped spores (Fig. 1d–f) (Whisler, 1968; Mendoza *et al*., 2002), with dimensions 8.7–16.9 (average 10.6) × 3.4–5.9 (average 4.5) μm, close to the dimensions of *A. appalchense* (White *et al*., 2006). No amoebal cysts were observed (Whisler, 1968), but for *A. parasiticum*, the production of such cysts occurs only when the host moults or is injured (White *et al*., 2006). In some cases, *A. parasiticum* has been misidentified as the former chlorophyte (now considered a lower fungus) *Hyaloraphidium curvatum* (Ustinova *et al*., 2000), because of the similarities in the morphological features. Nevertheless, our phylogenetic analysis revealed no phylotypes similar to any *Hyaloraphidium*-like clones. The high number of *Amoebidium* sp. individuals on 11 September 2004 possibly adds to the potential parasitic hazards imposed on Lake Koronia's organisms.

Two phylotypes belonged to the *Ciliophora*. Phylotype kor-110904-3 was related to *Enchelys polynucleata*/*Spathidium stammeri*. These genera include known free-swimming predators of flagellates and other ciliates as well, commonly found in freshwater systems. Phylotype kor-250804-25 was closely related to *Pattersoniella vitiphila*. This genus consists of free-swimming protists with cosmopolitan distributions in marine, freshwater and terrestrial habitats, which feed on bacteria, microalgae and smaller protists, but several of the genera included are macrophagous carnivores on other ciliates and even smaller metazoa (Lynn, 2008). It seems that these phylotypes represent the members of the higher microbial loop of the lake; however, their relative abundance was low both in the clone library and in the microscopic examination.

Phylotype kor-250804-8 fell within the ubiquitous heterotrophic nanoflagellate (HNFs) group of the *Jakobida* (Fig. 3). This phylotype could not be assigned to a specific morphospecies of HNFs observed under microscopy. This phylotype possibly represents a new member of the jakobids as it was distantly related to any known species of this group.

Finally, clone kor-110904-15 grouped within the *Apicomplexa* protists, a group of endoparasites of animals, which includes toxoplasmosis- and malaria-causing agents (Vivier & Desportes, 1990), but was phylogenetically distant from any known representative.

In the present study, we investigated the microeukaryotic diversity of an extreme lentic environment (Lake Koronia, Greece) during an ecological catastrophe with a mass kill of birds and fish in August–September 2004 at the morphological and molecular level. Only three taxa, including the most dominant ones, were commonly found by both the 18S rRNA gene diversity and the microscopic observation of live material. This suggests that the chlorophyte *P. boryanum*, the haptophyte *P. parvum* and the mesomycetozoan *Amoebidium* sp. dominate microscopic eukaryote populations under the extreme conditions of Lake Koronia. Based on the inferred ecophysiology of the found taxa, the > 1-μm fraction of the microbial food web seems to be characterized by cosmopolitan higher trophic levels (i.e. ciliates) that thrive on smaller organisms (i.e. flagellates and fungi) belonging to taxa of organisms, which are known to be toxic, parasitic and pathogenic, thus rendering Lake Koronia a hostile and dangerous environment for animals including humans, as has been suggested earlier for this lake (Moustaka-Gouni *et al*., 2004; Michaloudi *et al*., 2009) but also for other lakes with a similar microbial community organization (Lefèvre *et al*., 2008).

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