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Molecular data unravels three freshwater peridinioids in the Baghdad Island Tourist Lake, reporting *Palatinus apiculatus* (Peridiniopsidaceae, Peridiniales) as a new record for the Iraqi algal flora

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Molecular data unravels three freshwater peridinioids in the Baghdad Island Tourist Lake, reporting *Palatinus apiculatus* (Peridiniopsidaceae, Peridiniales) as a new record for the Iraqi algal flora

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In the past few decades, molecular phylogenetic approaches have been proven to be crucial techniques to precisely delimit the taxonomic position of the algal species complex. Species of dinoflagellates have been traditionally identified based on morphotaxonomic traits, but molecular evidence accumulated over the past decades emphasized that some morphologically based descriptions need to be more accurate. This recognition led to an increasing reliance on sequencing data to delimit species identities. Our current knowledge of the diversity and distribution of dinoflagellates in Western Asian countries is still poorly known, particularly in less-studied developing countries like Iraq. Herein, the nuclear internal transcribed spacer (ITS) rRNA phylogenetic approach, as well as the morphotaxonomic features and ecological preferences, was used to refine the taxonomic assessment of three peridinioids (Peridiniales, Dinophyceae) isolated from the Baghdad Island Tourist Lake, central Iraq. As inferred from the ITS molecular phylogeny, the strain ZaFiAm50 was placed within the strongly supported *Palatinus* clade inside the Peridiniopsidaceae family. Considering the integrative ITS molecular and morphological traits, this species was identified as *Palatinus apiculatus*. This freshwater peridinioid is considered a new record for the Iraqi algal flora based on available literature. The remaining two isolates phylogenetically belonged to the Peridiniaceae family clade, where the strain ZaFiAm80 was identified as *Peridinium willei* while the strain ZaFiAm10 received high branch support and resolved inside *Peridinium cinctum* lineage. Each species was given a short description, geographical distribution, and ecological preferences.

Keywords: Dinophyceae, Iraq, ITS rRNA phylogeny, new records, morphotaxonomy

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INTRODUCTION

Microalgae represent a great and yet unraveled diversity of the biosphere and have functional roles in the nitrogen and carbon cycles, in the freshwater and marine food webs, as well as in the global production of oxygen and CO_2 sequestration (Somogyi et al., 2022). Dinoflagellates are a diverse group of single-celled eukaryotic microalgae found in brackish, marine, and freshwater habitats. Dinoflagellates' genome is unique and considered the largest among other eukaryotic microorganisms (Aranda et al., 2016). Taxonomically, dinoflagellates are a complex but functionally diverse algal group, and, therefore, the application of molecular approaches is crucial to delimit the species identity due to their genetic crypticity and morphological variability 'ecomorphotypes' (López et al., 2018; Bolch, 2022; Holzer et al., 2022). They have a major lineage of unicellular eukaryotes with unparalleled diversity and complexity in morphological features (Taylor et al., 2008). Although the dinoflagellate monophyly has been successfully demonstrated with a combination of molecular phylogenetic data sets, the interrelationships across the lineages remain unresolved (Murray et al., 2005). Integrative molecular and modern taxonomic studies have recently established several new genera, species, and families (e.g., Gottschling et al., 2017; Luo et al., 2021; Dawut et al., 2023). For instance, the new genus *Palatinus* Craveiro, Calado, Daugbjerg et Moestrup, with the type species *P. apiculatus* (Ehrenberg) Craveiro, Calado, Daugbjerg et Moestrup (order Peridiniales, family Peridiniopsidaceae) (Craveiro et al., 2009), has been recently erected from the *Peridinium* group palatinum and typical *Peridinium* based on its discriminative LSU rRNA phylogenetic placement, besides the differences in plate numbers and ornamentation, and a suite of internal cell features. From an ecological standpoint, dinoflagellates are essential because they act as primary producers in their habitats (Hinder et al., 2012; Borowitzka, 2018).

In the original description of the family Peridiniopsidaceae (Gottschling et al., 2017), the presence of six cingular plates and up to two intercalary plates in the epitheca are two of the key diagnostic features to discriminate members of this family from other members of the family Peridiniaceae. The fine structure descriptions for several family members of Peridiniopsidaceae have been summarized by Pandeirada et al. (2023). Taxonomically, the family Peridiniopsidaceae mainly includes the five major genera: *Peridiniopsis* Lemmermann, *Palatinus*, *Parvodinium* Carty, *Johsia* Luo, Na Wang, Mertens et Gu, and *Chiharadinium* Dawut et Horiguchi (Dawut et al., 2023; Pandeirada et al., 2023). The genus *Palatinus* is only represented by two taxonomically accepted freshwater peridinioids: *P. apiculatus* and *P. laevis* (Huitfeldt-Kaas) Gottschling, Kretschmann et Zerdoner Calasan. The key diagnostic features for *Palatinus* include: (1) a smooth or slightly granulate, but not areolate, plate surface, (2) a large central pyrenoid penetrated by cytoplasmic channels and radiating into chloroplast lobes, and (3) the presence of a peduncle-homologous microtubular strand. *Palatinus* cells exit the theca through the antapical-postcingular area (Craveiro et al., 2009; Kretschmann et al., 2018).

The current classification of the genus *Peridinium* Ehrenberg, based on features of the theca, holds together species with and without an apical pore complex and with varying numbers of plates in epithecal and cingular Kofoidean series and excludes species with several anterior intercalary plates smaller than two (Moestrup & Calado, 2018). The genus *Peridinium* (Peridiniaceae, Peridiniales) has approximately 113 taxonomically accepted species and infraspecies, most of them inhabiting fresh and brackish water environments (Guiry & Guiry, 2024). Morphologically, the delineation of the genus *Peridinium* and its related infraspecies mainly depends on their morphological characteristics, specifically the arrangement of the plates and apical pore complex (Moestrup & Calado, 2018). The type species *P. cinctum* (O.F. Müller) Ehrenberg is distinguished by having five cingular plates, three apical intercalary plates in an asymmetrical arrangement, and no apical pore (Carty, 2008). *P. cinctum* has been reported to be variable in both morphology and genotype (Romeika et al., 2019). Ecologically, *P. cinctum* is widespread in distribution and usually present in nutrient-rich, freshwater habitats.

Our knowledge about the diversity and distribution of freshwater dinoflagellates in Iraq is very poor. Furthermore, all the previous studies mainly depended on morphological identification, necessitating updated molecular approaches in species and infraspecies delimitation. As a result, some of these identifications must be more accurate morphologically due to needing more experience in morphotaxonomy standards of dinoflagellates. For instance, Maulood and co-workers (2013) reported thirty-five different dinoflagellates in their Iraq algal flora checklist. All these taxa were only morphologically identified by the traditional classification systems. Toma & Aziz (2021) recorded four species of dinoflagellates in different ponds and streams in Shaqlawa district-Erbil.

The assessment of ecological status in lentic habitats, such as lakes, is primarily based on assessing biological elements, such as phytoplanktons. Among these organisms, dinoflagellates could be excellent bioindicators for water quality assessment (Narale & Anil, 2017). In general, phytoplanktons, including dinoflagellates, as the first link in the trophic chain, respond quickly to dramatic changes in the aquatic environment, and for this reason, it is an excellent indicator of water quality biomonitors (Dembowska, 2021).

As part of our ongoing project on studying and recognizing the algal diversity in Baghdad Island Tourist Lake (Iraq), three different dinoflagellates, namely *Palatinus apiculatus*, *Peridinium cinctum,* and *P. willei*, were isolated and characterized by applying nuclear internal transcribed spacer (ITS) rRNA phylogenetic analysis and state-of-the-art morphotaxonomy. The findings obtained in this work not only improved our poor understanding of the hidden diversity of dinoflagellates and ecological status of this touristic Lake but also documented, for the first time, the freshwater species *P. apiculatus* as a new record for the Iraqi algal flora.

MATERIALS AND METHODS

Study area

Baghdad Island Tourist Lake is one of the amusement destinations in Iraq. It is an artificial freshwater lake located in the Al-Fahama region, about 20 km north of Baghdad. It has a total area of ~20 km^2. The Lake is situated on the right side of the tourist island. It is severely exposed to different human pressures as a touristic place. Two sites were monthly sampled during the winter season of 2020 (i.e., in December 2020, January 2021, and February 2021) to observe the phytoplanktonic diversity, particularly the dinoflagellates, of the Lake. Site 1 is located at the upper side of the Lake (33° 27' 39.6" N, 44° 20' 32.4" E), while site 2 is located at the end of the Lake (33° 26' 36.6" N, 44° 19' 47.4" E) (Fig. 1).

Sampled algal materials and light microscopy

Near the surface, five phytoplanktonic samples were collected in the morning with a phytoplankton net (mesh-size 20 µm) at the upper and lower sides of the Baghdad Island Tourist Lake. Each composite sample was placed in a 100 mL sterile, clean polyethylene (PET) bottle for transport. The specimens were transported on ice to the laboratory for further studies. Dinoflagellates were morphologically identified using a Genex® microscope (model GX-140105) at the Algal and Environmental Laboratory, Department of Biology, College of Science for Women, Baghdad University, and a BEL® photonics biological microscope at the Abd El-Salam M. Shaaban's Phycology Lab, the Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. A total of 20 measurements were made for the taxonomically important morphometric features. Specimens were morphologically identified using the relevant literature of Craveiro et al. (2009) and Moestrup & Calado (2018).

Hydrochemical characterization

The sampling campaign has been achieved during the three months of winter 2020. Water sampling was conducted using clean polyethylene bottles. All the bottles were filled airtight with no bubbles and kept at 4 °C until analyses. Water temperature, pH, electrical conductivity, and total dissolved solids in situ were measured with a calibrated HANNA HI 991301 meter. Dissolved oxygen (D.O.) was determined using the in-field calibrated dissolved oxygen meter Lutron® YK-22DO. Alkalinity measurements were also performed using a digital titrator (HACH). Detailed hydrochemical characteristics, including major ions, nutrients, trace elements, and metals, were measured following standard procedures and methods (Chapman & Pratt, 1978; Clesceri et al., 2000). Metals were analyzed with a PerkinElmer Optima 5300 inductively coupled plasma optical emission spectrometer (ICP–OES).

Culturing and isolation

The dinoflagellates identified during the study period were isolated and grown on BG-11 (Rippka et al., 1979) in a growth chamber at 20 ±1 °C and 12:12 h L/D photoperiod using cool white, fluorescent lamps at an irradiance of 175 µmol photons m^{-2} s^{-1}. The strains were isolated from the mixed plankton samples by the standard micro pipetting technique (Andersen and Kawachi, 2005).

Sequencing and phylogenetic analysis

For the DNA analysis, the cultures were harvested during the exponential growth phase and concentrated via centrifugation. Sequences of the nuclear internal transcribed spacer (ITS) rRNA were obtained for species identification. DNA was extracted from living cells, as described previously by Echt et al. (1992), with some modifications described in Abdullin et al. (2021). ITS rRNA was amplified using primer combinations and temperature profiles following Abdullin et al. (2021) in a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., USA). The PCR products were sequenced in both directions with the same primers at the FSCEATB FEB RAS using an ABI 3500 genetic analyzer (Applied Biosystems, USA) with a BigDye terminator v. 3.1 sequencing kit (Applied Biosystems, Maryland, USA). Sequences were assembled with the Staden Package v.1.4 (Bonfield et al., 1995).

The sequences of the ITS rRNA of the peridinioids were compared with those from authentic and reference strains available at the National Center for Biotechnology Information (NCBI, Bethesda, USA) using a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on June 1st, 2023) for estimation of their taxonomic position. The selection of representative NCBI accessions for phylogenetic analyses was followed by Luo et al. (2021) and Dawut et al. (2023). The dataset focused on closely related species of Peridiniopsidaceae Gottschling, Kretschmann & Zerdoner Casalan and Peridiniaceae Ehrenberg. For alignment constitution, we used sequences including SSU, ITS1-5.8S-ITS2, and LSU rRNA, according to Luo et al. (2021). The dataset included 30 accessions (3743 aligned positions). Three taxa representing phylogenetically distant lineage were added as an outgroup. The sequences (taxa, accession number, and strain name given as listed in the NCBI) were aligned in the SeaView program (Galtier et al., 1996) with manual corrections.

The best evolutionary model, GTR+I+G, was determined using jModelTest 2.1.1 (Darriba et al., 2012). Phylogenetic trees were constructed using the maximum likelihood (ML) method in RAxML v.7.2.6 (http://embnet.vital-it.ch/raxml-bb/; accessed on June 2nd, 2023) (Kozlov et al., 2019) and Bayesian inference (BI) in MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001). In BI, four runs of four Markov chains were executed for 5 million generations, sampling every 100 generations for 50000 samples.

Convergence of the chains was assessed, and stationarity was determined according to the "sump" plot, with the first 12500 samples (25%) discarded as burn-in; posterior probabilities were calculated from trees sampled during the stationary phase. The robustness of the ML trees was estimated by examining the bootstrap percentages (BPs; Stamatakis et al., 2008) and posterior probabilities (PPs) in BI. Those with BPs <50% and PPs <0.95 were not considered. The new ITS rRNA sequences from this study were submitted to the NCBI GenBank database and have the following accession numbers: MT873521 for *Peridinium cinctum* ZaFiAm10, MT873522 for *Palatinus apiculatus* ZaFiAm50, and MT873523 for *Peridinium willei* ZaFiAm80.

RESULTS

In the present study, three freshwater peridinioids were identified and discussed from the standpoints of ITS rRNA phylogenetics, state-of-the-art morphotaxonomy, and ecological characterization. Out of these taxa, *Palatinus apiculatus* became a new record for the Iraqi algal inventory. General cell morphology and the arrangement of plates in the three freshwater peridinioids identified in the present study were consistent with the protologues. Detailed descriptions, ecological preferences, and biogeography of all these taxa are given below. Average values of the hydrochemical characteristics of the Baghdad Island Tourist Lake during the study period are provided in Table 1.

Environmental variables

The values of the environmental variables measured and analyzed are presented in Table 1. The water temperature varied between 11.6 and 23.4 °C, with an average value of 21.5 ± 8.2 °C. The pH was relatively neutral to alkaline, with an average value of 7.70 ± 0.4. The lake water was turbid, with an average value of 5.58 ± 2.6 NTU. The Baghdad Island Tourist Lake is an artificial freshwater ecosystem with an average TDS value of 526 ± 27.4 mg. L^{-1}. Dissolved oxygen ranged between 5.0 to 9.7 mg. L^{-1}. The water hardness varied from moderately hard to very hard during the study period. SRP, nitrate, and ammonia concentrations were remarkably high. Ni, Zn, and Fe were the dominant metals, respectively.

Phylogenetic affinities of the specimens investigated

To aid the morphology-based identification process, we assembled the dataset with sequences of Peridiniopsidaceae and Peridiniaceae representing major genera and 3 outgroup taxa (Figure 2). Trees of consistent topology reconstructed by ML and BI methods agreed with the previous results and placed our strain ZaFiAm50 within the strongly supported *Palatinus* genus clade (100/1.00, for BP and PP, respectively) inside the Peridiniopsidaceae family (Figure 2). *Palatinus pseudolaevis* is placed basally in the clade, while the lineage of *Palatinus apiculatus* has a terminal position. Two isolates, ZaFiAm80 and ZaFiAm10, were attributed to the Peridiniaceae family clade (100/1.00). The former strain was closely related to *Peridinium willei* accession (97/0.99). The latter strain also received high branch support (100/1.00) and resolved inside *the Peridinium cinctum* lineage (100/1.00). Removing SSU and/or LSU regions did not affect the tree topology (results not shown), but branch support decreased slightly.

Description of the identified taxa

The three freshwater peridinioids identified in the Baghdad Island Tourist Lake showed morphological diagnostic features that coincided largely with their original descriptions. Morphological variability has not been observed under the study circumstances.

Palatinus apiculatus **(Ehrenberg) Craveiro, Calado, Daugbjerg et Moestrup**

Description: Cells ovoid, dorsoventrally flattened, 35–54 µm long x 30–45 µm wide; epitheca bell-shaped, longer than the rounded hypotheca, separated by distinct cingulum offset by half a cingulum width; the sulcus wide and widens as it descends into hypotheca, narrows in its short entry to epitheca; cell wall composed of plates, ornamented with ridges along margins and irregularly arranged small spines, more pronounced on the hypotheca; first apical plate narrow, short, sutures pronounced with delicate striations and occasionally flanged on either side, those reaching apex give an overall impression of an apical horn; epithecal plates asymmetrically arranged; chloroplasts numerous and radially arranged.

General distribution and ecology*:* Worldwide; found in pools and ponds, mainly in winter and spring.

Peridinium cinctum **(O.F.Müller) Ehrenberg**

Description: Cells rounded to ovoid, sometimes dorsoventrally flattened, 43–75 µm long x 33–70 µm wide; hypotheca shorter than epitheca, separated by narrow, flanged cingulum; the flanged sulcus extending about one-third length of epitheca, and descending to the antapex of hypotheca; cell wall consists of thick thecal plates with coarsely net-like

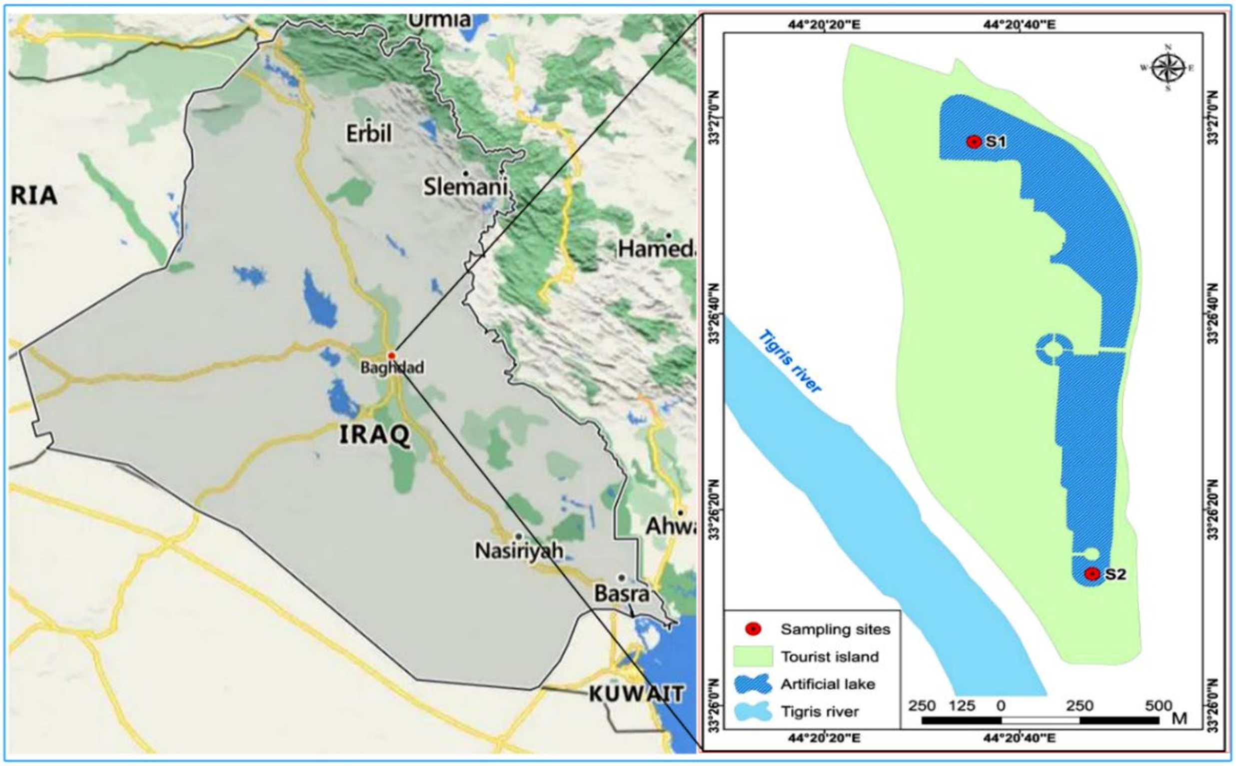

Figure 1. Sampling sites were studied at the Baghdad Island Tourist Lake, central Iraq.

Table 1. Physical and chemical variables of the Baghdad Island Tourist Lake where the freshwater peridinioid taxa were found during the winter season of 2020.

Parameters	**Minimum**	**Maximum**	**Mean ± SD**
Water temperature (°C)	11.6	23.4	21.5 ± 8.2
pH	6.98	8.53	7.70 ± 0.4
Turbidity (NTU)	1.56	10.6	5.58 ± 2.6
Conductivity ($\mu S.cm^{-1}$)	346	922	638.5 ± 20
T.D.S. ($mg.L^{-1}$)	252	800	526 ± 27.4
Dissolved Oxygen ($mg.L^{-1}$)	5.0	9.7	7.21 ± 1.2
Chemical Oxygen Demand (COD) ($mg.L^{-1}$)	2.0	3.87	2.89 ± 0.36
Alkalinity (as $CaCO_3$ $mg.L^{-1}$)	98.0	380	136.8 ± 51.2
Total hardness ($mg.L^{-1}$)	100	503	313.8 ± 91.2
Ca^{2+} ($mg.L^{-1}$)	28.4	124.3	68.7 ± 21.3
Mg^{2+} ($mg.L^{-1}$)	13.6	93.7	59.2 ± 19.1
Cl^- ($mg.L^{-1}$)	12.0	81.97	59.4 ± 14.6
Silicate (as SiO_2) ($mg.L^{-1}$)	0.44	10.6	4.09 ± 2.62
Soluble Reactive Phosphate (SRP) ($\mu g.L^{-1}$)	200	1850	930 ± 91
NH_4^+ ($\mu g.L^{-1}$)	0.0	1000	320 ± 27
NO_3^- ($\mu g.L^{-1}$)	0.0	2525	1730 ± 44.7
Fe ($\mu g.L^{-1}$)	0.0	30.4	5.58 ± 7.2
Ni ($\mu g.L^{-1}$)	0.0	98.4	36.97 ± 11.4
Zn ($\mu g.L^{-1}$)	0.0	37.0	19.9 ± 8.2

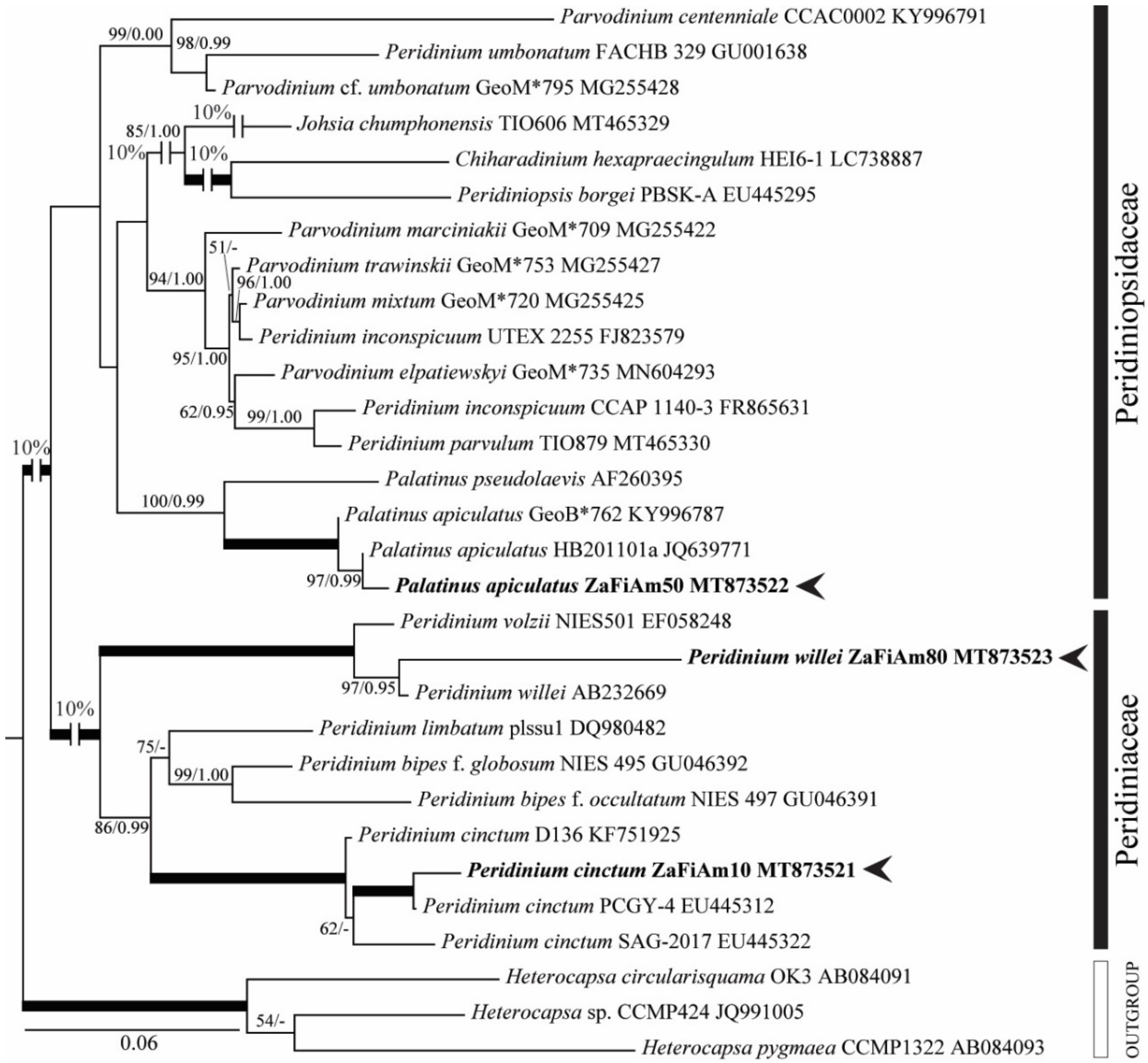

Figure 2. ML phylogenetic tree inferred from concatenated (SSU-ITS-LSU rRNA) genes of 30 peridinialean dinoflagellates showing the phylogenetic position of our isolates (boldface, with arrowhead; 3743 aligned positions; GTR+I+G model). Support [(BP) ≥ 50% and (PP) ≥ 0.95: ML/BI] are given above/below the branches. The sequences have strain names (if provided) and GenBank accession numbers. Scale bar – substitutions per nucleotide position. Some branches were reduced in length (only 10% shown).

ornamentation and in older cells ornamentation extending to small spines; antapical plates frequently uneven in size; apical pore absent; chloroplasts parietal, numerous, dark brown, and arranged around the periphery of cells.

General distribution and ecology: Cosmopolitan in pools, lakes, ponds, and reservoirs.

Peridinium willei **Huitfeldt-Kaas**

Description: Cells rounded, often more comprehensive than more prolonged, and dorsoventrally flattened, 35–77 µm long x 40–80 µm wide; hypotheca shorter than epitheca, separated by a deep, narrow cingulum that may be flanged; sulcus indented, protrudes into epitheca and widens as it descends to base of hypotheca; one or two teeth-like structures may be present on the sulcus margins; cell wall composed of fine net-like ornamentation on thecal plates; apical pore absent; first apical plate relatively large and asymmetric, intercalary and third apical plates narrow and laterally elongate; antapical plates different in size; apical and antapical plates sometimes bearing very pronounced wings; chloroplasts parietal, numerous, dark brown, and arranged around the periphery of cells.

General distribution and ecology: Cosmopolitan in pools, lakes, reservoirs, and peat bogs in all seasons.

DISCUSSION

The taxonomy of freshwater dinoflagellates has been extensively refined during the recent decades, using the combined polyphasic approaches including the molecular phylogeny and the modern taxonomy standards, confirming the taxonomic position of some cryptic species and also led to several nomenclatural changes, new generic propositions, and a redefinition of the phylogenetic relationships among the taxa (Moestrup & Daugbjerg, 2007; López et al., 2018; Holzer et al., 2022). Such revision was based on the populations sampled in Europe, East Asia, and North America. So far, little information is known about the freshwater dinoflagellates from developing countries in Western Asia, such as Iraq (Maulood et al., 2013; Toma & Aziz, 2021). In general, the study of freshwater dinoflagellates in Iraq has been sporadic and partial, and, therefore, our knowledge of the freshwater dinoflagellate species from Iraq is still poorly known. Most of the work above only listed the dinoflagellate species without any taxonomic and genetic information.

In the present study, and as part of our in-depth survey on the algal and cyanobacterial diversity in Iraq, the polyphasic approach applied unraveled three freshwater peridinioids: *Palatinus apiculatus*, *Peridinium cinctum* and *P. willei*. Based on the available literature, the first species is considered a new Iraq record (e.g., Maulood et al., 2013; Toma & Aziz, 2021). Taxonomically, it shares the cell dimensions and ecology of the populations from Europe (Moestrup & Calado, 2018). Regarding *P. cinctum* and *P. willei*, our specimens' morphotaxonomic features and ecological preferences also coincide with the diagnosis of populations illustrated in Carty (2014) and Moestrup & Calado (2018). However, further in-depth research using scanning electron microscopy is still necessary to uncover the presence or absence of morphotaxonomic differences in response to their ecological habitat. Ecologically, *P. cinctum* was a predominant species in the nutrient-rich water of the Baghdad Island Tourist Lake in the winter season. Accordingly, Lewis & Dodge (2011) pinpointed that this dinoflagellate species is widely distributed and usually prefers winter and spring seasons. Concerning *P. willei*, this meso-eutraphentic peridinioid seems to be widely distributed in freshwater biotopes across the globe based on information available on its biogeographical distribution and ecological preferences (Lewis & Dodge, 2011). *P. willei* might be taxonomically confused with *P. volzii*; however, these two species are still distinct morphologically, ecologically (Olrik, 1992) and genetically (Logares et al., 2007).

The ITS rRNA molecular phylogeny presented in Figure 2 complements the morphological features outlined in the results. In the past years, the exploitation of molecular approaches to unravel the phylogeny of the dinoflagellates has progressed considerably (Craveiro et al., 2009; Luo et al., 2021; Dawut et al., 2023). In the present study, comparing ITS rRNA sequences combined with detailed light microscopical observations led to accurately identifying these three morphologically close peridinioids. In agreement with this hypothesis, López et al. (2018), for instance, observed two main variations from the abundant plate pattern in *P. cinctum*, namely an unusual position of the 2^a plate and the irregular shape of the 1^a plate, and linked these unusual features to the geographic or ecological evidence.

Little is known about the ecology of freshwater dinoflagellates (e.g., Lewis & Dodge, 2011). Most freshwater dinoflagellates are found in nutrient-rich smaller water bodies and even temporary pools and artificial lakes, and the cells may be most abundant at different positions within the water body at various times of day. Lewis & Dodge (2011) noted that some freshwater species could thrive in cold waters while others are only found in summer when the water is warm (>15 °C). Accordingly, dinoflagellate species were predominant in the Baghdad Island Tourist Lake during winter.

Based on the hydrochemical variables under this study, the Baghdad Island Tourist Lake can be classified as "eutrophic" because of its SRP and N contents (Table 1). In a similar study, Bartolelli et al. (2005) investigated the hydrobiological characteristics of the Lago di Castreccioni reservoir in the central Apennines (Italy), designed for drinking and irrigation purposes as well as for tourist recreation, and they reported the prevalence of *P. cinctum*, like our study, with other microalgal taxa, indicating the mesotrophic condition of the reservoir. This study also confirmed the broad limits of *P. willei* to the P and N concentrations. Olrik (1992) studied the ecological niches of *P. willei* in Danish lakes and compared its ecological preferences with the closest species, *P. volzii*. She also emphasized that *P. willei* is most often confined to lakes with concentrations of TP <150 $\mu g.L^{-1}$, with the majority of occurrence at PO_4^{3-}-P concentration between 20–40 $\mu g.L^{-1}$. In the present study, *P. willei* showed much more tolerance to the elevated levels of SRP (200–1850 µg.L^{-1}), with an average value of 930 µg.L^{-1}. The same observation has been reported herein for N concentrations, where it tolerated high concentrations of NH_4^+ and NO_3^- with average values of 320 and 1730 µg.L^{-1}, respectively. Accordingly, Olrik (1992) stated that *P. willei* has a wide range of NH_4^+-N tolerance and an almost even distribution in Danish lakes at values between 5 and 420 mg NO_2+NO_3–N L^{-1}. According to the pH niches, the water of the studied Lake was circumneutral to slightly alkaline (6.98–8.53, with an average value of 7.70), and this condition usually prefers the prevalence of this freshwater peridinioid. Accordingly, Olrik (1992) highlighted that *P. willei* is usually found at pH values between 4.2 and 8.5.

This study not only provided the ITS rRNA sequencing data for three freshwater dinoflagellate species from Iraq for the first time but also confirmed the biogeographical distribution of *P. apiculatus* as a new record for Iraq. It has also improved our understanding of the ecological niches for these truly "eutraphentic" dinoflagellates, which could be used as water quality bioindicators.

CONCLUSIONS

The state-of-the-art taxonomic assessment of three freshwater peridinioids, namely *Palatinus apiculatus, Peridinium cinctum*, and *P. willei* isolated from the Baghdad Island Tourist Lake (central Iraq), was refined by applying the ITS rRNA molecular phylogeny, modern morphotaxonomy, and ecological criteria. Based on our findings and the available literature, *P. apiculatus* is reported as a new record for the Iraqi algal flora. Overall, all taxa identified tolerate high nutrient concentrations (i.e., eutraphentic species), reflecting different human pressures on the Baghdad Island Tourist Lake. Although the present study only focused on the most common dinoflagellates in this Lake, the entire flora will be presented in upcoming publications. We believe that the dinoflagellate flora of Iraq is still far from complete, and more detailed studies are needed to expand our poor knowledge of the hidden algal diversity and ecology in this less-studied Asian country.

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CONFLICTS OF INTEREST

No conflicts of interest have been declared.

AUTHOR CONTRIBUTIONS

Conceptualization, Z.J.K., F.M.H., A.M.S.A. and A.M.J.A-O.; methodology, A.M.S.A., A.M.J.A-O. and A.A.S.; software, F.M.H., Z.J.K., A.Y.N. and M.M.E.; validation, A.Y.N., M.M.E., F.M.H. and A.A.S.; formal analysis, Z.J.K., F.M.H., A.M.S.A. and A.M.J.A-O; investigation, Z.J.K., A.M.J.A-O. and A.A.S.; resources, Z.J.K., F.M.H., A.M.S.A., and A.M.J.A-O.; data curation, A.Y.N., M.M.E., F.M.H., and A.A.S.; writing—original draft preparation, Z.J.K., A.M.S.A., A.M.J.A-O., A.Y.N., and A.A.S.; writing—review and editing, F.M.H. and M.M.E.; visualization, F.M.H., M.M.E., and A.A.S.; supervision, F.M.H. and M.M.E.; project administration, F.M.H.; funding acquisition, F.M.H. and A.Y.N. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

Data are available upon request from the authors.

ETHICAL APPROVAL

Not applicable

REFERENCES

Abdullin SR, Nikulin AY, Bagmet VB, Nikulin VY, Gontcharov AA (2021). New cyanobacterium Aliterella vladivostokensis sp. nov. (Aliterellaceae, Chroococcidiopsidales), isolated from temperate monsoon climate zone (Vladivostok, Russia). Phytotaxa 527:221-233. https://doi.org/10.11646/phytotaxa.527.3.7

Andersen R.A. and Kawachi M (2005). Traditional microalgae isolation techniques. In: “Algal Culturing Techniques”. Andersen, R.A. (Ed.), pp. 83–100, Elsevier Academic Press, Inc.

Aranda M, Li Y, Liew YJ, Baumgarten S, Simakov O, Wilson MC, Piel J, Ashoor H, Bougouffa S, Bajic VB (2016). Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. Scientific Reports 6(1):1-15. https://doi.org/10.1038/srep39734

Bartolelli K, Cocchioni M, Dell’Uomo A, Scuri S (2005). Hydrobiological study of a reservoir in the central Apennines (Italy). Annales de Limnologie - International Journal of Limnology 41(2):127-139.

Bolch CJS (2022). Impact of molecular approaches on dinoflagellate taxonomy and systematics. In: Clementson LA, Eriksen RS, Willis A (Eds). Advances in phytoplankton ecology: Applications of emerging technologies. Elsevier pp 81-117.

Bonfield J, Smith KF, Staden R (1995). A new DNA sequence assembly program. Nucleic Acids Research 23:4992-4999. https://doi.org/10.1093/nar/23.24.4992

Borowitzka MA (2018). Biology of microalgae. In: Levinme IA, Fleurence J. (Eds). Microalgae in health and disease prevention. Academic Press, USA pp 23-72.

Carty S (2008). Parvodinium gen. nov. for the umbonatum group of Peridinium (Dinophyceae). Ohio Journal of Science 108:103-107.

Carty S (2014). Freshwater dinoflagellates of North America. Comstock Publishing Associates, Ithaca/London

Chapman HD, Pratt PF (1978). Methods of analysis for soils, plant and water. Division of Agriculture Science, University of California, California, USA

Clesceri LS, Greenberg AE, Eaton AD (2000). Standard methods for the examination of water and wastewater (20th ed). American Public Health Association, Washington D.C., USA

Craveiro SC, Calado AJ, Daugbjerg N, Moestrup Ø (2009). Ultrastructure and LSU rDNA-based revision of Peridinium group Palatinum (Dinophyceae) with the description of Palatinus gen. nov. Journal of Phycology 45(5):1175-1194. https://doi.org/10.1111/j.1529-8817.2009.00739.x

Darriba D, Taboada GL, Doallo R, Posada D (2012). jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9:772. https://doi.org/10.1038/nmeth.2109

Dawut M, Yamaguchi A, Horiguchi T (2023). Establishing a new genus, Chiharadinium gen. nov. (Peridiniales, Dinophyceae) for a tidal pool dinoflagellate formerly known as Scrippsiella hexapraecingula. Phycological Research 71:90-99. https://doi.org/10.1111/pre.12513

Dembowska EA (2021). The Use of phytoplankton in the assessment of water quality in the lower section of Poland's largest river. Water 13:3471. https://doi.org/10.3390/w13233471

Echt CS, Erdahl LA, McCoy TJ (1992). Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Genome 35:84-87. https://doi.org/10.1139/g92-014

Galtier N, Gouy M, Gautier C (1996). SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. Bioinformatics 12:543-548. https://doi.org/10.1093/bioinformatics/12.6.543

Gottschling M, Kretschmann J, Čalasan AŽ (2017). Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae). Phytotaxa 299(2):293-296. https://doi.org/10.11646/phytotaxa.299.2.16

Guiry MD, Guiry GM (2024). AlgaeBase. Worldwide electronic publication, National University of Ireland, Galway. https://www.algaebase.org; searched on 2nd February 2024

Hinder S, Hays G, Edwards M, Roberts EC, Walne AW, Gravenor MB (2012). Changes in marine dinoflagellate and diatom abundance under climate change. Nature Climate Change 2:271-275. https://doi.org/10.1038/nclimate1388

Holzer VJC, Kretschmann J, Knechtel J, Owsianny PM, Gottschling M (2022). Morphological and molecular variability of Peridinium volzii Lemmerm. (Peridiniaceae, Dinophyceae) and its relevance for infraspecific taxonomy. Organisms Diversity & Evolution 22:1-15. https://doi.org/10.1007/s13127-021-00514-y

Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755. https://doi.org/10.1093/bioinformatics/17.8.754

Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A (2019). RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35:4453-4455. https://doi.org/10.1093/bioinformatics/btz305

Kretschmann J, Čalasan AZ, Kusber W-H, Gottschling M (2018). Still curling after all these years: Glenodinium apiculatum Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany). Systematics and Biodiversity 16:200-209. https://doi.org/10.1080/14772000.2017.1375045

Lewis JM, Dodge JD (2011). Dinophyta (Dinoflagellates). In: John DM, Whitton, BA, Brook AJ (Eds). The Freshwater algal flora of the British Isles: An identification guide to freshwater and terrestrial Algae (2nd ed). Cambridge University Press, Cambridge, UK pp 250-274.

Logares R, Shalchian-Tabrizi K, Boltovskoy A, Rengefors K (2007). Extensive dinoflagellate phylogenies indicate infrequent marine–freshwater transitions. Molecular Phylogenetic and Evolution 45:887-903. https://doi.org/10.1016/j.ympev.2007.08.005

López AI, Kretschmann J, Čalasan AŽ, Gottschling M (2018). The many faces of Peridinium cinctum (Peridiniaceae, Peridiniales): morphological and molecular variability in a common dinophyte. European Journal of Phycology 53(2):156-165. https://doi.org/10.1080/09670262.2017.1397198

Luo Z, Mertens KN, Gu H, Wang N, Wu Y, Uttayarnmanee P, Pransilpa M, Roeroe KA (2021). Morphology, ultrastructure and molecular phylogeny of Johsia chumphonensis gen. et sp. nov. and Parvodinium parvulum comb. nov. (Peridiniopsidaceae, Dinophyceae). European Journal of Phycology 56:324-336. https://doi.org/10.1080/09670262.2020.1829078

Maulood BK, Hassan FM, Al-Lami AA, Toma JJ, Ismail AM (2013). Checklist of algal flora in Iraq. Ministry of Environment, Baghdad

Moestrup Ø, Calado AJ (2018). Dinophyceae. In: Süßwasserflora von Mitteleuropa. Freshwater flora of central Europe, Volume 6. Springer Spektrum, Berlin, Germany

Moestrup Ø, Daugbjerg N (2007). On dinoflagellate phylogeny and classification. In: Brodie J, Lewis J (Eds). Unravelling the algae: The past, present and future of algal systematics. The Systematics Association Special Volume Series 75, CRC Press, Boca Raton pp 215-230.

Murray S, Flø Jørgensen M, Ho SYW, Patterson DJ, Jermiin LS (2005). Improving the analysis of dinoflagellate phylogeny based on rDNA. Protist 156:269-286. https://doi.org/10.1016/j.protis.2005.05.003

Narale DD, Anil AC (2017). Spatial distribution of dinoflagellates from the tropical coastal waters of the South Andaman, India: implications for coastal pollution monitoring. Marine Pollution Bulletin 115:498-506. https://doi.org/10.1016/j.marpolbul.2016.11.035

Olrik K (1992). Ecology of Peridinium willei and P. volzii (Dinophyceae) in Danish lakes. Nordic Journal of Botany 12:557-568. https://doi.org/10.1111/j.1756-1051.1992.tb01834.x

Pandeirada MS, Craveiro SC, Daugbjerg N, Moestrup Ø, Calado AJ (2023). Cell fine structure and phylogeny of Parvodinium: towards an ultrastructural characterization of the Peridiniopsidaceae (Dinophyceae). European Journal of Phycology 58(2):169-189. https://doi.org/10.1080/09670262.2022.2091798

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 111:1-61. https://doi.org/10.1099/00221287-111-1-1

Romeikat C, López AI, Tietze C, Kretschmann J, Gottschling M (2019). Typification for reliable application of subspecific names within Peridinium cinctum (Peridiniales, Dinophyceae). Phytotaxa 424:147-157. https://doi.org/10.11646/phytotaxa.424.3.2

Somogyi B, Felföldi T, Boros E, Szabó A, Vörös L (2022). Where the little ones play the main role–picophytoplankton predominance in soda and hypersaline lakes of the Carpathian Basin. Microorganisms 10(4):818. doi:10.3390/microorganisms10040818

Stamatakis A, Hoover P, Rougemont J (2008). A Rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57:758-771. https://doi.org/10.1080/10635150802429642

Taylor FJR, Hoppenrath M, Saldarriaga JF (2008). Dinoflagellate diversity and distribution. Biodiversity and Conservation 17:407-418. https://doi.org/10.1007/s10531-007-9258-3

Toma JJ, Aziz FH (2021). New records of algae in Shaqlawa district, Erbil, Kurdistan region of Iraq. Al-Nahrain Journal of Science 24(3):55-62. DOI:10.22401/ANJS.24.3.09.