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Introducing *Cyanodorina* gen. nov. and *Cyanodorina ovale* sp. nov. (Microcystaceae, Chroococcales), a Novel Coccoid Cyanobacterium Isolated from Caohai Lake in China Based on a Polyphasic Approach

Wei Chen [1], Shuyin Li [2], Yuanzhao Xu [2], Ruozhen Geng [3], Gaofei Song [4,*] and Peiming Ma [1,*]

[1] Key Laboratory of Ecological Impacts of Hydraulic Projects and Restoration of Aquatic Ecosystem of Ministry of Water Resources, Institute of Hydroecology, Ministry of Water Resources & Chinese Academy of Sciences, Wuhan 430079, China
[2] Yangtze River Basin Ecological Environment Monitoring and Scientific Research Center, Yangtze River Basin Ecological Environment Supervision and Administration Bureau, Ministry of Ecological Environment, Wuhan 430010, China
[3] College of Life and Environmental Sciences, Wenzhou University, Wenzhou 325035, China
[4] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
* Correspondence: song@ihb.ac.cn (G.S.); pablomaming@gmail.com (P.M.)

Abstract: The Chroococcales is one of the least studied cyanobacterial orders comprising the non-baeocyte-producing coccoids cyanobacteria with stacked and fasciculated thylakoids. During a survey of aquatic biodiversity in Caohai Lake in Guizhou Province, China, a coccoid-like cyanobacterium was isolated. It was characterized using a polyphasic approach, based on morphology, electron microscopy, and molecular phylogenetic analyses. This species' colonies exhibited morphological similarity to those of *Microcystis* species but differed in their larger colony sizes and widely oval cells. The 16S rRNA gene sequence of this species had the maximum homology, corresponding to 93.10%, to that of the genus *Microcystis*. The results of 16S rRNA gene threshold value and 16S rRNA phylogenetic analyses confirmed that the studied species belongs to the family Microcystaceae but is phylogenetically distinct from the other species of Microcystaceae. Furthermore, The D1–D1′, Box–B helix, and V3 helix of the 16S–23S ITS region were also different from those previously described in Microcystaceae taxa. Combining the morphological, ecological, and molecular features of the coccoid-like cyanobacterium, we here propose the establishment of the *Cyanodorina* gen. nov. and the *Cyanodorina ovale* sp. nov.

Keywords: coccoid cyanobacteria; morphology; phylogeny; 16S rRNA gene; 16S–23S ITS

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1. Introduction

Cyanobacteria are the most diverse group of prokaryotes, with morphologies including simple unicellular bacteria, colonies of coccoid cells and filaments associated with special cells, such as heterocytes or akynetes, and false or true branching [1]. The systematics and taxonomy of unicellular cyanobacteria represent a major challenge due to the scarcity of distinct morphological traits and a relatively high level of evolutionary convergence [2]. Komárek et al. and Mareš classified the coccoid cyanobacteria into five major groups, i.e., the Gloeobacterales, the Synechococcales, the Pleurocapsales, the Chroococcidiopsidales, and the Chroococcales [3,4].

The non-baeocyte-producing coccoid cyanobacteria with stacked and fasciculated thylakoids mostly belong to the Chroococcales, which is probably the least studied major group of cyanobacteria [2,3]. The members of this order remain taxonomically neglected and poorly understood, with the possible exception of the genus *Microcystis*, because some

species in this genus can form blooms and are harmful for human health [5]. Chroococcales are difficult to cultivate in the laboratory because of their uncharacteristic morphology and the loss of mucilage and colonial features in culture, which may lead their misidentification both on the species and the genus levels [2].

Polyphasic taxonomic approaches have been widely applied in modern cyanobacterial taxonomy, allowing constructing monophyletic genera, such as several important groups of heterocytous cyanobacteria and numerous nonheterocytous filamentous types [2]. The whole classification of cyanobacteria has undergone extensive restructuring and revisions at the species, genus, family, order level in recent years [3]. Despite such progress, only a few studies have dealt with the modern taxonomy of the Chroococcalean genera such as *Chroococcus*, *Cryptococcum*, *Inacoccus*, *Halothece*, *Aphanothece*, *Cyanobacterium*, *Synechocystis*, *Sphaerocavum*, *Geminocystis*, *Chalicogloea*, *Gloeothece*, *Gloeocapsa*, *Cyanothece*, and *Euhalothece* [2,6–13]. Rigonato et al. [13] combined morphological and molecular analyses and revealed that *Sphaerocavum* is a morphotype of *Microcystis*. Other studies established taxa via gathering all available informative data on morphology, cell ultrastructure, ecology, physiology, or biochemical traits [2,6–12].

However, a new proposal for cyanobacterial taxonomic classification above the genus level was presented based on a robust phylogenetic analysis of recently available genomic data and the broadly sampled 16S rRNA gene phylogeny [14]. In the latest cyanobacteria taxonomy system, to achieve the monophyly at order rank, Strunecký (2022) [14] divided Cyanophyceae into 20 orders: Gloeobacterales, Thermostichales, Aegeococcales, Pseudanabaenales, Gloeomargaritales, Acaryochloridales, Prochlorotrichales, Synechococcales, Nodosilineales, Oculatellales, Leptolyngbyales, Geitlerinematales, Desertifilales, Oscillatoriales, Coleofasciculales, Spirulinales, Chroococcales, Gomontiellales, Chroococcidiopsidales, and Nostocales. The Chroococcales previously underwent several fundamental taxonomic reorganizations, revealing its complexity. The family Microcystaceae is now in the order Chroococcales. Up to now, 34 genera have been deposited under the family Microcystaceae.

In the present study, several complementary methods, including morphological observation, TEM, phylogenetic analysis of the 16S rRNA gene and ITS (16S-23S ITS) secondary structure prediction, and assessment of the source habitat type were applied for in-depth analyses of isolated cyanobacteria. Above all, we propose that the examined bacteria represent a new genus within Microcystaceae.

2. Materials and Methods

2.1. Study Area and Sample Collection

The samples were collected by a Peterson grab sampler from the bottom of Caohai Lake in Guizhou Province, China (26°49′ N, 104°15′ E, 2171.7 m a.s.l.), on 18 April 2022. The grab sampler collected a 10 cm thick sediment layer. The collected samples were stored in a sample bottle and then divided into four parts: the first part was fixed with formaldehyde, the second part was fixed with Lugol's reagent, the third part was used for field preculture, and the remaining one was kept cool in a portable fridge and brought back to the lab within 3 days. The globular population of the collected samples was inoculated into sterile BG11 medium as a preculture in 5 mL centrifuge tubes. During the field investigation period, the preculture tubes were exposed to sunlight indoors by temporarily placing them by a window, and room temperature was maintained to preserve the biological activity of the inoculum. In the laboratory, single balls were inoculated in sterile BG11 medium in screw-neck glass tubes using a lab-made Pasteur pipette under an inverted microscopy with 100× magnification (Olympus CKX31, Tokyo, Japan). The tubes were placed at 25 °C, alternating 12 h of light (35 μmol photons·m^{-2}·s^{-1}) and 12 h of darkness. The cultures have been maintained for five months now.

2.2. Morphological Characterization

In the laboratory, the samples were observed under a light microscope (Nikon Eclipse 80i, Japan). Morphological and morphometrical studies were carried out according to the

description of Boone and Castenholz and Komárek and Anagnostidis [15,16]. The cell size was measured in more than 50 individuals using a Nikon eclipse 80i light microscope with a DS-Ri1 digital camera (Nikon, Tokyo, Japan). The images were analyzed using the NIS-Elements D 3.2.

The ultrastructure of the studied species was examined by transmission electron microscopy (TEM). The samples were fixed and dehydrated according to Geng et al. [17]. Fresh samples were fixed using 2.5% glutaraldehyde in 0.1 M phosphate buffer at a pH 7.2 and 4 °C for 3 days. Then, these samples were washed using 0.1 M phosphate buffer, after which they were post-fixed using 1% osmium tetroxide for 2 h and washed again using 0.1 M phosphate buffer to remove osmium tetroxide. Next, they were dehydrated using a sequential ethanol gradient (30, 50, 70, 90, and 100%) and embedded in Spurr's resin. Uranyl acetate (2%) and lead citrate were used for staining. The samples were sliced using an ultramicrotome (Leica UC7, Weztlar, Germany). The slices were fixed on a copper net. Then, images of the processed samples were finally observed using a transmission electron microscope (Hitachi HT-7700, Tokyo, Japan) at an accelerating voltage of 80 kV.

2.3. DNA Extraction and PCR Amplification

A single cultured ball was picked under the stereoscope (Nikon SMZ 1500, Japan), washed in sterile water for several times, and then transferred into a 1.5 mL centrifuge tube. Total genomic DNA was extracted from the cyanobacterial cells using the Clarke's method [18]. The 16S rRNA gene and the 16S–23S ITS region, 2000 bp fragments, were PCR-amplified in a PCR Thermal Cycler (Applied Biosystems 2720, Waltham, MA, USA), using the primer sets pA and B23S [19,20]. The PCR reaction, with a total volume of 20 μL, contained: 8 μL of sterile water, 1 μL of genomic DNA (100 ng/μL), 0.5 μL of each primer (10 μmol/L), and 10 μL of 2× PCR mix with Taq polymerase (Cat TSE001, Beijing Tsingke Biotech Co. Ltd., Beijing, China). The positive PCR products were purified with a PCR purification kit (Omega, USA) and cloned into the pMD18-T vector (Takara, Kusatsu, Japan) according to the manufacturer's instructions. Five positive bacterial clones were randomly chosen per clone library and cultured for 12–14 h at 37 °C. Plasmids were extracted with the TIANprep Rapid Mini Plasmid Kit according to the manufacturer's instructions. The plasmids, including the target fragment, were sequenced using the ABI 3730 automated sequencer (Applied Biosystems). Thereafter, the available nucleotide sequences were deposited in the GenBank database, with accession numbers OP382354–OP382358.

2.4. Phylogenetic Analysis

Sequences of the putative relatives were obtained from GenBank. All the sequences were aligned using MAFFT 7.037, and ambiguous gap regions were manually adjusted [21]. The final phylogenetic trees were constructed using neighbor-joining (NJ), maximum likelihood (ML), and Bayesian inference (BI). The NJ analysis using the Kimura-2 model upon default parameters with 1000 bootstrap replicates was run via the MEGA5 program package [22]. The ML analysis was performed on the IQ-TREE web server with 10,000 bootstrap replicates by using ultrafast bootstrapping [23]. The best fitting models, K2P + I + G4 and GTR + F + I + G4, were selected for the ML and BI analyses via the Akaike Information Criterion (AIC) in ModelFinder [24]. The BI analysis was conducted with MrBayes v3.2.6 in the CIPRES Science Gateway V 0.3.3 [25,26]. In the BI analyses, two runs of eight Markov chains were run for 10,000,000 generations, sampling every 100 generations, with 25% of the sampled trees discarded as burn-in. The consensus phylogenetic trees thus obtained were visualized in MEGA5, with *Gloeobacter violaceus* as the outgroup.

Calculation of the *p*-distance in the 16S rRNA was carried out by MEGA5 and used to calculate the sequence similarity ($100 \times (1 - p)$) for the 16S rRNA data.

2.5. ITS Secondary Structure Prediction

The secondary structure folding analysis was carried out with 16S–23S ITS sequences [27]. The folding of conserved regions (D1–D1′, Box B and V3 helix) was analyzed using RNA structure (version 6.4) and compared [28].

3. Results

3.1. Morphological Description

Class: Cyanophyceae
Order: Chroococcales
Family: Microcystaceae
Cyanodorina W. Chen et G. Song & P. Ma gen. nov.

Description: Coccoid cyanobacteria. Colonies macroscopic, benthic epipelic cyanobacteria, with irregularly and densely arranged cells in a common sheath, dark green when fresh, yellow when old. Unicellular bacteria without envelopes, blue-green when fresh, yellow or yellow green when old. Stacked thylakoids formed fascicles of short sections in different number crossing the entire cell.

Etymology: The epithet *Cyanodorina* indicates that the colony forms a ball in Cyanobacteria.
Type species: *Cyanodorina ovale*
Cyanodorina ovale W. Chen et G. Song & P. Ma sp. nov. (Figures 1 and 2).

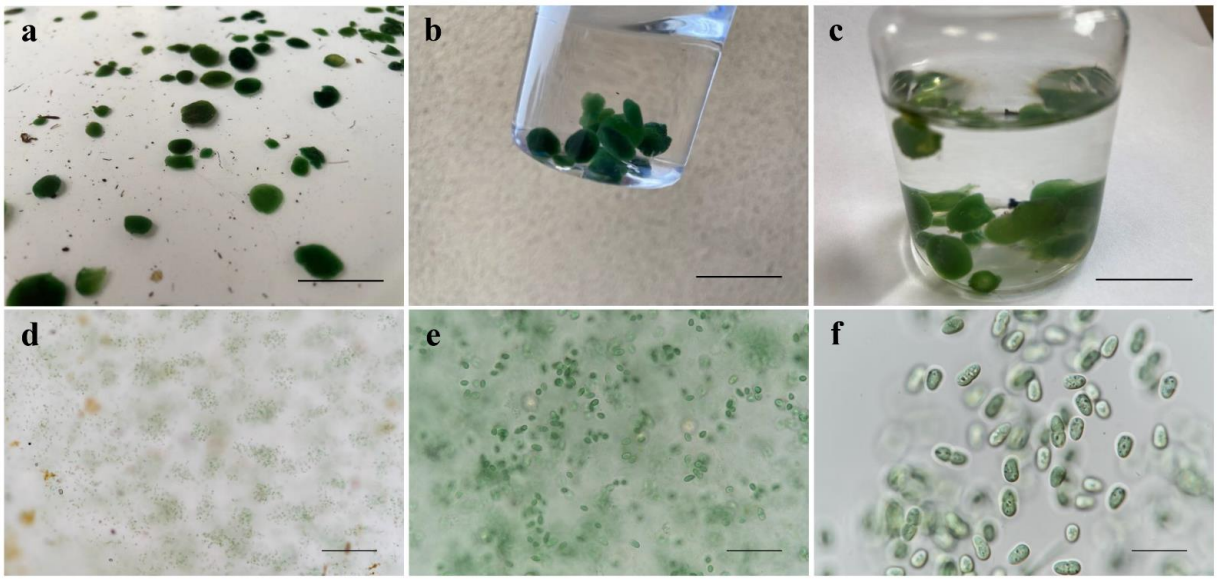

Figure 1. Field sample morphology of *Cyanodorina ovale*. (**a**) *Cyanodorina ovale* in the tray after sediment cleaning. (**b**,**c**) *Cyanodorina ovale* in liquid BG11 medium. (**d**–**f**) Colonies at different magnification. Scale bars: (**a**–**c**) 2 cm, (**d**) 200 μm, (**e**) 50 μm, (**f**) 20 μm.

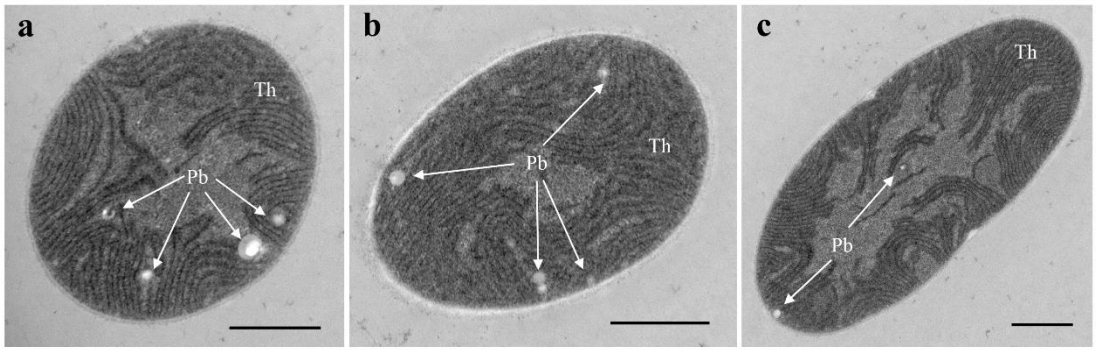

Figure 2. TEM micrographs of *Cyanodorina ovale*. (**a**) Cross section of a cell. (**b**) Longitudinal section of a cell. (**c**) Dividision of a cell. Pb, polyphosphate body, Th, thylakoids. Scale bars: 1 μm.

Diagnosis: By macroscopic and microscopic observation, this species colonies exhibited morphological similarity to the *Microcystis* species but differed in their larger sizes and the presence of widely oval cells. However, phylogeny based on the 16S rRNA gene showed that this species had a unique position close to some coccoid cyanobacterial genera such as *Microcystis*, *Chalicogloea*, *Cyanoarbor*. Moreover, the lower similarity in the 16S rRNA gene sequence of this species to those of coccoid cyanobacterial genera and the significant differences between this species and those of coccoid cyanobacterial genera, as regards the length and the secondary structure of the 16S–23S ITS region, supported it as a new cyanobacterial genus.

Description: Colonies more or less irregularly spherical, macroscopic, usually composed of small groups of cells (subcolonies), embedded in a wide, fine, homogeneous, colorless sheath. Cells appeared green to blue-green, widely oval, longer than wide, 6.54–8.21–8.92 µm long, and 4.51–5.08–5.69 µm wide, with a length/width ratio of 1.26–1.60–1.80; unicells dividing by simple binary fission, absence of clearly delimited and concentrically lamellated mucilaginous envelopes around individual cells. Stacked thylakoids formed fascicles of short sections in different number crossing the entire cell, as documented by TEM analysis (Figure 2).

Etymology: The name of the species was chosen for the oval shape of the cells.

Type locality: Isolated from a water sample in Caohai Lake, Guizhou Province, China (18 April 2022, 26°49′ N, 104°15′ E, 2171.7 m a.s.l.).

Holotype designated here: Dry and fixed samples are stored at the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China, as specimens No. GZ202213 (dry), No. GZDX202213 (formaldehyde), No. GZDL202213 (Lugol's).

Habitat: The surface sediment. Caohai Lake is a light eutrophic lake. In April 2022, the mean values of water temperature, pH, transparency, conductivity of electricity, dissolved oxygen, turbidity were 15.1 °C, 8.2, 53.25 cm, 410.5 µs cm^{-1}, 6.66 mg L^{-1}, and 10.45 NTU, respectively. The mean values of total nitrogen, total phosphorus, nitrate nitrogen, ammonia nitrogen, orthophosphate were 1.51 mg L^{-1}, 0.08 mg L^{-1}, 0.133 mg L^{-1}, 0.53 mg L^{-1}, 0.013 mg L^{-1}, respectively. The mean concentration of chlorophyll *a* was 21.25 µg L^{-1}.

3.2. Molecular and Phylogenetic Analysis

For the calculation of the 16S rRNA *p*-distance matrix, we used sequences of representative taxa from families within the order Chroococcales. The sequences of *Cyanodorina* appeared to share 99.73–100.00% similarity to all five clones, and the five clones' maximum similarity between *Microcystis* and other coccoid cyanobacterial genera was 93.10% (Table 1). *Cyanodorina* was found to share 93.10% sequence similarity with *Microcystis aeruginosa*, 93.00% with *Cyanoarbor violascens*, 91.30% with *Aphanothece sacrum*, 92.40% with *Chalicogloea cavernicola*.

In total, 187 representative taxa sequences were included in the phylogenetic analysis to assess the placement of the coccoid cyanobacterial (Figure 3 and Table S1). NJ, ML, and Bayesian inference analyses produced similar tree topologies in our phylogenies. The 16s rRNA phylogeny indicated that the studied species was distinct from the species of the other genus of Chroococcales (67% and 98% NJ and ML bootstrapping percentage (BP) and 0.98 posterior probability (PP)). *Cyanodorina* appeared as sister or parallel to other genera.

3.3. Comparison of ITS Regions between 16S and 23S rRNA Genes and Secondary Structures

The full-length of the ITS sequence of this species was 460 bp (Table 2), containing only one $tRNA^{Ile}$. The ITS secondary structures of the isolated *Cyanodorina ovale* were compared with the ITS structures of representative taxa from four genera in Microcystaceae, which were *Gloeothece aequatorialis*, *Chalicogloea cavernicola*, *Microcystis aeruginosa*, and *Aphanothece sacrum*. The ITS region of *Cyanodorina ovale* was different from those of the other taxa both in nucleotide sequence and in length of some regions. The D1-D1′ helix exhibited five distinct patterns within these five taxa (Figure 4a–e). All the outgroups differed from *Cyanodorina ovale* in the number of unilateral and bilateral bulges and base pairs. The Box-B helix was

relatively conserved in *Cyanodorina ovale* (Figure 4f–j). This structure of *Cyanodorina ovale* differed from those of all other four taxa; all taxa possess highly variable helices and share no similar patterns with each other. The V3 helices of *Cyanodorina ovale* CH01 appeared conspicuously different from those of other taxa in the four genera in sequence length and stem–loop structures (Figure 4k–o, Table 2). These results provided strong evidence for the phylogenetic conclusion obtained above, further indicating that this species belongs to the new genus.

Figure 3. Maximum likelihood (ML) phylogenetic tree of cyanobacteria based on 16S rRNA sequences (1020 bp) representing the current GenBank data for Chroococcalean cyanobacteria. Bootstrap values greater than 50% are shown on the ML tree for the NJ/ML methods and Bayesian posterior probabilities. * indicates bootstrapping values of 100 for NJ, ML, and BI posterior probabilities of 1.00. Sequences from GenBank are indicated by accession numbers. The main clades are indicated by numbers. Sequences from this study are denoted with solid circles.

Table 1. Sequence similarity comparison of the 16S rRNA gene between *Cyanodorina ovale* CH01 and species of close genera.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *Cyanodorina ovale* CH01 clone1																				
2. *Microcystis aeruginosa* NIES298	93.10																			
3. *Neochroococcus gongqingensis* CHAB4018	93.00	91.50																		
4. *Cyanoarbor violascens* C1	93.00	92.30	91.90																	
5. *Chalicogloea cavernicola* CCALA975	92.40	91.30	91.10	94.80																
6. *Cryptochroococcus tibeticus* TP201716.4	92.20	91.20	94.50	92.00	91.20															
7. *Pseudochroococcus couteii* PMC885.14	92.10	91.50	94.40	92.00	91.10	99.10														
8. *Gloeothece aequatorialis* SAG36.87 clone7	91.90	91.90	92.40	91.80	91.40	91.10	91.00													
9. *Inacoccus carmineus* CCIBt3475	91.60	90.80	94.20	91.20	90.00	93.90	94.00	91.30												
10. *Gomphosphaeria aponina* SAG52.96	91.50	89.90	94.00	90.70	90.50	93.20	93.20	91.20	93.80											
11. *Pannus brasiliensis* CCIBt3594	91.30	94.90	90.20	90.50	90.70	89.90	90.10	91.10	89.50	89.30										
12. *Aphanothece sacrum* H5	91.30	90.50	92.60	91.60	91.60	92.10	92.60	91.20	92.90	93.00	90.80									
13. *Eucapsis minor* SAG 14.99	91.10	93.50	91.90	91.50	90.80	90.10	90.20	90.70	90.90	90.30	92.80	90.90								
14. *Radiocystis geminata* MAC1214	90.80	91.00	91.50	91.40	91.20	90.10	90.10	91.40	92.20	91.30	89.70	91.30	91.40							
15. *Chroococcus subviolaceus* CCIBt3549	90.80	91.50	92.80	91.80	90.20	92.30	92.70	91.20	93.40	91.70	89.70	91.20	89.30	90.10						
16. *Cryptococcum komarkovae* CCALA54	90.30	88.90	93.00	90.10	90.00	92.20	92.20	89.90	91.90	91.60	89.10	91.20	89.80	89.70	91.30					
17. *Geitlerinema splendidum* CCALA1004	90.00	87.80	90.30	88.50	88.80	90.20	90.50	88.10	90.40	89.80	88.50	90.10	87.60	88.20	91.20	89.90				
18. *Anagnostidinema amphibium* NRERC452	88.90	89.70	89.80	88.50	87.90	89.50	89.60	89.00	89.30	90.00	88.10	89.10	88.00	89.40	90.10	87.40	87.60			
19. *Geminocystis herdmanii* PCC6308	88.00	86.60	88.70	87.00	87.10	90.00	89.90	87.00	89.10	90.20	86.80	89.10	86.70	87.30	87.30	88.40	87.50	86.70		
20. *Alborzia kermanshahica* S2	86.90	85.90	86.50	89.90	91.50	85.80	85.80	86.60	84.90	85.60	86.00	86.70	86.90	86.60	84.70	85.00	83.40	82.20	82.50	
21. *Cyanobacterium stanieri* MAC3217	86.50	84.60	86.50	84.30	84.70	87.70	87.90	85.30	87.70	88.90	84.70	87.60	84.80	85.90	86.00	86.50	86.80	85.10	92.80	80.00

Table 2. Analyses of the ITS of the 16S–23S region for *Cyanodorina ovale* CH01 and other related strains.

Organisms	GenBank	ITS Total Length (nt)	D1–D1′ Helix Length (nt)	D2 Region	$tRNA^{Ile}$	$tRNA^{Ala}$	Box B Helix Length (nt)	Box A Spacer	V3 Helix Length (nt)
Cyanodorina ovale CH01	OP382354	460	58	CTTTCAAACTCT	+	-	37	GCACCTTGAAAA	48
Aphanothece sacrum FPU3	AB116658.2	453	93	CTTTCAAACTAG	+	-	40	GCACCTTGAAAA	16
Gloeothece aequauorialis SAG 36.87	MF781064.1	449	60	CTTTCAAACTTA	-	-	43	GAACCTTGAAAA	36
Chalicogloea sp. BACA0589	OM732250.1	481	67	CTTTCAAACTCT	+	-	35	GCACCTTGAAAA	20
Microcystis aeruginosa UUEX 'B 2667	HQ625424.1	404	63	CTTTCAAACTAG	+	-	39	GAACCTTGAAAA	12

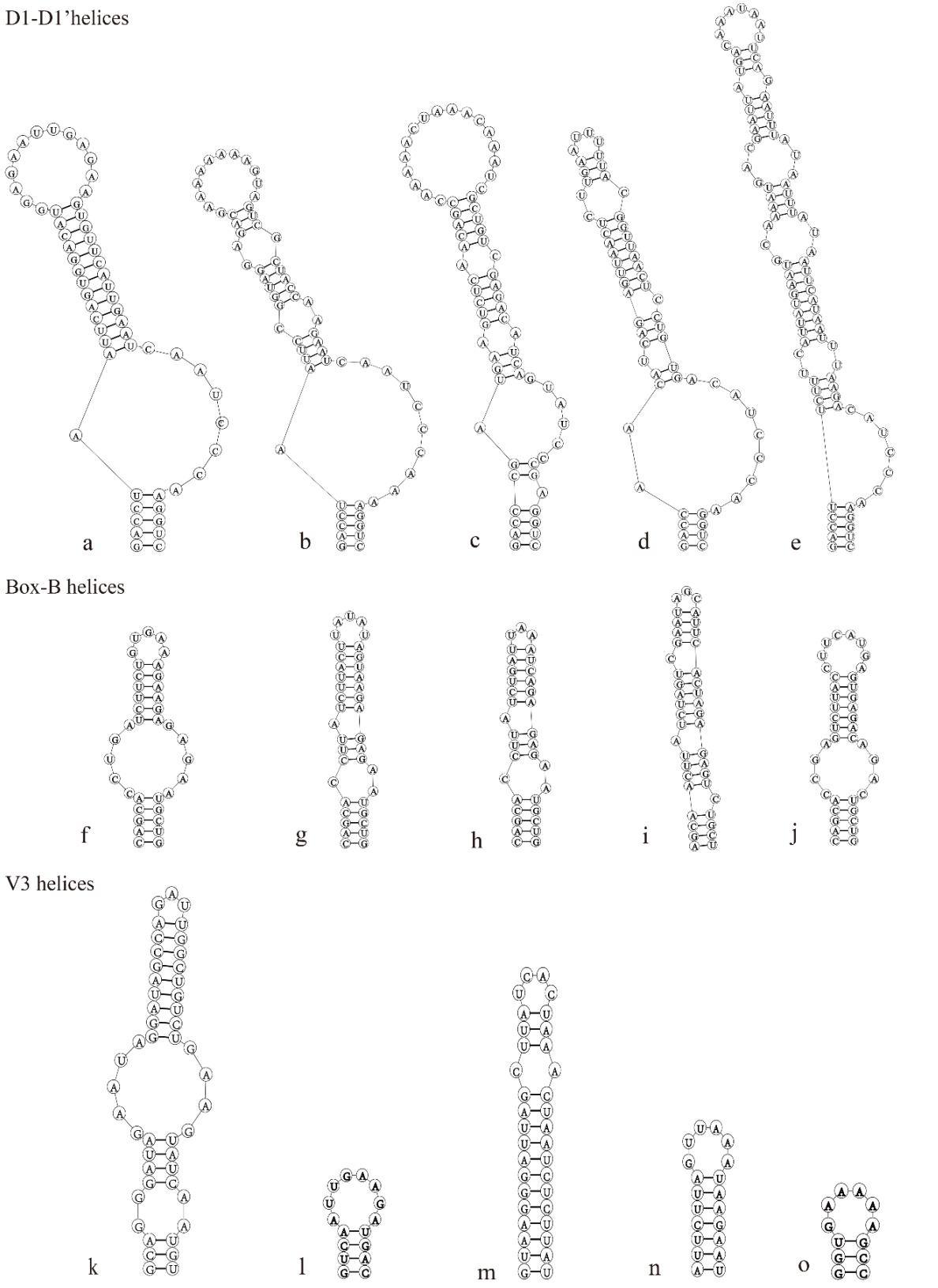

Figure 4. Secondary structures of D1–D1′ helices (**a**–**e**), Box-B helices (**f**–**j**) and V3 helices of various taxa. (**a**,**f**,**k**) *Cyanodorina ovale* CH01. (**b**,**g**,**l**) *Aphanothece sacrum* FPU3. (**c**,**h**,**m**) *Gloeothece aequauorialis* SAG 36.87. (**d**,**i**,**n**) *Chalicogloea* sp. BACA0589. (**e**,**j**,**o**) *Microcystis aeruginosa* UUEX 'B 2667.

4. Discussion

Cyanobacteria are one of the most studied groups of aquatic microbes because of their importance in fishery, biological, azotification, environmental protection, and research on classifying systems [29]. Simultaneously, with the availability of 16S rRNA and ITS regions, α-level taxonomy and revision of extant taxa are rapidly progressing at the species and genus level [14]. In modern cyanobacterial taxonomy, the genus should be a monophyletic cluster, and the species should be well characterized using a polyphasic approach. The polyphasic approach has become the gold standard in cyanobacterial taxonomy by providing all available informative data on morphology, cell ultrastructure, ecology, physiology, and biochemical traits [30–33].

Coccoid cyanobacteria show high heterogeneity and include various developmental lines, which differ in important phenotypic and ultrastructural markers [6]. Of the five widely accepted groups in the coccoid cyanobacteria, Gloeobacterales and Synechococcales either lack thylakoids or possess parietal thylakoids, and Pleurocapsales, Chroococcales, and Chroococcidiopsidales show complex arrangements of thylakoids [34]. As regards Chroococcales with fasciculated thylakoids, only a few studies have chosen the polyphasic approach [6–9,35]. A large number of essential taxa are still lacking molecular data which would enable a comprehensive phylogenetic evaluation [36–39]. Despite such fuzzy status quo, a robust phylogenetic backbone based on multilocus analysis strongly supports the re-integration of Chroococcales and Pleurocapsales into a single taxonomic unit to sustain monophyly and define the orders on a comparable level of phylogenetic branching through all the Cyanophyceae [14].

The present study described a new coccoid cyanobacterial taxon isolated from Caohai Lake, China. We compared the characteristics of the 13 previously described genera of Chroococcales (11 of these genera are Microcystaceae) (Table 3). It was found that *Gloeothece*, *Cyanothece*, *Rippkaea*, *Aphanothece*, *Cyanoaggregatum* possess a similar cell shape to *Cyanodorina ovale* but favor different habitats. *Cyanodorina ovale* is morphologically similar to species of the genus *Microcystis* in colonial form and cellular ultrastructure. As described, *Microcystis* cells are often spherical or spheroidal with a diameter of 1.7–8.1 µm and colonies from 40 µm to 3 mm. the colonial forms are gelatinous, free-floating, and spherical, discoid, or irregular [40]. The ultrastructure of *Microcystis* cells showed that stacked thylakoids formed fascicles of short sections in different numbers, crossing the entire cell [41]. Therefore, the new coccoid cyanobacterial taxon appeared different from *Microcystis* in cell morphology, cell size, and colonial size. Moreover, this new coccoid cyanobacterial taxon grows on the surface of the sediment. It is different from the floating *Microcystis*.

Meanwhile, the highest homology between 16S rRNA gene sequences with respect to the existing cyanobacterial taxa appeared to be 93.10% (*Microcystis aeruginosa* NIES298), and the unique phylogenetic position indicated a high possibility of a novel genus. The observation that the secondary structures D1–D1′ helix, Box B helix, and V3 helix in the 16S–23S ITS region containing two tRNAs appeared different from those of phylogenetically close coccoid representative taxa of four genera supports the independence of the isolated species at the genus level.

Cyanodorina ovale possesses a spherical, oval, or cylindrical shape with widely rounded ends and stacked and fasciculated thylakoids, which is typical of Microcystaceae [14]. This species' 16S rRNA appeared to share less than 93.10% genetic similarity with the other taxa that were the focus of this study, and this value was well below the genetic cut-off proposed by Yarza et al. of 94.5% [40]. Simultaneously, Yarza et al. [40] proposed a value of 86.5% to delineate families in regard to bacterial and archaeal species based on 16S rRNA gene sequences similarity [34]. In our study, the 16S rRNA gene phylogeny of this new taxon presented similarities with that of the known cyanobacterial genera in Microcystaceae, grouped in a distinct isolated clade (branch supports $\geq$ 98%). Following the conventional taxonomic research procedures, the observed species would belong to a new Microcystacean genus based on the obvious difference between *Cyanodorina ovale* and other Microcystacean species.

Table 3. Comparison of the characteristics of the 13 previously described genera of Chroococcales ("-" indicates that the feature is unknown).

Genus	Cell Shape	Cell Size (μm)	Colony Formation	Colony Size (μm)	Sheaths	Thylakoid Arrangement	Habitat
Cyanodorina	oval	6.54–8.92 × 4.51–5.69	Yes	5000–20,000	Yes	Irregular fascicles	Freshwater, benthic
Microcystis	spherical or hemispherical	1.7–8.1	Yes	40–3000	Yes	Irregular fascicles	Freshwater, free floating
Pannus	round	1–4	Yes	126.5–314.0	Yes	Irregular fascicles	Planktonic in brackish bays and reservoirs, benthic in stagnant freshwater
Eucapsis	semicircle	8–14	Yes	<50	Yes	-	Freshwater, benthic
Chalicogloea	spherical	2.6–4.3	Yes	<100.0	Yes	Irregular fascicles	Salpetre Cave
Cyanoarbor	subspherical	1–5	Yes	200.0–10,000.0	Yes	Irregular fascicles	Salty and alkaline waters, high mts lakes,
Gloeothece	oval to cylindrical	5–20 × 3–12	Yes, rarely absent	13.0–40.0 long, 10.0–26.0 wide	Yes	Irregular fascicles	Freshwater, terrestrial
Cyanothece	oval to cylindrical	2.5–5 × 7.5–10	No		No	Special (radial with a central network)	Freashwater, terrestrial
Crocosphaera	spherical to cylindrical	2.8–7.1 × 1.2–1.8	No		No	Irregular fascicles	Marine
Zehria	spherical to cylindrical	2.0–5.6 × 2.0–4.0	No		No	Irregular fascicles	Marine
Rippkaea	oval to cylindrical	4–9 × 3–5	No		No	Irregular fascicles	Semi-terrestrial (soil of rice field)
Aphanothece	oval to cylindrical	1–12	Yes	micro to macroscopic	Yes	Irregular fascicles	Freshwater, terrestrial
Cyanoaggregatum	oval or cylindrical	2.4–3.8 × 1.4–2.0	Yes	92.0–340.0 long, 62.0–160.0 diameter	Yes	-	lagoon

In conclusion, one new species of Microcystaceae was separated on the basis of a combination of different analyses, i.e., of the morphology, 16S rRNA gene dissimilarity and phylogeny, and secondary structures of the 16S-23S ITS region. On the basis of this evidence, we established a new Microcystacean genus—*Cyanodorina*—to correctly accommodate the new species *Cyanodorina ovale* within the family Microcystaceae.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15030329/s1, Table S1: List of all strains examined in this study, with NCBI Genbank accession numbers.

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