

## *Johannesbaptistia floridana* sp. nov. (Chroococcales, Cyanobacteria), a novel marine cyanobacterium from coastal South Florida (USA)

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**Abstract:** Mat dwelling species of cyanobacteria are diverse and integral to coastal marine sediment systems. Though observed throughout the globe, much of this diversity remains undescribed, especially on South Florida coast where cyanobacteria are pronounced. To help elucidate this diversity, one species of cyanobacteria from the marine sediments of South Florida was studied. Typical morphological features including uniseriate pseudofilaments with facultative intercellular gaps and cell division in one plane, transversely to the longer cell axis, indicated that the species belonged to the genus *Johannesbaptistia* De Toni. The combination of 16S rRNA gene and 16S–23S rRNA internal transcriber spacer (ITS), along with the diagnosable morphological characteristics suggest uniqueness from previously described species, thus supports the erection of the novel species *Johannesbaptistia floridana* sp. nov.

**Key words:** benthic, Cyanothrichaceae, ecology, epipellic, morphology, new species, North America, subtropical, 16S rRNA, 16S–23S rRNA ITS

## INTRODUCTION

*Johannesbaptistia* De Toni (synonym *Cyanothrix* Gardner, *Heterohormogonium* Copeland) is a cosmopolitan genus of cyanobacteria described from many regions throughout Europe, the Americas, Asia and the Caribbean (COPELAND 1936; MARSHALL 1981; WENZEL & HALPERIN 1987; HINDÁK 1988; GOLUBIĆ & ABED 2010). The genus inhabits lakes, brackish or estuarine pools and rivers, and can be found entangled within cyanobacterial mats (RICHERT et al. 2006; KOMÁREK & KOMÁRKOVÁ-LEGNEROVÁ 2007; ROSEN & MAREŠ 2016) and epipellic on benthic coastal substrata (this study). Currently, only one species has been studied in depth: *Johannesbaptistia pellucida* (Dickie) Taylor et Drouet and a total of five species are accepted: *Johannesbaptistia desikacharyi* Gupta et Das, *J. pellucida*, *J. primaria* (Gardner) De Toni (type species), *J. schizodichotoma* (Copeland) Komárek et Anagnostidis, and *J. whitfordii* Thomas et Gonzalves (GUIRY & GUIRY 2020). Several other species with similar characteristics to *Johannesbaptistia* have been described but remain synonymous (KOMÁREK & ANAGNOSTIDIS 1998) to the currently described species or unrevised.

*Johannesbaptistia pellucida* was originally

described as *Hormospora pellucida* (DICKIE 1874). GARDNER (1927) designated this genus as *Cyanothrix*, while TAYLOR (1928) erroneously named it *Nodularia* (?) *fusca*. However, the assignment to *Cyanothrix* was improper, as it was already in use by SCHMIDLE (1897) to designate Cyanophyceae that belonged to the genus *Hapalosiphon* Nägeli. Consequently, DE TONI (1934) proposed amending the genus *Cyanothrix* to *Johannesbaptistia*. In 1937, Taylor recognized the similarity between the originally ascribed *Nodularia* (?) *fusca* with *Cyanothrix* and informed Drouet, and in 1938, they established a new generic combination inclusive of all the of previous synonyms and species: *J. primaria* (the type species), *J. pellucida*, *J. willei*, and *J. gardneri* (DROUET 1938).

The genus *Johannesbaptistia* remains poorly understood systematically, as there is only one species (*J. pellucida*) that contains a short sequence of the 16S rRNA gene in GenBank (# AJ621836). To fill this information gap and further uncover the diversity within this genus, we isolated and cultured a unique strain of *Johannesbaptistia* from the coast of South Florida and are proposing it as a new species to science: *Johannesbaptistia floridana* sp. nov. It has characteristics dissimilar to other currently described species of the genus, and we present data on

the morphology, ecology, 16S rRNA gene and 16S–23S rRNA internal transcribed spacer (ITS).

## MATERIALS AND METHODS

**Isolation and culture.** Benthic sediment samples were collected from the South Florida coast (25°27'53.01"N, 80°20'3.264"W), and the cyanobacterium was first isolated using soilidified filtered (0.22µm) source water and micromanipulation of filaments. Single filaments were transferred into tubes containing sterile source water and sediment. To successfully culture this cyanobacterium, BG11 marine (35 g.l<sup>-1</sup> Instant Ocean) was modified by doubling the CaCl<sub>2</sub>, MgSO<sub>4</sub>, and NaCO<sub>3</sub> concentrations, adding NaSiO<sub>3</sub> (30 g.l<sup>-1</sup>), pH adjusted to 9 (0.1M NaOH dropwise), and adding sterile organic sand. The cyanobacterium is maintained in both modified liquid and solid media at 25 °C on a 12:12-h light:dark cycle under cool white (6500K) fluorescent lighting (100 µEs<sup>-1</sup>.cm<sup>-2</sup>). Herbarium voucher specimens are deposited in the US National Herbarium of the National Museum of Natural History, Smithsonian Institution (NMNH–SI, Washington, DC, USA) under the accession number # US 227647. Live cultures are maintained in the BLCC (Berthold–Laughinghouse Culture Collection; Ft. Lauderdale Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, Davie, Florida, USA) with strain ID# BLCC–M67.

**Morphological, molecular, and phylogenetic analyses.** Microphotographs were taken using a DIC microscope (Leica DM5500 B with LAS V4.12; Leica Microsystems, Switzerland) and an Epifluorescent microscope (Amscope, XYL–606). Fluorescent microscopy was used to image strain characteristics overlooked in light microscopy by using either inherent cyanobacterial autofluorescence or DAPI (4',6–Diamidino–2–Phenylindole, Dihydrochloride) staining for visualization of bacteria.

For molecular analyses, DNA was extracted from 10 mg of fresh unicyanobacterial material using a Plant Mini Kit (Qiagen) with modified protocols. To maximize extraction efficiency, the biomass was first washed three times with diH<sub>2</sub>O then macerated with a sterile pestle. The 16S rRNA gene and 16S–23S rRNA ITS region were amplified by PCR (polymerase chain reaction) on a ProFlex (Applied Biosystems) thermocycler using the primers 359F and 1487R for the 16S rRNA gene and 1337F and 23S30R for the 16S–23S rRNA ITS region (WILMOTTE et al. 1993; NÜBEL et al. 1997), using a 50 µl PCR reaction of 5 µl buffer, 5 µl BSA (bovine serum albumin), 2.5 µl MgCl<sub>2</sub>, 1.25 µl each primer, 1 µl dNTPs, 0.3 µl GoTaq G2 (Promega), and remainder nuclease-free H<sub>2</sub>O. The following conditions were used for amplification: an initial denaturation for 3 min 95.5 °C, then 35 cycles of denaturation for 30 sec at 95 °C, annealing for 1 min at 57 °C, extension for 1.20 min at 72 °C, with a final extension for 5 min at 72 °C.

A QIAquick PCR Purification Kit was used to clean PCR products which were subsequently visualized on a 1% agarose gel. The amplified products of the 16S rRNA gene were sequenced directly; however, the 16S–23S rRNA ITS products were cloned using TOP10 chemically competent cells (TOPO–TA, Invitrogen) prior to sequencing, following manufacture protocols. Cloned plasmids were extracted from clonal libraries using a PureLink Quick Plasmid Miniprep Kit (Invitrogen). Sanger sequencing of plasmid DNA was carried out by Eurofins Genomics (Kentucky, USA) using

BigDye Terminator v3.1 (Applied Biosystems). Sequences for both the 16S rRNA gene and 16S–23S rRNA ITS are deposited in GenBank (National Center for Biotechnology Information, NCBI) under the accession numbers MT321576 and MT321577, respectively.

BLAST (Basic Local Alignment Search Tool, NCBI) was used to identify 16S rRNA gene sequences similar to our isolate. A total of 42 sequences were aligned with our isolate using MUSCLE (SeaView v4.4) (EDGAR 2004). The alignment of the 16S rRNA gene included 1349 sites and *Gloeobacter violaceus* (AY485484) as the outgroup. The best-fit nucleotide model was assessed using jModelTest through MEGA (v10.1.7) (GUINDON & GASCUEL 2003; POSADA 2008; TAMURA et al. 2011). Phylogenetic trees were constructed using Bayesian inferencing (BI) and maximum likelihood (ML) with MrBayes (v3.3.6) and RaxML (7.2.7), respectively, through the CIPRES network (v.3.3) (GUINDON & GASCUEL 2003; MILLER et al. 2010). The ML analysis was carried out using the GTR+I model using 1,000 bootstrap resampling replicates. The BI analysis was conducted using MrBayes 3.6 (RONQUIST & HUELSENBECK 2003) with 1.5 × 10<sup>6</sup> generations, a 0.25 burn-in rate, and resampling every 100 generations.

## RESULTS AND DISCUSSION

### Species Description

*Johannesbaptistia floridana* D.E. Berthold et Laughinghouse sp. nov.

(Figs 1–3, Suppl. Fig. S1)

**Diagnosis:** Cells of pseudofilaments are much larger than previous species including *Johannesbaptistia pellucida*, *J. primaria*, and *J. willei*.

**Description:** Thallus light to dark blue green, coiled, epipellic on sand/mud, embedded in clear gelatinous matrix, pseudofilaments ± 1 mm long, curved and wavy, 24.0–29.2 (30) µm diam. Unicellular pseudofilaments, mostly uniseriate, sometimes pseudopolyseriate, often pseudodichotomously divaricated. Sheath hyaline, gelatinous, 2.6 µm thick, bacterial film often embedded. Healthy, dividing cells and divaricating pseudofilaments contain little to no space between cells. Slight or clear distance between cells in older pseudofilaments. Cell content light to dark green to blue–green, very finely granular, homogeneous, without gas vesicles. Cells discoid, 4.0–7.8 (8.8) µm long and 18.5–23.3 (23.7) µm in width. Apical cell rounded, light green to yellow. Reproduction through filament disintegration, necridia. Forms club-shaped, expansion of cells (8–12 cells) that are twice wider than vegetative cells.

**Holotype:** US 227647 (US National Herbarium, Smithsonian Institution, Washington, DC, USA) – metabolically inactive herbarium preparation [dried and preserved] of reference strain BLCC–M67. Collected by David E. Berthold on 25–August–2018.

**Type locality:** Marine sediment in Biscayne Bay, Homestead, Florida, USA (25°27'53.9"N, 80°20'05.8"W).

**Habit and habitat:** Benthic and epipellic on marine

Table 1 Morphological characteristics of several representative species within the genus *Johannesbaptistia*. Cell morphometrics are presented as cell length and width min–max (outlier).

	<i>Johannesbaptistia pri- maria</i> (Gardner) De Toni	<i>J. willet</i> (Gardner) De Toni	<i>J. pellucida</i> (Dickie) Taylor et Drouet	<i>J. gardneri</i> Frémy	<i>J. schizodichotoma</i> (Copeland) Komárek et Anagnostidis	<i>J. floridana</i> sp. nov.
<b>Filament width (µm)</b>	18–22	9–11	13–20 (22)	9–22	± 2	24–29.2 (30)
<b>Cell</b>						
<b>shape</b>	–	–	Discoid	–	Spherical or discoid	Discoid
<b>width (µm)</b>	10–15	6–6.5	6.6–8.3 (10)	5.4–16.3	1–2.5	18.5–23.3 (23.7)
<b>length (µm)</b>	3.6–5	3.5–4.5	1.5–5.5 (6.5)	3–4.5	1.2–1.6	4.0–7.8 (8.8)
<b>Apical Cell</b>	–	–	Rounded, conical	–	–	Rounded
<b>Cell Content</b>	–	Pale blue, olive green Finely granular	–	–	Green to blue–green Finely granular	–
<b>Reproduction</b>	–	–	Disintegration of filament, facultative liberation of solitary cells	–	–	Disintegration of filament, hormogonia–like; no soli- tary cells
<b>Occurrence</b>	Freshwater lake; Puerto Rico	Freshwater lake; Puerto Rico	Freshwater to saline; cosmo- politan	–	Thermal mineral springs; Yellowstone, USA	Coastal marine; Florida, USA
<b>Habit</b>	Metaphytic	Metaphytic	Benthic and metaphytic	–	–	Benthic
<b>Reference</b>	GARDNER (1927)	GARDNER (1927)	KOMÁREK & ANAGNOSTIDIS (1999)	FRÉMY (1935)	KOMÁREK & ANAGNOSTIDIS (1999)	This paper



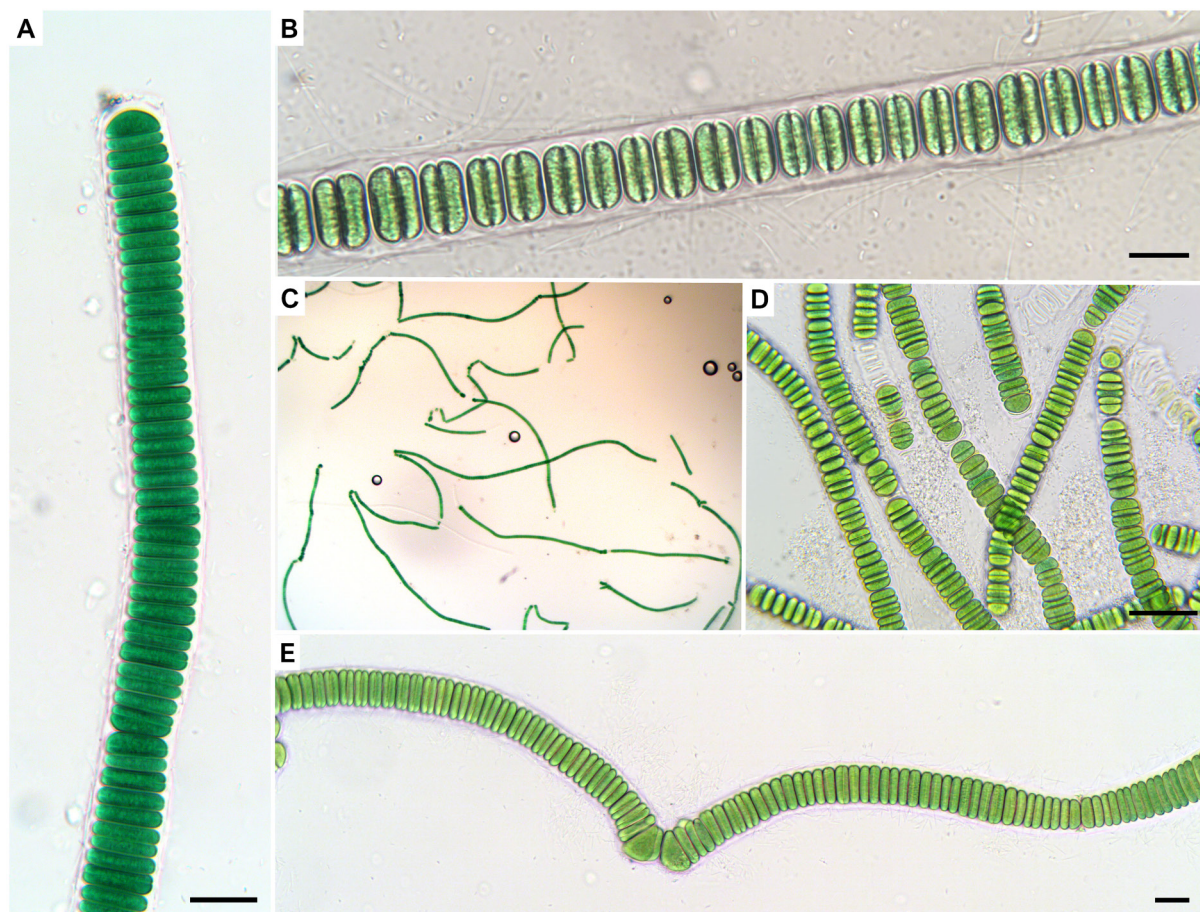


Fig. 1. Microscopic images of *Johannesbaptistia floridana* sp. nov. (strain BLCC-M67 [reference strain]) showing characteristic morphological traits including: (A) blue–green uniseriate pseudofilament; (B) clear spaces between dividing daughter cells; (C) extensive length of divaricated pseudofilaments; (D) disintegration and necridia of pseudofilaments, and (E) pseudodichotomous divarication of pseudofilaments. Scale bars 20 µm.

intertidal substrata.

**Etymology:** “*floridana*” is to honor the State of Florida (USA) where the cyanobacterium was collected.

**Reference strain:** BLCC-M67 (University of Florida/IFAS, FLREC, Davie, FL, USA) and ULC590 (BCCM/ULC Cyanobacteria Collection, University of Liège, Liège, Belgium).

**Gene Sequences:** GenBank accession numbers MT321576 for the 16S rRNA gene and MT321577 for the 16S–23S rRNA ITS.

### Morphological analyses

*Johannesbaptistia floridana* sp. nov. demonstrated characteristic morphological features of the genus *Johannesbaptistia* including blue–green uniseriate pseudofilaments, clear spaces between dividing cells, pseudodichotomously divaricated pseudofilaments and cell division transversely to the longer axis of cells (Figs 1A–C, E). Other typical morphological traits included necridia (Figs 1D, Fig. 2B) of cells within pseudofilaments, cellular anastomization (Fig. 2F), and fine cellular granulation (Fig. 2G).

Besides traditional morphological traits common to *Johannesbaptistia*, *J. floridana* has atypical characters

not yet described in currently described species. The apical cell of *J. floridana* consistently appears light yellow in comparison to the deeper blue–green of nearby vegetative cells (Fig. 2C). Other observations made throughout the life cycle of *J. floridana* include a club-shaped cellular deformity where cells can become twice as wide (Fig. 3C). Teratogenic forms in cultures have previously been observed in *Johannesbaptistia*, where culture conditions shift from unfavorable to favorable (RICHERT et al. 2006).

*Johannesbaptistia floridana* sp. nov. has an extensive network of relatively large filamentous heterotrophic bacteria that form a texturized mat over the surface of the cyanobacterium sheath (Fig. 3A, B). This bacterial layer is further accentuated along with morphological oddities of *J. floridana* using fluorescent microscopy (Suppl. Fig. 1A). This bacterial layer is a feature that may or may not be artificial from culturing. DAPI and natural fluorescence of cells show cellular divarication at cell to cell junctions and following cell necrosis. Furthermore, a combination of autofluorescence and DAPI stained cyanobacteria show cellular division in one plane (Suppl. Figs 1B–E).

*Johannesbaptistia floridana* sp. nov. and *J. pellucida* share some similarities in morphological traits (Table 1).





Fig. 2. Microscopic images of *Johannesbaptistia floridana* sp. nov. (strain BLCC-M67 [reference strain]) showing non-traditional morphological traits of this genus including: (A) cell division in single plane within apical cells; (B) necridia of pseudofilaments; (C) characteristic yellowing of apical cells; (D) pseudobranching at the apical cell junction; (E) cell division; (F) anastomization of cells, and (G) fine cell granulation with large yellow dividing cell before divarication. Scale bars 50 µm.

These two species contain similar cell shape and content with discoid cells and fine granulation, and pseudofilaments that are capable of reproduction by disintegration. The two species differ in terms of reproduction, where *J. pellucida* facultatively releases single cells, a characteristic not observed in *J. floridana*. The most noticeable difference between *J. floridana* and all other described species of *Johannesbaptistia* is the pseudofilament and cell dimensions. *Johannesbaptistia floridana* is larger than all other currently described species of this genus, with cells of 24.0–29.2 (30.0) µm (Table 1).

#### Molecular and phylogenetic analyses

Molecular data for representatives of the genus *Johannesbaptistia* are currently scarce, with only one partial 16S rRNA gene sequence deposited in GenBank of *J. pellucida* kopara–GC (RICHERT et al. 2006). *Johannesbaptistia floridana* sp. nov. forms a well-supported clade (BI: 1.0; ML: 100) with *J. pellucida* kopara–GC (Fig. 4). Our data also show that the genus *Johannesbaptistia* is sister to the genus *Limnococcus* (BI: 1.0; ML: 100), and more distantly related to *Gomphosphaeria* (BI: 0.97; ML:

–). Pairwise distance (data not shown) for *J. pellucida* kopara–GC and *J. floridana* is 98.0%.

The 16S–23S rRNA ITS secondary structure of *J. floridana* is presented in Figure 5. Since molecular data for *Johannesbaptistia* is limited, we present the secondary structures of closely related strains for comparison. Compared to closely related taxa with available molecular data, including *Crocospaera*, *Inacoccus*, *Pleurocapsa*, and *Synechocystis*, the D1–D1' region of *J. floridana* is different in structure; the D1–D1' is extensive with 93 nucleotides similar to that of *Crocospaera* with 92 nucleotides, while the remaining taxa contain only 64 nucleotides. Alternatively, while disparate from that of *Crocospaera*, *Pleurocapsa*, and *Synechocystis*, the Box B region of *J. floridana* is identical to that of *Inacoccus* with a total of 33 nucleotides.

The new taxon *Johannesbaptistia floridana* proposed in this study is based on criteria including monophyly and a combination of molecular and morphological traits previously outlined by JOHANSEN & CASAMATTA (2005). The 16S rRNA of *J. floridana* has enough dissimilarity (p-distance) to *J. pellucida*, to separate the two species

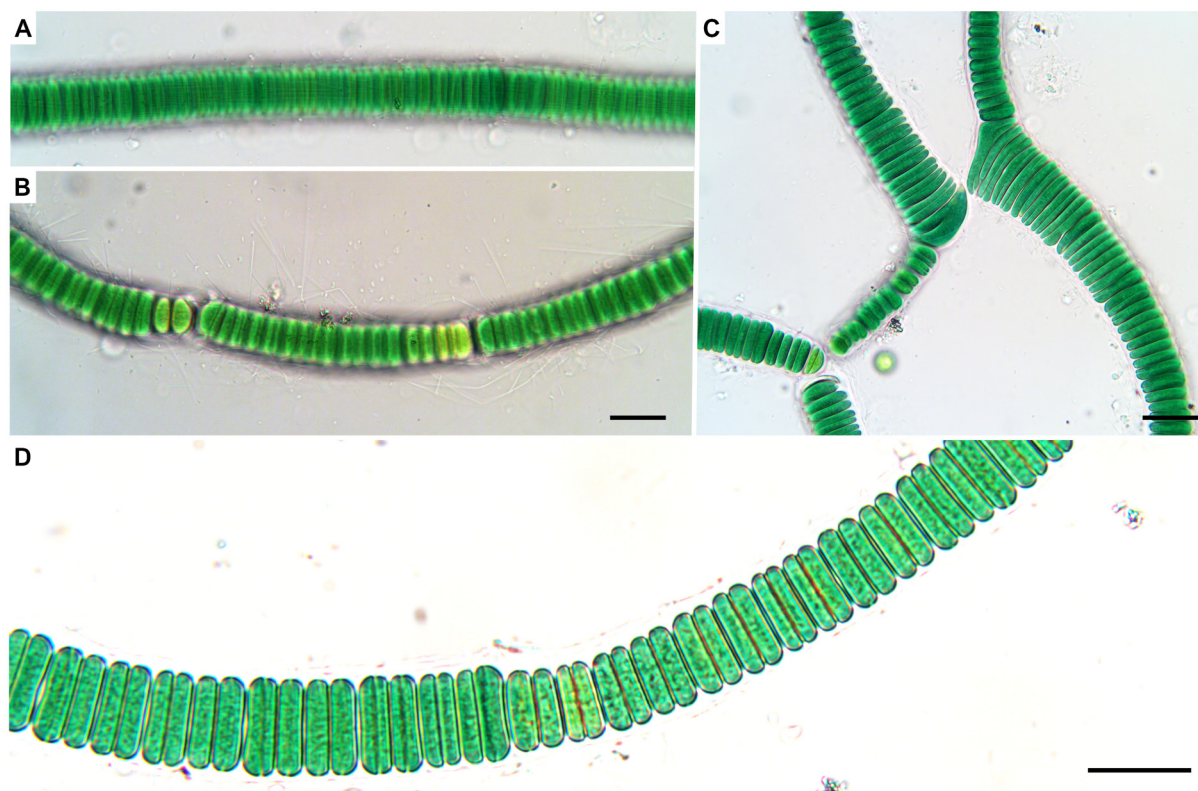


Fig. 3. Microscopic images of *Johannesbaptistia floridana* sp. nov. (strain BLCC-M67 [reference strain]) showing remarkable characteristics including: (A) texturized appearance of gelatinous sheath; (B) extent of bacterial biofilms covering sheath; (C) club-shaped cell teratology, and (D) cell size variation within a pseudofilament. Scale bars 30μm.

based on the < 98.7% suggested cutoff (YARZA et al. 2014). The 16S rRNA gene phylogeny shows high support for *J. floridana* as a novel species. Morphological traits of *J. floridana* include contrasting cellular dimensions. The combination of 16S rRNA gene phylogeny and sequence similarity support, along with the diagnosable morphological characteristics supports the erection of the novel species *J. floridana*.

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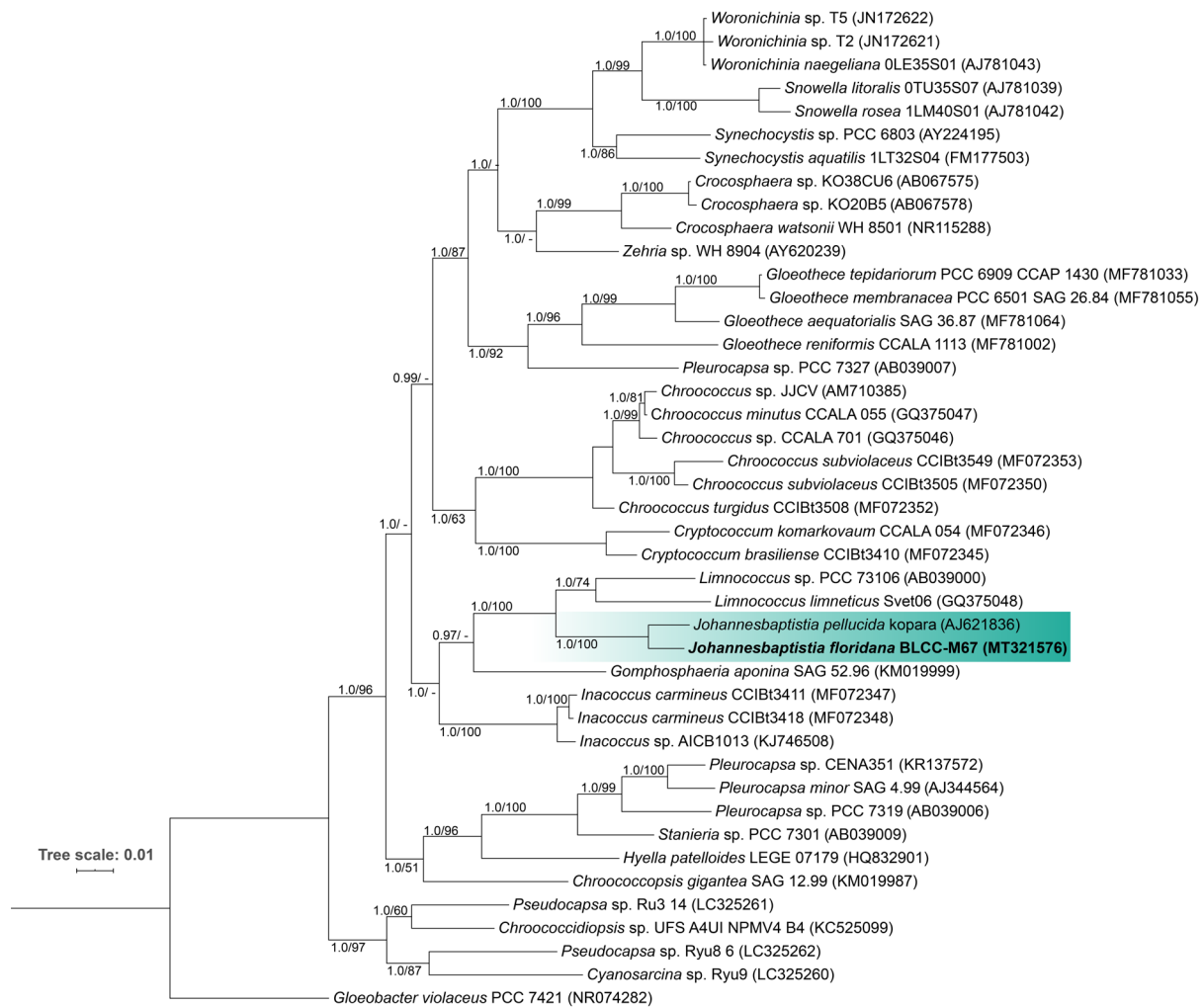


Fig. 4. Maximum likelihood and Bayesian inference (16S rRNA) of the phylogenetic relationship of *Johannesbaptistia floridana* sp. nov. (strain BLCC-M67) and 41 taxa of Chroococcales plus *Gloeobacter violaceus* PCC 7421 (NR074282) as the outgroup. Posterior probabilities (PB) and bootstrap support (BS) are indicated at the nodes. A – indicates less than 0.50 (PB) or 50% (BS) support.

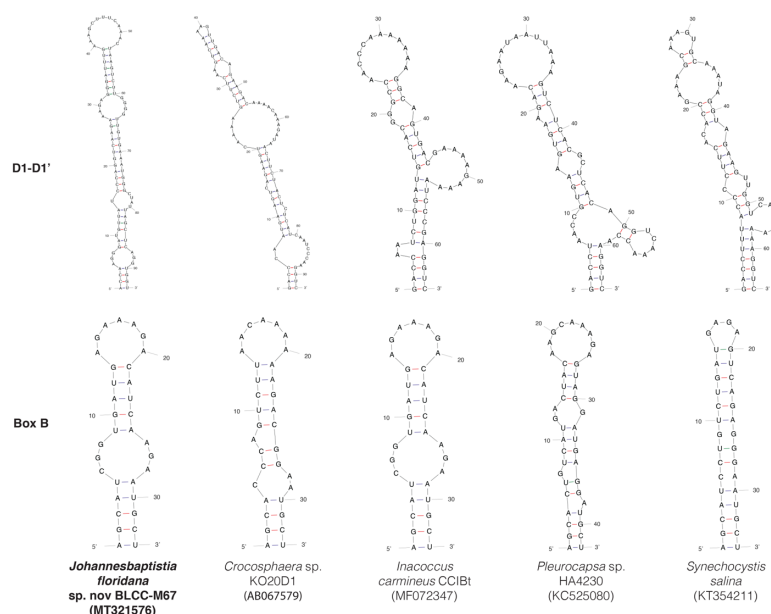


Fig. 5. Secondary structures of the 16S–23S rRNA internal transcribed spacer (ITS) of *Johannesbaptistia floridana* BLCC-M67 and other taxa of the Order Chroococcales.

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#### Supplementary material

The following supplementary material is available for this article:

Fig. S1. Fluorescent microphotographs of *Johannesbaptistia floridana* sp. nov.

This material is available as part of the online article (<http://fottea.czechphycology.cz/contents>)

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