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Recognizing novel cyanobacterial diversity in marine benthic mats, with the description of Sirenicapillariaceae *fam. nov.*, two new genera, *Sirenicapillaria gen. nov.* and *Tigrinifilum gen. nov.*, and seven new species

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ABSTRACT
Marine benthic proliferations are increasing in occurrence, range and duration by way of anthropogenic nutrient loading and climate change. The spread of these cyanobacteria within a variety of regions has made it fundamental to recognize the diversity and the species involved in these blooms. To expand knowledge on marine cyanobacterial diversity and reveal their phylogenetic relationships, sampling and isolation of benthic proliferative events from underexplored regions were conducted in the USA (Florida) and France (Loire-Atlantique). Cyanobacteria were described with the polyphasic approach using morphology, 16S rRNA gene phylogeny, 16S–23S rRNA ITS secondary structures and pairwise distances. Sampling of marine cyanobacteria from seagrasses, lagoons and marine coastal waters from nine Florida localities, in addition to Florida freshwater canals and French salt flats, revealed floating, epipsammic and epiphytic mats. A total of 30 cyanobacteria were isolated of which 21 represented two novel genera, *Sirenicapillaria* and *Tigrinifilum*. *Sirenicapillaria* is a genus that is found in massive benthic blooms throughout the western and southern Florida Coast.

INTRODUCTION

Coastal marine areas are notably changing through anthropogenic nutrient loading, temperature fluctuations due to climate change, and acidification (Pearce & Feng 2013; Di Lorenzo & Mantua 2016; Oliver *et al.* 2017). With warming weather and nutrients in favour, benthic cyanobacterial mats (BCMs) can form dense proliferations over shallow and deeper regions that were once dominated by seagrass beds or hard-bodied corals, respectively (Paerl & Paul 2012; Gurgel *et al.* 2020; Urrutia-Cordero *et al.* 2020; Benny *et al.* 2021). These dense BCMs are increasing and becoming more prevalent (Coleman *et al.* 2020; Wood *et al.* 2020; Ford *et al.* 2021) where they can dominate reefs and coastal and littoral regions (Paul *et al.* 2005; Sneed *et al.* 2017; Ford *et al.* 2018; Lydon *et al.* 2020; Berthold *et al.* 2021; FDEP 2019–2021). BCMs can also be a nuisance due to producing toxins or unknown secondary compounds, emitting noxious odours, decreasing dissolved oxygen when decomposing leading to hypoxia and fish kills, or harbouring microorganisms that can cause skin irritation (Ford *et al.* 2018; Reilly 2020; Wood *et al.* 2020). With the expansion of BCMs causing negative ecological effects, it is necessary to recognize and understand their biological and chemical diversity (Sharp *et al.* 2009; Engene *et al.* 2011; Sendall & McGreggor 2018).

Although benthic marine cyanobacterial proliferations (i.e. benthic blooms) are frequently observed, the extent of their spatial and temporal occurrence as well as the species involved

remain largely unexplored. To further compound the issues, BCM proliferations covering seagrasses, corals and the benthos are often misidentified and reported as '*Lyngbya*', '*Lyngbya majuscula*', '*Microseira wollei*', '*Moorena producens*', or '*Dapis*', an indication for the need of more taxonomic studies (Engene *et al.* 2012, 2013, 2018; FDEP 2021). With proper cyanobacterial descriptions and taxonomic designations, our understanding of cyanobacteria that bloom on the coast is strengthened, which is also important for public outreach (Collins 2021).

Accurate identification of cyanobacteria within mats can be challenging as BCMs are usually entangled masses of several cyanobacterial species (Biessy *et al.* 2021; Berthold *et al.* 2021). Since marine BCMs are highly diverse, integrative molecular techniques are often necessary for proper specific and generic delimitations (Caires *et al.* 2018a; Lefler *et al.* 2021). The combination of molecular and ultrastructural approaches together with ecological and morphological analyses is necessary to unravel cyanobacterial diversity (Komárek 2016). This polyphasic method is especially important in delimiting simple homocytous trichal cyanobacteria in the order Oscillatoriales, such as *Lyngbya*, *Oscillatoria* and *Phormidium*, which have few morphological features and still include polyphyletic assemblages of species (Komárek 2016; Mühlsteinová *et al.* 2018). In addition to describing cyanobacteria on a generic and specific level, family level taxonomy is equally necessary. Recent genetic delimitation of *Oscillatoria princeps* Vaucher *ex* Gomont by Mühlsteinová

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et al. (2018) provided a reliable taxonomic reference point for the genus *Oscillatoria* and thus the family Oscillatoriaceae, and revealed a need for the revision of the genera that have been traditionally classified as Oscillatoriaceae, because these are polyphyletic as currently circumscribed (Suda *et al.* 2013; Berthold *et al.* 2021; Zimba *et al.* 2021).

To investigate cyanobacteria that form BCMs, benthic proliferations from coastal western Florida, South Florida and western France were studied. From the sampled cyanobacterial mats, we propose two novel genera: *Sirenicapillaria gen. nov.*, represented by three new species (*S. glauca sp. nov.*, *S. rigida sp. nov.* and *S. stauglerae sp. nov.*), and *Tigrinifilum gen. nov.*, represented by two species (*T. floridanum sp. nov.* and *T. guerandense sp. nov.*). Two new species of *Capilliphycus*, *C. guerandensis sp. nov.* and *C. flaviceps sp. nov.* are also described.

MATERIAL AND METHODS

Cyanobacteria sampling, isolation and cultivation

Cyanobacterial benthic mats which possessed filamentous 'Lyngbya-like' taxa were sampled from various marine locations around Florida (USA), including the west (Gulf) coast near Lemon Bay, the east (Atlantic) coast in Biscayne Bay, and the Florida Keys. A total of 10 sampling events were conducted in Florida coastal waters between August 2017 and July 2020 (Table S1). Samples were collected mostly from epilithic or epipsammic mats, dislodged floating mats and among seagrass beds. Cyanobacterial mats and dried mat material found in hypersaline flats from western France (Guérande) and freshwater canals in Florida were also included in this study for robust taxon sampling of BCMs.

Cyanobacteria were isolated with traditional microbiological methods using solidified (15 g l^{-1} agar) sterile source water and micromanipulation of single filaments onto solid BG-11 media with added aquarium salts (35 g l^{-1}) (SWBG-11). Cyanobacterial isolates were treated with cycloheximide to remove contaminating eukaryotes (355 mM; SIGMA) and cultured in liquid SWBG-11 (35 ppt) at 25°C under 12:12 h light cycles. Live cultures are maintained in the BLCC (University of Florida, Fort Lauderdale Research and Education Center, Davie, Florida, USA) and the BCCM/ULC (University of Liège, Liège, Belgium); both dried and preserved (4% formaldehyde) type material for each novel taxon is deposited in the US National Herbarium (National Museum of Natural History, Smithsonian Institution, Washington, DC, USA).

Morphological and phylogenetic analyses

Images of cyanobacterial thalli were taken using a DSLR camera. Morphometric data were derived from images captured on a DIC (differential interference contrast) compound microscope (Leica DM5500 B) and corresponding LAS X software (Life Sciences; Leica Microsystems, Wetzlar, Germany).

Prior to DNA extraction, fresh biomass (100 mg) of each unialgal culture was washed twice with sterile deionized water to remove salts. Total genomic DNA was extracted with a DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany) and then quantified using NanoDrop Lite (Thermo Scientific). The 16S rRNA gene sequence and 16S–23S rRNA internal transcribed spacer (ITS) region were amplified by polymerase chain reaction (PCR) on a thermocycler (ProFlex PCR system; Applied Biosystems; Life Technologies) using the primers 359F and 1487R, and 1337F and 23S30R, respectively (Wilmotte *et al.* 1993; Nübel *et al.* 1997) following protocols outlined in Berthold *et al.* (2021).

PCR products were purified with a QIAquick PCR Purification Kit (Qiagen) and were subsequently visualized on a 0.8% agarose gel. The amplified products of the 16S rRNA gene were directly sequenced; however, the 16S–23S rRNA ITS products were cloned using a TOPO TA cloning kit with TOP10 chemically competent cells (Invitrogen; Life Technologies) according to manufacturer protocols. Recombinant plasmids from several positive transformants for each sample were extracted from clonal libraries and purified (PureLink® Quick Plasmid Miniprep Kit). Sanger sequencing of both 16S rRNA PCR products and plasmid DNA was carried out by Eurofins Genomics (Kentucky, USA) using BigDye Terminator v3.1 (Applied Biosystems). Sequences are deposited in GenBank (National Center for Biotechnology Information, NCBI) under the accession numbers listed in Table S1.

The Basic Local Alignment Search Tool (BLASTN), NCBI, was used for identifying sequences of representative strains similar to the 16S rRNA gene of the cyanobacterial isolates. A total of 141 sequences were used to align with the 30 isolates from our study using MUSCLE (SeaView v4.4; Edgar 2004), and then annotated manually based on conserved regions, with *Gloeobacter violaceus* (AY485484) as an outgroup. A total of 1013 sites of the 16S rRNA gene were used in the alignment. The best fit model was assessed using jModelTest through MEGA v10.1.7 (Stecher *et al.* 2020). Phylogenetic trees were constructed using Bayesian inferencing (BI) with MrBayes v3.2.7a and maximum likelihood (ML) using RAxML v8.2.12, through the CIPRES network v3.3 and MEGA, respectively (Miller *et al.* 2010). The ML analysis was carried out using the K2 + G + I model with 1,000 bootstrap resampling replicates. The BI analysis was conducted with MrBayes v3.2.7a (Ronquist & Huelsenbeck 2003) using 1.5×10^6 generations, a 0.25 burnin rate and resampling every 100 generations.

Predicted secondary structures including the D1-D1' and Box-B helices of the 16S–23S rRNA ITS region were generated using Mfold (Zuker 2003) with default settings and the structures were drawn using untangle with loop fix setting. Pairwise distances for the 16S rRNA gene and 16S–23S rRNA ITS sequences between isolates and representative related clades were calculated using MEGA. Mean *p*-distance between and within cyanobacterial families based on 16S rRNA gene sequences were also calculated using MEGA, and taxonomic family classification was based on Guiry & Guiry (2021) and Hauer & Komárek (2021).

RESULTS

Taxonomic treatments

Subclass Oscillatoriophycidae L. Hoffmann, Komárek & Kaštovský
Order Oscillatoriales J.H. Schaffner

Sirenicapillariaceae D.E. Berthold, Lefler & Laughinghouse *fam. nov.*

DESCRIPTION: Homocytous, uniseriate, isopolar, straight to slightly curved to coiled, filamentous. Thalli planktonic and/or attached to substrata and vegetation, prostrate, erect, fascicular or not. Euryhaline. Filaments with or without sheaths. Trichomes with or without constriction at cross wall. Cell content granulated typically at cross-walls. Cells discoid, apical cells range from bluntly rounded, to conical rounded, to rounded, calyptra present or not. Reproduction by single cell release, hormogonia, and/or diagonal fragmentation. False branching is rare. Currently, this family includes the genera *Affixifilum*, *Capilliphycus*, *Limnoraphis*, *Limnospira*, *Neolyngbya*, *Sirenicapillaria* and *Tigrinifilum*, which differ by 16S rRNA phylogeny.

DIAGNOSIS: Monophyletic group of marine/brackish/freshwater genera based on the 16S rRNA gene phylogeny, sister to the Laspinemataceae clade.

COMMENT: Genera are commonly found in marine coastal areas, with some found in inland waters. Some genera display desiccation tolerance and growth in hypersalinity.

ETYMOLOGY: Name based on the genus *Sirenicapillaria*, a rigid marine filamentous cyanobacterium that forms nuisance benthic blooms across the Florida western and southern coasts.

TYPE GENUS: *Sirenicapillaria gen. nov.*

Subclass Oscillatoriophycidae L. Hoffmann, Komárek & Kaštovský
Order Oscillatoriales J.H. Schaffner
Family Sirenicapillariaceae D.E. Berthold, Lefler & Laughinghouse *fam. nov.*

Sirenicapillaria D.E. Berthold, Lefler & Laughinghouse *gen. nov.*

DESCRIPTION: Homocytous filamentous cyanobacteria, forming dark brown, blue green erect thallus on benthos. Long coarse filaments (*c.* 10 cm) with thick, sometimes facultative and lamellate hyaline sheath. Trichomes cylindrical, thicker in the centre and slowly attenuated towards apices, uniseriate, constricted at cross-walls. Cells discoid, shorter than wide. Cell content light to dark brown, greyish green and blue green. Apical cell rounded, narrowed at times, with occasional calyptra. Reproduction by release of individual cells, straight or diagonal trichome fragmentation, necridia, hormogonia. Rarely false branches.

TYPE SPECIES: *Sirenicapillaria stauglerae* D.E. Berthold, Lefler & Laughinghouse *sp. nov.*

ETYMOLOGY: From Latin *Siren* (Greek Σειρήν), a Siren, and Latin *capillus*, hair, in reference to the long thalli and 'mermaid's hair' appearance of these marine cyanobacteria.

Sirenicapillaria glauca D.E. Berthold, Lefler & Laughinghouse *sp. nov.*
Figs 1–6

DESCRIPTION: Thallus erect, light to dark blue green. Filaments straight and somewhat flexuous, longer than 10 cm, 18.4–25.2(–28) µm wide with thick hyaline sheath. Trichomes cylindrical, (14–)15–20(–21) µm wide, thicker in the centre and gradually attenuated towards the ends. Cells slightly constricted at cross walls. Cells discoid, wider than long, 1.3–2.8 µm long. Cell content light to dark green, blue green to greyish, with lightly pigmented cells towards apices. Apical cell rounded, at times conical, with occasional calyptra. Reproduction by straight or diagonal trichome fragmentation, hormogonia, and necridia.

TYPE LOCALITY: USA. Florida: Englewood, seagrass beds within Lemon Bay Aquatic Preserve (26°54.503′N, 82°20.365′W).

HABITAT: Epipsammic, epiphytic and floating on marine water surface.

ETYMOLOGY: Latin adjective *glaucus, -a, -um*, in reference to the 'sea green' cell and thalli colour of this cyanobacterium.

HOLOTYPE: US 227772 (dried material in a metabolically inactive state of reference strain BLCC-M125), deposited in US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

REFERENCE STRAIN: BLCC-M125.

MATERIALS ANALYSED: BLCC-M125.

Sirenicapillaria rigida D.E. Berthold, Lefler & Laughinghouse *sp. nov.*
Figs 7–15

DESCRIPTION: Thallus dark brown, tangled, web-like. Filaments straight and rigid, 5 cm long, 28–83(–94) µm wide with thick, lamellate hyaline sheath. Trichomes cylindrical, 22.8–63.3 µm wide, thicker in the middle and gradually attenuated towards apices. Cells slightly constricted at cross walls. Cells discoid, wider than long, 1.8–3.9 µm long. Cell content light to dark brown, with pigmentless cells towards apices. Apical cell rounded, at times conical, with occasional calyptra. Reproduction by release of individual cells, straight or diagonal trichome fragmentation, necridia, hormogonia.

COMMENT: Individual filaments observed with naked eye and reminiscent of the freshwater *Microseira wollei*. Hormogonia form attached clumps on individual filaments.

TYPE LOCALITY: USA. Florida: Duck Key (24°46.635′N, 80°54.527′W).

HABITAT: Benthic, wrapped around seagrasses in marine coasts.

ETYMOLOGY: Latin adjective *rigidus, -a, -um*, in reference to the physical rigidity of the filaments.

HOLOTYPE: US 227691 (dried material in a metabolically inactive state of reference strain BLCC-M116), deposited in US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

REFERENCE STRAIN: BLCC-M116.

MATERIALS ANALYSED: BLCC-M116 (US 227691), BLCC-M134 (US 227777).

Sirenicapillaria stauglerae D.E. Berthold, Lefler & Laughinghouse *sp. nov.*
Figs 16–26

DESCRIPTION: Thallus erect, light to dark brown, almost black. Filaments straight and flexuous, 5 cm long, 12.5–33(–35) µm wide with thick hyaline sheath. Trichomes cylindrical, 10–26.8 µm wide, thicker in the centre and gradually attenuated towards apices. Cells slightly constricted at cross walls. Cells discoid, wider than long, 1.0–2.4 µm long. Cell content light to dark brown, grey to red, with pigmentless cells towards apices. Apical cell rounded, at times conical, with occasional calyptra.

Reproduction by straight or diagonal trichome fragmentation, hormogonia and necridia. Rarely false branches.

COMMENT: Filament ends usually do not have thick sheaths, while mid-filaments usually have thick lamellate hyaline sheaths.

TYPE LOCALITY: USA. Florida: Englewood, seagrass beds within Lemon Bay Aquatic Preserve (26°54.503′N, 82°20.365′W).

HABITAT: Benthic on marine coasts.

ETYMOLOGY: The epithet *stauglerae* is in honour of Elizabeth 'Betty' Staugler for her committed work in studying and surveying coastal marine habitats and the 'Eyes on Seagrass' Florida Sea Grant Extension programme, which first noted the emergence of this cyanobacterium blanketing areas that had previously been inhabited by seagrasses.

HOLOTYPE: US 227770 (dried material in a metabolically inactive state of reference strain BLCC-M121), deposited in US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

REFERENCE STRAIN: BLCC-M121.

MATERIALS ANALYSED: BLCC-M121, BLCC-M122, BLCC-M123, BLCC-M138.

Subclass Oscillatoriophycidae L. Hoffmann, Komárek & Kaštovský
Order Oscillatoriales J.H. Schaffner
Family Sirenicapillariaceae D.E. Berthold, Lefler & Laughinghouse *fam. nov.*

Tigrinifilum D.E. Berthold, Lefler & Laughinghouse *gen nov.*

DESCRIPTION: Homocytous filamentous cyanobacteria forming solitary and free-floating, straight, wavy to slightly flexuous filaments, with or without thin hyaline sheath. Trichomes cylindrical. Cells slightly constricted between meristematic zones, highly constricted between dividing cells in older filaments. Cross-walls densely granulated and darkened except at the filament ends. Cells discoid, wider than long. Cell content light to dark green, blue green, brown, and grey. Apical cell rounded, with occasional clear calyptra. Reproduction by fragmentation or hormogonia.

TYPE SPECIES: *Tigrinifilum floridanum* D.E. Berthold, Lefler & Laughinghouse *sp. nov.*

ETYMOLOGY: From Latin *tigrinus*, striped like a tiger, and Latin *filum*, a thread, in reference to the resemblance of a 'banded tiger' characteristic of the filaments.

COMMENT: Species of this genus are halotolerant-halophilic and resistant to desiccation.

Tigrinifilum floridanum D.E. Berthold, Lefler & Laughinghouse *sp. nov.*
Figs 27–29, 31–33, 36–38

DESCRIPTION: Filaments solitary and free-floating, or attached to substrate. Filaments straight, wavy, to flexuous, (9–)10.2–19 μm wide with thick hyaline sheath. Trichomes cylindrical, (7–)8.0–19.2 μm wide. Cells slightly constricted between meristematic zones, highly constricted between dividing cells in older filaments. Cross-walls densely granulated and darkened. Cells discoid, wider than long, rarely isodiametric, 1.0–2.7 μm long. Cell content light to dark green, blue green, brown, and grey. Apical cell rounded, with occasional clear calyptra. Reproduction by fragmentation or hormogonia.

TYPE LOCALITY: USA. Florida: Elliot Key (Biscayne Bay) (25°23.810′N, 80°14.043′W).

HABITAT: Entangled within larger benthic cyanobacteria or dislodged floating mats.

ETYMOLOGY: Latin *floridanum*, in reference to the widespread occurrence of this cyanobacterium throughout the southern Florida coast.

HOLOTYPE: US 223427 (dried material in a metabolically inactive state of reference strain BLCC-M48), deposited in US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

REFERENCE STRAIN: BLCC-M48.

MATERIALS ANALYSED: BLCC-M48, BLCC-M50, BLCC-M51, BLCC-M57, BLCC-M66, BLCC-M73, BLCC-M77, BLCC-M85, BLCC-M87, BLCC-M102, BLCC-M107, BLCC-M118, BLCC-M119, BLCC-M120.

Tigrinifilum guerandense D.E. Berthold, Lefler & Laughinghouse *sp. nov.*
Figs 30, 34, 35

DESCRIPTION: Thallus solitary, floccose, free-floating, light to dark blue green. Filaments straight, wavy to flexuous, without sheath. Trichomes cylindrical, 5.3–6.2 μm wide. Cells slightly constricted between meristematic zones, highly constricted between dividing cells in older filaments. Cross-walls densely granulated and darkened. Cells discoid, wider than long, rarely isodiametric, 1.5–2.9 μm long. Cell content light to dark green, blue green, brown and grey. Apical cell rounded. Reproduction by filament disintegration or fragmentation.

TYPE LOCALITY: FRANCE. Loire-Atlantique: Guérande, Terre de Sel (47°18.938′N, 2°27.197′W).

HABITAT: Entangled within cyanobacterial mats of inland salt flats.

ETYMOLOGY: The epithet *guerandense*, is a reference to the salt flats in Guérande where the species originates.

HOLOTYPE: US 227675 (dried material in a metabolically inactive state of reference strain BLCC-M99), deposited in US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

REFERENCE STRAIN: BLCC-M99.

MATERIALS ANALYSED: BLCC-M99.

Subclass Oscillatoriophycidae L. Hoffmann, Komárek & Kaštovský
Order Oscillatoriales J.H. Schaffner
Family Sirenicapillariaceae D.E. Berthold, Lefler & Laughinghouse *fam. nov.*

Capilliphycus flaviceps Lefler, D.E. Berthold & Laughinghouse *sp. nov.*
Figs 39–44

DESCRIPTION: Thallus amorphous and entangled, mat-like. Filaments straight, 14–18.9 μm in diameter, rarely coiled. Sheath thick, facultative, hyaline, not lamellate. Cells slightly constricted. Cross walls densely granulated. Cells discoid, wider than long, 10.7–14 μm wide, 1.4–2.4 μm long. Cell content green, green-yellow, and yellow at apices. Apices conical to rounded, without calyptra. Hormogonia formed by straight fragmentation, with or without necridic cells. Diagonal fragmentation occurs with or without necridic cells.

TYPE LOCALITY: USA. Florida: Key Biscayne (25°43.588′N, 80°09.513′W).

HABITAT: Found within floating marine mats of larger filamentous cyanobacteria.

ETYMOLOGY: Latin adjective *flavus, -a, -um*, yellow, and Latin ending -*ceps* (*caput*), head, in reference to the tendency for yellowing of the apices of the trichome.

HOLOTYPE: US 227779 (dried material in a metabolically inactive state of reference strain BLCC-M137), deposited in US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

REFERENCE STRAIN: BLCC-M137.

MATERIALS ANALYSED: BLCC-M137, BLCC-M53 (US 227634).

Capilliphycus guerandensis Lefler, D.E. Berthold & Laughinghouse *sp. nov.*
Figs 45–48

DESCRIPTION: Thallus light to dark forest green, and yellow to orange when exposed to air (drying). Filaments straight to flexuous, 18.5–29.2 μm in diameter, rarely coiled. Sheath thin, facultative, hyaline, not lamellate. Cells not constricted. Cells discoid, wider than long, 15.8–24.9 μm wide, 1–2.2 μm long. Cell content green. Apices conical to rounded, attenuating, without calyptra. Apical cell yellow. Hormogonia formed by straight fragmentation, with or without necridic cells. Diagonal fragmentation occurs with or without necridic cells.

COMMENT: This species grows partially submerged.

TYPE LOCALITY: FRANCE. Loire-Atlantique: Guérande, Terre de Sel (47°18.938′N, 2°27′11.850″W)

HABITAT: Hypersaline pools within inland salt flats.

ETYMOLOGY: The epithet *guerandensis* is a reference to the salt flats in Guérande where the species originates.

HOLOTYPE: US 227655 (dried material in a metabolically inactive state of reference strain BLCC-M76), deposited in US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

REFERENCE STRAIN: BLCC-M76.

MATERIALS ANALYSED: BLCC-M76, BLCC-M92 (US 227668).

Capilliphycus tropicalis T.A. Caires, Sant'Anna & J.M. Nunes

COMMENT: Our material fits the original description of this species in Caires *et al.* (2018b) and clusters together with *Capilliphycus tropicalis* in the 16S rRNA gene sequence phylogenetic tree.

SPECIES FEATURES: Thallus green and entangled, mat-like. Filaments straight, 11.8–16.8 μm in diameter. Sheath hyaline. Cells light to dark green. Cells discoid, wider than long, 8.4–12 μm wide, 1–2.1 μm long. This is the first report of this species outside of Brazil.

MATERIALS ANALYSED: BLCC-M106 (US 227681).

Cyanobacterial isolation and morphological characteristics

A total of 30 unicyanobacterial isolates were used in this study (Table S1). All 30 cyanobacterial strains contained discoid cells with or without a sheath. Sampling of marine cyanobacteria from seagrasses, lagoons and marine coastal waters from nine Florida localities revealed floating, epipsammic and epiphytic mats. From Florida BCMs, a total of 24 unicyanobacterial strains were isolated, of which 21 isolates represent two novel cyanobacterial genera, *Sirenicapillaria* and *Tigrinifilum*. In addition, three novel cyanobacterial isolates of *Capilliphycus* and *Tigrinifilum* from France (Guérande) are described, as well as the phylogenetic information (16S rRNA gene and 16S–23S rRNA ITS) of three isolates of *Limnoraphis* from freshwater canals in Florida.

Morphological analyses of the 24 Floridian marine cyanobacterial strains demonstrated cyanobacteria with cell dimensions ranging between 10.2 and 83 μm for filament width and discoid cells ranging between 5.3–63.3 and 1.0–3.9 μm for both trichome cell width and length (Table 1). *Sirenicapillaria* has cyanobacteria with the largest cell size and sheath, especially *S. rigida*.

Morphological analyses demonstrated that *Sirenicapillaria* is composed of taxa with very long thalli (*c.* 20 cm), filaments with facultative sheaths, and three species: *S. glauca* (Figs 1–6), *S. rigida* (Figs 7–15), and *S. stauglerae* (Figs 16–26). In a healthy lab environment, this genus has concentrated pigments and appears dark (Figs 1, 16), whereas in the field, in high light, or with unfavourable conditions, it turns light brown to gold colours (Fig. S2). *Sirenicapillaria* filaments are very long (up to 10 cm), with a thick sheath at the middle of the filament (Fig. 10) and a gradual attenuation of the filament towards the ends with a thin sheath or none at all (Figs 6, 9). The largest species in terms of filament width is *S. rigida*, often observed with a lamellate or thickened gelatinous sheath (Figs 13, 14). Compared to its sister species, *S. rigida* has extremely tough filaments (Fig. 7), forming an intertwined, rigid and net-like mat; this is potentially the justification for the misidentification of *Sirenicapillaria* as *Microseira* [*Lyngbya*] in local reports.

Sirenicapillaria stauglerae and *S. glauca* overlap in both cell width and length. Although *S. stauglerae* and *S. glauca* have similar dimensions, these species are easily differentiated based on thallus colour and cell colour; *S. stauglerae* is generally brown and golden brown while *S. glauca* maintains a greyish, blue-green pigmentation (Fig. 3). The thallus of *Sirenicapillaria* is reminiscent of that of *Lyngbya majuscula* Harvey *ex* Gomont and *Dapis*, where a very long thalli resembles fine hair underwater (Figs 1, 16). Unlike *L. majuscula* that was originally described as black hair from the coast of England (Gomont 1892), *Sirenicapillaria* is more gold to brown and was discovered from the Florida Gulf coast through the Florida Keys. While the morphology of *S. rigida* overlaps with *L. majuscula*, the latter reaches larger widths and, together with their different type regions, we do not suggest this to be *L. majuscula*.

The genus *Tigrinifilum* is somewhat comparable to previously described cyanobacterial taxa in *Lyngbya*, including *L. aestuarii* F. Liebman *ex* Gomont and *L. salina* Kützing *ex* Starmach (Komárek & Anagnostidis 2005). Shared morphological features between *T. floridanum* and *L. aestuarii* include rare false branching, pronounced cross wall granulation and a

Table 1. Morphological characteristics of known species of *Sirenicapillaria* gen. nov., *Tigrinifilum* gen. nov. and *Capilliphycus*. Measurements (µm) are represented as min–max and mean ± st. dev.

Genus	*Sirenicapillaria*	*Sirenicapillaria*	*Sirenicapillaria*	*Tigrinifilum*	*Tigrinifilum*	*Capilliphycus*	*Capilliphycus*
Species	*rigida*	*stauglerae*	*glauca*	*floridanum*	*guerandense*	*flaviceps*	*guerandensis*
Thallus	rigid and loose, tangled knots	flexuous and erect	loose, tangled knots	entangled, mat-like	floccose	entangled, mat-like	entangled, mat-like
Filament width (µm)	28.0–83.0(–94) 47.6 ± 17.2	12.5–33(–35) 19.5 ± 4.6	18.4–25.2(–28) 22.4 ± 2	(9–)10.2–19.0 14.2 ± 1.5	-	14–18.9 16.5 ± 1.4	18.5–29.2 22.3 ± 3.1
Sheath	hyaline, thick, lamellate	hyaline, thin to thick	hyaline, thin	hyaline, facultative	no sheath	hyaline, facultative	thick, facultative
Trichome	progressive attenuation	progressive attenuation	slight attenuation	no attenuation	no	no	no
Cell shape	discoid	discoid	discoid	discoid	discoid	discoid	discoid
Cell length (µm)	1.8–3.9 2.9 ± 0.5	1.0–2.4 1.5 ± 0.3	1.3–2.8 1.8 ± 0.4	1.0–2.7 1.7 ± 0.3	1.5–2.9 2.2 ± 0.4	1.4–2.4 1.7 ± 0.2	1.0–2.2 1.6 ± 0.3
Cell width (µm)	22.8–63.3 37.9 ± 11.9	10–26.8 15.2 ± 3.9	(14–)15–20(–21) 17.2 ± 1.8	(7–)8.0–19.2 11.2 ± 1.4	5.3–6.2 5.8 ± 0.3	10.7–14 12.4 ± 1.1	15.8–24.9 19 ± 2.5
Apical cell	bluntly rounded	bluntly rounded	bluntly rounded	bluntly rounded	conical to bluntly rounded	conical	conical
Cell content	homogeneous	homogeneous	homogeneous	homogenous	homogeneous	homogenous	homogenous
Cell colour	red, light to dark brown	light to dark brown	forest green	green, blue green, grey, brown, gold,	blue green, green	green to greenish yellow, yellow at apices	green, yellow at apices
Constriction	yes	yes	yes	yes	no	slightly	no
Cross-wall	facultative granulation	facultative granulation	facultative granulation	very granulated	very granulated	granulated	no
Reproduction	release of single cells, hormogonia by straight or diagonal trichome fragmentation, helped or not by necridic cells	release of single cells, hormogonia by straight or diagonal trichome fragmentation, helped or not by necridic cells	release of single cells, hormogonia by straight or diagonal trichome fragmentation, helped or not by necridic cells	hormogonia by straight or diagonal trichome fragmentation, helped or not by necridic cells	trichome fragmentation, helped or not by necridic cells	hormogonia by straight or diagonal trichome fragmentation, helped or not by necridic cells	hormogonia by straight or diagonal trichome fragmentation, helped or not by necridic cells
Occurrence	marine and benthic in seagrasses	marine and benthic in seagrasses	marine and benthic in seagrasses	marine and benthic and epipsammic	hypersaline, marine planktonic	marine and benthic and epipsammic	hypersaline, benthic
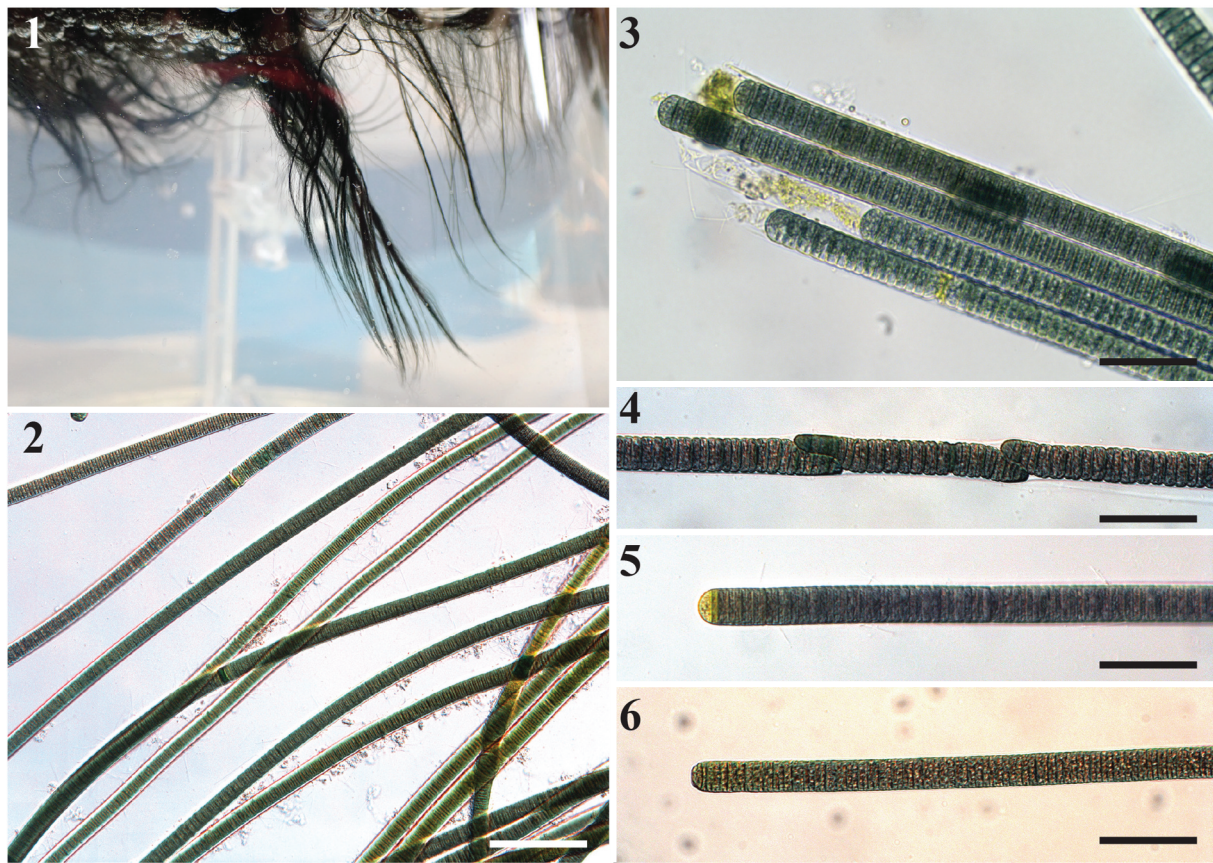
Figs 1–6. Images of *Sirenicapillaria glauca* (reference strain BLCC-M125).
Fig. 1. Images showing hair-like thalli.
Fig. 2. Microscope images showing variation in filament pigmentation. Scale bar = 100 μm.
Fig. 3. Blue green cell colour. Scale bar = 50 μm.
Fig. 4. Filament diagonal fragmentation Scale bar = 50 μm.
Fig. 5. Yellow apical cell. Scale bar = 50 μm.
**Fig. 6**. Filament ends without sheath. Scale bar = 50 μm.

marine habitat. However, *T. floridanum* and *L. aestuarii* differ considerably in climatic conditions of the type locality, which for *L. aestuarii* is the North Sea off the coast of present day Friesland in northwestern Germany. Furthermore, there is a large difference in filament and cell sizes between *T. floridanum* and *L. aestuarii*, with the latter being up to 10 μm wider. *Tigrinifilum floridanum* also resembles *L. salina* in having similar filament width and cell length, as well as in the marine habitat. However, the type locality for *L. salina* is a thermal spa in Kissingen, Germany, and morphological differences including the presence of a layered and stratified sheath and a lack of constriction between cells within *L. salina* suggest that *T. floridanum* is novel. Additionally, *T. floridanum* demonstrates a calyptra while *L. salina* does not.

16S rRNA phylogeny, intergeneric and family *p*-distance analyses

BLASTN analysis of the 16S rRNA gene sequence of the 30 marine cyanobacterial isolates shows them as closely related to the genera *Affixifilum, Capilliphycus, Limnoraphis, Limnospira* and *Neolyngbya* with 97% or lower identity. BLASTN analysis of isolates from *Tigrinifilum* indicated 99.4% or lower identity

to uncultured cyanobacteria found in coastal sediments of the Mediterranean Sea (Païssé *et al.* 2010) and in sulfidic springs of western USA (Headd & Engel 2014). Analysis of the 16S rRNA gene sequence of *Sirenicapillaria* isolates indicated high similarity to sequences of 'cf. *Lyngbya* sp. BAN TS02' (99.2%; HQ419195) from Australia and 97.1% or lower similarity to *Limnoraphis hieronymusii* (JN854140), *Capilliphycus tropicalis* (MF190468), cyanobacteria found in the guts of marine fish (Jones *et al.* 2018), and uncultured cyanobacteria 'cf. *Lyngbya*' or 'cf. *Limnoraphis*' from the freshwater Clear Lake in California (Kurobe *et al.* 2013) and from Lake Atitlán, Guatemala (Rejmánková *et al.* 2011; Komárek *et al.* 2013), respectively.

BI and ML phylogenetic analyses of the 16S rRNA gene sequence of the 30 isolates demonstrated a large clade containing other taxa including *Affixifilum, Capilliphycus, Limnoraphis, Limnospira* and *Neolyngbya* with high support (BS: 89%; PP: 1.0) (triangle in Fig. 49). The large Sirenicapillariaceae clade is sister to the Laspinemataceae clade (BS: –; PP: 0.99) and closely related to the *Lyngbya confervoides* clade (BS: –; PP: 1.0). Within Sirenicapillariaceae, *Sirenicapillaria* (BS: 88%; PP: 1.0) and *Tigrinifilum* (BS: 76%; PP: 1.0) formed distinct clades (Fig. 49). The BI and ML analyses also showed clades of described genera including *Capilliphycus* (BS: –; PP: 1.0) and *Limnoraphis* (BS:
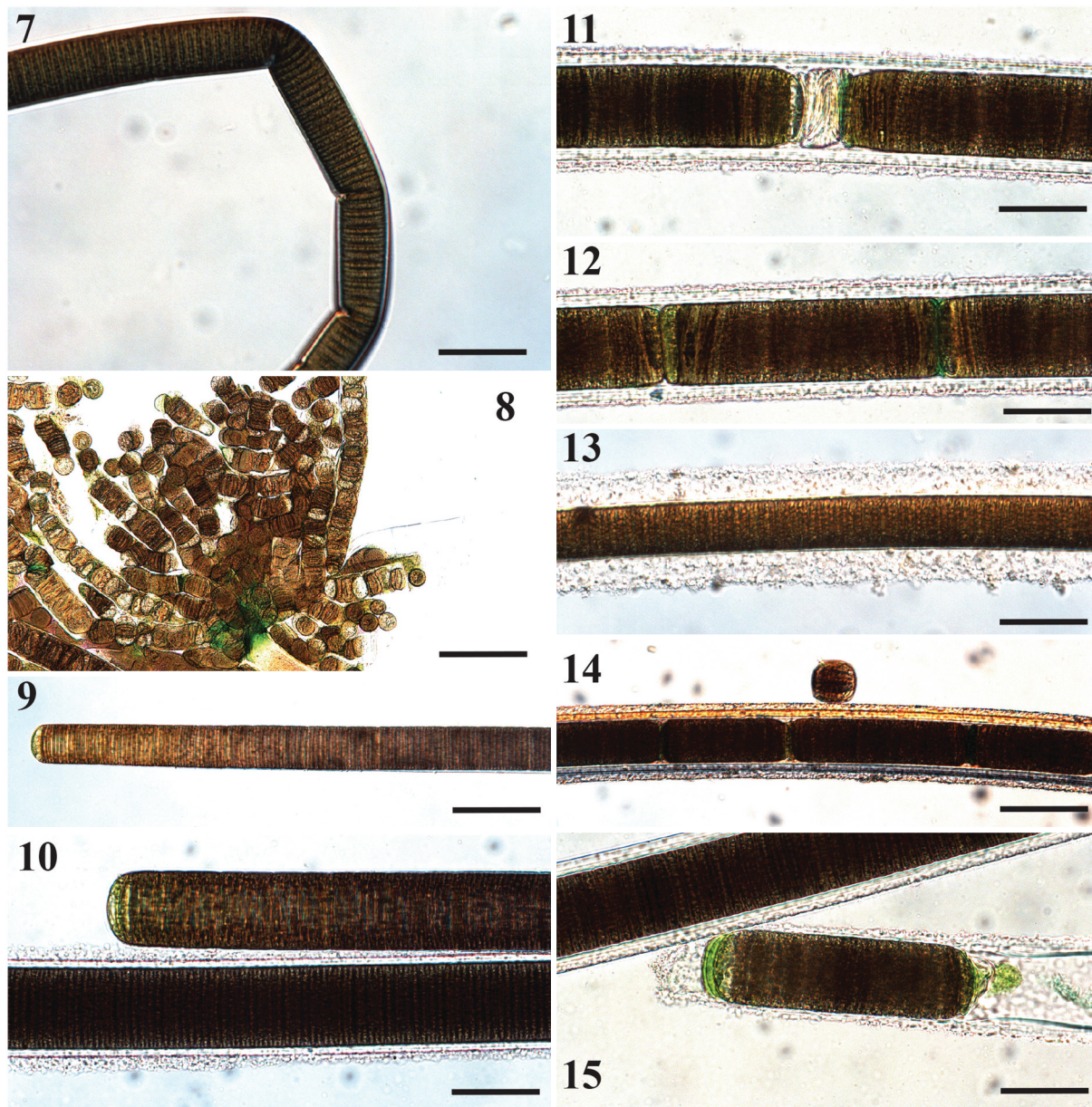
Figs 7–15. Microscope images of *Sirenicapillaria rigida* (reference strain BLCC-M116).
**Fig. 7**. Rigid filament sheath and bending. Scale bar = 100 µm.
Fig. 8. Clump of hormogonia usually attached to filaments. Scale bar = 100 µm.
Fig. 9. Reduced pigmentation towards the apices. Scale bar = 100 µm.
Fig. 10. Difference between a long filament centre with a sheath and apices without. Scale bar = 50 µm.
Fig. 11. Necridia. Scale bar = 50 µm.
Fig. 12. Filament slight diagonal fragmentation. Scale bar = 50 µm.
Fig. 13. Extensive mucilaginous sheath. Scale bar = 50 µm.
Fig. 14. Coarse and lamellate sheath. Scale bar = 50 µm.
Fig. 15. Hormogonium with calyptra. Scale bar = 50 µm.

100%; PP: 1.0). Within *Capilliphycus, C. flaviceps* (BS: 97%; PP: 1.0) as well as *C. guerandensis* (BS: 99%; PP: 1.0) formed separate subclades. Within *Sirenicapillaria*, three separate clades formed including *S. glauca* (BS: 88%; PP: 1.0), *S. rigida* (BS: 77%; PP: 0.93) and *S. stauglerae* (BS: 91%; PP: 1.0). The clade that included *Tigrinifilum* had two subclades of *T. floridanum* (BS: 100%; PP: 0.66) and *T. guerandense* (BS: 74%; PP: 1.0).

Intergeneric pairwise distances of the 16S rRNA gene sequence of the genera within the family Sirenicapillariaceae revealed genetic distances with 93.62%–97.97% among *Affixifilum, Capilliphycus, Limnospira, Limnoraphis, Neolyngbya, Sirenicapillaria* and *Tigrinifilum* (Table S2). *Sirenicapillaria* and *Tigrinifilum* showed ≤97.03% and ≤96.08% genetic identity to all other genera within Sirenicapillariaceae, respectively (Table S2).

The genetic similarity analyses of the 16S rRNA gene sequence across analysed established homocytous cyanobacterial families demonstrated ≤94.5% similarity for the novel
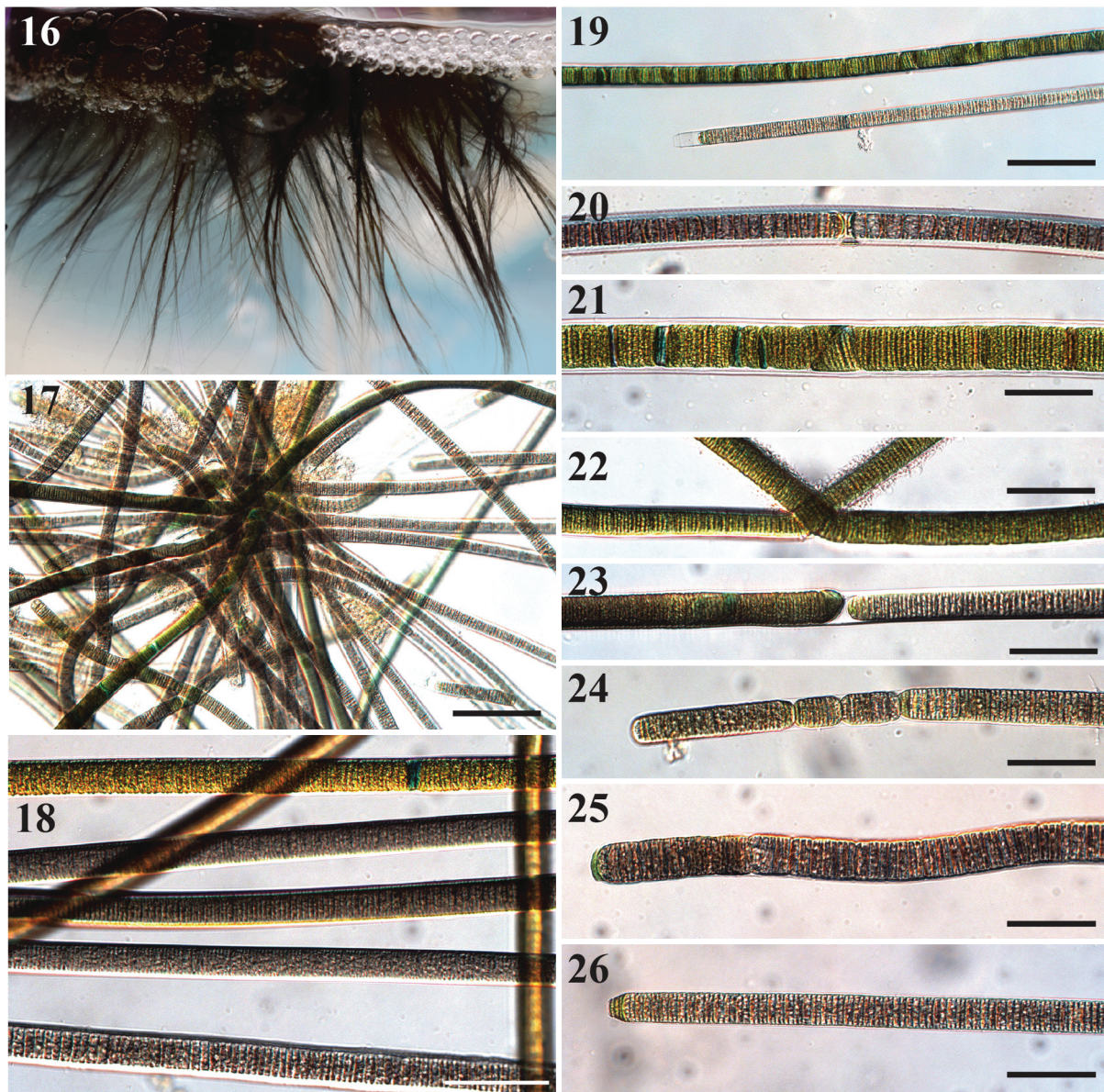
Figs 16–26. Images of *Sirenicapillaria stauglerae* (reference strain BLCC-M121).
Fig. 16. Thallus.
Fig. 17. Microscope image showing filament clumping. Scale bar = 100 μm.
Fig. 18. Microscope image showing filament morphological plasticity. Scale bar = 50 μm.
Fig. 19. Width and colour difference between filament centre and apex. Scale bar = 100 μm.
Fig. 20. Necridia and textured sheath. Scale bar = 50 μm.
Fig. 21. Diagonal fragmentation. Scale bar = 50 μm.
Fig. 22. Rare false branching. Scale bar = 50 μm.
Fig. 23. Filament prior to false branching. Scale bar = 50 μm.
Fig. 24. Hormogonia formation. Scale bar = 50 μm.
Fig. 25. Calyptra. Scale bar = 50 μm.
Fig. 26. Slight conical apical cell shape. Scale bar = 50 μm.

family Sirenicapillariaceae (Table 2). Sirenicapillariaceae is closely related to Coleofasciculaceae *sensu stricto* (94.5%), Microcoleaceae (94.5%) and Laspinemataceae (94.4%), but comparatively distant to Oscillatoriaceae *sensu stricto* (92.6%). The within family genetic similarity of Sirenicapillariaceae was 97.2% while the within similarities of the established families were comparatively lower for Spirulinaceae (94.7%) and Desertifilaceae (95.3%) and ≥98.0% for the remaining families including Microcoleaceae, Oscillatoriaceae, Laspinemataceae, Vermifilaceae and Coleofasciculaceae. Morphological descriptions of the genera within Sirenicapillariaceae are summarized in Table 3.
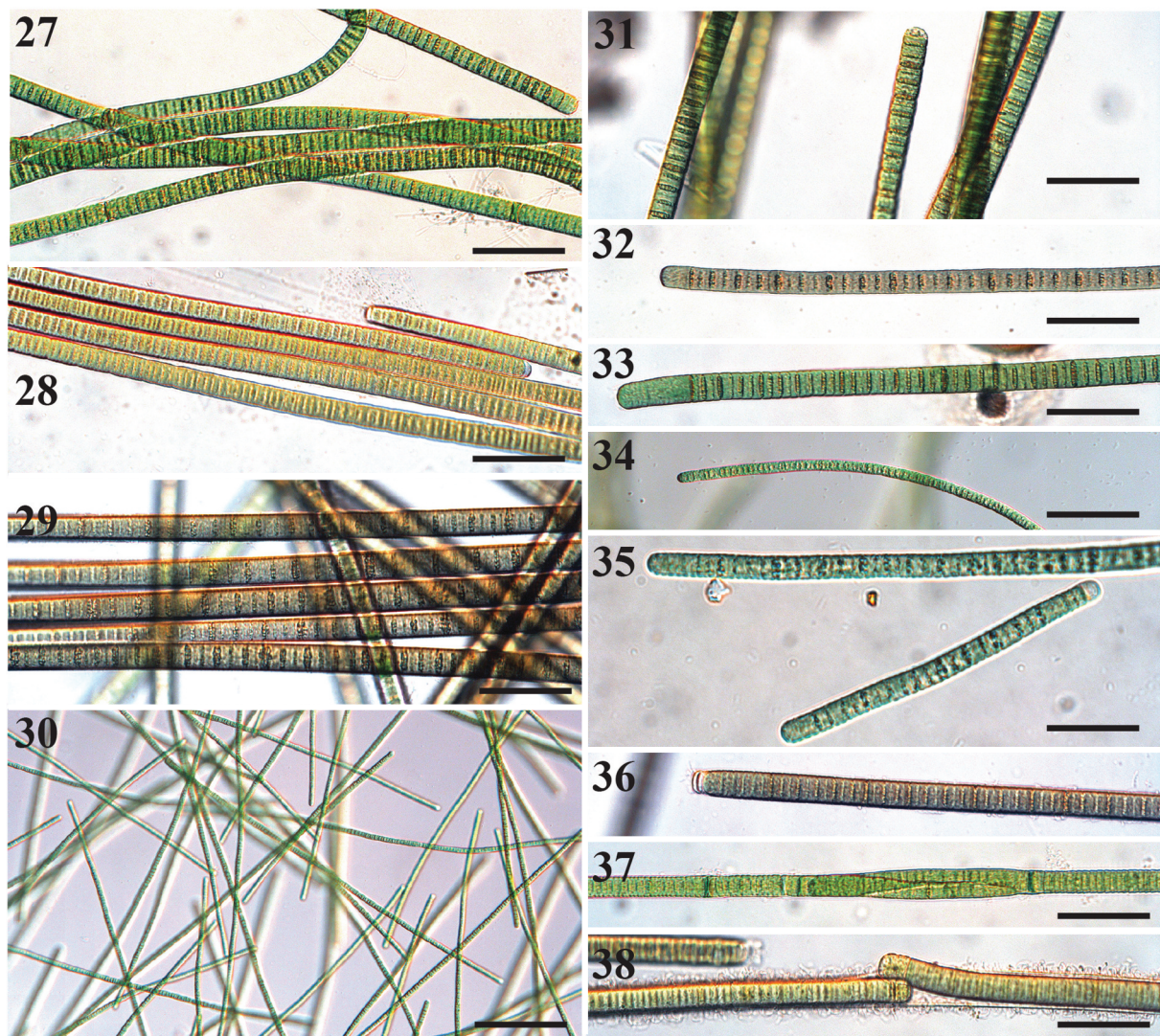
Figs 27–38. Microscope images of species of *Tigrinifilum*.
Fig. 27. Variation in filament colours: *T. floridanum* strain BLCC-M51. Scale bar = 50 µm.
Fig. 28. Variation in filament colours: *T. floridanum* strain BLCC-M119. Scale bar = 50 µm.
Fig. 29. Variation in filament colours: *T. floridanum* strain BLCC-M118. Scale bar = 50 µm.
Fig. 30. Floccose thalli of *T. guerandense* (reference strain BLCC-M99). Scale bar = 50 µm.
Fig. 31. Extreme granulation at the crosswalls resembling tiger stripes in *T. floridanum*. Scale bar = 50 µm.
Fig. 32. Lack of granulation at apical crosswalls of *T. floridanum*. Scale bar = 50 µm.
Fig. 33. Lack of granulation at apical crosswalls of *T. floridanum*. Scale bar = 50 µm.
Fig. 34. Extensive granulation at crosswalls and lack of granulation in apices in *T. guerandense*. Scale bar = 50 µm.
Fig. 35. Extensive granulation at crosswalls and lack of granulation in apices in *T. guerandense*. Scale bar = 20 µm.
Fig. 36. Calyptra of *T. floridanum*. Scale bar = 50 µm.
Fig. 37. Filaments growing past each other of *T. floridanum*. Scale bar = 50 µm.
Fig. 38. Rare false branching of *T. floridanum*. Scale bar = 50 µm.

16S–23S rRNA internal transcribed spacer (ITS) secondary structure and *p*-distance analyses

Lengths of the identifiable domains in the 16S–23S rRNA ITS sequence of all genera can be found in Table 4. Within *Capilliphycus*, the 16S–23S rRNA ITS secondary structure of both the D1-D1′ (Fig. 50) and the Box-B (Fig. 51) showed contrasting lengths and structures among both proposed species. Two D1-D1′ helices of *C. guerandensis* were recovered, tRNA dependent. Both helices were comparable in length (60 nucleotides) and were characterized by a six-nucleotide clamp (5′- GACCUA—UAGGUC -3′) followed by a unilateral bulge on the 3′ side. A unilateral bulge before the terminal loop caused by a single nucleotide on the 5′ side was seen in the helix from the operon lacking tRNA. In the helix for the operon with tRNA, this unilateral bulge is present as a bilateral bulge (5′- GA—A -3′). *Capilliphycus flaviceps* BLCC-M53 had a five-nucleotide clamp (5′- GACCU—AGGUC -3′) followed by a unilateral bulge on the 3′ side. A bilateral bulge (5′- AG—A -3′) occurs in the main stem before the large terminal loop. *Capilliphycus flaviceps* BLCC-M137 possessed two operons, with and without tRNAs, but only one D1-D1′ helix, which had a six-nucleotide clamp (5′- GACCUA—UAGGUC -3′)
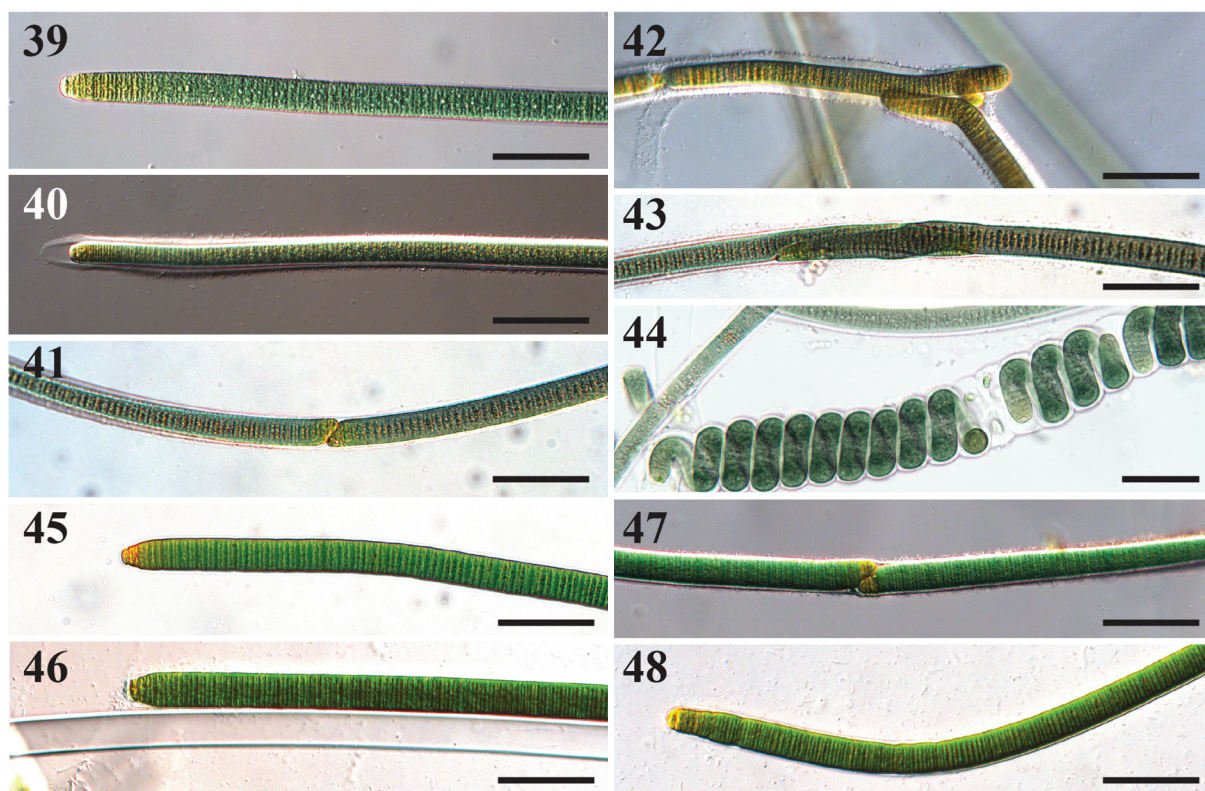
Figs 39–48. Microscope images of *Capilliphycus*. Scale bars = 50 μm.
Fig. 39. Yellow apical region of *C. flaviceps* (reference strain BLCC-M137).
Fig. 40. Extended sheath at the apices in *C. flaviceps* (reference strain BLCC-M137).
Fig. 41. Diagonal fragmentation in *C. flaviceps* (reference strain BLCC-M137).
Fig. 42. False branching in *C. flaviceps* (reference strain BLCC-M137).
Fig. 43. Filaments growing past another in *C. flaviceps* (reference strain BLCC-M137).
Fig. 44. Spiral morphology of some filaments of *C. flaviceps* (reference strain BLCC-M137).
Fig. 45. Yellow apical cells of *C. guerandensis* (reference strain BLCC-M76).
Fig. 46. Facultative sheath in *C. guerandensis* (reference strain BLCC-M76).
Fig. 47. Diagonal fragmentation in *C. guerandensis* (reference strain BLCC-M76).
Fig. 48. Tapering of apices in *C. guerandensis* (reference strain BLCC-M76).

followed by a unilateral bulge on the 3′ side. A bulge of unmatched base pairs (5′- A—G -3') occurs before a bilateral bulge (5′- AA—A -3'), which is followed by the large terminal loop.

The Box B helices of all *Capilliphycus* species had a four-nucleotide clamp (5′- AGCA—UGCU -3′), although the helices varied in length and structure. The Box B of *C. guerandensis* is 40 nucleotides with a unilateral bulge caused by a single nucleotide on the 3′ side as well as a bilateral bulge (5′- GA—A -3′) before the terminal loop. The Box B of *C. flaviceps* BLCC-M53 is shorter (25 nucleotides) with a unilateral bulge (5′- C—AA -3′) in the main stem. *Capilliphycus flaviceps* BLCC-M137 contained two, tRNA dependent, Box B helices characterized by a unilateral bulge caused by a single nucleotide on the 3′ side and a terminal loop. The Box B helix of the tRNA operon was longer, 28 nucleotides compared to 25 nucleotides. No V3 helices could be identified.

Within *Sirenicapillaria*, the 16S–23S rRNA ITS secondary structure of both the D1-D1′ and the Box-B showed different lengths and structures among all proposed species. *Sirenicapillaria glauca* had a five-nucleotide clamp (5′-GACCU—AGGUC -3′) followed by a unilateral bulge on the 3′ side. A bulge of unmatched base pairs occurs in the stem after the first bulge (5′- GA—GA -3′) and again (5′- C—A -3′) before a unilateral bulge on the 5′ side before the terminal loop. Although operons with and without tRNAs were recovered, *Sirenicapillaria rigida* only possessed one D1-D1′ helix with a five-nucleotide clamp (5′- GACCU—AGGUC -3′) followed by a unilateral bulge on the 3′ side. A bulge of unmatched base pairs occurs in the stem after the first bulge (5′- GA—GA -3′) and again (5′- C—A -3′) before a unilateral bulge on the 5′ side. This is followed by a unilateral bulge on the 3′ side before a small terminal loop. *Sirenicapillaria stauglerae* possessed two operons, with and without tRNAs. The operon without tRNAs had a five-nucleotide clamp (5′- GACCU—AGGUC -3′) followed by a unilateral bulge on the 3′ side. A bulge of unmatched base pairs occurs after the first bulge (5′- A—A -3′). Two unilateral bulges on the 5′ side before the terminal loop. The D1-D1′ helix of the operons with tRNAs was similar to that of the helix without differing only at nucleotide 44, although this did not affect the structure. The helix is characterized by a five-nucleotide clamp (5′- GACCU—AGGUC -3′) followed by a unilateral bulge on the 3′ side. A bulge of unmatched base pairs occurs after the first bulge (5′- A—A -3′). Two unilateral bulges on the 5′ side before the terminal loop.
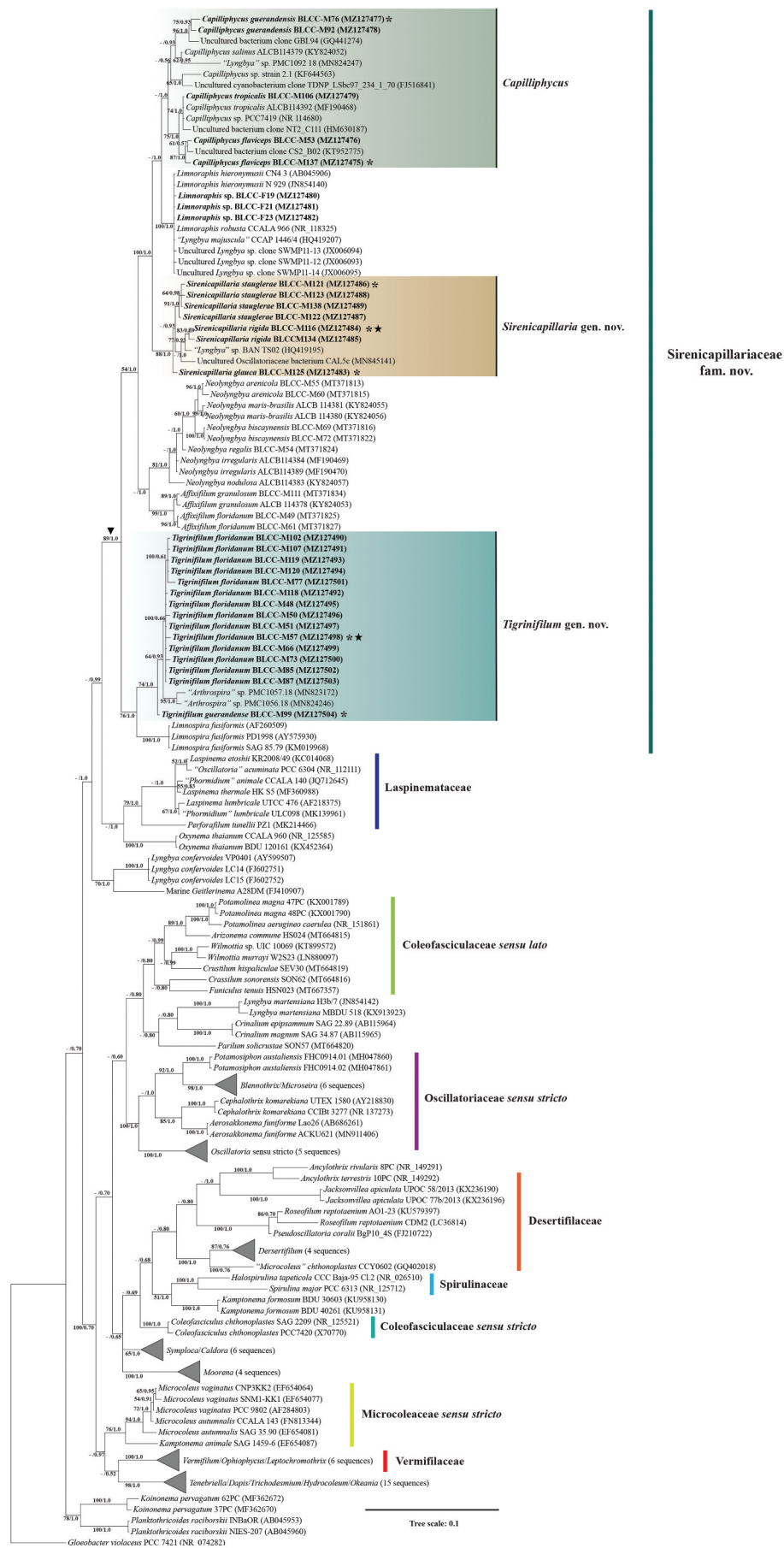
Fig. 49. Maximum likelihood and Bayesian inference, showing Bayesian topology of the phylogenetic relationship of the 16S rRNA gene sequence of the 30 cyanobacterial strains presented in this work and 141 cyanobacterial taxa including *Gloeobacter violaceus* PCC 7421 (NR074282) as outgroup. Bootstrap support and posterior probabilities ≥50 and 0.5 are shown at consensus nodes of both inferential analyses, respectively. (*) represents the reference strains for the species; triangle indicates the branch where the family Sirenicapillariaceae is delimited; stars denote the type species of the genus.

Table 2. Mean *p*-distance (percentage of genetic similarity) between and within (last column) established cyanobacterial families, based on 16S rRNA gene sequences. Values are based on an alignment of 91 taxa and 7 established cyanobacterial families including the novel family Sirenicapillariaceae with eight genera: *Affixifilum, Capilliphycus, Lyngbya, Limnospira, Limnoraphis, Neolyngbya, Sirenicapillaria* and *Tigrinifilum*. Taxonomic family classifications were based on Guiry & Guiry (2021) and Hauer & Komárek (2021).

		1	2	3	4	5	6	7	Within
1	Sirenicapillariaceae								97.20
2	Oscillatoriaceae *sensu stricto*	92.62							98.35
3	Laspinemataceae	94.39	93.65						98.41
4	Microcoleaceae	94.51	93.98	94.77					98.68
5	Desertifilaceae	93.13	91.83	91.69	92.11				95.25
6	Vermifilaceae	93.96	92.43	92.82	94.67	91.63			98.01
7	Spirulinaceae	92.97	91.58	92.81	93.05	92.33	91.87		94.66
8	Coleofasciculaceae	94.54	93.56	93.64	94.54	93.63	93.61	93.87	99.80

The Box B helices of all *Sirenicapillaria* species contained a four-nucleotide clamp (5′- AGCA—UGCU -3′), although the helices varied in length and structure. The Box-B of *Sirenicapillaria glauca* BLCC-M125 was 29 nucleotides long with a bilateral bulge after the clamp (5′- GCAC—AA -3′), causing a rightward lean. Only one BoxB helix of *Sirenicapillaria rigida* was recovered, tRNA independent, it contained a five base pair clamp (5′- AGCAG—CUGCU -3′) followed by a unilateral bulge after the clamp (5′- CAA—A -3′), causing a rightward lean, and a large terminal loop. Only one Box B helix from *Sirenicapillaria stauglerae* was recovered, tRNA independent. This BoxB helix was the shortest at 24 nucleotides and a unilateral bulge after the clamp (5′- C—AA -3′), causing a leftward lean, and a small (3 nucleotide) loop. V3 helices were recovered for all *Sirenicapillaria* species (data not shown).

Within *Tigrinifilum*, the 16S–23S rRNA ITS secondary structure of both the D1-D1′ and the Box-B showed dissimilar lengths and structures between both proposed species. *Tigrinifilum floridanum* had two D1-D1′ helices, which were 62 nucleotides and 63 nucleotides. The longest D1-D1′ helix is characterized by a five-nucleotide clamp (5′- GACCU—AGGUC -3′) followed by a large unilateral bulge on the 3′ side. A unilateral bulge caused by a single nucleotide on the 3′ occurs followed by two unilateral bulges by a single nucleotide on the 5′ side before the terminal loop, there were four variable bases throughout the helix, although they had no effect on the structure. The shorter D1-D1′ helix possessed a shorter, four base pair, clamp (5′- GACC—GGUC -3′) followed by a unilateral bulge on the 3′ side. A pair of unmatched nucleotides occurs after the first bulge (5′- U—C -3′). Both helices were recovered from different clones of the same strains in several isolates. *Tigrinifilum guerandense* contained one D1-D1′ helix characterized by a four-nucleotide clamp (5′- GACC—GGUC -3′) followed by a unilateral bulge on the 3′ side. A pair of unmatched nucleotides (5′- A—A -3′) occurs before a unilateral bulge by a single nucleotide on the 5′ side. A unilateral bulge of three nucleotides on the 3′ side occurs before a small terminal loop.

Each species of *Tigrinifilum* possessed one Box B helix. *Tigrinifilum floridanum* had a short three nucleotide clamp (5′- AGC—GGU -3′) followed by a unilateral bulge on the 3′ side caused by a single nucleotide. A bilateral bulge (5′- GA—A -3′) occurs within the helix before the small terminal loop. The Box

B helix of *T. guerandense* was shorter than that of *T. floridanum* (34 vs 40 nucleotides). *Tigrinifilum guerandense* contained a four-nucleotide clamp (5′- UCCU—AGGA -3′) with a bilateral bulge (5′- CAA—GTT -3′). A unilateral bulge on the 5′ side caused by a single nucleotide occurs before the short terminal loop. The V3 helix of *T. guerandense* was recovered (data not shown) but no V3 helix was recovered from *T. floridanum*.

We also present the first full 16S–23S rRNA ITS sequence of *Limnoraphis* sp. from Florida, USA. Operons were recovered with and without tRNA but did not affect the D1-D1′ nor BoxB helices. The D1-D1′ helix is 58 nucleotides long and possessed a five-nucleotide clamp (5′- GACCU—AGGUC -3′) with a unilateral bulge caused by a single nucleotide on the 3′ before the terminal loop. The Box B helix is 38 nucleotides long and had a four-nucleotide clamp (5′- AGCA—UGGU -3′) followed by a unilateral bulge on the 3′ side caused by a single nucleotide. There is a bilateral bulge before the terminal bulb (5′- C—AA -3′); a V3 helix was not recovered.

The 16S–23S rRNA ITS sequence dissimilarity between species of *Capilliphycus* (without tRNA) including *C. tropicalis, C. salinus* T.A. Caires, Sant'Anna & J.M. Nunes, *C. flaviceps* and *C. guerandensis* was ≥14.8% (Table S3). The sequence dissimilarity between species of *Sirenicapillaria* including *S. glauca, S. rigida* and *S. stauglerae* was ≥11.6% (Table S4). The 16S–23S rRNA ITS region (with tRNAs) dissimilarity between the two species of *Tigrinifilum, T. floridanum* and *T. guerandense*, was ≥5.8% (Table S5).

DISCUSSION

Sampling of marine cyanobacteria in this work from seagrasses, lagoons, salt flats, and marine coastal areas, in addition to freshwater canals, yielded a total of 30 unicyanobacterial isolates (Table S1). The isolates in this work were used to demonstrate the taxonomic relationship among filamentous homocytous cyanobacteria using the 16S rRNA gene sequence phylogeny as well as the 16S–23S rRNA ITS region genetic distances and secondary structure of the Box-B and D1-D1′ helices. The 16S rRNA gene sequence phylogeny, reinforced by both BI and ML analyses and morphological analyses, supported the delineation of two novel cyanobacterial genera, *Sirenicapillaria* and *Tigrinifilum*. Pairwise distance analyses of the 16S rRNA gene sequence demonstrated similarities of ≤97.01% among the two novel genera *Sirenicapillaria* and

Table 3. Morphological characteristics of the genera *Affixifilum, Neolyngbya, Limnoraphis, Capilliphycus, Sirenicapillaria gen. nov.* and *Tigrinifilum gen. nov.* within the family Sirenicapillariaceae *fam. nov.*

Characteristics	Affixifilum	Neolyngbya	Limnoraphis	Capilliphycus	Sirenicapillaria gen. nov.	Tigrinifilum gen. nov.
Thallus	thick compact mats, rarely solitary	small green mats or large brown fasciculate mats	filaments solitary, free-floating, or growing in small aggregations	extensive fasciculate mats or small clusters	rigid and loose, tangled knots	floccose or entangled, mat-like
Filament	8.1–29.8 μm diameter, filaments straight, or slightly curved to slightly waved	6.9–26.7 μm diameter, filaments straight	5–25 μm diam; straight or slightly curved	(8–)12–30(–46) μm diam.; straight	12.5–83(–94.0); straight and rigid	(9–)10.2–19.0; straight
Sheath	hyaline or laminate, thin or thick	hyaline, thin or thick, sometimes lamellated	firm, colourless, hyaline, thin, or slightly thick	hyaline or bright yellow to yellow-brown, firm, thin or thick, sometimes lamellated	hyaline or gelatinous, lamellate at times, thin to very wide	facultative, or not present
Trichome width	10.1–32.4 μm diameter	7.8–24.7 μm diameter	15–19 μm diameter	(6.6–)10–21(–24) μm	10–63 μm	5–20 μm
Cell content	contains gas vesicles and numerous cyanophycin granules	containing numerous gas vesicles	gas vesicles facultative	containing numerous gas vesicles usually forming aerotopes	heavily pigmented	homogeneous
Constriction	slightly constricted	not or slightly constricted	not or slightly constricted	not or slightly constricted	slightly constricted	facultative
Cross-wall	heavily granulated	frequently granulated	not informed	often granulated	granulated	very heavily granulated
Attenuation	attenuation present	trichomes not attenuated towards end	not attenuated	not or slightly attenuated towards ends	slight attenuation across filament length towards ends	
Cells	short discoid, 0.95–3.8 μm long	short; 1.3–4.5 μm long	cylindrical, short, always wider than long	short, 1.4–5.6(–6) μm long, 1.2–11 times larger than long	discoid, 1–4 μm long, 6–21 times wider than long	discoid, 1–3 μm long, twice as wide
Apical cell	usually rounded or conical-rounded, calyptra common, attenuation when forming hormogonia, may form many at once	usually rounded or conical-rounded, thickened rare, without calyptra	without clear calyptra	rounded, conical, conical-rounded, flat-rounded, truncate, rarely capitate, sometimes with thickened, without calyptra	bluntly rounded, at times slightly conical	conical to bluntly rounded
Reproduction	hormogonia formed within trichomes by straight fragmentation with or without necridia. Terminal hormogonia formed by attenuation of the apices and formation of several hormogonia.	hormogonia formed by diagonal, straight, or irregular fragmentation pattern of the trichome	intense hormogonia production	hormogonia formed by straight or diagonal fragmentation of the trichome, helped or not by necridic cells	release of single cells, hormogonia formed by straight or diagonal fragmentation of the trichome, helped or not by necridic cells	hormogonia by straight or diagonal trichome fragmentation, helped or not by necridic cells
Occurrence	epilithic or epipsammic mats in tropical marine environments	epilithic or epipsammic in tropical to subtropical environments	planktic in mesotrophic waters in Lake Atitlán, Guatemala, Florida freshwater canal	marine and halophilic, and in inland saline biotopes	marine and benthic in seagrasses	marine/hypersaline and benthic, planktonic
Reference	Lefler *et al.* (2021)	Caires *et al.* (2018a)	Komárek *et al.* (2013)	Caires *et al.* (2018b); this paper	this paper	this paper

Table 4. Lengths of identifiable domains in the 16S–23S rRNA Internal Transcribed Spacer (ITS) regions of strains of *Capilliphycus*, *Limnoraphis*, *Sirenicapillaria* gen. nov. and *Tigrinifilum* gen. nov. Duplicated strain numbers indicate different ITS patterns (with or without tRNAs). Multiple values indicate variable operon length within species and/or strains. A dash (-) denotes not observed.

Species/Strain(s)	Leader	D1-D1' helix	Spacer + D2 + Spacer	D3 + Spacer	tRNA^{Ile}	Spacer+ V2 + Spacer	tRNA^{Ala}	Spacer	Box-B Helix	Spacer	BoxA	D4 to ITS end
Capilliphycus flaviceps BLCC-M53	8	58	39	22	-	-	-	-	25	17	11	57
C. flaviceps BLCC-M137	8	58	39	26	-	-	-	-	25	17	11	51
C. flaviveps BLCC-M137	8	58	39	13	74	24	73	83	28	17	11	51
C. guerandensis BLCC-M76; BLCC-M92	8	60	39	22	74	24	73	66	40	17	11	55
C. guerandensis BLCC-M76	8	60	44	20	-	-	-	-	40	17	11	55
C. tropicalis	8	58	39	22	-	-	-	-	24	17	11	53
C. salinus	8	61	39	22	-	-	-	-	34	17	11	45
Sirenicapillaria stauglerae	8	62	75	30	-	-	-	-	27	17	11	47
S. stauglerae BLCC-M121	8	62	45	10	74	17	73	40	27	17	11	47
S. rigida BLCC-M116; BLCC-M134	8	63	41	13	74	18	73	40	31	17	11	47
S. rigida BLCC-M134	8	63	73	33	-	-	-	-	28/31	17	11	47
S. glauca BLCC-M125	8	66	73	30	-	-	-	-	29	17	11	47
Limnoraphis sp. BLCC-F19; BLCC-F23	8	58	38/39	62	-	-	-	-	38	17	11	54
Limnoraphis sp. BLCC-F23	8	58	39	13	74	24	73	61	38	17	11	54
Tigrinifilum floridanum	8	62/63	38	12	74	15	73	70	40	18	11	62
Tigrinifilum guerandense BLCC-M99	8	62	38/39	12	74	15	73	99	34	22	11	56

Tigrinifilum and other closely related cyanobacterial genera, namely *Affixifilum*, *Capilliphycus*, *Limnospira*, *Limnoraphis* and *Neolyngbya*. Although the genetic distance is above the suggested generic cut-off rate of 94.5% (Yarza *et al.* 2014), additional genetic regions, especially the 16S–23S rRNA ITS region, are often helpful for delimiting genera of cyanobacteria along with morphological and ecological data.

The percentage of dissimilarity of the 16S–23S rRNA ITS region is another standard, in addition to gene phylogenies, that is used to delimitate cyanobacterial species (Pietrasiak *et al.* 2014; Vázquez-Martínez *et al.* 2018; González-Resendiz *et al.* 2019; Berthold *et al.* 2021; Lefler *et al.* 2021). The intrageneric percent dissimilarity within *Capilliphycus* and the two novel genera *Sirenicapillaria* and *Tigrinifilum* demonstrated high support for the delimitation of the seven novel species presented herein (Tables S3–S5). Based on the value of 7% dissimilarity of the 16S–23S rRNA ITS region for strong support in separating species, the novel species within the genera *Capilliphycus* (*C. flaviceps* and *C. guerandensis*) and *Sirenicapillaria* (*S. glauca*, *S. rigida* and *S. stauglerae*) demonstrate high support for their delimitations in addition to the robust 16S rDNA phylogenetic analyses, morphological differences and 16S–23S rRNA ITS region secondary structures. Although the intrageneric *p*-distance within *Tigrinifilum* was below the cut-off (5.8%), where 3%–7% dissimilarity requires additional lines of evidence for species delimitation (González-Resendiz *et al.* 2019), the strong support from the 16S rRNA gene phylogenetic analyses in addition to the morphological disparities support two novel species, *T. floridanum* and *T. guerandense*.

Sirenicapillaria is responsible for many coastal BCMs, especially on the western Florida coast in Lemon Bay where the reference strain was originally sampled (FDEP 2019–2021; Fig. S2). The cyanobacterium forms huge floating brownish mats, especially in spring and summer, which are reported to produce noxious odours when decomposing, with consequences

for human health and coastal economies (Reilly 2019; Lane 2021). Although *Sirenicapillaria* is a prevalent bloomer, it is often reported as '*Lyngbya*', '*Lyngbya*-like', '*Lyngbya majuscula*' or even the freshwater '*Microseira wollei*'. *Sirenicapillaria* is often found proliferating along with *Vaucheria* and other green algae (FDEP 2021), wrapped around turtlegrass (*Thalassia testudinum* Banks *ex* König) and manatee grass (*Syringodium filiforme* Kützing) or floating and decomposing around red mangrove (*Rhizophora mangle* Linnaeus) prop roots or marinas (Fig. S2). Lemon Bay is an aquatic preserve supporting mangrove, seagrass and oyster communities, and *Sirenicapillaria* can grow across several miles of benthos. Once *Sirenicapillaria* reaches large densities, through high productivity and oxygen bubble production, the mats can raise the seagrasses out of the sediments. The benthic blooms can inhibit coral larval settlement, produce secondary compounds that deter fish herbivory and indirectly affect aquatic mammal health by displacing seagrasses (Lydon *et al.* 2020; Ritson-Williams *et al.* 2020). Though some physical effects of *Sirenicapillaria* proliferations have been observed on seagrasses, the synergistic physical or chemical effects on the marine flora and fauna of these regions remain largely unknown. Further research is warranted to uncover potential toxicity and novel secondary compounds within *Sirenicapillaria*.

We found two species of *Tigrinifilum*, *T. floridanum* from the Florida coast and *T. guerandense* from salt flats in France (Fig. S2). *Tigrinifilum* is a genus that is distinctive by the highly granulated crosswalls that give the cyanobacterium a striped tiger appearance (Fig. 27). Another unique morphological feature of *Tigrinifilum* is the lack of granulations at the filament ends (Figs 32–35). The species within *Tigrinifilum* are morphologically distinct, where *T. guerandense* is much smaller than *T. floridanum*, with no overlap in cell widths. Both species are slightly motile and tolerate high levels of salinity, especially *T. guerandense*, which was isolated from salt flats containing pools of briny seawater (>60 psu).
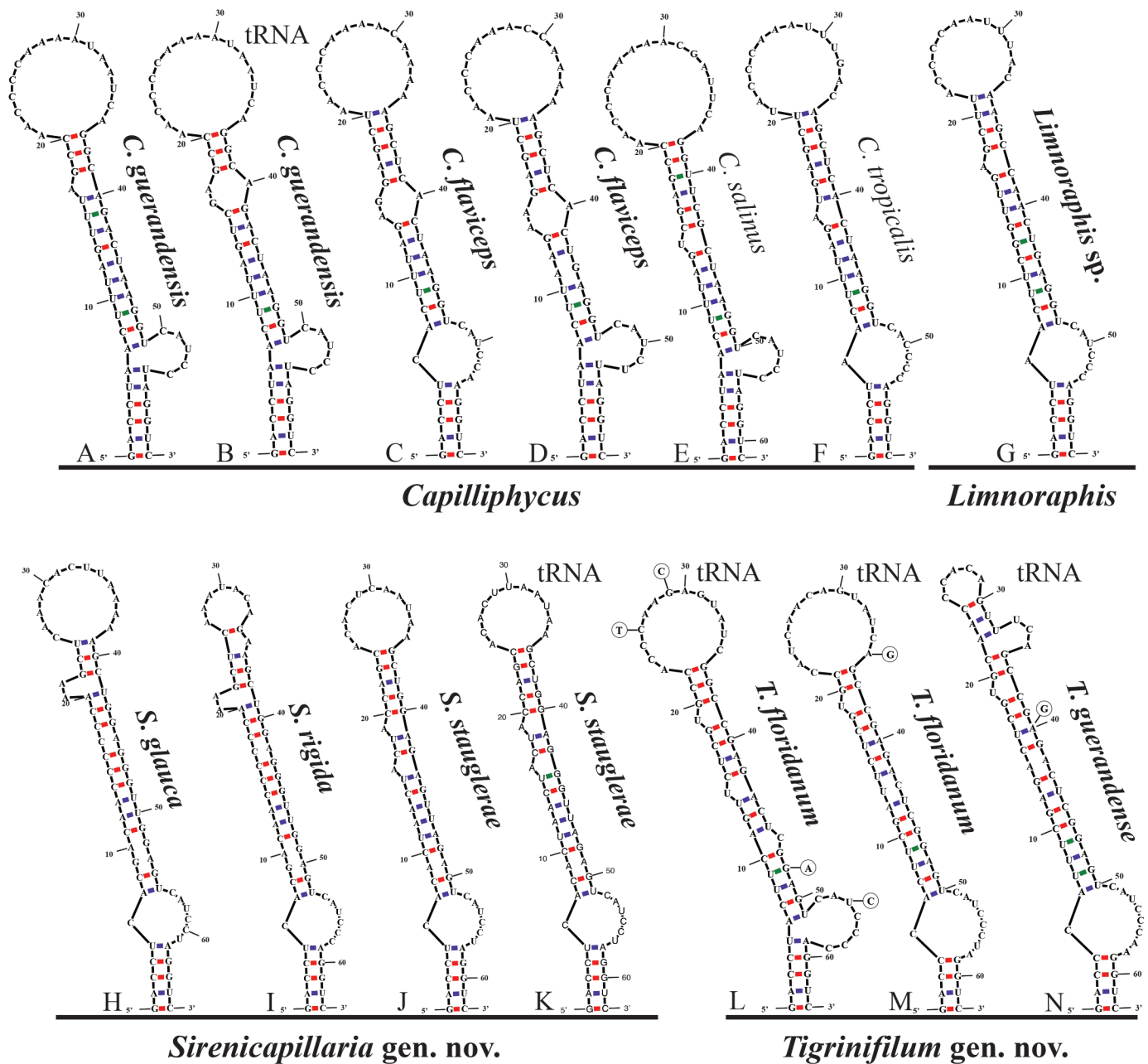
Fig. 50. The 16S–23S rRNA ITS sequence secondary structures of the D1-D1′ of four cyanobacterial genera (*Capilliphycus, Limnoraphis, Sirenicapillaria, Tigrinifilum*) presented in this work. Nonbolded taxa represent previously published data. A: *C. guerandensis* BLCC-M76 & BLCC-M92. B: *C. guerandensis* BLCC-M79 & BLCC-M92 with tRNA. C: *C. flaviceps* BLCC-M53. D: *C. flaviceps* BLCC-M137 with and without tRNA. E: *C. salinus*. F: *C. tropicalis*. G: *Limnoraphis* sp. BLCC-F19 & BLCC-F23 with and without tRNA. H: *S. glauca* BLCC-M125. I: *S. rigida* BLCC-M116 & BLCC-M134 with and without tRNA. J: *S. stauglerae* BLCC-M121, BLCC-M122, BLCC-M123, & BLCC-M138. K: *S. stauglerae* BLCC-M121 with tRNA. L: *T. floridanum* BLCC-M48, BLCC-M50 clone A, BLCC-M57 clone C, BLCC-M66 clone B, BLCC-M77 clone C, BLCC-M87 clone C, BLCC-M107, BLCC-M118 clone B, BLCC-M119 clone B, & BLCC-M120 clones A & B, with tRNA. M: *T floridanum* BLCC-M50, BLCC-M57 clones A & B, BLCC-M66 clones A & C, BLCC-M73, BLCC-M87 clones A & B, BLCC-M118 clones A & C, BLCC-M119 clone C, & BLCC-M120 clone C, with tRNA. N: *T. guerandensis* BLCC-M99, with tRNA.

Tigrinifilum is especially desiccation tolerant; mats of *T. floridanum* withstand drying for extensive periods (up to two months in laboratory cultures) and *T. guerandense* was also isolated from completely dried cyanobacterial mat material (Fig. S2). *Tigrinifilum floridanum* is a species that varies widely in pigmentation (Figs 27–29) and trichome width (8–19 μm; Figs 4, 32–34), and is also widespread across the southeastern Florida coast, from Hollywood down to the Florida Keys.

Two species of *Capilliphycus* were previously known, including the type, *C. salinus*, and *C. tropicalis*. The novel species *C. guerandensis* from French salt flats differs by being wider than the other described species in the genus. *Capilliphycus flaviceps* overlaps in filament width with previously described *Capilliphycus* species, though *C. flaviceps* has a yellowing pigmentation at the apices and an extension of apical sheaths (Fig. 40). This yellowing feature can also be seen in *C. guerandensis*, although confined to the apical cells
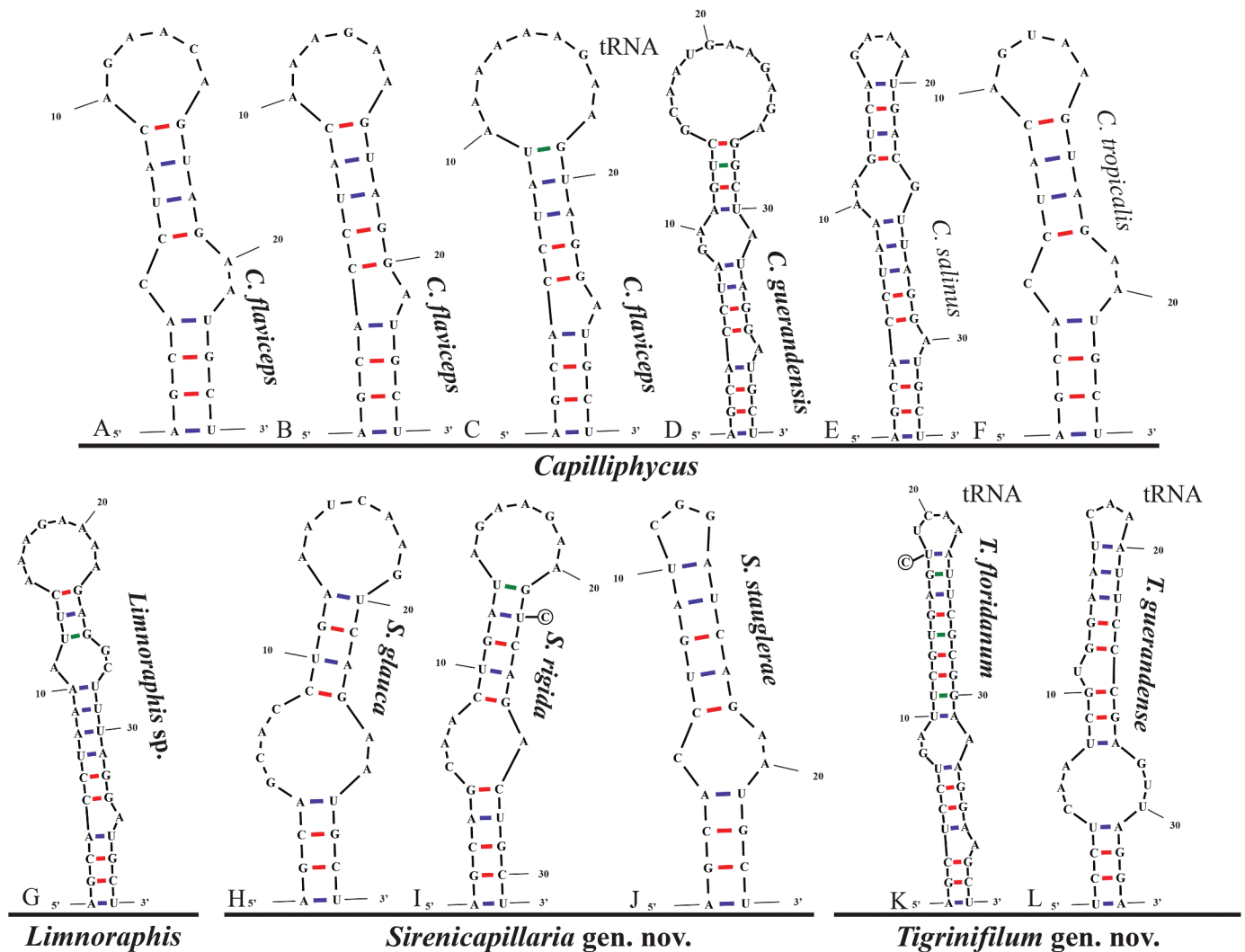
Fig. 51. The 16S–23S rRNA ITS sequence secondary structures of the Box-B of the four cyanobacterial genera (*Capilliphycus, Limnoraphis, Sirenicapillaria, Tigrinifilum*) presented in this work. Nonbolded taxa represent previously published data. A: *C. flaviceps* BLCC-M53. B: *C. flaviceps* BLCC-M137. C: *C. flaviceps* BLCC-M137 with tRNA. D: *C. guerandensis* BLCC-M76 & BLCC-M92 with and without tRNA. E: *C. salinus*. F: *C. tropicalis*. G: *Limnoraphis* sp. BLCC-F19 & BLCC-F23 with and without tRNA. H: *S. glauca* BLCC-M125. I: *S. rigida* BLCC-M116 & BLCC-M134 with and without tRNA. J: *S. stauglerae* BLCC-M121, BLCC-M122, BLCC-M123, & BLCC-M138 with and without tRNA. K: *T. floridanum* BLCC-M48, BLCC-M50, BLCC-M57, BLCC-M66, BLCC-M73, BLCC-M77, BLCC-M87, BLCC-M107, BLCC-M118, BLCC-M119, & BLCC-M120, with tRNA. L: *T. guerandensis* BLCC-M99, with tRNA.

(Fig. 45), and not extending into a region as in *C. flaviceps* (Fig. 39). A feature shared between species in *Capilliphycus* is the spiralling or contortion of trichomes or the rearrangement of cells within a filament, as seen in both *C. tropicalis* (Caires *et al.* 2018b, p. 297, fig. 3E) and *C. flaviceps* (Fig. 44).

In agreement with previous observations, *Capilliphycus* contains cyanobacteria with tolerance for high salt concentrations. Caires *et al.* (2018b) indicated that *C. salinus* was isolated from tidal pools of 50 psu. Comparatively, *C. guerandensis* was isolated from hypersaline ponds and tidal flats (>60 psu) and grew in media with a salinity of up to 100 psu. Herein we also present a strain of *C. tropicalis* (BLCC-M106) with a full 16S–23S rRNA ITS region isolated from Florida marine waters, extending the known range of this species from Brazil to Florida. The occurrence of *Capilliphycus* in both countries is another indication of the similarities between (sub)tropical Florida marine coastal cyanobacterial diversity and that of tropical northern Brazil (Caires & Affe

2021; Lefler *et al.* 2021). In our study, the *Capilliphycus* species that were isolated from Florida were components of benthic proliferations mainly formed by other larger homocytous filamentous cyanobacteria, such as *Sirenicapillaria* and *Okeania*.

In addition to the novel genera and species presented herein, we also recognize a new family, Sirenicapillariaceae. The new family is supported by its monophyly (Fig. 49) as well as genetic *p*-distance similarities using the 16S rRNA gene sequences of taxa from established families including Microcoleaceae, Coleofasciculaceae *sensu stricto*, Vermifilaceae and Oscillatoriaceae *sensu stricto* (Table 2). Sirenicapillariaceae is currently composed of seven genera of marine and freshwater cyanobacteria: *Affixifilum, Capilliphycus, Limnoraphis, Limnospira, Neolyngbya, Sirenicapillaria* and *Tigrinifilum* (Fig. 49; Table 3).

CONCLUSION

Cyanobacterial mats from the coast of Florida were sampled and isolated along with cyanobacteria from a French salt flat. A total of 30 cyanobacterial isolates are presented herein, of which 21 represent two novel genera isolated from Florida coasts and seagrasses: *Sirenicapillaria* and *Tigrinifilum*. Three isolates from the French salt flat led to the erection of two novel species: *Capilliphycus guerandensis* and *Tigrinifilum guerandense*. Additionally, three freshwater isolates of *Limnoraphis* were included for a more robust taxon sampling of the novel family Sirenicapillariaceae.

To describe the novel cyanobacterial diversity presented in this work, a combination of techniques was applied including morphology, 16S rRNA gene phylogeny and genetic similarities, along with 16S–23S rRNA ITS secondary structure and genetic *p*-distances. The polyphasic approach demonstrated congruency among analyses and strong support for the delimitation of the two novel genera *Sirenicapillaria* and *Tigrinifilum*, and the new species of *Capilliphycus*. Family level phylogenetic analysis of the 16S rRNA gene sequence supported the erection of Sirenicapillariaceae which includes *Capilliphycus, Limnoraphis, Limnospira, Neolyngbya, Sirenicapillaria* and *Tigrinifilum*.

A most crucial taxonomic establishment within this research was the identification of the frequent proliferating benthic cyanobacterial genus *Sirenicapillaria*, which has been commonly misidentified as the freshwater *Microseira* ('*Lyngbya*') *wollei* or the marine *L. majuscula*, or species of *Dapis* or *Okeania*. *Sirenicapillaria* proliferates across the western Florida Coast and is also found in floating mats in the Florida Keys. Large swaths of seagrasses succumb annually to blooms of these cyanobacteria, especially in spring and summer, imposing large constraints on local economies and negative impacts on marine biota. Recognizing *Sirenicapillaria*, a large player in benthic cyanobacterial bloom events, is a fundamental step in deciphering the diversity and putative emerging threats within marine environments.

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