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The novel marine cyanobacterium *Nunduva sanctimaloensis* sp. nov. (Nostocales, Cyanobacteria) from rocky shores and its reproduction through modified monocytes

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Abstract: Rocky intertidal regions are diverse as they provide many unique habitats for the growth of cyanobacteria. From outcrops battered by waves, tidal pools, and periods of desiccations due to tides, these regions offer distinct environments and much of their cyanobacterial diversity remains unexplored. To help elucidate cyanobacteria from this habitat, one nostocalean cyanobacterium from Brittany, France was isolated and cultured. Typical morphological features including filaments with heteropolar trichomes, basal or intercalary heterocytes, false branching, and long hyaline apical hairs suggested the genus *Nunduva*. The combination of 16S rRNA gene and 16S–23S rRNA internal transcribed spacer (ITS), along with diagnosable morphological characteristics supports a distinct taxon from previously described species. Herein, the novel false branching cyanobacterium *Nunduva sanctimaloensis* sp. nov. is described along with its reproduction through modified monocytes.

Key Words: 16S rRNA, 16S–23S rRNA ITS, Branching, Caespitose, Life cycle, Rivulariaceae

Introduction

Rocky shores of marine intertidal zones of temperate and tropical regions are potential areas of high cyanobacterial diversity (Bano & Siddiqui 2003). These zones are often inhabited by cyanobacteria, especially heterocytous and/or branched morphotypes (González–Resendiz et al. 2015). Though many of these marine environments have been extensively studied regarding their macroalgal (i.e., seaweed) diversity, much of the cyanobacterial diversity remains unexplored.

A cyanobacterial family with a high level of diversity with especially cryptic genera is the Rivulariaceae, composed of cyanobacteria with heteropolar filaments and tapering hyaline apical hair (León–Tejera et al. 2016; González–Resendiz et al. 2018b). Though substantial work has been carried out to elucidate novel taxa from cryptic species, much cyanobacterial diversity remains unexplored (Nabout et al. 2013; Hinchliff et al. 2015). Marine species of cyanobacteria with heteropolar, attenuated trichomes, and geminate false branching have often been lumped together erroneously with morphologically similar freshwater or terrestrial taxa including *Brasilonema*, *Calothrix* and *Rivularia* (Sihvonen et al. 2007). The genus *Nunduva* was recently delimited from freshwater *Rivularia* types based on trichome geminate and single false branching and their marine ecology (González–Resendiz et al. 2018a), as well as distant phylogenetic placement from taxa of the family Scytonemataceae, such as the freshwater *Calothrix*.

Nunduva is composed of five tropical species isolated from the marine waters of Mexico including the type species: *N. biania* González–Resendiz, León–Tejera et J.R.Johansen, *N. fasciculata* González–Resendiz, León–Tejera et J.R.Johansen, *N. kania* González–Resendiz, León–Tejera et J.R.Johansen, *N. komarkovae* González–Resendiz, León–Tejera et J.R.Johansen, *N. sanagustinensis* González–Resendiz, León–Tejera et J.R.Johansen, while a sixth species, *N. britannica* Hauer, was isolated from an estuary in England (Miranda in Guiry & Guiry 2021; Johansen et al. 2021). A seventh potential species of *Nunduva* from the coast of Portugal (*Nunduva* [="*Rivularia*"] LEGE 07159) has been suggested but lacks proper morphological evaluation and

16S–23S rRNA internal transcribed spacer (ITS) sequence to assist in species delimitation (Vázquez–Martínez et al. 2018).

In 2018, a cyanobacterium was collected and isolated from the rocky marine shores of Brittany, France. Based on ecological and morphological similarities, the isolate resembled a nostocalean cyanobacterium, with heteropolar trichomes, basal heterocytes and false branching, reminiscent of *Nunduva* (González–Resendiz et al. 2018a) but dissimilar to described species. The aim of this study was to characterize the isolate by applying the polyphasic approach and combining ecological, morphological, 16S rRNA phylogeny, and 16S–23S ITS rRNA gene sequence phylogeny and secondary structure. Here we present a new species of heterocytous marine cyanobacteria, *Nunduva sanctimaloensis* sp. nov., and a type of reproduction not yet described in the Nostocales.

Materials and Methods

Sampling, isolation, and culturing. Attached epilithic samples were collected from rock outcrops across the coast at Grande Plage du Sillon, Saint–Malo, France (48°39'11.880"N 2°1'28.890"W). The cyanobacterium was first separated using micromanipulation of filaments under a Stereomicroscope (Amscope) on solid SW–BG11 (35 g.l^{-1} Instant Ocean). The isolate was successfully 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 (50 µE.s^{-1}.cm^{-2}) with shaking at 120rpm.

Live cultures are maintained in the BLCC (BLCC–M94) at the Fort Lauderdale Research and Education Center, University of Florida – Institute of Food and Agricultural Sciences in Davie, Florida (University of Florida/IFAS, FLREC, Davie, FL, USA) and at the BCCM/ULC (ULC648) of the University of Liège (Liège, Belgium). Herbarium voucher specimens are deposited in the US National Herbarium of the National Museum of Natural History, Smithsonian Institution (NMNH, Washington, DC, USA) under the accession number US 227670.

Morphological, molecular, and phylogenetic analyses. Microphotographs were captured using a DIC microscope (Leica DM5500 B with LAS V4.12; Leica Microsystems, Switzerland) and an epifluorescent microscope (Amscope, XYL–606).

For molecular analyses, DNA was extracted from 100 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_2O to remove salts, sand, polysaccharides, and 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 23S30R for the 16S rRNA and 16S–23S rRNA ITS region (Wilmotte et al. 1993; Nübel et al. 1997), using protocols outlined by Mühlsteinová et al. (2018).

The PCR product was cleaned using a QIAquick PCR Purification Kit and subsequently verified on a 0.8% agarose gel. Amplified products of the 16S rRNA gene and 16S–23S rRNA ITS region were cloned using TOP10 chemically competent cells (TOPO–TA, Invitrogen) prior to sequencing, following manufacturer protocols. Cloned plasmids of several colonies (n=5) 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). All sequenced clones possessed the same sequence for the 16S rRNA gene and 16S–23S rRNA ITS region. The sequence for the 16S rRNA and 16S–23S rRNA ITS is deposited in GenBank (National Center for Biotechnology Information, NCBI) under the accession number MW981328.

BLAST (Basic Local Alignment Search Tool, NCBI) was used to identify 16S rRNA gene sequences similar to our isolate. A total of 73 sequences were aligned with the isolate using MUSCLE (SeaView v4.4) (Edgar 2004). The alignment of the 16S rRNA gene included 1063 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. 2021). 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 K2+G+I model with 1,000 bootstrap resampling replicates. The BI analysis was conducted using MrBayes 3.6 (Ronquist & Huelsenbeck 2003) with 1.5×10^6 generations, a 0.25 burn–in rate, and resampling every 100 generations.

Results and Discussion

Molecular and phylogenetic 16S rRNA gene analyses
The Bayesian inferential phylogenetic tree of the 16S rRNA gene sequence showed high support (BS: 62%; PB: 0.98) for the genus *Nunduva* as previously reported in González–Resendiz et al. (2018a) and Johansen et al. (2021) (Fig. 1). There was also high support for the delimitation of the species *Nunduva sanctimaloensis* (BS: 76%; PB: 1.0) along with a previously analyzed strain cf. *Nunduva* LEGE 07159, sister to *N. britannica*. González–Resendiz et al. (2018a) suggested that the strain LEGE 07159 was a possible new species of *Nunduva* which our data support. The strain *Nunduva* LEGE 07159 was isolated from the intertidal zone of the coast of Portugal and *N. sanctimaloensis* was isolated from that of France, with a matching temperate environment, differing from the tropical species of the genus.

16S–23S rRNA internal transcribed spacer (ITS) secondary structure and p–distance analyses
The 16S–23S rRNA ITS sequence showed dissimilar lengths between each species (Table 1). The Box B helix varied between each species, with *N. sanctimaloensis* having the shortest helix, 32 nucleotides, with a large bilateral bulge after the clamp (Fig. 2). The D1–D1' helix of *N. sanctimaloensis* is comparable to that of *N. britannica* in shape and length, 94 nucleotides, but differs from the other species (Fig. 2). While the length and shape of the D1–D1' helix is similar between *N. sanctimaloensis* and

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N. britannica, there are various nucleotides that differ between them throughout the helices.

Besides the secondary structure, the percent dissimilarity of the aligned orthologous 16S–23S rRNA ITS gene sequence is another practical criterion used to separate cyanobacterial species (Pietrasiak et al. 2014; Vázquez–Martínez et al. 2018) (Table 2). The percent dissimilarity among *Nunduva* species (*N. biana*, *N. britannica, N. fasciculata*, *N. kania*) and *N. sanctimaloensis* was between 9.5–18.2%, higher than the suggested percent dissimilarity for species cutoff (3%) (González–Resendiz et al. 2019), and strongly supports the novel species. With the additional dissimilarity in secondary structure for both D1–D1' and Box–B helices and phylogenetic and morphological differences, there is strong evidence for the erection of *Nunduva sanctimaloensis*.

Species description and morphological analyses

Nunduva sanctimaloensis revealed similar morphological traits shared by other species of the genus, including mucilaginous caespitose colonies on rocks in marine environments (Fig. 3b), constituted of heteropolar filaments, trichomes with single/double false branching that sometimes form characteristic apical hyaline hairs (Fig. 3c–f), solitary or serial heterocytes (basal or intercalary), and trichome fragmentation with or without necridia, or by heterocyte formation (Fig. 4). There are also morphological variations among *Nunduva* species, mainly the cell dimensions; *N. sanctimaloensis* has the largest vegetative cell width amongst the species, followed by *N. sanagustinensis*, *N. fasciculata*, *N. kania*, *N. britannica*, and lastly *N. biania* and *N. komarkovae*. Though *N. sanctimaloensis* has the largest cell width, *N. komarkovae*, *N. fasciculata* and *N. sanagustinensis* have the longest cells, leaving *N. biania* with the shortest cells. *Nunduva sanctimaloensis, N. britannica*, and one undescribed strain (*Nunduva* LEGE 07159) share a temperate locality while the remaing are tropical species from Mexico (Table 3); Although the two described temperate species share geographic localities, they differ morpholigcally where *N. sanctimaloensis* filaments usually attenuate into a thin long hair while that of *N. britannica* does not contain such hairs. Furthermore, both species differ in apical cells where *N. sanctimaloensis* has more triangular and rounded apical cells than that of *N. britannica* which are rounded.

Species description

Subclass: Nostocophycidae
Order: Nostocales
Family: Rivulariaceae
***Nunduva sanctimaloensis* D.E. Berthold, Werner et Laughinghouse sp. nov.**
(Figs 3–5, Suppl. Fig. 1–2)

Description: Epilithic or free–floating in spherical clusters. Thallus dark green to blue green, caespitose in the environment, fasciculate or not in culture. Filaments heteropolar, straight or flexuous, ± 2 mm long, 16.4–21.1 (19 ± 1.5) µm wide, with single or double false branching. Sheath hyaline, colorless, sometimes thick, lamellate and constricted, and sometimes expanded towards filament ends. Trichomes cylindrical, usually slightly to deeply constricted at cross–walls, 10.5–17.7 (14.5 ± 2.7) µm wide, very tapered only at the ends, usually ending in apical hyaline hairs. Cells in young developing trichomes (7.4–13.4 µm wide) are longer than wide, while older trichomes (10.5–17.7 µm wide) have discoid to flattened oval cell, always shorter than wide, 1.7–3.7 (2.4 ± 0.5) µm long; apical cells spherical, rounded to triangular, at times elongated to include several cells (±6) as seen throughout reproduction cycle. Cell content blue–green to forest green, finely granulated, cross–walls sometimes granulated. Heterocyte discoid, quadratic, cylindrical, basal or intercalary, in single (usually basal or intercalary) or serially intercalary in double, or triple, 8.8–17.3 µm length, 11–13 µm wide. Reproduction by monocytes released through vacuolized apical cells individually released from sheath or by isopolar or heteropolar hormogonia. Reproduction also facilitated by trichome fragmentation, with or without necridic cells, cell constriction or heterocyte breakup. Akinetes not observed.

Holotype: US 227670 (US National Herbarium, Smithsonian Institution, Washington, DC, USA) – herbarium preparation [dried and preserved] of reference strain BLCC–M94. Collected by David E. Berthold on 25–August–2018.
Type locality: France. Brittany: Saint–Malo, marine rocky beach in Plage du Sillon. (48°39'11.880"N 2°1'28.890"W).
Habit and habitat: Benthic and epilithic in marine intertidal.
Etymology: "*sanctimaloensis*" is to honor the French port, Saint–Malo, Ille–et–Vilaine, Brittany where the cyanobacterium was sampled.
Reference strain: BLCC–M94 (University of Florida/ IFAS, FLREC, Davie, FL, USA); ULC648 (University of Liège, Liège, Belgium).
GenBank Number: MW981328.

Unlike the tropical *Nunduva* species like *N. komarkovae*, *N. sanagustinensis*, *N. biania*, and *N. kania*, *N. sanctimaloensis* is morphologically, phylogenetically, and geographically most closely related to *N. britannica*. Although *N. sanctimaloensis* and *N. britannica* are the most closely related species, the strong phylogenetic support (Fig. 1) and the disparity in morphology (Table 3) supports the erection of two separate temperate *Nunduva* species.

Nunduva sanctimaloensis sometimes displays a lamellate sheath (Fig. 3c) similar to that of *N. biania*, and frayed sheath (Fig. 4e) similar to *N. britannica* (González–Resendiz et al. 2018a). One morphological aspect of *N. sanctimaloensis* that is not reported in other *Nunduva* species and a potential diacritical marker of the species, is the constriction of the sheath covering basal

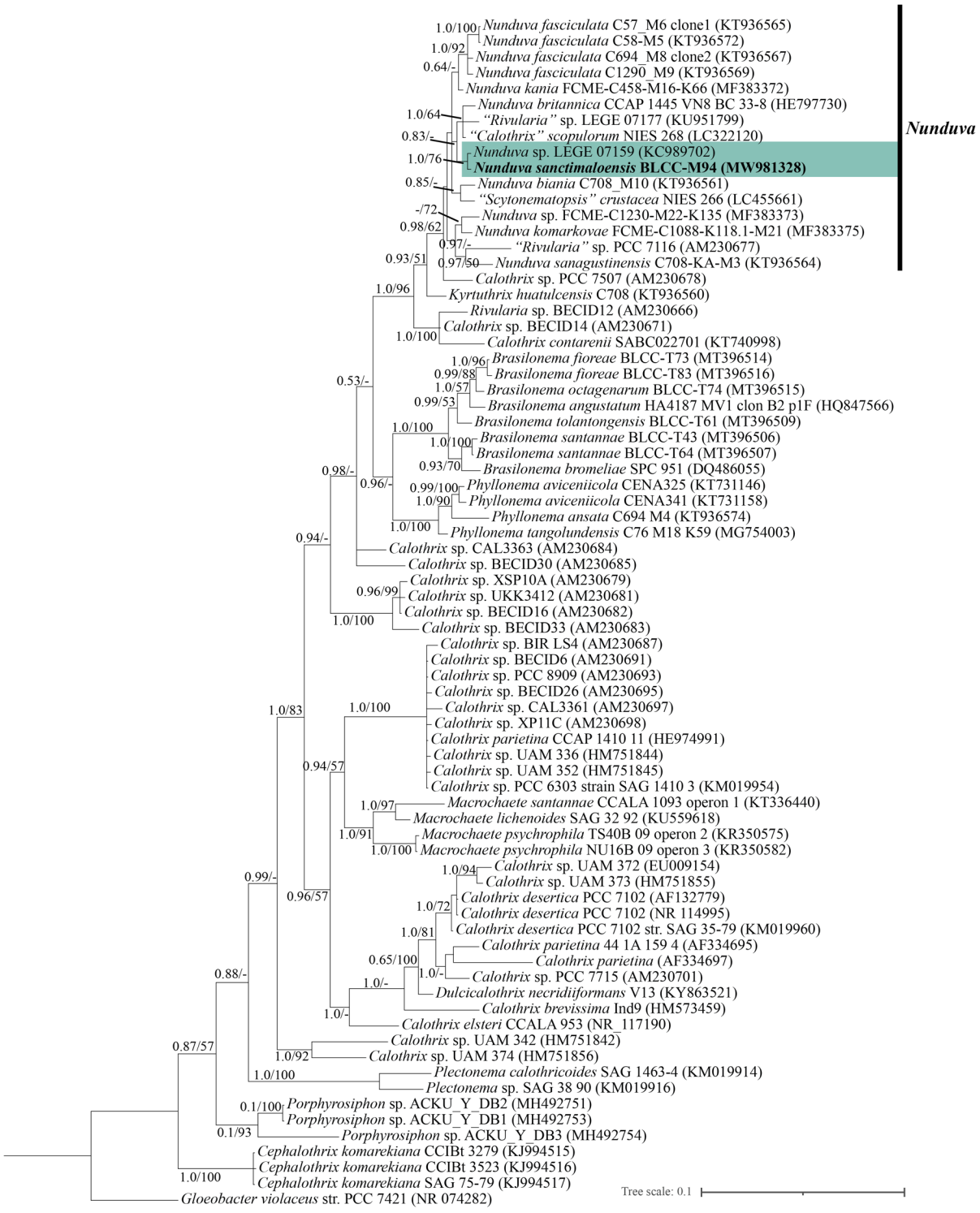

Fig. 1. Bayesian topology showing both Bayesian and Maximum likelihood values of the phylogenetic relationship of the 16S rRNA gene sequence *Nunduva sanctimaloensis* and 73 cyanobacterial strains using *Gloeobacter violaceus* PCC 7421 (NR074282) as an outgroup. Bootstrap support and posterior probabilities at and above 50 and 0.5 are shown at consensus nodes of both inferential analyses, respectively.

heterocytes, mature filaments, or developing hormogonia (Fig. 4 d, f, g; respectively). Similar to *N. biania* and *N. britannica*, *N. sanctimaloensis* has intercalary and basal heterocytes, but unlike the described species, *N. sanctimaloensis* demonstrates single and double serial heterocytes in both basal and intercalary positions (Suppl. Fig 1).

A most notable feature in *N. sanctimaloensis* is reproduction through budding of apical cells through the open sheath, or monocytes (such as those of *Stauromatonema nigrum* Frémy; Geitler 1942), a form of reproduction analogous to exocytes of chroococcalean cyanobacteria, such as *Chamaesiphon* or *Clastidium* (Komárek & Anagnostidis 1998). Reproduction by monocytes occurs

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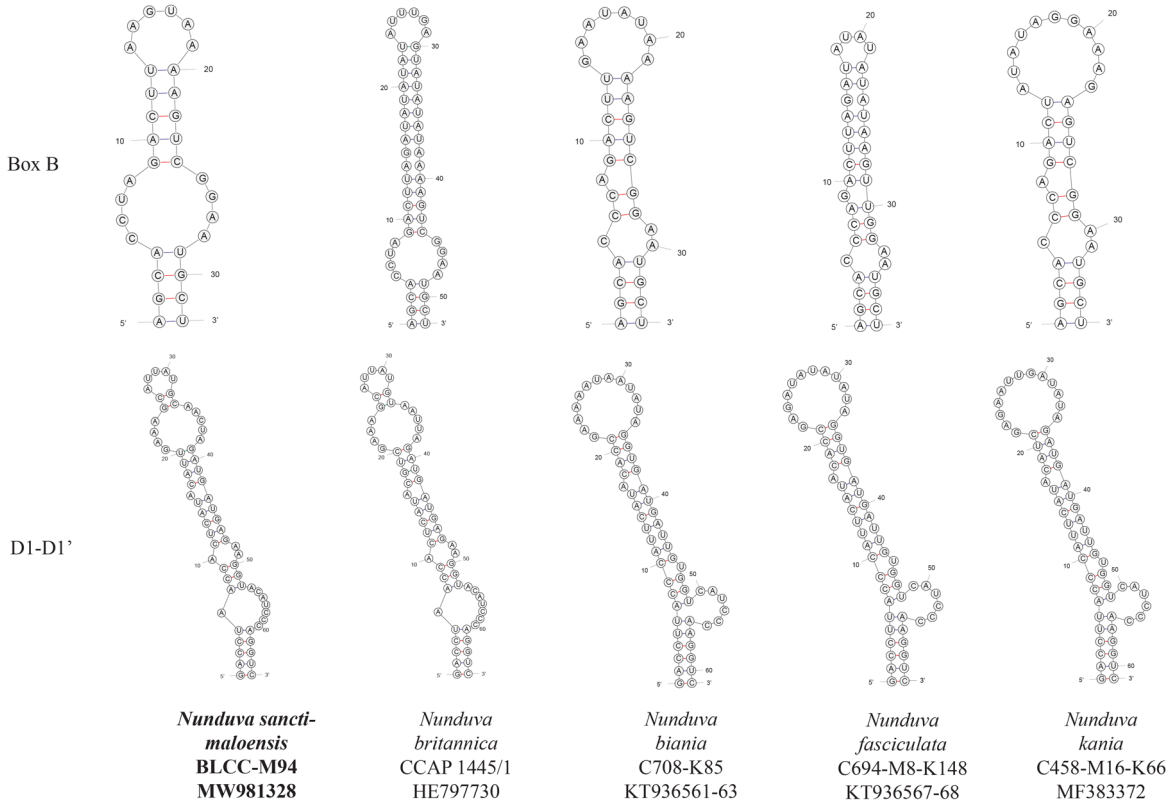

Fig. 2. 16S-23S rRNA internal transcribed spacer (ITS) secondary structure showing the Box B and D1-D1' regions of the species of the genus *Nunduva*.

through the binary fission of apical sessile cells where the apex region of the cell separates from the attached cell. Monocytes differ from described reproductive features including hormogonia and hormocytes (single–celled hormogonia), based on their method of production and/or release. While monocytes are produced and released from attached sessile apical cells through an attenuated sheath, hormocytes, such as the ones described from *Mojavia pulchra* Reháková et Johansen and *Rexia erecta* Casamatta, Gomez et J.R.Johansen, are released after necridia or filament disintegration through an opened sheath (see Casamatta et al. 2006, pg 18 Fig. 2 b–d) (Řeháková et al. 2007; Casamatta et al. 2006).

The production of monocytes by *N. sanctimaloensis* occurs only in one division such as seen in *Geitleribactron*, perpendicular to the cell axis, whereas exocytes may divide again in other genera (e.g., *Chroococcus*), or occur by multiple fission in the case of nanocyte and baeocyte production (as seen in *Stanieria*) (Komárek & Anagnostidis 1998). After separation, monocytes may remain attached to the trichome, open sheath, other filaments, or attach to the substrate, from where polarized growth of a filament follows (Komárek & Anagnostidis 1998).

Cell budding or monocyte formation in *N. sanctimaloensis* begins with filaments at the apices, affecting the first 15 cells (Fig. 5a). The apical cells first elongate through binary fission and simultaneously become vacuolized where the cytoplasm is reduced leaving only the cell wall (Suppl. Fig. 2). As the apical cells continuously vacuolize inside the sheath, the sheath and cells at the apices elongate into a long, thin hyaline hair (Fig. 3f). The vacuolized apical cells, or modified monocytes, eventually rupture through the filament apices and open sheath and are released (Fig. 5 b–c). These modified and released monocytes then begin to expand in dimension (attached or not), and attach to other filaments or materials, in pieces or as a whole. Upon attachment (or not) the monocytes develop a cytoplasm and sheath (Fig. 5d). Monocytes may not rupture through the apices but remain trapped within the sheath or form from necridic regions and shift perpendicular to the trichome (Fig. 5b).

From unattached or attached developed monocytes, further division and elongation results into what falsely appears to be released mature hormogonia (Fig. 5 f–e) or false branching from necridia, respectively (Fig, 5g; arrow in Suppl Fig. 2o). In addition to the false branching, this cyanobacterium may also produce what appears as false branching from developed and attached monocytes which could be termed "fake branching", not to be confused with either false branching, where adjacent cells (or through heterocytes or necridia) within a trichome are diverted outside a filament, or pseudobranching where individual cells within a sheath change direction, found in Nostocales and Chroococcales, respectively (Suppl. Fig. 2 n–o).

The apical bead–like cell of *N. sanctimaloensis* (Fig. 3c) is similar to that of *N. fasciculata* (González–Resendiz

Table 1. Lengths of identifiable domains within the 16S-23S rRNA Internal Transcribed Spacer (ITS) regions of *Nunduva* species. Not determined (n.d.) due to missing available genetic data.

Species	GenBank Accession	Leader	D1-D1' helix	Spacer + D2 + Spacer	D3 + Spacer	tRNA^{Ile}	Spacer+ V2+ Spacer	tRNA^{Ala}	Spacer	Box-B Helix	Spacer	BoxA	D4 + Spacer	V3 helix	To ITS end
N. sanctimaloensis BLCC–M94	MW981328	8	64	90	20	74	39	73	54	32	17	11	26	39	47
N. biania C708_K85	KT936563	8	61	91	20	74	68	73	28	34	17	11	26	39	47
N. britannica CCAP1445	HE797730	8	64	94	20	74	78	73	59	52	17	11	26	53	45
N. fasciculata C694_M8	KT936568	8	60	48	24	74	87	73	41	38	17	11	27	59	46
N. kania	MF383372	8	61	46	29	74	90	73	25	35	17	11	26	39	52
N. sanagustinensis C708 M3	KT936564	8	64	42	52	74	88	73	49	31	17	11	27	39	34
N. komarkovae C1088	MF383375	8	62	41	59	74	87	73	47	36	18	11	28	39	51
N. komarkovae C923	MF383374	8	62	41	59	74	87	73	54	36	18	11	28	39	51

et al. 2018a, pg 90 fig. D–E). The similarity in apical cell in both *Nunduva* species suggests that *N. fasciculata* may also reproduce using monocytes. The presence of an attached cell covered with a sheath (González–Resendiz et al. 2018a, p. 90, fig. F) may also indicate the type *N. fasciculata* reproduces using monocytes.

Reproduction through monocytes by *N. sanctimaloensis* is a reproductive method usually ascribed to only few Nostocales or the cyanobacterial order Chroococcales. Finding this reproduction mode in *Nunduva sanctimaloensis* suggests that this form of reproduction is more widespread than previously thought and the extent of this form of reproduction across other *Nunduva* species or other closely related Rivulariaceae taxa remains untested. Similarly, this form of reproduction could have undergone several loss and gain events over the evolution of cyanobacteria. Discovering this previously unkwnown mode of reproduction within *Nunduva* cyanobacteria can aid in higher taxonomical classification, especially at the family or order level.

Classical morphological scrutiny is important for cryptic taxa, as morphological traits can facilitate phylogenetic and evolutionary studies. This is especially important when analyzing life cycles and reproduction. This can help our understanding of how these organisms spread in the environment (Cottingham et al. 2021), though, only few recent studies describe reproduction and spread (e.g., Mateo et al. 2011; McGregor & Sendall 2017; Berthold et al. 2021). Observing cyanobacterial development over time is a necessary aspect in describing these organisms. Preservation and culturing of strains are also important aspects of studying cyanobacteria and highlight the importance of algal culture collections (Ramos et al. 2018).

Conclusion

Morphological features often overlap between phylogenetically disparate generic entities (Lahr et al. 2014) especially notable within the Rivulariceae. The combination of taxonomic tools including morphology, ecology, molecular phylogeny, and ultrastructural approaches has proven indispensable in cyanobacterial taxonomy, leading to the description of many new genera, and deciphering many cryptic taxa (e.g., León–Tejera et al. 2016; McGregor & Sendall 2017; González–Resendiz et al. 2018a,b; Lefler et al. 2021; Berthold et al. 2021). *Nunduva sanctimaloensis* is a novel cyanobacterium isolated from the marine rocky shores of France. Together with the 16S rRNA gene sequence phylogeny and the 16S–23S rRNA ITS secondary structures and pairwise distance results, *N. sanctimaloensis* is highly supported as a novel species. Along with molecular genetics, morphological disparities among the *Nunduva* species also suggest *N. sanctimaloensis* is a new species to science.

Table 2. Percent dissimilarity (%) of 16–23S rRNA ITS sequence among species of the genus *Nunduva*.

	***Nunduva* species/strains**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	*N. sanctimaloensis* BLCC–M94							
2	*N. britannica* CCAP1445	9.45						
3	*N. sanagustinensis* C708 M3	10.98	13.7					
4	*N. fasciculata* C694 M8	13.93	14.55	18.25				
5	*N. kania*	12.11	16.02	15.05	9.76			
6	*N. biania* C708 K85	10.17	13.95	13.05	12.86	12.71		
7	*N. komarkovae* FCME–C1088-M21–K118	15.01	18.07	16.3	15.29	12.08	13.22	
8	*N. komarkovae* FCME–C923–M12	16.11	18.2	16.64	14.62	11.86	13.2	2.69

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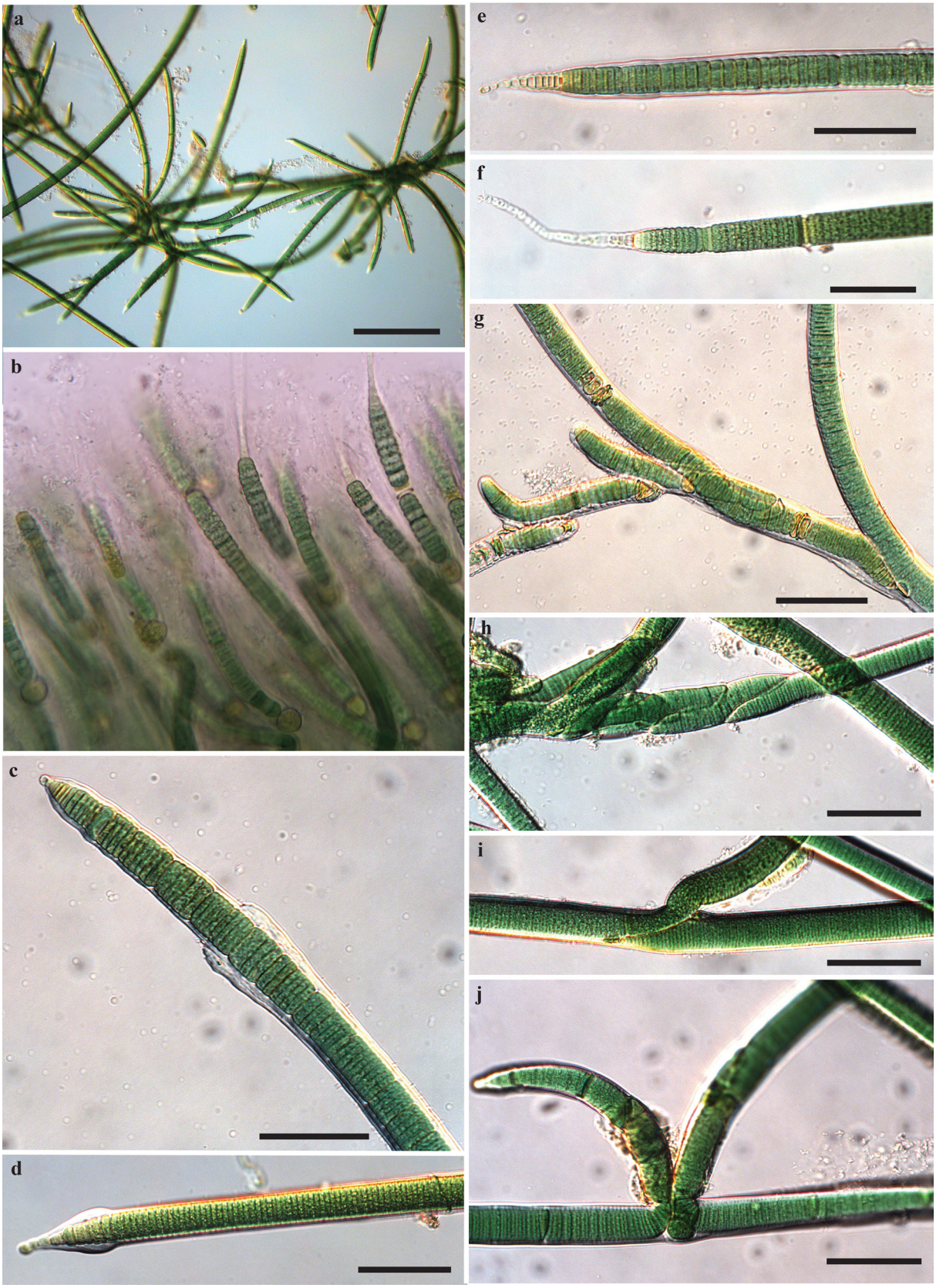

Fig. 3. Light microscope images of *Nunduva sanctimaloensis* sp. nov. (reference strain BLCC-M94) showing morphological characteristics: (A) clustering thalli from cultured material; (B) caespitose thalli from environmental material; (C) bead-like appearance of apical cell; (C-F) different phases of apical cell development that result in hyaline apical hair; (G-J) filaments with false branching – from heterocytes (G), filament diagonal fragmentation (H), single false branching (I), double false branching (J). Scale bars 100 µm (A), 50 µm (C-J).

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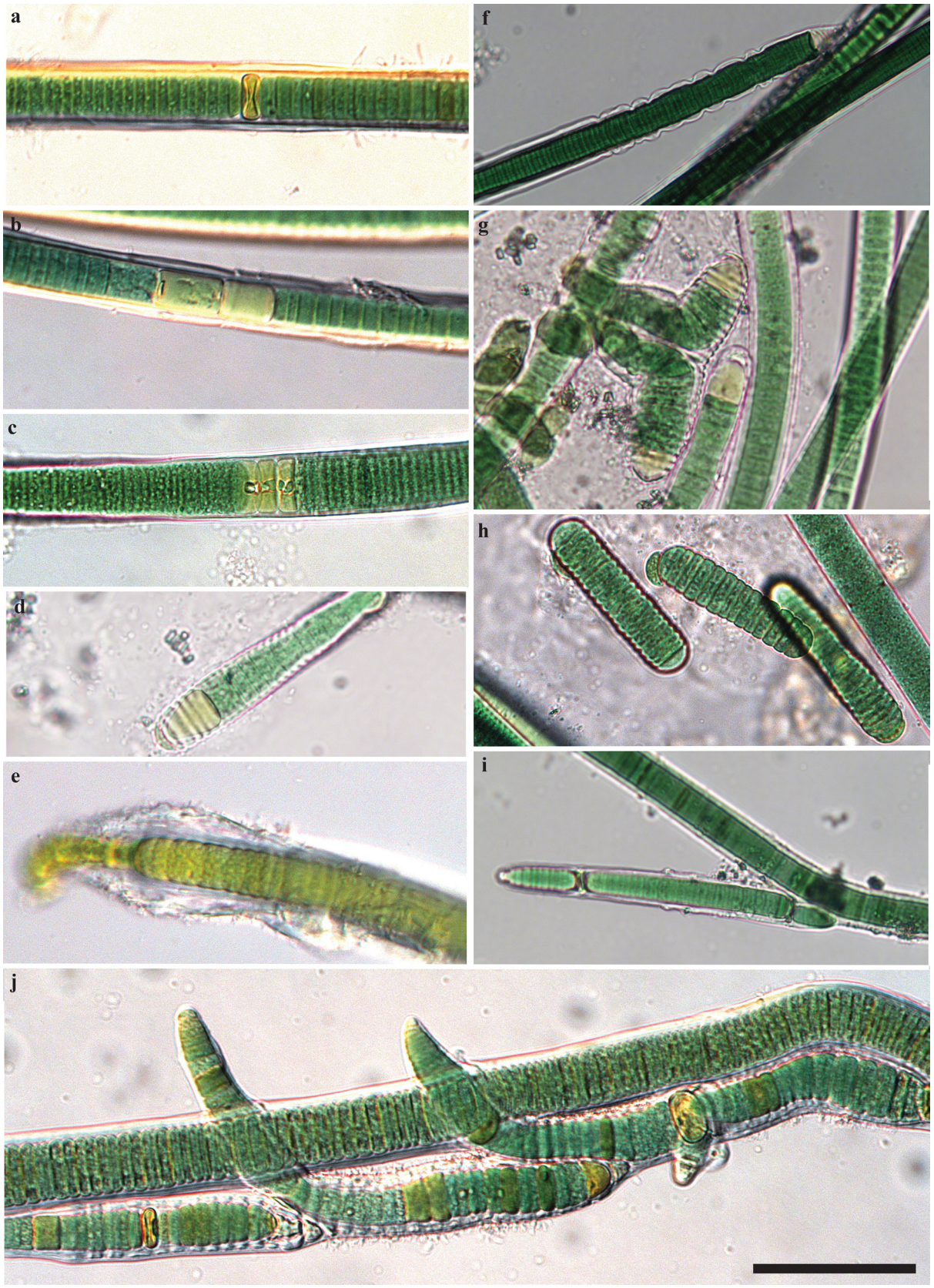

Fig. 4. Light microscope images of *Nunduva sanctimaloensis* sp. nov. (reference strain BLCC-M94) showing morphological characteristics: (A-D) filaments demonstrating morphological variations including (A) single oval, (B) double cylindrical serial, (C) triple serial heterocytes, and (D) conical heterocyte; (E) frayed apical sheath; (F-G) filaments with thick, lamellate and constricted sheath; (H-I) hormogonia from necridia cells - isopolar (H) and heteropolar (I); (J) filament with thick, and unconstricted sheath, and false branching. Scale bars 50 µm.

Table 3. Morphological characteristics among *Nunduva* species. (--) indicates not described. Cell morphometrics are demonstrated as min-max in µm. * = mean ± standard deviation.

	Thallus	**Filament features / width**	**Trichome**	**Shape**	**Cell length × width**	**Apical Cell**	**Cell content**
N. fasciculata León–Tejera, González–Resendiz et Johansen (Type)	caespitose, blackish green	erect, densely fasciculate, rare false branching 9–15	cylindrical, constricted or not, slightly tapered	isodiametric	5–8 × 6–10	colorless, short, rounded, bead-like	blue-green, olive-green granulated
N. biania León–Tejera, González–Resendiz et Johansen	caespitose, yellowish–brown	heteropolar, erect, rare single false branching 3–8	cylindrical, constricted or not, tapered, slightly swollen at base, without hair	shorter than wide	1–2.7 × 2–7	tapered	olive-green granulated
N. kania León–Tejera, González–Resendiz et Johansen	filamentous	heteropolar, isopolar, frequent false branching	constricted, tapered, with hair	wider than long, longer than wide in the apical zone	2–5 × 7.5–9	tapered, long with semi-hyaline, vacuolated hair	olive-green granulated
N. britannica Hauer	--	heteropolar, isopolar, straight or wavy, false branching 5–13	clearly constricted, tapered or not, swollen at basal part, without hair	shorter than wide up to isodiametric	-- × 5–9	spherical	granulated, large granules
N. komarkovae González–Resendiz, León–Tejera et J.R. Johans.	caespitose, formed by blue-green to dark green entangled filaments	heteropolar, isopolar, slightly curved, double and single false branching (in nature) heteropolar (in culture) 20–30	not to slightly constricted, abruptly tapered (in nature); constricted, thickened at the base, gradually tapered, with hair (in culture)	shorter than wide	9–15 × 3-6 (in nature) -- × 3–7 (in culture)	--	granulated
N. sanagustinensis González–Resendiz, León–Tejera et J.R. Johans.	caespitose, formed by blue-green to dark green, densely entangled filaments	heteropolar, isopolar, slightly curved, double and single false branching, 10–30 µm wide, wider at basal parts (in nature) heteropolar (in cultue)	not to slightly constricted, tapered, sometimes erect, frequently with hair (in nature) constricted, gradually tapered, thickened at the base, with hair (in culture)	shorter than wide	4–9 × 8–16	slightly attenuated, sometimes forming a short, nonhyaline hair	granulated
N. sanctimaloensis D.E. Berthold, Werner et Laughinghouse	caespitose, fasciculate, dark green to blue green	heteropolar, straight or flexuous, densely arranged and radiating from center of (hemi)spherical colonies, rare or frequent false branching, usually single 16.4–21.1	cylindrical, constricted, tapered, usually with hair	discoid to flattened oval (six times wider than long)	1.7–3.7 × 10.5–17.7	yellowish, rounded to triangular, long semi-hyaline, vacuolated apical hair	blue-green to forest green, finely granulated, cross-walls granulated or not

Table 3. Cont.

	Heterocyte position and number	Heterocyte shape	Heterocyte length × width	Reproduction	Habitat and habit	Type locality	Reference
N. fasciculata León–Tejera, González–Resendiz et Johansen (Type)	intercalary contiguous or near one another	elongated, flattened, discoid cylindrical	8–15 × 1.8–4.8	isopolar hormogonia, by necridia,heterocytes formation	supratidal and intertidal fringe, epilithic on granitic rock	Mexico, Oaxaca, San Agustín Bay	González--Resendiz et al. (2018a)
N. biania León–Tejera, González–Resendiz et Johansen	intercalary, basal	elongated, discoid, cylindrical	4–4.5 × 4–6	iso- or heteropolar hormogonia by cell constriction, heterocyte formation, separation of branches	intertidal fringe; epilithic on granitic rock	Mexico, Oaxaca, San Agustín Bay	
N. kania León–Tejera, González–Resendiz et Johansen	basal, single	conical, hemispherical	2.5–2.6 × 4.9–5.4	iso- or heteropolar hormogonia, by separation of branches, cell constriction, without necridia	intertidal fringe, epilithic on basaltic rock	Mexico, Veracruz, Playa Hermosa	
N. britannica Hauer	intercalary, basal, single or pairs	hemispherical, trapezoid, cylindrical	-- × up to 10	isopolar hormogonia, by trichome disintegration	brackish, estuarine	United Kingdom, England, Isle of Wright, Newtown	
N. komarkovae González–Resendiz, León–Tejera et J.R.Johans.	intercalary, basal, single, scarce	hemicircular (basal), and subquadrate to longer (intercalary) – (in nature); hemispherical, spherical to oval-cylindrical (in culture)	-- × 7–10 (in culture)	mainly isopolar hormogonia, by trichome fragmentation or by biconcave lens-shaped necridia (in nature); iso and heteropolar hormogonia (in culture)	intertidal fringe, epilithic on granitic rock, in partially exposed rock crevices	Mexico, Veracruz, Port of Veracruz	Johansen et al. (2021)
N. sanagustinensis González–Resendiz, León–Tejera et J.R.Johans.	intercalary, basal, single or pairs, scarce	usually shorter than wide (in nature); hemispherical, spherical to oval-cylindrical, complanate (in culture)	3–6 × -- (in nature) 12–14 × -- (in culture)	terminal or basal hormogonia, iso- or heteropolar, mainly by necridia or trichome fragmentation (in nature); basal hormogonia, iso- or heteropolar, consisting of flattened or irregularly twisted or contorted cells enclosed in a widened sheath (in culture)	supratidal fringe epilithic on granitic rock, both in partially sunny and exposed surfaces as well as slightly shadowed rock crevices	Mexico, Oaxaca, San Agustín Bay	
N. sanctimaloensis D.E. Berthold, Werner et Laughinghouse	intercalary, basal single or in series of double or triple	conical, rectangular, discoid, quadratic, cylindrical	8.8–17.3 × 11–13 *(9.9 ± 1.6) × (11.6 ± 0.8)	monocytes isopolar or heteropolar hormogonia, by cell constriction, h heterocyte formation, with or without necridia	intertidal benthic and epilithic	France, Brittany, beach in Plage du Sillon	This paper

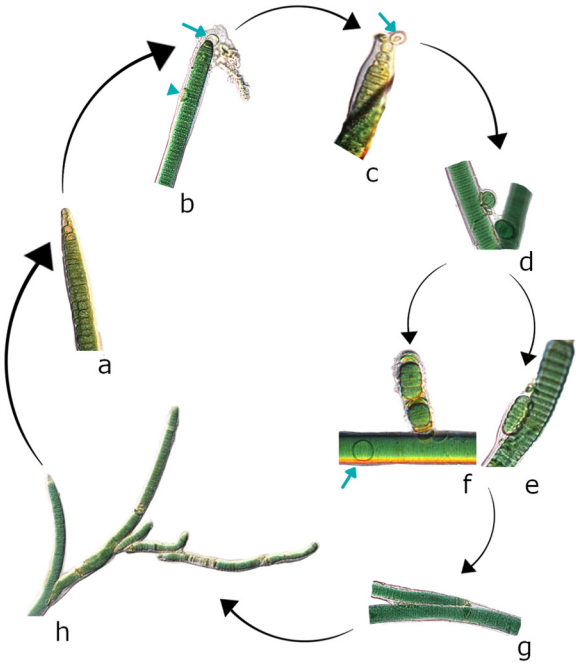

Fig. 5. Reproduction cycle by monocytes of *Nunduva sanctimaloensis* sp. nov. (reference strain BLCC–M94): (A) apical cells extension and vacuolization; (B) monocyte not sessile, but still trapped in sheath (arrow), and trapped inner monocyte (arrowhead); (C) rupturing from the apical open sheath and releasing monocytes (arrow); (D) development of a cytoplasm and sheath over monocyte; (E-F) growth of attached monocyte into small filaments: outside the sheath (E), and trapped inner monocyte (E-arrow) or inside the sheath (F); (G) resulting of growth of the filament; (H) extension of heteropolar filament from developed monocyte with false branching.

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Supplementary material

The following supplementary material is available for this article:

Fig. S1. Microscope images of *Nunduva sanctimaloensis* sp. nov. (reference strain BLCC–M94) showing double serial basal heterocytes.

Fig. S2. Microscope images of *Nunduva sanctimaloensis* sp. nov (reference strain BLCC–M94) showing the sequence in which reproduction by modified monocytes occurs where individual filament apices extend in length and apical cells begin to vacuolize

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

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