diversity

Article

Terrestrial Aerophytic Cyanobacteria in the Canary Island Laurel-Forest (Laurisilva): Discovery of *Brasilonema novocanariensis* sp. nov. and *Rhizonema melkonianarum* sp. nov. from the *Laurus* Phyllosphere

Nereida M. Rancel-Rodríguez [1,*], **Christophe Vieira** [2] **and Marta Sansón** [1]

[1] Departamento de Botánica, Ecología y Fisiología Vegetal, Facultad de Ciencias, Sección Biología, Universidad de La Laguna, Avenida Astrofísico Francisco Sánchez, 38206 La Laguna, Spain; msanson@ull.edu.es
[2] Research Institute for Basic Sciences, Jeju National University, Jeju 63243, Republic of Korea; cvcarp@gmail.com
* Correspondence: nrrodri@ull.edu.es

Abstract: Cyanobacteria, known for their remarkable adaptability, are a diverse group of oxygenic photoautotrophic organisms found in various habitats worldwide. While their presence in aquatic ecosystems has been extensively studied, research on terrestrial and aerophytic cyanobacteria remains relatively limited, particularly concerning their taxonomic diversity and molecular characteristics. In this study, utilizing morphological and molecular data derived from the 16S rRNA gene, we unveiled and described two new species of filamentous cyanobacteria growing epiphytically on *Laurus novocanariensis* in the laurel forest (laurisilva) of the Canary Islands. The first species, *Brasilonema novocanariensis* sp. nov., belongs to a genus of aerophytic and subaerophytic nostocalean cyanobacteria. The second, *Rhizonema melkonianarum* sp. nov., belongs to the genus *Rhizonema*. The discovery of a new species of *Brasilonema* from the Canary Islands related to species initially found on the opposite side of the Atlantic Ocean, and recently in Asia, Europe, and Portugal, raises questions about their dispersal mechanisms, whether through natural means or human-mediated processes, prompting the need for future studies to investigate these propagation patterns. In the case of *Rhizonema melkonianarum* sp. nov., the transition from adapting to a basidiolichen host to inhabiting the phyllosphere of angiosperms raises ecological and evolutionary adaptation questions regarding cyanobacterial adaptability and their ecological roles in diverse habitats. This research expands our understanding of the diversity and geographic distribution of these terrestrial phyllosphere-associated cyanobacteria.

Keywords: Atlantic region; molecular systematics; new species; Nostocales; phyllosphere; 16S rRNA gene

check for updates

Citation: Rancel-Rodríguez, N.M.; Vieira, C.; Sansón, M. Terrestrial Aerophytic Cyanobacteria in the Canary Island Laurel-Forest (Laurisilva): Discovery of *Brasilonema novocanariensis* sp. nov. and *Rhizonema melkonianarum* sp. nov. from the *Laurus* Phyllosphere. *Diversity* **2024**, *16*, 625. https://doi.org/10.3390/d16100625

Academic Editor: Glenn B. McGregor

Received: 23 May 2024
Revised: 12 September 2024
Accepted: 1 October 2024
Published: 10 October 2024

1. Introduction

Cyanobacteria, pivotal organisms in various ecosystems, are currently undergoing taxonomic transformations due to the accessibility of 16S rRNA gene sequencing and refined protocols for taxon characterization [1]. Despite these advancements, traditional morphological taxonomy continues to exert influence in the categorization of the majority of cyanobacterial taxa [2,3]. The diversity of cyanobacteria in tropical regions, especially in extreme habitats, remains inadequately explored [4,5]. While cyanobacteria are commonly associated with aquatic environments, they also fulfill crucial functions in terrestrial and aerophytic habitats, including soil, rocks, and aerial surfaces such as plant rhizosphere, bark, and phyllosphere [6–8].

Although terrestrial and aerophytic cyanobacteria are ecologically significant, they have received less attention compared to their aquatic counterparts [7,9,10]. Recent methodological advances that integrate morphological and molecular techniques (i.e., polyphasic approach) have facilitated the discovery of new cyanobacterial taxa over the past decades [9,11,12].

The Nostocales, heterocytes, forming cyanobacteria, stand out as one of the most diverse groups within the cyanobacteria phylum. This order represents a substantial portion of the overall generic diversity, accounting for at least one-third of the total genera within it (108 out of 300 genera; [13]). In his studies, Komárek [11,12] identified 12 families within the order Nostocales, a classification also upheld by Strunecky in 2023 [1]. Phylogenetically, the Nostocales constitute a monophyletic lineage, supported by analysis of their 16S rDNA sequences [11,14]. Morphologically characterized by filamentous morphology and adept nitrogen fixation abilities [1,15], they serve as crucial contributors to global carbon dioxide fixation [16]. The Nostocales order, named according to the International Code of Nomenclature for Algae, Fungi, and Plants (ICN or ICNafp; [17]), is recognized as displaying a diverse thallus, that includes unbranched and isopolar forms, as well as false-branched or true-branched types [1,11]. They possess specialized cells, such as heterocytes, for nitrogen fixation, and akinetes [1,11].

Within the order Nostocales, the Scytonemataceae family, which consists of approximately 24 taxonomically accepted genera [1] and up to 39 genera according to Guiry and Guiry in 2024 [13], plays a notable role, thriving across various terrestrial, aerial, and soil habitats [7,8,18]. These habitats include caves and sporadically, the surfaces of higher plants, albeit with less frequency in freshwater bodies [9].

This study focuses on two genera, *Brasilonema* Fiore et al. and *Rhizonema* Lücking and Barrie, within the Scytonemataceae and Rhizonemataceae family, respectively, in the Canary Island laurel forest (Laurisilva, Figure 1A).

Figure 1. The Tenerife laurel forest is the endemic evergreen forest of the archipelago. (**A**) Laurisilva forest on the island of Tenerife; Anaga Rural Park. (**B**) Leaf of *Laurus novocanariensis*, as a habitat revealed by cyanobacteria.

Brasilonema, a genus notably found in subtropical, tropical, and temperate regions, comprises 40 species [7,19–24], characterized by cylindrical trichomes, not or very slightly constricted at cross walls and not attenuated towards ends. Heterocytes are solitary, intercalary, and cylindrically diskoid, more long than wide [21]. However, their occurrence and diversity in regions such as the Canary Islands remain underexplored. *Rhizonema*, another notable genus, comprises a single species of lichenized cyanobacterium, *Rhizonema interruptum* Lücking and Barrie. Described in 2014 [25], this genus is distinguished by cylindrical trichomes with intercalar heterocytes and occasional true branching, sharing morphological similarities with many members of the Scytonemataceae family, but Lücking and Barrie reintroduce the genus into the novel Rhizonemataceae family [25].

The Canary Islands laurel forest is one of the most biologically distinctive ecosystems in the Canarian archipelago [26]. This dense forest thrives in areas of orographic cloud formation, with towering trees reaching up to 30 m in height, reflecting a rich and complex biogeographic history. This unique ecosystem is mainly restricted to the semi-humid mid-elevations of the mountainous western islands, especially on the windward slopes [26,27]. Among these islands, Tenerife, the largest of the archipelago, hosts a wide variety of protected areas and natural habitats. Tenerife's laurel forests have their largest presence in the Anaga Rural Park and the Teno Rural Park. The Anaga Rural Park (Figure 1) is located in the northeast of the island and covers an area of more than 14,000 hectares, the largest area of laurel forest on the island, which has been preserved in part due to its rugged terrain and challenging foot access in various areas [27].

This study investigated the taxonomic identity of *Brasilonema* and *Rhizonema* species discovered during an opportunistic sampling of laurel tree leaves in the Canary Island Laurisilva (Figure 1).

2. Materials and Methods

Sampling site, isolation, and culturing: Leaves, representing the phyllosphere of the targeted cyanobacteria, were collected from the laurel tree *Laurus novocanariensis* Rivas-Mart., et al. The laurel leaves were collected in March 2012 from the Laurisilva within the Anaga rural Park (28°32′57″ N–16°11′09″ W (Figure 2); altitude: 727 m), Tenerife; Canary Islands, Spain. The collected leaves were individually placed in plastic zip-loc bags and labeled. Sampling was conducted under a permit Sigma 415/2012, granted by the Department of Environment, Territorial Sustainability, and Water of the Cabildo de Tenerife.

The leaves were meticulously dissected under sterile conditions on a laminar flow bench, resulting in small, uniform pieces of 1 × 1 cm^2, and placed in Petri dishes with specific sterile culture medium BG11-H with and without a source of combined nitrogen BG11-Ho (Modified by Naumann in 2004) for a twin-layer culture system based on [28]. Enrichments were incubated at 25 °C, with a light intensity of 2–20 µmol photons m^{-2} s^{-1}, and a light/dark cycle of 14/9 h for a minimum of 3 months. Enrichment cultures were checked regularly, every 2 weeks, using an inverted Olympus microscope equipped with phase contrast optics (CK-41, Olympus, Hamburg, Germany). Upon detecting heterocytes-forming cyanobacteria, clonal cultures were initiated. To obtain unialgal cultures, isolation of single cells, short filaments, or hormogonia was performed using the micropipette technique according to [29] from the same leaf enrichment for both strains.

Maintenance of clonal cultures: Isolated single cells were transferred to microtiter plates (24 wells, filled with 1 mL of BG11-Ho) and maintained in a growth chamber at the Spanish Bank of Algae Culture Collection (BEA) under controlled conditions (20 °C, light intensity of 5–20 µmol photons m^{-2} s^{-1} and a light/dark cycle of 14/9 h). The cultures were maintained in BG11-Ho in 50 mL Erlenmeyer flasks and on agar slants, with regular transfers every 16–20 weeks. Back-up cultures were kept in Petri dishes (35 mm in diameter). Deposed strains established clonal cultures were assigned collection numbers BEA 1759B–1761B.

Morphological analyses: Every two weeks, morphology and development observations of isolated strains were performed using a Nikon 90i (Nikon, Tokyo, Japan) microscope equipped with phase-contrast optics. Quantitative evaluations were carried out with three-week-old cultures. Defined characters were counted in at least 20 different specimens. Size measurements, conducted at least 20 times, were performed utilizing the built-in tools of the Nikon 90i.

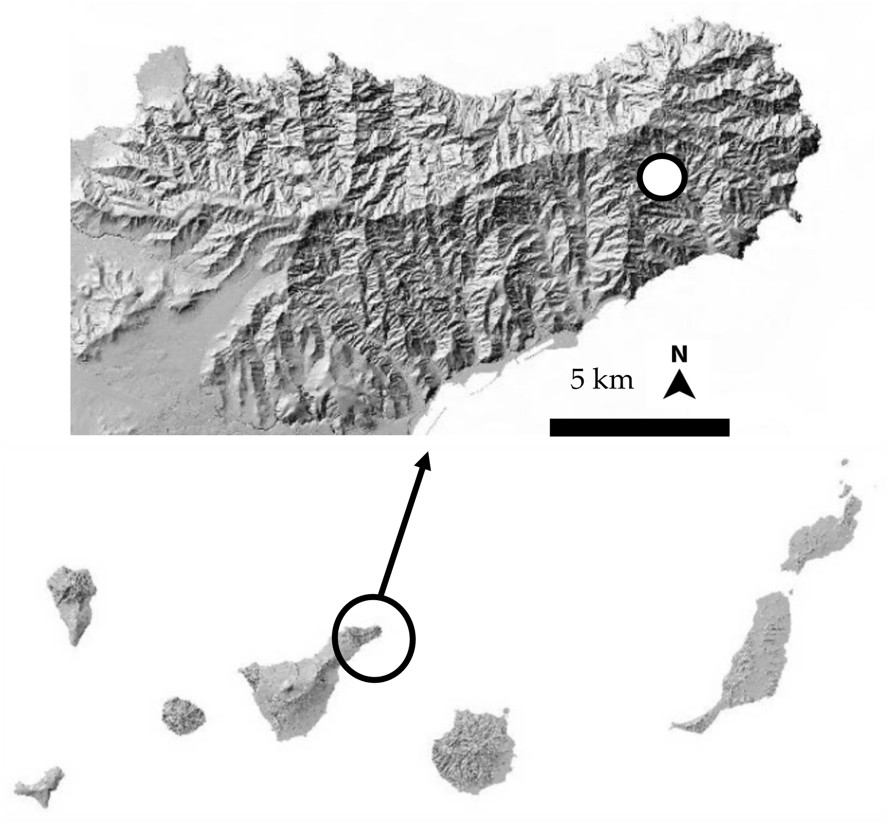


Figure 2. Map of the Canary Islands; the sampling site is highlighted in the Anaga Rural Park, in the laurel forest of the island of Tenerife. White circle indicates the coordinates of the sampling.

Molecular Analyses: DNA extractions were carried out using a Qiagen DNeasy plant mini kit following the manufacturer´s instructions, with some modifications; to harvest the cells, 1.5 mL of algal culture was centrifuged for 10 min at 10,000× *g* in a 2 mL microcentrifuge tube, and the supernatant was discarded afterward. To mechanically disrupt the cells, they were first frozen at −20 °C for at least one day. After that, sterile glass beads (∅ 5 mm) were added to the 20 μg frozen pellet. All further steps were performed according to the manufacturer´s protocol until the elution step, where DNA was eluted in a twice-heated 50 μL elution buffer (65 °C) instead of 100 μL, which led to a significant increase in the final DNA concentration of the eluate. The DNA content was quantified via NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and DNA was stored at −20 °C afterwards. DNA was amplified with a Hot Start PCR and High Fidelity DNA Polymerase Thermo Scientific® Phusion. High-Fidelity DNA Polymerase offers both high fidelity and robust performance and thus can be used for all PCR applications. The primers designed by [30] were used to generate cyanobacteria-specific primers to avoid accidental amplification of bacterial DNA Operon (as all cultures contained bacteria). The specific positions at the 3′ ends were incorporated into ptLSU C-D-rev (5′-GCCGGCTCATTCTTCAAC-3′) and ptLSU B19-forw (5′-CACGTGRAATYCCGTGTGAATCWGC-3′). In the PCR reactions, these primers were paired with universal SSU rRNA primers (SSU-4-forw, 5′-GATCCTKGCTCAGGATKAACGCTGGC-3′). The sizes of the bands are about 1500 bp for the SSU. To compare the obtained bands the DNA ladder (Molecular Weight Marker) of NIPPON Genetics was used.

Sequencing was performed by Macrogen Company (Amsterdam, The Netherlands; www.macrogen.com) The resulting sequences were deposited in GenBank under the accession numbers PP409594–PP409596. Consensus sequences were generated in Mesquite v3.02 (build 681) [31] and aligned in SeaView 5.0 [31]. Newly obtained rDNA sequences were integrated with sequences from existing databases to construct an alignment that includes genes for SSU rRNA, tRNA-Ile, and tRNA-Ala. The conserved secondary structures of rRNA and tRNA, as elucidated on https://crw2-comparative-rna-web.org/ and https://rnacentral.org/search?q=has_secondary_structure, played a key role in determining the architecture of the alignment. SSU sequence lengths from the three clonal cultures of heterocytes cyanobacteria varied between 1357 and 1420 nucleotides. The 16S rRNA alignment contained a total of 55 cyanobacteria sequences, including three new ones from the laurel forest, 51 relative sequences from GenBank, in addition to one non-heterocytes-forming cyanobacteria as the outgroup. If any uncertainty regarding secondary structure arose, the sequences were reassessed in Mesquite [32]. Hypervariable gene regions were analyzed on "the Mfold Web Server" [33]. In all datasets, a mask was applied, which was manually adjusted and excluded those positions/hypervariable regions that could not be unambiguously aligned and were thus not taken into consideration for phylogenetic analyses.

Phylogenetic Analyses: The analysis involved the alignment of a total of 104 16S rRNA sequences, comprising 1428 base pairs (bp). This alignment included publicly available sequences from both *Brasilonema* and *Rhizonema*, as well as sequences from related genera such as *Scytonema* and *Rivularia*, and some other Nostocales closely related to both clades. *Gloeobacter violaceus* PCC 7421 was selected as the outgroup, and additional Nostocales, including *Calothrix* sp. UAM 374 strain and HA4292 MV4 *Scytonematopsis contorta* M.A.Vaccarino and J.R. Johansen, were included in the analysis. The dataset was phylogenetically analyzed using both Maximum Likelihood (ML) and Bayesian Posterior Probabilities (BPPs) methods.

Phylogenetic reconstructions were performed using both maximum likelihood (ML) and Bayesian inference (BI) methods. For both the ML and BI analyses, the model GTR + I + Γ was used. ML analyses were conducted using the PTHREADS version of RAxML 7.2.6 [34]. BI was performed in MrBayes v.3.1.2 [35] with two Markov-chain Monte Carlo (MCMC) chains for 50 M generations, using default options with the covarion model and autocorrelation. Trees were sampled every 1000 generations, and the burn-in was determined by the convergence criterion. Statistical support for branches was calculated by bootstrapping with 1000 replicates using ML [36]. All supporting values, including posterior probabilities (BIs), were plotted onto the best ML topology if the respective branches were present (ML: $\geq$70, BI: $\geq$0.95). The tree was rooted with *Gloeobacter violaceus* Rippka, J.B. Waterbury, and Cohen-Bazire, a member relating to the closest genus relative to our study group.

The secondary structures of the 16S–23S rRNA ITS region were analyzed for our isolates and compared with previously published sequences. We used the Mfold web server [33] to delineate and fold the secondary structures. After folding, the structures were downloaded from the web server and edited with Affinity Designer 1.5.4 Serif Europe Ltd. Apple, West Bridgford, UK.

3. Results

3.1. Phylogenetic Analyses

Two novel 16S rRNA sequences obtained from cyanobacteria growing epiphytically on laurel leaves in the laurel forest of Tenerife (Canary Islands) were identified as belonging to the genera *Brasilonema* and *Rhizonema* (see Figure 3).

The resulting ML tree topology is presented in Figure 3, showing support for both methods. Maximum likelihood support is shown as bootstrap (BS), and Bayesian support is shown as BPP.

The dataset of the genus *Brasilonema* shows the presence of all currently accepted species, 71 sequences from *Brasilonema* species, available on Genbank. Those marked with an asterisk (*) on the tree (Figure 3) belong to the new species described in this manuscript. These species belong to the family Scytonemataceae as classified by Rabenhorst ex Bornet and Flahault, characterized by sheathed false-branched filaments with predominantly intercalary heterocytes. Notably, sequences for *B. epidendron* Fiore et al. and *B. ornatum*

Fiore et al. are currently unavailable [21]. As has been seen in previous studies, *Brasilonema* forms a rather strongly supported clade (100% BS–100% BPP).

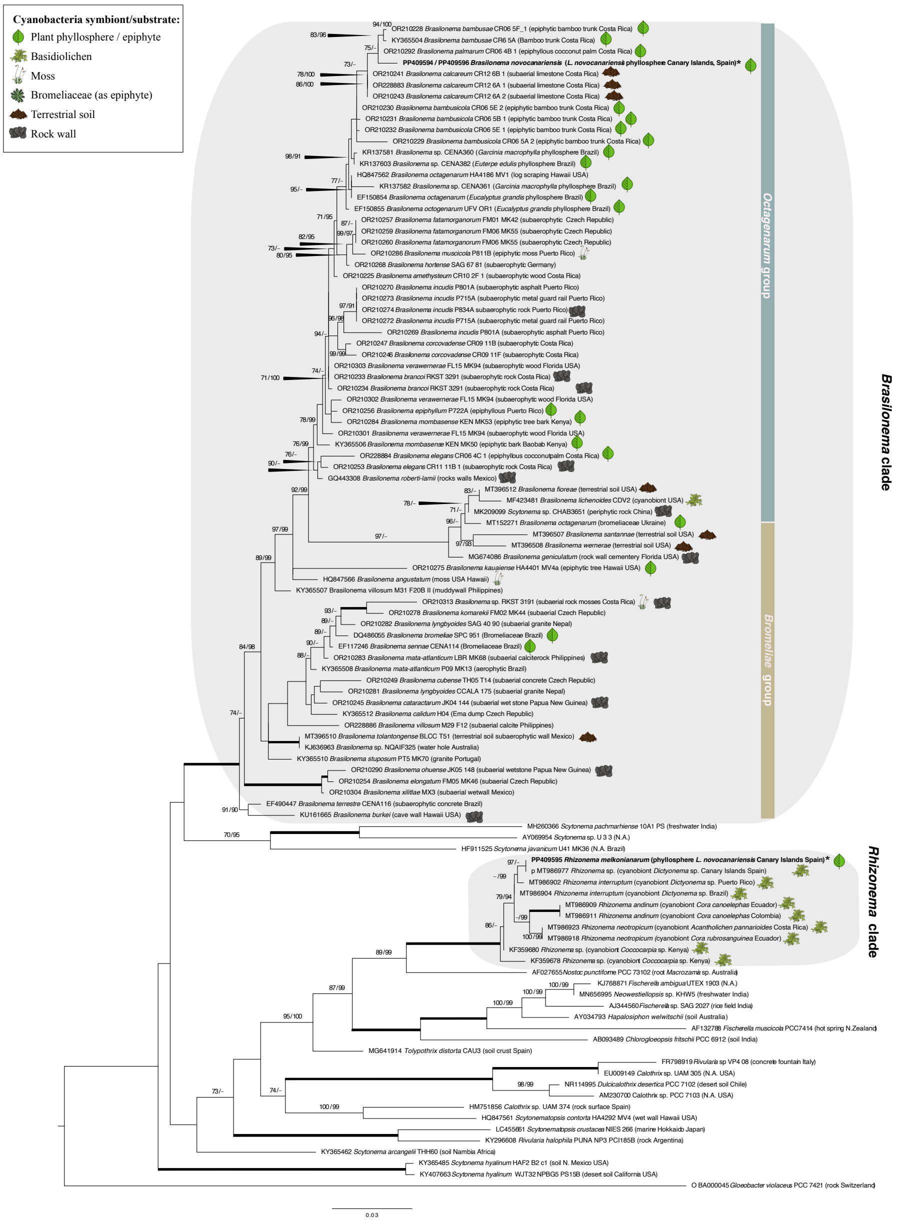


Figure 3. Maximum likelihood (ML) tree topology, constructed using the GTR + I + Γ evolution model, contains 104 sequences (1428 bp) of 16S rRNA. Bootstrap (BS) values ≥ 70 for ML and Bayesian inference posterior probability values ≥ 90 are displayed on each branch. Full support for both methods is denoted by black bold lines. The new sequences presented here of each species are marked with * and in bold letters, and the gray box indicates the placement of the new sequences presented in this work. Right-side blue bar "*Octagenarum* group" or right-side beige bar "*Bromeliae* group" named following Bohunicka et al. [24].

The Canarian strains of *Brasilonema*, BEA 1760B and BEA 1761B (Acc. nº PP409594 and PP409596), isolated from a laurel leaf in the Laurisilva forest of Tenerife (Canary Islands, Spain), are closely related to *Brasilonema bambusae* (OR210228; KY365504) and *B. palmarum* (OR210292) as described by Bohunicka et al. [24]. This clustering is supported by 75% bootstrap (BS) on the phylogenetic tree. The pairwise divergences were 0.007 between *B. novocanariensis* and *B. bambusae*, and 0.006 with *B. palmarum* (see Supplementary Material, Table S1). Notably, *B. bambusae* was recently described as growing epiphytically on bamboo trunks, while *B. palmarum* was found growing on coconut palms, with both species occurring in Costa Rica.

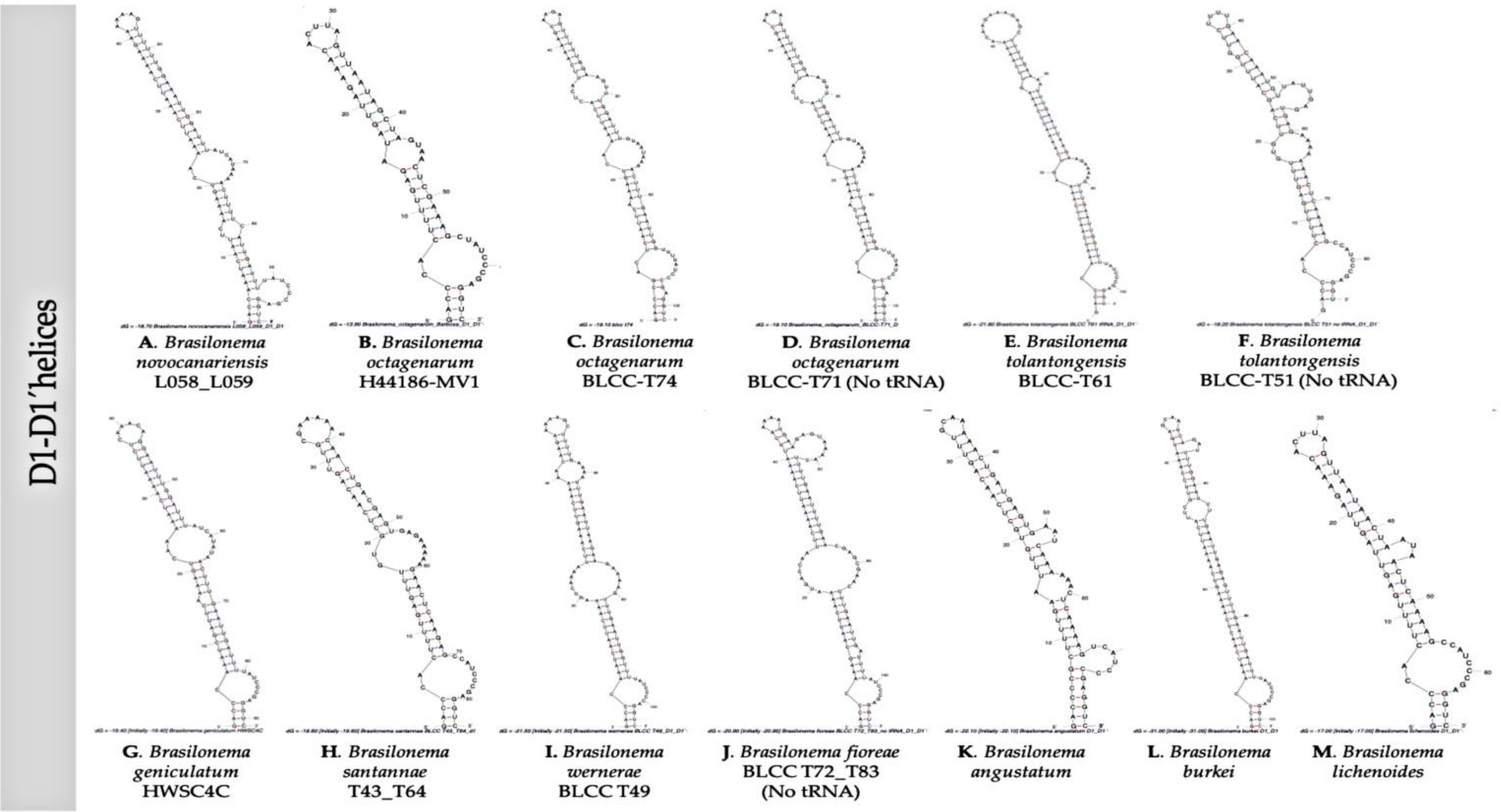
In comparison, sequences from *B. calcareum* (OR210241; OR228883; OR210243), a strain isolated from subaerial limestone in Costa Rica, form a sister clade, showing a 73% supported relationship in maximum likelihood when compared to the *Brasilonema* strains from the Canary Islands (pairwise divergence: 0.009, Table S1). Other species of the genus *Brasilonema* also appear as sister clades in the phylogenetic tree (Figure 3). According to a recent publication by Bohunická et al. [24], the Canarian *Brasilonema* species belong to the *Octagenarum* group, defined as the clade within the genus that contains *B. octagenarum*, with 16S rRNA gene similarities above 98.7%. This group currently includes *B. octagenarum*, *B. amethysteum*, *B. bambusae*, *B. bambusicola*, *B. brancoi*, *B. calcareum*, *B. corcovadense*, *B. elegans*, *B. epiphyllum*, *B. fatamorganum*, *B. fioreae*, *B. geniculatum*, *B. incudis*, *B. lichenoides*, *B. mombasense*, *B. muscicola*, *B. palmarum*, *B. roberti-lamii*, and *B. verawernerae*.

The placement of the sequence from the other cyanobacteria isolated from the laurel phyllosphere of the Canary Islands positioned within a fully supported clade of genus *Rhizonema*, by both methods (100% BS and BPP), related to lineages labeled as *R. interruptum*. It appears that only the species *R. interruptum* has a valid name in Algaebase [13]. However, two additional sequences, *R. andinum* and *R. neotropicum,* are available in GenBank (positioned within a clade with moderate BS support in the ML tree and BPP support in the Bayesian tree (79 and 94, respectively). However, when considering its association with environmental basidiolichen sequences of *Rhizonema* sp. from Tenerife (Supplementary Material [37]), it is crucial to note that the species is represented by a relatively short sequence, mainly 211 base pairs (bp) for 16S rRNA and 454 bp for ITS. In contrast, our sequence is 1410 bp in length, including both the SSU and the ITS regions. Importantly, these sequences lack substantial clade support when compared to *R. interruptum* from South America, as reported in [37,38]. It is important to emphasize that strain BEA 1759B, the sequence (PP409595) newly presented here has a unique feature: it was identified from the population of free-living cyanobacteria, isolated growing epiphyllous on an *L. novocanariensis* leaf, a phenomenon not previously observed on this genus.

3.2. 16S–23S rRNA Internal Transcribed Spacer (ITS) Secondary Structure

The B-Box and D1-D1′ helices of the 16S–23S ITS secondary structure region were examined across 13 *Brasilonema* isolates, representing 10 species, as well as other published sequences. The D1-D1′ helices (Figure 4) showed identical structures in *B. octagenarum* BLCC-T71 (Figure 4D) and BLCC-T74 (Figure 4C) but varied in sequence and length with *B. octagenarum* H44186-MV1 (Figure 4B). *B. novocanariensis* (Figure 4A) had a length similar to BLCC-T71 and BLCC-T74 but differed in nucleotide sequence and structure, showing an extra loop. The B-Box region displayed similar lengths and configurations, though the sequences varied among the strains (Figure 5). Strains of (Figure 5A) had a structure similar to *B. octagenarum* (Figure 5B–D), with only a few base pairs differing (At this time, the authors are unable to compare the secondary structure of Brasilonema with the new species in Bohunicka et al. [24], as their publication remains ahead of print and the Supplementary Material has not yet been made available from the Journal).

For *Rhizonema melkonianarum*, the B-Box and D1-D1′ helices of the 16S–23S ITS secondary structure region (Figure 6) could not be compared to other species, as this is the first instance of secondary structure analysis for this genus.



A. *Brasilonema novocanariensis* L058_L059

B. *Brasilonema octagenarum* H44186-MV1

C. *Brasilonema octagenarum* BLCC-T74

D. *Brasilonema octagenarum* BLCC-T71 (No tRNA)

E. *Brasilonema tolantongensis* BLCC-T61

F. *Brasilonema tolantongensis* BLCC-T51 (No tRNA)

G. *Brasilonema geniculatum* HWSC4C

H. *Brasilonema santannae* T43_T64

I. *Brasilonema wernerae* BLCC T49

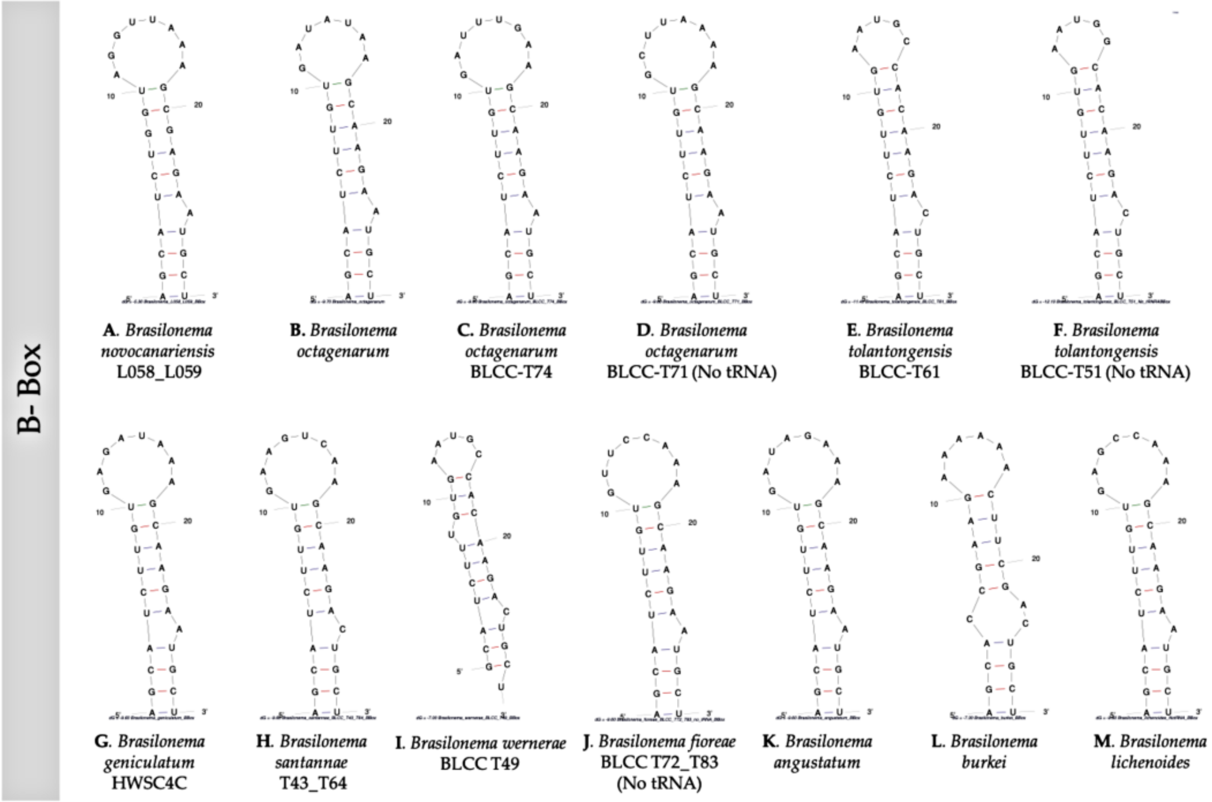
J. *Brasilonema fioreae* BLCC T72_T83 (No tRNA)

K. *Brasilonema angustatum*

L. *Brasilonema burkei*

M. *Brasilonema lichenoides*

Figure 4. Secondary structures of the D1-D1′ helices from the 16S–23S rRNA ITS regions of 13 isolates and nearest relatives from *Brasilonema* for which 16S–23S rRNA ITS sequence data are available: (**A**). *B. novocanariensis* sp. nov; (**B**). *B. octagenarum* H44186-MV1; (**C**). *B. octagenarum* BLCC-T74; (**D**). *B. octagenarum* BLCC-T71 no tRNA; (**E**). *B. tolantongensis* BLCC-T61; (**F**). *B. sennae* BLCC-T49; (**G**). *B. geniculatum* HWSC4C (**H**). *B. santannae* T43_T64 (**I**). *B. wernerae* BLCCT49 (**J**). *B. fioreae* BLCC-T72-T83 no tRNA; (**K**). *B. angustatum* HA4187-MV1; (**L**). *B. burkei* HA4348-LM4; (**M**). *B. lichenoides* 168/2015.



A. *Brasilonema novocanariensis* L058_L059

B. *Brasilonema octagenarum*

C. *Brasilonema octagenarum* BLCC-T74

D. *Brasilonema octagenarum* BLCC-T71 (No tRNA)

E. *Brasilonema tolantongensis* BLCC-T61

F. *Brasilonema tolantongensis* BLCC-T51 (No tRNA)

G. *Brasilonema geniculatum* HWSC4C

H. *Brasilonema santannae* T43_T64

I. *Brasilonema wernerae* BLCC T49

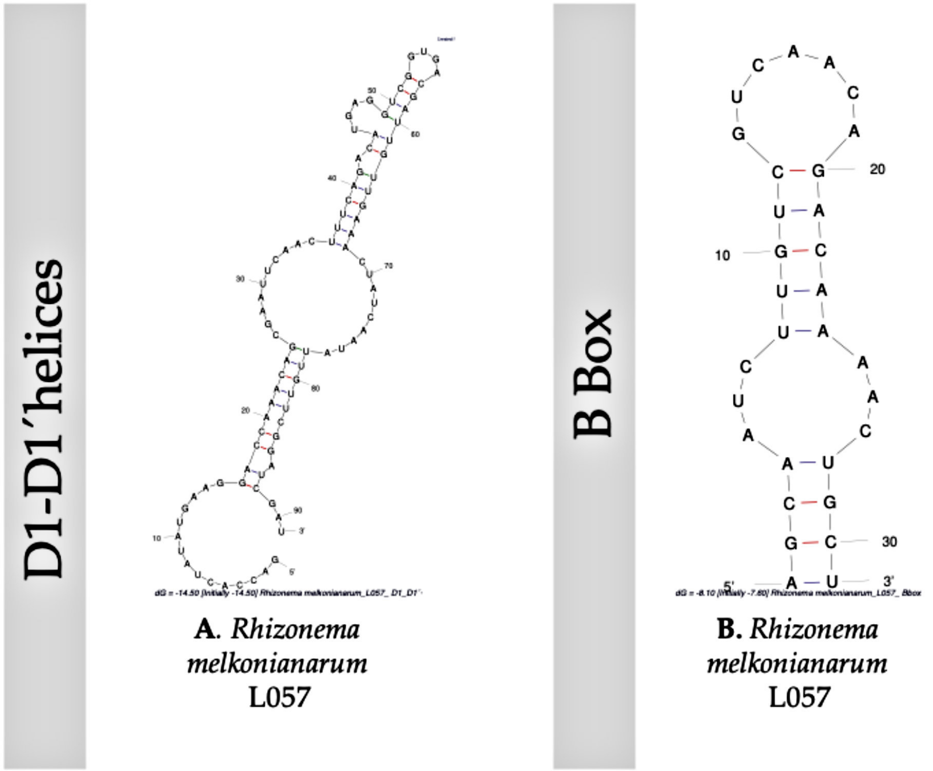
J. *Brasilonema fioreae* BLCC T72_T83 (No tRNA)

K. *Brasilonema angustatum*

L. *Brasilonema burkei*

M. *Brasilonema lichenoides*

Figure 5. Secondary structures of the B-Box region from the 16S–23S rRNA ITS regions of 13 isolates and their nearest relatives within *Brasilonema*, for which 16S–23S rRNA ITS sequence data are available: (**A**). *B. novocanariensis* sp. nov.; (**B**). *B. octagenarum* H44186-MV1; (**C**). *B. octagenarum* BLCC-T74; (**D**). *B. octagenarum* BLCC-T71 no tRNA; (**E**). *B. tolantongensis* BLCC-T61; (**F**). *B. sennae* BLCC-T49; (**G**). *B. geniculatum* HWSC4C (**H**). *B. santannae* T43_T64 (**I**). *B. wernerae* BLCCT49 (**J**). *B. fioreae* BLCC-T72-T83 no tRNA; (**K**). *B. angustatum* HA4187-MV1; (**L**). *B. burkei* HA4348-LM4; (**M**). *B. lichenoides* 168/2015.



A. *Rhizonema melkonianarum* L057

B. *Rhizonema melkonianarum* L057

Figure 6. Secondary structures of the D1-D1′ and B-Box regions from the 16S–23S rRNA ITS regions of *Rhizonema melkonianarum*; there are not more 16S–23S rRNA ITS sequence data available: (**A**). *R. melonianarum* D1-D1′ region; (**B**). *R. melkonianarum* B-Box region.

3.3. Morphological Examination

One of the species presented here belongs to the family Scytonemataceae as classified by Rabenhorst ex Bornet and Flahault, characterized by sheathed false-branched filaments with predominantly intercalary heterocytes [1]. One of them fits the generic morphological description of the *Brasilonema* genus. To comprehend the distinctions among each species within the genus and ensure consistency, a table was compiled (Supplementary Material Table S2 [39,40]) detailing all morphological differences observed in accepted species. Following these criteria and aided by phylogenetic analysis (Figure 3), it is possible to establish the isolate from the Canary Islands as a novel species, described here as *B. novocanariensis* sp. nov. Table 1 shows the morphological differences between the closest relative species. The four *Brasiolonema* species—*B. novocanariensis*, *B. bambusae*, *B. palmarum*, and *B. calcareum*—share key morphological characteristics but exhibit distinct differences, with *B. novocanariensis* displaying unique features that support its classification as a new species. While all species generally possess cylindrical cells, *B. novocanariensis* is distinguished by the presence of lenticular (lens-shaped) cells, a trait absent in *B. bambusae*, *B. palmarum*, and *B. calcareum*, which all have consistently cylindrical cells. In terms of filament diameter, all species fall within a similar range (7–21 µm), but *B. novocanariensis* tends to have slightly narrower trichomes. Thallus coloration also helps differentiate these species: *B. novocanariensis* typically exhibits violet coloration, while *B. bambusae*, *B. palmarum*, and *B. calcareum* present more dark-black–brown tones, with *B. calcareum* leaning toward olive-green. Cell color in the Costa Rica species is rarely violet and more commonly brown or green, whereas the Canarian species typically exhibit violet cells, rarely appearing green or brown. Additionally, all four species have intercalary heterocytes, but the structure of terminal heterocytes is always present in the Canarian species. Heterocytes also vary in size. The Canarian species exhibits spherical terminal heterocytes and lenticular to sublenticular intercalar heterocytes, whereas the three Costa Rica species show cylindrical heterocytes following the description by Bohunicka et al. [24].

Table 1. Morphological comparison of the new *Brasilonema novocanariensis* sp. nov. versus *B. bambusae; B. palmarum* and *B. calcareum* described by [24]; and also with *B. octagenarum* described by [19].

	***B. novocanariensis* sp. nov.**	***B. bambusae***	***B. palmarum***	***B. calcareum***	***B. octagenarum***
Filament width (μm)	17–19	10–22	11–19	13–23	9.8–18.5
Trichome diameter (μm)	10–17	(7) 10–17 (21)	11–19	8–20	9.5–14.9–18.4
Cells colors	Brownish to violet to grayish green	Gray–brown; (pale) blue–green; (pale) brown; dull purple; olive-brown or olive-green; green; yellow–green or gray	Brown; gray–purple; olive-green; gray–green	Olive-green, gray–green or green	Brownish, olive-green, rarely violet
Cell shape	Lenticular to sublenticular	Cylindrical to disk-like	Cylindrical	Cylindrical to disk-like	Cylindrical
Cell length (μm)	5–9	2–20	2–12	2–17 (20)	1.5–13.3
Thallus form	Heteropolar rarely isopolar free filaments	N.A.	N.A.	N.A.	Isopolar
Thallus color	Brownish-violet	Black, brown, to olive-green	Olive-black	Black; brown–black; black-green	Dirty-green, brownish or blackish-green
Heterocytes form	Terminal: Spherical to subspherical; Intercalar: lenticular to sublenticular	Terminal: N.A. Intercalar: cylindrical	Terminal: N.A. Intercalar: cylindrical rounded	Terminal: hemispherical Intercalar: cylindrical rounded	Discoid or +/− cylindrical
Heterocytes (wide × long μm)	7–10 × 7–9	10–16 × 5–15	7–16 × 6–12	7–20 × 3–14	5.4–15.6 × 10–17.6
Young trichomes shape	Straight	N.A.	N.A.	N.A.	Lightly curved
Hormogonia development	Subcubic cells without constriction at the cross wall, movement without heterocyst	Cylindrical cells, narrowing towards the growing tips	Short cells	Cylindrical cells, often with terminal heterocytes	Present
Ecology	Subaerophytic, epiphytic on living leaves of *Laurus novocanariensis*	Subaerophytic, forming black mat on bamboo trunks in bamboo grove	Subaerophytic, on a Palm leaf	Subaerophytic, on limestones	Epiphytic on damaged leaves, stems, and buds of *Eucalyptus grandis*
Type locality	Spain, Canary Islands (Tenerife laurel forest)	Costa Rica, Puntarenas (Costa Ballena)	Costa Rica, Puntarenas (Costa Ballena)	Costa Rica, Guanacaste (Barra Honda)	Brazil (MG), Timoteo
Acc. Number	PP409594/PP409596	KY365504; OR210228	OR210292	OR210241; OR228883; OR210243	
Reference	This publication	[24]	[24]	[24]	[19]

N.A.: no data available.

The other species presented here belongs to *Rhizonema.* The genus is known as a cyanobiont of basidiolichens within the genus *Dictyonema*, as originally described by Lücking and Barrie in 2014. In that study, they validated and reintroduced a nomen nudum family [41] as Rhizonemataceae, characterized by lichenized cyanobacterium [1] with the only species being *Rhizonema interruptum*. To better understand the morphology of the genus, a comparative table of the morphological characteristics of the species known to date was prepared (Table 2). However, it is noteworthy that all their descriptions and 16S rRNA sequences were derived from environmental samples. *R. interruptum* exhibits cylindrical trichomes with intercalar heterocytes (12–18 μm wide and 4–8 μm long) and occasional true branching [25]. In contrast, the Canarian species also display terminal heterocytes (subcubic shaped) and intercalar heterocytes (lenticular to sublenticular shape), with longer ones measuring 6–13 μm in length and narrower heterocytes ranging from 9 to 12 μm in width. The former species described by Lücking and Barrie [25] features low rectangular vegetative cells (15–20 μm wide and 9–10 μm long), whereas the newly discovered free-living *Rhizonema* from the laurel forest exhibits lenticular to sublenticular cells with varying sizes (6–13 μm long and 9–12 μm wide). The Canarian species was studied as outlined in the Materials and Methods section, with observations conducted biweekly, and it never exhibited true branching as observed in *R. interruptum* [25]; instead, false branching was frequently observed.

Table 2. Comparison of generic and specific morphological characteristics of the species within the genus *Rhizonema*. (N.A.: not available in the publication).

	***Rhizonema melkonianarum* sp. nov**	***Rhizonema interruptum***	***Rhizonema andinum***	***Rhizonema neotropicum***
Filament width (µm)	9–12	15–20	10–12	10–15
Trichome diameter (µm)	10–14	N.A.	N.A.	N.A.
Sheath morphology	Thin, firm, closed at one end of the filament	Thin	N.A.	N.A.
Sheaths color	Hyaline	Hyaline	N.A.	N.A.
Cells colors	Green	Blue–green	Olive to blue–green	Yellowish green
Cell shape	Lenticular to sublenticular	Low rectangular	Flattened rectangular	Flattened rectangular
Cell length (µm)	6–13	5–10	5–10	5–10
Thallus form	Heteropolar and isopolar	Isopolar	Isopolar filamentous to chroococcoid	Isopolar filamentous to chroococcoid
Thallus color	Green to rare red coloration	Blue–green	Olive to blue–green	Yellowish green
Heterocytes form	Terminal: spherical to subspherical; Intercalar: lenticular to sublenticular	N.A.	N.A.	N.A.
Heterocytes (wide × long µm)	9–12 × 6–13	12–18 × 3–7	8–10 × 4–6	8–12 × 4–7
Heterocytes positions	Terminal and intercalar	Intercalar	Intercalar	Intercalar
False branching type	Double or single	N.A.	N.A.	N.A.
True branching type	N.A.	Rare	Rare	Rare
Young trichomes shape	Not constricted at the cross wall	N.A.	N.A.	N.A.
Trichomes	Slightly curved	N.A.	N.A.	N.A.
Tapering	Slightly tapering	N.A.	N.A.	N.A.
Hormogonia development	Subcubic cells without constriction at the cross–wall	Rare	Not observed	Not observed
Ecology	Subaerophytic, epiphytic on living leaves of *Laurus novocanariensis* (free-living)	Lichen–forming by *Dictyonema* spp.	Lichen—forming by *Dictyonema huaorani*; *Acantholichen sorediatus* and *Cora elephas*	Lichen—forming by *Dictyonema sericeum*; *D. haptiferum*; *Corella* ap.
Type locality	Spain, Canary Islands (Tenerife; laurel—forest)	Killarney, Turk Cascade (Ireland)	Ecuador, Costa Rica, Colombia	Guatemala, Bolivia; Colombia
Acc. Number	PP409595	N.A.	MT987440 (Ecuador); MT987460 (Costa Rica); MT987398 (Colombia)	MT987477 (Guatemala); MT987478 (Bolivia); MT987494 (Colombia)
Reference	This publication	[24]	[36]	[36]

3.4. Habitat

The genus Brasilonema was originally described in tropical and subtropical regions of Brazil [19–23]. To date, 24 new species have been identified and documented, establishing it as a pantropical genus [24]. According to Bohunicka et al., it also thrives in artificial environments such as tropical greenhouses, power-plant cooling towers, and other microhabitats that replicate subtropical to tropical conditions in non-native climatic regions. The authors also concluded that the widespread distribution of *Brasilonema* supports its classification as a pantropical genus with few geographical barriers to its dispersal. Its absence in natural habitats within temperate and polar zones may be attributed to its intolerance of desiccation and freezing winter conditions [24]. The type species, *Brasilonema bromeliae*, is epiphytic on bromeliads, and the first time the authors described it was from Brazil; that time was a novel description of the genus for the first time [21]. Other species show preferences for environments such as moist wood or stone (Table S2) as *B. tolantogense*, among others. For the first time, the genus has been discovered in the phyllosphere of the Macaronesian geographic region, growing epiphyllous on the leaves of *L. novocanariensis* from the Parque Rural de Anaga close to Reserva Natural El Pijaral, a special area within the laurel forest of the island of Tenerife in the Canarian archipelago. It is designated here as *Brasilonema* *novocanariensis* sp. nov., identified as a free-living cyanobacterium, alongside the newly described *Rhizonema melkonianarum* sp. nov. in this study. Until the present study, the *Rhizonema* genus was found exclusively within the consortium of basidiolichens (Table 2). This work broadens the understanding by showing the possibility of encountering it also as a free-living cyanobacterium colonizing the phyllosphere of *L. novocanariensis* tree.

3.5. Taxonomic Description

Class: Cyanophyceae
Order: Nostocales
Family: Scytonemataceae

Brasilonema. novocanariensis **N.M.Rancel-Rodríguez, C.W.Vieira, and M.Sansón sp. nov.**

Diagnosis: *Brasilonema novocanariensis* differs significantly from B. bambusae, *B. palmarum*, and *B. calcareum* from Costa Rica in several key morphological and ecological traits. *B. novocanariensis* is characterized by its narrower filament width and trichome diameter, as well as differences in cell shape and color. In particular, its cell length varies notably from those of the other three species. *Brasilonema novocanariensis* exhibits a more heteropolar thallus, in contrast to the isopolar thalli observed in *B. bambusae*, *B. palmarum*, and *B. calcareum*. Additionally, the heterocytes of *B. novocanariensis* show unique differences in both shape and size compared to those of *the Costa Rican species* (see Table 1). Ecologically, while all four species exhibit subaerophytic characteristics, *B. novocanariensis* is found exclusively in the phyllosphere, growing on the undamaged leaves of *Laurus novocanariensis* in the Canary Islands. In contrast, *B. bambusae* thrives on bamboo trunks, *B. palmarum* on coconut palms, and *B. calcareum* on limestone substrates. Overall, *B. novocanariensis* is set apart by its lenticular cell shape, narrower trichomes, violet thallus coloration, and specific ecological association with L. novocariensis, underscoring its status as a distinct species.

Description: Thallus-dense, prostrate, or erect red–brown filaments in culture. Filaments with sheath closed at one end, fasciculate, straight, with Scytonemataceae-type false branching. Filaments 17–19 µm wide. Sheath thin, firm, hyaline. Trichomes cylindrical, straight, not constricted at cross-wall, slightly tapering, 10–17 µm wide. Heteropolar rarely isopolar-free trichomes. Cells lenticular to sublenticular (wider than long), brown–red to green in color, granulated, vacuolated, 5–9 µm long × 10–17 µm wide. Apical cells rounded; calyptra not present. Heterocytes common, terminal (spherical to subspherical), and intercalar (lenticular to sublenticular), 7–10 µm wide × 7–9 µm long. Akinete not present. Reproduction by motile hormogonia (subcubic cells without constriction at the cross wall) (Figure 7).

Holotype: TFC-Phyc 16969 (Herbario TFC Universidad de la Laguna, Tenerife, Canary Island, Spain).

Type locality: The Anaga Rural Park, located on the island of Tenerife in the Canary Islands, Spain; 28°32′57″ N–16°11′09″ W.

Habitat: Colonizing the phyllosphere of the laurel tree *Laurus novocanariensis.*

Etymology: "novocanariensis" (L.) is derived from the discovery substrate, the leaf of the *L. novocanariensis* tree.

Reference strain: BEA 1760 B (Banco Español de Algas, Gran Canaria, Canary Islands, Spain).

Isotypes: TFC-Phyc 16970; BEA 1761 B; CCAC 7000 B and CCAC 7001 B

GenBank accession numbers: 16S rRNA: PP409594 (BEA 1760B); PP409596 (BEA1761B).

Class: Cyanophyceae
Order: Nostocales
Family: Rhizonemataceae

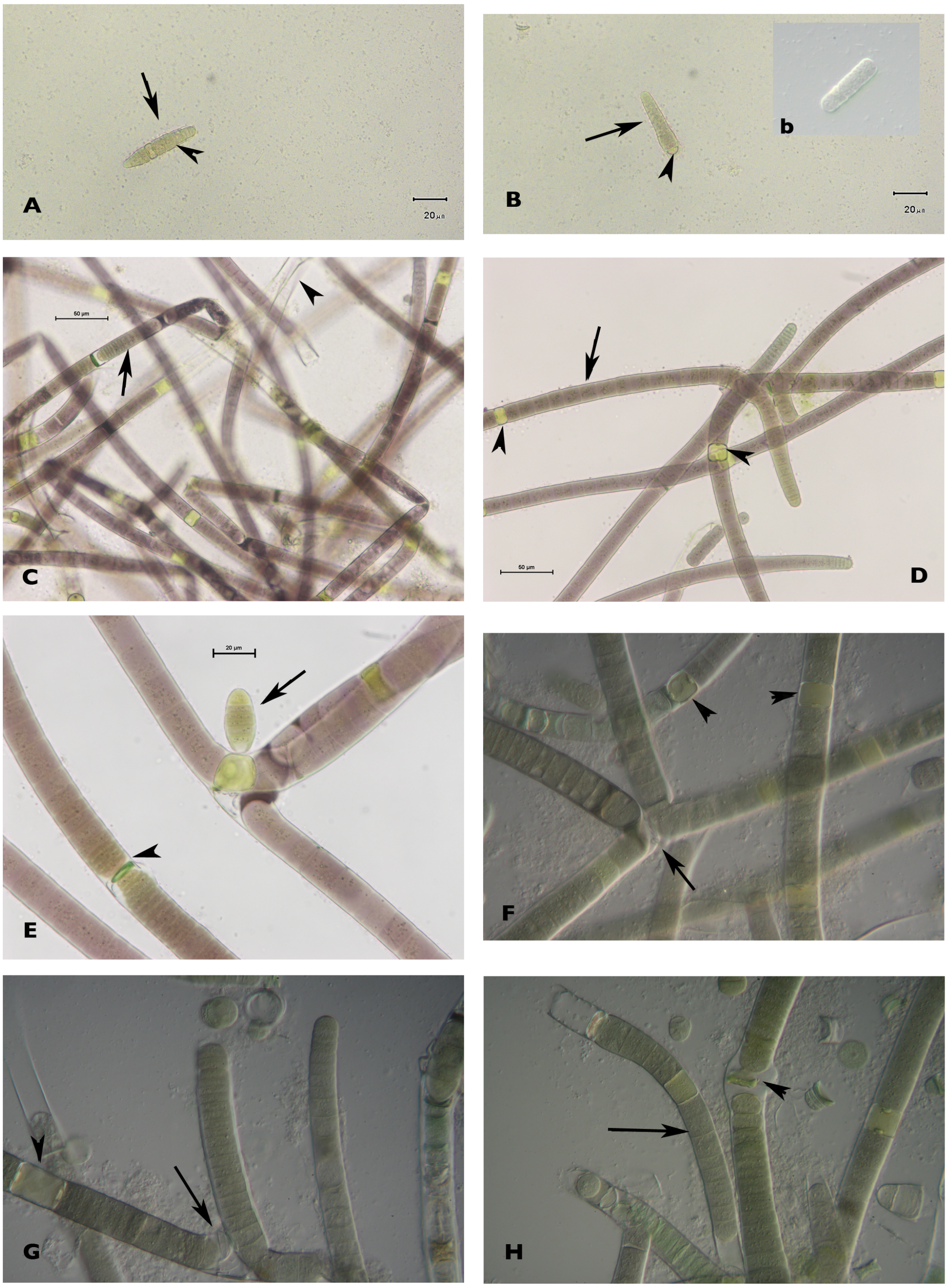


Figure 7. Photographic documentation of *Brasilonema. novocanariensis* sp. nov. BEA 1760B and 1761B (PP409596 and PP409594, respectively). (**A**) Isopolar filament (arrow) and lenticular to sublenticular vegetative cells (arrowhead). Intercalar heterocyte with sublenticular shape. (**B**) Heteropolar filament (arrow). Terminal heterocyte (arrowhead). (**b**) Hormogonia are characterized by subcubic cells. (**C**) Formation of a hormogonium within the sheath (arrow). Empty sheath (arrowhead). (**D**) Filament showing lenticular to sublenticular vegetative cells (arrow). Intercalar heterocytes exhibiting lenticular to sublenticular morphology (arrowheads). (**E**) Short filament (arrow). Necrotic cells (arrowheads). (**F**) False branch arising next to a necrotic cell distant from an intercalar heterocyte (arrow). Intercalar heterocytes (arrowheads). (**G**) False-branch (arrow). Intercalar heterocyte (arrowhead). (**H**) Green, uniseriate filament (arrow). False branch arising next to a necrotic cell, initial steps (arrowhead). Scale bar: 20 µm (**A**,**B**,**E**,**F**,**G**,**H**), 50 µm (**C**,**D**).

Rhizonema melkonianarum **N.M.Rancel-Rodríguez, C.W.Vieira and M.Sansón sp. nov.**

Diagnosis: *Rhizonema melkonianarum* sp. nov. differs from *Rhizonema interruptum* in several morphological and ecological characteristics. *R. melkonianarum* is described by a distinct width and trichome diameter, along with variations in cell shape and color. In particular, consistent cell size in the terminal parts of the trichome, and cell length show differences between both species. Furthermore, *R. melkonianarum* presents longer heterocysts compared to the wider ones in *R. interruptum*. Ecologically, *R. melkonianarum* was identified from a population of free-living cyanobacteria isolated from the phyllosphere of *L. novocanariensis*, a phenomenon not previously observed in this genus. Hence, we consider this fact to be significant, underscoring the importance of using a free-living population as the type species for the genus.

Description: Thallus-dense, prostrate, or erect green filaments in culture. Filaments with sheath closed at one end, fasciculate, straight or slightly bent, with Scytonemataceae-type false branching (true branching type not shown). Filaments 9–12 µm wide. Sheath thin, firm, hyaline, close at one end. Trichomes cylindrical, straight, not constricted at cross-wall, slightly tapering, 10–14 µm wide. Heteropolar and isopolar free trichomes. Cells lenticular to sublenticular (wider than long), green in color, granulated, 6–13 µm long × 4–12 µm wide. Apical cells rounded; calyptra not present. Heterocytes common, terminal (spherical to subspherical) and intercalar (lenticular to sublenticular), 9–12 µm wide × 6–13 µm long. Akinete not present. Reproduction by motile hormogonia (subcubic cells without constriction at the cross wall) (Figure 8).

Holotype: TFC-Phyc 16968 (Herbario TFC Universidad de la Laguna, Tenerife, Canary Island, Spain).

Type locality: The Anaga Rural Park, located on the island of Tenerife in the Canary Islands, Spain, 28°32′57″ N–16°11′09″ W.

Habitat: Colonizing the phyllosphere of the laurel tree *Laurus novocanariensis*.

Etymology: "melkonianarum" (L.) is an epithet that honors the couple Michael and Barbara Melkonian.

Reference strain: BEA 1759B (Banco Español de Algas, Gran Canaria, Canary Islands, Spain)

Isotypes: CCAC 6999B

GenBank accession numbers: 16S rRNA: PP409595.

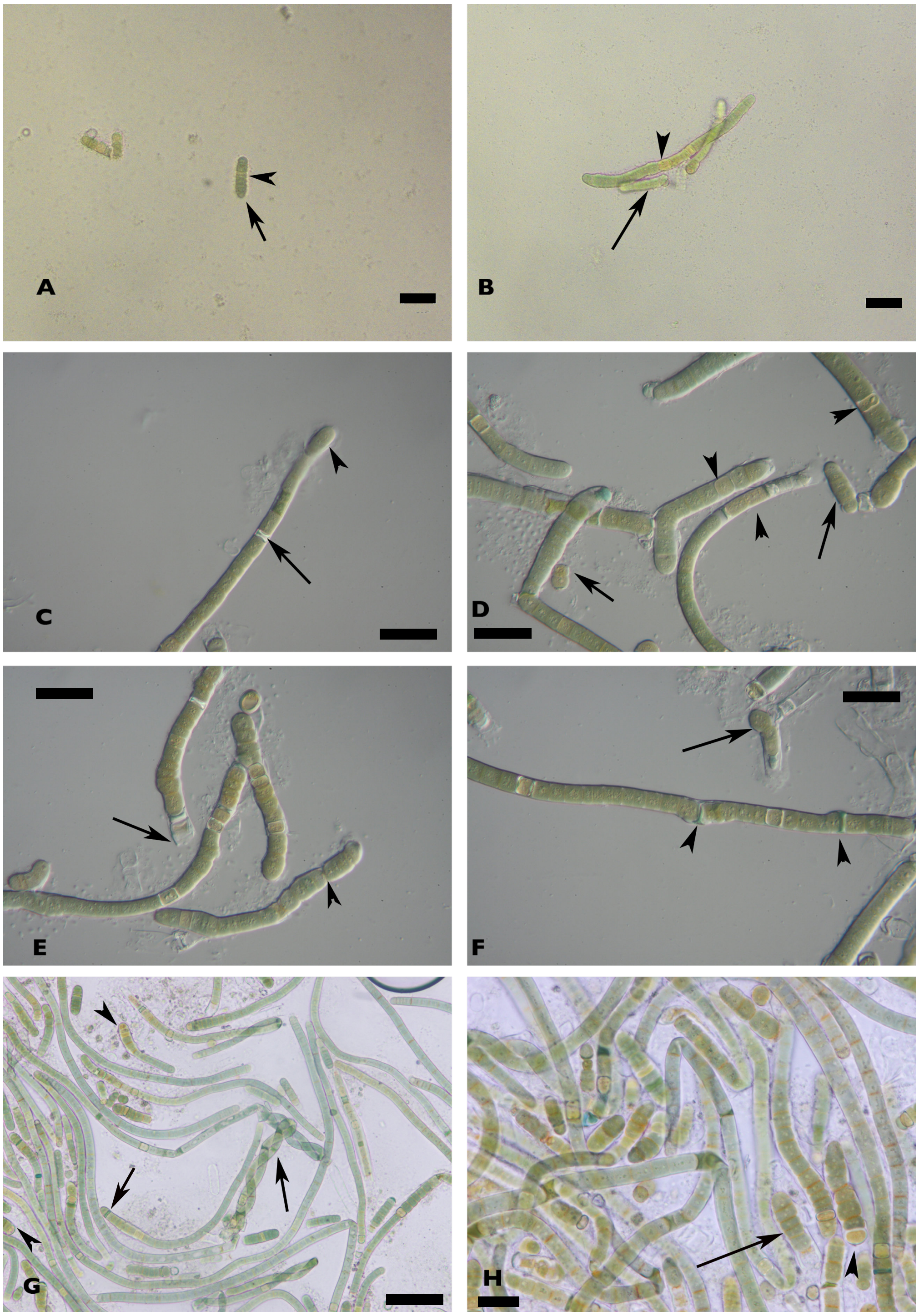


Figure 8. Photographic documentation of *Rhizonema melkonianarum* sp. nov. BEA 1759B (PP409595). Heteropolar as well as isopolar, uniseriate, straight filaments. (**A**) Hormogonium with subcubic cells (arrow). (**B**) Hormogonium (arrow) Vegetative cell transforming into a intercalary heterocyste (arrowhead). (**C**) Necrotic cell (arrow). Hormogonium falling separating from the apex (arrowhead). (**D**) Hormogonia (arrow). Intercalar heterocytes showing different morphologies (arrowhead). (**E**) Firm sheath (arrow). False branch initial step development (arrowhead). (**F**) Necrid cell (arrow). False branch formation (arrowhead). (**G**) Green filament growth into the sheath (arrow). Subcubic terminal heterocyte at one end of the filament (arrowhead). (**H**) Filaments are composed of lenticular to sublenticular vegetative cells (arrow). Scale bar: 20 µm (**A**,**B**,**H**), 50 µm (**C**,**D**,**E**,**F**,**G**).

4. Discussion

This study unveils two novel cyanobacterial taxa from the laurel forest (laurisilva) of the Canary Islands, expanding our understanding of cyanobacterial diversity and their ecological roles. The findings include *Brasilonema novocanariensis* sp. nov., a newly documented cyanobacterium in the Macaronesian region, and *Rhizonema melkonianarum* sp. nov., which marks a significant ecological transition within the genus *Rhizonema*. Below, we discuss the taxonomic, geographic, and ecological implications of these discoveries.

4.1. Brasilonema novocanariensis sp. nov.

4.1.1. Taxonomic Insights

Brasilonema novocanariensis sp. nov. represents a significant addition to the genus *Brasilonema*, known for its adaptability to diverse environments [7,8,19–24,39,40]. It is characterized by unique features, including vertical fascicles of trichomes, false branching, and a sub-aerophilic habitat [19–24]. Morphologically, this species fits within the characteristics of the genus but also presents unique features that warrant its classification as a new species (Table 1; Table S2), based on morphological traits that do not perfectly match with *Brasilonema octagenarum* described from Brazil by Aguiar et al., [19], or with *B. bambusae and*, *B. palmarum* by [24]. Although genetically related to these species (Figure 3. OR210228; KY 365504; OR210292), *B. novocanariensis* is not identical. According to Bohunicka et al. [24], in the group division, the novel species belongs to the *Octagenarum* group (Figure 3, indicated by the blue bar on the right side). A comparison between *B. novocanariensis* from the Canaries and the species documented by [24], collected from Costa Rica, based on the results presented in Table 1, reveals notable differences in measurements and some morphological characters. The use of 16S rRNA gene sequencing and the 16S–23S rRNA ITS region secondary structure (Figures 4 and 5) have confirmed its distinctiveness from other *Brasilonema* species, solidifying its taxonomic status [19–23].

4.1.2. Geographic Distribution

The discovery of *B. novocanariensis* in the Canary Islands marks the first documentation of this genus in the Macaronesian region. Previously, *Brasilonema* species were predominantly recorded in tropical and subtropical regions of Brazil [21] and other parts of North and South America [8]. Recently, 24 new species have been identified by [24], including some from temperate regions such as the Czech Republic and Germany. This novel finding significantly extends the known geographic range of the genus, indicating its potential for wider distribution than previously understood.

4.1.3. Ecological Adaptations

Brasilonema has been described in a wide variety of habitats, hosting several species/lineages. These include subaerophytic concrete, cave walls, terrestrial soil, temporary water holes, moss, epiphytes on Bromeliaceae, the phyllosphere of angiosperms, periphytic rocks, cyanobionts, and rock walls, underscoring their adaptability to various ecological niches (Table S2) [19,21,24,39]. Of particular interest is our focal species, *B. octagenarum*, which was primarily documented inhabiting the phyllosphere of angiosperms [19], recorded mainly in Brazil, with additional sightings in the USA (Hawaii) and Ukraine [7,8,22]. *Brasilonema novocanariensis* thrives epiphyllously on the leaves of *Laurus novocanariensis* within the laurel forest of Tenerife. The phyllosphere, or leaf surface, serves as a unique habitat that supports its growth. This species was first described from the phyllosphere of *E. grandis* in Brazil as *B. octagenarum*, where it was found on damaged leaves [19]. In contrast, the species found in the Canary Islands was never observed on damaged leaves, but on healthy ones. This discovery of a new tree leaf colonization highlights the ecological flexibility of *Brasilonema*, which has been documented in various habitats, including sub-aerophytic concrete, cave walls, and the phyllospheres of other angiosperms. The potential introduction routes of *B. novocanariensis*, possibly linked to historical *Eucalyptus* imports from Brazil [42,43], highlight the intricate pathways of cyanobacterial dispersal and colonization.

4.2. Rhizonema melkonianarum sp. nov.

4.2.1. Taxonomic Insights

Rhizonema melkonianarum sp. nov. is a newly described species within the family Rhizonemataceae. This species is distinguished by its lack of true branching and its unique morphological traits, which differ from other *Rhizonema* species traditionally associated with basidiolichens (Table 2). Molecular analysis, particularly the 16S rRNA gene sequencing, has been pivotal in confirming its taxonomic placement and distinctiveness from other known *Rhizonema* species. An interesting commonality of all species in this genus is their role as photobionts for various basidiomycetes [25,37,38]. The newly isolated sequence from the *L. novocanariensis* phyllosphere appears to be related to a photobiont species described by Lücking, associated with a basidiolichen from Tenerife within the genus *Dictyonema*, although the cyanobacteria-specific species are identified directly from the basidiolichen association as *Rhizonema interruptum* with a short sequence and only in the supplementary material of [37]. The authors [25] described the species type of this genus as *R. interruptum* from a waterfall in Ireland, not from the Canarian archipelago, as the typical photobiont from the lichen-forming fungi *Dictyonema coppinsii* Lücking and Barrie, but the accession number of the cyanobacteria was not available on their publication or Genbank. Comparing the morphology of the free-living isolate on the laurel leaf with the original description reveals differentiating morphological characteristics (Table 2). Additionally, the only available sequence for the 16S rRNA gene in Genbank is just 211 bp [37], which limits the comparison. However, it is important to emphasize that, as mentioned in their publication [25], the description was not based on clonal cultures. Similarly, in [37], for *R. neotropicum* or *R. andinum,* the sequences of the photobiont belong to the environmental lichen-forming and not from a cyanobacteria clonal culture. This paper is also the first record of the 16S–23S rRNA ITS region secondary structure (Figure 6) for the genus.

4.2.2. Geographic Distribution

The identification of *R. melkonianarum* sp. nov. in the Canary Islands represents a notable geographic extension for the genus *Rhizonema*, primarily known from another European island (Ireland), Central and South America, and some parts of Africa [37,38]. The presence of this species in the Canary Islands emphasizes the region's role as a hotspot for cyanobacterial diversity and biogeographic dispersal.

4.2.3. Ecological Adaptations

Rhizonema melkonianarum sp. nov. exhibits a significant ecological shift from its genus counterparts by inhabiting the phyllosphere of *Laurus novocanariensis*, rather than its usual association with basidiolichens [25,37,38]. This adaptation highlights the species' ecological flexibility and the potential for cyanobacteria to transition between different ecological niches. The ability of *R. melkonianarum* sp. nov. to colonize the phyllosphere underscores its potential role in the ecosystem dynamics of the laurel forest.

4.3. Future Research Directions and Conclusions

This research highlights the importance of investigating cyanobacterial diversity in unique habitats like the laurisilva of the Canary Islands. Future studies should further investigate the cyanobacterial diversity associated with the laurisilva phyllosphere, as well as focus on elucidating the ecological roles and interactions of *Brasilonema* and *Rhizonema* species with their host organisms and the broader ecosystem. Investigating the potential for natural or human-mediated dispersal of these cyanobacteria will provide valuable insights into their colonization capabilities and evolutionary adaptations.

In conclusion, the discovery of *Brasilonema novocanariensis* sp. nov. and *Rhizonema melkonianarum* sp. nov. broadens our understanding of cyanobacterial diversity and their ecological roles in terrestrial environments. These findings underscore the adaptability of cyanobacteria in diverse habitats and highlight the significance of the Canary Islands as a region of interest for future cyanobacterial research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16100625/s1, Table S1. Morphological comparison of accepted Brasilonema species; Table S2: Comparison of *Brasilonema novocanariensis* sp. nov. and related species.

Author Contributions: Conceptualization, N.M.R.-R., C.V. and M.S.; methodology, N.M.R.-R.; software, N.M.R.-R. and C.V.; validation, N.M.R.-R., C.V. and M.S.; formal analysis, N.M.R.-R. and C.V.; investigation, N.M.R.-R.; resources, N.M.R.-R. and C.V.; data curation N.M.R.-R. and C.V.; writing—original draft preparation, N.M.R.-R.; writing—review and editing, N.M.R.-R. and C.V.; visualization, N.M.R.-R. and C.V.; supervision, N.M.R.-R., C.V. and M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The culture collection data are available in Banco Español de Algas (https://marinebiotechnology.org/es/) and Central Collection of Algal Cultures (CCAC https://www.uni-due.de/biology/ccac/). The genome sequence data that support the findings of this study are openly available in GenBank of the NCBI at https://www.ncbi.nlm.nih.gov (accessed on 28 February 2024) under accession no. PP409595, PP99409596 and PP459594.

Acknowledgments: The first author acknowledges Michael Melkonian for his invaluable guidance and mentorship throughout her doctoral studies under his supervision. Additionally, she acknowledges Barbara Melkonian for her mentorship in microalgae culture collection techniques, unwavering support, and warmth during her time in Cologne, as well as her role as a reference in her scientific journey and enduring friendship.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Strunecký, O.; Ivanova, A.P.; Mareš, J. An updated classification of cyanobacterial orders and families based on phylogenomic and polyphasic analysis. *J. Phycol.* **2023**, *59*, 12–51. [CrossRef] [PubMed]
2. McGregor, G.B.; Sendall, B.C. Cyanobacterial diversity and taxonomic uncertainty: Polyphasic pathways to improved resolution. In *Advances in Phytoplankton Ecology*, 1st ed.; Clementson, L., Eriksen, R., Willis, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 7–45.
3. Singh, P.K.; Kumar, A.; Singh, V.K.; Shrivistava, A.K. (Eds.) *Advances in Cyanobacterial Biology*, 3rd ed.; Academic Press: London, UK, 2020; pp. 154–196.
4. Gaysina, L.A.; Saraf, A.; Singh, P. Cyanobacteria in diverse habitats. In *Cyanobacteria*; Singh, P.K., Kumar, A., Singh, V.K., Shrivistava, A.K., Eds.; Academic Press: London, UK, 2019; pp. 1–28.
5. Genuário, D.B.; Vaz, M.G.; Santos, S.N.; Kavamura, V.N.; Melo, I.S. Cyanobacteria from Brazilian extreme environments: Toward functional exploitation. In *Microbial Diversity in the Genomic Era*; Tiquia-Arashiro, S.M., Rodrigues, D.F., Eds.; Academic Press: London, UK, 2019; pp. 265–284.
6. Dvořák, P.; Poulíčková, A.; Hašler, P.; Belli, M.; Casamatta, D.A.; Papini, A. Species concepts and speciation factors in Cyanobacteria, with connection to the problems of diversity and classification. *Biodivers. Conserv.* **2015**, *24*, 739–757. [CrossRef]
7. Vaccarino, M.A.; Johansen, J.R. *Brasilonema angustatum* sp. nov. (Nostocales), a new filamentous cyanobacterial species from the Hawaiian islands. *J. Phycol.* **2012**, *48*, 1178–1186. [CrossRef] [PubMed]
8. Villanueva, C.D.; Hašler, P.; Dvořák, P.; Poulíčková, A.; Casamatta, D.A. *Brasilonema lichenoides* sp. nov. and *Chroococcidiopsis lichenoides* sp. nov. (Cyanobacteria): Two novel cyanobacterial constituents isolated from a tripartite lichen of headstones. *J. Phycol.* **2018**, *54*, 224–233. [CrossRef]
9. Cordeiro, R.; Luz, R.; Vasconcelos, V.; Gonçalves, V.; Fonseca, A. Cyanobacteria Phylogenetic Studies Reveal Evidence for Polyphyletic Genera from Thermal and Freshwater Habitats. *Diversity* **2020**, *12*, 298. [CrossRef]
10. Mühlsteinová, R.; Hauer, T. Pilot survey of cyanobacterial diversity from the neighborhood of San Gerardo de Rivas, Costa Rica with a brief summary of current knowledge of terrestrial cyanobacteria in Central America. *Braz. J. Bot.* **2013**, *36*, 299–307. [CrossRef]
11. Komárek, J. Modern classification of cyanobacteria. In *Cyanobacteria: An Economic Perspective*; Sharma, N.K., Rai, A.K., Stal, L.J., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2014; pp. 21–39.
12. Komárek, J. A polyphasic approach for the taxonomy of cyanobacteria: Principles and applications. *Eur. J. Phycol.* **2016**, *51*, 346–353. [CrossRef]
13. Guiry, M.D.; Guiry, G.M. AlgaeBase. World-Wide Electronic Publication, University of Galway. 2024. Available online: https://www.algaebase.org (accessed on 1 October 2023).

14. Willame, R.; Boutte, C.; Grubisic, S.; Wilmotte, A.; Komárek, J.; Hoffmann, L. Morphological and molecular characterization of planktonic cyanobacteria from Belgium and Luxembourg. *J. Phycol.* **2006**, *42*, 1312–1332. [CrossRef]
15. Rajaniemi, P.; Hrouzek, P.; Kaštovska, K.; Willame, R.; Rantala, A.; Hoffmann, L.; Sivonen, K. Phylogenetic and morphological evaluation of the genera Anabaena, Aphanizomenon, Trichormus and Nostoc (Nostocales, Cyanobacteria). *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 11–26. [CrossRef]
16. Whitton, B.A. (Ed.) *Ecology of Cyanobacteria II: Their Diversity in Space and Time*; Springer Science & Business Media: Dordrecht, The Netherlands, 2012; 760p. [CrossRef]
17. Turland, N.J.; Wiersema, J.H.; Barrie, F.R.; Greuter, W.; Hawksworth, D.L.; Herendeen, P.S.; Smith, G. *International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code), Proceedings of the Nineteenth International Botanical Congress Shenzhen, China, 29 July 2017*; Koeltz Botanical Books: Oberreifenberg, Germany, 2018. [CrossRef]
18. Álvarez, C.; Jiménez-Ríos, L.; Iniesta-Pallarés, M.; Jurado-Flores, A.; Molina-Heredia, F.P.; Ng, C.K.Y.; Mariscal, V. Symbiosis between cyanobacteria and plants: From molecular studies to agronomic applications. *J. Exp. Bot.* **2023**, *74*, 6145–6157. [CrossRef]
19. Aguiar, R.; Fiore, M.F.; Franco, M.W.; Ventrella, M.C.; Lorenzi, A.S.; Vanetti, C.A.; Alfenas, A.C. A novel epiphytic cyanobacterial species from the genus *Brasilonema* causing damage to Eucalyptus leaves. *J. Phycol.* **2008**, *44*, 1322–1334. [CrossRef] [PubMed]
20. Barbosa, M.; Berthold, D.E.; Lefler, F.W.; Laughinghouse, H.D., IV. Diversity of the genus *Brasilonema* (Nostocales, Cyanobacteria) in plant nurseries of central Florida (USA) with the description of three new species: *B. fioreae* sp. nov., *B. santannae* sp. nov. and *B. wernerae* sp. nov. *Fottea* **2021**, *21*, 82–99. [CrossRef]
21. Fiore, M.F.; Sant'Anna, C.L.; Azevedo, M.T.P.; Komárek, J.; Kaštovský, J.; Sulek, J.; Lorenzi, A.S. The cyanobacterial genus *Brasilonema*, gen. nov., a molecular and phenotypic evaluation. *J. Phycol.* **2007**, *43*, 789–798. [CrossRef]
22. Rodarte, B.; Becerra-Absalon, I.; Montejano, G.A.; Osorio-Santos, K.; Alba-Lois, L.; Leon-Tejera, H.; Segal-Kischinevzky, C. Morphological and molecular characterization of *Brasilonema roberti-lamii* (Cyanophyceae, Nostocales, Scytonemataceae), from Central Mexico. *Phytotaxa* **2014**, *164*, 255–264. [CrossRef]
23. Sant'Anna, C.L.; Azevedo, M.P.; Fiore, M.D.F.; Lorenzi, A.S.; Kaštovský, J.; Komárek, J. Subgeneric diversity of *Brasilonema* (Cyanobacteria, Scytonemataceae). *Braz. J. Bot.* **2011**, *34*, 51–62. [CrossRef]
24. Bohunická, M.; Johansen, J.R.; Villanueva, C.D.; Mareš, J.; Štenclová, L.; Becerra-Absalón, I.; Hauer, T.; Kaštovský, J. Revision of the pantropical genus *Brasilonema* (Nostocales, Cyanobacteria), with the description of 24 species new to science. *Fottea* **2024**, *24*, 137–184. [CrossRef]
25. Lücking, R.; Barrie, F.R.; Genney, D. *Dictyonema coppinsii*, a new name for the European species known as Dictyonemainterruptum (Basidiomycota: Agaricales: Hygrophoraceae), with a validation of its photobiont Rhizonema (Cyanoprokaryota: Nostocales: Rhizonemataceae). *Lichenologist* **2014**, *46*, 261–267. [CrossRef]
26. Nogué, S.; de Nascimento, L.; Fernández-Palacios, J.M.; Whittaker, R.J.; Willis, K.J. The ancient forests of La Gomera, Canary Islands, and their sensitivity to environmental change. *J. Ecol.* **2013**, *101*, 368–377. [CrossRef]
27. del Arco Aguilar, M.J.; Rodríguez Delgado, O.; del Arco Aguilar, M.J.; Rodríguez Delgado, O. *Vegetation of the Canary Islands*; Springer International Publishing: Berlin/Heidelberg, Germany, 2018; pp. 83–319. [CrossRef]
28. Stanier, R.Y.; Kunisawa, R.; Mandel, M.C.B.G.; Cohen-Bazire, G. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev.* **1971**, *35*, 171–205. [CrossRef]
29. Surek, B.; Melkonian, M. CCAC Culture Collection of Algae at the University of Cologne: A new collection of axenic algae with emphasis on flagellates. *Nova Hedwig.* **2004**, *79*, 77–92. [CrossRef]
30. Marin, B.; Nowack, E.C.; Melkonian, M. A plastid in the making: Evidence for a second primary endosymbiosis. *Protist* **2005**, *156*, 425–432. [CrossRef] [PubMed]
31. Maddison, W.P.; Maddison, D.R. Mesquite: A Modular System for Evolutionary Analysis. Version 3.81. Available online: http://www.mesquiteproject.org (accessed on 16 May 2024).
32. Gouy, M.; Tannier, E.; Comte, N.; Parsons, D.P. *Seaview Version 5: A Multiplatform Software for Multiple Sequence Alignment, Molecular Phylogenetic Analyses, and Tree Reconciliation*; Methods in Molecular Biology; Humana: New York, NY, USA, 2021; Volume 2231, pp. 241–260. [CrossRef]
33. Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **2003**, *31*, 3406–3415. [CrossRef] [PubMed]
34. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef]
35. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef]
36. Posada, D.; Crandall, K.A. Felsenstein Phylogenetic Likelihood. *J. Mol. Evol.* **2021**, *89*, 134–145. [CrossRef]
37. Dal Forno, M.; Lawrey, J.D.; Sikaroodi, M.; Gillevet, P.M.; Schuettpelz, E.; Lücking, R. Extensive photobiont sharing in a rapidly radiating cyanolichen clade. *Mol. Ecol.* **2021**, *30*, 1755–1776. [CrossRef]
38. Masumoto, H.; Sanders, W.B. The lichen photobiont genus *Rhizonema* (Cyanobacteria) exhibits diverse modes of branching, both false and true. *J. Phycol.* **2022**, *58*, 612–625. [CrossRef] [PubMed]
39. Becerra-Absalon, I.; Rodarte, B.; Osorio, K.; Alba-Lois, L.; Segal-Kischinevzky, C.; Montejano, G. A new species of *Brasilonema* (Scytonemataceae, Cyanoprokaryota) from Tolantongo, Hidalgo, Central Mexico. *Fottea Olomouc.* **2013**, *13*, 25–38. [CrossRef]

40. Miscoe, L.H.; Johansen, J.R.; Vaccarino, M.A.; Pietrasiak, N.; Sherwood, A.R. II. Novel cyanobacteria from caves on Kauai, Hawaii. *The Diatom Flora and Cyanobacteria from Caves on Kauai, Hawaii* Bibliotheca Phycologica. **2016**, *Volume 123*, 75–152.
41. Büdel, B.; Kauff, F. Prokaryotic Algae, Blue-green Algae. In *Syllabus of Plant Families*, 13th ed.; Frey, W., Ed.; Borntraeger: Stuttgart, Germany, 2012; Part 1/1; pp. 5–40.
42. Silva-Pando, F.J. Eucalyptus in South Europe: Searching for the Promised Land–Introduction and Dissemination of Eucalyptus in Southwestern Europe. In *Invasive Alien Species: Observations and Issues from Around the World*; Wiley: Hoboken, NJ, USA, 2021; Volume 3, pp. 278–316.
43. Arevalo, J.R.; Delgado, J.D.; Fernández-Palacios, J.M. Regeneration of potential laurel-forest under a native canopy vs. exotic canopy, Tenerife (Canary Islands). *For. Syst.* **2011**, *20*, 255–265. [CrossRef]