

## Article

# *Drouetiella elegans* (Oculatellaceae, Cyanobacteriota): A New Species of the Recently Established Genus from the Russian Arctic

Denis Davydov \*  and Anna Vilnet 

Polar-Alpine Botanic Garden-Institute—Separate Subdivision of Federal Research Centre ‘Kola Science Centre’, 184209 Apatity, Russia; a.vilnet@ksc.ru

\* Correspondence: d.davydov@ksc.ru

## Abstract

The species diversity of the recently described genus *Drouetiella* (Oculatellaceae, Cyanobacteriota), including thin filamentous cyanobacteria, has been rapidly increasing due to the subsequent discovery of new-to-science species in the last several years. This study focuses on one more strain that was isolated from a small lake on the Chukotka Peninsula in the Russian Arctic and tested by an integrative approach. In the result of molecular phylogenetic analysis of the 16S rRNA gene and the 16S–23S ITS rRNA region, this strain was found in a separate clade in the genus *Drouetiella*. The strain is characterized by unique secondary structures of conserved regions D1–D1′, Box–B, V2, and V3 helices of the 16–23S ITS rRNA. Morphologically, the tested strain was distinct from other *Drouetiella* species by long cells in mature trichomes, absence of false branching, and hormogonia. *Drouetiella elegans* occurred in the plankton of a small lake and shared ecological similarities with some aquatic strains of *Drouetiella lurida*. We provide a taxonomic description of a new species, *Drouetiella elegans* sp. nov.

**Keywords:** the Arctic; diversity; filamentous cyanobacteria; molecular phylogeny; 16S rRNA; 16S–23S ITS rRNA



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## 1. Introduction

Thin filamentous cyanobacteria are among the most easily cultivated groups, which is why research into their diversity and phylogeny is progressing rapidly. However, the limited set of differentiating features and their high plasticity make it impossible to morphologically identify not only species but also entire genera. The integrative approach is the most reliable method for studying their diversity and refining the systematics of this group. Only five years have passed since the family Oculatellaceae Mai & J.R. Johansen was separated in 2018 [1] on the basis of the previously described five genera and six new ones, and it was separated into an independent order Oculatellales Strunecký & Mareš [2] with 17 genera. At present, the family Oculatellaceae includes 24 genera with 66 species [3,4]. The most species-rich genus is *Oculatella* Zammit, Billi & Albertano, which, since its description in 2012 [5], counts 14 species. The younger genera *Timaviella* Sciuto & Moro [6], *Pegethris* Mai, J.R. Johansen & Bohunická [1], and *Albertania* Zammit [7] each include six species. While the 14 genera are presently monotypic, the rapid pace of biodiversity discovery and the frequent description of new species in recent genera make it certain that the genera of the Oculatellaceae family will be expanded with new species.

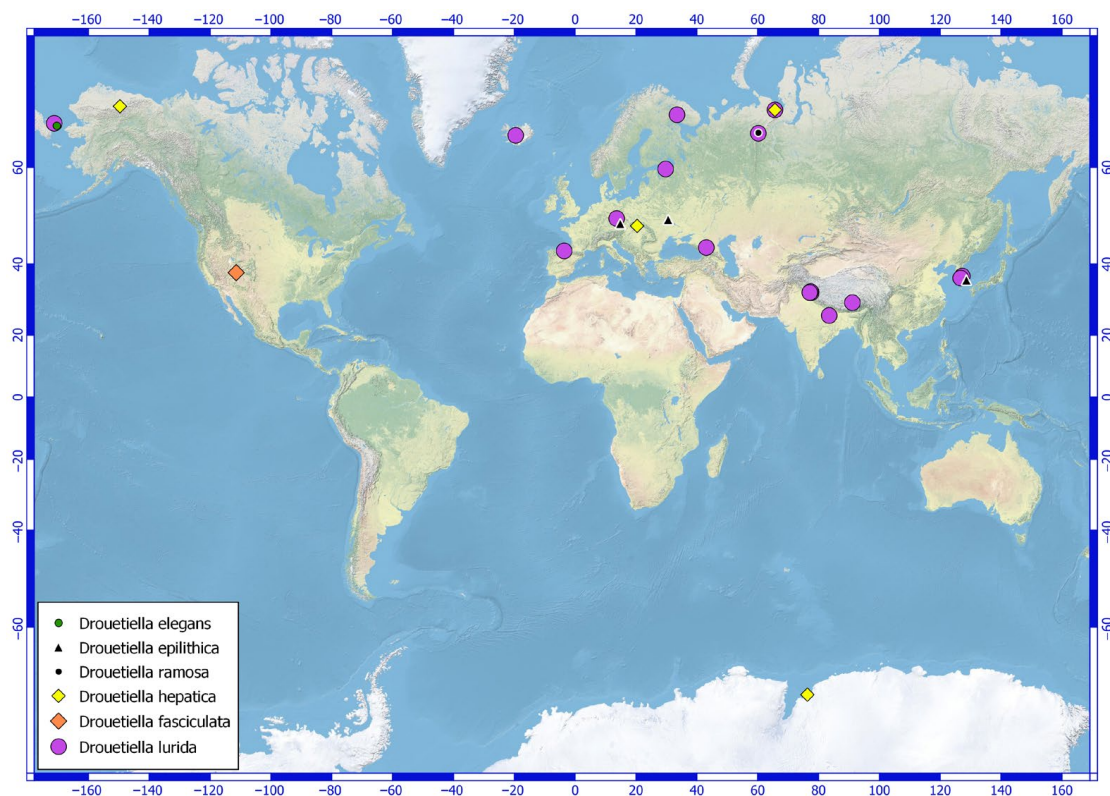
The genus *Drouetiella* can serve as a successful example of increasing species diversity by describing new taxa from diverse biomes and ecotopes. This genus is represented by thin, solitary filamentous, often with false-branching trichomes covered by colorless sheaths that are untapered and slightly constricted at the cross-walls. *Phormidium luridum* Gomont (*Leptothrix lurida* Kützing) with the type strain Lukesova 1986/6 from the tailings of Cainozoic clay in the Czech Republic was chosen as the type of species of the genus *Drouetiella* [1]. Simultaneously, *Drouetiella fasciculata* Mai, J.R. Johansen & Bohunická from a large seep wall and waterfall in Navajo, Utah, U.S.A., and *D. hepatica* Mai, J.R. Johansen & Pietrasiak from subaerial limestone in the National Park Slovak Paradise were described [1]. Five years later, the description of two new species was published: *Drouetiella ramosa* D. Davydov, Vilnet, Novakovskaya & Patova in biological soil crusts from the Urals, Russia [8], and *D. epilithica* D.-H. Kim, N.-J. Lee, H.-R. Wang, A.S. Lim & O.-M. Lee on a stone monument from the Republic of Korea [9].

The study presents the results of an integrative analysis of a strain isolated from a small lake on the Chukotka Peninsula in the Russian Arctic. The strain's morphological and ecological traits were examined, and molecular analyses were performed using 16S rRNA and 16–23S internal transcribed spacer (ITS) sequences. Based on the results of a comprehensive approach, one more new species of the genus *Drouetiella* has been proposed.

## 2. Materials and Methods

### 2.1. Origin of the Strain and Culture Conditions

A strain of *Drouetiella* was isolated from a small, unnamed lake (66°10.591' N, –170°22.359' E) located in the lower course of the Uuchynveem River (Figure 1). The lake is situated 20 km west of the Uelen rural locality, Chukotsky District, Chukotka Autonomous Area, Russia.



**Figure 1.** Distribution of molecularly confirmed *Drouetiella* specimens across various geographic regions.

Field samples were collected by Dr. E.V. Chemeris on 8 August 2019. The natural sample consisted of hollow translucent mucous balls found in the plankton. The sample was dried in a sterile paper bag and later transferred to liquid Z8 medium in a flask in the laboratory, following the method described by Kotai [10] and Waterbury [11]. The accumulated culture was initially seeded onto solid BG-11 medium in Petri dishes. Unialgal cultures were subsequently obtained by isolating material from the edges of discrete colonies that had been growing for three weeks on the solid agar medium.

The strain was maintained at 22 °C under artificial illumination with a 16:8 h light/dark photoperiod and a photon flux density of 35  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . The isolated strain has been deposited in the Collection of Cyanoprokaryotes of the Polar-Alpine Botanical Garden-Institute (KPABG) and assigned the accession number LID-150280. A portion of the type material was dried and deposited in the KPABG herbarium. The label information was included in the 'L.' database [12] and assigned the accession number KPABG 4519! for future reference.

## 2.2. DNA Extraction and Sequencing

DNA was extracted from the unialgal culture using the DNeasy Plant Mini Kit (Qiagen, Venlo, Limburg, The Netherlands) according to the manufacturer's protocol. The partial 16S rRNA gene and the 16S–23S ITS rRNA region were amplified using a pair of primers: one modified by [13] after [14] (1: 5'–CTC TGT GTG CCT AGG TAT CC–3') and 27F (5'–AGA GTT TGA TCC TGG CTC AG–3') suggested by [15]. PCR amplification was performed in 20  $\mu\text{L}$  reaction volumes with MasDDTaqMIX (Dialat Ltd., Moscow, Russia), 10 pmol of each oligonucleotide primer, and 1 ng of DNA. The amplification protocol included an initial denaturation step at 94 °C for 3 min, followed by 40 cycles of 30 s at 94 °C, 40 s at 56 °C, and 60 s at 72 °C, with a final extension at 72 °C for 2 min. The amplified fragments were purified using the Cleanup Mini Kit (Evrogen, Moscow, Russia) and subsequently sequenced using the ABI PRISM® BigDye™ Terminator v3.1 Sequencing Ready Reaction Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the standard protocol provided for the Applied Biosystems 3730 DNA Analyser (Applied Biosystems, Waltham, MA, USA). Additionally, the internal primer 2 (5'–GGG GGA TTT TCC GCA ATG GG–3') [16] was used for sequencing. A single amplicon was obtained without ambiguities for the entire 16S–23S rRNA region. To avoid sequencing errors, the amplicon for the 16S–23S ITS rRNA region of the tested strain was amplified and sequenced again under the same conditions; the nucleotide sequence was identical to that obtained the first time.

## 2.3. Molecular Analyses

The nucleotide sequence of 16S–23S rRNA for strain LID-150280 was assembled in the program BioEdit 7.0.1 [17]. The BLAST 2.17.0 searches the most similar accessions of 16S rRNA to clarify the identification of the tested strain. The type strain of the recently described *Drouetiella epilithica* (NR186875) provided the highest nucleotide sequence similarity. The taxonomic position of strain LID-150280 and its genetic divergence from close relatives were estimated through phylogenetic reconstructions. The dataset included 32 accessions from the genus *Drouetiella* with reference strains of five known species, as well as accessions of 31 species from 22 genera of the family Oculatellaceae and an accession of *Pantanalinema rosanae* V. Vaz, Genuário, Andreote, C.F. Malone, C.L. Sant'Anna, L. Barbiero & M.F. Fiore from the phylogenetically allied family Leptolyngbyaceae J. Komárek, Kaštovský, Mareš & J.R. Johansen chosen as an outgroup according to current taxonomical views [2,4]. The 16S rRNA gene alignment consists of 65 specimens and has a length of 1179 sites. The alignment of the 16S–23S ITS rRNA region of the *Drouetiella* species includes 26 specimens with a total length of 554 sites.

The maximum likelihood (ML) method using IQ-TREE [18] and the Bayesian approach with MrBayes v3.2.1 [19] were employed to reconstruct the molecular phylogeny based on the 16S rRNA gene and the 16S–23S ITS rRNA region. The ML analysis was performed with the best-fit evolutionary model of nucleotide substitutions and ultrafast bootstrapping [20] using 1000 replicates. ModelFinder [21] identified K2P+R4 as the best model for the 16S rRNA gene and TPM2u+F+I+G4 for the 16S–23S ITS rRNA region. For the Bayesian analysis, the GTR+I+G model was applied to both datasets, as recommended by the software’s developers. Two independent runs of the Metropolis-coupled MCMC were conducted to sample parameter values according to their posterior probability. Each run included three heated chains and one unheated chain, with two starting trees chosen randomly. The chains were run for two million generations for the 16S rRNA gene analysis and one million generations for the 16S–23S ITS rRNA region analysis, with trees sampled every 100th generation. In the 16S rRNA gene analysis, the first 5000 trees from each run were discarded as burn-in, resulting in 30,000 sampled trees. For the 16S–23S ITS rRNA region analysis, the first 2500 trees from each run were discarded as burn-in, with 15,000 trees sampled from both runs. The average standard deviation of split frequencies between the two runs was 0.008053 for the 16S rRNA gene and 0.005849 for the 16S–23S ITS rRNA region. Bayesian posterior probabilities (BA PP) were calculated from trees sampled after the burn-in period. Majority rule (MJ) consensus trees were obtained after combining the runs, excluding the 25% burn-in.

The nucleotide sequence similarity of the 16S rRNA gene was estimated using the formula:  $S = 100 \times (1 - p)$ , where  $p$  represents the average pairwise  $p$ -distances. These distances were calculated using MEGA 11 [22] with the pairwise deletion option to account for gaps. The dissimilarity of the 16S–23S ITS region was also determined as the average pairwise  $p$ -distances.

To search for conserved helices D1–D1′, Box–B, V2, and V3 in the 16S–23S ITS regions, the model proposed by [23] was followed. The hypothetical secondary structures of all conserved helices were reconstructed using Mfold [24] specifically for reference strains of the genus *Drouetiella* and strain LID–150280.

### 3. Results

#### 3.1. Taxonomic Description

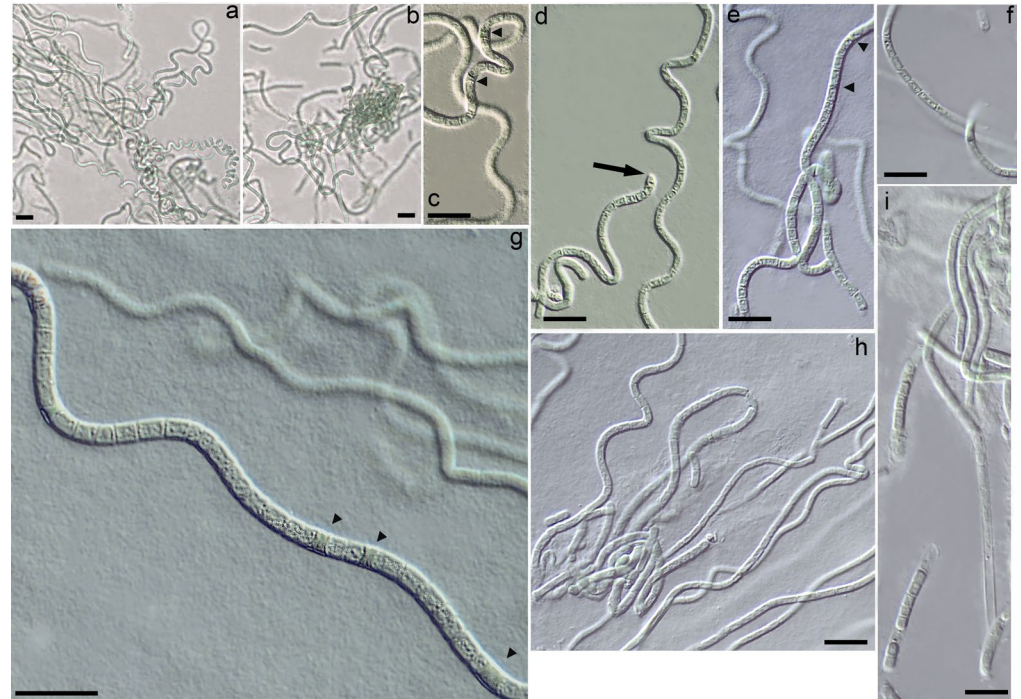
*Drouetiella elegans* D. Davydov & Vilnet *sp. nov.* (Figure 2).

Diagnosis: *Drouetiella elegans* is phenotypically distinct from other *Drouetiella* species due to its long cells in mature trichomes and the absence of false branching, hormogonia, and meristematic zones. It is characterized by distinct phylogenetic positions based on 16S rRNA and 16S–23S ITS rRNA gene analyses and the unique hypothetical secondary structures of the D1–D1′, Box–B, V2, and V3 helices. The 16S rRNA similarity with other *Drouetiella* species varies from 96.31% to 98.97%, and the dissimilarity in the 16S–23S ITS rRNA exceeds 12%.

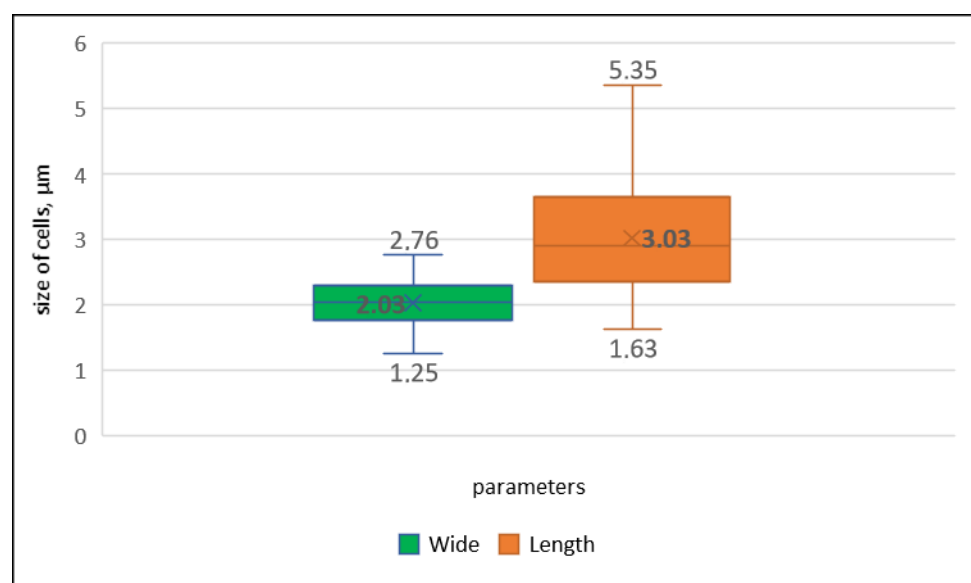
Description: Colonies on solid media formed bright blue-green growths on the agar surface, which gradually transitioned to an olive-green color with continued cultivation. The trichomes were curved, wavy, sometimes coiled, and less frequently straight, exhibiting a blue-green hue and a thin structure (Figure 2a–d). In young colonies, the trichomes displayed irregular curving, forming tangled knots reminiscent of the growth pattern observed in *Pegethrix* species (Figure 2a). There was no false branching in the trichomes, and hormogonia were absent. The trichomes maintained a consistent width throughout their length, appearing cylindrical and either not constricted or only slightly constricted at the cross-walls (Figure 2e–g). The sheath was firm, colorless, usually attached to the trichome, and occasionally distinct (Figure 2i), but absent in immature filaments or hormogonia. The



cells were elongated and varied in length (Figure 2d,h), measuring (1.6) 2.5–4.5 (5.35)  $\mu\text{m}$  in length and (1.25) 1.76–2.3 (2.7)  $\mu\text{m}$  in width (Figure 3). Cell length varied between young and mature trichomes. In young filaments, the cells were shortened or square, with elongation observed only at the ends of the trichomes. As the trichomes matured, the cells elongated, resulting in trichomes with predominantly elongated cells. The terminal cells were untapered and rounded (Figure 2d). Necridic cells were present and frequent (Figure 2c,e,g).



**Figure 2.** Light micrographs of the *Drouetiella elegans* LID-150280. Scale bar = 10  $\mu\text{m}$ : (a,b) Variation in the degree of trichomes coiling from bent, flexuous to spiral coils; (c,e) Necridic cells in trichomes; (d) The terminal cells are untapered and rounded; (f) Elongated cells in mature trichomes; (g) Necridic cells in flexuous trichomes; (h) Irregular length of cells in mature trichomes; (i) Sheath tightly embraces trichome.



**Figure 3.** Distribution of the average (in bold), maximum (above boxes), and minimum (under boxes) values of length and width of cells in the *Drouetiella elegans* strain LID-150280.

Holotype: KPABG–4519 (dried cultured material, preserved in a metabolically inactive state), collected on 8 August 2019 by E.V. Chemeris, deposited in the herbarium of Polar-Alpine Botanical Garden-Institute (KPABG), Apatity, Russia.

Type locality: 66°10.591' N, –170°22.359' E; the lower course of the Uchynveem River, a small lake, planktic; Chukotsky District, Chukotka Autonomous Area, Russia.

Etymology: From Latin adjective *elegans*, elegant, fine.

Nucleotide sequence: GenBank accession number is PQ589721 for the 16S and 16S–23S ITS rRNA genes.

### 3.2. Molecular Analyses

The assembled nucleotide sequence of the entire 16S–23S rRNA region for strain *Drouetiella elegans* LID–150280 had been deposited in the NCBI database. The Maximum Likelihood (ML) analysis of the 16S rRNA gene dataset yielded a tree with a log-likelihood of –7878.117. In the Bayesian analysis (BA) of the 16S rRNA gene, the arithmetic mean log-likelihoods for each sampling run were –7964.64 and –7962.25. The trees obtained by both methods displayed congruent topologies (Figure 4). The ML analysis of the 16S–23S ITS rRNA region produced a tree with a log-likelihood of –2747.981, and the log-likelihoods after Bayesian estimation were –2766.24 and –2767.24. The tree topologies for the 16S–23S ITS rRNA region obtained from both methods were similar (Figure 5).

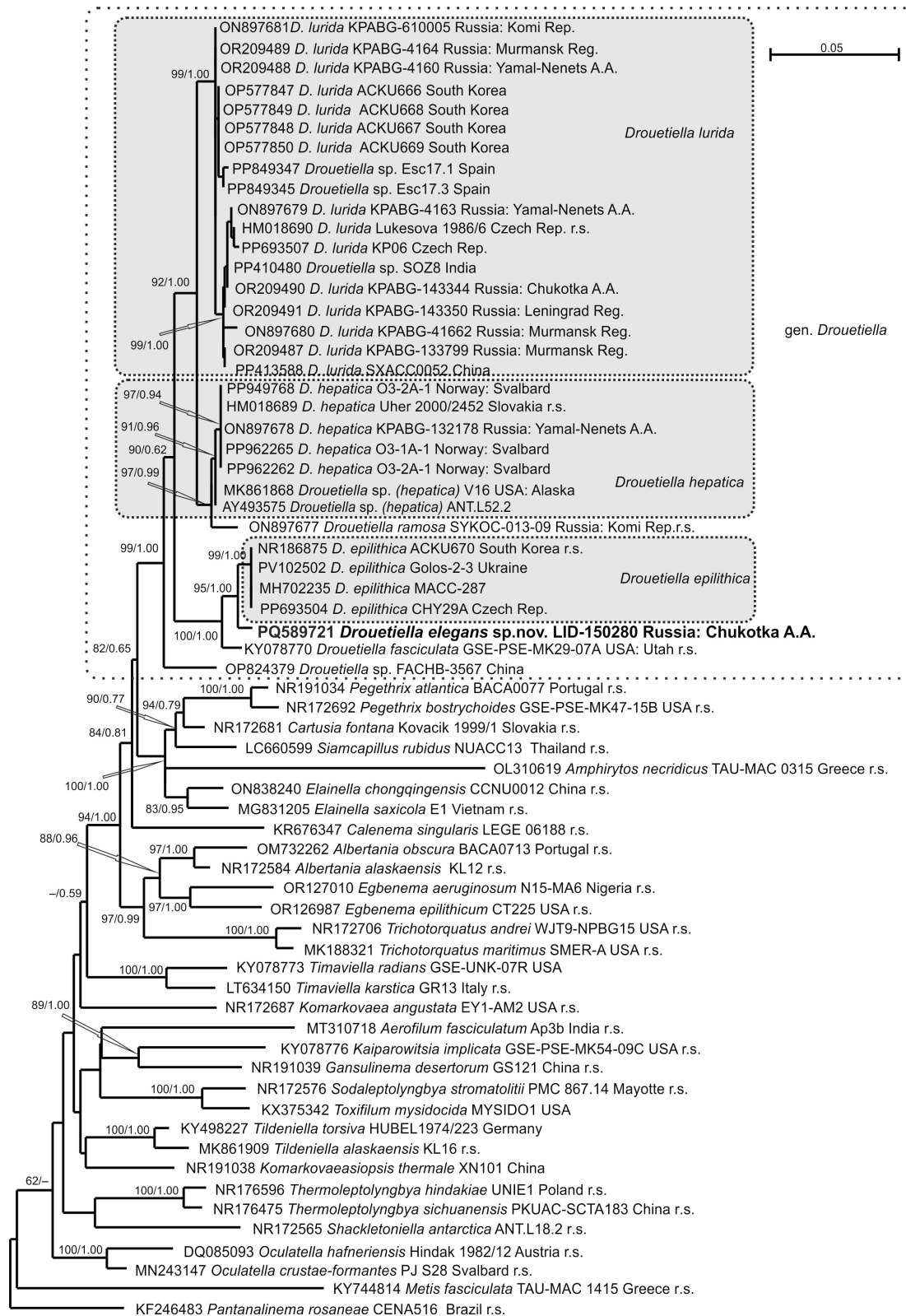
The strain LID–150280 was found within the clade of the genus *Drouetiella*, which received the highest support (bootstrap support (BS) 99% ML/posterior probability (PP) 1.00 BA).

The genus *Drouetiella* clade was positioned in relation (82/0.65) to a clade composed of the genera *Pegethrix*, *Cartusia*, *Elainella*, and the recently described *Siamcapillus* and *Amphiry-tos* (100/1.00) [9,25,26]. Within the *Drouetiella* clade, strain *D. elegans* LID–150280 was placed in a sister relationship to four strains of the species *D. epilithica* (95/1.00). The subsequent relation was composed of strain of *Drouetiella fasciculata* (100/1.00). Recently described *Drouetiella ramosa* kept its relation to the clade composed of seven strains of *D. hepatica* (91/0.96). Eighteen sequencing to date strains of *Drouetiella lurida* were located in one highly supported clade (99/1.00). The topology of the 16S–23S ITS rRNA region indicated a relation of strain *Drouetiella elegans* LID-150280 to *D. fasciculata*, but with poor support (84/0.84). This pair was subsequently related to *Drouetiella epilithica* with support (67/0.89). *Drouetiella ramosa* was changed to a sister relation (100/1.00) of the clade composed of *D. lurida* and *D. hepatica* compared with the previously shown affinity to *D. hepatica*-clade [8].

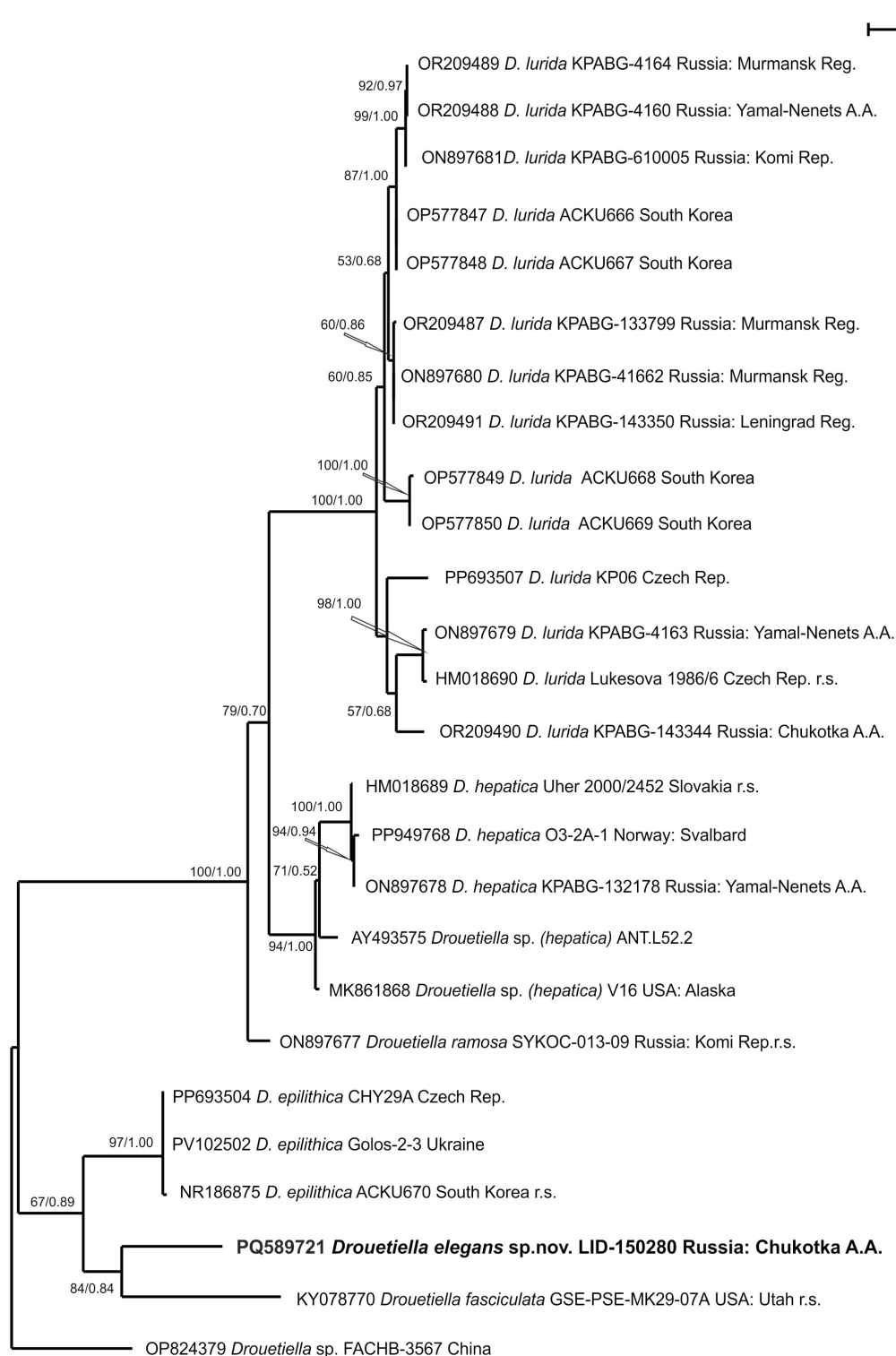
The similarity of the 16S rRNA gene of strain *Drouetiella elegans* LID–150280 to reference strains of *Drouetiella* species varied from 96.31% to 98.97%, while the dissimilarity of the 16S–23S ITS rRNA region ranged from 12.27% to 20.43% (Table 1). The highest similarity was observed between strain *Drouetiella elegans* LID–150280 and *D. epilithica* ACKU670 (98.97%/12.27%), followed by *D. fasciculata* GSE-PSE-MK29-07A (98.28%/15.80%). The infraspecific nucleotide sequence similarity for 16S of *Drouetiella lurida* is 99.43%, *D. hepatica*—99.84%, *D. epilithica*—100%. The nucleotide sequence dissimilarity the 16S–23S ITS rRNA region of *Drouetiella lurida* is 4.04%, *D. hepatica*—3.43%, *D. epilithica*—0.29%.

The sequence length of the 16S–23S ITS rRNA region was robustly estimated for reference strains of *Drouetiella hepatica* Uher 2000/2452, *D. ramosa* SYKOA C-013-09, *D. epilithica* ACKU670, and *D. fasciculata* GSE-PSE-MK29-07A. However, incomplete data were available for *Drouetiella lurida* Lukesova 1986/6 in GenBank (Table 2). Strain *Drouetiella elegans* LID–150280 exhibited a D1–D1' region (63 bp) that was 1–2 bp shorter than that of other species. The V2 region (10 bp) was reduced and similar in length to that of *Drouetiella epilithica* and *D. fasciculata*. The Box-B helix length was similar only to that of

*Drouetiella epilithica* (35 bp), and the V3 region was characterized by a stable length (52 bp) for the genus.



**Figure 4.** Phylogram obtained under the maximum likelihood approach for 64 accessions of Oculatellaceae based on the 16S rRNA gene. Bootstrap support values from the maximum likelihood and Bayesian posterior probabilities of more than 50% (0.50) are indicated. The reference strains are marked as r.s.



**Figure 5.** Phylogram obtained under the maximum likelihood approach for 26 accessions of the genus *Drouetiella* based on the 16S-23S ITS rRNA region. The reference strains are marked as r.s.



**Table 1.** Values of nucleotide sequence similarity/dissimilarity (%) for the genus *Drouetiella*, based on 16S rRNA gene (1179 bp) and 16S–23S ITS rRNA region (555 bp).

Taxon	Intraspecific Nucleotide Sequence Similarity 16S/Dissimilarity 16S–23S ITS, %	Interspecific Nucleotide Sequence Similarity 16S/Dissimilarity 16S–23S ITS, %					
		1	2	3	4	5	6
1. <i>D. elegans</i>	n/c/n/c						
2. <i>D. lurida</i>	99.43/4.04	96.86/20.80 96.31/19.02 <sup>1</sup>					
3. <i>D. hepatica</i>	99.84/3.43	97.12/19.50 96.63/20.43 <sup>1</sup>	98.26/13.60				
4. <i>D. ramosa</i>	n/c/n/c	97.18/20.00 <sup>1</sup>	98.12/12.50				
5. <i>D. epilithica</i>	100/0.29	98.97/12.20 98.97/12.27 <sup>1</sup>	96.69/21.60	96.84/19.50	97.05/18.30		
6. <i>D. fasciculata</i>	n/c/n/c	98.28/15.80 <sup>1</sup>	96.59/21.20	97.00/18.90	97.00/19.30	98.57/15.70	
7. <i>Drouetiella</i> sp. FACHB-3567	n/c/n/c	96.63/16.20	96.34/17.90	96.39/16.90	96.27/17.00	95.91/14.30	96.71/17.50

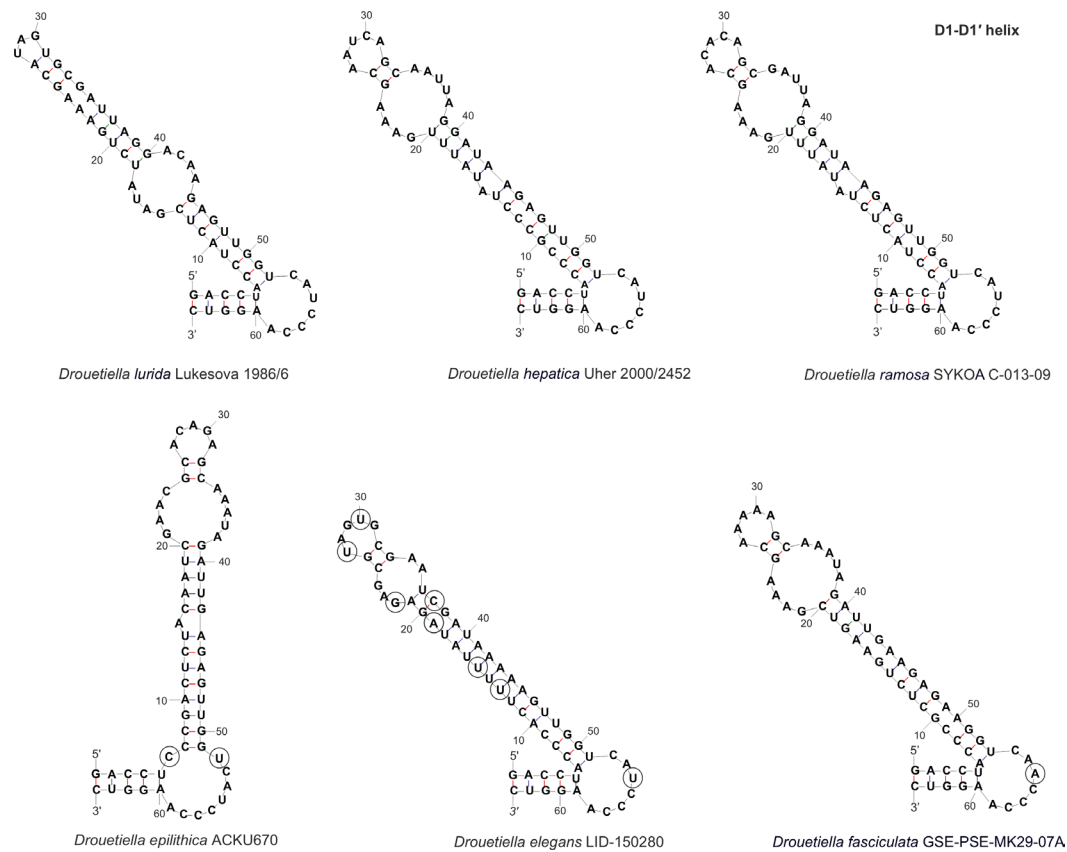
<sup>1</sup> The values obtained for reference strains of *Drouetiella* species.**Table 2.** Sequence length variability (base pairs, bp) within 16S–23S ITS rRNA among reference strains of *Drouetiella*.

Taxon	ITS Total Length in Current Alignment	D1–D1'	Box–B	V2	V3
<i>D. elegans</i> LID-150280	490	63	35	10	52
<i>D. lurida</i> Lukesova 1986/6 <sup>1</sup>	456	64	33	16	26
<i>D. hepatica</i> Uher 2000/2452	505	64	34	20	52
<i>D. ramosa</i> SYKOA C-013-09	507	64	34	23	52
<i>D. epilithica</i> ACKU670	487	64	35	10	52
<i>D. fasciculata</i> GSE-PSE-MK29-07A <sup>1</sup>	489	65	39	10	52

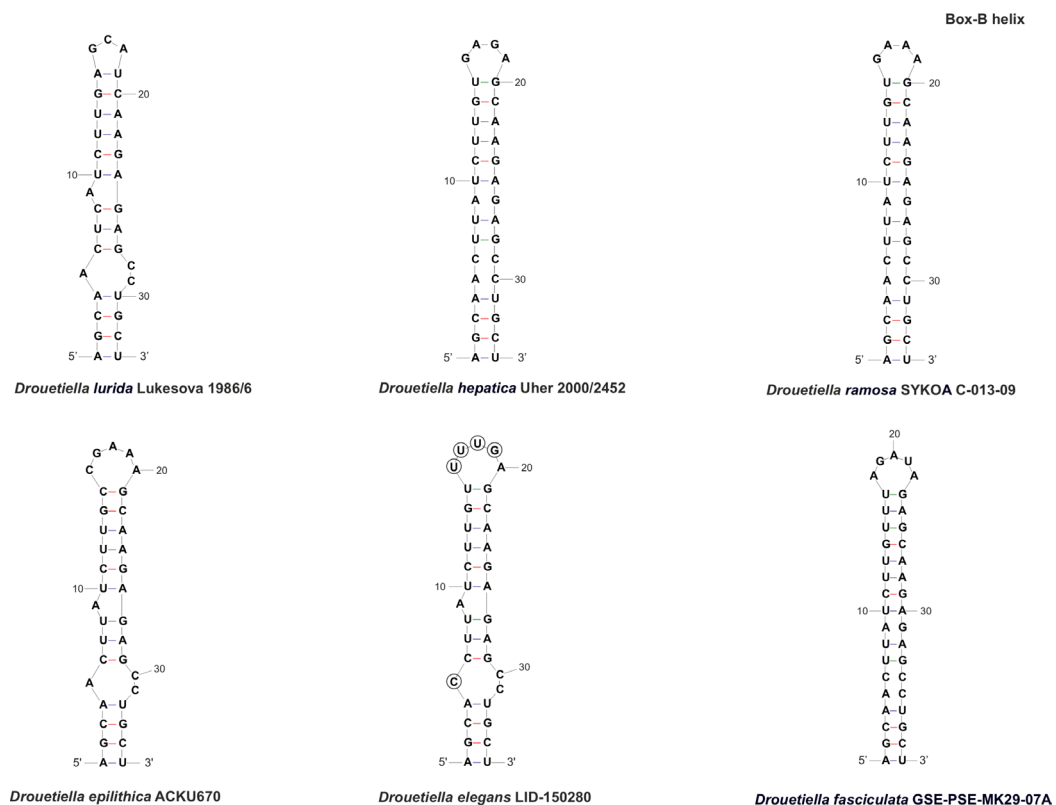
<sup>1</sup> There are incomplete sequences from GenBank.

The secondary structure of the D1–D1' helix of strain *Drouetiella elegans* LID–150280 was common with other *Drouetiella* species and contained three loops (Figure 6). The basal stem structure was consistent with that of the genus *Drouetiella*, and the nucleotide sequence of the basal loop was similar to that of *D. lurida*, *D. hepatica*, and *D. ramosa*, differing in both length and sequence from the most closely related species, *D. epilithica* (C–A substitution) and *D. fasciculata* (U–A substitution). The middle stem was the longest, with 16 steps; the middle loop was the shortest (5 bp); and the terminal loop had a unique motif due to seven substitutions compared with other species.

The Box–B helix of strain *Drouetiella elegans* LID–150280 resembled those of *D. lurida* and *D. epilithica*, with two loops and a bulb composed of a single unpaired nucleotide (A) in the middle part of the stem (Figure 7). The basal loop of strain *Drouetiella elegans* LID–150280 differed in the C–A substitution compared to *D. lurida* and *D. epilithica*, and the terminal loop had a unique sequence among other species due to four substitutions (UUUG).

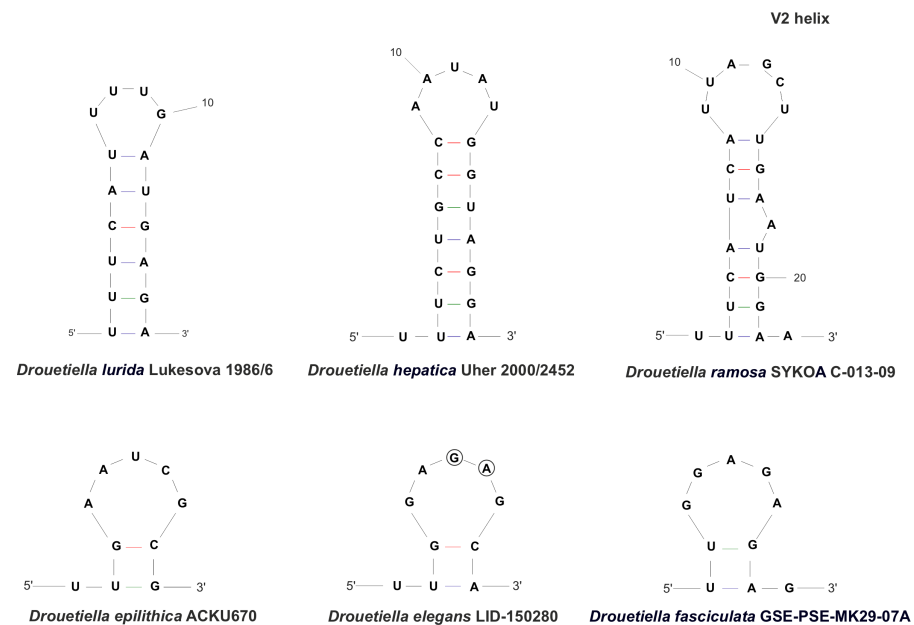


**Figure 6.** The secondary structure of D1–D1' helices of the 16S–23S ITS rRNA region of *Drouetiella* strains. Circles indicate differences.



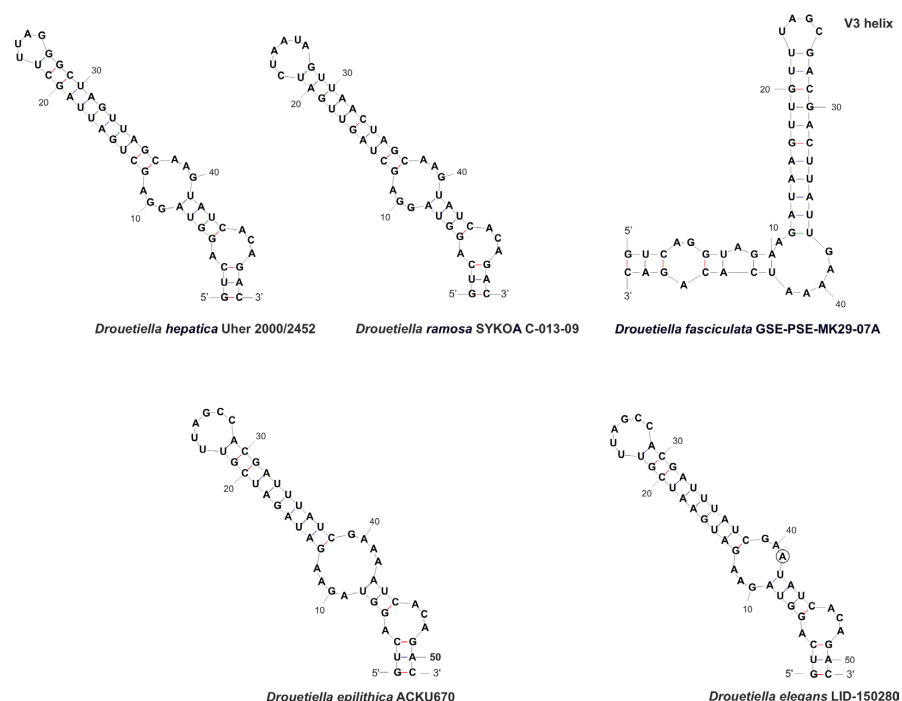
**Figure 7.** The secondary structure of Box–B helices of the 16S–23S ITS rRNA region of *Drouetiella* strains. Circles indicate differences.

The V2 region was reduced in strain *Drouetiella elegans* LID-150280, similar to *D. epilithica* and *D. fasciculata* (Figure 8). It had a stem only two steps long and a terminal loop with five nucleotides, with only two nucleotides clearly distinguishing strain *Drouetiella elegans* LID-150280 from *D. epilithica* and *D. fasciculata*. The V2 region in *Drouetiella lurida*, *D. hepatica*, and *D. ramosa* had a stem length of 6–7 steps and a variable loop region, both in length and nucleotide sequence.



**Figure 8.** The secondary structure of V2 helices of the 16S-23S ITS rRNA region of *Drouetiella* strains. Circles indicate differences.

The V3 region of strain *Drouetiella elegans* LID-150280 was similar to that of *D. epilithica*, *D. hepatica*, and *D. ramosa* in the nucleotide sequence of the basal stem and loop but differed in the sequence of the middle loop (Figure 9).



**Figure 9.** The secondary structure of V3 helices of the 16S-23S ITS rRNA region of *Drouetiella* strains. Circles indicate differences.

The length of the stem between the middle and terminal loops and the sequence of the terminal loop of strain *Drouetiella elegans* LID–150280 were similar to those of *D. epilithica*. However, a single substitution at position 43 led to differences in the middle loop motif and the length of the stem between the middle and basal loops.

Thus, the separate position on constructed trees, the similarity of the 16S rRNA gene, the dissimilarity in the 16S–23S ITS rRNA region, and the features of secondary structures of conserved helices in the 16S–23S ITS region, together with morphological, ecological, and geographical traits, allowed us to consider the strain LID–150280 as a new species for science: *Drouetiella elegans*.

#### 4. Discussion

Thin filamentous non-heterocytous cyanobacteria exhibit significant taxonomic diversity within the family Oculatellaceae. Delimitation of taxa within this family is reliably achievable only through molecular genetic data. The genus *Drouetiella* is a notable example of cryptic diversity. The morphological characteristics typical of *Drouetiella* species are not sufficient for unambiguous species identification. Therefore, species within this genus are primarily identified through the analysis of 16S rRNA and 16S–23S ITS rRNA sequences.

In a previous study, we attempted to compare the morphology of strains from different *Drouetiella* species [8]. Considering the newly described species *Drouetiella epilithica* and *D. elegans*, it can be stated that the main characteristics of this genus include thin cylindrical trichomes with a width ranging from 1.25 to 3.1 (4.3)  $\mu\text{m}$ , without constrictions at the cross-walls, the presence of necridic cells that facilitate trichome disintegration, and a rounded terminal cell. Another important anatomical feature for cyanobacterial taxonomy is the arrangement of thylakoids. As with all members of the family Oculatellaceae, species of the genus *Drouetiella* are characterized by a parietal thylakoid arrangement [1,9].

The publishing of new data from strains gathered from remote localities allows for the correction of initial species and genus descriptions to clarify variability and diversification. The *Drouetiella lurida* strains from Yamal-Nenets Autonomous Area, Komi Republic, and Murmansk Region of Russia [8] and from the Republic of Korea [9] are characterized by the presence of false branching, necridic cells, and hormogonia, which were not found in the type strain Lukesova 1986/6. Newly marked morphological features provide misleading separation of *Drouetiella lurida* from other species. Opposite strain *Drouetiella hepatica* from Yamal-Nenets Autonomous Area does not have false branching, which is also rare in type strain Uher 2000/2452 from Slovakia. Other species are still presented by type strain only, and their morphological variability is not sufficiently studied. Key features for species differentiation are summarized in Table 3. False branching is very often observed in *Drouetiella ramosa* and *D. epilithica*, rarely in *D. hepatica* and *D. lurida*, and is absent in *D. fasciculata* and *D. elegans*. Meristematic zones in the trichomes, characterized by highly shortened cells, are typical of *Drouetiella hepatica* and *D. epilithica*. Hormogonia are absent in *Drouetiella hepatica* and *D. elegans*, or are very rarely observed in *D. lurida*, *D. ramosa*, and *D. fasciculata*, being common only in *D. epilithica*. *Drouetiella elegans* differs from phylogenetically allied *D. epilithica* by the absence of false branching, meristematic zones, and hormogonia, and more narrow and elongate cells. Less pronounced differences were found between *Drouetiella elegans* and *D. fasciculata*, consisting of the absence of constrictions on the transverse walls and rarely forming hormogonia in the latter species. Newly described species *Drouetiella elegans* is similar to strain *D. hepatica* V16 from Alaska by coiled trichomes [27].

Table 3. Morphological comparison among named *Drouetiella* strains.

Characteristics/ Species	<i>D. lurida</i>		<i>D. hepatica</i>		<i>D. ramosa</i>	<i>D. fasciculata</i>	<i>D. epilithica</i>	<i>D. elegans</i>
	Lukesova 1986/6 <sup>1</sup>	KPABG 4163	Uher 2000/2452 <sup>1</sup>	KPABG 132178	SYKOA C-013-09 <sup>1</sup>	GSE-PSE- MK29-07A <sup>1</sup>	ACKU670 <sup>1</sup>	LID-150280
False branching	-	+	very rare	-	common	-	+	-
Cells elongated	+	+	+	+	-	+	+	+
Constricted at cross-walls	not or slightly	distinctly	not or slightly	not or slightly	not or slightly	not	not or slightly	not or slightly
Necridia	-	+	frequent	+	+	+	+	+
Width of cells (µm)	1.7–2.1	1.8–2.5	1.5–3.0	1.6–3.1	2.3–4.3	1.5–2.4 (3.0)	2.0–3.0	1.25–2.7
Length of cells (µm)	(2.1) 2.9–3.8 (5.4)	1.2–2.6	(2.2) 3.1–4.5	2.0–4.0	1.9–2.6	3.1–4.4 (5.4)	2.0–3.2	(1.6) 2.5–4.5 (5.357)
Meristematic zones	-	-	+	-	-	-	+	-
Hormogonia	-	-	-	-	rare	rare	+	-
Coloration	liver-brown	olive-green The Russian	brownish Slovakia, temperate	olive-green	olive-green, blue-green	blue-green	blue-green	blue-green
Locality/habitat	The Czech, temperate for- est/aerophytic, soil	Arc- tic/epilithic on a boulder in a river, underwater	forest/on subaerial seep wall and waterfall	The Russian Arctic/seep wall	The Russian Subarctic/ aerophytic, soil	The USA, semi-arid/ wet wall and waterfall	The Republic of Korea/on the base of the stone monument	The Russian Arctic/ planktic in a lake

<sup>1</sup> The values obtained for reference strains of *Drouetiella* species.



As mentioned earlier, *Drouetiella elegans* cannot definitely be differentiated from other species by subtle variations in morphological traits. Therefore, due to the minor overlap in morphological characteristics among *Drouetiella* species, their primary distinction relies on molecular data.

As a result of phylogenetic estimations, *Drouetiella elegans* was found in a separate clade clustered in sister relation with the clade containing the type strain of *D. epilithica* ACKU670 in the 16S rRNA calculation or in poorly supported relation with *D. fasciculata* GSE-PSE-MK29-07A in the 16S–23S ITS rRNA region. The distinct position among known taxa supported the description of the obtained genetic unit as a new species.

The 16S rDNA sequence similarity of *Drouetiella elegans* varies from 96.31% with *D. lurida* Lukesova 1986/6 to 98.97% with *D. epilithica* ACKU670, which fits well into the 98.7–99.00% threshold for Cyanobacteriota species delimitation [28,29]. The highest value of 16S–23S ITS rRNA sequence dissimilarity of *Drouetiella elegans* was registered with *D. epilithica* ACKU670 (12.27%), and the lowest was 20.43% with *D. hepatica* Uher 2000/2452. The threshold of sequence dissimilarity in the 16S–23S ITS rRNA region of more than 7% suggested strains as belonging to distinct species [30], which additionally supports species status for *Drouetiella elegans*. The species *Drouetiella lurida*, *D. hepatica* and *D. epilithica* demonstrates the high level of infraspecific 16S gene similarity (99.43–100%) and low level of 16–23S ITS dissimilarity (0.2–4%) among specimens from remote localities, which characterizes all species as genetically stable units and further confirms the molecular genetic differentiation of *D. elegans* at the species level.

The secondary structures of the conserved helices in the 16S–23S ITS rRNA region are species-specific. The main feature of phylogenetically allied species *Drouetiella elegans*, *D. epilithica*, and *D. fasciculata* is a highly reduced V2 helices compared to other species in the genus.

The distribution map (Figure 1) highlights the locations where various *Drouetiella* strains have been identified, offering insight into the global dispersal and diversity of this genus. The species *Drouetiella lurida* has been known since 1849 [31]; numerous records of its occurrence have been documented in the literature. Based on these data, the species is characterized by a multizonal distribution. However, it is important to note that the number of findings confirmed by molecular data is relatively small, and most of the records should be considered provisional. Nevertheless, even the confirmed records indicate that the species exhibits broad ecological plasticity, thriving in both aerophytic and subaerophytic conditions as well as underwater as an epilithic organism.

The type specimen was found in soil in a temperate forest in the Czech Republic [1]. In the Leningrad Region, *Drouetiella lurida* was discovered in flowing spring water [32]. The species was also identified as an epilithic organism on a submerged boulder in a river in the Polar Ural Mountains [8]. In the Murmansk Region, it was collected from an anthropogenic habitat at the ash dump of the Apatity Power Station, and in the Subpolar Ural Mountains, it was found on the soils of a grass-moss community [8]. The species has also been reported on gravels from water in the Republic of Korea [9] and China (GenBank accession number is PP413588). Strains of *Drouetiella lurida* were also collected from high-altitude lakes in the cold desert region of Himachal Pradesh, India [33], or soil (PP410480) and in the water of a floodplain lake in Chukotka [32]. *Drouetiella lurida* was also found in biofilms from Spanish caves (PP849347 and PP849345).

Besides the locus classicus in a temperate forest in Slovakia, *Drouetiella hepatica* has been found in Antarctica [34], Alaska [27], Svalbard [35], and the Polar Urals [8]. *Drouetiella epilithica* was gathered from the base of the stone monument in Daegu (the Republic of Korea) [9], and recently was suggested from caves of the Czech Republic [36] and pine bark from Ukraine [37]. As for *Drouetiella fasciculata* and *D. ramosa*, these species have not

yet been identified in locations other than those where the type specimens were collected. *Drouetiella fasciculata* is known from a large seep wall and waterfall in Navajo Sandstone (Utah, USA), where the species composes mats with other filamentous algae [1]. *Drouetiella ramosa* was found on a quartz mine on damp quartz sand in the Subpolar Urals Mountains (Komi Republic, Russia) [8]. The majority of species of the genus *Drouetiella* prefer aerophyte or subaerophyte habitats. *Drouetiella elegans* lives as a plankton in the water of a small lake (Chukotka, Russia) and in its ecology partly resembles some aquatic strains of *D. lurida* from Russia or the Republic of Korea.

Thin filamentous non-heterocytic cyanobacteria of the order Oculatellales are widespread in various ecotopes and have the ability to be successfully cultivated in laboratory conditions, which, apparently, is a reliable basis for studying their diversity and clarifying their taxonomy. In addition to the *Drouetiella elegans* described here, another new species can be proposed from the *Drouetiella* sp. strain FACHB-3567 based on the results of the provided molecular evaluation of all currently published nucleotide data.

Strains of this genus show considerable promise for bioremediation applications, particularly in harsh environments in Antarctica, Alaska, Svalbard, and the Russian Arctic. As pioneer species, they possess a remarkable ability to colonize various anthropogenic substrates, including mining waste from ore processing, overburden materials, and other industrially disturbed landscapes. Their ecological resilience is well-documented by their widespread distribution in extreme Arctic ecosystems and successful establishment on ash deposits at the Apatity Thermal Power Plant [8,38].

The capacity to form viable populations under such extreme conditions indicates the presence of robust metabolic adaptations to stressful environments. This combination of traits—including stress tolerance, rapid colonization ability, and survival in nutrient-poor conditions—positions these strains as particularly valuable for remediation efforts in northern regions where conventional approaches often prove ineffective.

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