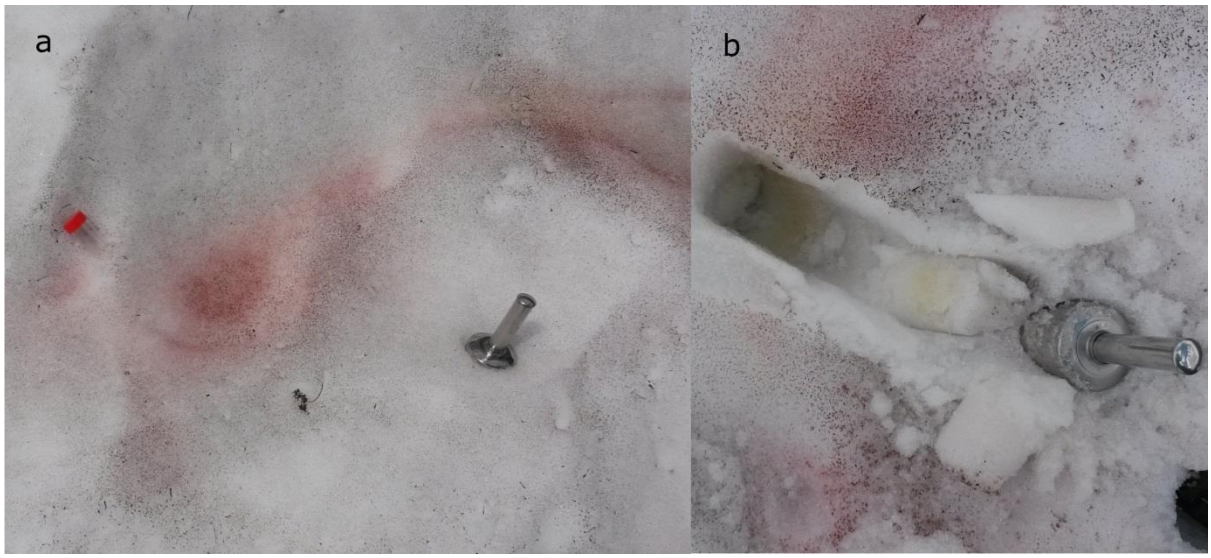


Fig. S1. a) Red snow surface (sample 1) caused by microalgae at Kühtai, Tyrolean Alps, Austria. b) The discoloration reached several centimetres down, changing from red to green.

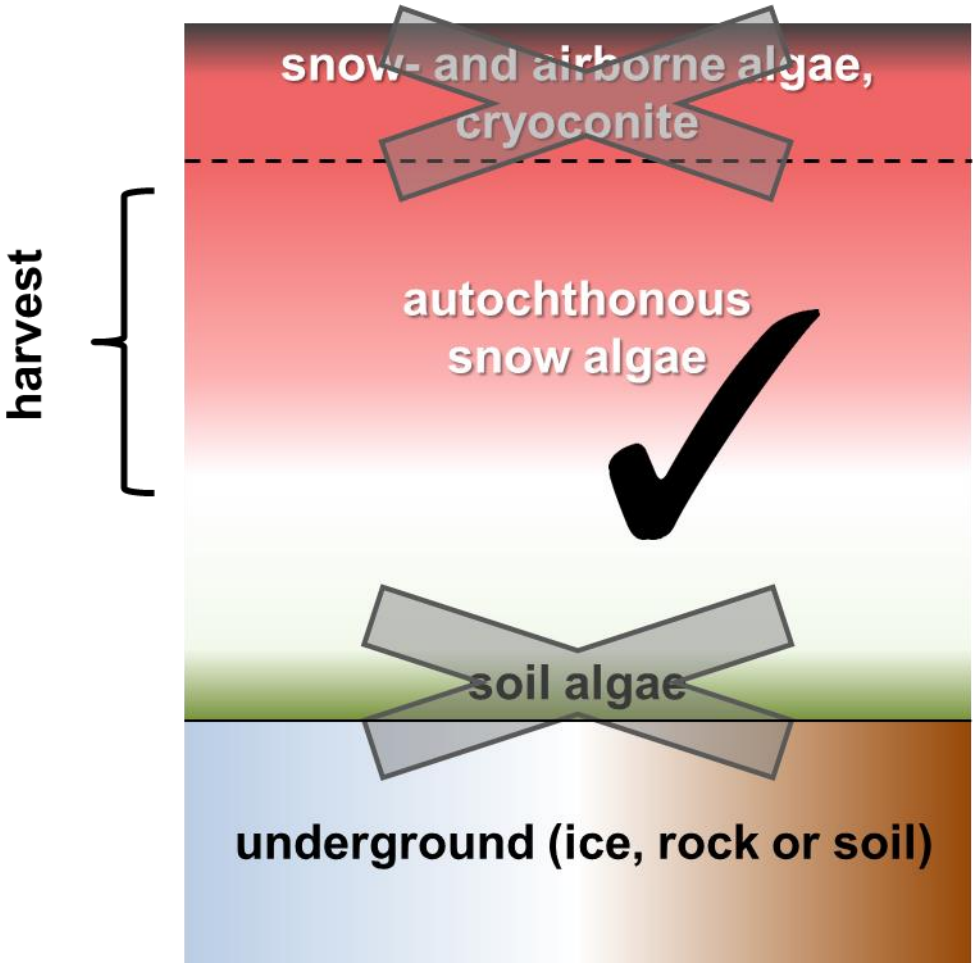


Fig. S2. Vertical scheme of a melting snowfield showing the best practice of 'true' (autochthonous) snow algae sampling. In order to avoid allochthonous organisms (airborne, soil bound), the uppermost surface and lowermost ground centimeters of snow should not be harvested. A consistent sampling strategy is important when the aim is to compare microbial diversity and abundances between different sites. Exceptions may be steep slopes in polar regions, where snow algae populations are surface based, and thus, surface layer cannot be omitted.

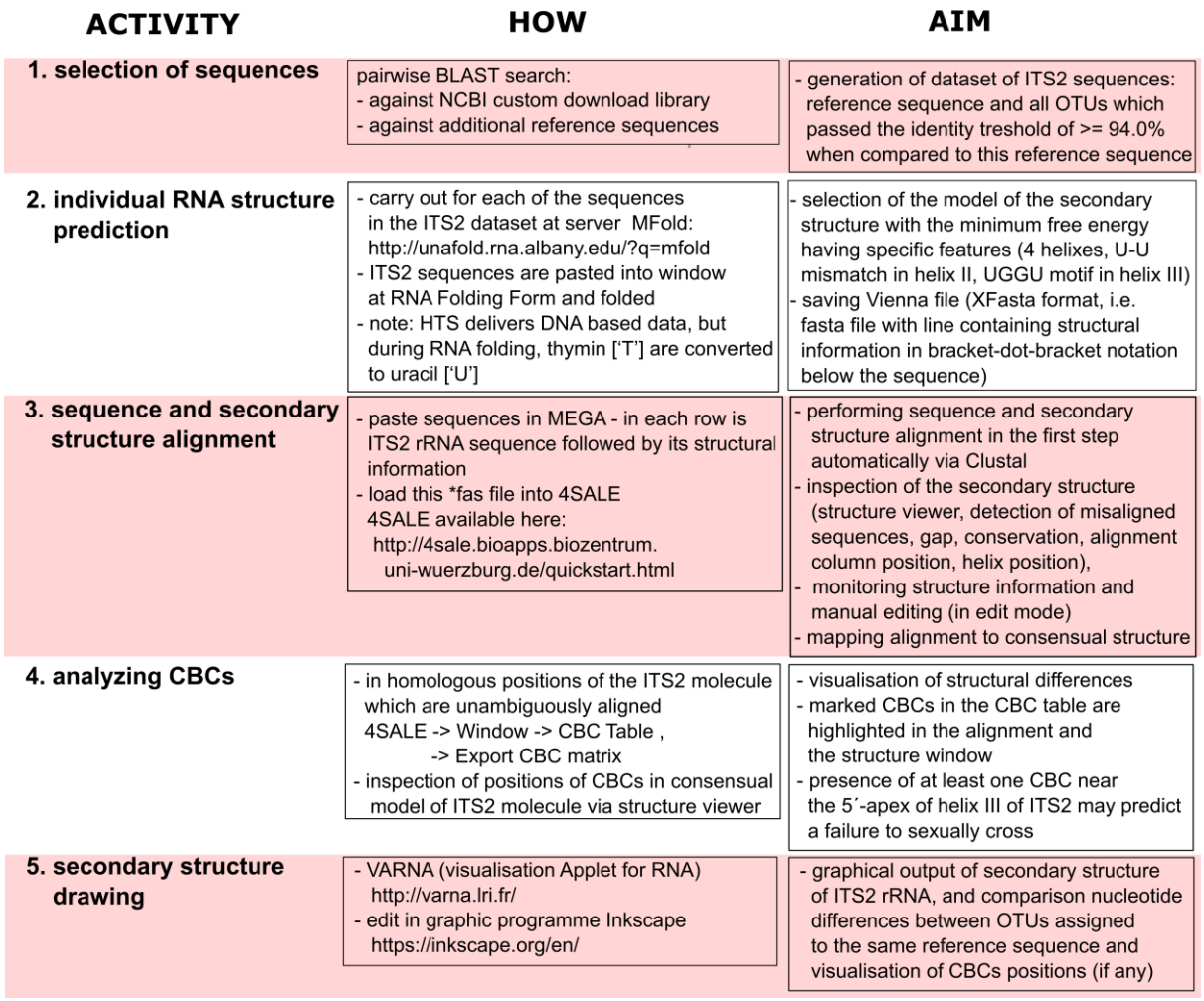


Fig. S3. Schematic workflow for taxonomic assignments of environmental ITS2 OTUs from environmental samples of snow algal communities where Chlamydomonadacae prevail. For this taxonomic group the CBC species concept (in frame of a polyphasic approach) was successfully applied (e.g., MATSUZAKI et al.2015). The aim of the process is to get sequence-structure alignments of selected OTUs with their reference sequences and then search for compensatory base changes (CBCs) in homologous positions near the 5´- apex of helix III encompassing the YGGY motif (the most conserved region of the ITS2 secondary structure of eukaryotes).

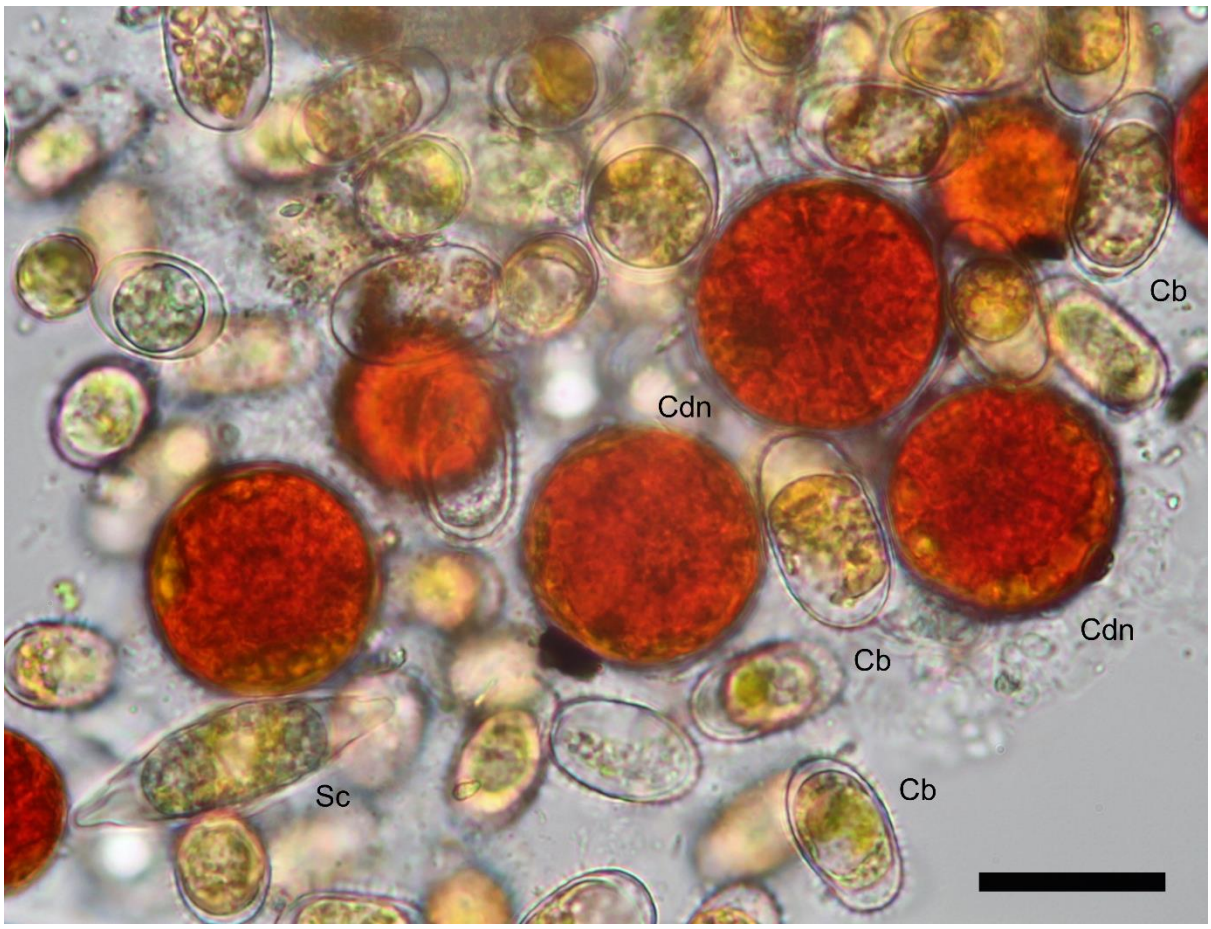
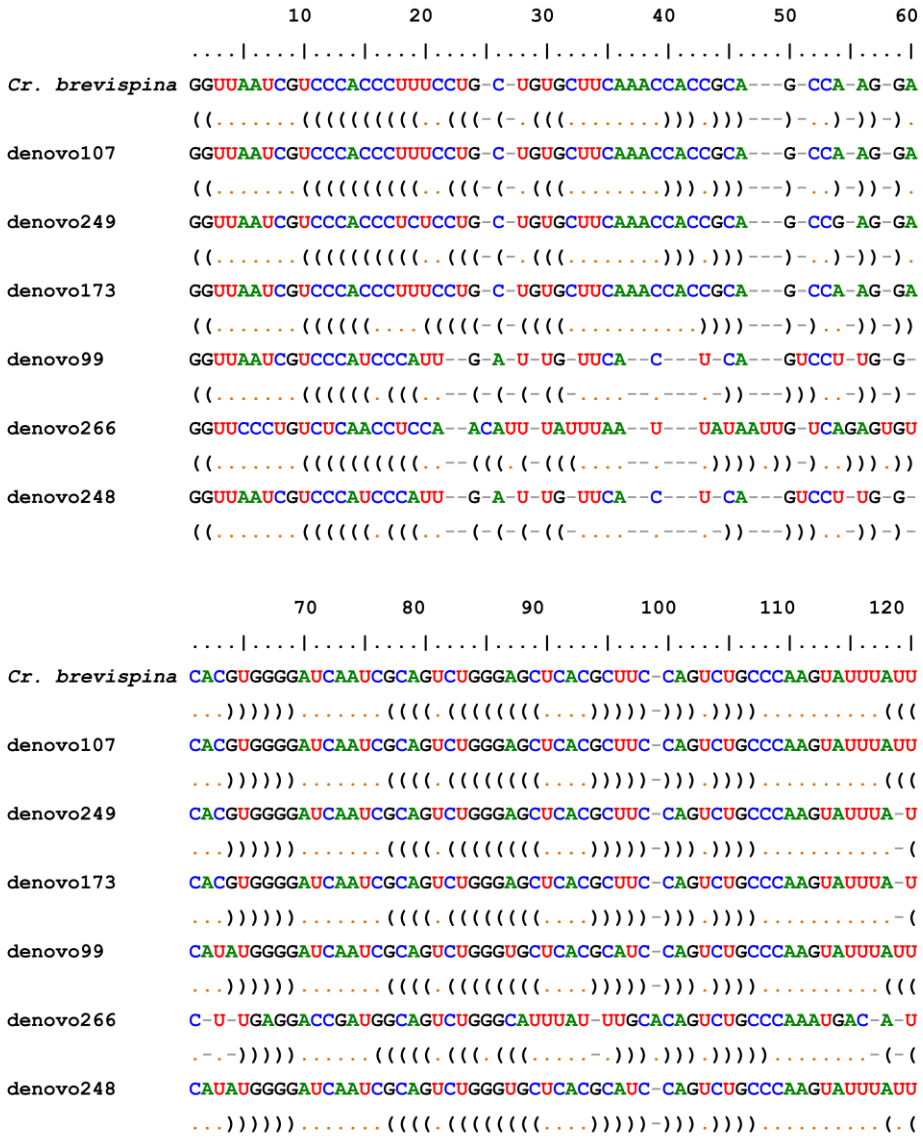


Fig. S4. Light micrograph of cells in field sample 2. The three locally abundant snow algae identified using morphological features were *Cr. brevispina* (Cb; later identified by HTS and secondary structure prediction as an undescribed species - OTU denovo99), *Scotiella cryophila* K-1 (Sc) and *Cd. nivalis* (Cdn). Scale bar: 20 μm.


10 20 30 40 50 60
Cr. brevispina GGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGCA---G-CCA-AG-GA
denovo107 GGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGCA---G-CCA-AG-GA
denovo249 GGUUAAUCGUCCCACCCUCUCCUG-C-UGUGCUUCAAACCACCGCA---G-CCG-AG-GA
denovo173 GGUUAAUCGUCCCACCCUUUCCUG-C-UGUGCUUCAAACCACCGCA---G-CCA-AG-GA
denovo99 GGUUAAUCGUCCCAUCCCAUU--G-A-U-UG-UUCA--C---U-CA---GUCCU-UG-G-
denovo266 GGUUCCCUGUCUCAACCUCCA--ACAUU-UAUUUAA--U---UAUAAUUG-UCAGAGUGU
denovo248 GGUUAAUCGUCCCAUCCCAUU--G-A-U-UG-UUCA--C---U-CA---GUCCU-UG-G-
70 80 90 100 110 120
Cr. brevispina CACGUGGGGAUCAAUCGCAGUCUGGGAGCUCACGCUUC-CAGUCUGCCCAAGUAUUUAUU
denovo107 CACGUGGGGAUCAAUCGCAGUCUGGGAGCUCACGCUUC-CAGUCUGCCCAAGUAUUUAUU
denovo249 CACGUGGGGAUCAAUCGCAGUCUGGGAGCUCACGCUUC-CAGUCUGCCCAAGUAUUUA-U
denovo173 CACGUGGGGAUCAAUCGCAGUCUGGGAGCUCACGCUUC-CAGUCUGCCCAAGUAUUUA-U
denovo99 CAUAUGGGGAUCAAUCGCAGUCUGGGUGCUCACGCAUC-CAGUCUGCCCAAGUAUUUAUU
denovo266 C-U-UGAGGACCGAUGGCAGUCUGGGCAUUUAU-UUGCACAGUCUGCCCAAAUGAC-A-U
denovo248 CAUAUGGGGAUCAAUCGCAGUCUGGGUGCUCACGCAUC-CAGUCUGCCCAAGUAUUUAUU

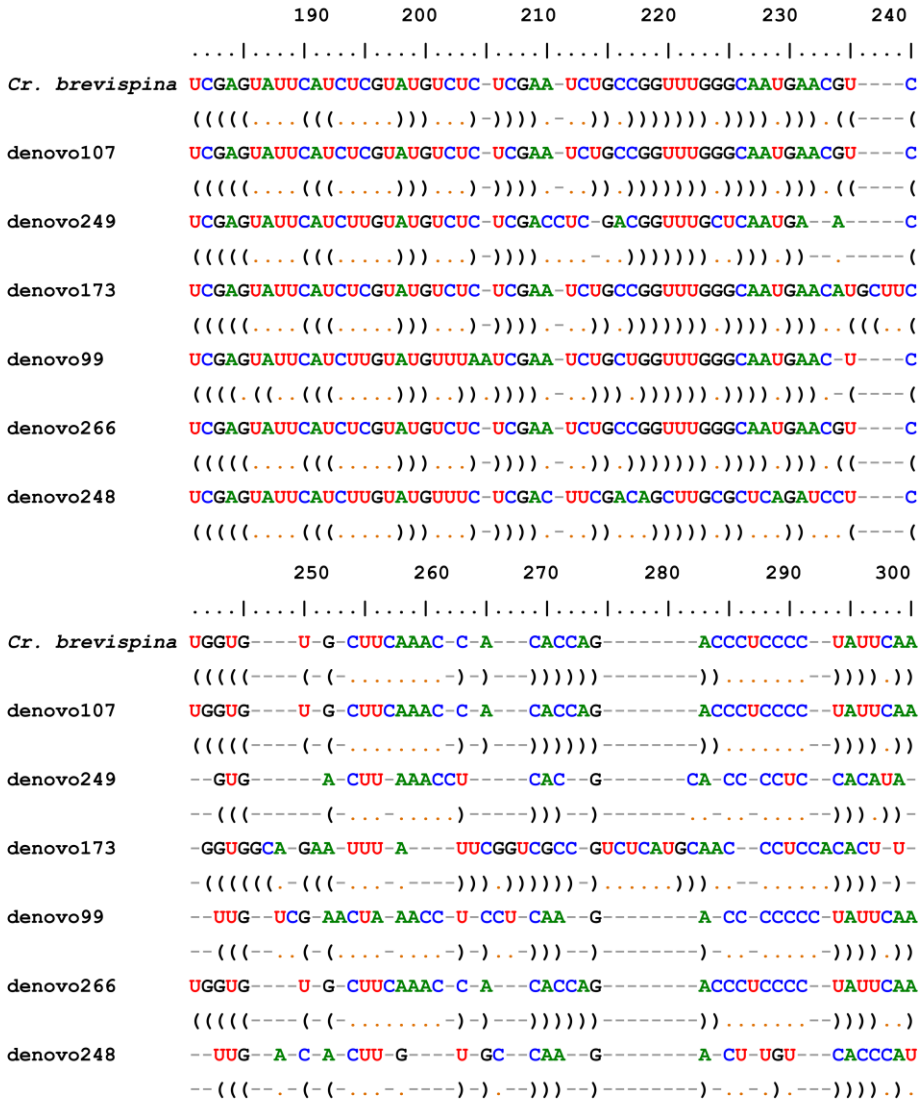

190 200 210 220 230 240
Cr. brevispina
denovo107
denovo249
denovo173
denovo99
denovo266
denovo248
250 260 270 280 290 300
Cr. brevispina
denovo107
denovo249
denovo173
denovo99
denovo266
denovo248

Fig. S5. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Chloromonas brevispina* K-2 (accession number MG791868) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’]).

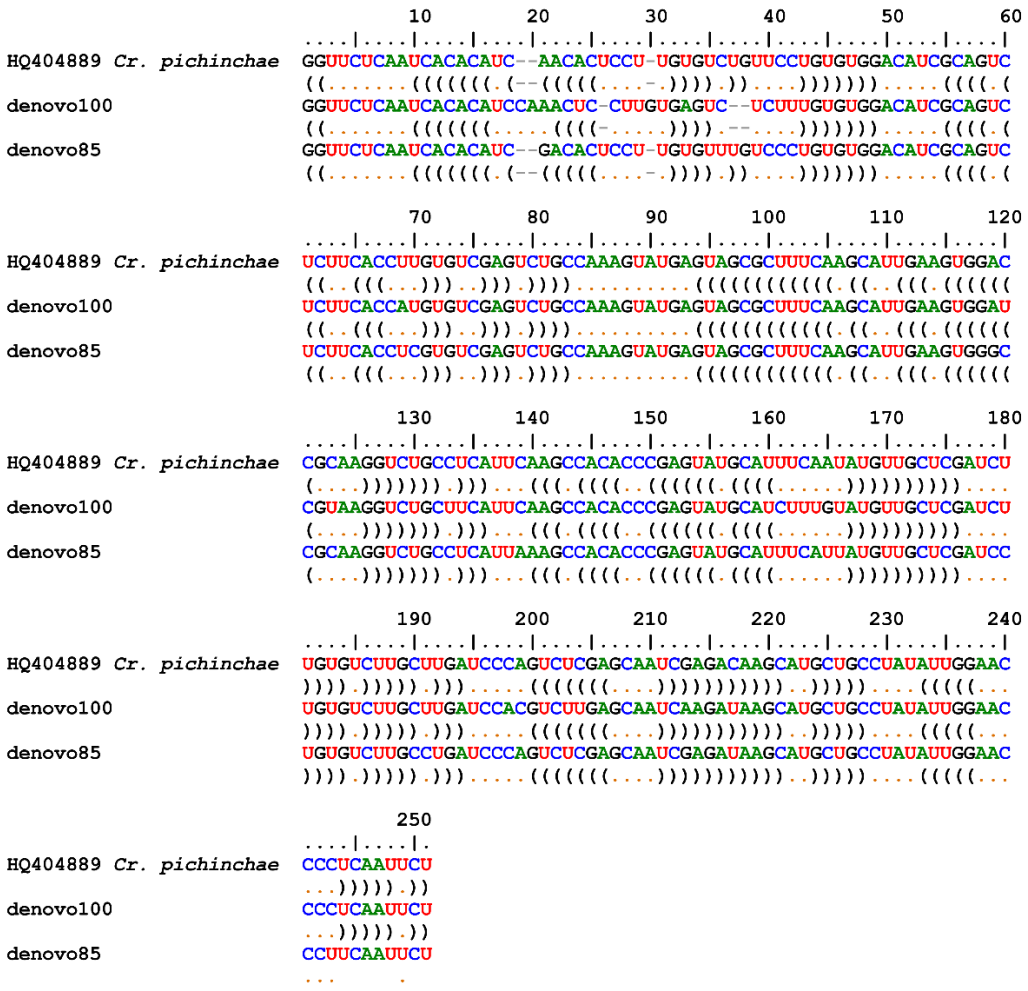


Fig. S6. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Chloromonas pichinchae* (accession number HQ404889.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

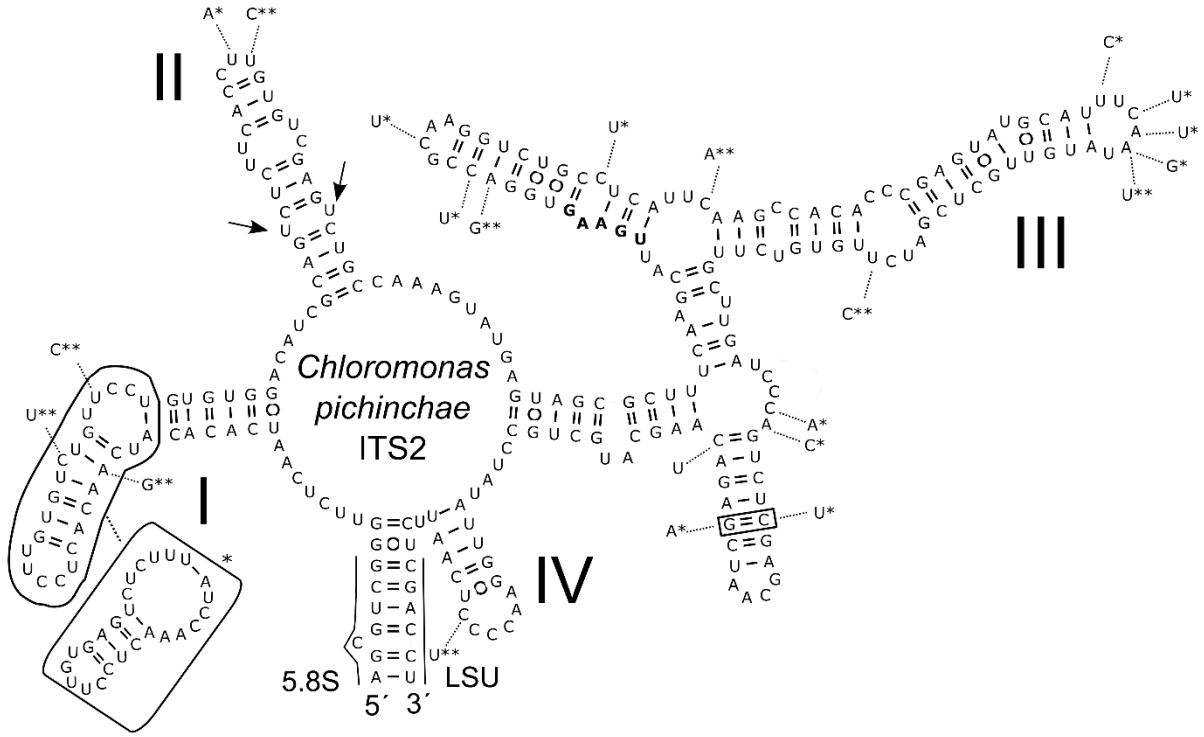


Fig. S7. Comparison of the secondary structure of ITS2 rDNA transcripts between *Chloromonas pichinchae* CCCryo 261-06 (accession number HQ404889.1) and the closely related OTU ‘denovo100’ and OTU ‘denovo85’. Differences characteristic for both OTUs are shown by nucleotides outside the structure and are linked by dotted lines. One asterisk means that the difference was detected only in OTU ‘denovo100’, and double asterisks imply that the difference were detected in OTU ‘denovo85’; middle and top part of helix I (encircled) represent an expansion segment, whose length is not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al.2013). Therefore, this part of helix I characteristic for OTU ‘denovo100’ is shown outside the structure and is linked by dotted lines. A single compensatory base change (CBC) in comparison with the reference sequence was found in OTU ‘denovo100’ but outside the most conserved part of helix III (the most conserved is close to the 5′end of III helix) and, therefore, this CBC represents intraspecific variability of *Chloromonas pichinchae*. OTU ‘denovo85’ had no CBC when compared to the reference species, and thus, was assigned to *Chloromonas pichinchae.* (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

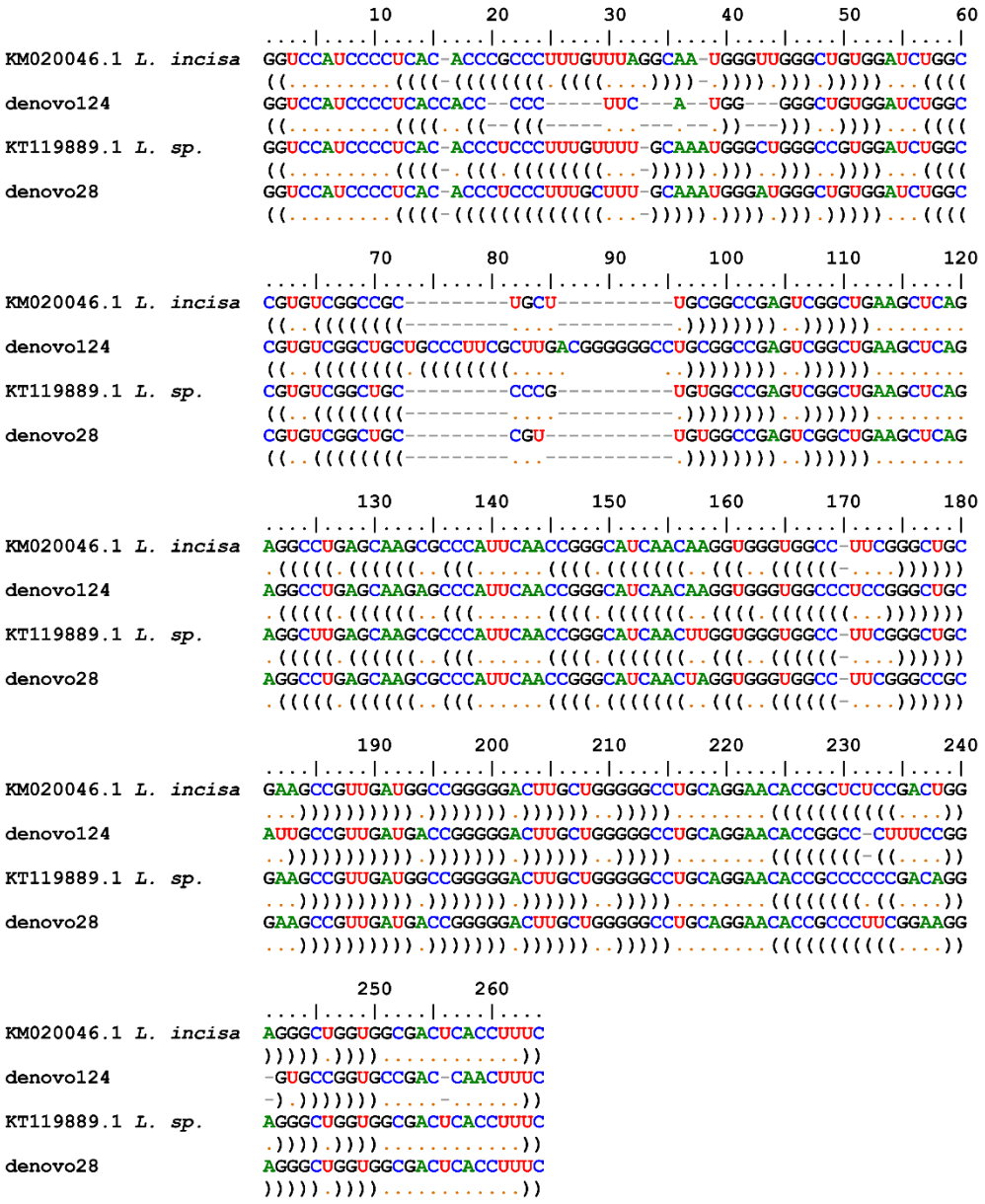


Fig. S8. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Lobosphaera incisa* SAG 2466 (accession number KM020046.1), OTU ‘denovo124’, *Lobosphaera* sp. K-1 (accession number KT119889.1) and OTU ‘denovo28’. No compensatory base change (CBC) was found in the dataset. Thus, both OTUs were assigned to species *Lobosphaera incisa* (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

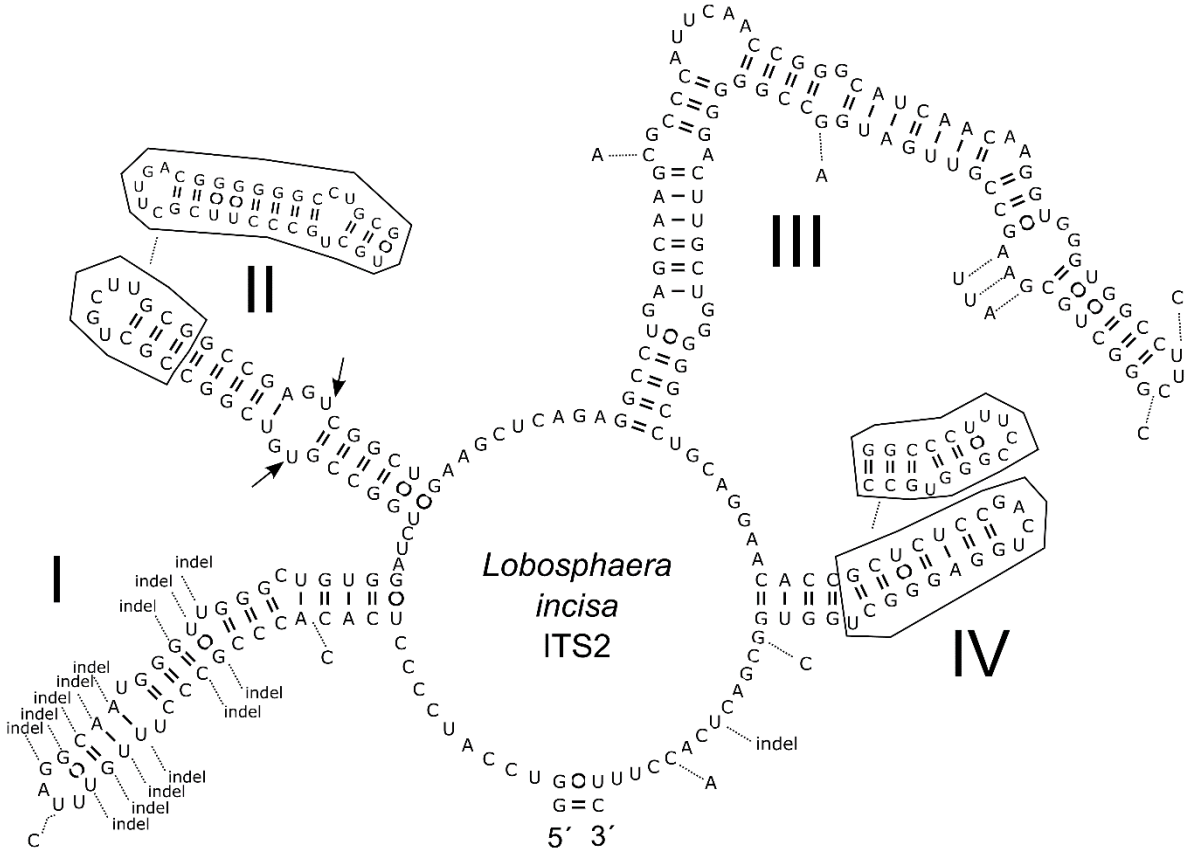


Fig. S9. Comparison of the secondary structure of ITS2 rDNA transcripts between *Lobosphaera incisa* SAG 2466 (accession number KM020046.1) and the closely related OTU ‘denovo124’. Differences characteristics for the latter are shown by nucleotides outside the structure and are linked by dotted lines. Top of helix II and helix IV (encircled) represent one of expansion segments, whose length is not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al. 2013). Therefore, this part of helix II and helix IV characteristic for OTU ‘denovo124’ is shown outside the structure and is linked by dotted lines. No CBC was found between *Lobosphaera incisa* SAG 2466 and OTU ‘denovo124’ suggesting that OTU ‘denovo124’ can be assigned to species *Lobosphaera incisa*. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

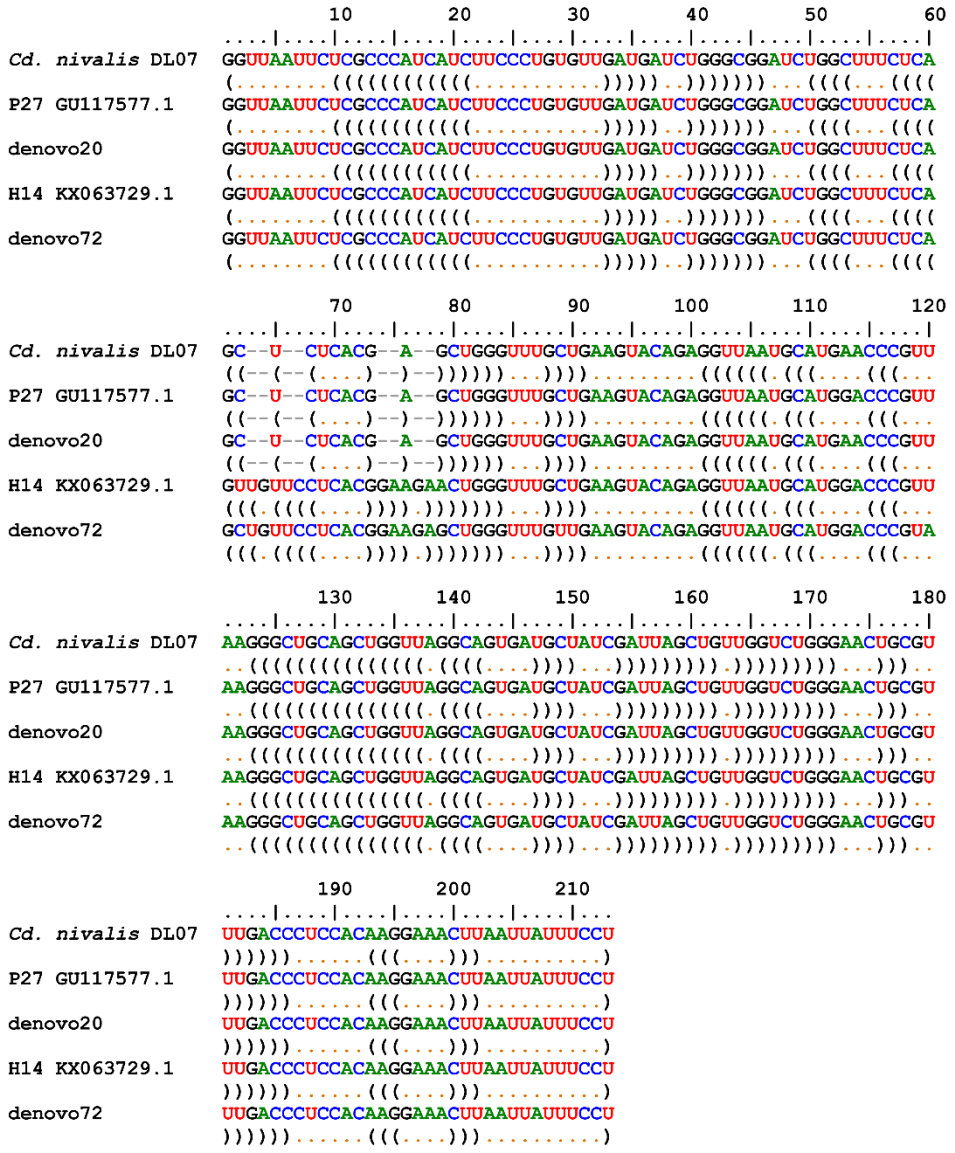


Fig. S10. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Chlamydomonas nivalis* DL07 (accession number MF803749.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. *Chlamydomonas nivalis* P27 (accession number GU117577.1) picked up by Qiime differs from *Chlamydomonas nivalis* DL07 by single nucleotide position in a single strand region in helix III. Note that the sequence of 'denovo20' is identical with the reference sequence of DL07. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

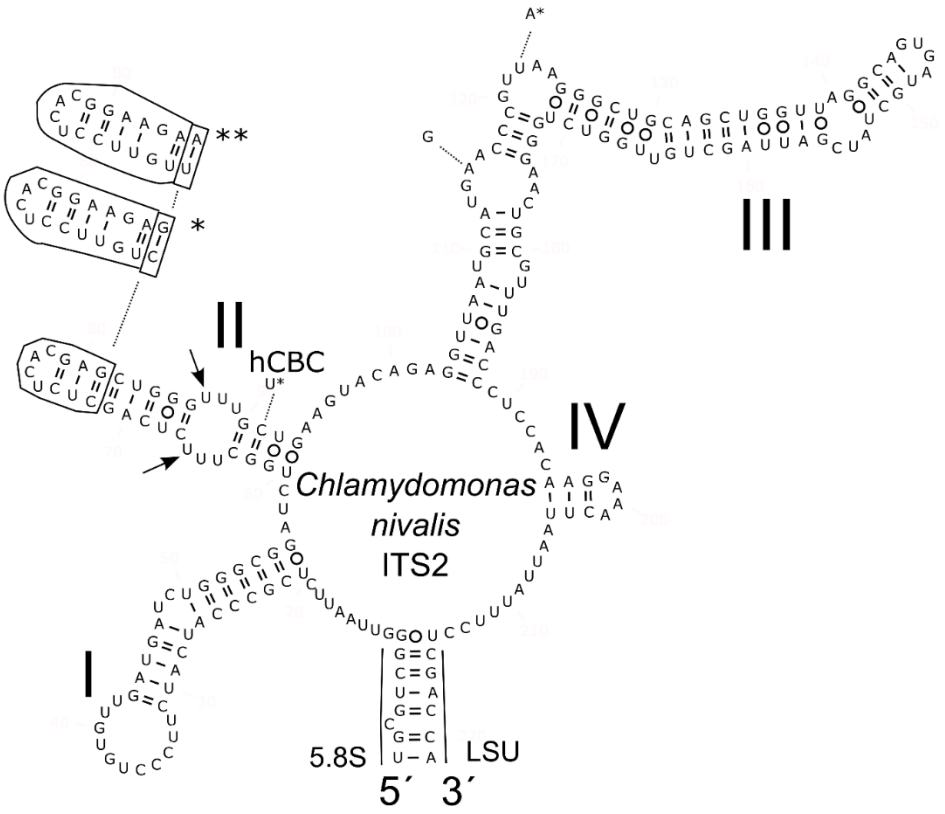


Fig. S11. Comparison of the secondary structure of ITS2 rDNA transcripts between *Chlamydomonas nivalis* DL07 (accession number MF803749.1), *Chlamydomonas nivalis* P27 (accession number GU117577.1), *Chlamydomonas* clone H14 (accession number KX063729.1), and the closely related OTU 'denovo20' and OTU 'denovo72'. OTU 'denovo20' was 100% identical with the reference sequence of *Chlamydomonas nivalis* DL07. Differences characteristic for *Chlamydomonas nivalis* P27, OTU denovo72 and clone H14 are shown by nucleotides outside the structure and are linked by dotted lines. Single nucleotide difference in helix III was shared in all three specimens (i.e., 'G' instead of 'A'). One asterisk means that the difference was detected only in OTU 'denovo72', and double asterisks imply that the difference was detected in clone H14. Top part of helix I (encircled) represent an expansion segments, whose length are not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al. 2013). Therefore, this part of helix I characteristic for OTU 'denovo72' and clone H14 are shown outside the structure and are linked by dotted lines. There was no CBC between 'denovo72' and reference sequence DL07, the sequence identity was 93%. Interestingly, ITS2 of 'denovo72' has higher sequence similarity of 98% with uncultured *Chlamydomonas* clone H14 (accession number KX063729.1) but there is one CBC in helix II between 'denovo72' and clone H14 (in the 13th nucleotide in helix II). A CBC at the same position in the structure is present also when reference sequence DL07 and clone H14 are compared. Single hemi-CBC (hCBC) in the basal part of helix I was found when compared *Chlamydomonas nivalis* DL07 and OTU 'denovo72'. Absence of any CBC in the helix III among all these sequences suggest that all these OTUs can be assigned to the same species. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

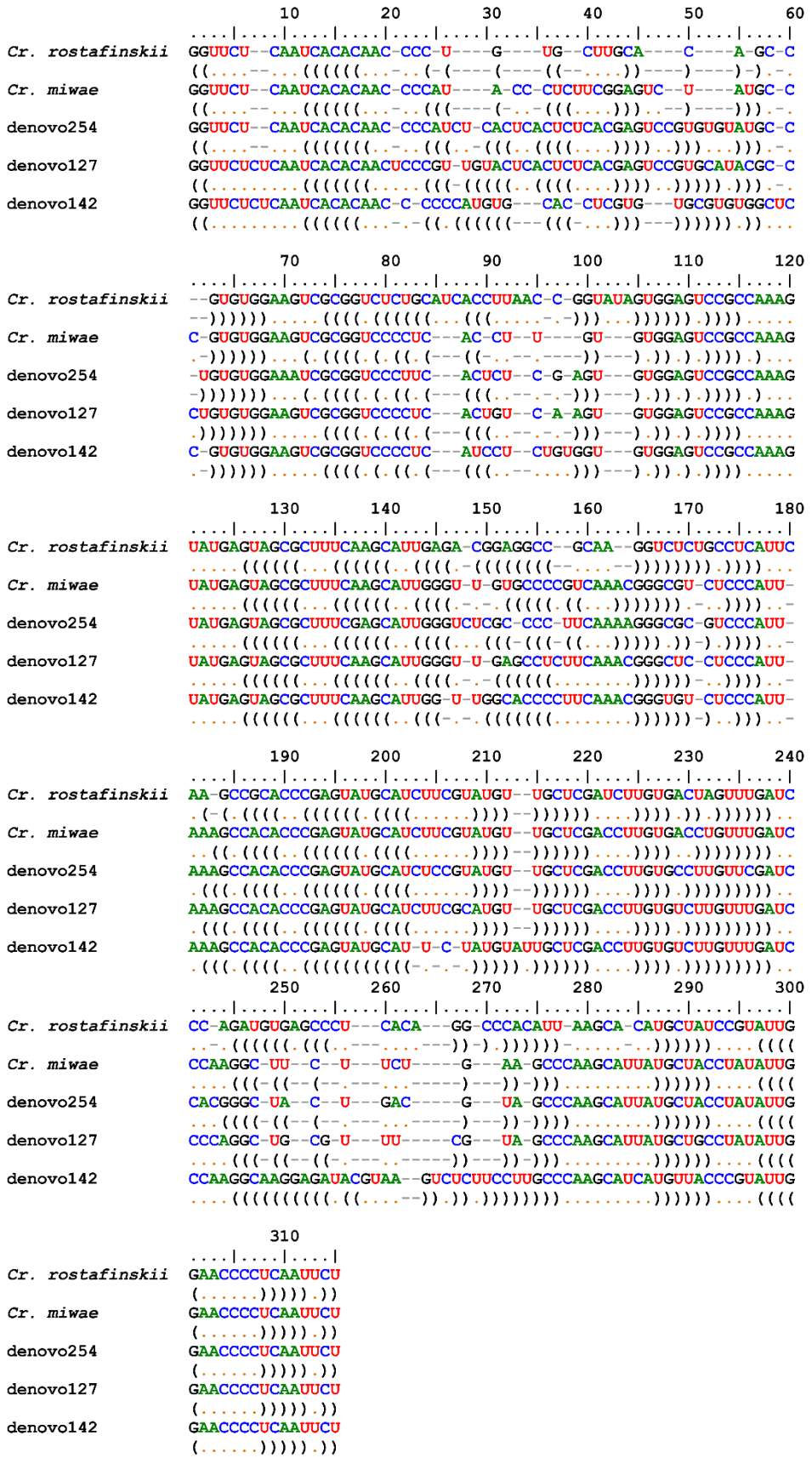


Fig. S12. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Chloromonas rostafinskii* CCCryo 010-99 (accession number

HQ404863.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. Further search in NCBI revealed that a better hit in term of higher sequence identity of 86-90% for these OTUs was represented by *Chloromonas miwae* NIES-2379 (accession number LC012762.1). As a contrast ITS2 of *Chloromonas rostafinskii* had sequence similarity with these three OTUs between 77-85% only. Compensatory base changes (CBC) in comparison with the reference sequence of *Chloromonas miwae* were found in helix III in OTU ’denovo254’ (three CBCs), OTU ‘denovo127’ (two CBCs) and OTU ’denovo142’ (four CBCs). Thus, three OTUs represent probably independent taxa. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

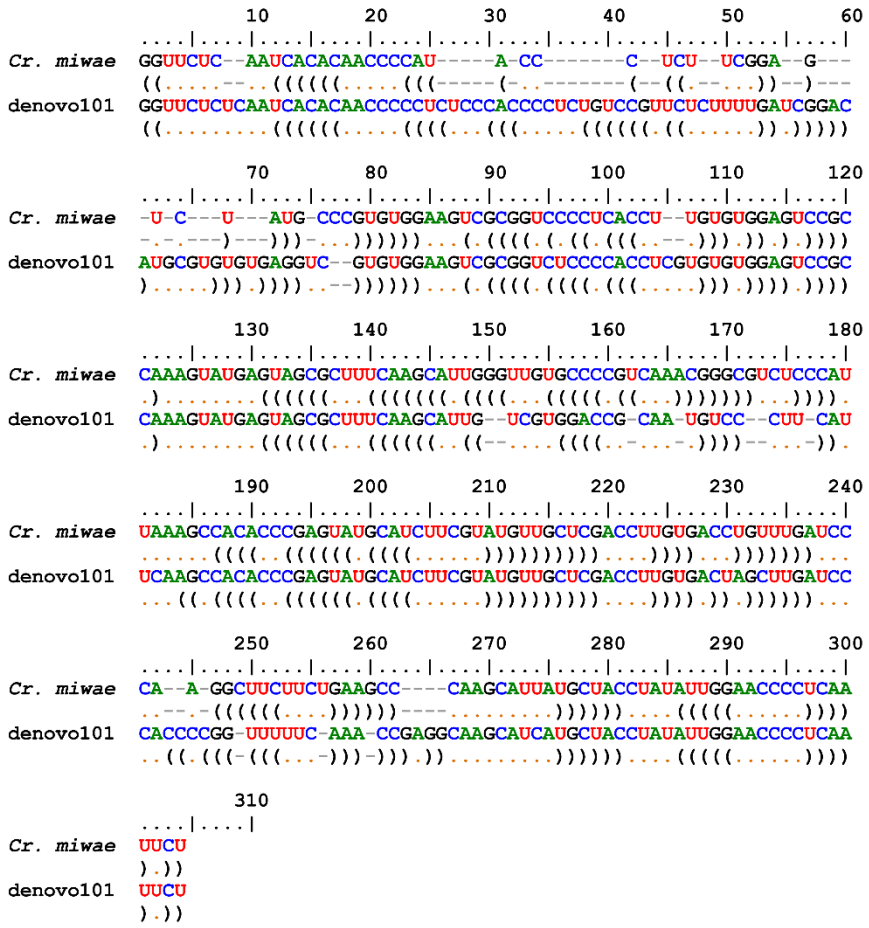


Fig. S13. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Chloromonas miwae* NIES-2379 (accession number LC012762.1) and OTU ’denovo101’. No blast hit was initially found against custom download database using Qiime. Further manual search in NCBI revealed 84% sequence similarity shared with *Chloromonas miwae* NIES-2379. Three compensatory base changes (CBCs) in comparison with the reference sequence of *Chloromonas miwae* were found in helix III, two out of the most conserved part of the structure, i.e., in the top close to the 5′end of III helix. Thus OTU ’denovo101’ represents likely an independent taxon. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

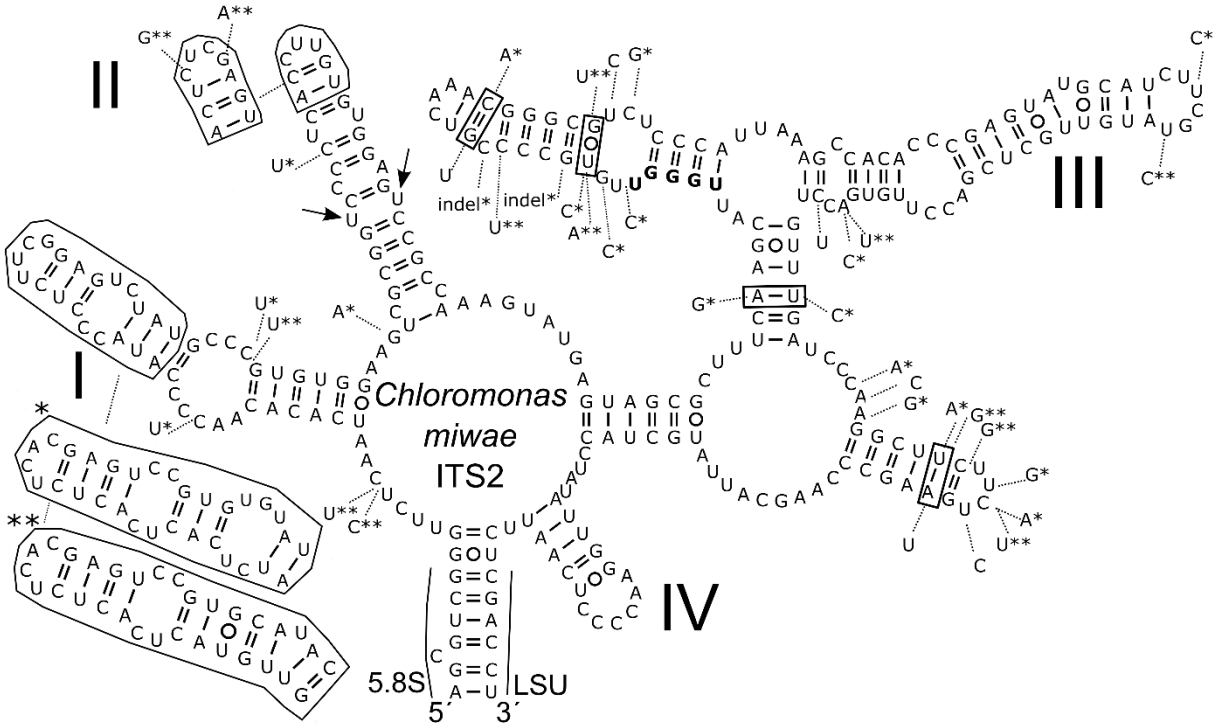


Fig. S14. Comparison of the secondary structure of ITS2 rDNA transcripts between *Chloromonas miwae* NIES-2379 (accession number LC012762) and the closely related OTU ‘denovo254’ and OTU ‘denovo127’. Differences characteristic for both OTUs are shown by nucleotides outside the structure and are linked by dotted lines. One asterisk means that the difference was detected only in OTU ‘denovo254’, and double asterisks imply that the difference were detected in OTU ‘denovo127’. Middle and top part of helix I and top part of helix II (encircled) represent expansion segments, whose length are not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al. 2013). Therefore, these parts of helix I and II characteristic for OTU ‘denovo254’ and ‘denovo127’ are shown outside the structure and are linked by dotted lines. CBSs between ITS2 sequences of *Chloromonas miwae* NIES-2379, OTU ‘denovo254’ and OTU ‘denovo127‘ are indicated by rectangles in the helix III, one out of these CBC is located in the most conserved part of helix III suggesting that both OTUs represent probably two independent species. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

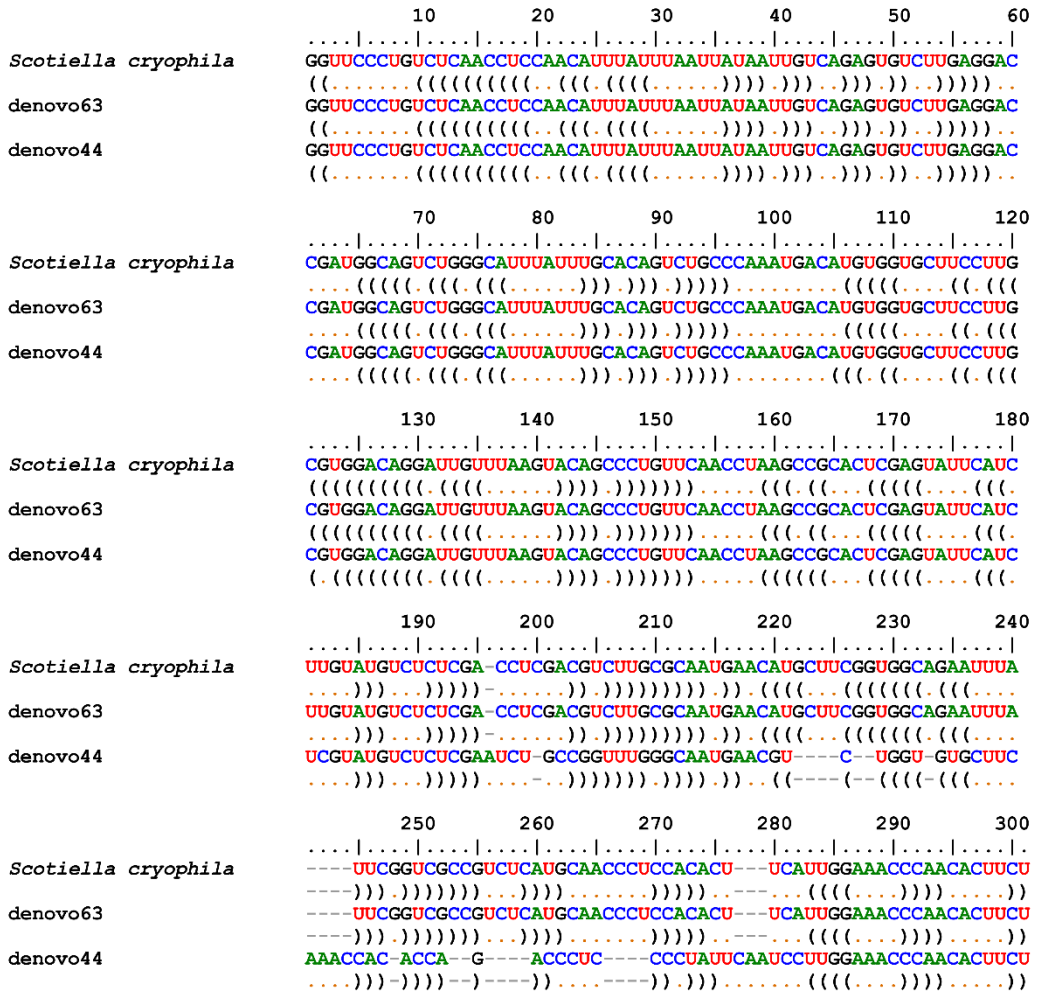


Fig. S15. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Scotiella cryophila* K-1 (accession number MG253843.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. Note that the sequence of 'denovo63' is identical with the reference sequence. (Note: HTS delivers DNA based data, but during RNA folding, thymine ['T'] is converted to uracil ['U'])

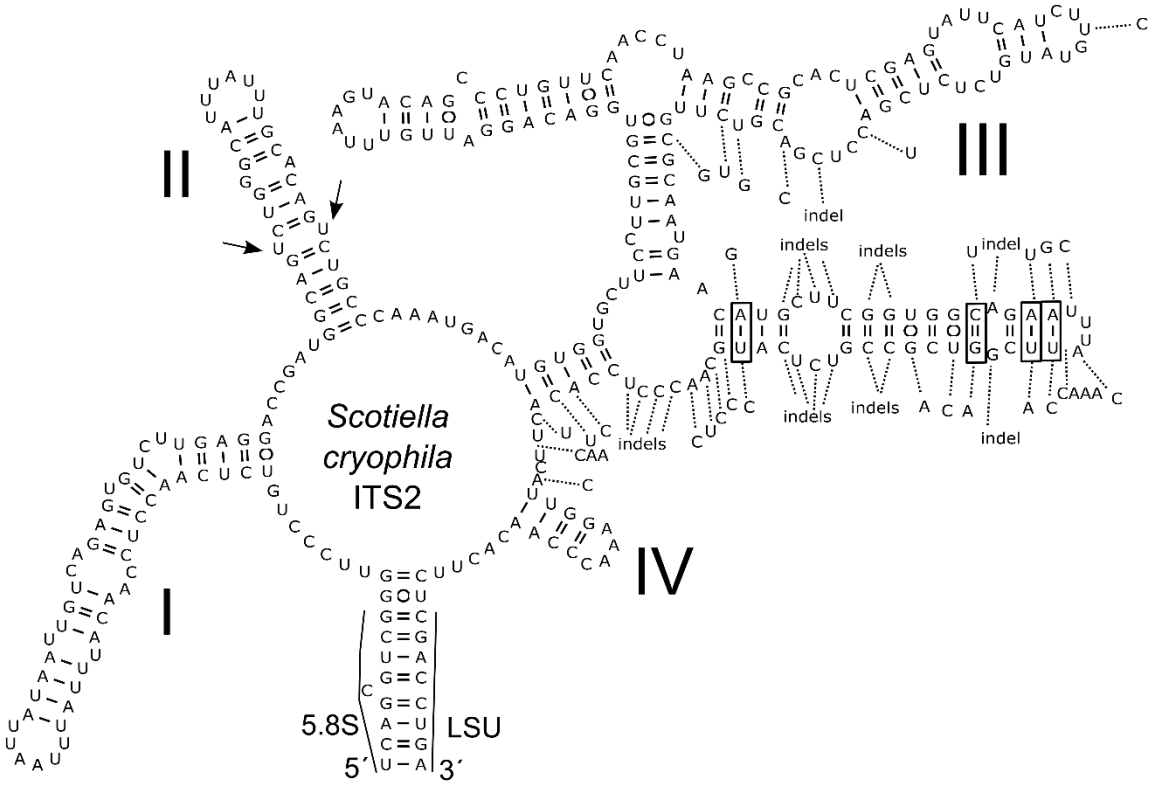


Fig. S16. Comparison of the secondary structure of ITS2 rDNA transcripts between *Scotiella cryophila* K-1 (accession number MG253843.1) and the closely related OTU ‘denovo63’ and OTU ‘denovo44’. OTU ‘denovo63’ was 100% identical with reference sequence. Differences characteristic for the OTU ‘denovo44’ are shown by nucleotides outside the structure and are linked by dotted lines. Each out of four compensatory base changes between ITS2 sequences of *Scotiella cryophila* K-1 and OTU ‘denovo44’ is indicated by a rectangle in the helix III and are found outside the most conserved part of helix III (the most conserved is close to the 5′end of III helix), therefore, ‘denovo44’ can be assigned to *Scotiella cryophila*. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

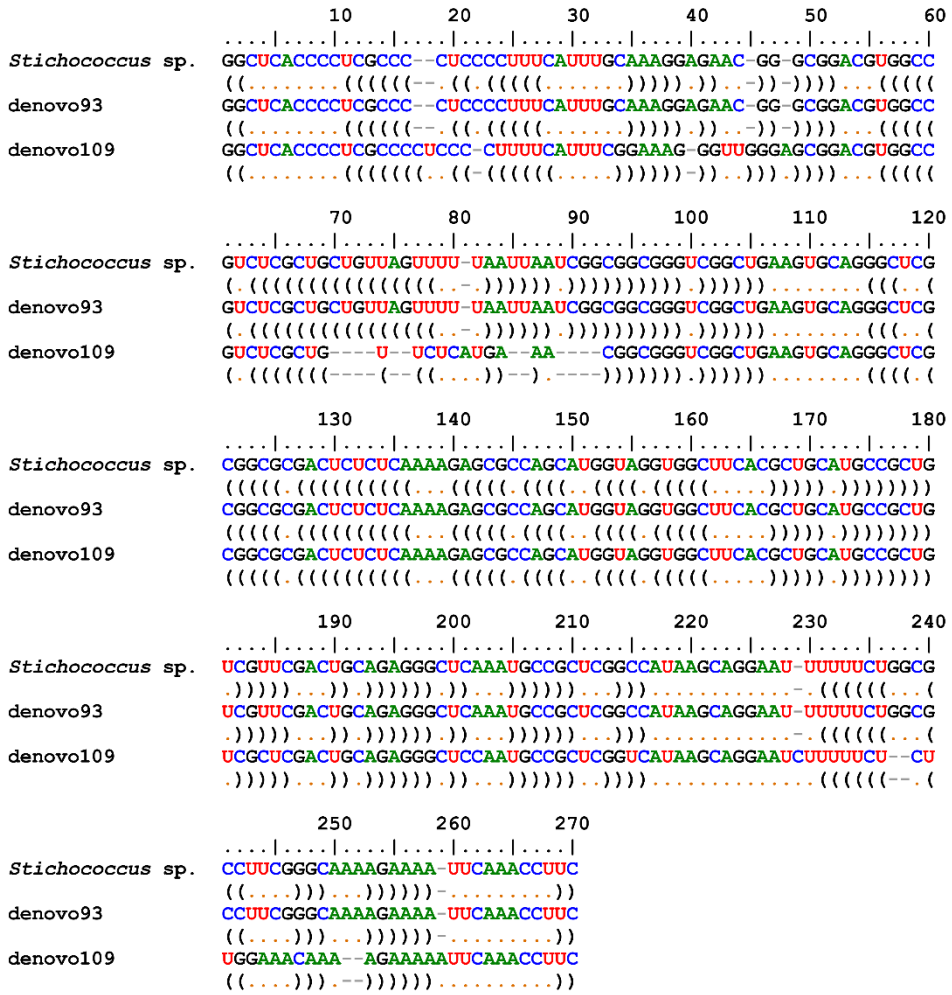


Fig. S17. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Stichococcus* sp. SAG 2482 (accession number KX094848.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. Note that the sequence of OTU ‘denovo93’ is identical with the reference sequence. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

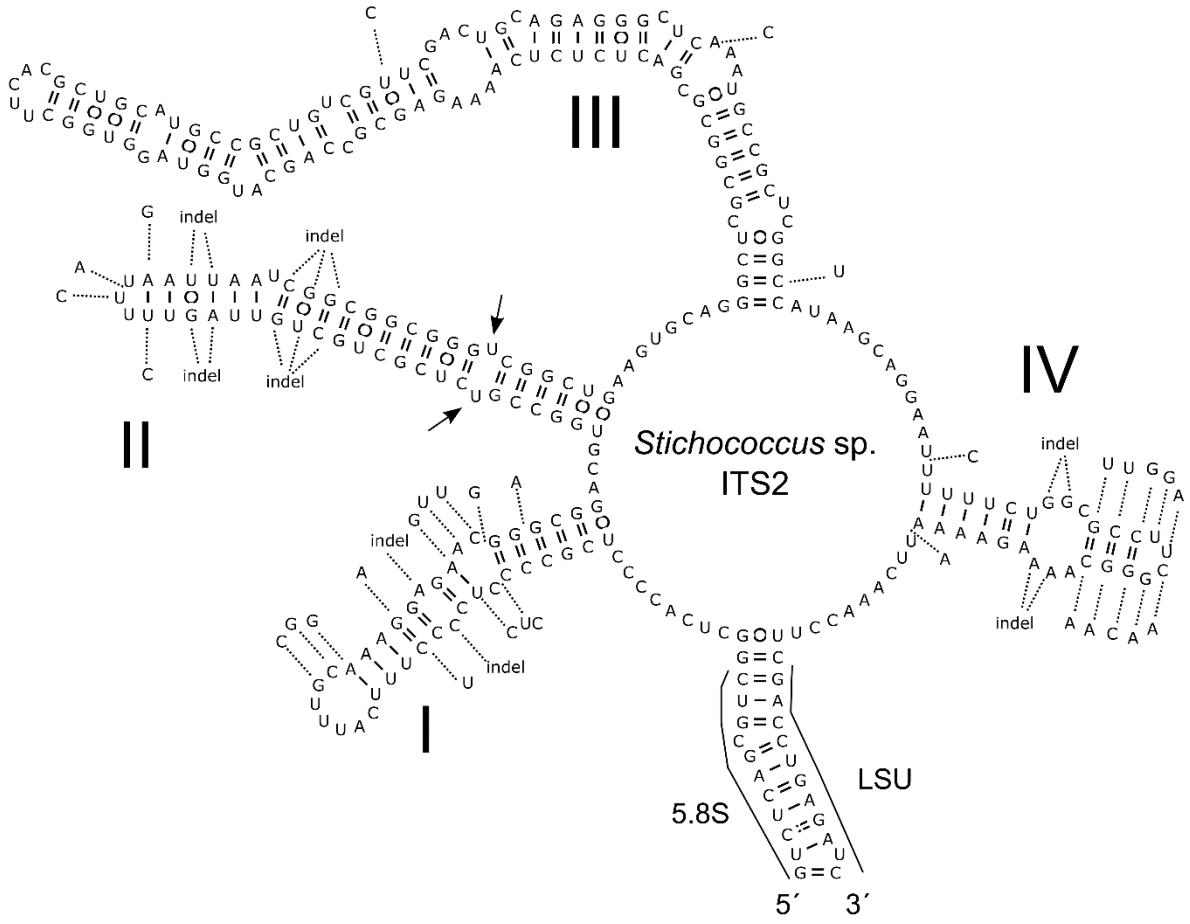


Fig. S18. Comparison of the secondary structure of ITS2 rDNA transcripts between *Stichococcus* sp. (accession number KX094848.1) and the closely related OTU ‘denovo93’ and OTU ‘denovo109’. OTU ‘denovo93’ was 100% identical with reference sequence. Differences characteristic for the OTU ‘denovo109’ are shown by nucleotides outside the structure and are linked by dotted lines. For OTU ‘denovo109’ vs. reference species – six pairs of nucleotide differences at both side of structures keeping pairing were found in variable parts of the structure (the middle part of helix I, the terminal part of helix II, helix IV, i.e. no true CBCs). However, one hemi-CBC (a change at one side of the structure in the conserved part) was found in the conserved bottom part of helix III (this site was reported to be conserved across all 56 ITS2 sequences of *Stichococcus* spp. analyzed in study of (HODAČ et al. 2016). (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

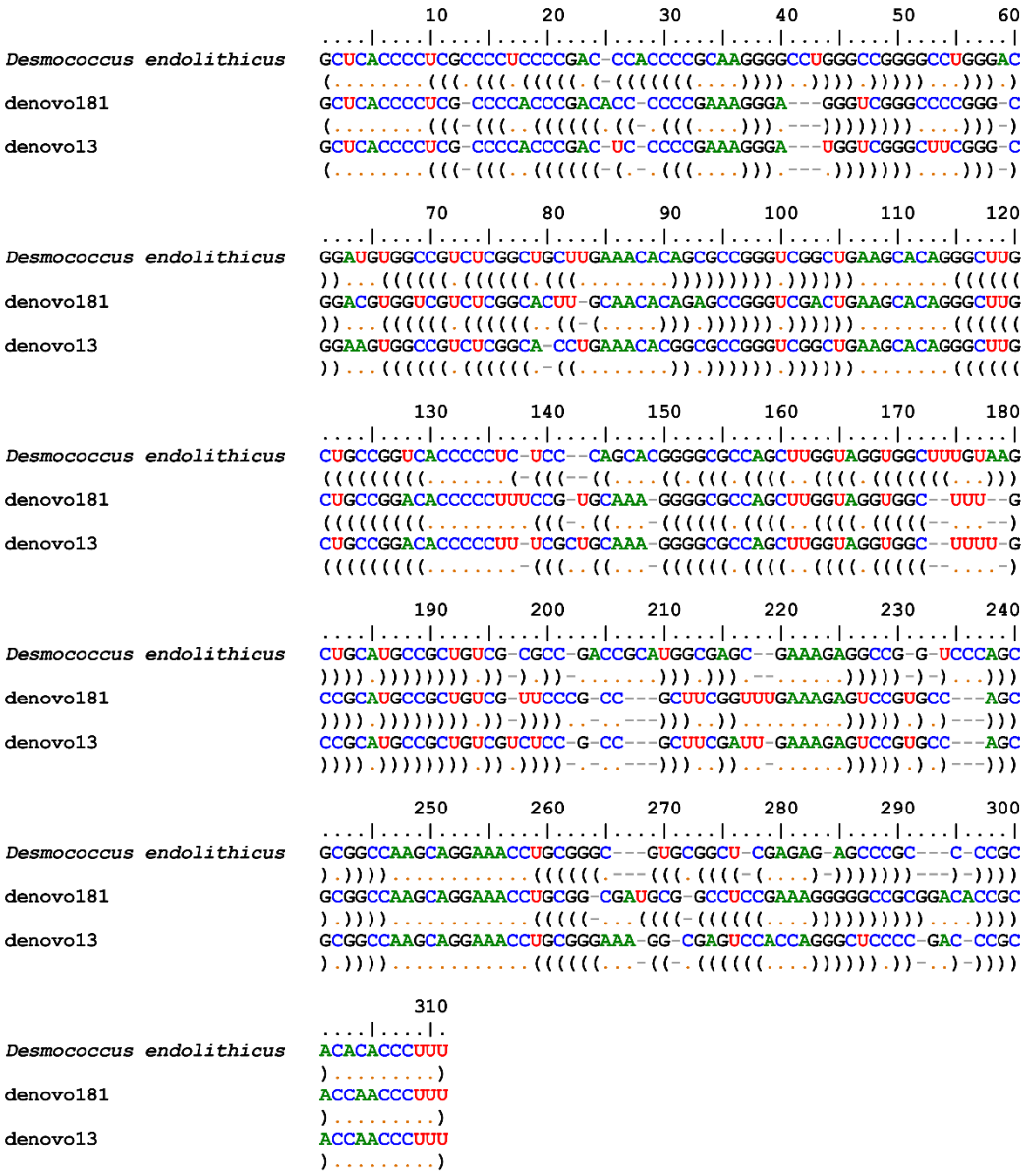


Fig. S19. Sequence-structure alignment of nuclear ribosomal DNA ITS2 transcripts from *Desmococcus endolithicus* SAG 25.92 (accession number KX094830.1) and the most abundant OTUs, which were preliminary assigned to this reference taxon using Qiime. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

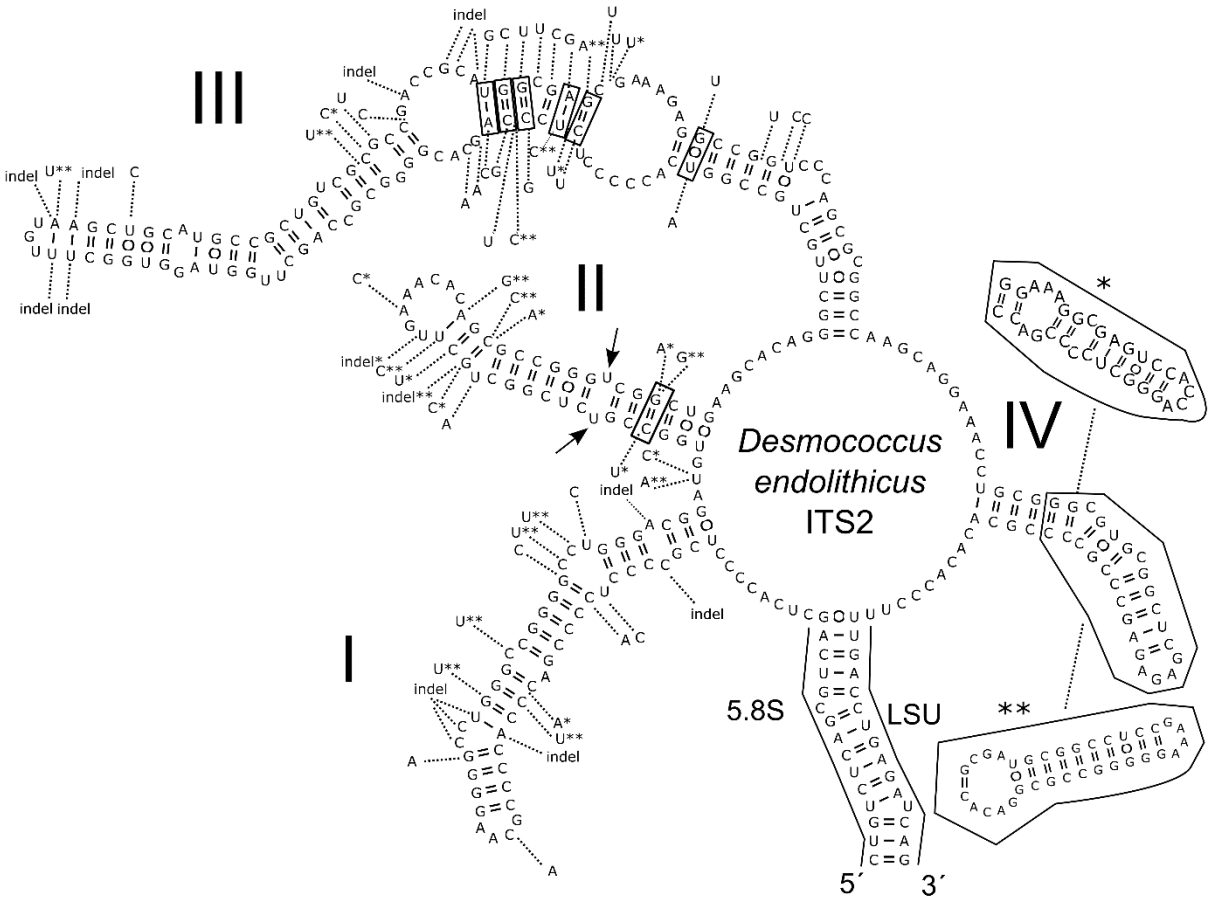


Fig. S20. Comparison of the secondary structure of ITS2 rDNA transcripts between *Desmococcus endolithicus* SAG 25.92 (accession number KX094830.1) and the closely related OTU ‘denovo181’ and OTU ‘denovo13’. Differences characteristic for these OTUs are shown by nucleotides outside the structure and are linked by dotted lines. One asterisk means that the difference was detected only in OTU ‘denovo181’, and double asterisks imply that the difference were detected in OTU ‘denovo13’; middle and top part of helix IV (encircled) represent an expansion segment, whose length is not conserved and in which positions are <70% conserved according to consensual secondary structure model of Chlorophyceae (CAISOVÁ et al. 2013). Therefore, this part of helix IV characteristic for both OTUs is shown outside the structure and is linked by dotted lines. Compensatory base changes between ITS2 sequences of *Desmococcus endolithicus* SAG 25.92 and OTU ‘denovo181’, and between *Desmococcus endolithicus* SAG 25.92 and OTU ‘denovo13’ are indicated by rectangles in the helix II and in the helix III. OTUdenovo181 and OTUdenovo13 had five and four CBCs, respectively, in conserved parts of the ITS2 secondary structure in comparison with the reference sequence. Moreover, ITS2 sequence similarities between *Desmococcus endolithicus* SAG 25.92 vs. OTU ‘denovo181’ and OTU ‘denovo13’ were only 82% and 84%, respectively (e.g. below the suggested identity threshold <89%). (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

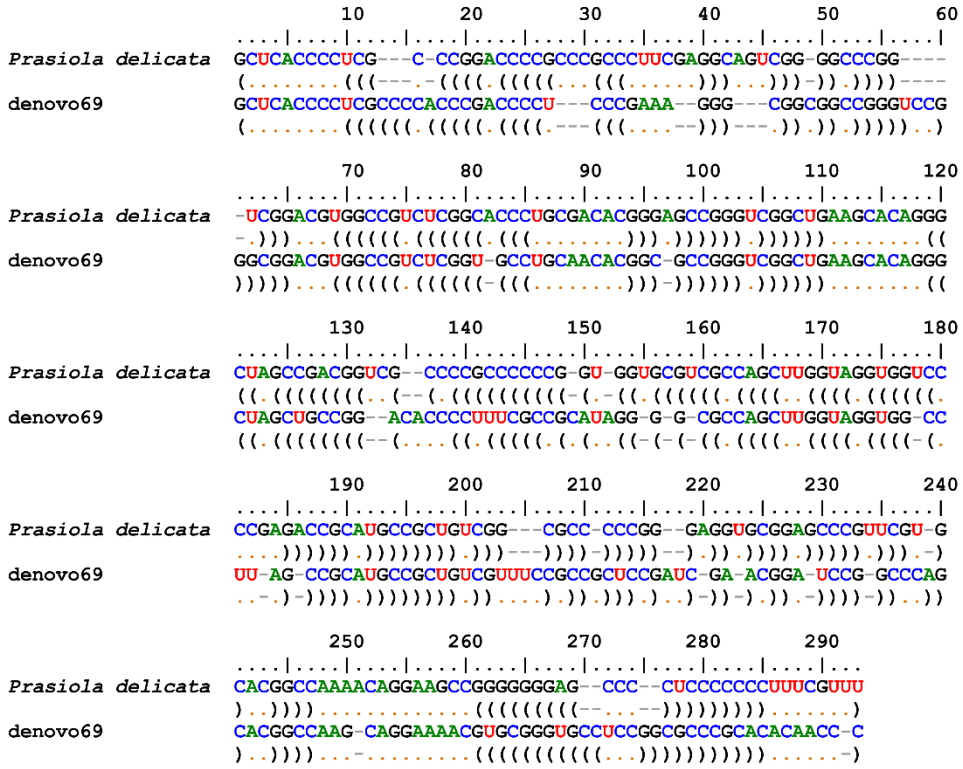


Fig. S21. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Prasiola delicata* isolate I34S (accession number MF801375.1) and OTU ’denovo69’. No blast hit was initially found against custom download database using Qiime. Further manual search in NCBI revealed 82% sequence similarity shared with *Prasiola delicata* isolate I34S. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

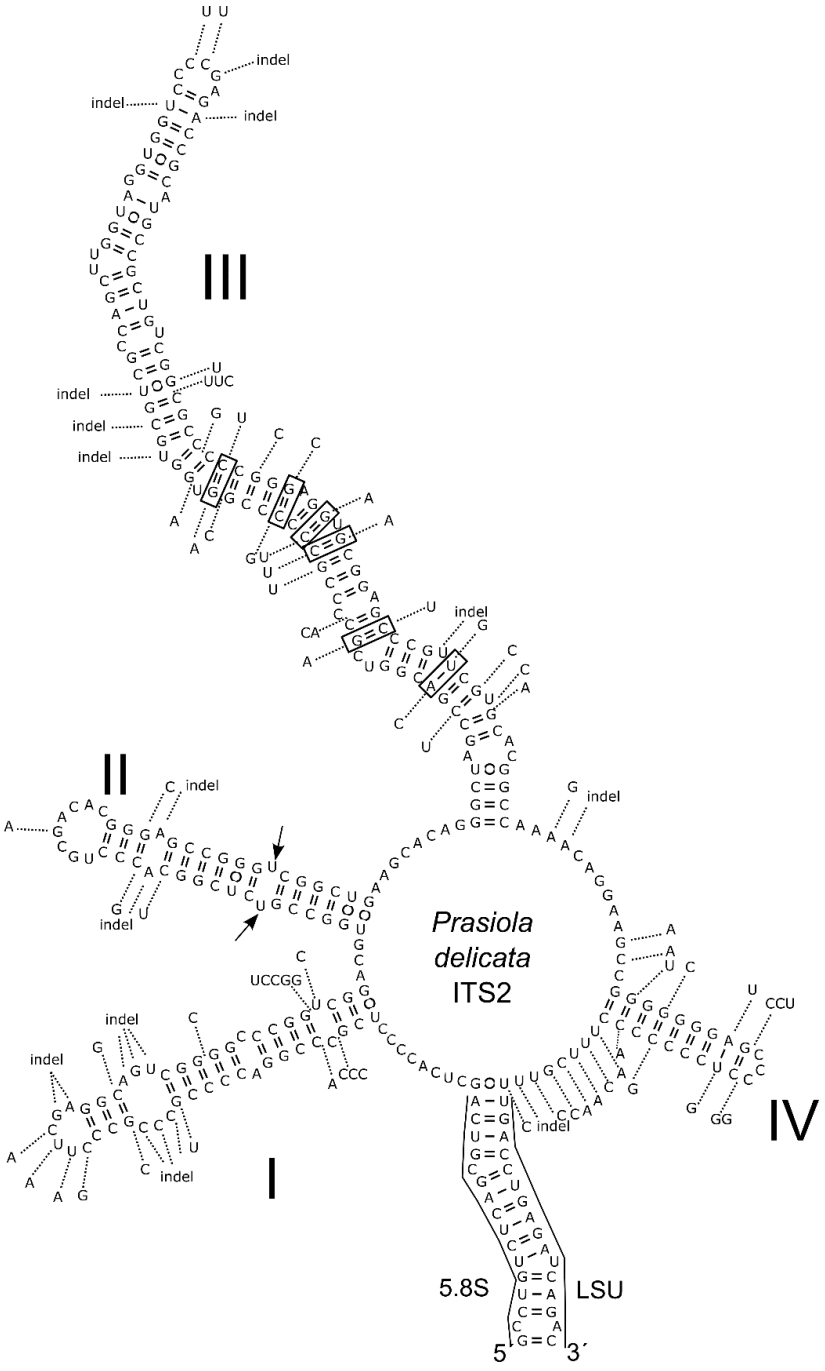


Fig. S22. Comparison of the secondary structure of ITS2 rDNA transcripts between *Prasiola delicata* isolate I34S (accession number MF801375.1) and OTU ‘denovo69’. Differences characteristic for this OTU are shown by nucleotides outside the structure and are linked by dotted lines. Compensatory base changes in conserved parts of the structure are indicated by rectangles. OTU ‘denovo69’ had six CBCs in helix III of the ITS2 secondary structure in comparison with the reference sequence. Moreover, ITS2 sequence similarities between *Prasiola delicata* isolate I34S and OTU ’denovo69’ were only 82% (i.e. below the suggested identity threshold <89%). (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

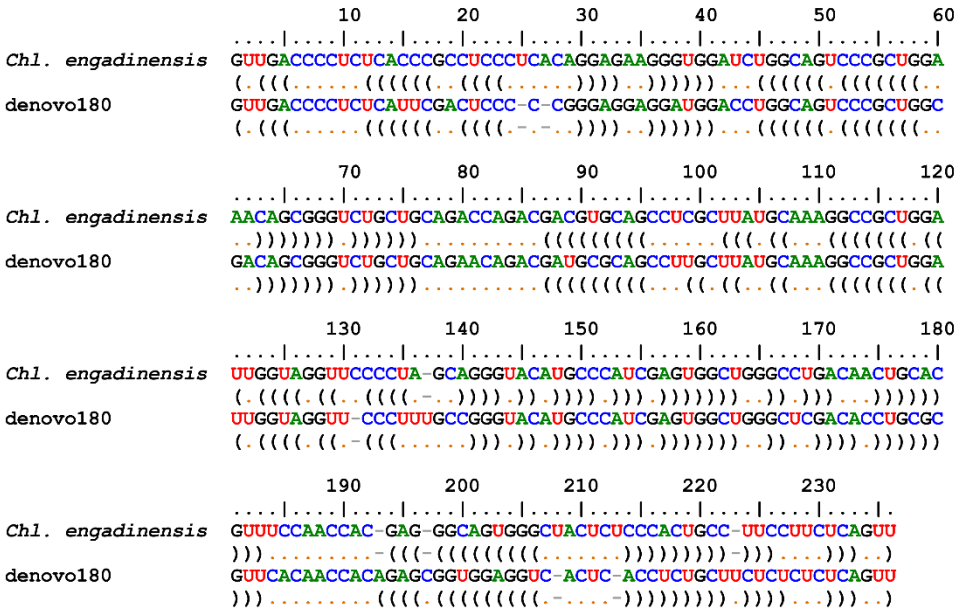


Fig. S23. Sequence-structure alignment of nuclear ribosomal DNA internal transcribed spacer 2 transcripts from *Chlorocloster engadinensis* SAG 812-1 (accession number FM946011.1) and OTU ’denovo180’. No blast hit was initially found against custom download database using Qiime. Further manual search in NCBI revealed 84% sequence similarity shared with *Chlorocloster engadinensis* SAG 812-1. (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

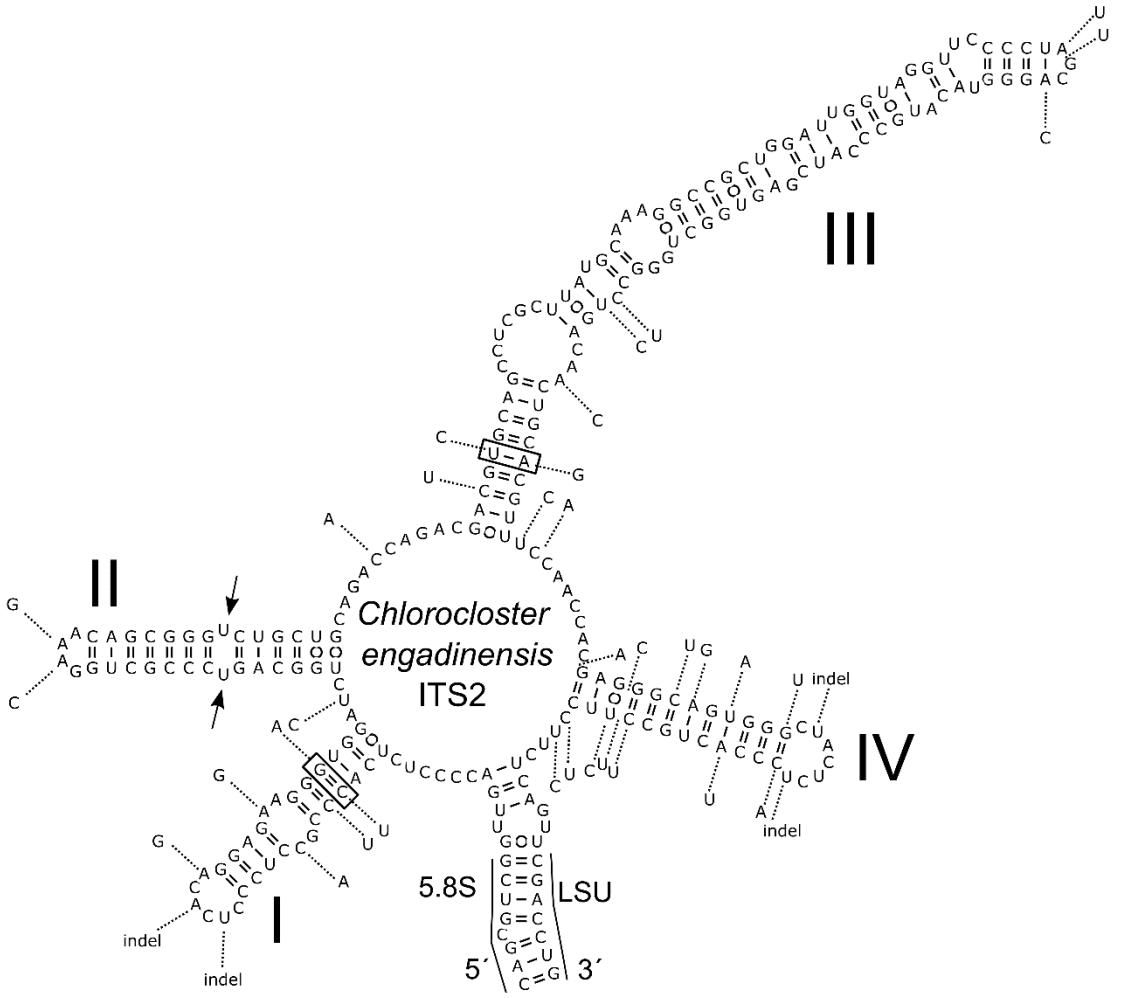


Fig. S24. Comparison of the secondary structure of ITS2 rDNA transcripts between *Chlorocloster engadinensis* SAG 812-1 (accession number FM946011.1) and OTU ‘denovo180’. Differences characteristic for this OTU are shown by nucleotides outside the structure and are linked by dotted lines. Compensatory base changes in conserved parts of the structure are indicated by rectangles. OTU ‘denovo180’ had one CBC in basal part of helix I and one CBC in basal part of helix III in comparison with the reference sequence. Moreover, ITS2 sequence similarities between OTU ’denovo180’ and its closest hit in NCBI, *Chlorocloster engadinensis* SAG 812-1, were only 84% (i.e. below the suggested identity threshold <89%). (Note: HTS delivers DNA based data, but during RNA folding, thymine [‘T’] is converted to uracil [‘U’])

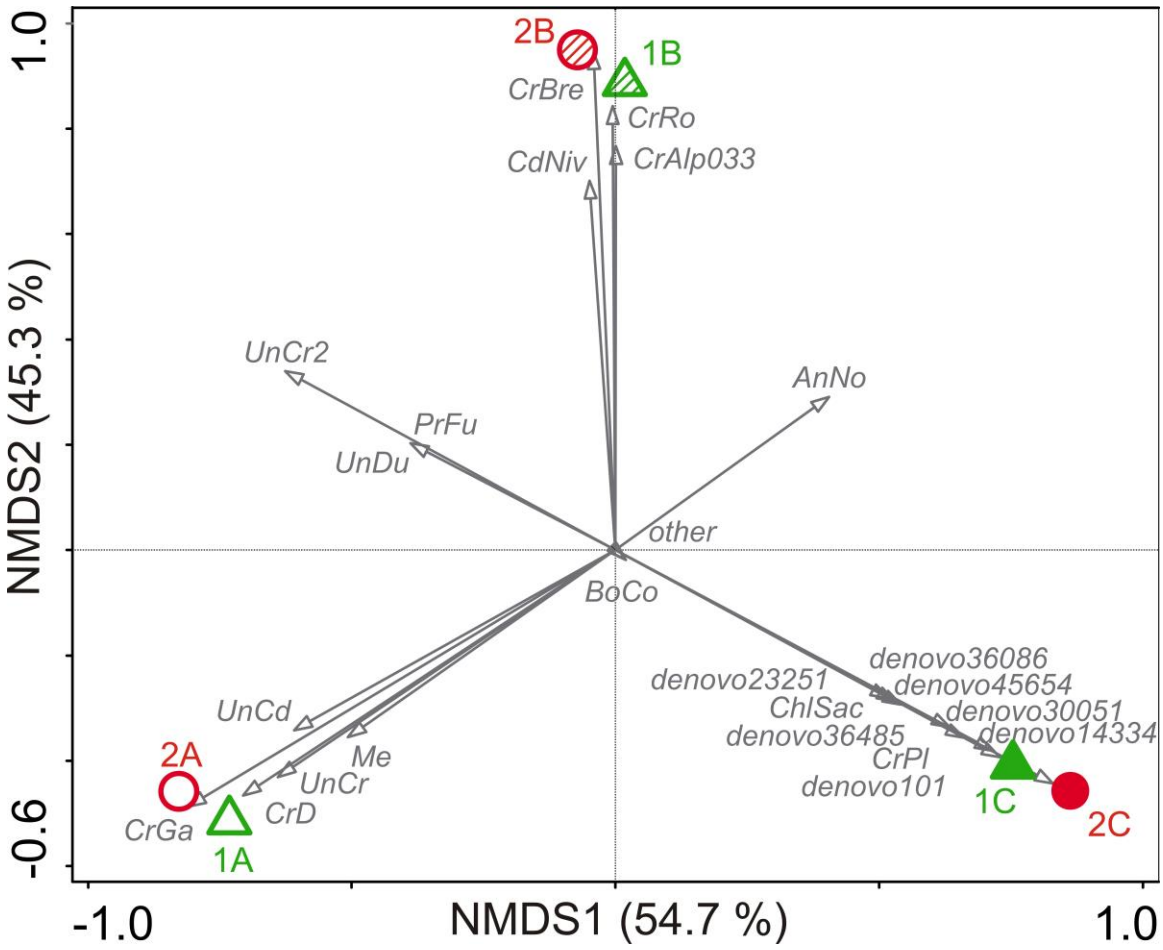


Fig. S25. Nonmetric multidimensional scaling (NMDS) ordination describing the differences in taxonomic composition of the two samples (sample 1 – green, sample 2 – red) based on 18S rDNA data analysed using the three strategies of taxonomic assignment (A, B, C – for details, see the text). Values within parentheses show % of variance explained by each axis. Source of the data for NMDS ordination: Table 5.
Acronyms:

AnNo	*Ancylonema nordenskioeldii* AF514397.2
BoCo	*Botrydiopsis constricta* AJ579339.1
CdNiv	*Chlamydomonas nivalis* DL07 MF803748
CrAlp033	*Chloromonas* cf. *alpina* CCCryo 033-99 HQ404865.1
CrBre	*Chloromonas brevispina* K-2 MG791867
CrD	*Chloromonas* sp. D-CU581C AF517086.1
CrGa	*Chloromonas* sp. Gassan-A LC012753.1
CrPl	*Chloromonas platystigma* strain CCCryo 020-99 AF514401.1
CrRo	*Chloromonas* cf. *rostafinskii* CCCryo 025-99 AF514402.1
ChlSac	*Chloroidium saccharophilum* isolate HST10K KX024691.1
Me	*Mesotaenium* sp. AG-2009-1 FM992335.1
PrFu	*Prasiola furfuracea* AF189073.1
UnCd	Uncultured Chlamydomonadaceae AB902971.1
UnCr	Uncultured *Chloromonas* AB903008.1
UnCr2	Uncultured *Chloromonas* AB902984.1
UnDu	Uncultured Dunaliellaceae EF023287.1

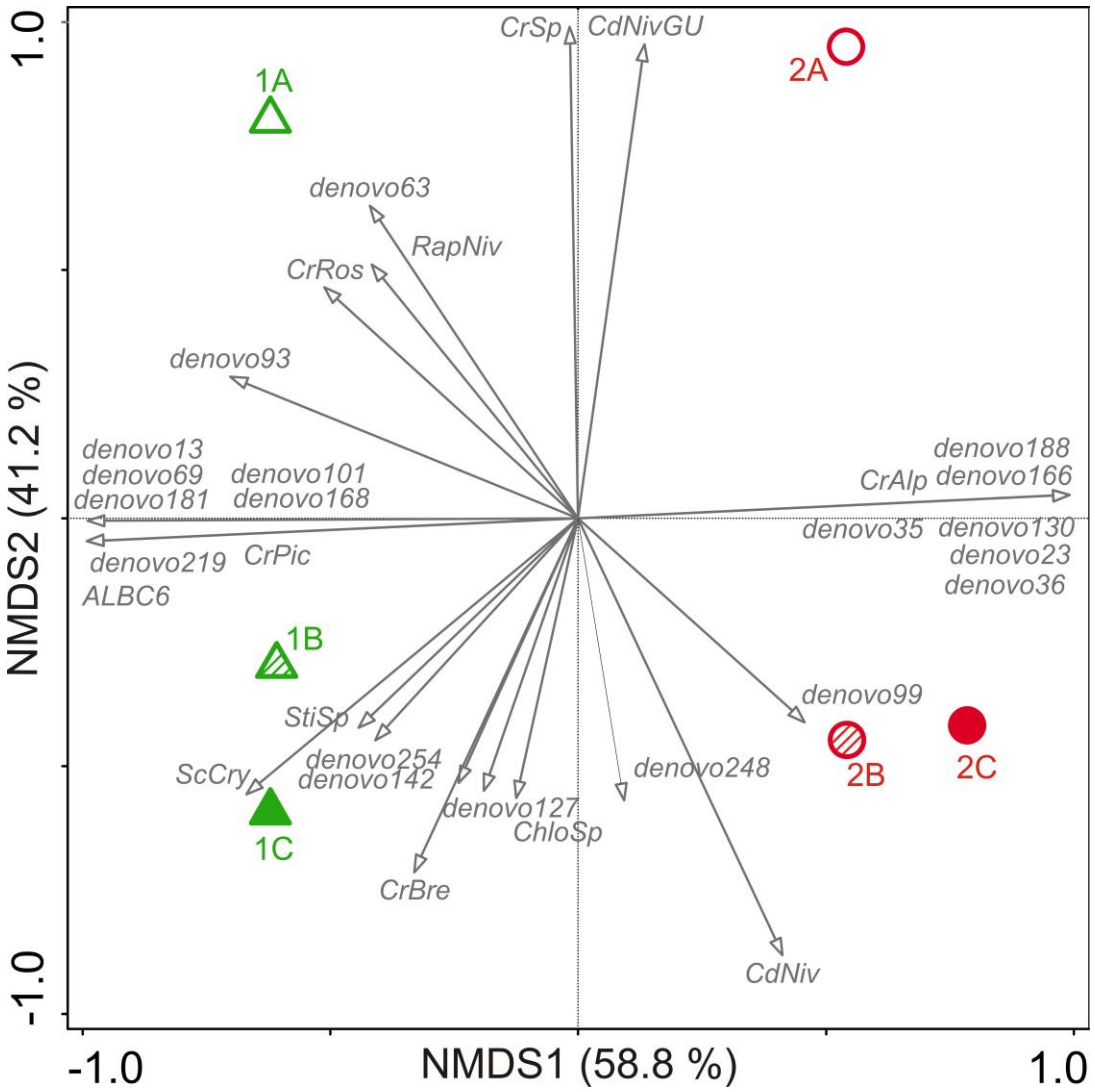


Fig. S26. Nonmetric multidimensional scaling (NMDS) ordination describing the differences in taxonomic composition of the two samples (sample 1 – green, sample 2 – red) based on ITS2 data analysed using the three strategies of taxonomic assignment (A, B, C – for details, see the text). Values within parentheses show % of variance explained by each axis. Source of the data for NMDS ordination: Table S7.

Acronyms:

ALBC6	Uncultured Chlorophyte clone ALBC6 JX435348
CdNiv	*Chlamydomonas nivalis* DL07 MF803749
CdNivGU	*Chlamydomonas nivalis* GU117577
CrAlp	*Chloromonas alpina* CCCryo032-99 HQ404864
CrBre	*Chloromonas brevispina* K-2 MG791868
CrPic	*Chloromonas pichinchae* CCCryo 2616-06 HQ404889
CrRos	*Chloromonas rostafinskii* HQ404863
CrSp	*Chloromonas* sp. CCCryo 289-06 HQ404893
ChloSp	uncultured *Chlorella* sp.5 AB90311
RapNiv	*Raphidonema nivale* AJ431676
ScCry	*Scotiella cryophila* K-1 MG253843
StiSp	*Stichococcus* sp. SAG 2482 KX094848

Table S1. List of primers used for amplification and Sanger sequencing reactions of 18S rDNA and ITS2 rDNA markers; (F) forward; (R) reverse.

Primer	Marker	Direction	Sequence	Reference
P2	18S	F	CTGGTTGATTCTGCCAGT	(MOON-VAN DER STAAY et al. 2000)
P4	18S	R	TGATCCTTCYGCAGGTTCAC	(MOON-VAN DER STAAY et al. 2000)
FA	18S	F	AACCTGGTTGATCCTGCCAGT	(MATSUZAKI et al. 2015)
RD	18S	R	GCTGGCACCAGACTTGCCCTC	(MATSUZAKI et al. 2015)
FC	18S	F	GGGAGGTAGTGACAATAAATA	(MATSUZAKI et al. 2015)
RF	18S	R	CCCGTGTTGAGTCAAATTAAG	(MATSUZAKI et al. 2015)
FE	18S	F	GGGAGTATGGTCGCAAGGCTG	(MATSUZAKI et al. 2015)
RB	18S	R	TGATCCTTCTGCAGGTTCACCTAC	(MATSUZAKI et al. 2015)
AL1500af	ITS	F	GCGCGCTACACTGATGC	(HELMS et al. 2001)
LR3	ITS	R	GGTCCGTGTTTCAAGACGG	(VILGALYS & HESTER 1990)
ITS5	ITS	F	GGAAGTAAAAGTCGTAACAAGG	(WHITE et al. 1990)
ITS4	ITS	R	TCCTCCGCTTATTGATATGC	(WHITE et al. 1990)
SSU	ITS	F	CTGCGGAAGGATCATTGATTC	(PIERCEY-NORMORE & DEPRIEST 2001)
LSU	ITS	R	AGTTCAGCGGGTGGTCTTG	(PIERCEY-NORMORE & DEPRIEST 2001)

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Table S2. ITS2 reference sequences downloaded from NCBI for the custom-built database

Taxon	Specimen/strain designation	Accession number ITS2 rDNA
Chlamydomonas reinhardtii	CC620	U66954.2
Chlamydomonas sp.		AF033295.1
Raphidonema nivale	CCAP 470/4	AJ431676.1
Raphidonema sempervirens	CCAP 470/6	AJ431674.1
Spirogyra sp.	GRS2	KM453687.1
Klebsormidium sp.	SAG 2065	EU434032.1
Mesostigma viride		EC732140.1
Chloromonas nivalis	P24/DR4	GU117576.1
Chloromonas nivalis	CCCryo_005-99	HQ404862.1
Chloromonas pichinchae	CCCryo_261-06	HQ404889.1
Chloromonas sp.	CCCryo 207-05	HQ404883.1
Chloromonas alpina	CCCryo 033-99	HQ404865.1
Chloromonas alpina	CCCryo 032-99	HQ404864.1
Chloromonas sp.	CCCryo 289-06	HQ404893.1
Chloromonas rostafinskii	CCCryo 010-99	HQ404863.1
Chloromonas pichinchae	CCCryo 192-04	HQ404880.1
Chlamydomonas nivalis	P27/DR1	GU117577.1
Chloromonas sp.	JP13	AB902970.1
Uncultured Chlorophyta	clone ALBC6	JX435348.1

Table S3. List of reference species locally abundant in the studied Austrian alpine snowfields and the GenBank accession numbers of encoded 18S rDNA and ITS2 rDNA markers.

	accession number	
Taxon	18S rDNA	ITS2 rDNA
Chlamydomonas nivalis DL07	MF803748	MF803749
Scotiella cryophila K-1	MG253843	MG253843
Chloromonas brevispina K-2	MG791867	MG791868

Table S4. Algal community compositions based on the 18S rDNA data sets comprising the 50 most abundant OTUs that made up >87% of the community. The table shows the discrepancies between OTU assignments using three strategies: (A) basic version using Qiime and the publicly available Silva database, (B) extended version using Qiime and additional reference sequences of the locally abundant taxa (underlined), and (C) further extended version using the final manual verification of taxa assignments in NCBI, only allowing a 2 bp nucleotide difference (≥99.4% identity; sequences below this threshold were recorded as "no blast hit"). With the respective reference sequence in order to be recorded as a match. Ambiguous hits are sequences that share all the same level of similarity with an inspected OTU, and thus they cannot be assigned unambiguously. Our results reveal that the manual verification in NCBI is essential and, thus, highly recommended.

OTU ID	Sample 1 [%]	Sample 2 [%]	(A) Qiime + Silva	(B) Qiime + Silva + local references	(C) Qiime + Silva + local references + manual verification
denovo14334	33.0	66.8	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	Ambiguous hits: *Chloromonas brevispina* K-2 *Scotiella cryophila* K-1 *Chloromonas sp.* TA 8 AB902996 *Chloromonas* sp. Gassan-B LC012714
denovo45654	18.7	0.1	*Mesotaenium* sp. AG-2009-1 FM992335.1	*Ancylonema nordenskioeldii* AF514397.2	*Ancylonema nordenskioeldii* AF514397.2
denovo36485	0.9	13.6	Uncultured Chlamydomonadaceae AB902971.1	*Chlamydomonas nivalis* DL07	*Chlamydomonas nivalis* DL07
denovo40226	8.2	0	*Botrydiopsis constricta* AJ579339.1	*Botrydiopsis constricta* AJ579339.1	*Botrydiopsis constricta* AJ579339.1
denovo15070	4.6	1.0	Uncultured *Chloromonas* AB903008.1	*Chloromonas* cf. *alpina* CCCryo 033-99 HQ404865.1	*Chloromonas platystigma* strain CCCryo 020-99 AF514401.1
denovo20542	4.6	0	Uncultured Dunaliellaceae EF023287.1	Uncultured Dunaliellaceae EF023287.1	*Chloroidium saccharophilum* isolate HST10K KX024691.1
denovo101	3.2	1.1	*Chloromonas* sp. D-CU581C AF517086.1	*Chloromonas* cf. *rostafinskii* CCCryo 025-99 AF514402.1	Ambiguous hits: *Chloromonas* sp. NIES-2379 AB906350.1, *Chloromonas rostafinskii* strain CCCryo 025-99 AF514402.1
denovo23251	3.0	<0.1	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	Ambiguous hits: *Chloromonas brevispina* K-2 *Chloromonas* sp. Hakkoda-1 LC012710.1, *Chloromonas* sp. Gassan-A LC012709.1
denovo30051	0.4	1.9	*Chloromonas* sp. JP15 AB902984.1	*Chloromonas* sp. JP15 AB902984.1	No blast hits
denovo36086	2.1	0	*Prasiola furfuracea* AF189073.1	*Prasiola furfuracea* AF189073.1	No blast hits
denovo2551	1.6	0.1	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas* sp. TA 8 AB902996.1	Ambiguous hits: *Scotiella cryophila* K-1 *Chloromonas* sp. Gassan-B LC012714
denovo44194	0.9	0.4	*Chloromonas* sp. D-CU581C AF517086.1	*Chloromonas tughillensis* UTEX_SNO_88 AB734116.1	No blast hit

denovo30674	0.1	1.0	*Prototheca cutis* AB470468.1	*Prototheca cutis* AB470468.1	No blast hit
denovo32383	1.1	0	*Chloromonas tughillensis* UTEX_SNO_88 AB734116.1	*Chloromonas tughillensis* UTEX_SNO_88 AB734116.1	*Chloromonas fukushimae* AB906342
denovo12840	0	1.0	*Chloromonas* sp. JP15 AB902984.1	*Chloromonas* sp. JP15 AB902984.1	Ambiguous hits: >40 sequences including *Chloromonas* sp. TA 9 AB903024.1
denovo12413	0	1.0	*Chlamydomonas* sp. GL1 AB902971.1	*Chlamydomonas nivalis* DL07	*Chlamydomonas nivalis* DL07
denovo14574	0	0.9	*Chlamydomonas* sp. GL1 AB902971.1	*Chlamydomonas nivalis* DL07	*Chlamydomonas nivalis* DL07
denovo18060	0	0.8	*Chloromonas* sp. GL4 AB903027.1	*Chloromonas* sp. GL4 AB903027.1	Uncultured eukaryote clone ALA5117773
denovo44928	0.1	0.7	Uncultured eukaryote clone TE107A KM870650.1	Uncultured eukaryote clone TE107A KM870650.1	Uncultured eukaryote clone TE107A KM870650.1
denovo25214	0.8	0	*Botrydiopsis callosa* AJ579340.1	*Botrydiopsis callosa* AJ579340.1	*Botrydiopsis callosa* AJ579340.1
denovo18262	0	0.7	*Hydrurus* sp. Sva 10_3 HE820740.1	Uncultured *Hydrurus* HE820740.1	*Hydrurus foetidus* FM955256.1
denovo12567	0.1	0.5	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	Ambiguous hits 7 sequences including: *Chloromonas brevispina* K-2 *Scotiella cryophila* K-1 *Chloromonas* sp. Gassan-B LC012714.1 *Chloromonas polyptera* JQ790556.1
denovo40835	0	0.4	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	No blast hit
denovo40500	0.1	0.2	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	Ambiguous hits: *Chloromonas brevispina* K-2 *Scotiella cryophila* K-1 *Chloromonas* sp. Gassan-B LC012714.1
denovo46231	0	0.3	*Chloromonas* sp. AL4 AB903027.1	*Chloromonas* sp. AL4 AB903027.1	*Chloromonas* sp. AL4 AB903027.1
denovo48297	0	0.3	*Chloromonas* sp. Hakkoda-1 LC012710.1	*Chloromonas brevispina* K-2	No blast hit
denovo45502	0	0.3	*Botrydiopsis constricta* AJ579339.1	*Botrydiopsis constricta* AJ579339.1	Botryidiopsidaceae sp. AM695636.1
denovo24516	0.3	0	*Raphidonema sempervirens* AJ309939.1	*Raphidonema sempervirens* AF514410.2	Ambiguous hits: >40 sequences including *Raphidonema sempervirens* AF514410.2 *Stichococcus* sp. KP08194
denovo21882	0.3	0	*Heterococcus fuornensis* AM490821.1	*Heterococcus fuornensis* AM490821.1	Ambiguous hits: 6 sequences including *Heterococcus chodatii* AM490822.1 *Heterococcus fuornensis* AM490821.1 *Heterococcus pleurococcoides* AJ579335.1
denovo29189	0.2	0	*Stichococcus bacillaris* JQ315605.1	*Stichococcus jenerensis* DQ275461.1	Ambiguous hits 4 sequences including *Stichococcus* sp. SAG 2482 KP081395.1 *Stichococcus* sp. KP081396.1
denovo34115	0.2	0	*Chloromonas* sp. JP15 AB902984.1	*Chloromonas* sp. JP15 AB902984.1	Ambiguous hits: >40 sequences including

					Chloromonas sp. TA9 AB903024.1 *Chloromonas* sp. SV3 AB903022.1 *Chloromonas* sp. PA2 AB903020.1
denovo16044	0.2	0	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas* sp. Hakkoda-1 LC012710.1	Ambiguous hits: *Chloromonas* sp. Hakkoda-1 LC012710.1 *Chloromonas* sp. Gassan-A LC012709.1
denovo4533	0.1	0.2	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	Ambiguous hits: 8 sequences including *Chloromonas brevispina* WP124 *Scotiella cryophila* K-1 *Chloromonas* sp. Gassan B LC012714.1 *Chloromonas polyptera* JQ790556.1
denovo31489	0.2	0	*Prasiola furfuracea* AF189073.1	*Stichococcus bacillaris* SAG 379-1b AJ311637.1	No blast hit
denovo17414	0	0.2	*Chloromonas* sp. D-CU581C AF517086.1	*Chloromonas nivalis* CCCryo-005-99	No blast hit
denovo47016	0	0.2	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	Ambiguous hits: 8 sequences including *Scotiella cryophila* K-1 *Chloromonas* sp. Gassan-B LC012714.1 *Chloromonas polyptera* JQ790556.1
denovo38221	0.2	0	*Prototheca cutis* AB470468.1	*Prototheca cutis* AB470468.1	No blast hit
denovo13893	0	0.2	*Chlamydomonas* sp. GL1 AB902971.1	*Chlamydomonas nivalis* DL07	No blast hit
denovo16269	0	0.2	uncultured Chlamydomonadaceae HW01/1 GU117574.1	*Chlainomonas* sp. MF803743	*Chlainomonas* sp. DL06 MF803743
denovo43924	0	0.2	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	No blast hit
denovo34049	0.2	0	*Chloromonas* sp. Hakkoda-1 LC012710.1	*Chloromonas* sp. Hakkoda LC012710.1	No blast hit
denovo24547	0.1	0	*Mesotaenium* sp. AG-2009-1 FM992335.1	*Ancylonema nordenskioeldii* AF514397.2	*Ancylonema nordenskioeldii* AF514397.2
denovo15255	0.1	0	uncultured Dunaliellaceae EF023287.1	uncultured Dunaliellaceae EF023287.1	No blast hit
denovo28329	0.1	0	*Heterococcus fuorensis* AM490821.1	*Heterococcus fuorensis* AM490821.1	No blast hit
denovo23345	0.1	0	*Mesotaenium* sp. AG-2009-1 FM992335.1	*Ancylonema nordenskioeldii* AF514397.2	*Ancylonema nordenskioeldii* AF514397.2
denovo18826	0	0.1	*Raphidonema sempervirens* AJ309939.1	*Raphidonema sempervirens* AF514410.2	Ambiguous hits: >20 sequences including *Raphidonema sempervirens* AF514410.2 *Raphidonema nivale* AB488604.1 *Koliella sempervirens* AM412750.1
denovo34529	0	0.1	*Chlamydomonadas* sp. GL1 AB902971.1	*Chlamydomonas nivalis* DL07	*Chlamydomonas nivalis* DL07
denovo9133	0	0.1	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas brevispina* K-2	No blast hit
denovo44422	0	0.1	*Hydrurus foetidus* FM955256.1	*Hydrurus foetidus* FM955256.1	*Hydrurus foetidus* FM955256.1
denovo8805	0	0.1	*Chloromonas* sp. Gassan-A LC012753.1	*Chloromonas* cf.*rostafinskii* HQ404863	No blast hit
Other	13.7	4.9			

Table S5. Oligotype sequences of the three most abundant 18S rDNA OTUs .

OTU ID	Oligotype ID	Sequence
denovo14434	TTT	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGTTGCAATGGTCCGGTTCGCTGTGTACTGTT GCGGCCTTCCTTTCTGCCGGGGACGGGCTCTTGGGCTTCATTGTCTGGGATCCGGAGTCGGCGAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAAGCTTA CGCTCTGAATACATTAGCATGGAATAACACGATAGGACTCTGGCCTATCTTGTTGGTCTGTAGGACTGGAGTAATGATTAAGAGGGACAGTCGGGGGCATTCG TATTTCATTGTCAGAGGTGAAATTCTTGGA
	TTC	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGTTGCAATGGTCCGGTTCGCTGTGTACTGTT GCGGCCTTCCTTTCTGCCGGGGACGGGCTCTTGGGCTTCATTGTCTGGGATCCGGAGTCGGCGAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAAGCTTA CGCTCTGAATACATTAGCATGGAATAACACGATAGGACTCTGGCCTATCTTGTTGGTCTGTAGGACTGGAGTAATGATTAAGAGGGACAGTCGGGGGCATTCG TATTTCATTGTCAGAGGTGAAATTCTCGGA
	TCT	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGTTGCAATGGTCCGGTTCGCTGTGTACTGTT GCGGCCTTCCTTTCTGCCGGGGACGGGCTCTTGGGCTTCACTGTCTGGGATCCGGAGTCGGCGAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAAGCTTA CGCTCTGAATACATTAGCATGGAATAACACGATAGGACTCTGGCCTATCTTGTTGGTCTGTAGGACTGGAGTAATGATTAAGAGGGACAGTCGGGGGCATTCG TATTTCATTGTCAGAGGTGAAATTCTTGGA
	CTT	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGTTGCAACGGTCCGGTTCGCTGTGTACTGTT GCGGCCTTCCTTTCTGCCGGGGACGGGCTCTTGGGCTTCATTGTCTGGGATCCGGAGTCGGCGAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAAGCTTA CGCTCTGAATACATTAGCATGGAATAACACGATAGGACTCTGGCCTATCTTGTTGGTCTGTAGGACTGGAGTAATGATTAAGAGGGACAGTCGGGGGCATTCG TATTTCATTGTCAGAGGTGAAATTCTTGGA
denovo36485	T	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGTTCGGTCGGTCCGCCTCTGGTGTGCACTGA TTGAGCCTTCCTTTCTGCCGGGGACGGGTTCCTGGGCCTCACGGTTTGGGACTCGGAGTCGGCGAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAAGCCT ACGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGCCTATCTTGTTGGTCTGTAGGACTGGAGTAATGATTAAGAGGGACAGTCGGGGGCATTC GTATTTCATTGTCAGAGGTGAAATTCTTGG
	C	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGGGTGGGTTCGGTCGGTCCGCCTCTGGTGTGCACTGA TTGAGCCTTCCTTTCTGCCGGGGACGGGTTCCTGGGCCTCACGGTTTGGGACTCGGAGTCGGCGAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAAGCCT ACGCTCTGAATACATTAGCATGGAATAACGCGATAGGACTCTGGCCTATCTTGTTGGTCTGTAGGACTGGAGTAATGATTAAGAGGGACAGTCGGGGGCATTC GTATTTCATTGTCAGAGGTGAAATTCTCGG
denovo45654	C	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGGTTGGTGTGGTCGGTCTGCTTTCTAGTTGAACTGG CTACTCCATCCTTCTTGCCGGGGACGCGTTCTGGCCTTCATTGGCTGGGACGCGGAGTCGGCGATGTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTA CGCTCTGAATACATTAGCATGGAATAACGTGATAGGACTCTGGTCCTATTGTGTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACAGTTGGGGGCATTC GTATTTCATTGTCAGAGGTGAAATTCTTGG
	A	GCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTTGGGTTGGTGTGGTCGGTCTGCTTTCTAGTTGAACTGG CTACTCCATCCTTCTTGCCGGGGACGCGTTCTGGCCTTAATTGGCTGGGACGCGGAGTCGGCGATGTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTA CGCTCTGAATACATTAGCATGGAATAACGTGATAGGACTCTGGTCCTATTGTGTTGGTCTTCGGGACCGGAGTAATGATTAATAGGGACAGTTGGGGGCATTC GTATTTCATTGTCAGAGGTGAAATTCTTGG

Table S6. Output of the ITSx software. ITS2 regions were extracted from all sequences to avoid the inclusion of the highly conserved neighbouring genes (i.e., 5.8S and 28S). Inclusion of these regions in the identification process would otherwise lead to misleading results. HMMER was used to predict the origin of the sequences (e.g., Chlorophyta, Fungi) based on Hidden Markov Models.

Number of sequences in input file:	273404
Sequences detected as ITS by ITSx:	273309
On main strand:	273239
On complementary strand:	70
Sequences detected as chimeric by ITSx:	0
ITS sequences by preliminary origin	
Alveolates:	35443
Amoebozoa:	0
Bacillariophyta:	1
Brown algae:	2
Bryophytes:	0
Euglenozoa:	0
Eustigmatophytes:	0
Fungi:	47293
Green algae:	189922
Liverworts:	0
Metazoa:	11
Microsporidia:	0
Oomycetes:	1
Prymnesiophytes:	0
Raphidophytes:	0
Red algae:	0
Rhizaria:	0
Synurophyceae:	0
Tracheophyta:	636

Table S7. Algal community composition based on the ITS2 data set and comprising the 38 most abundant OTUs that made up >98% of the community. Table shows the discrepancies between OTU assignments using three strategies: (A) basic version using Qiime and a custom database downloaded from NCBI, (B) extended version using Qiime and additional reference sequences of the locally abundant taxa (underlined), and (C) further extended version using final manual verification of taxa assignments. The latter one comprised the comparison of the representative sequences of the OTUs with their respective reference sequences in terms of sequence identities (≥89.0% similarity required; sequences below this threshold were recorded as "no blast hit") and their secondary structures (absence of compensatory base changes (CBC) in homological positions near the 5´- apex of helix III [the most conserved region of the ITS2 secondary structure of eukaryotes] required). Our results reveal that the manual verification including secondary structure prediction and CBC search is essential, and thus, highly recommended.

OTU ID	WP79 [%]	WP99 [%]	(A) Qiime + NCBI database	(B) Qiime + NCBI database + local references	(C) Qiime + NCBI database + local references + manual verification (sequence similarity [%], sequence cover [%])
denovo99	1.8	59.1	*Chloromonas* sp. CCCryo289-06 HQ404893.1	*Chloromonas brevispina* K-2	No blast hit (88%, 83%, 6 CBC when compared denovo99 and *Chloromonas brevispina* K-2 – one CBC out of it is located in the most conserved part of the structure, i.e., in the top close to the 5´end of III helix, see Fig. 4)
denovo20	5.5	28.2	*Chlamydomonas nivalis* GU117577.1	*Chlamydomonas nivalis* DL07	*Chlamydomonas nivalis* DL07 (100%, 100%)
denovo107	39.8	<0.1	*Chloromonas* sp. CCCryo289-06 HQ404893.1	*Chloromonas brevispina* K-2	*Chloromonas brevispina* K-2 (100% identical except for one nucleotide - instead of ‘R‘ in reference sequence, there was ‘G‘, 100%)
denovo100	13.7	<0.1	*Chloromonas pichinchae* HQ404889.1	*Chloromonas pichinchae* HQ404889.1	*Chloromonas pichinchae* HQ404889.1 (92%, 100%, 1 CBC in helix III [outside the most conserved part] when compared denovo100 and *Chloromonas pichinchae*, see Figs S6, S7)
denovo63	`10.9	0.2	No blast hit	*Scotiella cryophila* K-1	*Scotiella cryophila* K-1 (100%, 100%)
denovo142	8.2	1.7	*Chloromonas rostafinskii* HQ404863.1	*Chloromonas rostafinskii* HQ404863.1	No blast hit (79%, 60% - denovo142 vs. *Chloromonas rostafinskii*: 86%, 77% - denovo142 vs. *Chloromonas miwae* LC012762.1, four CBCs [one CBC out of it is located in the most conserved part of the structure, i.e., in the top close to the 5´end of III helix] when compared denovo142 and *Chloromonas miwae*, sequence-structure alignment in Fig. S12)
denovo130	1.4	3.5	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo181	4.1	0	No blast hit	No blast hit	No blast hit (82%, 87%, *Desmococcus endolithicus* KX094830.1; five CBCs – one in helix II and four CBCs in helix III, see Figs S19, S20)
denovo254	3.1	0.1	*Chloromonas rostafinskii* HQ404863.1	*Chloromonas rostafinskii* HQ404863.1	No blast hit (82%, 48% - denovo254 vs. *Chloromonas rostafinskii*: 88%, 84% - denovo254 vs. *Chloromonas miwae* LC012762.1, three

					CBCs [one out of in the most conserved part of the structure] when compared denovo254 and *Chloromonas miwae*, see Figs S12, S14)
denovo23	0.1	2.2	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo259	2.6	0.2	Uncultured Chlorophyta clone ALBC6 JX435348.1	Uncultured Chlorophyta clone ALBC6 JX435348.1	Uncultured Chlorophyta clone ALBC6 JX435348.1 (100%, 100%)
denovo76	0.1	1.9	*Chloromonas alpina* CCCryo032-99	*Chloromonas alpina* CCCryo032-99 HQ404864.1	*Chloromonas alpina* CCCryo032-99 HQ404864.1 (100%, 100%)
denovo219	1.8	0	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo85	1.5	<0.1	*Chloromonas pichinchae* CCCryo 2616-06 HQ404889.1	*Chloromonas pichinchae* CCCryo 2616-06 HQ404889.1	*Chloromonas pichinchae* CCCryo 2616-06 HQ404889.1 (95%, 100%), see Figs S6, S7
denovo166	0.1	0.8	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo74	0.6	0.3	*Raphidonema nivale* AJ431676.1	*Raphidonema nivale* AJ431676.1	uncultured *Chlorella* sp. 5 AB903011.1 (100%, 100%)
denovo93	0.7	0	No blast hit	No blast hit	*Stichococcus* sp. SAG 2482 KX094848.1 (100%, 100%)
denovo35	<0.1	0.5	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo69	0.6	0	No blast hit	No blast hit	No blast hit (82%, 52%, *Prasiola delicata* MF801375.1, six CBCs in helix III, see Figs S21, S22)
denovo36	<0.1	0.5	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo127	0.3	0.1	*Chloromonas rostafinskii* HQ404863.1	*Chloromonas rostafinskii* HQ404863.1	No blast hit (85%, 48% - denovo127 vs. *Chloromonas rostafinskii*: 90%, 82% - denovo127 vs. *Chloromonas miwae* LC012762.1, two CBCs in helix III [one CBC out of it in the most conserved part of the structure] when compared denovo127 and *Chloromonas miwae*, see Figs S12, S14)
denovo13	0.3	0	No blast hit	No blast hit	No blast hit (84%, 85%, *Desmococcus endolithicus* KX094830.1; four CBCs in helix III, see Figs S19 and S20).
denovo44	0.2	0	Uncultured Chlorophyta clone ALBC6 JX435348.1	*Scotiella cryophila* K-1	*Scotiella cryophila* K-1 (96%, 100%, 4CBCs in helix III [outside the most conserved part] when compared denovo44 and *Scotiella cryophila* K-1, see Figs S15, S16)
denovo249	0.2	0	Uncultured Chlorophyta clone ALBC6 JX435348.1	*Chloromonas brevispina* K-2	*Chloromonas brevispina* K-2 (96%, 82%, one CBC in helix III [outside the most conserved part] when compared denovo249 and *Chloromonas brevispina* K-2, sequence-secondary structure alignment in Fig. S5)
denovo188	<0.1	0.1	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo101	0.2	<0.1	No blast hit	No blast hit	No blast hit (84%, 75%, *Chloromonas miwae* LC012762.1; three CBCs in helix III [two out of in the most conserved part of the

					structure] when compared denovo101 and *Chloromonas miwae*, see Fig. S13)
denovo168	0.2	0	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo143	0.1	0	No blast hit	No blast hit	*Stichococcus* sp. KX094857.1 (99%, 100%)
denovo109	0.1	0	No blast hit	No blast hit	*Stichococcus* sp. KX094848.1 (89%, 89%, four changes at both side of secondary structure found in less conserved part of the structure, likely no CBC, Figs S17, S18)
denovo28	0.1	0	No blast hit	No blast hit	*Lobosphaera* sp. K-1 KT119889.1 (94.0%, 100%), no CBC found when compared denovo28, *Lobosphaera* sp. K-1 KT119889.1 and *Lobosphaera incisa* KM020046.1, so denovo28 can be assigned to *Lobosphaera incisa*, sequence-structure alignment in Fig. S8
denovo124	0.1	0	No blast hit	No blast hit	*Lobosphaera incisa* KM020046.1 (94%, 100%), see Figs S8, S9
denovo45	0.1	0	No blast hit	No blast hit	No blast hit (no significant similarity found)
denovo72	<0.1	0.1	*Chlamydomonas nivalis* GU117577.1	*Chlamydomonas nivalis* GU117577.1	*Chlamydomonas nivalis* GU117577.1 (94%, 100%), no CBC when denovo72 compared to *Chlamydomonas nivalis* DL07; [interestingly, ITS2 of denovo72 has higher sequence similarity with Uncultured *Chlamydomonas* clone H14 KX063729.1 (98%, 100%) but there is one CBC in helix II between denovo72 and clone H14], see Fig. S10, S11.
denovo180	0.1	0	No blast hit	No blast hit	No blast hit (84%, 100%, *Chlorocloster engadinensis* FM946011.1; one CBC in basal part of helix I and one CBC in the basal part of helix II, see Figs S23, S24)
denovo212	<0.1	0.1	No blast hit	No blast hit	*Trebouxia* sp. MG098229 (100%, 100%)
denovo173	0.1	0	*Chloromonas* sp. CCCryo289-06 HQ404893.1	*Chloromonas brevispina* K-2	*Chloromonas brevispina* K-2 (100%, 75%, two CBCs in helix III [outside the most conserved part] when compared denovo173 and *Chloromonas brevispina* K-2, sequence structure alignment in Fig. S5)
denovo266	0.1	0	Uncultured Chlorophyta clone ALBC6 JX435348.1	*Chloromonas brevispina* K-2	*Chloromonas brevispina* K-2 (96%, 69%, 2 CBCs in basal part of helix II and 1 CBC in helix II when compared denovo266 and *Chloromonas brevispina* K-2, sequence structure alignment in Fig. S5)
denovo248	<0.1	<0.1	*Chloromonas* sp. CCCryo289-06 HQ404893.1	*Chloromonas brevispina* K-2	No blast hit (92%, 54%, 6 CBCs when compared denovo248 and *Chloromonas brevispina* K-2, one CBC out of it is located in the most conserved part of the structure, sequence structure alignment in Fig. S5)
Other	1.2	0.4			