Taxonomic changes in the genus *Ctenocladus* (*Ulvales, Ulvophyceae*)

Tatyana Darienko, *Experimental Phycology and Culture Collection of Algae, University of Göttingen, Nikolausberger Weg 18, D-37073 Göttingen, Germany*

Thomas Pröschold, *Research Department for Limnology at Mondsee, University of Innsbruck, Mondseestr. 9, A-5310 Mondsee, Austria* (correspondence: Thomas.Proeschold@uibk.ac.at)

Recently, the species *Ctenocladus verrucariae* was described by Darienko & Pröschold (2022: 2) based on investigations of two strains (SAG 2039 and SAG 2052), photobionts of the lichens *Hydropunctaria scabra* (Vězda) Keller, Gueidan & Thüs and *Verrucaria margacea* (Wahlenberg) Wahlenberg (*Verrucariaceae*). A cryopreserved sample of the latter strain was designated as holotype for this species. The same strain was used to cryopreserve a holotype for the newly described genus and species, *Rindifilum ramosum* Malavasi, Klimešová, Lukešová & Škaloud (Malavasi & al. 2022: 129). In accordance with Art. 14.1 of the ICN (Turland & al. 2018), *Ctenocladus verrucariae* has priority over *Rindifilum ramosum* (publication dates: 19th of June *vs*. 23rd of June 2022).

Assignment to a different genus raises questions about generic concepts within the *Ctenocladiaceae* Borzì. Škaloud & al. (2018) assigned three genera to this family: *Ctenocladus* Borzì, *Pseudopleurococcus* J.W.Snow, and *Spongioplastidium* Vischer. Malavasi & al. (2022) then added the genus *Rindifilum*. Darienko & Pröschold (2017, 2022) questioned the genus *Pseudopleurococcus* and transferred *P. printzii* Vischer to the genus *Ctenocladus* (see details in Darienko & Pröschold 2022). The newly described species *Ctenocladus verrucariae* is phylogenetically closely related to *Ctenocladus circinnatus* Borzì and *C. printzii* (Vischer) Darienko & Pröschold. All taxa belonging to the *Ctenocladiaceae* have similar morphological features (bilateral branched filaments forming pseudoparenchymatic thalli, parietal chloroplast with pyrenoids, e.g. Darienko & Pröschold 2017; 2022 and Malavasi & al. 2022), and it is almost impossible to identify them at species level solely on morphology, as explained in Darienko & Pröschold (2017, 2022). These morphological features are even not restricted to the *Ctenocladiaceae*. The genus *Pseudendoclonium* Wille and related genera have similar morphologies (e.g. Darienko & Pröschold 2017). Thüs (2002) identified the photobionts of both lichens as *Dilabifilum incrustans* (Vischer) Tschermak-Woess, a species which was transferred to the genus *Pseudendoclonium* Wille based on phylogenetic analyses of SSU and ITS rDNA sequences. Malavasi & al. (2022) described "hammer-shaped cells" as a diagnostic morphological feature for the genus *Rindifilum*. However, this feature was reported by Vischer for *Ctenocladus printzii* (see figs 1–11 in Vischer 1933) and by Darienko & Pröschold for *Ctenocladus circinnatus* as well as species of the genera *Pseudendoclonium*, *Halofilum*, *Paulbroadya* and *Lithotrichon* (see figs 4–13 in Darienko & Pröschold 2017). Morphological plasticity when grown in different culture conditions clearly shows the similarity of these genera and species and these can only be distinguished in combination with molecular data.

SSU rDNA sequences are currently favoured in establishing generic concepts for many morphologically simple green algae. For example, the sequences of the members belonging to the *Ctenocladiaceae* and the *Phaeophila* clade, its sister clade, have been investigated (Darienko & Pröschold 2017; Škaloud & al. 2018). As already described in Darienko & Pröschold (2017), the SSU rDNA sequences of the *Ctenocladiaceae* showed only little variability (3.1%) and if adding the new species, the variability increased to 4.3%. In contrast, the four sequences of *Phaeophila dendroides* (P.Crouan & H.Crouan) Batters varied in 6.6% of the SSU despite that these isolates were morphologically indistinguishable (O'Kelly & al. 2004). For the phylogenetic analyses, the SSU rDNA sequences were aligned and included into a data set of a total of 15 sequences (1778 bp)

of both lineages. GenBank accession numbers of all sequences used are given in Fig. 1. The phylogenetic analyses were conducted using the program PAUP, version 4.0b169 (Swofford 2002) with the automated model selection tool. The robustness of the tree was calculated using the methods described in Darienko & Pröschold (2017, 2022).

The genetic variability is also reflected the phylogenetic analyses (Fig. 1). The branches in the *Phaeophila* clade are longer showing greater evolutionary distances. Even in the highly variable regions V4 and V9, commonly used markers for high-throughput sequencing approaches, this clade showed a higher genetic variability among the strains of *P. dendroides* compared to the *Ctenocladiaceae* (V4: 21.1% *vs*. 9.1%; V9: 19.3% *vs.* 13.8%). Within this family, the secondary structures of V4 showed only variable regions in the helices E23_1 & E23_2 and E23_4 E23_7 (Fig. 2). The strains of the *Phaeophila* also differed in the other regions of V4 (not shown). In the V9 region of the SSU (Helix 49), only the ends of the loop varied among the species of *Ctenocladus* (highlighted in white boxes in Fig 3).

Summarizing, all these data (morphological similarity, low genetic variability) clearly demonstrate that the above strains and specimen of the *Ctenocladiaceae* belonging the one genus, *Ctenocladus*. Therefore, we emend the generic description and propose to treat the genus *Rindifilum* as a synonym as follows:

Ctenocladus Borzì emend. Darienko & Pröschold, Borzì 1883, *Studi Algologici* I: 27–50.
Synonym: *Rindifilum* Malavasi, Klimešová, Lukešová & Škaloud 2022, *Cryptogamie, Algologie* 43, 129.
Pseudoparenchymatic thalli marginally forming bilateral branched filaments. Cells cylindrical, uninucleate, possessing a parietal chloroplast with pyrenoids. Asexual reproduction by zoospores. Akinetes present. Zoosporangia usually formed by the basal pyriform cells, containing biflagellated zoospores. Akinetes spherical-subspherical, produced terminally at the end of lateral branches, or in rows. Sexual reproduction if known isogamous with biflagellate gametes.
Type species: *Ctenocladus circinnatus* Borzì 1883

Ctenocladus circinnatus Borzì, *Studi Algologici* I: 28, figs. 3: 1-10, 4: 11-20, 1883.
Note: For lectotypification and epitypification: see Darienko & Pröschold (2017).

Ctenocladus verrucariae Darienko & Pröschold 2022, *Notulae Algarum* 241: 2, figs. 2-3.
Synonym: *Rindifilum ramosum* Malavasi, Klimešová, Lukešová & Škaloud 2022, *Cryptogamie, Algologie* 43: 129.

Ctenocladus printzii (Vischer) Darienko & Pröschold
Basionym: *Pseudopleurococcus printzii* Vischer 1933, *Beihefte zum botanischen Centralblatt* 51(1): 34, figs. 11: 1–11, 12: 1–8, 1933
Synonym: *Dilabifilum printzii* (Vischer) Tschermak-Woess, *Österreichische botanische Zeitschrift* 118: 452, 1970.
Notes: For lectotypification and epitypification: see Darienko & Pröschold (2017). Transfer to the genus *Ctenocladus* was not accepted by Škaloud & al. (2018) despite the morphological similarity to this genus. This species was originally assigned to the genus *Pseudopleurococcus* Snow, a genus which is questioned by several authors (see details in Darienko & Pröschold 2017; 2022). Vischer (1933) only assigned this species to that genus (with reservation) because of the lack of zoospores.

Interestingly, as shown in Figs 1-3, the two specimens originally assigned to *C. circinnatus* by Liu & al. (2016) differed in SSU rDNA sequences to the other *Ctenocladus* strains. Unfortunately, no ITS sequences are available from these specimens, but Liu & al. (2016) sequenced the *tuf*A gene in addition. To establish if the Tibetan herbarium specimens represent *C. circinnatus* as determined by Liu & al. (2016) or a new entity, we sequenced the plastid-coding gene *tuf*A of all studied strains. The plastid-coding gene *tuf*A was amplified using the primer combination t*uf*GF4/*tuf*AR following the protocol of Famá & al. (2002) and Saunders & Kucera (2010). This gene was considered as species-specific by Hall & al. (2010) and Saunders & Kucera (2010). As already demonstrated for the SSU rDNA sequences above, these two specimens collected from lakes in Tibet were also different in the *tuf*A phylogeny and separated by two amino acid changes from *C. circinnatus* (Fig. 4). The other three species also differed in changes among the amino acid profile of *tuf*A (marked in yellow in Fig. 4). As consequence of our findings, we propose the two specimens described by Liu & al. (2016) as new species, as follows.

Ctenocladus tibetensis Darienko & Pröschold, *sp. nov.* (Fig. 1 A-F in Liu & al. 2016)

Description (according to Liu & al. 2016): Thalli composed of numerous radially arranged filaments with unilateral branching, without mucilage. Cells cylindrical, 6–8 µm wide and 28–85 µm long, uninucleate, with a parietal plastid and one to three pyrenoids. Terminal vegetative cells usually producing thick-walled, spherical or approximately spherical akinetes with a diameter of 10–21 µm, giving rise to chain-like rows. Zoosporangia irregularly spherical, with eight or more zoospores released at the apical end of the cell.

Diagnosis: Differs from other species of *Ctenocladus* genetically by SSU (KU362724) and *tuf*A (KU362726) sequences.

Holotype: Herbarium specimen TB2014012 deposited in the Freshwater Algal Herbarium (**IHB**), Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China.

Type locality: Lake Dongcuo, (31.593611, 91.125000**),** Nagqu, Amdo County, Tibet.

This research was funded by the Austrian Science Fund (FWF): grant number P34416-B. We thank Michael Guiry and an anonymous reviewer for their valuable comments to the manuscript.

Borzì, A. (1883). Saggio di ricerche Sulla biologia delle alghe. *Ctenocladus*, gen. nov. *Studi Algologici* 1: 27-50.

Darienko, T. & Pröschold, T. (2017). Towards a monograph of non-marine Ulvophyceae using an integrative approach (Molecular phylogeny and systematics of terrestrial Ulvophyceae II). *Phytotaxa* 324: 1-41.

Darienko, T. & Pröschold, T. (2022). The new photobiont *Ctenocladus verrucariae* sp. nov. (Ulvales, Ulvophyceae) found in various lichens of the family Verrucariaceae (Eurotiomycetes). *Notulae Algarum* 241: 1-10.

Famà, P., Wysor, B., Kooistra, W.H.C.F. & Zuccarello, G.C. (2002). Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tuf*A gene. *Journal of Phycology* 38: 1040-1050.

Hall, J.D., Fucíková, K., Lo, C., Lewis, L.A. & Karol, K.G. (2010). An assessment of proposed DNA barcodes in freshwater green algae. *Cryptogamie, Algologie* 31: 529-555.

Liu, B., Liu, X., Hu, Z., Zhu, H. & Liu, G. (2016). Phylogenetic position and morphological observation of the *Ctenocladus circinnatus* Borzì, a rare green alga from Changtang Plateau, China. *Phytotaxa* 260: 75-82.

Malavasi, V., Klimešová, M., Lukešová, A. & Škaloud, P. (2022). *Rindifilum ramosum* gen. nov., sp. nov., a new freshwater genus within the Ulvales (Ulvophyceae, Chlorophyta). *Cryptogamie, Algologie* 43: 125-133.

O'Kelly, C.J., Wysor, B. & Bellows, W.K. (2004). Gene sequence diversity and the phylogenetic position of algae assigned to the genera *Phaeophila* and *Ochlochaete* (Ulvophyceae, Chlorophyta). *Journal of Phycology* 40: 789-799.

Saunders G.W. & Kucera, H. (2010). An evaluation of *rbc*L, *tuf*A, UPA, LSU and ITS as DNA barcode markers for marine green macroalgae. *Cryptogamie, Algologie* 31: 487-528.

Škaloud, P., Rindi, F., Bödecker, C. & Leliaert, F. (2018). Chlorophyta: Ulvophyceae. In *Süßwasserflora von Mitteleuropa* 13, Büdel, B., Gärtner, G., Krienitz, L., Schagerl, M. (eds.), Springer Spektrum: Berlin/Heidelberg, Germany, pp. 1-288.

Swofford, D.L. (2002). *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10*. Sinauer Associates: Sunderland, MA, USA.

Thüs, H. (2002). Taxonomie, Verbreitung und Ökologie silicoler Süßwasserflechten im außeralpinen Mitteleuropa. *Bibliotheca Lichenologica* 83: 1-214.

Tschermak-Woess, E. (1970). Über wenig bekannte und neue Flechtengonidien V. Der Phycobiont von *Verrucaria aquatilis* und Fortpflanzung von *Pseudopleurococcus arthopyreniae*. *Österreichische Botanische Zeitschrift* 118: 443-455.

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.J. & Smith, G.F., editors (2018). *International code of nomenclature for algae, fungi, and plants (Shenzhen Code)* adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile*, Vol. 159. pp. [i]-xxxviii, 1-253. Glashütten: Koeltz Botanical Books.

Vischer, W. (1933). Über einige kritische Gattungen und die Systematik der Chaetophorales. *Beihefte zum Botanischen Centralblatt* 51/I: 1-100.

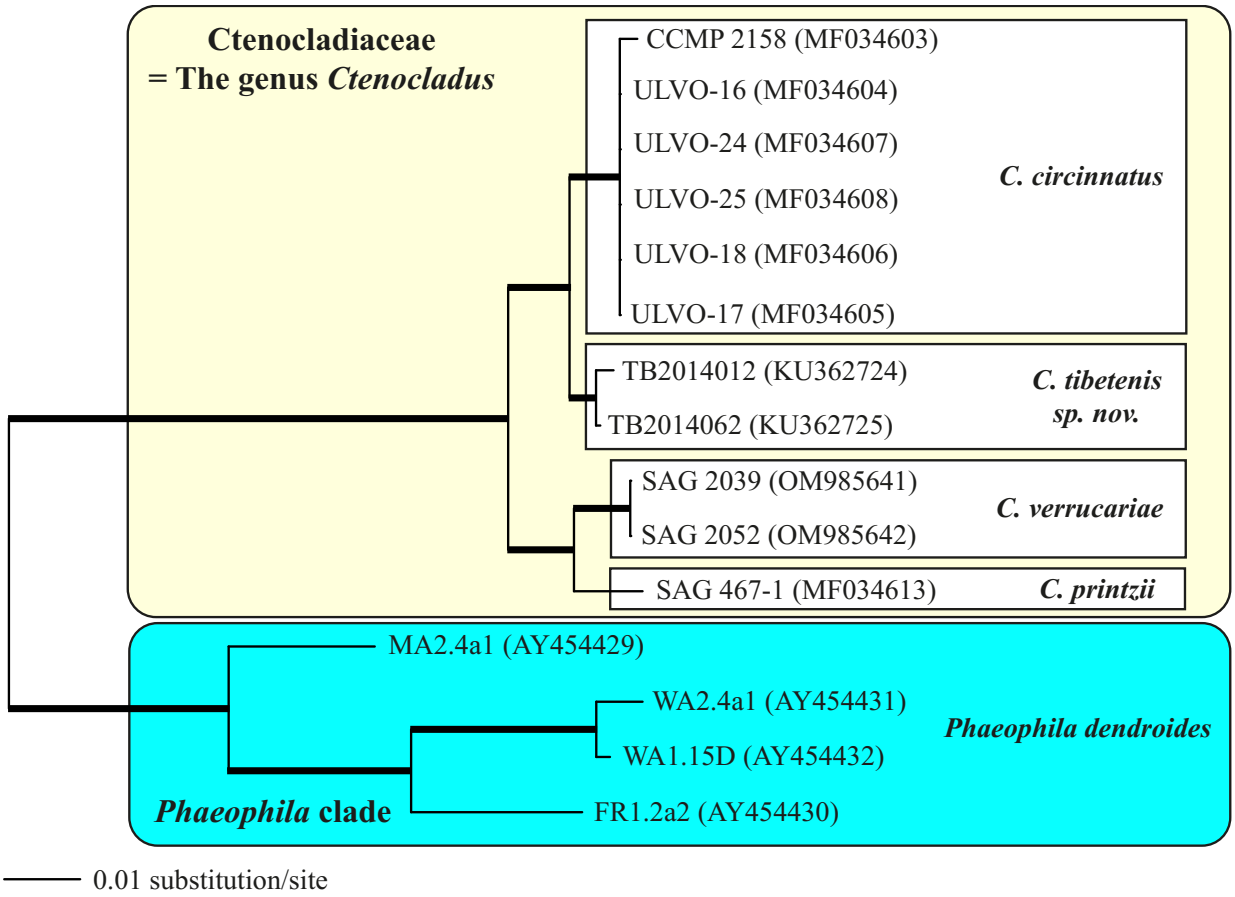

Fig. 1. Molecular phylogeny of the *Ctenocladiaceae* and *Phaeophila* clade (*Ulvales, Ulvophyceae*) based on SSU rDNA sequence comparisons. The phylogenetic tree shown was inferred using the maximum likelihood method based on a data set of 1778 aligned positions of 15 taxa using PAUP 4.0a build169. For the analysis, the TrN+I+G (base frequencies: A 0.2467, C 0.2236, G 0.2784, U 0.2513; rate matrix A-C 1.0000, A-G 1.7478, A-U 1.0000, C-G 1.0000, C-U 4.1220, G-U 1.0000) with the proportion of invariable sites (I = 0.6495) and gamma shape parameter (G = 0.6263) was chosen, which was calculated as the best model by the automated model selection tool implemented in PAUP. The branches in bold are highly supported in all analyses (Bayesian values > 0.95 calculated with PHASE and MrBayes; bootstrap values > 70% calculated with PAUP using maximum likelihood, neighbour- joining, maximum parsimony, and RAxML using maximum likelihood).

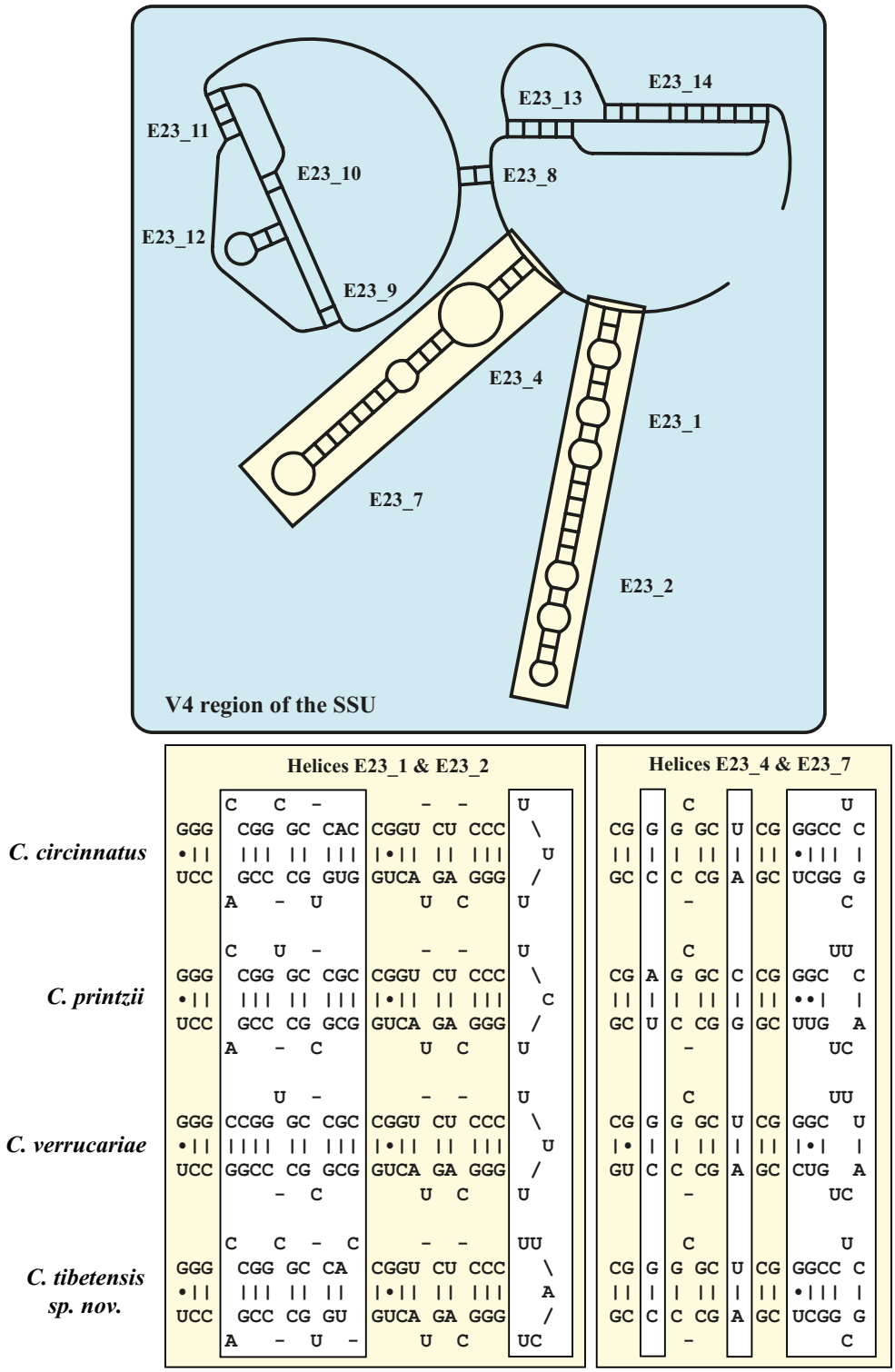

Fig. 2. Secondary structure of the V4 region of the SSU rDNA among the *Ctenocladus* species. The variable regions within the V4 are highlighted in white boxes.

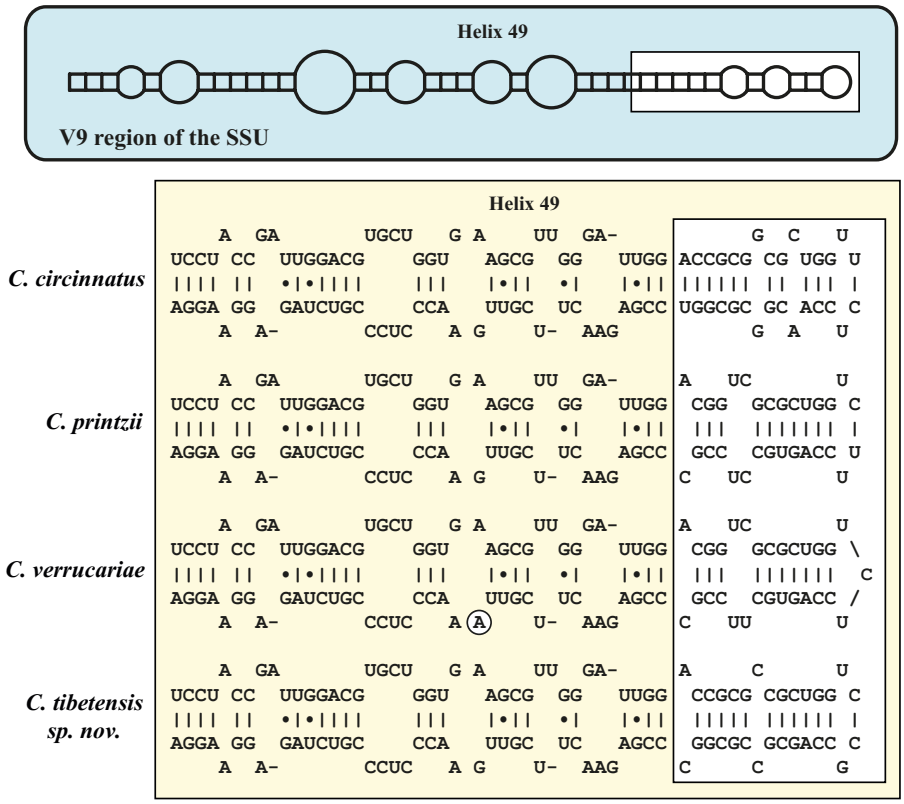

Fig. 3. Secondary structure of the V9 region (Helix 49) of the SSU rDNA among the *Ctenocladus* species. The variable regions within the V9 are highlighted in white boxes.

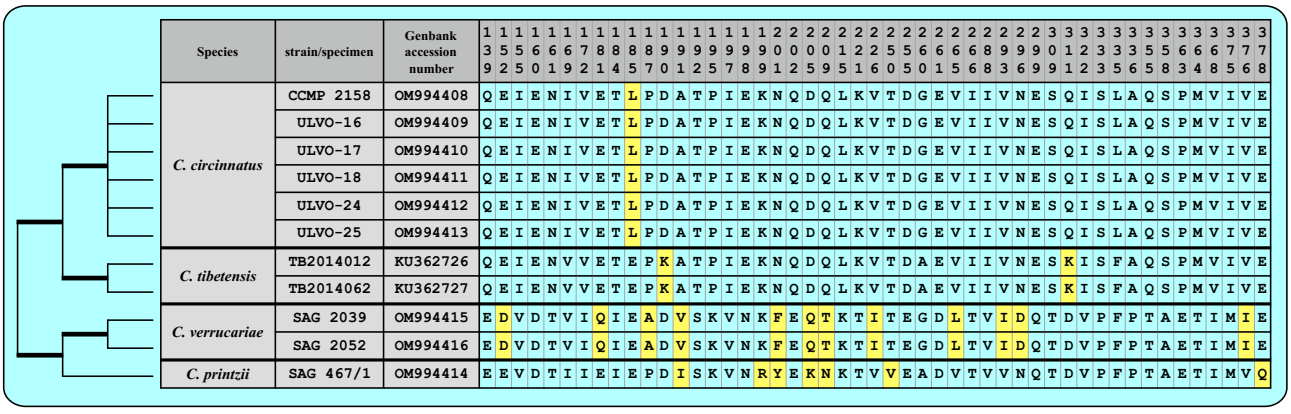

Fig. 4. *tuf*A-phylogeny of *Ctenocladus* and the variable amino acid positions among *C. circinnatus*, *C. printzii*, *C. tibetensis*, and *C. verrucariae*. The characteristic amino acids for each species are highlighted in yellow. The phylogenetic tree of *tuf*A shown was inferred using the maximum likelihood method based on a data set of 855 aligned positions of 11 taxa using PAUP 4.0a build169. For the analysis, the TrN+I (base frequencies: A 0.3446, C 0.1574, G 0.2026, U 0.2954; rate matrix A-C 1.0000, A-G 2.2998, A-U 1.0000, C-G 1.0000, C-U 4.9704, G-U 1.0000) with the proportion of invariable sites (I = 0.6746) and gamma shape parameter (G = equal) was chosen, which was calculated as the best model by the automated model selection tool implemented in PAUP. The branches in bold are highly supported in all analyses (Bayesian values > 0.95 calculated with PHASE and MrBayes; bootstrap values > 70% calculated with PAUP using maximum likelihood, neighbour-joining, maximum parsimony, and RAxML using maximum likelihood).