

***Valeriella*, a new genus of terrestrial coccoid green algae previously assigned to *Spongiochloris*  
R.C.Starr (*Chlorophyceae*, *Chlorococcaceae*)**

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The genus *Spongiochloris* was described by R.C. Starr (1924–1998) in 1955 based on: production of zoospores with two equal flagella and with bodies that become spherical when quiescent; and the presence of a net-like chloroplast with a single pyrenoid (Starr 1955: 68). The authentic strain of type species *S. spongiosa* (Starr 1955: 70; SAG 280-2b = CCAP 3/1 = UTEX 1) was isolated by W. Vischer (1890–1960) in 1945 from soil collected at Il Fuorn, Switzerland, but originally assigned to *Asterococcus spongiosus* Vischer (Vischer 1945: 47). The second species, *S. excentrica* R.C.Starr (Starr 1955: 72; UTEX 108 = CCAP 280/1 = SAG 280-1), was isolated by H.C. Bold (1909–1987) from soil collected near Rock Island, Caney Fork River, Tennessee, USA. Later, six additional species of *Spongiochloris* were described: *S. lamellata* Deason & H.C.Bold (1960: 34; authentic strain UTEX 977), *S. minor* Chantanachat & H.C.Bold (1962: 26; authentic strain UTEX 1184) and *S. incrassata* Chantanachat & H.C.Bold (1962: 27; authentic strain UTEX 1182), *S. gigantea* H.W.Bischoff & H.C.Bold (1963: 25; authentic strain UTEX 1243), *S. llanoensis* H.W.Bischoff & H.C.Bold (1963: 27; authentic strain UTEX 1245), and *S. typica* Trainor & R.J.McLean (1964: 58; authentic strain UTEX 1238). Most of the described *Spongiochloris* species are found in arid habitats. Well after its original description, it is obvious that *S. minor* is a cosmopolitan species found also in hot arid areas as well as in temperate zones (e.g., Mikhailyuk & al. 2011, 2019). The other species are less common. However, they are reported as common members of biological soil crusts in deserts of Africa and North America (Flechtner & al. 2008, 2013; Büdel & al. 2009).

Despite the genus *Spongiochloris* being widely distributed, a phylogenetic study of the authentic strains is lacking. Only the SSU rDNA of the authentic strains of *S. spongiosa* (UTEX 1, U63107) and *S. excentrica* (SAG 280-1, KM020086) have been sequenced, and showed that both investigated strains are not closely related (Fig. 1). Saber & al. (2018) described a new genus *Pharao* Saber, Fucíková, McManus, Guella & Cantonati characterized by similar morphology as *Spongiochloris*, but an investigation of the authentic strains was not carried out.

Here we sequenced the SSU rDNA of all the authentic strains of *Spongiochloris*. DNA extraction, PCR, and PCR purification were carried out as described in Darienko & al. (2019). The sequences were included into a dataset of representatives belonging to the *Chlorophyceae* and *Chlamydoephyceae*. The alignment was constructed according to the SSU secondary structures. The phylogenetic analyses were inferred using the methods described in Darienko & al. (2019).

As shown in Fig. 1, the strains belong to two groups of the Chlorophyceae: The *Chlamydoephyceae* (Clockwise-group) and the Direct-Opposite (DO) group of the *Chlorophyceae sensu* Mattox & Stewart (1984). The type species of *Spongiochloris*, *S. spongiosa*, together with *S. gigantea* and *S. lamellata* are members of the *Stephanosphaera* clade *sensu* Pröschold & al. (2001), whereas the other strains were either closely related to the genus *Pharao* (*S. llanoensis*, *S. typica*) or forming as a separate lineage within the DO group (*S. excentrica*, *S. minor* and *S. incrassata*).

All taxa described morphologically could be separated in two groups: the first group includes cells with diameters from 70–200  $\mu\text{m}$  and the second group which includes species with diameters  $>70$   $\mu\text{m}$ . Another morphological feature that separates members of the genus is the structure of pyrenoid matrix. *Spongiochloris spongiosa*, *S. lamellata* and *S. gigantea* have a pyrenoid matrix separated into several portions. Other species have a homogenous pyrenoid matrix (Ettl & Gärtner, 2014).

As a result of the phylogenetic analyses and subsequent morphological comparisons, we emend the description of the genus *Spongiochloris* and propose new combinations for the species not closely related to the generitype:

*Spongiochloris* R.C.Starr, 1955 *emend.* Darienko & Pröschold

Emended description: Cells solitary, spherical with smooth cell wall. Immature cells uninucleate, mature cells multinucleate, with one parietal, cup-shaped chloroplast in young cells becoming reticulate to spongiform with age. A single pyrenoid in immature cells and several pyrenoids in mature cells. Pyrenoid matrix split into several pieces. Size of mature cells above 80  $\mu\text{m}$ .

Reproduction by zoospores of the “*Protosiphon*” type (without cell walls) or autospores. Type: *Spongiochloris spongiosa* (Vischer) R.C.Starr, 1955.

*Spongiochloris spongiosa* (Vischer) R.C.Starr, *Indiana University Publications. Science Series* 20: 70, 1955.

Basionym: *Asterococcus spongiosus* Vischer, *Verhandlungen der Naturforschenden Gesellschaft in Basel* 61: 47, fig. 5: 1-11, 1955.

**Lectotype (designated here):** Fig. 5: 1-11 in Vischer (1945: 48).

**Epitype (designated here for the lectotype above):** The authentic strain SAG 280-2b cryopreserved in a metabolically inactive state at the Culture Collection of Algae, University of Göttingen (SAG), Germany.

Note: This species was described from near Il Fuorn, Switzerland but a type was not designated, and no herbarium material seems to have been preserved. In the absence of such material, we have designated a lectotype above and an epitype for the lectotype from the original culture.

*Spongiochloris gigantea* H.W.Bischoff & H.C.Bold, *University of Texas Publication* 6318: 25, 1963.

**Lectotype (designated here):** Fig. 104 in Bischoff & Bold (1963: 91).

Notes: The authentic strain UTEX 1243 is available from the Culture Collection of Algae at the University of Texas at Austin, USA. According to Bischoff & Bold (1963: 60), an herbarium specimen was deposited in the Chicago Natural History Museum (presently The Field Museum, F); however, such type material is not to be found in the on-line index. In the absence of such material, we here designate a lectotype. This species was described from a culture isolated from soil from Enchanted Rock, Llano County, Texas, collected in July, 1960.

*Spongiochloris lamellata* Deason & H.C.Bold 1960, *University of Texas Publication* 6022: 34, 1960.

Holotype: A herbarium specimen (only marked as “Texas”) formerly in the Chicago Natural History Museum (F) now located in the New York Botanical Garden Herbarium as NY 03685146.

Note: The authentic strain, UTEX 977, is available at the Culture Collection of Algae at the University of Texas at Austin, USA. This species was described from a culture isolated from soil from “Carrizo Sands, Caldwell County, Texas” on 11 September 1958 and thus represent an “single gathering”. The type material is marked Nov. 15, 1960 and appears to be a glass slide with some culture material.

***Valeriella*** Darienko & Pröschold, *gen. nov.*

Description: Cells solitary, spherical, with smooth cell walls. Immature cells uninucleate, mature cells multinucleate, with one parietal, cup-shaped chloroplast in immature cells becoming reticulate to spongiform with age. One pyrenoid in young cells to several pyrenoids in mature cells. Pyrenoid matrix homogeneous. Size of mature cells less than 80 µm. Reproduction by zoospores of “*Protosiphon*” type (without cell walls) or autospores.

Diagnosis: Differing from other *Spongiochloris* species by its SSU rDNA sequences.

Type: *Valeriella excentrica* (R.C.Starr) Darienko & Pröschold, *comb. nov.*, below.

Etymology: Named for Dr Valerie R. Flechtner, a diligent investigator of biological soil crusts in arid habitats of North America.

***Valeriella excentrica*** (R.C.Starr) Darienko & Pröschold, *comb. nov.*

Basionym: *Spongiochloris excentrica* R.C.Starr, *Indiana University Publications. Science Series* 20: 72, figs 165-186, 1955.

**Lectotype (designated here):** A herbarium specimen derived from CCAP 280/1 (= SAG 280-1) and deposited in the Chicago Natural History Museum (F) is now located in the New York Botanical Garden Herbarium as NY 03053344.

**Epitype (designated here for the above lectotype):** The authentic strain SAG 280-1 cryopreserved in a metabolic inactive state at the Culture Collection of Algae, University of Göttingen (SAG), Germany.

Note: This species was described from a culture, which was isolated from a soil sample collected near Rock Island, Caney Fork River, Tennessee by Harold C. Bold before 1951. No type was designated but a type prepared from a culture derived from the original culture was deposited in F and is now at NY 03053344. This material is dated 9 June 1953 and is labelled “Violet M. Diller”. This sheet is designated here as the lectotype as it does not appear to have been prepared by Richard C. Starr. As this material is little more than some smears on two pieces of paper, an epitype is designated here.

***Valeriella minor*** (Chantanachat & H.C.Bold) Darienko & Pröschold, *comb. nov.*

Basionym: *Spongiochloris minor* Chantanachat & H.C.Bold, *University of Texas Publication* 6218: 26, 1962.

Holotype: A herbarium sheet deposited in the Chicago Natural History Museum (F) is now located in the New York Botanical Garden Herbarium as NY 03685147.

Notes: The authentic strain UTEX 1184 is no longer available at the Culture Collection of Algae at the University of Texas at Austin, USA. The name was originally introduced for a “single gathering” from soil collected 6 miles from North St. John, Arizona, and while the authors did not specifically designate a type, this sheet, made from 1-month-old cultured material and dated “Sept. 22, 1962” represents the holotype (Art. 40.2; Turland & al. 2018).

***Valeriella incrassata*** (Chantanachat & H.C.) Darienko & Pröschold, *comb. nov.*

Basionym: *Spongiochloris incrassata* Chantanachat & H.C. Bold, *University of Texas Publication* 6218: 27, 1962.

Holotype: A herbarium specimen deposited in the Chicago Natural History Museum (F) is now located in the New York Botanical Garden Herbarium as NY 03685145.

Comment: An authentic strain UTEX 1182 is available at the Culture Collection of Algae at the University of Texas at Austin, USA. The name was originally introduced for an “entire gathering”, a soil sample from “As-Sanam, Lat. 21°46' N, Long. 53°10' E Saudi Arabia” and while the authors did not specifically designate a type, NY 03685145, made from 1-month-old cultured material, and dated “Sept. 22, 1962”, represents the holotype (Art. 40.2; Turland & al. 2018).

The two species *Spongiochloris llanoensis* and *S. typica* are closely related to the genus *Pharao*. Morphologically they differ only in cell sizes and presence of zoospores from the latter genus. Zoospore formation was not observed for the type species *Pharao desertorum* (Saber & al. 2018). As consequence of our phylogenetic analyses, we emend the generic description of the genus *Pharao* and proposed two new combinations:

*Pharao* Saber, Fuciková, McManus, Guella & Cantonati, 2018 *emend.* Darienko & Pröschold.  
Emended description: Cells solitary, spherical, with smooth cell walls. Immature cells uninucleate, mature cells multinucleate, with one parietal, cup-shaped chloroplast in immature cells becoming reticulate to spongiform with age. One pyrenoid in young cells to several pyrenoids in mature cells. Pyrenoid matrix homogenous. Size of mature cells less than 80 µm. Reproduction by zoospores of “*Protosiphon*” type (without cell walls), aplanospores, or autospores.

Diagnosis: Differing from *Spongiochloris* species by SSU rDNA sequences.

Type: *Pharao desertorum* Saber, Fuciková, McManus, Guella & Cantonati 2018: 346.

***Pharao llanoense*** (H.W.Bischoff & H.C.Bold) Darienko & Pröschold, *comb. nov.*

Basionym: *Spongiochloris llanoensis* H.W.Bischoff & H.C.Bold, *University of Texas Publication* 6318: 27, 1963.

**Lectotype (designated here):** Fig. 106 in Bischoff & Bold (1963: 93).

Comment: The authentic strain UTEX 1245 is available at the Culture Collection of Algae at the University of Texas at Austin, USA. According to Bischoff & Bold (1963: 60), a herbarium specimen was deposited in the Chicago Natural History Museum (presently The Field Museum, **F**); however, such type material is not listed in the **NY** on-line index. In the absence of such material, we here designate a lectotype. This species was described from a culture isolated from soil from Enchanted Rock, Llano County, Texas; July, 1960.

***Pharao typicum*** (Trainor & R.J.McLean) Darienko & Pröschold, *comb. nov.*

Basionym: *Spongiochloris typica* Trainor & R.J.McLean, *American Journal of Botany* 51: 58, 1964.

**Lectotype (designated here):** Fig. 1 in Trainor & McLean (1964: 59).

Comments: Unfortunately, the authentic strain UTEX 1238 is no longer available at the Culture Collection of Algae at the University of Texas at Austin, USA. While Trainor & McLean (1964) did not specify a type, the alga was “... isolated from a soil sample collected from a cornfield in Storrs, Connecticut, in October, 1958”, which represents an “entire gathering” as specified in ICN Art. 40.2 (Turland & al. 2018), thus fulfilling the requirements for valid publication. The authors do not mention depositing any specimen, and, as such, the original collection can be presumed lost and a lectotype designated (above) from the protologue.

In addition, our phylogenetic analyses (Fig. 1) indicate that the two genera *Herndonina* (Watanabe 2020: 406) and *Chlororustica* (Watanabe & al. 2021: 173) are closely related to the genus *Pharao*. Despite the morphological similarity of these genera, their taxonomic status requires further comparative studies. If the species of these genera are considered that they belong to one genus the generic name *Pharao* would have priority in accordance with ICN Art. 14.1 (Turland & al. 2018).

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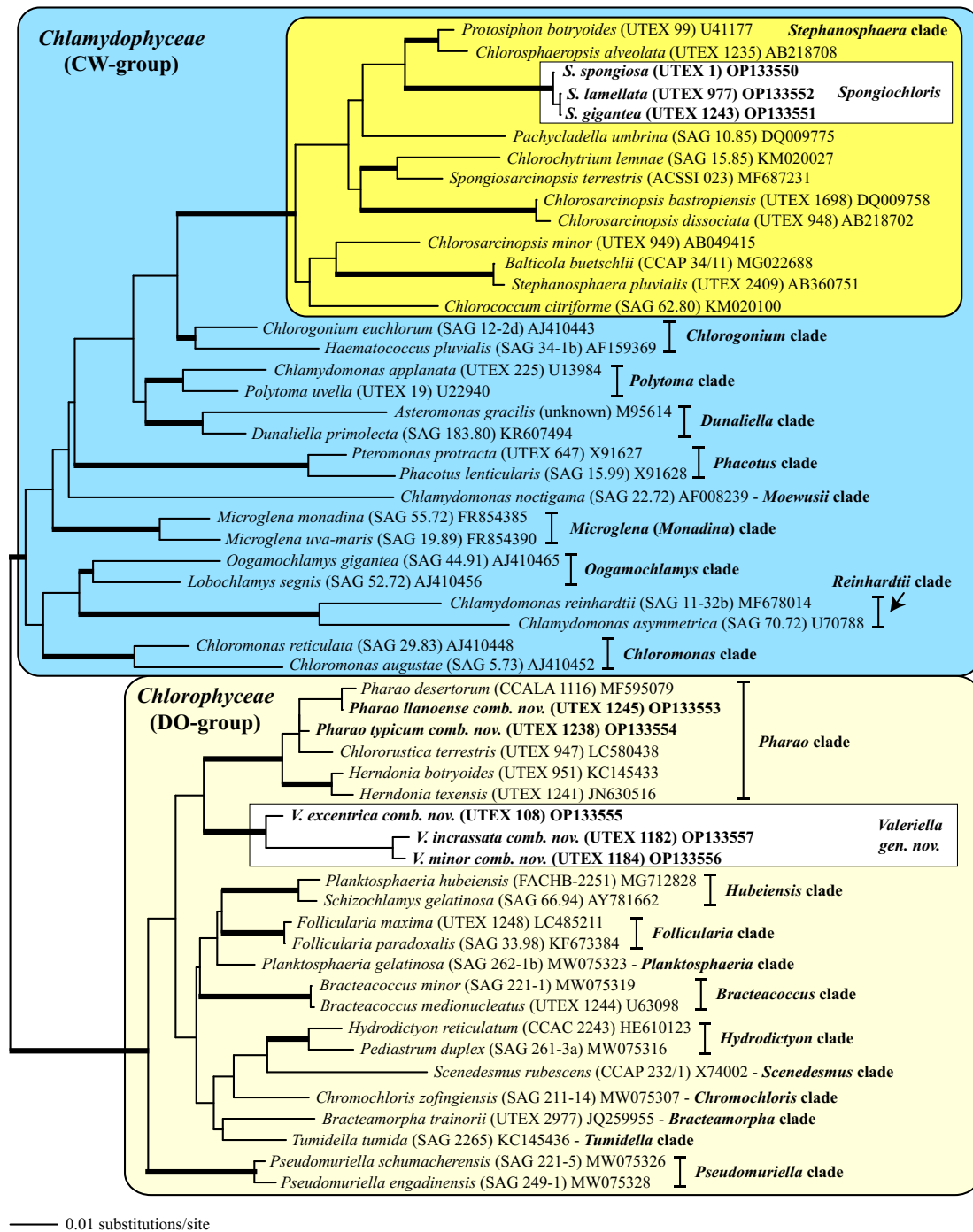


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**Fig. 1.** Molecular phylogeny of the *Chlamydomyceae* and *Chlorophyceae* (*Chlorophyta*) based on SSU rDNA sequence comparisons. The phylogenetic tree shown was inferred using the maximum likelihood method based on a data set of 1775 aligned positions of 78 taxa using PAUP 4.0a build169. For the analysis, the GTR+I+G (base frequencies: A 0.2466, C 0.2081, G 0.2703, U 0.2750; rate matrix A-C 1.1930, A-G 2.8438, A-U 1.5141, C-G 0.9225, C-U 5.0653, G-U 1.0000) with the proportion of invariable sites ( $I = 0.5613$ ) and gamma shape parameter ( $\Gamma = 0.4882$ ) 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).