



Occurrence of true branches in *Rhizoclonium* (Cladophorales, Ulvophyceae) and the reinstatement of *Rhizoclonium pachydermum* Kjellman

ZHI-JUAN ZHAO^{1,2}, HUAN ZHU^{1,2}, ZHENG-YU HU¹ & GUO-XIANG LIU^{1*}

¹Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, People's Republic of China

²University of Chinese Academy of Sciences, Beijing 100039, People's Republic of China

* Corresponding author (liugx@ihb.ac.cn)

Abstract

The phylogenetic position of the freshwater green alga *Rhizoclonium pachydermum* (Ulvophyceae: Cladophorales) was investigated using nuclear 18S rRNA gene and internal transcribed spacer 2 (ITS2) sequences. This alga has been referred to as *Cladophora pachyderma*. Based on its morphology, it was formerly classified in the section *Affines* in the genus *Cladophora*. However, this classification was not supported by the current phylogenetic analyses, where *Rhizoclonium pachydermum* formed a well-supported clade with other *Rhizoclonium* species. We consider that *Rhizoclonium* possesses real branches and the most important criteria that characterize the genus are: long unbranched filaments only with rhizoid branches, or only branched at the basal region of the thallus; and cylindrical cells with few or limited numbers of nuclei.

Key words: 18S rDNA, Cladophorales, ITS2, phylogeny, *Rhizoclonium pachydermum*, taxonomy

Introduction

Rhizoclonium pachydermum Kjellman (1877: 55) is a filamentous green alga (Chlorophyta: Cladophoraceae) that usually grows on the wet surfaces of rocks, the walls of wells, and the upper or lower sides of stones, being a fairly shade-loving species (van den Hoek 1963). This alga was established by Kjellman (1877) originally based on a sample he collected on the west coast of Novaya Zemlya. The alga has been recorded from Germany, Sweden, West Greenland, China, and other countries (Jao 1947, Kann 1947, van den Hoek 1963, Christensen 1991, Liu & Hu 1999). Brand (1909) transferred it to the genus *Cladophora* Kützing (1843: 262) because it had real branches and a disc-like holdfast. Since then, *R. pachydermum* Kjellman has been called *Cladophora pachyderma* (Kjellman) Brand (1908: 72) (Hoek 1963, Liu & Hu 1999).

The genus *Cladophora* is a rather heterogeneous assemblage of species, and one of the most species-rich genera among the green macroalgae (van den Hoek 1982, 1984, van den Hoek and Chihara 2000). Van den Hoek (1963) placed *C. pachyderma* in the first group of *Cladophora*: Section *Affines* Brand (1909:70). This section is often characterized as follows: i) long filaments, which grow via frequent intercalary cell divisions; ii) scattered branches, concentrated in the basal region of the plants, inserts laterally, often deflecting the axes over a wide angle; iii) attachment by a disc-like holdfast formed by the lower cell wall of the basal cell; and iv) cells relatively short, length/width ratio mostly 1–2 (van den Hoek 1963).

The two species in this group were *Cl. basiramosa* Schmidle in Wittrock, Nordsted & Lagerheim (1896: 13–14, fasc. 26, no. 1225) (Schmidle 1897) and *Cl. pachyderma*. Van den Hoek supplemented Kjellman's illustrations with some new drawings, which agreed with the taxonomic opinion of Brand. However, he also noted that both algae were very similar. In his opinion, the lack of moniliform chains of zooidangia and the very different ecology appeared to justify the perhaps provisional separation of *Cl. pachyderma* from *Cl. basiramosa* Schmidle. When Christensen (1991) re-examined Kjellman's type material, he found a thin *Oedogonium* and some diatoms that proved to be freshwater species, as well as representatives of the freshwater genera *Gomphonema* and *Eunotia*.

TABLE 3 (continued)

Taxon	Locality	Voucher, culture	GenBank No.	
			SSU	ITS2
<i>Siphonocladus tropicus</i>	-	UTEX LB 2369	Z35313	*
<i>Valonia utricularis</i>	-	VuF	Z35323	*
<i>Cladophoropsis vaucheriiformis</i>	Japan: Okinawa, Gushikawa	-	AB062719	*
<i>Chamaedoris peniculum</i>	-	CMP5	Z35417	*
<i>Chaetomorpha moniligera</i>	Japan:Hokkaido,Oturu	-	AB062703	*
<i>Chaetomorpha antennina</i>	Japan:Shizuoka,Shimoda	-	AB062700	*
<i>Chaetomorpha crassa</i>	Japan:Ishikawa,Shika	-	AB062701	*
<i>Chaetomorpha ligustica</i>	Japan	SAP:114369	AB807622	*
<i>Chaetomorpha linum</i>	Japan:Kochi	-	AB062702	*
<i>Chaetomorpha</i> sp.	-	WC	Z35420	*
<i>Rhizoclonium</i> sp.	USA	LB1523	*	AB259959
<i>Rhizoclonium riparium</i>	China	AST2010021	*	JN399202
<i>Aegagropila linnaei</i>	United Kingdom: Scotland, Loch Watten, Caithness	L0793543	*	GU325821
<i>Pithophora</i> sp.	Wuhan, Hubei, China	HB1204	*	KC898955
<i>Pithophora</i> sp.	Wuhan, Hubei, China	HB1201	*	KC898954
<i>Cladophora vagabunda</i>	Japan:Fukui, Mihama, Lake Kugushi	vag-1	*	AB665562
<i>Cladophora laetevirens</i>	Japan:Fukui, Mihama, Lake Hiruga	lae-1	*	AB665564
<i>Cladophora fascicularis</i>	China: Qingdao	AST2010014	*	JQ308255
Outgroup				
<i>Ulothrix zonata</i>	-	SAG 38.86	Z47999	*
<i>Ulva fasciata</i>	Japan: Kochi, Usa	#1	AB425964	*
<i>Ulothrix zonata</i>	Russia:Irkutsk	WELT:A032277	*	HE860526

“-” missing data.

“*”not used in the phylogenetic trees.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No. 31270252). We are grateful to Dr. Saúl Blanco Lanza (University of Leon) and other referees, for editorial corrections and their comments leading to improve the manuscript.

References

- Agardh, C.A. (1827) Aufzählung einiger in den österreichischen Ländern gefundenen neuen Gattungen und Arten von Algen, nebst ihrer Diagnostik und beigefügten Bemerkungen. *Flora* 10: 625–640.
- Balakrishnan, M.S. (1961) A cytotaxonomical investigation of Indian members of the Cladophoraceae. *In: Mahabale, T.S. (ed.) Professor S.P. Agharkar Commemoration Volume*. University of Poona, Poona, pp. 34–40.
- Boedeker, C. (2012) Phylogenetic, taxonomic and biogeographical studies in the Pithophoraceae (Cladophorales, Chlorophyta). *Journal of Phycology* 48: 808–825.
<http://dx.doi.org/10.1111/j.1529-8817.2012.01145.x>
- Borzi, A. (1883) *Studl algologici. Fasc. I*. Messina, 112 pp.
- Brand, F. (1909) Zur Morphologie und Biologie des Grenzgebietes zwischen den Algengattungen *Rhizoclonium* und *Cladophora*. *Hedwigia* 48: 45–73.
- Brand, F. (1913) Ueber *Cladophora humida* n. sp., *Rhizoclonium lapponicum* n. sp., und deren bostrychoide verzweigung.

- Hedwigia* 57: 179–183.
- Chapman, V.J. (1956) The marine algae of New Zealand, part I, Myxophyceae and Chlorophyceae. *Botanical Journal of the Linnean Society* 55: 333–501.
<http://dx.doi.org/10.1111/j.1095-8339.1956.tb00019.x>
- Christensen, T. (1991) On some Cladophoraceae (Chlorophyta) with long unbranched filaments. *Archiv für Protistenkunde* 139: 9–19.
[http://dx.doi.org/10.1016/s0003-9365\(11\)80003-5](http://dx.doi.org/10.1016/s0003-9365(11)80003-5)
- Delile, A.R. (1813). *Florae Aegyptiacae illustratio. In France (Commission d’Egypte). Description de l’Egypte ou recueil des observations et des recherches qui ont été faites en Egypte pendant l’expédition de l’armée française (1798 – 1801), Histoire naturelle* 2, pp. 49–82, 145–320 + atlas, pl. 1–62.
- Doyle, J.J. & Dickson, E.E. (1987) Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon* 36: 715–722.
<http://dx.doi.org/10.2307/1221122>
- Gardavsky, A. (1993) *Rhizoclonium fractiflexum* sp. nova, a new member of Cladophorales (Chlorophyta) described from freshwater aquaria. *Archiv für Protistenkunde* 143: 125–136.
[http://dx.doi.org/10.1016/S0003-9365\(11\)80281-2](http://dx.doi.org/10.1016/S0003-9365(11)80281-2)
- Gay, F. (1891) Le genre *Rhizoclonium*. *Journal of Botany (Paris)* 5: 53–58.
- Hall, T.A. (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Harvey, W.H. (1849) *Phycologia Britannica*. London, pl. 121–240.
- Hayakawa, Y., Ogawa, T., Yoshikawa, S., Ohki, K. & Kamiya, M. (2012) Genetic and ecophysiological diversity of *Cladophora* (Cladophorales, Ulvophyceae) in various salinity regimes. *Phycological Research* 60: 86–97.
<http://dx.doi.org/10.1111/j.1440-1835.2012.00641.x>
- Huelsenbeck, J.P. & Crandall, K.A. (1997) Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology, Evolution and Systematics* 28: 437–466. <http://dx.doi.org/10.1146/annurev.ecolsys.28.1.437>
- Huelsenbeck, J.P. & Ronquist F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Ichihara, K., Shimada, S. & Miyaji K. (2013) Systematics of *Rhizoclonium*-like algae (Cladophorales, Chlorophyta) from Japanese brackish waters, based on molecular phylogenetic and morphological analyses. *Phycologia* 52: 398–410. <http://dx.doi.org/10.2216/12-102.1>
- Jao, C.C. (1947) Studies on the freshwater algae of China XVII: Ulotrichales, Siphonocladiales, and Siphonales from Kwangsi. *Botanical Bulletin of Academia Sinica* 1: 257–269.
- Kann, E. (1947) Zur Ökologie der Litoralalgen in ostholsteinischen Waldseen. *Archiv für Hydrobiologie* 41: 14–42.
- Kjellmann, F.R. (1877) Ueber die Algenvegetation des Murmanschen Meeres an der Westküste von Nowaja Semlja und Wajgatsch. *Nova Acta Regiae Societatis Scientiarum Upsaliensis Volumen Extraordinem* 12: 1–85.
<http://dx.doi.org/10.5962/bhl.title.64252>
- Kützing, F.T. (1843) *Phycologia generalis*. Leipzig, 458 pp.
- Kützing, F.T. (1845) *Phycologia germanica*. Nordhausen, 240 pp.
- Kützing, F.T. (1849) *Species Algarum*. Brockhaus, Leipzig, 922 pp.
- Leliaert, F., & Boedeker, C. (2007) Cladophorales. In: Brodie, J., Maggs, C.A. & John, D.M. (eds.) *Green seaweeds of Britain and Ireland*. Natural History Museum Publications, London, pp. 131–183.
- Leliaert, F., De Clerck, O., Verbruggen, H., Boedeker, C. & Coppejans, E. (2007) Molecular phylogeny of the Siphonocladales (Chlorophyta: Cladophorophyceae). *Molecular Phylogenetics and Evolution* 44: 1237–1256.
<http://dx.doi.org/10.1016/j.ympev.2007.04.016>
- Liu, G.X. & Hu, Z.Y. (1999) Three new records of freshwater Cladophorales from China. *Acta Hydrobiologica Sinica* 23: 191–194.
- Luo, W., Pflugmacher, S., Pröschold, T., Walz, N. & Krienitz, L. (2006) Genotype versus phenotype variability in *Chlorella* and *Micractinium* (Chlorophyta, Trebouxiophyceae). *Protist* 157: 315–333.
<http://dx.doi.org/10.1016/j.protis.2006.05.006>
- Mathews, D.H.; Sabina, J., Zuker, M. & Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *Journal of Molecular Biology* 288: 911–940.
<http://dx.doi.org/10.1006/jmbi.1999.2700>
- Nienhuis P.H. (1975) *Biosystematics and ecology of Rhizoclonium riparium (Roth) Harvey (Chlorophyceae: Cladophorales) in the estuarine area of the rivers Rhine, Meuse, and Scheldt*. Bronder Offset B.Y., Rotterdam, 240 pp.
- Parodi, E.R. & Caceres, E.J. (1993) Life history of freshwater populations of *Rhizoclonium hieroglyphicum* (Cladophorales, Chlorophyta). *European Journal of Phycology* 28: 69–74.
<http://dx.doi.org/10.1080/09670269300650111>
- Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
<http://dx.doi.org/10.1093/bioinformatics/14.9.817>
- Prescott, G.W. (1982) *Algae of the western Great Lakes area*. Otto Koeltz Science Publishers, Koenigstein, 998 pp.
- Roth, A.G. (1806) *Catalecta botanica quibus plantae novae et minus cognitae describuntur atque illustrantur. Fasciculus*

tertius cum tabulis aenaeis XII. Leipzig, 350 pp.

- Scagel R.F. (1966) *Marine algae of British Columbia and Northern Washington, Part I: Chlorophyceae (green algae)*. National Museum of Canada Bulletin 207, Ottawa, vii + 257 pp.
- Schmidle, W. (1897) Beiträge zur Algenflora des Schwarzwaldes und Oberrheins VI. *Hedwigia* 36: 1–25.
- Setchell, W.A. & Gardner, N.L. (1920) The marine algae of the Pacific coast of North America. Part. 2, Chlorophyceae. *University of California of publications in Botany* 8: 139–374.
- Swindell, S.R. & Plasterer, T.N. (1997) SEQMAN: Contig assembly. *Methods in Molecular Biology* 70: 75–89.
<http://dx.doi.org/10.1385/0-89603-358-9:75>
- Swofford, D.L. (2003) *PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b 10*. Sinauer Associates, Sunderland.
- van den Hoek, C. & Chihara, M. (2000) *A taxonomic revision of the marine species of Cladophora (Chlorophyta) along the coasts of Japan and the Russian Far-East*. National Science Museum, Tokyo, 242 pp.
- van den Hoek, C. (1963) *Revision of the European species of Cladophora*. Brill, Leiden. 294 pp.
- van den Hoek, C. (1982) *A taxonomic revision of the American species of Cladophora (Chlorophyceae) in the north Atlantic ocean and their geographic distribution*. North-Holland Publishing, Amsterdam, 329 pp.
- van den Hoek, C. (1984) The systematics of the Cladophorales. In: Irvine, D.E.G. & John, D.M. (eds.) *Systematics of the green algae*. Academic Press, London and Orlando, pp. 157–178.
- Weber, F. & Mohr, D.M.H. (1804) *Naturhistorische Reise durch einen Theil Schwedens*. Göttingen, pp. [i]-xii, [13] -207 [-208].
- West, W. & West, G.S. (1897) Welwitsch's African freshwater algae. *Journal of Botany, British and Foreign* 35: 1–7, 33–42, 77–89, 113–122, 172–183, 235–243, 264–272, 297–304, pls. 365–369.
- Wittrock, V.B., Nordstedt, C.F.O. & Lagerheim, G. (1896) *Algae aquae dulcis exsiccatae praecipue scandinavicae quas adjectis algis marinis chlorophyllaceis et phycochromaceis* 26–29: 1201–1400.
- Womersley, H.B.S. (1984) *The marine benthic flora of southern Australia Part I*. Government Printer, Adelaide, South Australia, 329 pp.