

Molecular Phylogeography and Species Discrimination of Freshwater *Cladophora* (Cladophorales, Chlorophyta) in North America

Sara Ross¹, Robert Sheath² & Kirsten Müller¹

¹Department of Biology, University of Waterloo, Waterloo, ON, Canada

²Provost's Office, California State University, San Marcos, California, USA

The genus *Cladophora* (Cladophorales, Chlorophyta) is cosmopolitan in temperate and tropical regions in freshwater, brackish and marine habitats (Marks and Cummings, 1996; John, 2002). The freshwater species, *Cl. glomerata*, is frequently observed in eutrophic waters and can cause nuisance blooms under favorable conditions (van den Hoek, 1963) and considerable research has been done (and is ongoing) to prevent or reduce such problem blooms (Reynolds et al. 2000). Despite the considerable research on the ecological and physiological aspects of freshwater *Cladophora*, very little has been done to address the complex taxonomic issues within this genus. In fact, a practical and meaningful taxonomic framework for species within the genus *Cladophora* has yet to be determined (John, 2002). The “species” is the fundamental unit in taxonomic biology and many studies assume correct species identification. However, as in the case with many algal groups species are not easily delineated. Species exist because they fill discrete ecological niches and possibly differ in their physiological responses to the environment. Hence, being able to determine species (or genotype) is essential for interpreting both ecological and physiological studies as well as comparison between studies. This may have a considerable impact on management of nuisance blooms of members of this genus in different bodies of water.

In 1753, Linnaeus established the genus *Conferva* and subsequent to this, Kützing (1843), moved a large number of this genus to a new genus, *Cladophora*. With the renaming came a large number of new species, subspecies and varieties. In fact there are over 650 existing names for species of *Cladophora*. However, the genus *Cladophora* demonstrates a great degree of phenotypic plasticity due to varying environmental factors and age of the alga and therefore there are only 170 currently recognized species epithets. In 1963, van den Hoek reviewed the European species of this genus largely through the use of cultured *Cladophora* material and type specimens using both morphological and reproductive characteristics. He determined that there were 11 separate freshwater species belonging to six sections. The freshwater species listed were composed of six separate sections (species shown in brackets): *Aegagropila* [*Cl. aegagropila* (L.) Rabenh.], *Glomeratae* [*Cl. fracta* var. *fracta* (Mull. Ex Vahl) Kutz., *Cl. fracta* var. *intricata* (Mull ex Vahl) Kutz., *Cl. glomerata* var. *glomerata* (L.) Kutz., and *Cl. glomerata* var. *crassior* (L.) Kutz.], *Cladophora* [*Cl. rivularis* (L.) v.d. Hoek], *Cornuta* (*Cl. cornuta* Brand), *Affines* (*Cl. kosterae* Hoffm. & Tild.) and *Basicladia* [*Cl. basiramosa* Schmidle, and *Cl. pachyderma* (Kjellm.) Brand.].

The morphological characteristics used to differentiate these six sections and eleven species by van den Hoek (1963) include thallus organization, pattern and amount of branching, type of main axes growth (intercalary or acropetal), mode of reproduction, length/width ratio of main axes cell, apical cell diameter, attachment organ and finally if the organism is attached to a substrate or free-floating. For example, section *Aegagropila* is usually densely and irregularly branched, taking on a ball like formation and section *Cladophora* is composed of usually free-floating plants, which are sparsely branched. In addition, section *Glomeratae* demonstrates the greatest amount of phenotypic plasticity as they can be densely branched, or not; free-floating, or not; with different types of growth, main axes organization, and cell length-width ratios.

Closer inspection of these morphological traits shows that there are a number of overlapping taxonomic characteristics that are to be used to distinguish species within and among these different species and sections (Figure 1). This confusion is most evident in the *Glomeratae* section. *Cl. glomerata* var. *glomerata* differs from *Cl. glomerata* var. *crassior* in the fact that one is attached and one is floating, but each is readily confused with *Cl. fracta* var. *fracta* and *Cl. fracta* var. *intricata* which also only differ

based on their attachment or lack thereof. *Cl. glomerata* has been misidentified as *Cl. fracta* var. *fracta*, another genus, *Rhizoclonium*, and *Cl. barismosa*, all in their unbranched form. Though cell size can be used as a determinant, certain environments may permit each to have a very similar main axes cell diameter. It is very well known and has been for many years that the *Cladophora* species display a high degree of phenotypic plasticity, which is the ability of an individual's genotype to respond to environmental influences and generating different phenotypes.

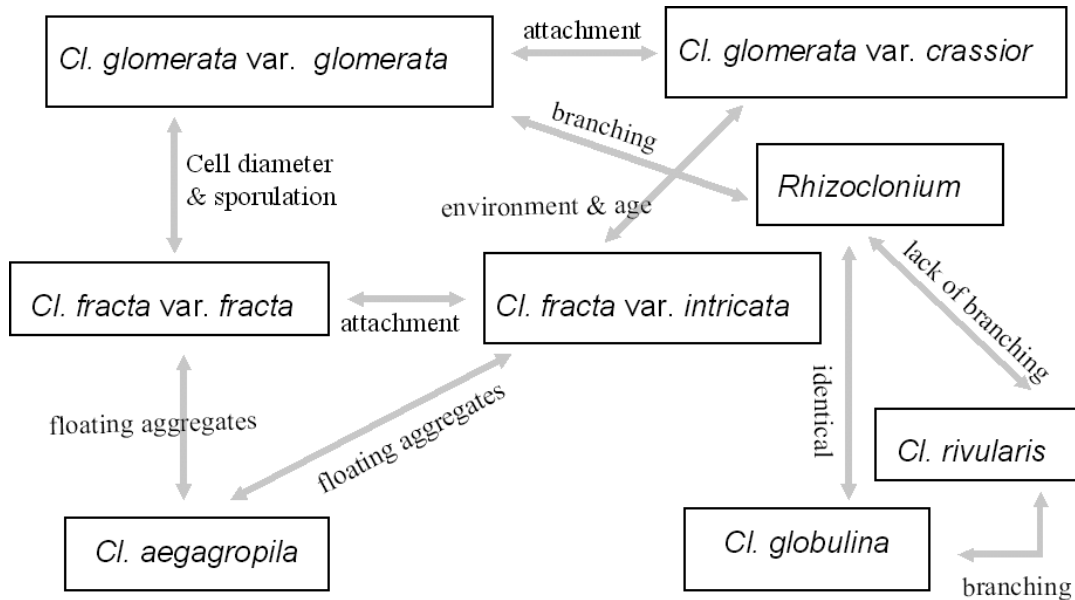


Figure 1. Diagram illustrating how the taxonomic characters used for identifying species of *Cladophora* are overlapping. Many of these characteristics are dependent on the environmental conditions under which the plant is observed.

Considering the tremendous morphological diversity and the issues arising with species identification, it is necessary to re-examine the taxonomic status of these freshwater species using molecular markers. Molecular markers are polymorphic protein or DNA sequences that can be used as indicators of genome-wide variations. There are a number of advantages to using molecular markers, namely that markers and marker variation can be quantified with great precision. Secondly the use of molecular markers allows for qualitative statistical analysis. Initially, we examined the internal transcribed spacers (ITS 1 and ITS 2) of the nuclear ribosomal DNA (rRNA) cistron to determine sequence divergence among North American populations of *Cladophora*. Typically, the ITS regions are beneficial marker for differentiating species and has been used extensively in other algal phyla to address such relationships (Bakker *et al.*, 1992). However, we noted that all sequences were identical or nearly identical. Hence, we are now examining the utility of other molecular markers. This is similar to what was noted by Marks and Cummings (1996) who examined freshwater *Cladophora glomerata* in Europe. Although analysis of ITS regions is used for phylogenetic relationships, it is still a conserved region compared to analysis of the intersimple sequence (ISSR) molecular marker. The ISSR marker is used to determine genetic variation within and among populations. In fact we have observed that there do appear to be differences among populations in the Great Lakes as well as populations from other lakes and streams in North America. While the preliminary data looks promising we have not yet completed this study.

By far the most informative molecular markers currently being used are Microsatellites. Microsatellites are simple sequence repeats, and are tandemly repeated nucleotide stretches of DNA. The repeats may be mono-, di-, tri-, tetra-, or even penta-nucleotide units (i.e. GCGGCGGCGGCG) and are located throughout the genome. Variability is determined by polymorphic length variation. This method

is used extensively for DNA fingerprinting (Queller *et al.*, 1993) and is extremely sensitive and are frequently used to differentiate varieties or individuals, and can reveal parentage and identity (Karp *et al.*, 1996; Wattier *et al.*, 1997).

The primary focus of our research is to create a usable taxonomic scheme by addressing the following objectives: 1) Determine if *Cladophora* in the Great Lakes is all one clone or if different species/varieties are present, 2) Determine the relationship of Great Lakes *Cladophora* with other North American collections, 3) Delineate species of Freshwater *Cladophora* using morphology, chromosome and molecular analyses. We believe that delineation of the species of *Cladophora* that is in the Great Lakes may aid in helping to understand the recent resurgence of blooms as well as the origin of new populations.

Bakker, F. T., Olsen, J. L., Stam, W. T., van den Hoek, C. 1992. Nuclear Ribosomal DNA internal transcribed spacer regions (ITS 1 and ITS 2) define discrete biogeographic groups in *Cladophora albida* (Chlorophyta). *Journal of Phycology* (28): 839-845.

Bakker, F. T., Olsen, J. L., Stam, W. T. 1995. Global phylogeography in the cosmopolitan species *Cladophora vagabunda* (Chlorophyta) based on nuclear rDNA internal transcribed spacer sequences. *European Journal of Phycology* (30): 197-208.

Billot, C., Rousvoal, S., Estoup, A., Epplen, J. T., Saumitou-Laprade, P., Valero, M., Kloareg, B. 1998. Isolation and characterization of microsatellite markers in the nuclear genome of the brown alga *Laminaria digitata* (Phaeophyceae). *Molecular Ecology* (7): 1771-1788.

John, D.M. (2002). Order Cladophorales (=Siphonocladales). In: *The Freshwater Algal Flora of the British Isles. An identification guide to freshwater and terrestrial algae.* (John, D.M., Whitton, B.A. & Brook, A.J. Eds), pp. 468-470. Cambridge: Cambridge University Press.

Karp, A. & Edwards, K.J. 1997. DNA markers: a global overview. In: *DNA markers: Protocols, Applications and Overviews* (Caetano-Anollés, G. & Gresshoff, P.M., eds.), 1-14. Wiley-VCH, New York.

Marks, J. C., Cummings, M. P. 1996. DNA sequences variation in the ribosomal internal transcribed spacer region of freshwater *Cladophora* species (Chlorophyta). *Journal of Phycology* (32): 1035-1042.

Powell, W., Morgante, M., Rafalski, J. A., McDevitt R., Vendramin, G. C. 1995. Polymorphic simple sequence repeat regions in the chloroplast genome: applications to the population genetics of Pines. *Current Biology* (5): 1023-1029.

Queller, D. C., Strassmann, J. E., Hughes, C. R. 1993. Microsatellites and kinship. *Trends in Ecological Evolution* (8): 285-288.

Reynolds, C.S., Reynolds, S.N., Munawar, J.F. 2000. The regulation of phytoplankton population dynamics in the world's largest lakes. *Aquatic Ecosystem Health & Management*, (3): 1-21.

van den Hoek, C. (1963). *Revision of the European species of Cladophora. Proefschrift...Rijksuniversiteit te Leiden.* pp. XI + 248, 1 fig, 55 plates, 18 maps. Leiden: E. J. Brill.

Wattier, R., Dallas, J.F., Destombe, C., Saumitou-Laprade, P. & Valero, M. 1997. Single locus microsatellites in Gracilariales (Rhodophyta): High level of genetic variability within *Gracilaria gracilis* and conservation in related species. *J. Phycol.*, (33): 868-880.

