TAXONOMIC RESHUFFLING OF THE CLADOPHORACEAE

The notion that some groups of algae suffer from a “low-morphology problem” that masks their true species diversity has been recognized for at least two decades (van Oppen et al. 1996). This problem has become dramatically evident in recent years as DNA sequence data have taken center stage in species delimitation. The issue is usually associated with algal groups having simple morphologies and a limited set of morphological characters useful for morpho-taxonomic circumscriptions and in taxa experiencing habitat-induced phenotypic plasticity (Verbruggen 2014). The Cladophoraceae, a family of small filamentous green seaweeds, is clearly impacted by the low-morphology problem and is an excellent example to illustrate these difficulties. The paper of Boedeker et al. (2016) published in this issue represents a milestone in the systematic biology of this widespread group of chlorophytes. The authors propose a new classification of the Cladophoraceae based on a robust molecular phylogeny. The impressive sampling effort and geographical coverage lead to strong conclusions, making this study a major taxonomic advancement for green algae in general, not just the Cladophoraceae.

The Cladophoraceae contains three genera that are familiar to most marine biologists: *Cladophora*, *Chaetomorpha*, and *Rhizoclonium*. They all have thalli consisting of uniseriate filaments formed by large multinucleate cells. The distinction between genera is (apparently) straightforward: *Cladophora* has branched filaments, *Chaetomorpha* has unbranched thick filaments, and *Rhizoclonium* has unbranched thin filaments. In the field, algae of these genera are generally easy to recognize as green filamentous patches, bushes, or rope-like structures occurring in a wide range of habitats (Fig. 1). The Cladophoraceae is one of the few algal taxa that has crossed the borders between sea and freshwater, because it includes species distributed in marine, brackish, and freshwater environments (e.g., in the Mekong river in Laos, where *Cladophora* is harvested for consumption). Some species thrive in marine intertidal habitats and coastal lagoons, where they occasionally produce large blooms in situations where nutrients are abundant and competition is reduced (Zulkifly et al. 2013). From a geographic point of view, the family has a cosmopolitan distribution, ranging from tropical to polar waters, with generally higher diversity in temperate regions.

The Cladophoraceae is one of the longest-known and studied taxa of green algae (the first few species were described as early as the late 18th century), yet are still recognized as a taxonomically challenging group. Species delimitation has been particularly problematic, as demonstrated by the

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33 plethora of specific and intraspecific names that exist (1,072 listed in AlgaeBase only for
34 *Cladophora*, Guiry & Guiry 2016). A poor understanding of the biology of these algae has
35 contributed to this nomenclatural inflation. It is now known that thallus morphology is greatly
36 affected by phenotypic plasticity related to environmental conditions (Leliaert & Boedeker 2007)
37 and that this has been the major cause of taxonomic misinterpretations. Some morphological
38 characters considered taxonomically relevant may vary in relation to habitat. An excellent example
39 is found in one of the most detailed treatments available for this group: the monograph of van den
40 Hoek (1963) for European *Cladophora*. This study, which is still considered a key reference for the
41 family, reports that several common *Cladophora* species exhibit different apical cell sizes
42 depending on whether the alga grows in well-lit or shaded sites, yet apical cell size used to be an
43 important taxonomic character. At the same time, the shortage of morphological characters
44 available for these algae poses a limit to the number of species that can be diagnosed on a
45 morphological basis. It is therefore not surprising that convergent evolution of morphological traits
46 has been common in the Cladophoraceae; this phenomenon emerged in early molecular studies of
47 the family (Bakker et al. 1994, Hanyuda et al. 2002, Leliaert et al. 2003) and is now confirmed with
48 more substantial evidence.

49 The study by Boedeker et al. (2016) is an example of good practice in the establishment of a
50 natural classification of a taxonomically difficult group of algae. The authors use sequences of the
51 LSU and SSU rDNA genes obtained from a large number of species to infer a robust molecular
52 phylogeny that is then used to update the classification of the family. The number of samples used
53 and the geographic coverage of the study are among the largest seen in a phylogenetic study on
54 macroalgae, resulting from years of extensive collections made by the authors or received from
55 collaborators, and the sequence data are analyzed with sound, state-of-the-art phylogenetic methods.

56 Not unexpectedly, the study provides definitive evidence that none of the three main genera,
57 *Cladophora*, *Chaetomorpha*, and *Rhizoclonium* is monophyletic. This also suggests that a complete
58 overhaul of the classification of the group is needed. A factor complicating this matter is that
59 several old taxon names exist that are now considered synonyms of these genera, which need to be
60 considered in naming the groupings resolved in the phylogenies. In situations like this, uncertainty
61 about which clades should receive which names often abounds and can lead to decision paralysis.
62 Clearly, such paralysis represents a huge impediment for the establishment of a correct
63 nomenclature and its applications in algal biology and marine ecology. In theory, the logical
64 solution to this problem would be to sequence type specimens, the only ones to which taxonomic
65 names are unambiguously linked. This approach has become increasingly popular in recent years
66 (especially for red algae) and it is likely that high-throughput sequencing methods will make it part

67 of the core toolkit for algal taxonomy (*e.g.*, see Hughey et al. 2014). At present, however, obtaining
68 sequences from old herbarium specimens is still a very laborious and error-prone procedure for
69 which there is no guarantee of success. In reality, it is often necessary to go down a more pragmatic
70 path, letting experts familiar with the biology of the group link taxonomic names and molecular
71 groupings by examination of type specimens and consideration of the original descriptions. This is
72 the approach adopted by Boedeker et al. (2016), and we praise them for being decisive in the face of
73 uncertainty and assigning names to seven monophyletic genera: in addition to redefining
74 *Cladophora*, *Chaetomorpha* and *Rhizoclonium*, they describe the new genera *Lubrica* and
75 *Pseudorhizoclonium* and reinstate *Acrocladus* and *Willeella*.

76 The study is also an interesting example of the nomenclatural problems caused by
77 discrepancies between morphology-based and DNA-based classifications in algal groups with a
78 simple habit. If the nomenclatural rules were strictly adhered to, only a single species would remain
79 in *Chaetomorpha* (the generitype *C. melagonium* (F. Weber & D. Mohr) Kützing), and all other
80 species would need to be transferred to a different genus. We find that the authors make a sensible
81 decision to avoid such a nomenclatural ‘earthquake’ and propose to conserve *Chaetomorpha* against
82 its type in a future publication (transferring *C. melagonium* to a new genus).

83 Besides taxonomic advances, the study also contributes new insights into the evolutionary
84 history of the Cladophoraceae, showing that there have been at least three independent switches
85 between branched and unbranched morphologies, and that the family has colonized freshwater
86 environments on two separate occasions. This highlights the morphologically and physiologically
87 dynamic nature of these algae, which has likely been the basis of their evolutionary success.

88 Although this study represents a model for the establishment of natural taxonomic
89 classifications, the methods used here may not yield similarly conclusive results for other algal
90 groups. A serendipitous aspect of the study is that just two molecular markers yielded a robust
91 phylogeny of the group, with high support for most internal branches (see Figure 2). It was this
92 well-supported backbone that allowed the new classification to be built, but in many other algal taxa
93 two markers will not be sufficient to obtain an equally robust phylogeny, especially in poorly-
94 known groups for which taxon sampling is sparse (see, for example, a similarly well-designed study
95 by Fučíková et al. (2014a) that could not completely resolve the phylogeny of the Sphaeropleales
96 despite using seven concatenated markers). For many groups, obtaining robust phylogenies will
97 require combining multiple markers from different cell compartments and a similarly extensive
98 taxon sampling, which may be hard to achieve in the case of small-sized or morphologically cryptic
99 algae.

100 The Cladophoraceae are now better organized taxonomically, but they offer plenty more
101 challenges. An interesting point for those not familiar with the systematics of Cladophoraceae, is
102 that the study of Boedeker et al. (2016) is the first one to present a phylogeny of the family based on
103 more than one marker. In an era in which it is normal to see algal phylogenies based on many
104 markers or, more and more often, whole organellar genomes, this may appear strange. In particular,
105 it seems odd that slow-evolving plastid markers which are popular for phylogenetic inference and
106 DNA barcoding in green algae (*rbcL*, *tufA*, *atpA*, *psaA*, *psaB*, *psbA*) have not yet been sequenced
107 for the Cladophoraceae. For reasons that are still unknown, the amplification of these "easy target"
108 plastid markers in the Cladophoraceae has been an almost insurmountable challenge, with only one
109 *rbcL* sequence published thus far (for *Chaetomorpha valida* (J.D. Hooker & Harvey) Kützinger, Deng
110 et al. 2014). In molecular phylogenies, this sequence is highly divergent from those of other
111 Ulvophyceae (Fučíková et al. 2014b) and it should be confirmed based on additional data.
112 Presumably the amplification of plastid markers in these algae must be prevented by some atypical
113 features of the plastid genome. The structure of the plastid genome remains one of the most
114 mysterious and intriguing aspects of this group. This is suggested in part by the presence of actively
115 transcribed plasmid-like DNA that seems to have derived from plastid DNA (LaClaire et al. 1998).
116 The challenge will be to characterize these plasmids and their role in the biology of these algae, and
117 this knowledge will contribute to our understanding of the genetic and physiological features that
118 have made the Cladophoraceae such an adaptable and successful group.

119 Placement at higher taxonomic levels has also been a source of uncertainty and cannot be
120 considered fully settled yet. At the ordinal level, Cladophoraceae belong to the order Cladophorales,
121 a group that includes several lineages with rather different habits, but generally well supported in
122 molecular phylogenies and defined by the unifying character of having multinucleate cells with
123 nuclei arranged in regularly spaced cytoplasmic domains (siphonocladous organization; Leliaert et
124 al. 2012). At the class level, Cladophorales have been generally placed in the Ulvophyceae as
125 defined by Mattox & Stewart (1984). However, van den Hoek et al. (1995) considered the
126 cladophoralean algae distinctive enough to deserve a higher status and erected the class
127 Cladophorophyceae. In recent years, most authors have preferred their classification as an order in
128 the Ulvophyceae, supported by the fact that molecular phylogenies based mainly on nuclear
129 markers recovered the Ulvophyceae as a monophyletic group (López-Bautista & Chapman 2003,
130 Cocquyt et al. 2010) in which the Cladophorales were robustly placed. However, studies based
131 mainly on plastid DNA did not provide strong support for the monophyly of the Ulvophyceae, and
132 recent large-scale phylogenies based on plastid multigene data do not support a monophyletic
133 Ulvophyceae (Fučíková et al. 2014b). Needless to say, the Cladophorales, due to the difficulty of

134 amplifying chloroplast markers, have not been well-incorporated into such chloroplast
135 phylogenomic studies. Depending on future developments in the classification of the core
136 chlorophytes (cf. Fučíková et al. 2014b), the class-level placement of the Cladophorales may have
137 to be revised, and the possibility that the class Cladophorophyceae of van den Hoek et al. (1995)
138 may eventually be resurrected should not be discounted.

139 In an era in which algal genomics has taken off, it is also worthy to note that genomic data
140 from the Cladophoraceae are still in great shortage and limited to metagenomic data. This is
141 unfortunate, especially because genomic information, in combination with additional physiological
142 and biochemical studies, would greatly help clarify the processes regulating morphogenesis in these
143 algae and further explain the basis of their phenotypic plasticity. We hope that more work will be
144 carried out on these aspects of the biology of the Cladophoraceae and we remark that, even after
145 more than two centuries of studies, this group still provides a wealth of opportunities for creative
146 and exciting research.

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162 REFERENCES

163
164 Bakker, F. T., Olsen, J. L., Stam, W. T. & van den Hoek, C. 1994. The *Cladophora* complex
165 (Chlorophyta): new views based on 18S rRNA gene sequences. *Mol. Phylogen. Evol.*
166 3:365-82.

167 Boedeker, C. Leliaert, F. & Zuccarello, G. 2016. Molecular phylogeny of the Cladophoraceae
 168 (Cladophorales, Ulvophyceae), with the resurrection of *Acrocladus* Nägeli and *Willeella*
 169 Børgesen, and the description of *Lubrica* gen. nov. and *Pseudorhizoclonium* gen. nov. *J.*
 170 *Phycol.*

171 Cocquyt, E., Verbruggen, H., Leliaert, F. & De Clerck, O. 2010. Evolution and cytological
 172 diversification of the green seaweeds (Ulvophyceae). *Mol. Biol. Evol.* 27:2052-61.

173 Deng, Y., Zhan, Z., Tang, X., Ding, L. & Duan, D. 2014. Molecular cloning and expression analysis
 174 of *rbcL* cDNA from the bloom-forming green alga *Chaetomorpha valida*
 175 (Cladophorales, Chlorophyta). *J. Appl. Phycol.* 26:1853–1861.

176 Fučíková, K., Leliaert, F., Cooper, E. D., Škaloud, P., D'hondt, S., De Clerck, O., Gurgel, C. F. D.,
 177 Lewis, L. A., Lewis, P. O., Lopez-Bautista, J. M., Delwiche, C. F. & Verbruggen H.
 178 2014b. New phylogenetic hypotheses for the core Chlorophyta based on chloroplast
 179 sequence data. *Front. Ecol. Evol.* 2: 63.

180 Fučíková, K., Lewis, P.O. & Lewis, L. A. 2014a. Putting incertae sedis taxa in their place: a
 181 proposal for ten new families and three new genera in Sphaeropleales (Chlorophyceae,
 182 Chlorophyta). *J. Phycol.* 50:14–25.

183 Guiry, M. D. & Guiry, G. M. 2016. AlgaeBase. World-wide electronic publication, National
 184 University of Ireland, Galway. <http://www.algaebase.org>; searched on 4 July 2016.

185 Hanyuda, T., Wakana, I., Arai, S., Miyaji, K., Watano, Y. & Ueda, K. 2002. Phylogenetic
 186 relationships within Cladophorales (Ulvophyceae, Chlorophyta) inferred from 18S rRNA
 187 gene sequences, with special reference to *Aegagropila linnaei*. *J. Phycol.* 38:564-71.

188 Hughey, J.R., Gabrielson, P.W., Rohmer, L., Tortolani, J., Silva, M. Miller, K. A., Young, J. D.,
 189 Martell, C. & Ruediger, E. 2014. Minimally destructive sampling of type specimens of
 190 *Pyropia* (Bangiales, Rhodophyta) recovers complete plastid and mitochondrial genomes.
 191 *Sci. Rep.* 4: 5113.

192 La Claire, J. W., Loudenslager, C. M. & Zuccarello, G. C. 1998. Characterization of novel
 193 extrachromosomal DNA from giant celled marine green algae. *Curr. Genet.* 34:204–211.

194 Leliaert, F. & Boedeker, C. 2007. Cladophorales. In Brodie, J., Maggs, C. A. & John, D. [Eds.]
 195 *Green Seaweeds of Britain and Ireland*. Natural History Museum Publications, London,
 196 U.K., pp. 131-183.

197 Leliaert, F., Rousseau, F., de Reviers, B. & Coppejans, E., 2003. Phylogeny of the
 198 Cladophorophyceae (Chlorophyta) inferred from partial LSU rRNA gene sequences: is the
 199 recognition of a separate order Siphonocladales justified? *Eur. J. Phycol.* 38:233-46.

- 200 Leliaert, F., Smith, D. R., Moreau, H., Herron, M. D., Verbruggen, H., Delwiche, C. F. & De
201 Clerck, O. 2012. Phylogeny and molecular evolution of the green algae. *Crit. Rev. Plant*
202 *Sci.* 31:1–46.
- 203 Lopez-Bautista, J. M. & Chapman, R. L. 2003. Phylogenetic affinities of the Trentepohliales
204 inferred from small-subunit rDNA. *Int. J. Syst. Evol. Microbiol.* 53:2099-106.
- 205 Mattox, K. R. & Stewart, K. D. 1984. The classification of the green algae, a concept based on
206 comparative cytology. In Irvine, D. E. G. & John, D. M. [Eds.] *Systematics of the Green*
207 *Algae*. Academic Press, London & Orlando, pp. 29–72.
- 208 van den Hoek, C. 1963. *Revision of the European species of Cladophora*. Brill E.J., Leiden,
209 Netherlands, 248 pp.
- 210 van den Hoek, C., Mann, D. G. & Jahns, H. M. 1995. *Algae. An Introduction to Phycology*.
211 Cambridge University Press, Cambridge, UK, 623 pp.
- 212 van Oppen, M. J. H., Klerk, H., Olsen, J. L. & Stam, W. T. 1996. Hidden diversity in marine algae:
213 some examples of genetic variation below the species level. *J. Mar. Biol. Assoc. U.K.*
214 76:239–42.
- 215 Verbruggen, H. 2014. Morphological complexity, plasticity, and species diagnosability in the
216 application of old species names in DNA-based taxonomies. *J. Phycol.* 50:26-31.
- 217 Zulkifly, S. B., Graham, J. M., Young, E. B., Mayer, R. J., Piotrowski, M. J., Smith, I. & Graham,
218 L. E. The genus *Cladophora* Kützinger (Ulvophyceae) as a globally distributed ecological
219 engineer. *J. Phycol.* 49:1–17.

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222 FIGURE LEGEND

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224 Fig. 1. Habit of selected representatives of Cladophoraceae. (A) *Acrocladus herpesticus*. (B)
225 *Willeella ordinata*. (C) *Chaetomorpha antennina*. (D) *Cladophora* sp.

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