

# Transitions between marine and freshwater environments provide new clues about the origins of multicellular plants and algae

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1 **TRANSITIONS BETWEEN MARINE AND FRESHWATER ENVIRONMENTS**  
2 **PROVIDE NEW CLUES ABOUT THE ORIGINS OF MULTICELLULAR PLANTS**  
3 **AND ALGAE**

4

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13 **List of abbreviations:**

14 APG - Angiosperm Phylogeny Group

15 Ca - calcium

16 CaCO<sub>3</sub>- calcium carbonate

17 Cl<sup>-</sup> - chloride

18 Gya - billion years ago

19 K - potassium

20 Mya - million years ago

21 Na – sodium

22 Si - silicon

23 **Abstract**

24 Marine-freshwater and freshwater-marine transitions have been key events in the evolution of  
25 life, and most major groups of organisms have independently undergone such events at least  
26 once in their history. Here we first compile an inventory of bidirectional freshwater and  
27 marine transitions in multicellular photosynthetic eukaryotes. While green and red algae have  
28 mastered multiple transitions in both directions, brown algae have colonized fresh water on  
29 maximally six known occasions, and angiosperms have made the transition to marine  
30 environments only two or three times. Next we review the early evolutionary events leading  
31 to the colonization of current habitats. It is commonly assumed that the conquest of land  
32 proceeded in a sequence from marine to freshwater habitats. However, recent evidence  
33 suggests that early photosynthetic eukaryotes may have arisen in subaerial or freshwater  
34 environments and only later colonized marine environments as hypersaline oceans were  
35 diluted to the contemporary level. Although this hypothesis remains speculative, it is  
36 important to keep these alternative scenarios in mind when interpreting the current habitat  
37 distribution of plants and algae. Finally we discuss the roles of structural and functional  
38 adaptations of the cell wall, reactive oxygen species scavengers, osmoregulation, and  
39 reproduction. These are central for acclimatization to freshwater or to marine environments.  
40 We observe that successful transitions appear to have occurred more frequently in  
41 morphologically simple forms and conclude that, in addition to physiological studies of  
42 euryhaline species, comparative studies of closely related species fully adapted to one or the  
43 other environment are necessary to better understand the adaptive processes.

44 **Keywords:** Macroalgae, aquatic plants, marine-freshwater and freshwater-marine transitions,  
45 adaptation, evolution

## 46 1. FRESHWATER AND MARINE HABITATS

47 Water bodies cover 71% of the Earth's surface, either as oceans, lakes, rivers or as ice (Ott  
48 1988). Approximately 97.6% of all water on Earth is saline and only 2.4% is fresh water  
49 (although estimates vary depending on the author). Most of this fresh water is found as  
50 groundwater or as ice, and only 0.009% occurs within lakes and rivers (Wetzel 2001). Fresh  
51 water is defined as water with a salinity  $\leq 0.5$  g dissolved salts $\cdot$ L<sup>-1</sup> (International Symposium  
52 for the Classification of Brackish Waters 1958), and the global average for lakes and rivers is  
53 0.12 g $\cdot$ L<sup>-1</sup> of salt (Wetzel 2001). The reason for differences in salinity among water bodies is  
54 the water cycle: surface water (both salty and fresh) evaporates and precipitates as fresh  
55 water. A part of this fresh water precipitates on land and gradually flows back to the sea,  
56 dissolving and transporting salts on its way. In parallel, the evaporation of large basins in  
57 combination with the rise of land masses generates new terrestrial salt reserves. Ocean salinity  
58 has been modeled to have varied over the last 600 My between 30 to 60 g $\cdot$ L<sup>-1</sup> with a current  
59 mean of 35 g $\cdot$ L<sup>-1</sup> (Hay et al. 2006). Mainly the sodium(Na)/potassium(K) balance in seawater  
60 is thought to have shifted in favor of Na over time due to the higher potential of K to bind to  
61 clay and thus to be removed from seawater (MacIntyre 1970).

62 Seawater is not just a concentrated version of fresh water, as a number of processes impact  
63 ion distribution in each environment. For one, salts (including nutrients) are washed into the  
64 ocean at different rates. Thus, the availability of macro- and micronutrients differs  
65 significantly between freshwater and marine environments. Primary production in the  
66 majority of freshwater ecosystems is most often limited by the availability of phosphorus  
67 (Schindler 1977), whereas marine environments are primarily limited by nitrogen (Hecky and  
68 Kilham 1988; Zehr and Ward 2002) or trace elements such as iron (Martin et al. 1990). Also,  
69 once eluted from the soil, calcium ions (Ca<sup>2+</sup>) readily precipitate as calcium carbonate  
70 (CaCO<sub>3</sub>), whereas Na<sup>+</sup> remains in solution and is efficiently transported to the ocean.

71 Freshwater environments are therefore mainly  $\text{Ca}^{2+}$ -dominated whereas seawater is  $\text{Na}^{+}$ -  
72 dominated. Nevertheless, there is usually more total carbonate in marine environments,  
73 compared to fresh water (Table 1).

74 Fresh water composition depends more on the surrounding terrestrial environment and is  
75 highly variable compared to seawater. Factors that influence fresh water composition include  
76 chemical interactions with the soil, the geological context, weathering, precipitation (quantity  
77 and quality), temperature, stream discharge, nutrient uptake, and physical and biological  
78 transformations (Feller 2009). Geologists distinguish between rock-dominated and  
79 precipitation-dominated fresh water (Gibbs 1970). Rock-dominated fresh water is usually  
80 found at higher altitudes and salt concentrations are highly dependent on the material of the  
81 basin; it is usually rich in silicate (Si) and K. Precipitation-dominated fresh water occurs  
82 primarily in leached areas with high rainfall. Its ion composition reflects that of the rain, with  
83 ions occurring in similar proportions as in seawater, even in water bodies that are far inland.  
84 Finally, evaporation has a strong impact on the ion composition of fresh water, increasing  
85 salinity and removing  $\text{CaCO}_3$  due to precipitation from solution.

86 Life on Earth is generally thought to have originated ~4 billion years ago in primordial,  
87 hypersaline oceans (Martin et al. 2008) with salinities  $> 80 \text{ g}\cdot\text{L}^{-1}$  (Pinti 2005). From there,  
88 bacteria and eukaryotes gradually colonized freshwater and then terrestrial environments.  
89 Recently, an alternative scenario has been proposed in which life first arose in non-marine  
90 settings (*i.e.* subaerial or freshwater) (Wellman and Strother 2015) and only later colonized  
91 marine habitats. It is possible that early eukaryotes including the ancestors of the major  
92 macro-algal lineages also originated and diversified in this non-marine setting 2-1.1 Gya, and  
93 that the colonization of marine environments may have been stimulated during times of lethal  
94 land surface conditions. In either scenario, habitat transitions were key events in the early  
95 evolution of photosynthetic, multicellular eukaryotes.

96 However, most extant lineages - from archaeans to metazoans - are found in both fresh water  
97 and seawater, indicating that each of them also independently made a transition between these  
98 environments at least once in their recent evolutionary history. When, how often, and under  
99 what conditions these transitions have occurred (and can occur) are questions fundamental to  
100 our understanding of habitat diversification in the broadest sense and to explain the  
101 distribution of marine, freshwater, and terrestrial biodiversity.

102 In this review, we first compile an inventory of extant marine-freshwater and freshwater-  
103 marine transitions in multicellular, photosynthetic eukaryotes (Figure 1) and then discuss our  
104 findings in the context of current hypotheses about their evolutionary and geological history.  
105 We define multicellular photosynthetic protists/algae as a physically connected set of  
106 genetically identical cells with the ability to produce distinctive reproductive structures,  
107 although we also included some siphonous algae in our review. We consider an alga as  
108 marine if it naturally occurs and reproduces in seawater (see above), and as freshwater, if it  
109 naturally occurs and reproduces in fresh water (salinity  $\leq 0.5 \text{ g}\cdot\text{L}^{-1}$ ). Species found in brackish  
110 water are, in almost all cases, also able to tolerate marine conditions and were thus considered  
111 marine. Terrestrial species are those found outside of waterbodies, although some species also  
112 occur in fresh water, and some might require regular exposure to fresh water (e.g. abundant  
113 rain for reproduction). Please note that, across the different lineages examined in this review,  
114 particular taxonomic ranks (families, genera, species) are not always equivalent.

## 115 **2. CENSUS OF MARINE-FRESHWATER AND FRESHWATER-MARINE** 116 **TRANSITIONS**

### 117 **Unicellular and non-photosynthetic multicellular organisms**

118 Unicellular protists and bacteria occur in extremely large populations with generally short  
119 generation times and fast evolutionary rates (Baer et al. 2007). These properties facilitate

120 large-scale dispersal into different environments. Nevertheless, in a recent study Logares et al.  
121 (2009) demonstrated that among these organisms, separate clades have adapted to either  
122 marine or freshwater environments, a separation probably due in part to the energetic costs  
123 associated with osmoregulation and ion homeostasis.

124 Multicellular organisms (with exceptions) are generally characterized by comparatively  
125 smaller population sizes, longer generation times and a slower rate of evolution (Baer et al.  
126 2007). Nevertheless, many of these lineages too, are found in both marine and freshwater  
127 environments. In animals, marine to freshwater transitions (and vice versa) have been  
128 particularly well-studied in fish (Vega and Wiens 2012). In addition, several species of fish  
129 are also able to migrate between fresh water and seawater in the course of their lives. These  
130 diadromous fish rely on specialized membrane transporters and compatible osmolytes to  
131 adjust intracellular osmolarity and ion concentrations. Active osmoregulation, however,  
132 depends on a sufficient supply of energy. Plants and macroalgal species that live permanently  
133 submerged have to deal with reduced light quality and quantity and thus reduced energy  
134 supply. Adaptations in photosynthetic pigments (Dawes 1998) and/or specific structural and  
135 physiological adaptations related to osmotolerance for marine vs. freshwater conditions are  
136 therefore required (see section 4).

### 137 **Archaeplastida**

138 The supergroup Archaeplastida or Plantae (includes: Glaucophyta, the Viridiplantae, and the  
139 Rhodophyta) arose from a primary endosymbiosis event with a cyanobacterium and a  
140 heterotrophic eukaryote (McFadden 2001). It is the most diverse group of multicellular  
141 photosynthetic eukaryotes, reaching substantial abundances in marine, freshwater, and  
142 terrestrial habitats. The Glaucophytes are rare, unicellular organisms at the root of the  
143 Archaeplastida that occur predominantly in fresh water, although recent 18S metabarcoding  
144 data from the Tara Oceans Project revealed glaucophyte sequences in oceanic environments

145 (see Supplementary dataset W5 in de Vargas et al. 2015). The Viridiplantae (the green  
146 lineage) split into two monophyletic groups, the Streptophyta, and the Chlorophyta,  
147 approximately 1,200-750 million years ago (Becker and Marin 2009) and includes green  
148 algae, charophytes, and terrestrial plants. Rhodophytes (red algae) are mainly multicellular  
149 marine algae but also comprise freshwater and a very few, partially terrestrial species (Sheath  
150 and Vis 2015).

### 151 *Streptophyta*

152 The most basal multicellular streptophytes are the Klebsormidiophyceae (Figure 1A). They  
153 are small, filamentous freshwater and terrestrial algae; only 41 different species of this class  
154 are currently registered in AlgaeBase (Guiry and Guiry 2015). Some members of the  
155 Klebsormidiophyceae can survive and grow in saline waters up to 60 g·L<sup>-1</sup> (Karsten and Rindi  
156 2010), and *Klebsormidium marinum* (Deason) P.C.Silva, K.M.Mattox & W.H.Blackwell has  
157 been found close to the sea (Deason 1969). However, we are unaware of any confirmed  
158 findings in marine environments. The Zygnematophyceae (= Conjugatophyceae) is a group of  
159 small unicellular or filamentous algae with over 3000 species (Guiry and Guiry 2015).  
160 Among them, the Zygnematales are generally considered a freshwater order (Lee 2008),  
161 although a few findings of *Spirogyra* spp. have also been recorded from saline lakes (Hammer  
162 1986) and estuaries (Attrill 1998). The second order within this class, the Desmidiales,  
163 comprises thousands of microscopic freshwater species (Brook 1981; Hall and Mccourt  
164 2015). The multicellular representatives (*Spondylosium*, *Desmidium*, *Onychonema*,  
165 *Hyalotheca*, *Cosmocladium*, *Groenbladia*, *Sphaerososma*) (McCourt et al. 2000), are almost  
166 exclusively freshwater organisms. To our knowledge, the only (possible) exception is an  
167 unconfirmed report of *Sphaerososma vertebratum* Brébisson ex Ralfs from Indian coastal  
168 waters (Thayalan et al. 2013). The Coleochaetophyceae, with ~35 species, are also  
169 predominantly found in fresh water (Cook and Graham 2016).



170 Higher morphological complexity, including tissue differentiation and more elaborate body  
171 plans, is found in the Charophyceae, comprising only a single order, the Charales (Lee 2008).  
172 Charales, also called stoneworts due to the fact that some species in the group can become  
173 heavily calcified, are closely related to terrestrial plants. Their thalli can be up to 1 m long,  
174 and they form underwater meadows in freshwater ponds and streams. Although the vast  
175 majority of the ~700 known species of Charales (Guiry and Guiry 2015) grow in fresh water  
176 (John and Rindi 2015), there are also examples from brackish water, notably in the upper  
177 Baltic Sea (Schubert and Blindow 2004) and salt-contaminated lakes in Australia (Burne et al.  
178 1980). *Lamprothamnium* spp. generally inhabit estuaries and coastal pools where the salinity  
179 can vary rapidly between fresh and full strength seawater. *Tolypella salina* R. Corillion is  
180 found in salt marshes along the French Atlantic coast (Lambert et al. 2013), while the  
181 majority of *Tolypella* species occur in fresh water. It may be assumed that within the Charales  
182 at least two independent transitions from freshwater to marine environments have taken place.  
183 The largest group of streptophytes are the Embryophyta (land plants). They separated from  
184 the charophytes and the Zygnematophyceae (Wodniok et al. 2011), and colonized a terrestrial  
185 habitat approximately 470 million years ago (Sanderson et al. 2004). Embryophytes are  
186 currently estimated to comprise 200,000 to 450,000 species (Scotland and Wortley 2003).  
187 About 4,500 of them live in aquatic<sup>1</sup> environments. However, Les et al. (1997) estimated that  
188 only 130 species of land plants have entirely returned to fully submerged aquatic  
189 environments. This is presumed to have happened during several independent transitions,  
190 which have occurred in the Alismatidae, the Ceratophyllaceae (Ceratophyllales), and  
191 Callitrichaceae (Asterids). While in the cases of Ceratophyllaceae and Callitrichaceae the  
192 entire families are restricted to fresh water (possibly due to their lack of roots and rhizomes)  
193 (Arber 1920), five families within the lower monocot order Alismatales made the transition to

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<sup>1</sup> Please note that our use of aquatic includes both fresh water and seawater

194 marine environments on at least two occasions (Figure 1B). As the sister-group of each of the  
195 marine groups of seagrasses is found in fresh water, and as freshwater forms are clearly more  
196 common than marine forms, a transition from fresh water to seawater is more parsimonious  
197 than a direct transition from terrestrial environments as noted by Les et al. (1997). In addition,  
198 there may have been a secondary re-colonization of freshwater environments by a marine  
199 ancestor within the Potamogetonaceae (suggested in Figure 1).

## 200 *Chlorophyta*

201 Approximately 80% of the >6,000 green algal species (Guiry and Guiry 2015) are freshwater  
202 and about 20% are marine or brackish species (John and Rindi 2015). Microscopic species are  
203 usually found in fresh water and macrophytic taxa in marine waters (John and Rindi 2015).

204 Multicellular chlorophytes have developed in four lineages, the Palmophyllales  
205 (Mamiellophyceae), the Trebouxiophyceae, the Chlorophyceae, and the Ulvophyceae  
206 (Leliaert et al. 2012). Here, we attempt to give an overview of marine-freshwater or  
207 freshwater-marine transitions in these classes, keeping in mind that for many of the species,  
208 molecular phylogenies are lacking and highly convergent morphology severely limits the  
209 utility of morphological classification.

210 The Palmophyllales constitute a small group of early-branching chlorophytes with only three  
211 genera (*Palmophyllum*, *Verdigellas*, and *Palmoclathrus*) restricted to deep-water or low light  
212 marine environments (Zechman et al. 2010; Leliaert et al. 2011).

213 The Trebouxiophyceae comprise ~700 species (Guiry and Guiry 2015), of which some  
214 members enter symbiotic relationships with fungi to form lichens, while others are common  
215 in freshwater plankton. Although this group consists mainly of unicellular or simple colony-  
216 forming representatives, the Microthamniales, Phyllosiphonales, and Prasiolales also form  
217 filaments and simple thalli. Freshwater forms comprise the majority of the Trebouxiophyceae,

218 and multicellular representatives include only freshwater (Microthamniales) and terrestrial  
219 forms (Phyllosiphonales). To our knowledge, there are no known multicellular marine forms  
220 within the Trebouxiophyceae (Leliaert et al. 2012). At least one clade of the Prasiolales,  
221 however, frequently occurs in the supralittoral of cold-temperate and polar oceans, and a few  
222 species can be cultivated in vitro in full-strength seawater medium (Rindi et al. 2007; Heesch  
223 et al. 2016).

224 The Chlorophyceae comprise ~3,500 species (Guiry and Guiry 2015), many of which are  
225 unicellular or colonial (notably the Chlamydomonadales). These groups are not included in  
226 this review. Among the filamentous forms, most occur primarily in fresh water (John and  
227 Rindi 2015), although several freshwater to marine transitions have occurred. One freshwater  
228 to seawater transition probably arose in the Chaetopeltidales, a freshwater/terrestrial order that  
229 also comprises marine species in the genus *Pseudulvella* (Sanchez-Puerta et al. 2006). While  
230 mostly from fresh water, the Chaetophorales (Caisová et al. 2011 and references therein),  
231 comprise eleven marine or partially marine genera (*Arthrochaete*, *Didymosporangium*,  
232 *Elaterodiscus*, *Endophyton*, *Entodictyon*, *Gongrosira*, *Kymatotrichon*, *Protoderma*,  
233 *Sporocladopsis*, *Uronema*, *Thamniochloris*; see e.g. Lee 1980; Nielsen 1988; Stuercke and  
234 McDermid 2004).

235 The Ulvophyceae are a very diverse group of multicellular green algae with ~1,700 species  
236 (Guiry and Guiry 2015), including unicellular, multicellular, siphonous/coenocytic (single cell  
237 with multiple nuclei), and siphonocladous (several cells each with multiple nuclei) forms.  
238 Different groups are thought to have independently evolved multicellularity (Cocquyt et al.  
239 2010a). The Ulvophyceae are mainly marine, but some species are also found in freshwater  
240 (Ichihara et al. 2009a) or terrestrial habitats. A few orders of Ulvophyceae such as the  
241 Scotinosphaerales and the Oltmannsiellopsidales are not included here as they comprise only  
242 simple or unicellular algae (e.g. Škaloud et al. 2013 and references therein).

243 Within the multicellular Ulvophyceae, only the Trentepohliaceae (~100 species; Guiry and  
244 Guiry 2015) are thought to contain exclusively terrestrial/freshwater species (Lopez-Bautista  
245 2006). Its sister clade, comprising the Cladophorales, Bryopsidales, and Dasycladales  
246 (Cocquyt et al. 2010b), together accounting for >1,000 species (Guiry and Guiry 2015), is  
247 predominantly marine. The Bryopsidales include siphonous and siphonocladous algae that  
248 can form dense seabed meadows. All known members are marine. This order also comprises  
249 the invasive species *Caulerpa taxifolia* (M.Vahl) C.Agardh. The Cladophorales are generally  
250 considered a marine order, but freshwater taxa are known from both of its principal clades  
251 (Hanyuda et al. 2002), thus corresponding to at least two independent adaptations. One clade  
252 comprises the genera *Aegagropila* (freshwater, including the so-called lake balls or Marimo  
253 balls), *Pithophora* (freshwater), *Armodiella* (freshwater), *Wittrockiella* (marine), and some  
254 *Cladophora* (marine and freshwater). Of the species examined by Hanyuda et al. (2002), the  
255 second clade comprises only two species (*Rhizoclonium hieroglyphicum* (C.Agardh) Kützing  
256 and *Cladophora glomerata* (L.) Kützing) that occur in seawater and fresh water, and the  
257 remaining 29 are marine. The Dasycladales are mainly unicellular or siphonous and are  
258 dominantly marine (Lee 2008), although e.g. *Batophora oerstedii* J.Agardh was also found in  
259 freshwater environments (Valet 1979).

260 The Ulotrichales are a heterogeneous group of primarily uniseriate, filamentous green algae  
261 comprising ~170 species (Guiry and Guiry 2015). They are found in marine, brackish,  
262 freshwater, and terrestrial environments (e.g. Friedl and O’Kelly 2002; Lee 2008 and  
263 references therein). As the morphology of these species is highly variable and few molecular  
264 phylogenies are available, the number of transitions between freshwater and marine  
265 environments cannot be estimated. In some cases species thought to belong to the same genus  
266 have different habitat preferences (e.g. *Codiolum kuckuckii* Skottsberg & Levring (freshwater;  
267 Silva and Chacana 2005) vs. *Codiolum brevipes* Foslie (marine; Lokhorst and Trask 1981);

268 *Ulothrix zonata* (F.Weber & Mohr) Kützing (freshwater; Graham et al. 2004) vs. *Ulothrix*  
269 *implexa* (Kützing) Kützing (marine; Bartsch and Kuhlenkamp 2000)).

270 The Ulvales, represented by >300 species (Guiry and Guiry 2015), are characterized by a  
271 cylindrical or sheet-like morphology (Lee 2008). The Bolbocoleaceae and the Phaeophilaceae  
272 are considered marine (e.g. Hauck 1876; Wynne 2011), while the Cloniophoraceae are found  
273 only in freshwater or brackish environments. Members of the genus *Dilabifilum* are found  
274 mainly in freshwater or terrestrial habitats (John and Rindi 2015) but also hypersaline  
275 environments (Vinogradova and Darienko 2008). The largest families within the Ulvales, *i.e.*  
276 the Kornmanniaceae, Ulvaceae, and Ulvellaceae, are represented by both freshwater and  
277 marine taxa, though they are mainly marine (Wehr and Sheath 2003). In each of these  
278 families, it is likely that multiple independent colonizations of fresh water have occurred. For  
279 example, most members of the genus *Blidingia* are marine, but some populations of the  
280 otherwise marine *Blidingia marginata* (J.Agardh) P.J.L.Dangeard ex Bliding have been  
281 encountered in fresh water (as *Blidingia minima* var. *ramifera* Bliding, nom. inval.) (Iima et  
282 al. 2004). Four species in the genus *Pseudendoclonium* (*P. akinetum* Tupa, *P. basiliense*  
283 Vischer, *P. laxum* D.M.John & L.R.Johnson, and *P. prostratum* Tupa) have been described  
284 from fresh water (Tupa 1974; Whitton and John 2014). This may well constitute another  
285 transition, although the monophyly of the genus remains questionable (Mullins 2007). Most  
286 species within the Ulvaceae are marine or brackish water species, but several independent  
287 transitions to fresh water have occurred in the genus *Ulva*. *U. flexuosa* Wulfen (comprising *U.*  
288 *intestinalis* L.; Mareš et al. 2011) and its sister species *U. meridionalis* R.Horimoto &  
289 S.Shimada (Horimoto et al. 2011) constitute the most common freshwater clade, frequently  
290 forming blooms in eutrophicated or salt-contaminated waters. A second clade consists of *U.*  
291 *limnetica* K. Ichihara & S. Shimada (Ichihara et al. 2009a). It is only known from freshwater  
292 bodies in Japan. Finally, *U. maeotica* (Proshkina-Lavrenko) P.Tsarenko and *U. simplex*

293 (K.L.Vinogradova) H.S.Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland  
294 have been described in marine and freshwater habitats in the Ukraine (Burova et al. 2011), but  
295 their phylogenetic relationship has not been investigated.

296 Within the Ulvellaceae, only two genera have freshwater representatives: *Entocladia* and  
297 *Ulvella*. *Entocladia gracilis* Hansgirg is the only freshwater species in the genus (John and  
298 Rindi 2015), whereas at least three species of *Ulvella* have colonized fresh water, *i.e.*, *U.*  
299 *bullata* (C.-C.Jao) H.Zhu & G.Liu, *U. tongshanensis* H.Zhu & G.Liu, and *U. prasina* (C.-  
300 C.Jao) H.Zhu & G.Liu (Zhu et al. 2015).

### 301 *Rhodophyta*

302 Red algae occur in both freshwater and marine environments (Hirano 1965; Vis and Sheath  
303 1996). Approximately 97% of the ~7,000 described species (Guiry and Guiry 2015) are  
304 marine, occurring in both intertidal and subtidal zones. Compared with green algae, there are  
305 only very few unicellular representatives.

306 Freshwater species exist in almost all red algal orders (Sheath and Vis 2015): only one higher  
307 taxon of red algae, the subclass Ahnfeltiophycidae with its 11 described species, lacks  
308 freshwater representatives (Figure 1A). Most freshwater red algae live in rivers and streams  
309 rather than in lakes (Wehr and Sheath 2003). They are usually filamentous (Figure 2) and  
310 smaller than marine species, with a narrower size range from 1-10 cm vs. <1-30 cm for  
311 marine species (Sheath and Hambrook 1990).

312 There are also red algal species that occur in both fresh water and seawater, e.g. *Bangia*  
313 *atropurpurea* (Mertens ex Roth) C.Agardh and *Bostrychia moritziana* (Sonder ex Kützing)  
314 J.Agardh (Youngs et al. 1998). The related species, *Bostrychia scorpioides* (Hudson)  
315 Montagne, is common in saltmarshes worldwide. Members of the genus *Hildenbrandia* (but  
316 different species) are likewise present in both environments. Based on a molecular phylogeny

317 of North American taxa there are several independent marine and freshwater clades  
318 (Sherwood and Sheath 1999). In European *Hildenbrandia* spp., the freshwater species form a  
319 monophyletic group, suggesting that only one transition to fresh water took place in Europe  
320 (Sherwood et al. 2002) and that the ancestral state was marine.

321 The Porphyridiophyceae, the Stylonematophyceae, the Compsopogonophyceae, and the  
322 Rhodellophyceae (together comprising ~130 species; Guiry and Guiry 2015) contain species  
323 whose unicellular or simple filamentous morphologies occur in both marine and freshwater  
324 environments; as do members of the subclass Nemaliophycidae (~ 900 species; Guiry and  
325 Guiry 2015). Of the eight orders three are exclusively freshwater, one is mixed and four are  
326 marine (Lam et al. 2016). Only very few microscopic species of red algae have been reported  
327 as terrestrial, notably *Porphyridium purpureum* (Bory) K.M.Drew & R.Ross (Hoffmann  
328 1989), *P. sordium* Geitler, and *Rufusia pilicola* Wujek & Timpano (Sheath and Vis 2015).

329 Exclusively marine groups have mainly been confined to the Corallinophycidae (>700  
330 species; Guiry and Guiry 2015) since members of this group have calcified cell walls (Lee  
331 2008) (but see Charales). It was only in 2013 that the first freshwater species of  
332 Corallinophycidae, *Pneophyllum cetinaensis* Kaleb, Zuljevic & Peña, was discovered in a  
333 Croatian river characterized by extremely high pH and concentrations of calcium carbonate  
334 (Žuljević et al. 2016). Among most freshwater habitats, there is a general over-representation  
335 of red algae in softer water, however, it has been suggested that recent invaders of fresh water  
336 are restricted to ion-rich environments (Sheath and Hambrook 1990).

### 337 **Stramenopila**

#### 338 *Phaeophyceae*

339 The largest class among multicellular stramenopiles is the Phaeophyceae, which comprises  
340 forms from tiny filaments to giant kelps. Brown algae are very rare in fresh water. Of ~2,000

341 currently accepted brown algal species (Guiry and Guiry 2015), only seven (0.35%) have  
342 been found in fresh water, and all are small, filamentous or crust-forming (Figure 2; see  
343 below). They have been classified within three families (although one genus, *Porterinema*, is  
344 currently incertae sedis): the Ectocarpaceae, the Lithodermataceae, and the Sphacelariaceae,  
345 the latter two families both members of the order Sphacelariales (Silberfeld et al. 2014).

346 The Ectocarpaceae are small and filamentous. Two species have independently colonized  
347 fresh water. One transition was made by *Pleurocladia lacustris* A. Braun, which has been  
348 found in both marine and in freshwater environments, but more frequently in freshwater  
349 (Wehr et al. 2013). Its sister species, *P. lucifuga* (Kuckuck) Wilce, is exclusively marine. The  
350 second transition may have occurred in *Ectocarpus*. A strain of *E. subulatus* Kützing (Peters  
351 et al. 2015) isolated from a freshwater environment in Australia (West and Kraft 1996) is still  
352 able to grow in full marine medium (Dittami et al. 2012). It is currently the only freshwater  
353 report of *Ectocarpus*, with the exception of one report from a highly salt-contaminated river in  
354 Germany (Geissler 1983). Given the phylogenetic position of the freshwater strains of  
355 Ectocarpaceae, the most parsimonious explanation for these results would be two independent  
356 colonizations of fresh water within the family.

357 Two additional and probably independent transitions to fresh water have occurred within the  
358 Sphacelariales. At least one occurred in the Lithodermataceae, which comprises four genera of  
359 small crust- or tuft- forming algae; two of which (*Lithoderma* and *Pseudolithoderma*) are  
360 exclusively marine, and two others (*Bodanella* and *Heribaudiella*), with one species each, are  
361 restricted to fresh water. *Heribaudiella fluviatilis* (Areschoug) Svedelius can, at times, be one  
362 of the dominant species of benthic algae in smaller rivers (Wehr and Sheath 2003). Within the  
363 Sphacelariaceae, *Sphacelaria* has two freshwater species: *Sphacelaria lacustris* Schloesser &  
364 Blum reported from Lake Michigan, USA (Schloesser and Blum 1980); and *S. fluviatilis* C.-  
365 C. Jao recorded from China and the United States (McCauley and Wehr 2007; Necchi 2016).



366 A fifth marine-freshwater transition in brown algae probably occurred within the species  
367 *Porterinema fluviatile* (H.C.Porter) Waern, an alga which branches very early in the brown  
368 algal tree (McCauley and Wehr 2007) and is currently not attributed to a specific order. This  
369 species has a global distribution with populations occurring both in freshwater and in marine  
370 environments in North America and in Europe. A possible sixth example is a strain of  
371 *Ectocarpus*-like brown algae tentatively named *Ectocarpoides piscinalis* nom. nud. isolated  
372 from a domestic freshwater aquarium in Northampton, UK. Preliminary molecular analyses  
373 indicate that this isolate constitutes a yet undescribed brown algal species, possibly in a new  
374 order (Belcher et al. 2009).

375 Among the larger brown algae, especially among species belonging to the Fucales, temporary  
376 tolerance for low salinity is commonly observed. For example, *Fucus ceranoides* var.  
377 *limnicola* S.M.Baker & M.H.Bohling is frequently found in upper estuaries with high  
378 freshwater influence (Khfaji and Norton 1979) and *F. radicans* L.Bergström & L.Kautsky is  
379 permanently submerged in waters of low salinity (3–5 g·L<sup>-1</sup>) in the northern Baltic  
380 (Bergstrom et al. 2005). Also, populations of *F. vesiculosus* L. have been observed at low  
381 salinities in the Baltic, but have lost sexual reproduction (Tatarenkov et al. 2005). Several  
382 ecophenes of *Fucus* are also known from salt marshes and have previously been referred to as  
383 *F. cottonii* M.J.Wynne & Magne (Neiva et al. 2012). There is no documented occurrence of  
384 wracks/rockweeds in freshwater or terrestrial habitats. Likewise, the largest brown algae,  
385 belonging to the Laminariales (or kelps), are exclusively marine.

386 In summary, we know of maximally six marine-freshwater transitions in brown algae. None  
387 have led to diversification or wide-spread dominance in freshwater systems. Given the  
388 overwhelming dominance of marine representatives it is likely that the common ancestor of  
389 brown algae was indeed marine; however, this does not extend to all stramenopiles.

390 *Schizocladiphyceae, Phaeothamniophyceae, and Tribophyceae*

391 The Schizocladiphyceae comprise only one member, *Schizocladia ischiensis* E.C.Henry,  
392 K.Okuda & H.Kawai, which is marine and was collected at the Island of Ischia, Gulf of  
393 Naples, in the Mediterranean (Kawai et al. 2003).

394 The Phaeothamniales have previously been considered part of the Chrysophyceae, but based  
395 on molecular phylogenetic data have been erected as a new class, the Phaeothamniophyceae  
396 (Bailey et al. 1998). The three principal genera are *Phaeoschizochlamys*, *Phaeothamnion*, and  
397 *Stichogloea*, all of which have been described from fresh water. Other genera tentatively  
398 associated with this class are not available in culture, and their placement in this group  
399 remains uncertain (Bailey 2010). The only known marine species of Phaeothamniales is  
400 *Chrysophaeum lewisii* W.R.Taylor, but its position within this order would need to be  
401 confirmed by molecular data.

402 Among the Tribophyceae, which comprise mostly freshwater algae, three orders are known to  
403 form filaments or to be siphonous, the Botrydiales (multinucleate cells), the Tribonematales,  
404 and the Vaucheriales. All three of these orders mainly occur in fresh water, and several have  
405 also colonized terrestrial habitats. Within the Vaucheriales ~20 species have been found in  
406 marine or brackish environments (South and Whittick 2009). Based on a phylogenetic tree of  
407 32 *Vaucheria* strains (21 species; Andersen and Bailey 2002), the marine representatives form  
408 two groups: one comprising only *Pseudodichotomosiphon* sp., a genus closely related to  
409 *Vaucheria* (exact phylogenetic position uncertain; Fukushi-Fujikura et al. 1991), and the other  
410 comprising all other marine species in this group. The most parsimonious explanation for this  
411 distribution would be two separate transitions from freshwater to marine habitats, one in  
412 *Pseudodichotomosiphon* and one in *Vaucheria*.

413

### 414 3. MARINE AND FRESHWATER ORIGINS OF PHOTOSYNTHETIC 415 EUKARYOTES

416 In order to understand the directionality of transitions between fresh water and seawater in  
417 photosynthetic eukaryotes, we have to understand the evolution of these organisms in their  
418 deep time, ancestral environments. It is generally assumed that photosynthesis was first  
419 acquired by a common eukaryotic ancestor of the red, green and glaucophyte lineages; and  
420 then subsequently transmitted to the haptophyte, cryptophyte, and stramenopile lineages via  
421 secondary or tertiary endosymbiosis events (Archibald 2009). The timing of the primary  
422 endosymbiosis event is still a matter of debate but estimated at between 2.1 Gya and 900 mya,  
423 depending on the methods used and the interpretation of fossils (Han and Runnegar 1992;  
424 Cavalier-Smith 2009; Parfrey et al. 2011; McFadden 2014). The physical environment of the  
425 primary endosymbiosis event remains uncertain, but some indications can be derived from the  
426 cyanobacterium that became the plastid and from the host.

427 On the plastid side, phylogenetic analyses based on 30 different cyanobacterial taxa have  
428 show that the cyanobacterial group closest to plastids contains both freshwater/terrestrial (e.g.  
429 *Synechocystis*) and marine genera (e.g. *Trichodesmium*) (Ochoa de Alda et al. 2014).  
430 However, a more recent and comprehensive study by Ponce-Toledo et al. (2017) has  
431 identified the freshwater cyanobacterium *Gloeomargarita lithophora* as the closest known  
432 relative of plastids. Moreover, based on ancestral state reconstructions of early plastids and  
433 cyanobacteria, a freshwater origin seems to be more consistent (Blank 2013b). A recent  
434 comparison of cyanobacterial genomes (Dagan et al. 2013) concluded that a freshwater origin  
435 was probably necessary for water-splitting photosynthesis. It has also been suggested that  
436 early cyanobacteria (prior to the primary endosymbiosis event) likely lacked important genes  
437 involved in the synthesis of the compatible solutes trehalose, glucosylglycerol,  
438 glucosylglycerate and glycine betaine (Blank 2013a), implying that they may not have been

439 able to colonize seawater at that time. This view is supported by estimates of the chemical  
440 composition of the early oceans at the time of primary endosymbiosis in which ancient  
441 seawater was probably two to three times more saline than today (Huston et al. 2010) and  
442 much richer in Ca<sup>2+</sup> and iodine (Pinti 2005). Moreover, in the period from 2500 - 580 mya,  
443 the oceans were likely anoxic or only moderately oxic (Anbar et al. 2002; Johnston et al.  
444 2009).

445 On the host side, the earliest branching within the Archaeplastida is still not resolved with  
446 certainty (Jackson and Reyes-Prieto 2014) but is thought to have been between the ancestor of  
447 the green and red lineages, and the glaucophytes (Rodríguez-Ezpeleta et al. 2005). The fact  
448 that extant members of the glaucophytes occur almost exclusively in fresh water (Kies and  
449 Kremer 1986) suggests that ancestral glaucophytes may also have been freshwater organisms.  
450 However, even if this is true, it is still unclear whether this also applies to the ancestor of all  
451 Archaeplastida. In the case of red algae, the earliest branching group of the red algae, the  
452 Cyanidales, occurs in hot springs with varying salinity and chemical composition (Reeb and  
453 Bhattacharya 2010). Therefore, and based on their reduced genomes (Collén et al. 2013; Qiu  
454 et al. 2015), it has been suggested that the ancestor of red algae may have also emerged in a  
455 hot spring, *i.e.* non-marine environment (Qiu et al. 2015). Though it will always remain  
456 speculative, modern marine representatives of ancient Archaeplastida may well be  
457 descendants of non-marine organisms.

458 A traditional argument against an early freshwater origin of Archaeplastida is based on the  
459 supposition that lakes and rivers were ephemeral along with the probable absence of  
460 biological vectors able to transport algae or spores between freshwater systems at the time.  
461 The oldest known lakes and rivers, such as Lake Baikal (Russia, 25 My) or the Finke River  
462 (Australia, 350 My) are still “young” (Haines et al. 2001; Colman et al. 2003). Using  
463 geochemical proxies Wellman and Strother (2015) suggest that primary production in

464 terrestrial settings was probably established between 3.0 and 2.7 Ga. These aeroterrestrial  
465 forms may have created a link between freshwater habitats. Thus, non-marine aquatic and  
466 aeroterrestrial forms may have played the pivotal role in primordial times, with only later a  
467 link to the marine environment. Such early aeroterrestrial organisms would have needed  
468 protection against UV radiation (Mulkidjanian et al. 2006), which today comes from the  
469 ozone layer, *i.e.*, the interaction of photosynthetic oxygen with water. Even though oxygen  
470 was not yet plentiful, protection against UV could have been augmented by other UV  
471 absorbing gases, such as methane (Hessen 2008). Accordingly, the aeroterrestrial 'flora'  
472 would have been able to take advantage of an increasingly protective habitat and greater  
473 access to newly forming freshwater environments.

474 The main challenge to resolving the original ancestral habitats is that the earliest divergences  
475 are not known and the deep evolutionary radiations were rapid. The best we can do with class  
476 and ordinal level phylogenies of the three lineages is to examine the transitions that have  
477 occurred in extant taxa (which we do) and estimate directionality based on available taxon  
478 sampling and sister-group observations. Though coarse, it provides some additional  
479 indications for the aeroterrestrial/freshwater - marine sequence.

480 But what about the stramenopiles? Here the timing of the secondary or tertiary endosymbiosis  
481 events (at the origin of the stramenopiles) is important. We speculate that, if this event (or  
482 events) occurred in seawater, the genes required for fresh water tolerance were not transferred  
483 to the nucleus of the host (or hosts) and therefore lost. If so, this would provide one possible  
484 explanation for why so few brown algae have colonized fresh water. On the other hand,  
485 unicellular stramenopiles such as diatoms are found in both environments with many  
486 transitions, possibly in both directions. Most xanthophytes also occur in fresh water, so that  
487 no clear conclusions about the ancestral state of stramenopiles can be drawn.

488 Across all lineages, morphological complexity is clearly a factor that impacts the ease of  
489 environmental transitions. Microscopic (unicellular) representatives of all photosynthetic  
490 lineages have undergone numerous recent back-and-forth transitions between seawater and  
491 freshwater environments. Green algae have many unicellular representatives, and most of  
492 their multicellular morphologies remain simple; they have also frequently transitioned to  
493 marine environments and back (see section 2). In contrast, the red algae have relatively few  
494 unicellular representatives, and the multicellular forms are morphologically complex and  
495 dominant in marine environments; there have been few transitions to fresh water. The brown  
496 algae comprise the most morphologically complex algae and are almost exclusively marine.  
497 Finally, the streptophytes comprise the morphologically most complex plant species  
498 dominating terrestrial and freshwater habitats. Within this group, only the seagrasses (~60  
499 species) have made a permanent transition to the marine environment (Les et al. 1997; Olsen  
500 et al. 2016).

501

#### 502 **4. PHYSIOLOGICAL AND GENOMIC ADAPTATIONS TO SEAWATER AND** 503 **FRESH WATER**

504 It is clear that marine-freshwater or freshwater-marine transitions have been common in  
505 photosynthetic eukaryotes (Figure 1). Still, we have only rudimentary understanding of the  
506 genomic changes underlying the physiological adaptations. Canonical understanding is  
507 usually derived from comparative acclimation experiments with euryhaline species that are  
508 capable of growing in both environments. For example, a recent study in the freshwater green  
509 alga *Ulva limnetica* used suppression subtractive hybridization in combination with  
510 quantitative real-time-PCR to compare gene expression profiles of freshwater vs. seawater-  
511 grown cultures of the same strain (Ichihara et al. 2011). An up-regulation was observed in  
512 enzymes involved in the degradation of sorbitol, probably as a means of reducing intracellular

513 osmotic pressure, as well as the activation of reactive oxygen species scavengers. Also, an  
514 earlier study of the same strain under the same conditions detected the accumulation of lectin-  
515 like proteins in fresh water-grown cultures (Ichihara et al. 2009b), but the molecular role of  
516 these proteins for fresh water tolerance remains unknown.

517 Salt tolerant *Chara longifolia* C.B. Robinson and *Lamprothamnium* sp. were shown to  
518 respond to salinity changes by increasing vacuolar concentrations of K<sup>+</sup>, Cl<sup>-</sup> and sometimes  
519 sucrose, but not Na<sup>+</sup> (Beilby 2015). In both taxa, membrane potential is maintained by an  
520 increased activity of proton pumps. In an older study, *Lamprothamnium papulosum*  
521 (K.Wallroth) J.Groves was also shown to modify levels of cell wall sulfated polysaccharides  
522 with varying salinity (Davis and Lipkin 1986; Shepherd and Beilby 1999).

523 In red algae, the discovery of the coralline freshwater red alga, *Pneophyllum cetinaensis*,  
524 constitutes an interesting case study of a recent (probably within the last 120,000 years) but  
525 irreversible transition to fresh water (Žuljević et al. 2016), but so far the necessary genomic  
526 and physiological adaptations have not been studied. Another example is *Bangia*  
527 *atropurpurea*, which is able to grow in fresh water (Reed 1980). In acclimation experiments  
528 to full marine salinity, this species was shown to rapidly synthesize large quantities of  
529 floridoside, thought to serve as an osmoprotectant for the cells (Reed 1985). *B. atropurpurea*  
530 from the Great Lakes (USA) has been shown to exhibit reduced growth in seawater at a  
531 salinity of 26 g·L<sup>-1</sup>, but this effect was reversed (*i.e.* reduced growth in fresh water compared  
532 to seawater) after three generations of acclimation to seawater (Sheath and Cole 1980).

533 By analogy to the Baltic *Fucus vesiculosus* described in section 2 (Tatarenkov et al. 2005), the  
534 *Bangia* populations also lack sexual reproduction in fresh water, despite the fact that they  
535 have maintained their presence in the Great Lakes since the 1960s and probably originated  
536 from fresh water-adapted European populations (Shea et al. 2014). Indeed, sexual  
537 reproduction may merit particular attention when studying the adaptation to fresh water, as

538 has previously been argued by Raven (1999). Specifically, the fusion of gametes is highly  
539 sensitive to the surrounding osmotic conditions, and mechanisms to block polyspermy in  
540 marine algae frequently rely on the influx of Na<sup>+</sup>, while freshwater algae generally rely on the  
541 efflux of Cl<sup>-</sup>.

542 Within the brown algae, the aforementioned freshwater strain of *Ectocarpus subulatus*  
543 provides a model for the transition between both environments. Here a combination of  
544 metabolite and transcriptome profiling revealed that over half of all examined genes and most  
545 metabolites were differentially expressed between the two conditions (Dittami et al. 2012). In  
546 this case, changes included the upregulation of genes involved in cell wall sulphation in  
547 seawater, which was confirmed using stained antibodies (Torode et al. 2015). This freshwater  
548 strain of *E. subulatus* has never been observed to reproduce sexually in the laboratory (A.  
549 Peters, personal communication as well as eight years of observation in our laboratory).

550 Differential gene expression analyses and physiological characterization of euryhaline  
551 organisms can shed light on the physiological acclimations needed to cope with  
552 osmoregulation and ion homeostasis in fresh water vs. seawater. However, they provide little  
553 information on the adaptations to either environment and their evolution after the initial  
554 transition. It is these adaptations that, once completed, may prevent a move back to the former  
555 environment. Comparisons of sister taxa that are strictly freshwater or strictly marine would  
556 provide valuable information to answer these questions, but are still rare.

557 An important example for such adaptations to life in marine vs. freshwater environments is  
558 cell wall sulphation. This process is present in all marine algae (red, green, and brown) and  
559 seagrasses (Kloareg and Quatrano 1988), but has not been found in freshwater plants or  
560 freshwater algae. Both aforementioned euryhaline red and brown algal species, *L. papulosum*  
561 and *E. subulatus*, have been shown to regulate cell wall sulphation depending on their  
562 environment (Shepherd and Beilby 1999; Torode et al. 2015). Interestingly, in a recent



563 genome analysis of the marine angiosperm, the seagrass *Zostera marina*, Olsen et al. (2016)  
564 showed that the enzymes responsible for cell wall sulphation in *Z. marina* re-evolved from  
565 carbohydrate sulfatases, which may be active on a wider range of substrates. Such  
566 comparisons illustrate the value of comparative genomics in deciphering the adaptations  
567 related to freshwater-marine habitat preferences and may shed light on the evolutionary  
568 events that have led to or prevented marine-freshwater transitions more recently.

569

## 570 **5. CONCLUSIONS**

571 Bidirectional marine-freshwater transitions have occurred in multicellular photosynthetic  
572 eukaryotes all across the eukaryotic tree, but branches that are specialized to one or the other  
573 habitat have clearly emerged. At one extreme, streptophytes dominate freshwater and  
574 terrestrial habitats, with only a few species (the seagrasses) having colonized the sea from  
575 freshwater ancestors. At the other extreme, brown algae, with the exception of a few  
576 morphologically simple species, are found only in seawater. Green and red algae hold an  
577 intermediate position and have successfully made the transition between seawater and fresh  
578 water many times. As a general trend, successful transitions appear to occur more frequently  
579 in morphologically simple organisms, as illustrated in the case of brown algae, but also to a  
580 certain extent in green algae and plants. Unicellular organisms have not been treated in this  
581 review but follow in parallel with this observation, frequently found in both types of habitats.  
582 It is commonly assumed that the colonization of fresh water was the intermediate step that  
583 allowed a gradual colonization of land by a marine, green algal lineage (Becker and Marin  
584 2009). While there is little doubt that plants have colonized terrestrial environments from  
585 fresh water, we cannot rule out the possibility of an original freshwater/terrestrial origin of  
586 photosynthetic eukaryotes followed by secondary colonization of marine habitats. To learn  
587 more about these early and later evolutionary transitions and the underlying mechanisms

588 responsible for habitat constraint, the availability of omics resources, in combination with  
589 comparative and experimental approaches, will allow us to unravel the determinants of these  
590 fundamentally different evolutionary trajectories.

591

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## 600 **REFERENCES**

- 601 Anbar, A. D., et al. 2002. Proterozoic ocean chemistry and evolution: a bioinorganic bridge?  
602 *Science* 297:1137–42.
- 603 Andersen, R. A. & Bailey J. C. 2002. Phylogenetic analysis of 32 strains of *Vaucheria*  
604 (Xanthophyceae) using the *rbcL* gene and its two flanking spacer regions. *J. Phycol.* 38:583–  
605 592.
- 606 APG: The Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny  
607 Group classification for the orders and families of flowering plants:APG III. *Bot. J. Linnean*  
608 *Soc.* 161:105–121.
- 609 Arber, A. 1920. *Water plants. a study of aquatic angiosperms.*
- 610 Archibald, J. M. 2009. The puzzle of plastid evolution. *Curr. Biol.* 19:R81-8.

611 Attrill, M. J. [Ed.] 1998. *A Rehabilitated Estuarine Ecosystem*. Springer, Boston, USA.

612 Baer, C. F., Miyamoto, M. M. & Denver, D. R. 2007. Mutation rate variation in multicellular  
613 eukaryotes: causes and consequences. *Nature Rev. Genet.* 8:619–631.

614 Bailey J. C. 2010. Phaeothamniophyceae. *The Tree of Life Web Project*. Available at: <http://>  
615 <http://tolweb.org/> (last accessed 10 May 2017).

616 Bailey C. J., Bidigare R. R., Christensen S. J. & Andersen R. A. 1998. Phaeothamniophyceae  
617 Classis Nova: A new Lineage of Chromophytes based upon photosynthetic pigments, rbcL  
618 sequence analysis and ultrastructure. *Protist* 149:245–63.

619 Bartsch, I. & Kuhlenkamp, R. 2000. The marine macroalgae of Helgoland (North Sea): an  
620 annotated list of records between 1845 and 1999. *Helgol. Mar. Res.* 54:160–189.

621 Becker, B. & Marin, B. 2009. Streptophyte algae and the origin of embryophytes. *Ann. Bot.*  
622 103:999–1004.

623 Beilby, M. J. 2015. Salt tolerance at single cell level in giant-celled Characeae. *Front. Plant*  
624 *Sci.* 6:226.

625 Belcher, J. H., Carter, C. F. & Peters, A.F. 2009. An unknown filamentous brown alga  
626 (Phaeophyceae) from a domestic freshwater aquarium in Northampton UK. *Phycologist*  
627 77:13.

628 Bergstrom, L., Tatarenkov, A., Johannesson, K., Jonsson, R. B. & Kautsky, L. 2005. Genetic  
629 and morphological identification of *Fucus radicans* sp. nov. (Fucales, Phaeophyceae) in the  
630 brackish Baltic Sea. *J. Phycol.* 41:1025–1038.

631 Blank, C. E. 2013a. Phylogenetic distribution of compatible solute synthesis genes support a  
632 freshwater origin for cyanobacteria. *J. Phycol.* 49:880–895.

633 Blank, C. E. 2013b. Origin and early evolution of photosynthetic eukaryotes in freshwater  
634 environments: reinterpreting proterozoic paleobiology and biogeochemical processes in light  
635 of trait evolution. *J. Phycol.* 49:1040–1055.

636 Brook, A. J. 1981. *The biology of desmids*. California University Press, Berkley.

637 Brown, J. W & Sorhannus U. 2010. A molecular genetic timescale for the diversification of  
638 autotrophic stramenopiles (Ochrophyta): substantive underestimation of putative fossil ages.  
639 *PloS one* 5:e12759.

640 Burne, R. V., Bauld, J. & De Deckker, P. 1980. Saline lake charophytes and their geological  
641 significance. *J. Sediment. Res.* 50:281–293.

642 Burova, O. V., Tsarenko, P. M., Kovalenko, O. V., Mikhailyuk, T. I., Petlovany, O. A.,  
643 Lilitka, G. G. & Bilous, O. P. 2011. Ulvophyceae. *Algae of Ukraine: diversity,*  
644 *nomenclature, taxonomy, ecology and geography. Volume 3: Chlorophyta.* Gantner Verlag  
645 K.-G., Ruggell, Liechtenstein, pp. 20–61.

646 Caisová, L., Marin, B., Sausen, N., Pröschold, T. & Melkonian, M. 2011. Polyphyly of  
647 Chaetophora and Stigeoclonium within the Chaetophorales (Chlorophyceae), revealed by  
648 sequence comparisons of nuclear-encoded SSU rRNA genes. *J. Phycol.* 47:164–177.

649 Carrete Vega, G. & Wiens, J. J. 2012. Why are there so few fish in the sea? *Proc. Biol. Sci /*  
650 *Royal Soc.* 279:2323–9.

651 Cavalier-Smith, T. 2009. Megaphylogeny, cell body plans, adaptive zones: causes and timing  
652 of eukaryote basal radiations. *J. Euk. Microbiol.* 56:26–33.

653 Cocquyt, E., Gile, G. H., Leliaert, F., Verbruggen, H., Keeling, P. J. & De Clerck, O. 2010b.  
654 Complex phylogenetic distribution of a non-canonical genetic code in green algae. *BMC Evol.*  
655 *Biol.* 10:327.

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

658 Collén, J. et al. 2013. Genome structure and metabolic features in the red seaweed *Chondrus*  
659 *crispus* shed light on evolution of the Archaeplastida. *Proc. Natl. Acad. Sci. U. S. A.*  
660 110:5247–52.

661 Colman, S. M., Karabanov, E. B. & Nelson, C. H. 2003. Quaternary sedimentation and  
662 subsidence history of Lake Baikal, Siberia, based on seismic stratigraphy and coring. *J.*  
663 *Sediment. Res.* 73:941–956.

664 Cook, M. E. & Graham, L. E. 2016. Chlorokybophyceae, Klebsormidiophyceae,  
665 Coleochaetophyceae. *Handbook of the Protists*. Springer International Publishing, Cham,  
666 Germany, pp. 1–20.

667 Dagan, T. et al. 2013. Genomes of Stigonematalean cyanobacteria (subsection V) and the  
668 evolution of oxygenic photosynthesis from prokaryotes to plastids. *Genome Biol. Evol.* 5:31–  
669 44.

670 Davis, J. S & Lipkin, Y. 1986. *Lamprothamnium* prosperity in permanently hypersaline water.  
671 *Swiss J. Hydrol.* 48:240–246.

672 Dawes, C. J. 1998. *Marine Botany*. John Wiley & Sons, Hoboken, NJ, USA.

673 Deason, T. R. 1969. Filamentous and colonial soil algae from Dauphin Island, Alabama.  
674 *Trans. Am. Microscopical Soc.* 88:240–246.

675 Dittami, S.M., Gravot, A., Goulitquer, S., Rousvoal, S., Peters, A. F., Bouchereau, A., Boyen,  
676 C. & Tonon, T. 2012. Towards deciphering dynamic changes and evolutionary mechanisms  
677 involved in the adaptation to low salinities in *Ectocarpus* (brown algae). *Plant J.* 71:366–377.

678 Feller, M.C. 2009. Human impacts on streams and rivers. In Likens G. E. [Ed.] *River*  
679 *Ecosystem Ecology: a global perspective*. Academic Press, London, UK, pp. 221–236.

680 Friedl, T. & O’Kelly, C. J. 2002. Phylogenetic relationships of green algae assigned to the  
681 genus *Planophila* (Chlorophyta): evidence from 18S rDNA sequence data and ultrastructure.  
682 *Eur. J. Phycol.* 37:373–384.

683 Fukushi-Fujikura, Y., Harada, N., Othuru, O. & Maeda, M. 1991. Cell wall polysaccharides  
684 of *Pseudodichotomosiphon constrictus* with special reference to its systematics. *Phytochem.*  
685 30:201–203.

686 Le Gall, L. & Saunders, G. W. 2007. A nuclear phylogeny of the Florideophyceae  
687 (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA:  
688 establishing the new red algal subclass Corallinophycidae. *Mol. Phylogent. Evol.* 43:1118–30.

689 Geissler, U. 1983. Die salzbelastete Flusstrecke der Werra - ein Binnenlandstandort für  
690 *Ectocarpus confervoides* (Roth) Kjellmann. *Nova Hedwigia* 37:193–217.

691 Gibbs, R. J. 1970. Mechanisms controlling world water chemistry. *Science* 170:1088–90.

692 Goldberg, E. 1965. Minor elements in sea water. In Riley P., Skirrow G. [Eds.] *Chemical*  
693 *Oceanography*. Academic Press, New York, pp. 163–196.

694 Graham, J. M., Kranzfelder, J. A. & Auer, M. T. 2004. Light and temperature as factors  
695 regulating seasonal growth and distribution of *Ulothrix zonata* (Ulvophyceae). *J. Phycol.*  
696 21:228–234.

697 Guiry, M. D. & Guiry, G. M. 2015. AlgaeBase. World-wide electronic publication. Available  
698 at: <http://www.algaebase.org/> (last accessed 10 May 2017).

699 Haines, P. W., Hand, M. & Sandiford, M. 2001. Palaeozoic synorogenic sedimentation in  
700 central and northern Australia: A review of distribution and timing with implications for the  
701 evolution of intracontinental orogens. *Aus. J. Earth Sci.* 48:911–928.

702 Hall, J. & Mccourt, R. M. 2015. Conjugating green algae including desmids. In Wehr, J,  
703 Kociolek, P. & Sheath, R. [Eds.] *Freshwater algae of North America: Ecology and*  
704 *Classification*. Academic Press, London, pp. 429–457.

705 Hammer, T. U. 1986. *Saline lake ecosystems of the world*. Dr. W. Junk Publishers, Dordrecht,  
706 Netherlands.

707 Han, T. & Runnegar, B. 1992. Megascopic eukaryotic algae from the 2.1-billion-year-old  
708 neogaunee iron-formation, Michigan. *Science* 257:232–235.

709 Hanyuda, T., Wakana, I., Arai, S., Miyaji, K., Watano, Y. & Ueda, K. 2002. Phylogenetic  
710 relationships within Cladophorales (Ulvophyceae, Chlorophyta) inferred from 18s rRNA gene  
711 sequences, with special reference to *Aegagropila linnaei*. *J. Phycol.* 38:564–571.

712 Hauck, F. 1876. Verzeichniss der im Golfe von Triest gesammelten Meeralgen. (Fortsetzung).  
713 *Oesterr. Bot. Z.* 26:24–26.

714 Hay, W. W., Migdisov, A., Balukhovsky, A. N., Wold, C. N., Flögel, S. & Söding, E. 2006.  
715 Evaporites and the salinity of the ocean during the Phanerozoic: Implications for climate,  
716 ocean circulation and life. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 240:3–46.

717 Hecky, R. E & Kilham, P. 1988. Nutrient limitation of phytoplankton in freshwater and  
718 marine environments: A review of recent evidence on the effects of enrichment. *Limnol.*  
719 *Oceanogr.* 33:796–822.

720 Heesch, S., Pažoutová, M., Moniz, M. B. J. & Rindi, F. 2016. Prasiolales (Trebouxiophyceae,  
721 Chlorophyta) of the Svalbard Archipelago: diversity, biogeography and description of the new  
722 genera *Prasionella* and *Prasionema*. *Eur. J. Phycol.* 51:171–187.

723 Hesse, D. O. 2008. Solar radiation and the evolution of life. In Bjertness E. [Ed.] *Solar*  
724 *Radiation and Human Health*. Norwegian Academy of Science and Letters, Oslo, Norway,  
725 pp. 123–136.

726 Hirano, M. 1965. Freshwater algae in the Antarctic regions. In Van Miegham J., Van Oye P. &  
727 Schell J. [Eds.] *Biogeography and Ecology in Antarctica*. Junk, The Hague, pp. 127–193.

728 Hoffmann, L. 1989. Algae of terrestrial habitats. *Bot. Rev.* 55:77–105.

729 Horimoto, R., Masakiyo, Y., Ichihara, K. & Shimada, S. 2011. *Enteromorpha*-like *Ulva*  
730 (Ulvophyceae, Chlorophyta) growing in the Todoroki River, Ishigaki Island, Japan, with  
731 species reference to *Ulva meridionalis* Horimoto et Shimada sp. nov. *Bull. Natl. Sci. Mus.*  
732 *Ser. B* 37:155–167.

733 Huston, D. L., Pehrsson, S., Eglinton, B. M. & Zaw, K. 2010. The geology and metallogeny  
734 of volcanic-hosted massive sulfide deposits: variations through geologic time and with  
735 tectonic setting. *Econ. Geol.* 105:571–591.

736 Ichihara, K., Arai, S. & Shimada, S. 2009b. cDNA cloning of a lectin-like gene preferentially  
737 expressed in freshwater from the macroalga *Ulva limnetica* (Ulvales, Chlorophyta). *Phycol.*  
738 *Res.* 57:104–110.

739 Ichihara, K., Arai, S., Uchimura, M., Fay, E. J., Ebata, H., Hiraoka, M. & Shimada, S. 2009a.  
740 New species of freshwater *Ulva*, *Ulva limnetica* (Ulvales, Ulvophyceae) from the Ryukyu  
741 Islands, Japan. *Phycol. Res.* 57:94–103.

742 Ichihara, K., Mineur, F. & Shimada, S. 2011. Isolation and temporal expression analysis of  
743 freshwater-induced genes in *Ulva limnetica* (Ulvales, Chlorophyta). *J. Phycol.* 47:584–590.

744 Iima, M., Hanyuda, T., Ueda, K., Yoshizaki, M. & Ebata H. 2004. Morphology, ontogeny and  
745 phylogeny of freshwater green alga, *Blidingia minima* var. *ramifera* (Ulvales, Ulvophyceae)  
746 from Nikko, Tochigi prefecture, Japan. *Jpn. J. Phycol.* 52:177–182.



747 International Symposium for the Classification of Brackish Waters. 1958. The Venice system  
748 for the classification of marine waters according to salinity. *Limnol. Oceanogr.* 3:346–347.

749 Jackson, C. J & Reyes-Prieto, A. 2014. The mitochondrial genomes of the glaucophytes  
750 *Gloeochaete wittrockiana* and *Cyanoptyche gloeocystis*: multilocus phylogenetics suggests a  
751 monophyletic archaeplastida. *Genome Biol. Evol.* 6:2774–85.

752 John, D. M & Rindi, F. 2015. Filamentous (nonconjugating) and plantlike green algae. In  
753 Wehr, J. D, Sheath, R. G & Kociolek, J. P. [Eds.] *Freshwater algae of North America:*  
754 *ecology and classification*. Academic Press, pp. 375.

755 Johnston, D. T., Wolfe-Simon, F., Pearson, A. & Knoll, A. H. 2009. Anoxygenic  
756 photosynthesis modulated Proterozoic oxygen and sustained Earth's middle age. *Proc. Natl.*  
757 *Acad. Sci. U. S. A.* 106:16925–16929.

758 Karsten, U. & Rindi, F. 2010. Ecophysiological performance of an urban strain of the  
759 aeroterrestrial green alga *Klebsormidium* sp. (Klebsormidiales, Klebsormidiophyceae). *Eur. J.*  
760 *Phycol.* 45:426–435.

761 Kawai, H., Hanyuda, T., Draisma, S. G. A. & Müller, D. G. 2007. Molecular phylogeny of  
762 *Discosporangium mesarthrocarpum* (Phaeophyceae) with a reinstatement of the order  
763 Discosporangiales. *J. Phycol.* 43:186–194.

764 Kawai, H., Maeba, S., Sasaki, H., Okuda, K. & Henry, E. C. 2003. *Schizocladia ischiensis*: a  
765 new filamentous marine chromophyte belonging to a new class, Schizocladiophyceae. *Protist*  
766 154:211–28.

767 Khfaji, A. K. & Norton, T.A. 1979. The effects of salinity on the distribution of *Fucus*  
768 *ceranoides*. *J. Estuar. Coast. Mar. Sci.* 8:433–439.

769 Kies, L. & Kremer, B.P. 1986. Typification of the Glaucocystophyta. *Taxon* 35:128.

770 Kloareg, B. & Quatrano, R. S. 1988. Structure of the cell-walls of marine-algae and  
771 ecophysiological functions of the matrix polysaccharides. *Oceanogr. Mar. Biol.*26:259–315.

772 Lam, D. W., Verbruggen, H., Saunders, G.W. & Vis, M. L. 2016. Multigene phylogeny of the  
773 red algal subclass Nemaliophycidae. *Mol. Phylogenet. Evol.* 94:730–736.

774 Lambert, E., Desmots, D., Le Bail, J., Mouronval, J-B. & Felzines, J.-C. 2013. *Tolypella*  
775 *salina* R. Cor. on the French Atlantic coast: biology and ecology. *Acta Bot. Gallica* 160:107–  
776 119.

777 Lee, R. K. S. 1980. A catalogue of the marine algae of the Canadian Arctic. *Natl. Mus. Can.*  
778 *Publ. Bot.* 9:1–81.

779 Lee, R. E. 2008. *Phycology*. Cambridge University Press, Cambridge, UK.

780 Leliaert, F., Smith, D. R., Moreau, H., Herron, M. D., Verbruggen, H., Delwiche, C. F. & De  
781 Clerck, O. 2012. Phylogeny and molecular evolution of the green algae. *Crit. Rev. Plant Sci.*  
782 31:1–46.

783 Leliaert, F., Verbruggen, H. & Zechman, F. W. 2011. Into the deep: new discoveries at the  
784 base of the green plant phylogeny. *BioEssays* 33:683–92.

785 Les, D. H., Cleland, M. A. & Waycott, M. 1997. Phylogenetic studies in Alismatidae, II:  
786 Evolution of marine angiosperms (seagrasses) and hydrophily. *Syst. Bot.* 22:443.

787 Logares, R., Bråte, J., Bertilsson, S., Clasen, J. L., Shalchian-Tabrizi, K. & Rengefors, K.  
788 2009. Infrequent marine-freshwater transitions in the microbial world. *Trends Microbiol.*  
789 17:414–22.

790 Lokhorst, G. M. & Trask, B. J. 1981. Taxonomic studies on *Urospora* (Acrosiphoniales,  
791 Chlorophyceae) in Western Europe. *Acta Bot. Neerlandica* 30:353–431.

792 Lopez-Bautista, J. M. 2006. Molecular systematics of the subaerial green algal order  
793 Trentepohliales: an assessment based on morphological and molecular data. *Int. J. Syst. Evol.*  
794 *Microbiol.* 56:1709–1715.

795 MacIntyre, F. 1970. Why the Sea is Salt. *Sci. Am.* 223:110.

796 Mareš, J., Leskinen, E., Sitkowska, M., Skácelová, O. & Blomster, J. 2011. True identity of  
797 the European freshwater *Ulva* (Chlorophyta, Ulvophyceae) revealed by a combined molecular  
798 and morphological approach. *J. Phycol.* 47:1177–1192.

799 Martin, W., Baross, J., Kelley, D. & Russell, M. J. 2008. Hydrothermal vents and the origin of  
800 life. *Nature Rev. Microbiol.* 6:805–14.

801 Martin, J. H., Fitzwater, S. E. & Gordon, R. M. 1990. Iron deficiency limits phytoplankton  
802 growth in Antarctic waters. *Glob. Biogeochem. Cycles* 4:5–12.

803 McCauley, L. A. R. & Wehr, J. D. 2007. Taxonomic reappraisal of the freshwater brown  
804 algae *Bodanella*, *Ectocarpus*, *Heribaudiella*, and *Pleurocladia* (Phaeophyceae) on the basis of  
805 rbcL sequences and morphological characters. *Phycologia* 46:429–439.

806 McCourt, R. M., Karol, K. G., Bell, J., Helm-Bychowski, K. M., Grajewska, A.,  
807 Wojciechowski, M. F. & Hoshaw, R. W. 2000. Phylogeny of the conjugating green algae  
808 (Zygnemophyceae) based on rbcL sequences. *J. Phycol.* 36:747–758.

809 McFadden, G. I. 2001. Primary and secondary endosymbiosis and the origin of plastids. *J.*  
810 *Phycol.* 37:951–959.

811 McFadden, G. I. 2014. Origin and evolution of plastids and photosynthesis in eukaryotes.  
812 *Cold Spring Harb. Perspect. Biol.* 6:a016105.

813 Mulkidjanian, A. Y., Koonin, E. V., Makarova, K. S., Mekhedov, S. L., Sorokin, A., Wolf, Y.  
814 I., Dufresne, A., Partensky, F., Burd, H., Kaznadzey, D., Haselkorn, R. & Galperin, M.Y.

815 2006. The cyanobacterial genome core and the origin of photosynthesis. *Proc. Natl. Acad. Sci.*  
816 *U. S. A.* 103:13126–31.

817 Mullins, R. F. 2007. A molecular phylogenetic assessment of *Pseudendoclonium*. Master  
818 thesis, University of Massachusetts, 47:1-40. Available at:  
819 <http://scholarworks.umass.edu/theses/47/> (last accessed 10 May 2017).

820 Necchi, J.R.O [Ed.] 2016. *River Algae*. Springer International Publishing, Cham, Germany.

821 Neiva, J., Hansen, G. I., Pearson, G. A., Van De Vliet, M. S., Maggs, C. A. & Serrão, E. A.  
822 2012. *Fucus cottonii* (Fucales, Phaeophyceae) is not a single genetic entity but a convergent  
823 salt-marsh morphotype with multiple independent origins. *Eur. J. Phycol.* 47:461–468.

824 Nielsen, R. 1988. Marine algae within calcareous shells from New Zealand. *New Zealand J.*  
825 *Bot.* 25:425–438.

826 Ochoa de Alda, J. A. G., Esteban, R., Diago, M. L. & Houmard, J. 2014. The plastid ancestor  
827 originated among one of the major cyanobacterial lineages. *Nature Commun.* 5:4937.

828 Olsen, J. L. et al. 2016. The genome of the seagrass *Zostera marina* reveals angiosperm  
829 adaptation to the sea. *Nature* 530:331–335.

830 Ott, J. 1988. *Meereskunde*. Ulmer, Stuttgart, Germany.

831 Parfrey, L. W., Lahr, D. J. G., Knoll, A. H. & Katz, L. A. 2011. Estimating the timing of early  
832 eukaryotic diversification with multigene molecular clocks. *Proc. Natl. Acad. Sci. U. S. A.*  
833 108:13624–9.

834 Peters, A. F., Couceiro, L., Tsiamis, K., Küpper, F. C. & Valero, M. 2015. Barcoding of  
835 cryptic stages of marine brown algae isolated from incubated substratum reveals high  
836 diversity in Acinetosporaceae (Ectocarpales, Phaeophyceae). *Cryptogamie Algologie* 36:3–29.

837 Pinti, D. L. 2005. The origin and evolution of the oceans. In Gargaud, M., Barbier, B., Martin,  
838 H. & Reisse, J. [Eds.] *Advances in Astrobiology and Biogeophysics. Lect. Astrobiol.*  
839 Springer, Berlin, Heidelberg, pp. 83–112.

840 Ponce-Toledo, R. I., Deschamps, P., López-García, P., Zivanovic, Y., Benzerara, K. &  
841 Moreira, D. 2017. An early-branching freshwater cyanobacterium at the origin of plastids.  
842 *Curr. Biol.* 27:386–391.

843 Qiu, H., Price, D. C., Yang, E. C., Yoon, H. S. & Bhattacharya, D. 2015. Evidence of ancient  
844 genome reduction in red algae (Rhodophyta). *J. Phycol.* 51:624–636.

845 Raven, J. A. 1999. Constraints on marine algal invasion of low- salinity environments: sex in  
846 the Baltic. *J. Phycol.* 35:210–214.

847 Reeb, V., Bhattacharya, D. 2010. Red Algae in the Genomic Age. In Seckbach, J. &  
848 Chapman, D. J. [Eds.] *Cellular Origin, Life in Extreme Habitats and Astrobiology. Cellular*  
849 *Origin, Life in Extreme Habitats and Astrobiology.* Springer, Dordrecht, Netherlands, pp.  
850 409–426.

851 Reed, R. H. 1980. On the conspecificity of marine and freshwater *Bangia* in Britain. *Brit.*  
852 *Phycol. J.* 15:411–416.

853 Reed, R. H. 1985. Osmoacclimation in *Bangia atropurpurea* (Rhodophyta, Bangiales): the  
854 osmotic role of floridoside. *Brit. Phycol. J.* 20:211–218.

855 Rindi, F., McIvor, L., Sherwood, A. R. , Friedl, T., Guiry, M. D. & Sheath, R. G. 2007.  
856 Molecular phylogeny of the green algal order Prasiolales (Trebouxiophyceae, Chlorophyta). *J.*  
857 *Phycol.* 43:811–822.

858 Rodríguez-Ezpeleta, N., Brinkmann, H., Burey, S. C., Roure, B., Burger, G., Löffelhardt, W.,  
859 Bohnert, H. J., Philippe, H. & Lang, B. F. 2005. Monophyly of primary photosynthetic  
860 eukaryotes: green plants, red algae, and glaucophytes. *Curr. Biol.* 15:1325–1330.

861 Salminen, R., et al. 2005. Geochemical Atlas of Europe, Part 1, Background Information,  
862 Methodology and Maps.

863 Sanchez-Puerta, M. V., Leonardi, P. I., O’Kelly, C. J. & Cáceres, E. J. 2006. *Pseudulvella*  
864 *americana* belongs to the order Chaetopeltidales (class Chlorophyceae), evidence from  
865 ultrastructure and SSU rDNA sequence data. *J. Phycol.* 42:943–950.

866 Sanderson, M. J., Thorne, J. L., Wikström, N. & Bremer, K. 2004. Molecular evidence on  
867 plant divergence times. *Am. J. Bot.* 91:1656–65.

868 Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. *Science* 195:260–2.

869 Schloesser, R. E & Blum, J. L. 1980. *Sphacelaria lacustris* sp. nov., a freshwater brown alga  
870 from Lake Michigan. *J. Phycol.* 16:201–207.

871 Schubert, H. & Blindow, I. 2004. *Charophytes of the Baltic Sea*. Oberreifenberg: Koeltz.

872 Scotland, R. W. & Wortley, A. H. 2003. How many species of seed plants are there? *Taxon*  
873 52:101.

874 Scott, J., Yang, E.-C., West, J. A., Yokoyama, A., Kim, H.-J., De Goer, S. L., O’Kelly, C. J.,  
875 Orlova, E., Kim, S.-Y., Park, J.-K. & Yoon, H.-S. 2011. On the genus *Rhodella*, the emended  
876 orders Dixoniellales and Rhodellales with a new order Glaucosphaerales (Rhodellophyceae,  
877 Rhodophyta). *Algae* 26:277–288.

878 Shea, T. B, Sheath, R. G, Chhun, A., Vis, M. L, Chiasson, W. B & Müller, K. M. 2014.  
879 Distribution, seasonality and putative origin of the non-native red alga *Bangia atropurpurea*  
880 (Bangiales, Rhodophyta) in the Laurentian Great Lakes. *J. Great Lakes Res.* 40:27–34.

881 Sheath, R. G & Cole, K. M. 1980. Distribution and salinity adaptations of *Bangia*  
882 *atropurpurea* (Rhodophyta), a putative migrant into the Laurentian Great Lakes. *J. Phycol.*  
883 16:412–420.

884 Sheath, R. G. & Hambrook, J. A. 1990. Freshwater ecology. In Cole, K. M. & Sheath, R. G.  
885 [Eds.] *Biology of the red algae*. Cambridge University Press, Cambridge, UK, pp. 423–453.

886 Sheath, R. G. & Vis, M. L. 2015. Red algae. In Wehr, J. D., Kociolek, P. R. & Sheath, R.G.  
887 [Eds.] *Freshwater algae of North America: ecology and classification.*, Academic press,  
888 London, pp. 237–264.

889 Shepherd, V. A. & Beilby, M. J. 1999. The effect of an extracellular mucilage on the response  
890 to osmotic shock in the charophyte alga *Lamprothamnium papulosum*. *J. Membr. Biol.*  
891 170:229–242.

892 Sherwood, A. R., Shea, T. B. & Sheath, R. G. 2002. European freshwater *Hildenbrandia*  
893 (*Hildenbrandiales*, *Rhodophyta*) has not been derived from multiple invasions from marine  
894 habitats. *Phycologia* 41:87–95.

895 Silberfeld, T., Leigh, J. W., Verbruggen, H., Cruaud, C., de Reviers, B. & Rousseau, F. 2010.  
896 A multi-locus time-calibrated phylogeny of the brown algae (*Heterokonta*, *Ochrophyta*,  
897 *Phaeophyceae*): Investigating the evolutionary nature of the “brown algal crown radiation”.  
898 *Mol. Phylogenet. Evol.* 56:659–674.

899 Silberfeld, T., Racault, M.-F. L. P., Fletcher, R. L., Couloux, A., Rousseau, F. & de Reviers,  
900 B. 2011. Systematics and evolutionary history of pyrenoid-bearing taxa in brown algae  
901 (*Phaeophyceae*). *Eur. J. Phycol.* 46:361–377.

902 Silberfeld, T., Rousseau, F. & de Reviers, B. 2014. An updated classification of brown algae  
903 (*Ochrophyta*, *Phaeophyceae*). *Cryptogamie Algologie* 35:117–156.

904 Silva, P. C. & Chacana, M. 2005. Marine algae from Islas San Félix y San Ambrosio (Chilean  
905 Oceanic Islands). *Cryptogamie Algologie* 26:103–118.

906 Škaloud, P., Kalina, T., Nemjová, K., De Clerck, O. & Leliaert, F. 2013. Morphology and  
907 phylogenetic position of the freshwater green microalgae *Chlorochytrium* (Chlorophyceae)  
908 and *Scotinosphaera* (Scotinosphaerales, ord. nov., Ulvophyceae). *J. Phycol.* 49:115–129.

909 South, G. R. & Whittick, A. 2009. *An Introduction to Phycology*. John Wiley & Sons,  
910 Hoboken, NJ, USA.

911 Stuercke, B., McDermid & K. J. 2004. Variation in algal turf species composition and  
912 abundance on two Hawaiian shallow subtidal reefs. *Cryptogamie Algologie* 25:353–365.

913 Tatarenkov, A., Bergström, L., Jönsson, R. B., Serrão, E. A., Kautsky, L. & Johannesson, K.  
914 2005. Intriguing asexual life in marginal populations of the brown seaweed *Fucus*  
915 *vesiculosus*. *Mol. Ecol.* 14:647–51.

916 Thayalan, M., Priya, A., Hameetha Banu, V. N., Bhagavathi Roopini, M. G. & Sesh Serebiah,  
917 J. 2013. Comparative spatial assessment of phytoplankton and productivity in coastal fresh  
918 water pond, estuary and neritic water of Palk Bay, South East India. *Int. J. Adv. Res. Biol. Sci.*  
919 2:301–314.

920 Torode, T. A., Marcus, S. E., Jam, M., Tonon, T., Blackburn, R. S., Hervé, C. & Knox, J. P.  
921 2015. Monoclonal antibodies directed to fucoidan preparations from brown algae. *PloS One*  
922 10:e0118366.

923 Tupa, D. D. 1974. An investigation of certain chaetophoralean algae. *Nova Hedwigia Beiheft*  
924 46:1–155.

925 Valet, G. 1979. Approche paléocéologique du monde des Dasycladales à partir de l'écologie  
926 des formes actuelles. *Bull. cent. rech. explor. prod. Elf-Aquitaine* 2/3:859–866.

927 de Vargas, C. et al. 2015. Eukaryotic plankton diversity in the sunlit ocean. *Science*  
928 348:1261605.



- 929 Vinogradova, O. & Darienko, T. 2008. Terrestrial algae of hypersaline environments of the  
930 Central Syvash islands (Kherson Region, Ukraine). *Biologia* 63.
- 931 Vis, M. L. & Sheath, R. G. 1996. Distribution and systematics of *Batrachospermum*  
932 (*Batrachospermales*, *Rhodophyta*) in North America. 9. Section *Batrachospermum*:  
933 description of five new species. *Phycologia* 35:124–134.
- 934 Wehr, J. D. & Sheath, R. G. 2003. Freshwater habitats of algae. In Wehr, J. D. & Sheath, R.  
935 G. [Eds.] *Freshwater algae of North America: ecology and classification*. Academic Press,  
936 London, pp. 11–57.
- 937 Wehr, J. D. & Sheath, R. G. 2003. *Freshwater algae of North America: ecology and*  
938 *classification*. Academic Press, London.
- 939 Wehr, J. D., Stancheva, R., Truhn, K. & Sheath, R. G. 2013. Discovery of the rare freshwater  
940 brown alga *Pleurocladia lacustris* (*Ectocarpales*, *Phaeophyceae*) in California Streams. *West*.  
941 *N. Am. Nat.* 73:148–157.
- 942 Wellman, C. H. & Strother, P. K. 2015. The terrestrial biota prior to the origin of land plants  
943 (*embryophytes*): a review of the evidence. *Palaeontology* 58:601–627.
- 944 West, J. & Kraft, G. 1996. *Ectocarpus siliculosus* (Dillwyn) Lyngb. from Hopkins River  
945 Falls, Victoria - the first record of a freshwater brown alga in Australia. *Muelleria* 9:29–33.
- 946 Wetzel, R. G. 2001. *Limnology*. Elsevier, San Diego.
- 947 Whitton, B. A. & John, D. M. 2014. Coded List of Freshwater Algae 2014. Centre for  
948 Ecology & Hydrology.
- 949 Wodniok, S., Brinkmann, H., Glockner, G., Heidel, A. J., Philippe, H., Melkonian, M. &  
950 Becker, B. 2011. Origin of land plants: Do conjugating green algae hold the key? *BMC Evol.*  
951 *Biol.* 11:104.

952 Wynne, M. J. 2011. A checklist of benthic marine algae of the tropical and subtropical  
953 western Atlantic: third revision. *Nova Hedwigia Beihefte* 140:1–144.

954 Youngs, H. L., Gretz, M. R., West, J. A. & Sommerfeld, M. R. 1998. The cell wall chemistry  
955 of *Bangia atropurpurea* (Bangiales, Rhodophyta) and *Bostrychia moritziana* (Ceramiales,  
956 Rhodophyta) from marine and freshwater environments. *Phycol. Res.* 46:63–73.

957 Zechman, F. W., Verbruggen, H., Leliaert, F., Ashworth, M., Buchheim, M. A., Fawley, M.  
958 W., Spalding, H., Poeschel, C. M., Buchheim, J. A., Verghese, B. & Hanisak, M. D. 2010. An  
959 unrecognized ancient lineage of green plants persists in deep marine waters. *J. Phycol.*  
960 46:1288–1295.

961 Zehr, J. P. & Ward, B. B. 2002. Nitrogen cycling in the ocean: new perspectives on processes  
962 and paradigms. *Appl. Environ. Microbiol.* 68:1015–1024.

963 Zhu, H., Leliaert, F., Zhao, Z.-J., Xia, S., Hu, Z.-Y. & Liu, G.-X. 2015. *Ulvella tongshanensis*  
964 (Ulvellaceae, Chlorophyta), a new freshwater species from China, and an emended  
965 morphological circumscription of the genus *Ulvella*. *Fottea* 15:95–104.

966 Žuljević, A., Kaleb, S., Peña, V., Despalatović, M., Cvitković, I., De Clerck, O., Le Gall, L.,  
967 Falace, A., Vita, F., Braga, J. C. & Antolić, B. 2016. First freshwater coralline alga and the  
968 role of local features in a major biome transition. *Sci. Rep.* 6:19642.

969

970 **TABLE LEGENDS**

971 **Table 1:** Overview of the typical composition of seawater and fresh water in selected streams.

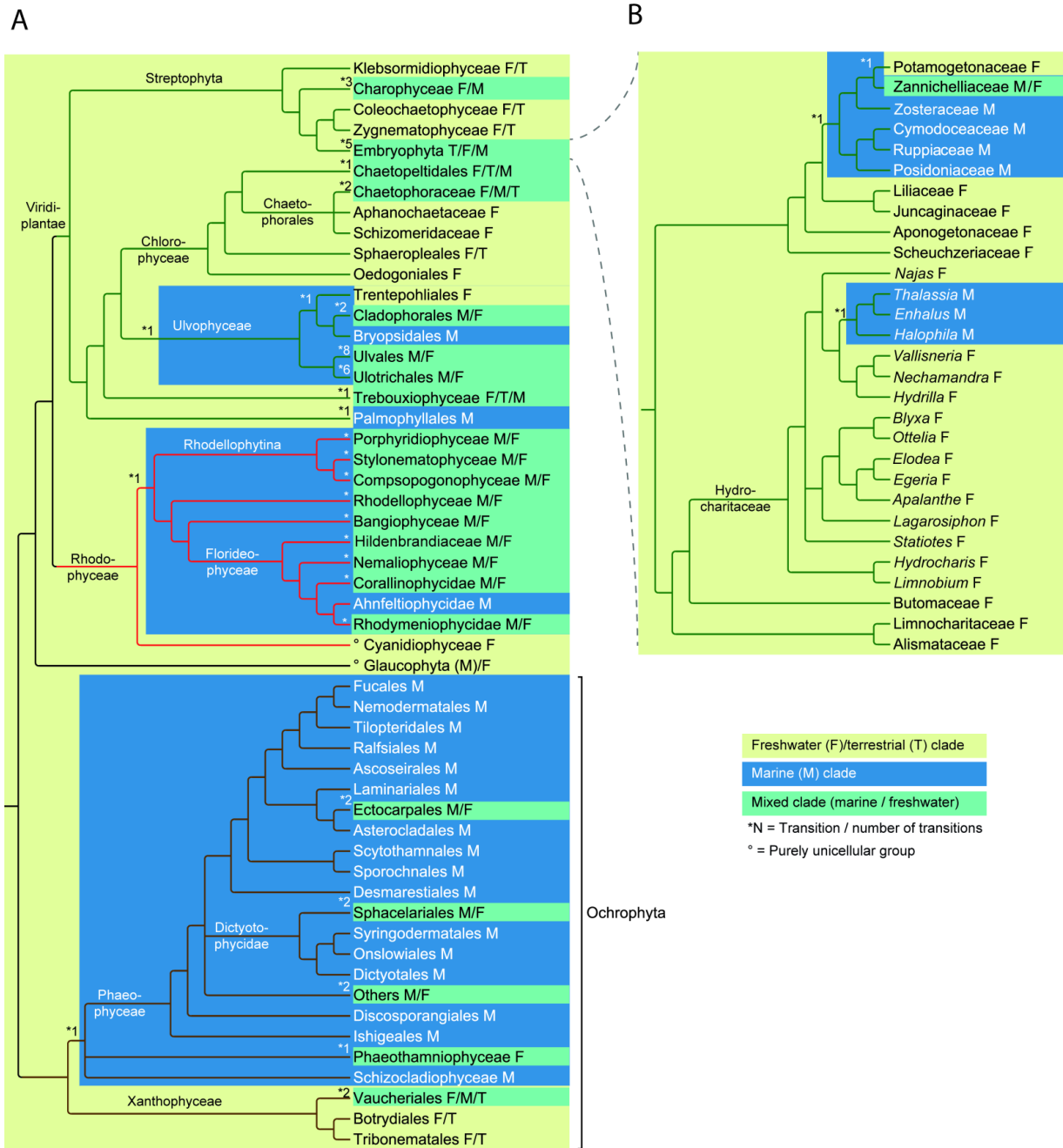
972 An asterisk (\*) indicates global averages of major ions in river waters according to Wetzel  
 973 (2001), followed by ranges found in the literature for different water bodies (Goldberg 1965;  
 974 Ott 1988; Wetzel 2001; Wehr and Sheath 2003; Salminen et al. 2005). The term brackish  
 975 water refers to a mixture of seawater and fresh water with salinities ranging from 0.5 to 30  
 976 g·L<sup>-1</sup>.

	Seawater	Fresh water
<b>Overall salinity</b>	<b>30-40 g·L<sup>-1</sup></b>	<b>&lt;0.5 g·L<sup>-1</sup></b>
Chloride (Cl <sup>-</sup> )	540 mM	0.22 mM* (< 8.6 mM)
Sodium (Na <sup>+</sup> )	500 mM	0.27 mM* (< 10 mM)
Sulfate (SO <sub>4</sub> <sup>2-</sup> )	28 mM	0.12 mM* (< 5 mM)
Magnesium (Mg <sup>2+</sup> )	54 mM	0.17 mM* (0.002-2 mM)
Calcium (Ca <sup>2+</sup> )	10 mM	0.37 mM* (0.005-10 mM)
Potassium (K <sup>+</sup> )	9.7 mM	0.06 mM* (< 1.3 mM)
Bicarbonate (HCO <sub>3</sub> <sup>-</sup> )	2.3 mM	< 16 mM
Bromide (Br <sup>-</sup> )	800 μM	< 12 μM
Boric acid (H <sub>3</sub> BO <sub>3</sub> )	400 μM	< 90 μM
Strontium (Sr <sup>2+</sup> )	90 μM	< 50 μM
Nitrate (NO <sub>3</sub> <sup>-</sup> )	0.01-50 μM	< 1 mM
Nitrite (NO <sub>2</sub> <sup>-</sup> )	0.01-5 μM	< 100 μM

Ammonium (NH <sub>4</sub> <sup>+</sup> )	0.1-5 μM	< 20μM
Phosphate(PO <sub>4</sub> <sup>3-</sup> )	2.5 μM	<10 μM
Silicate (SiO <sub>4</sub> <sup>4-</sup> )	0-180 μM	1-500 μM
Iodine (I)	0.5 μM	0.1-40 nM

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977

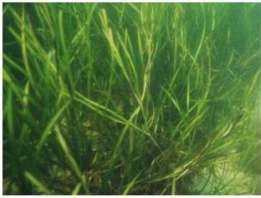




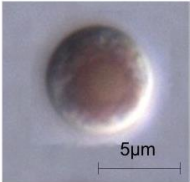

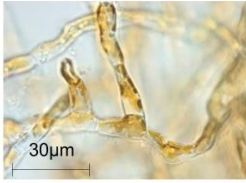


979

980 **Figure 1:** A) Backbone taxonomic relationships at the class and order levels according to  
 981 Kawai et al. (2007), Brown and Sorhannus (2010), Silberfeld et al. (2010, 2011) for  
 982 stramenopiles; Friedl and O’Kelly (2002), Cocquyt et al. (2010b), Leliaert et al. (2012) for the  
 983 green lineage; and Le Gall and Saunders (2007), Scott et al. (2011) for red algae. The tree  
 984 shows only the branching order; branch length has no significance and taxon sampling within

985 branches varies widely. Yellow background indicates a probable freshwater/terrestrial habitat,  
986 blue background a marine/brackish water habitat, and green the intermediate branches with  
987 both marine and freshwater representatives. Presumed marine-freshwater and freshwater-  
988 marine transitions are marked by \* and the minimum number of putative transitions is  
989 indicated where possible. ° denotes branches with no multicellular representatives; F,  
990 freshwater; M, marine; T, terrestrial. For example F/M means that both freshwater and marine  
991 representatives are found in a branch. B) Zoom on *Alismatales* after Les et al. (1997) with  
992 modifications according to (APG 2009). Note: There is no absolute proof for the ancestral  
993 habitats of the different lineages.

994

	Marine	Freshwater	Terrestrial
Green plants	 <p><i>Zostera marina</i><sup>1</sup></p>	 <p><i>Chara vulgaris</i><sup>*2</sup></p>	 <p><i>Pseudotsuga menziesii</i></p>
Rhodophytes	 <p><i>Furcellaria lumbricalis</i></p>	 <p><i>Batrachospermum atrum</i><sup>3</sup></p>	 <p><i>Porphyridium cruentum</i><sup>4</sup></p>
Phaeophytes	 <p><i>Sargassum muticum</i></p>	 <p><i>Pleurocladia lacustris</i><sup>5</sup></p>	none

995  
996 **Figure 2:** Examples of the morphological complexity reached by representatives of the green,  
997 red, and brown lineages in marine, freshwater, and terrestrial habitats, respectively. <sup>1</sup> Photo  
998 courtesy of Thorsten Reusch; <sup>2</sup> photo courtesy of Chris Carter; <sup>3</sup> photo courtesy of Marina  
999 Aboal Sanjurjo; <sup>4</sup> photo courtesy of the Roscoff Culture Collection (strain RCC 653); <sup>5</sup> strain  
1000 SAG 25.93