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► **To cite this version:**

Simon M Dittami, Svenja Heesch, Jeanine L. Olsen, Jonas Collén. Transitions between marine and freshwater environments provide new clues about the origins of multicellular plants and algae. *Journal of Phycology*, 2017, 10.1111/jpy.12547 . hal-01528781

HAL Id: hal-01528781

<https://hal.sorbonne-universite.fr/hal-01528781>

Submitted on 29 May 2017

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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 CaCO₃- calcium carbonate

17 Cl⁻ - 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 ≤ 0.5 g dissolved salts \cdot L⁻¹ (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⁻¹ 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⁻¹ with a current
59 mean of 35 g \cdot L⁻¹ (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 (Ca²⁺) readily precipitate as calcium carbonate
70 (CaCO₃), whereas Na⁺ remains in solution and is efficiently transported to the ocean.

71 Freshwater environments are therefore mainly Ca^{2+} -dominated whereas seawater is 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 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⁻¹ (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 Desmidiiales,
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 aquatic¹ 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

¹ 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⁻¹) 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^{2+} 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⁺, Cl⁻ and sometimes
519 sucrose, but not Na⁺ (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⁻¹, 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⁺, while freshwater algae generally rely on the
541 efflux of Cl⁻.

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

592 **ACKNOWLEDGEMENTS**

593 We thank the creators of AlgaeBase (<http://www.algaebase.org/>; Guiry and Guiry 2015) and
594 its funders for this valuable resource. We are also grateful to Dr. Akira Peters for bringing
595 important literature to our attention, Dr. Catherine Boyen and Dr. Thierry Tonon for critical
596 reading of the manuscript and helpful comments; Dr. Gabriel Markov and Dr. Catherine
597 Leblanc for helpful discussions; and the reviewers for the in-depth critique and suggestions.
598 This work benefited from the support of the French Government via the National Research
599 Agency investment expenditure program IDEALG (ANR-10-BTBR-04).

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969

970 **TABLE LEGENDS**

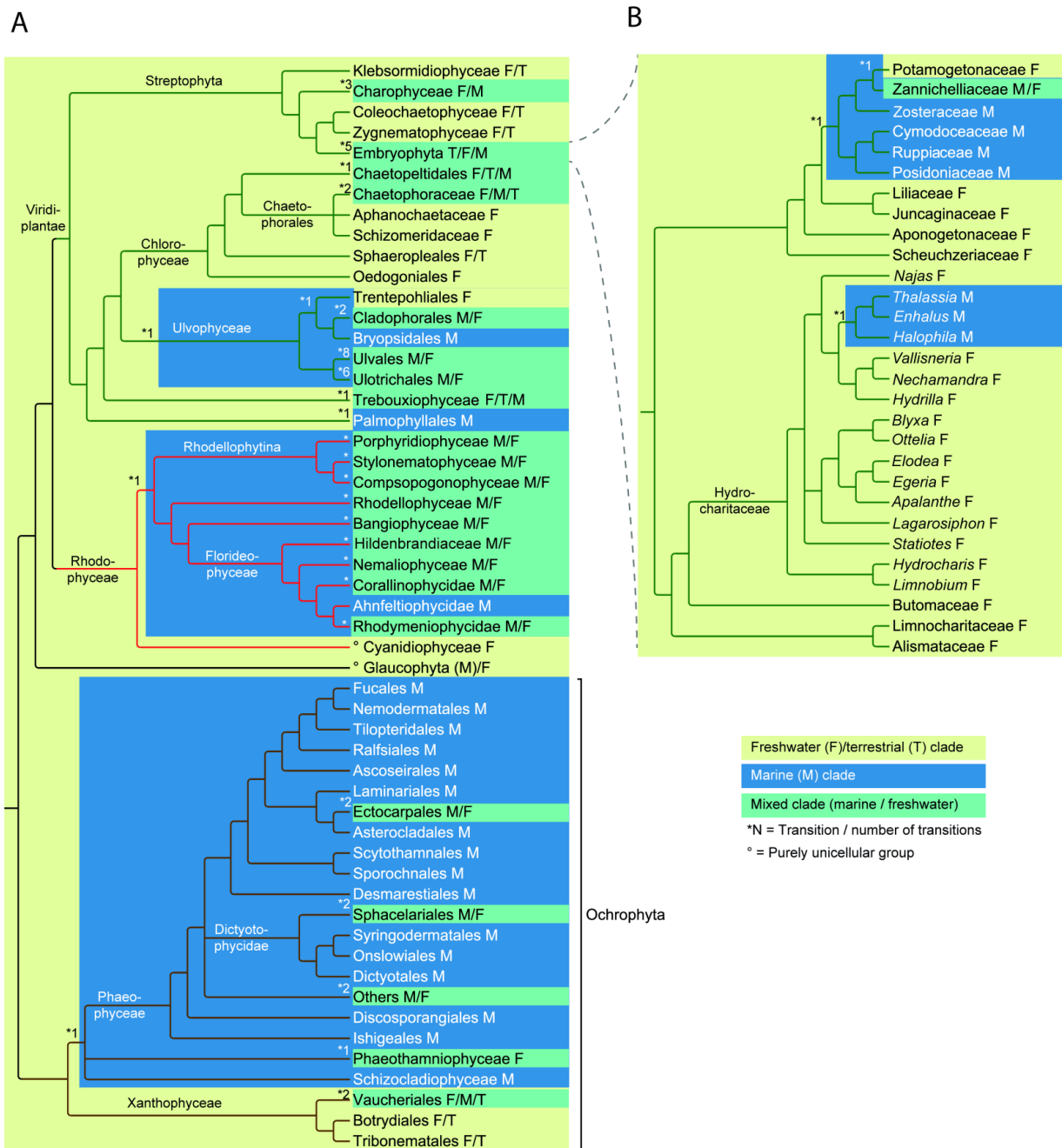
971 **Table 1:** Overview of the typical composition of seawater and fresh water in selected streams.

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⁻¹.

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

Ammonium (NH_4^+)	0.1-5 μM	< 20 μM
Phosphate(PO_4^{3-})	2.5 μM	<10 μM
Silicate (SiO_4^{4-})	0-180 μM	1-500 μM
Iodine (I^-)	0.5 μM	0.1-40 nM

977

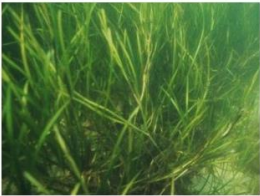




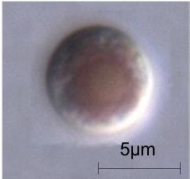

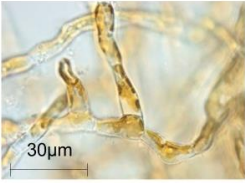


979

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>¹</p>	 <p><i>Chara vulgaris</i>^{*2}</p>	 <p><i>Pseudotsuga menziesii</i></p>
Rhodophytes	 <p><i>Furcellaria lumbricalis</i></p>	 <p><i>Batrachospermum atrum</i>³</p>	 <p><i>Porphyridium cruentum</i>⁴</p>
Phaeophytes	 <p><i>Sargassum muticum</i></p>	 <p><i>Pleurocladia lacustris</i>⁵</p>	<p>none</p>

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. ¹ Photo
998 courtesy of Thorsten Reusch; ² photo courtesy of Chris Carter; ³ photo courtesy of Marina
999 Aboal Sanjurjo; ⁴ photo courtesy of the Roscoff Culture Collection (strain RCC 653); ⁵ strain
1000 SAG 25.93