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    $CaCO_3$- 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

1. FRESHWATER AND MARINE HABITATS

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

Seawater is not just a concentrated version of fresh water, as a number of processes impact ion distribution in each environment. For one, salts (including nutrients) are washed into the ocean at different rates. Thus, the availability of macro- and micronutrients differs significantly between freshwater and marine environments. Primary production in the majority of freshwater ecosystems is most often limited by the availability of phosphorus (Schindler 1977), whereas marine environments are primarily limited by nitrogen (Hecky and Kilham 1988; Zehr and Ward 2002) or trace elements such as iron (Martin et al. 1990). Also, once eluted from the soil, calcium ions (Ca^{2+}) readily precipitate as calcium carbonate ($CaCO_3$), 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 $g \cdot L^{-1}$ (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 ≤ 0.5 g·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 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, Sphaerozosma*) (McCourt et al. 2000), are almost

166 exclusively freshwater organisms. To our knowledge, the only (possible) exception is an

167 unconfirmed report of *Sphaerozosma 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[1] 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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[1] Please note that our use of aquatic includes both fresh water and seawater

marine environments on at least two occasions (Figure 1B). As the sister-group of each of the

marine groups of seagrasses is found in fresh water, and as freshwater forms are clearly more

common than marine forms, a transition from fresh water to seawater is more parsimonious

than a direct transition from terrestrial environments as noted by Les et al. (1997). In addition,

there may have been a secondary re-colonization of freshwater environments by a marine

ancestor within the Potamogetonaceae (suggested in Figure 1).

Chlorophyta

Approximately 80% of the >6,000 green algal species (Guiry and Guiry 2015) are freshwater

and about 20% are marine or brackish species (John and Rindi 2015). Microscopic species are

usually found in fresh water and macrophytic taxa in marine waters (John and Rindi 2015).

Multicellular chlorophytes have developed in four lineages, the Palmophyllales

(Mamiellophyceae), the Trebouxiophyceae, the Chlorophyceae, and the Ulvophyceae

(Leliaert et al. 2012). Here, we attempt to give an overview of marine-freshwater or

freshwater-marine transitions in these classes, keeping in mind that for many of the species,

molecular phylogenies are lacking and highly convergent morphology severely limits the

utility of morphological classification.

The Palmophyllales constitute a small group of early-branching chlorophytes with only three

genera (*Palmophyllum*, *Verdigellas,* and *Palmoclathrus*) restricted to deep-water or low light

marine environments (Zechman et al. 2010; Leliaert et al. 2011).

The Trebouxiophyceae comprise ~700 species (Guiry and Guiry 2015), of which some

members enter symbiotic relationships with fungi to form lichens, while others are common

in freshwater plankton. Although this group consists mainly of unicellular or simple colony-

forming representatives, the Microthamniales, Phyllosiphonales, and Prasiolales also form

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), *Arnodiella* (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 Lithodermaceae, 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 $g \cdot L^{-1}$) 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 *Schizocladiophyceae, Phaeothamniophyceae, and Tribophyceae*

391 The Schizocladiophyceae 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

3. MARINE AND FRESHWATER ORIGINS OF PHOTOSYNTHETIC EUKARYOTES

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

On the plastid side, phylogenetic analyses based on 30 different cyanobacterial taxa have show that the cyanobacterial group closest to plastids contains both freshwater/terrestrial (e.g. *Synechocystis*) and marine genera (e.g. *Trichodesmium*) (Ochoa de Alda et al. 2014). However, a more recent and comprehensive study by Ponce-Toledo et al. (2017) has identified the freshwater cyanobacterium *Gloeomargarita lithophora* as the closest known relative of plastids. Moreover, based on ancestral state reconstructions of early plastids and cyanobacteria, a freshwater origin seems to be more consistent (Blank 2013b). A recent comparison of cyanobacterial genomes (Dagan et al. 2013) concluded that a freshwater origin was probably necessary for water-splitting photosynthesis. It has also been suggested that early cyanobacteria (prior to the primary endosymbiosis event) likely lacked important genes involved in the synthesis of the compatible solutes trehalose, glucosylglycerol, 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 aeroterresterial

465 forms may have created a link between freshwater habitats. Thus, non-marine aquatic and

466 aeroterresterial 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.

20

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

osmotic pressure, as well as the activation of reactive oxygen species scavengers. Also, an

earlier study of the same strain under the same conditions detected the accumulation of lectin-

like proteins in fresh water-grown cultures (Ichihara et al. 2009b), but the molecular role of

these proteins for fresh water tolerance remains unknown.

Salt tolerant *Chara longifolia* C.B. Robinson and *Lamprothamnium* sp. were shown to

respond to salinity changes by increasing vacuolar concentrations of K^+, Cl^- and sometimes

sucrose, but not Na^+ (Beilby 2015). In both taxa, membrane potential is maintained by an

increased activity of proton pumps. In an older study, *Lamprothamnium papulosum*

(K.Wallroth) J.Groves was also shown to modify levels of cell wall sulfated polysaccharides

with varying salinity (Davis and Lipkin 1986; Shepherd and Beilby 1999).

In red algae, the discovery of the coralline freshwater red alga, *Pneophyllum cetinaensis,*

constitutes an interesting case study of a recent (probably within the last 120,000 years) but

irreversible transition to fresh water (Žuljević et al. 2016), but so far the necessary genomic

and physiological adaptations have not been studied. Another example is *Bangia*

atropurpurea, which is able to grow in fresh water (Reed 1980). In acclimation experiments

to full marine salinity, this species was shown to rapidly synthesize large quantities of

floridoside, thought to serve as an osmoprotectant for the cells (Reed 1985). *B. atropurpurea*

from the Great Lakes (USA) has been shown to exhibit reduced growth in seawater at a

salinity of 26 g·L^{-1}, but this effect was reversed (*i.e.* reduced growth in fresh water compared

to seawater) after three generations of acclimation to seawater (Sheath and Cole 1980).

By analogy to the Baltic *Fucus vesiculosus* described in section 2 (Tatarenkov et al. 2005), the

Bangia populations also lack sexual reproduction in fresh water, despite the fact that they

have maintained their presence in the Great Lakes since the 1960s and probably originated

from fresh water-adapted European populations (Shea et al. 2014). Indeed, sexual

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

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TABLE LEGENDS

971 **Table 1:** Overview of the typical composition of seawater and fresh water in selected streams.
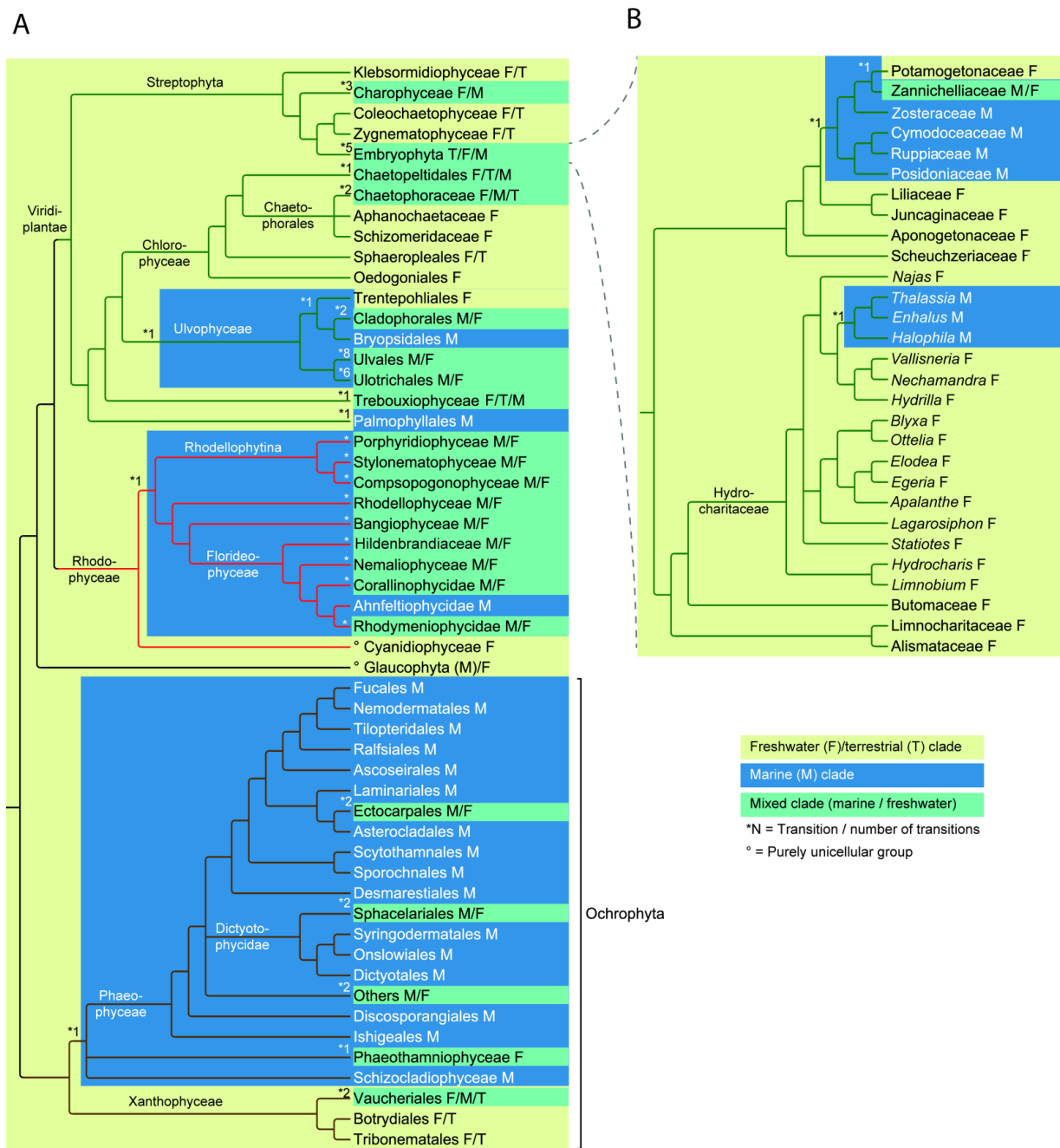
972 An asterisk (*) indicates global averages of major ions in river waters according to Wetzel

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 $g \cdot L^{-1}$.

	Seawater	Fresh water
Overall salinity	**30-40 $g \cdot L^{-1}$**	**<0.5 $g \cdot L^{-1}$**
Chloride (Cl^-)	540 nM	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_3BO_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	< 20µM
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

Figure 1

A

B

Streptophyta
Klebsormidiophyceae F/T
*3 Charophyceae F/M
Coleochaetophyceae F/T
Zygnematophyceae F/T
*5 Embryophyta T/F/M
*1 Chaetopeltidales F/T/M
*2 Chaetophoraceae F/M/T
Aphanochaetaceae F
Schizomeridaceae F
Sphaeropleales F/T
Oedogoniales F
Trentepohliales F
*2 Cladophorales M/F
Bryopsidales M
*8 Ulvales M/F
*6 Ulotrichales M/F
*1 Trebouxiophyceae F/T/M
*1 Palmophyllales M

Viridi-plantae
Chloro-phyceae
Chaeto-phorales
Ulvophyceae

Rhodellophytina
*1 Porphyridiophyceae M/F
* Stylonematophyceae M/F
* Compsopogonophyceae M/F
Rhodellophyceae M/F
* Bangiophyceae M/F
Hildenbrandiaceae M/F
* Nemaliophyceae M/F
* Corallinophycidae M/F
Ahnfeltiophycidae M
* Rhodymeniophycidae M/F

Rhodo-phyceae
Florideo-phyceae

° Cyanidiophyceae F
° Glaucophyta (M)/F

Fucales M
Nemodermatales M
Tilopteridales M
Ralfsiales M
Ascoseirales M
Laminariales M
*2 Ectocarpales M/F
Asterocladales M
Scytothamnales M
Sporochnales M
Desmarestiales M
*2 Sphacelariales M/F
Syringodermatales M
Onslowiales M
Dictyotales M
*2 Others M/F
Discosporangiales M
Ishigeales M
*1 Phaeothamniophyceae F
Schizocladiophyceae M
*2 Vaucheriales F/M/T
Botrydiales F/T
Tribonematales F/T

Phaeo-phyceae
Dictyoto-phycidae
Xanthophyceae
Ochrophyta

*1 Potamogetonaceae F
Zannichelliaceae M/F
*1 Zosteraceae M
Cymodoceaceae M
Ruppiaceae M
Posidoniaceae M
Liliaceae F
Juncaginaceae F
Aponogetonaceae F
Scheuchzeriaceae F
Najas F
Thalassia M
Enhalus M
Halophila M
Vallisneria F
Nechamandra F
Hydrilla F
Blyxa F
Ottelia F
Elodea F
Egeria F
Apalanthe F
Lagarosiphon F
Statiotes F
Hydrocharis F
Limnobium F
Butomaceae F
Limnocharitaceae F
Alismataceae F

Hydro-charitaceae

Freshwater (F)/terrestrial (T) clade
Marine (M) clade
Mixed clade (marine / freshwater)
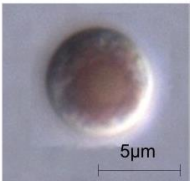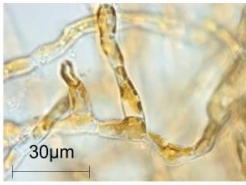*N = Transition / number of transitions
° = Purely unicellular group

Figure 1: A) Backbone taxonomic relationships at the class and order levels according to Kawai et al. (2007), Brown and Sorhannus (2010), Silberfeld et al. (2010, 2011) for stramenopiles; Friedl and O'Kelly (2002), Cocquyt et al. (2010b), Leliaert et al. (2012) for the green lineage; and Le Gall and Saunders (2007), Scott et al. (2011) for red algae. The tree 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	*Zostera marina[1]*	*Chara vulgaris*[2]	*Pseudotsuga menziesii*
Rhodophytes	*Furcellaria lumbricalis*	*Batrachospermum atrum[3]*	*Porphyridium cruentum[4]*
Phaeophytes	*Sargassum muticum*	*Pleurocladia lacustris[5]*	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. [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