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1 An alternative model for the earliest evolution of vascular plants

2

3 BORJA CASCALES-MINANA, PHILIPPE STEEMANS, THOMAS SERVAIS, KEVIN LEPOT

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5

6 Land plants comprise the bryophytes and the polysporangiophytes. All extant polysporangiophytes are
7 vascular plants (tracheophytes), but to date, some basalmost polysporangiophytes (also called
8 prottracheophytes) are considered non-vascular. Prottracheophytes include the Horneophytosida and
9 *Aglaophyton/Teruelia*. They are most generally considered phylogenetically intermediate between
10 bryophytes and vascular plants, and are therefore essential to elucidate the origins of current vascular
11 floras. Here, we propose an alternative evolutionary framework for the earliest tracheophytes. The
12 supporting evidence comes from the study of the Rhynie chert historical slides from the Natural History
13 Museum of Lille (France). From this, we emphasize that *Horneophyton* has a particular type of tracheid
14 characterized by narrow, irregular, annular and/or, possibly spiral wall thickenings of putative secondary
15 origin, and hence that it cannot be considered non-vascular anymore. Accordingly, our phylogenetic
16 analysis resolves *Horneophyton* and allies (i.e., Horneophytosida) within tracheophytes, but as sister
17 to eutracheophytes (i.e., extant vascular plants). Together, horneophytes and eutracheophytes form a
18 new clade called herein supereutracheophytes. The thin, irregular, annular to helical thickenings of
19 *Horneophyton* clearly point to a sequential acquisition of the characters of water-conducting cells.
20 Because of their simple conducting cells and morphology, the horneophytophytes may be seen as the
21 precursors of all extant vascular plant biodiversity.

22

23 Keywords: Rhynie chert, *Horneophyton*, Tracheophyte, Lower Devonian, Cladistics.

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55 Documenting the greening of the earliest land ecosystems requires a full understanding of the major
56 steps in plant evolution, most particularly the origin of vascular land plants. Land plants (embryophytes)
57 comprise the bryophytes (i.e., plant with parasitic unbranched sporophyte and a single sporangium) and
58 polysporangiophytes (i.e. plants with independent branched sporophyte and multiple sporangia)
59 [Kenrick & Crane 1997; Kenrick 2000). All living polysporangiophytes are vascular plants
60 (tracheophytes), but the basalmost representatives of the clade are considered non-vascular (Kenrick
61 2000) and structurally intermediate between bryophytes and vascular plants. Those non-vascular
62 polysporangiophytes, also called protracheophytes, include the Horneophytosida (*Caia*, *Horneophyton*
63 and *Tortilicaulis*) and *Aglaophyton/Teruelia* (Cascales-Miñana & Gerrienne 2017, and references
64 therein). Most of them are known in great detail because they come from the Early Devonian (mid-
65 Pragian-?earliest Emsian, Wellman 2006) Rhynie Lagerstätte, Aberdeenshire, in Scotland (see Kidston
66 & Lang 1917, 1920a, 1920b; Edwards *et al.* 2018), where the preservation of the fossils is exquisite.
67 The earliest preserved terrestrial ecosystem of Rhynie was indeed formed in a hot-spring environment
68 (Channing 2017; Edwards *et al.* 2018), which results in a unique and exceptional anatomical
69 preservation of the early land plants, among other organisms. Consequently, four Rhynie chert plants
70 (*Aglaophyton*, *Horneophyton*, *Rhynia* and *Asteroxylon*), showing different grades of complexity of
71 water-conducting cells, occupy key steps in current plant phylogeny (Kenrick 2000; Ligrone *et al.* 2012).
72 Those plants are essential to elucidate the origin of vascular plant biodiversity, especially *Horneophyton*
73 *lignieri* (Kidston & Lang) Barghoorn & Darrah, currently considered, together with *Caia* and
74 *Tortilicaulis*, the earliest lineage of polysporangiophytes.

75 *Horneophyton* is one of the historical plants firstly described by Kidson and Lang from the Rhynie
76 chert (Kidston & Lang 1920a; Barghoorn & Darrah 1938). Morphologically, *Horneophyton* is a small
77 leafless free-sporing plant characterized by bulbous rhizomes, erect dichotomous axes and terminal
78 sporangia with a central columella (Kidston & Lang 1920; Kenrick & Crane 1997). After Kidson and
79 Lang's original diagnosis, several researchers have focused attention on this plant, generating a wide
80 literature. For instance, there is well documented evidence of the anatomy of aerial axes and rhizomes
81 (Edwards 2004; Kerp 2017), of the structure of sporangia (Eggert 1974; El-Saadwy & Lacey 1979), as
82 well as of the *in situ* spores (Wellman *et al.* 2004; Wellman 2017). Even the gametophyte, *Langiophyton*

83 *mackiei*, is well known (Kerp 2017). However, the phylogenetic position of *Horneophyton* is
84 controversial. *Horneophyton* presents a mixture of characters typical of tracheophytes (e.g., branched
85 sporophyte, well-developed cuticles with stomata on axes; Kidston & Lang 1920a; Kerp 2017), but by
86 still retaining some bryophyte features (e.g., columellate sporangium; Kidston & Lang 1920a; Eggert
87 1974). Recent observations on the basal part of the upright axes further show the presence of water-
88 conducting cells with annular to slightly helical thickenings (Kerp 2017), which obviously calls into
89 question the non-vascular nature of *Horneophyton*. This paper aims to address this issue and its
90 evolutionary implications.

91

92 Material and methods

93 We studied for the first time the series of petrographic thin sections from the historical W. Hemingway
94 slides of the Natural History Museum of Lille, which belong to the same Rhynie chert collection as those
95 originally studied by Kidston & Lang (1917, 1920a, 1920b). Concretely, we studied 30 glass slides (8x
96 *Aglaophyton*, 8x *Rhynia*, 9x *Horneophyton*, and 5x *Asteroxylon*; see Table 1) showing typical
97 exquisitely well preserved reproductive and vegetative structures of the Rhynie flora. Photographs were
98 taken using a Nikon Df camera coupled to a Zeiss Stemi 2000-C stereomicroscope and with a Zeiss
99 Axioskop microscope coupled to a Zeiss AxioCam camera.

100 The phylogenetic position of *Horneophyton* was assessed via PAUP* 4.0 (Phylogenetic Analysis
101 Using Parsimony, and other Methods, <http://paup.phylosolutions.com/>). We codified a character matrix
102 (Data S1) including 21 core taxa of earliest land plants (Kenrick & Crane 1997; Kenrick 2000). Data
103 matrix was prepared on 32 morphological and anatomical characters (Table S1). List of characters (and
104 character states) was adapted from Kenrick & Crane (1997) and Cascales-Miñana & Gerrienne (2017),
105 with some modifications (Table S1). Bryophytes were transferred to outgroup, and treated as
106 monophyletic according to Puttick *et al.*'s (2018) recent maximum-likelihood and Bayesian estimations.
107 See Text S1 for implementation. The topology of resulted consensus tree (Consistency index = 0.875;
108 Homoplasy index = 0.125; Retention index = 0.9213, Rescaled consistency index = 0.8062; Text S2)
109 was plotted against stratigraphy using *strap* (Stratigraphic Tree Analysis for Palaeontology; Bell &

110 Lloyd 2104). The calibration of polysporangiophyte splits is based on to the temporal distribution of the
111 considered fossil plants (Text S3). *Strap* analysis was performed according to Bell and Lloyd's (2014)
112 tutorial.

113

114 Results and discussion

115 The observed diversity in the water-conducting cells of the four Rhynie chert plant studied here is shown
116 in Fig. 1. In *Aglaophyton* (Fig. 1A,E,I,M,N), the water-conducting cells are characterized by the
117 presence of chains and clusters of interconnected bubble-like structures in their lumen (Edwards 1986;
118 Edwards 2004). Whether those bubbles are of primary or secondary origin is unknown, but previous
119 studies have shown that they were most probably unlignified (Boyce *et al.* 2003). The conducting cells
120 of *Rhynia* (Fig. 1B,F,J,O,P) are of the S-type as defined by Kenrick & Crane (1991). S-type conducting
121 cells are elongate cells with large annular to helical thickenings including a spongy interior covered
122 towards the lumen by a thin decay-resistant microporate layer (Kenrick & Crane 1991, 1997), considered
123 unlignified by Boyce *et al.* (2003). The conducting cells of *Horneophyton* (Fig. 1C,G,K,Q,R; Fig. 2)
124 include thin irregular, annular to helical thickenings. Those thickenings are of unknown origin and
125 nature, but their dark colour is suggestive of the presence of a decay-resistant component that may have
126 locally allowed the preservation of abundant organic matter. The tracheids of *Asteroxylon* (Fig.
127 1D,H,L,S,T) are of the G-type as defined by Kenrick & Crane (1991). They show annular to helical
128 thickenings with pores distributed between the thickenings. Boyce *et al.* (2003) have shown that
129 *Asteroxylon* tracheids are most probably heavily lignified.

130 Two important observations are derived from the water-conducting cells diversity illustrated in Fig.
131 1. Firstly, the *Aglaophyton* and *Rhynia* conducting strands (Fig. 1A,E,B,F) are more closely similar to
132 each other than to those of *Horneophyton* and *Asteroxylon*, which show thicker cell walls in cross
133 sections (Fig. 1C,D,G,H; Fig. 2). Secondly, the complexity of the conducting cells obviously increases
134 from *Aglaophyton*, where there is an apparent lack of tracheid thickenings (Fig. 1I,M,N), to *Asteroxylon*,
135 which shows well-defined annular secondary thickenings (Fig. 1L,S,T). Most importantly, we note that
136 *Horneophyton* presents a genuine type of conducting cells characterized by irregular, annular and/or,

137 possibly spiral wall thickenings (Fig. 1K,R,S) of putative secondary origin. Accordingly, our
138 phylogenetic analysis resolves *Horneophyton* and allies within tracheophytes, but interestingly, not as
139 the basalmost lineage but as a sister clade to eutracheophytes (Fig. 3). The two plant groups constitute
140 a new clade that we call herein supereutracheophytes. This clade is characterized by narrow, irregular,
141 annular and/or, possibly spiral wall thickenings of putative secondary origin, while eutracheophytes
142 present a clear thick, lignified wall layer. Fig. 3 further shows that *Horneophyton* and allies are more
143 derived than the paratracheophytes (=Rhyniaceae), while *Aglaophyton* is resolved as the earliest
144 diverging polysporangiophyte, and the single lineage between the bryophytes and the vascular plants.

145 In 1920, Kidston & Lang (1920a) referred to *Horneophyton* (formerly *Hornea*) as "another vascular
146 plant from the Rhynie peat-bed" and documented cells with distinct narrow thickenings forming
147 irregularly connected rings or a spiral (Kidston & Lang 1920a, figs. 52-53). Indeed, the *Hornea* original
148 diagnosis describes the vascular strand as follows: "stele of stem with a zone of phloem surrounding the
149 xylem composed of small central and wider peripheral tracheids" (Kidston & Lang 1920a). The same
150 description appears in Barghoorn & Darrah's (1938) *Horneophyton* diagnosis. More recently, Edwards
151 (2004) pointed out that the *Horneophyton* terete strands "show unequivocal narrow interconnected
152 annular or spiral thickenings, with the smaller elements situated centrally", and further added "the
153 tracheids were surrounded by elongate, thin-walled cells interpreted as phloem". Likewise, Kerp (2017)
154 has recently illustrated the presence of "water-conducting cells with annular to slightly helical wall
155 thickenings in the basal part of the upright axis" for *Horneophyton*. Our observations also show annular
156 and/or spiral wall thickenings in *Honeophyton* conducting cells (Fig. 1Q,R). This is definitely consistent
157 with the original diagnosis (Kidston & Lang 1920a; Barghoorn & Darrah 1938), as well as with the
158 subsequent descriptions and the current interpretations (Edwards 2004; Kerp 2017), which provides
159 strong support for *Horneophyton* being a vascular plant.

160 Fundamental characters supporting a close relationship between *Horneophyton* and eutracheophytes
161 are (i) the decay resistance of the conducting cell wall and (ii) thickenings of cell wall (characters 16
162 and 17 from Table S1, respectively). Decay resistance is a character linked to the presence of lignin in
163 the secondary wall of the conducting cell, which accounts for an increased thickness of the wall (Kenrick
164 & Edwards 1988). Unthickened water-conducting cells are typical of mosses (Roberts *et al.* 2012). They

165 can be also observed in *Aglaophyton* (Fig. 1E,I). The wall of the conducting cells of the
166 paratracheophytes presents a thin decay-resistant layer facing the lumen (Kenrick & Crane 1991;
167 Kenrick *et al.* 1991a, 1991b), but it is unclear whether the decay-resistant layer is of the secondary nature
168 and whether it includes lignin. Recent evidence however suggests that the development of the wall
169 thickenings in *Rhynia* occurs shortly after elongation and hence that it is most probably of secondary
170 origin (Kearney *et al.* 2016). In another Rhynie chert plant, *Nothia aphylla*, for which decay resistance
171 is accepted (Kenrick & Crane 1997), water-conducting cells have no thickenings (Edwards 2003).
172 However, the walls of the conducting cells of *Nothia* appear remarkably thicker than those of
173 *Aglaophyton* and *Rhynia* (Kenrick & Crane 1997). The walls of *Horneophyton* water-conducting cells
174 (Fig. 1G) are also thicker than those of *Rhynia* (compare our Fig. 1F with Fig. 1G), and much thicker
175 than those of *Aglaophyton* (compare Fig. 1e with Fig. 1G). The thickness of the walls of *Horneophyton*
176 conducting cells is indeed much closer to that of the conducting cells of *Asteroxylon* (Fig. 1G,H). So, as
177 for *Asteroxylon*, *Horneophyton* decay resistance was scored as extensive in our analysis, while that of
178 *Rhynia* is scored as limited (character 16, Table S1).

179 Early polysporangiophytes mainly present water-conducting cells with annular or helical thickenings
180 (Kenrick & Crane 1991). These tracheid-like cells or tracheids are well known in paratracheophytes, as
181 well as in zosterophylls or basal lycopods such as *Asteroxylon* or *Baragwanathia* (Kenrick & Crane
182 1991; Kenrick *et al.* 1991a, 1991b). The presence of this type of thickenings in *Horneophyton* has been
183 described in several studies (Kidston & Lang 1920a; Barghoorn & Darrah 1938; Edwards 2004; Kerp
184 2017) and is again illustrated here (Fig. 1G,K). The thickenings are however irregular and less well-
185 developed than in *Asteroxylon* (Fig. 1S,T), or even in *Rhynia*, where we see a banded appearance (Fig.
186 1O,P). Nevertheless, because annular or helical thickenings, even if irregular, are obvious in
187 *Horneophyton*, the character “tracheid thickenings” was scored as present in our analysis (character 17,
188 Table S1).

189 The results of our cladistic analysis contrast with the currently accepted fossil-based plant phylogeny
190 (Fig. 4A) that presents two intermediate lineages between the bryophytes and tracheophytes. These
191 lineages correspond to the Rhynie chert plants *Horneophyton* and *Aglaophyton*. This conception mainly
192 derives from the presence of a columella in *Horneophyton* sporangium and of apparently uniform cell

193 walls in *Aglaophyton* conducting cells. According to this model, tracheophytes include two sister clades,
194 represented here by the Rhynie chert plants *Rhynia* (paratracheophytes, Fig. 3) and *Asteroxylon*
195 (eutracheophytes, Fig. 3), the latter being sister to euphyllophytes, i.e., the plants with “true leaves” or
196 megaphylls, a lineage that includes almost all living vascular plants. Our results however suggest an
197 alternative, and more parsimonious model for interpreting the basal relationships in land plant evolution
198 (Fig. 4B), with *Horneophyton* (and allies) being sister to eutracheophytes instead of being the earliest,
199 non-vascular polysporangiophyte.

200 The acquisition of more and more efficient water-conducting cells was crucial for plant evolution
201 and decisive during their terrestrialisation process. The presence of vascular tissues is therefore a key
202 synapomorphy for understanding polysporangiophyte evolution. Tracheids are defined in terms of form,
203 function and biochemical composition (Niklas & Smocovitis 1983), the latter character being the most
204 difficult to assess in fossils. All the features identifying tracheid (secondary wall deposition, presence
205 of lignin, pitting of wall) were presumably acquired sequentially; this is exemplified by the four plants
206 illustrated here that document the initial steps of the process. In fossil plants, the presence of lignified
207 conducting cells can only be assumed from the presence of a decay-resistant layer. This layer is
208 evaluated from comparative morphology with extant plants. There are no direct unequivocal measures
209 showing the presence of lignin neither for any Early Devonian tracheids nor for any early land plant.
210 However, the fossil record shows a great variety of conducting cells and not all of them possess a decay-
211 resistance nature. The best example is the S-type conducting cell. These cells, characteristics of
212 paratracheophytes such as *Sennicaulis*, *Rhynia* or *Huvenia* and related plants, are elongate, with
213 annular/helical thickenings, but with a very thin decay-resistant layer (Kenrick & Crane 1991) in which
214 the presence of lignin is still elusive (Boyce *et al.* 2003; Kearney *et al.* 2016). Interestingly, *Aglaophyton*
215 water-conducting cells strongly resemble the morphology found in *Rhynia* or *Huvenia* conducting
216 tissues (Remy & Hass 1996; Kearney *et al.* 2016). This fact suggests, together with similarity observed
217 between *Aglaophyton* and *Rhynia* central strands (see Fig. 1E,F), and the morphology of its reproductive
218 structures (Kenrick & Crane 1997; Cascales-Miñana & Gerrienne 2017), that *Aglaophyton* might be
219 included in the paratracheophytes, likely as its basalmost representative. This has already been discussed

220 by Edwards (2004), who suggested that similarities in xylem elements between *Aglaophyton* and *Rhynia*
221 would seem to narrow the differences between both plants.

222 The other key element in the currently accepted phylogenetic position of *Horneophyton* is the
223 presence of columellate sporangia (Eggert 19774; El-Saadawy & Lacey 1979). This character is
224 considered a bryophyte-like trait and it is the main feature advocating for the basalmost position of
225 *Horneophyton* within polysporangiophyte evolution (Kenrick 2000). However, columellate sporangium
226 is neither a character of all bryophytes, nor present in bryophytes only. This type of sporangium is absent
227 in liverworts, not present in all hornworts, and is characteristic of mosses only (Goffinet & Shaw 2009).
228 Importantly, the fossil record shows also collumellate sporangia in several Carboniferous lycopsids, i.e.,
229 in basal eutracheophytes, such as *Oxroadia* (Alvin 1965) or the sigillarian plant *Mezocarpon* (Pigg
230 1983). This means that the character “presence of a columella in the sporangium” might be
231 homoplasious, which reinforces the position of *Horneophyton* as a sister lineage to eutracheophytes
232 (Figs. 2,3B), and therefore, as a precursor of the living vascular plant biodiversity.

233 There is an important "time gap" between the earliest evidence for land plants, provided by the mid-
234 Ordovician dispersed spore fossil record (Rubinstein *et al.* 2010), and the earliest vascular plant
235 evidence, which corresponds to mid-Silurian *Cooksonia* macroremains (Libertín *et al.* 2018). This gap
236 could still be larger if we consider the recently suggested Morris *et al.*'s (2018) timescale. New
237 molecular-based results estimate indeed that land and vascular plants emerged during mid-Cambrian–
238 Early Ordovician and Late Ordovician-Silurian time intervals, respectively (Morris *et al.* 2018). No
239 fossil evidence confirms such intervals, but a series of small fossil plant remains have been recently
240 discovered from the uppermost Ordovician (Salamon *et al.* 2018). No unequivocal evidence of
241 tracheophytes is known before Silurian times. As shown in Fig. 3, *Cooksonia* is the oldest tracheophyte,
242 and importantly also, is the oldest and basalmost eutracheophyte, but it is not the most primitive vascular
243 plant. Due to its exceptional anatomical preservation, the Rhynie chert plants provide unique knowledge
244 about the earliest steps of polysporangiophyte evolution. This is the reason why they occupy key
245 positions in many models of plant evolution, especially tracheophytes. This fact also goes with an
246 underrepresentation and with an identification bias of basal tracheophytes, even more so if we take into

247 account that many Early Devonian plants are known from compression fossils only, with no information
248 about their vascular strand.

249

250 Conclusions

251 We show herein the water-conducting cells of four Lower Devonian plants from the Lagerstätte of
252 Rhynie (Aberdeenshire, Scotland) present in the slide collections of the Natural History Museum of
253 Lille (France). Those plants, namely the protracheophytes (considered non-vascular) *Aglaophyton* and
254 *Horneophyton*, the paratracheophyte *Rhynia* and the eutracheophyte *Asteroxylon*, are key taxa in the
255 earliest evolution of vascular plants. This work confirms previous descriptions and interpretations of the
256 water-conducting cells of *Aglaophyton*, *Rhynia* and *Asteroxylon*, but emphasizes that *Horneophyton* has
257 a particular type of tracheid characterized by narrow, irregular, annular and/or, possibly spiral wall
258 thickenings, and hence that it cannot be considered non-vascular anymore. Indeed, our new phylogeny
259 resolves *Horneophyton* within tracheophytes, but as sister to eutracheophytes in a larger clade called
260 supereutracheophytes. Results further suggest that the horneophytophytes are more derived than the
261 paratracheophytes. The Rhynie Chert plants lived some 20 million years after the earliest Silurian
262 records for vascular plants. Although based on a group of coeval plants, many of them displaying
263 ancestral characters, the alternative scenario for the basalmost evolution of vascular plants proposed
264 herein suggests a sequential acquisition of water-conducting cell characters. This results in a more
265 parsimonious model characterized by a progressive increase of the complexity of the conducting tissues.

266

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274

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366

367 **Figure legends**

- 368 *Fig. 1.* Rhynie chert water-conducting cells observed from the historical collections of the Natural
 369 History Museum of Lille. A-H. Transverse thin sections (TS). I-T. Longitudinal thin sections (LS). A.
 370 Conducting strand of *Aglaophyton majus* (TS, scale bar = 100 µm; MGLame-4). B. Vascular strand of
 371 *Rhynia gwynne-vaughanii* (TS, scale bar = 100 µm; MGLame-9). C. Vascular strand of *Horneophyton*
 372 *lignieri* (TS, scale bar = 100 µm; MGLame-18). D. Vascular strand of *Asteroxylon mackiei* (TS, scale
 373 bar = 100 µm; MGLame-27). E. Detail of water-conducting cells of *A. majus* (TS, scale bar = 20 µm;
 374 MGLame-4). F. Detail of S-type tracheids of *R. gwynne-vaughanii* (TS, scale bar = 20 µm; MGLame-
 375 9). G. Detail of water-conducting cells of *H. lignieri* (TS, scale bar = 20 µm; MGLame-18). H. Detail
 376 of tracheids of *A. mackiei* (TS, scale bar = 40 µm; MGLame-27). I. Water-conducting cells of *A. majus*
 377 (LS, scale bar = 20 µm; MGLame-8). J. Water-conducting cells of *R. gwynne-vaughanii* (LS, scale bar
 378 = 25 µm; MGLame-15). K. Water-conducting cells of *H. lignieri* (LS, scale bar = 50 µm; MGLame-
 379 18). L. Tracheids of *A. mackiei* (LS, scale bar = 50 µm; MGLame-29). M-N. Detail of water-conducting
 380 cells of *A. majus* (LS, scale bar = 20 µm; MGLame-8). O-P. Detail of water-conducting cells of *R.*

381 *gwynne-vaughanii* (LS, scale bar = 20 μm ; MGLame-15-16). Q-R. Detail of water-conducting cells of
 382 *H. lignieri* (LS, scale bar = 25 μm ; MGLame-18). S-T. Detail of tracheids of *A. mackiei* (LS, scale bar
 383 = 20 μm ; MGLame-29, MGLame-27).

384

385 *Fig. 2. Horneophyton* water-conducting cells observed from the Rhynie chert collections of Munster
 386 University (Slide P 5012). A. General view of the *Horneophyton* specimen illustrated in Kerp (2017,
 387 fig. 1h). B. Zoom view of A showing the structure of *Horneophyton* conducting tissues. Scale bar = 25
 388 μm . Pictures courtesy of Hans Kerp.

389

390 *Fig. 3.* Time-scaled phylogeny of earliest land plants. Major clades are in bold. Black boxes indicate the
 391 temporal distribution of involved taxa. Blue colour highlights the supereutracheophyte clade. See
 392 Material and methods for details.

393

394 *Fig. 4.* Synthetic comparison of the basalmost evolutionary relationships among land plants. A.
 395 Currently accepted most parsimonious plant phylogeny. Based on Kenrick (2000, fig. 1A). B. Proposed
 396 cladistics for the early land plants. Blue colour highlights the supereutracheophyte clade.

397

398 Tables

399 *Table 1.* List of slides of the Hemingway historical collection of the Rhynie chert housed at the Natural
 400 History Museum of Lille used in this study. For each slide, it is specified both the original and given
 401 code as well as the considered plant diversity.

402

403 Supplementary data

404 *Data S1.* Character matrix used in the phylogenetic analysis.

405

406 *Table S1.* List of characters and character states used for coding data matrix. Modifications from Kenrick
 407 and Crane [1] are in bold.

408

409 *Text S1.* Data matrix (Nexus format) of PAUP analysis I.

410

411 *Text S2.* Tree file (Newick format) of *strap* analysis.

412

413 *Text S3.* Age file (R package paleotree format) of *strap* analysis.