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1 Thirty-three years (1986–2019) of fossil plant cuticle studies using transmission electron
2 microscopy: a review

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17

18 **Abstract**

19

20 Although the cuticle is a very thin layer of a few micrometers, it is one of the main remains
21 found among fossil plants. Apparently very fragile, ultrathin cuticle sections of few dozen
22 nanometers thick are studied by using TEM studies and statistical measurements. The
23 ultrastructure details yielded are of significance not only for taxonomy, but also for
24 palaeoenvironment and evolution of fossil plant. After an historical introduction and a review
25 of the methods to obtain and observe the ultrathin sections, although data are still not enough

26 numerous, detailed information and interesting facts of cuticle ultrastructure emerge from the
27 studied plant groups. This paper gives a review of the main results of transmission electron
28 microscopy -TEM- of fossil plant cuticles since the pioneering study 33 years ago.

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35 Keywords: fossil plant cuticles; ultrastructure; transmission electron microscopy TEM;
36 taxonomy; palaeoenvironment; evolution

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39 **1 Introduction**

40 1.1 Historical insights

41

42 Our knowledge of the plant cuticle, including its naming, spelling, and wording (coticola,
43 cuticula, kutikula, cuticule, cuticle, etc.), progressed very slowly throughout centuries and
44 countries. It began with the term ‘cuticle’ used in a very wide sense for plants and/or humans
45 and animals, including formerly for plant cellular layer(s) beneath (epidermis and others...),
46 towards the sensu stricto ‘plant cuticle’ used in a modern sense as an extracellular membrane
47 over (or mixed with) the epidermis cell wall. The primary research to date on the subject,
48 besides probable omissions, has been discussed here.

49

50 The term cuticle originates from Latin (*cuticula* = *cutis* + *cula* = thin skin or small skin).
51 Identification of the outer parts of plants, or more precisely, allusions to the cuticle exist from
52 ancient Greek and Latin periods; however, it was not named ‘*cuticula*’. The French term
53 ‘cuticule’ was cited by Amigues’s Greek-French translation (1989) for the outermost part of
54 the oak acorn base part (pro parte for the Greek verb παραλιθάζειν, which means ‘make stony
55 around’) Luccioni (pers. com.), in the research on Theophrastus *Historia plantarum* (Book 3-
56 8-3, p. 22; see also Amigues 1999). The English term ‘cuticle’ was not found in the Greek-
57 English translation of Hort (1916), where ‘become stony at one end or the other’ was used.
58 However, in this English translation, Theophrastus’ Greek term *derma* (δέρμα, = skin) has
59 been translated to ‘skin’ when referring to the outer part of the olive (p. 399, Theophrastus
60 Book IV XIV 9–10), whereas it has been translated to ‘outer covering’ for the outer parts of
61 fruits in the general presentation of plants (p. 21, Theophrastus Book I 2 6; Luccioni pers.
62 com.). The particular impermeable property of the cuticle (without naming it) is clearly noted
63 in Book VII 141, for the frond of a fern (οἶον τὸ ἐπὶ τοῦ ἀδιάντου συμβαῖνον·νοῦδὲ γὰρ

64 ὑγραίνεται τὸ φύλλον βρεχόμενον, ὃ δὴ ἐπίδηλόν ἐστι διὰ τὸ μὴ τὴν νοτίαν ἐπιμένειν, ὅθεν
65 καὶ ἡ προσηγορία. γένη δὲ αὐτοῦ δύο, τὸ μὲν λευκὸν τὸ δὲ μέλαν, χρήσιμα δ' ἀμφοτέρω πρὸς
66 ἔκρυσιν κεφαλῆς τριχῶν ἐν ἐλαίῳ τριβόμενα). This text is translated into English as ‘...for
67 instance that which we find in wet-proof (maidenhair); the leaf does not even get wet when it
68 is watered, nor does it catch the dew, because the dew does not rest on it...’ (Hort, 1916, Vol 2
69 p. 135), whereas the French translation of Amigues (1989) is slightly different (Luccioni pers.
70 com): ‘...l’humidité n’adhère pas à sa surface...’ (= humidity does not adhere to its surface);
71 however, both translations imply the outermost part of the frond has impermeable properties.
72 Amigues (1996) commented on this fern, also used in Theocritus’ Idylls XIII poem (see also
73 Banks and Chapman 1853), and explained that the Greek name ἀδίαντον means ‘not wet’ and
74 uses the French words ‘cuticule cireuse’ (= waxy cuticle) in the comments. Although this is
75 not directly related to our subject, this plant seems somewhat difficult to identify as several
76 fern taxa have been proposed by the different translators; however, the species is very
77 probably *Adiantum capillus-veneris* L. (Luccioni pers. com.). This taxon is among the rare
78 ferns where the cuticle has been analysed using freeze-fracture (Wada and Staehelin 1981).
79 The term ‘cutis’ (= skin) has been used in ancient Latin texts: e.g. Pliny the Elder’s *Historia*
80 *naturalis* [Latin-French translation by Littré (1877)] used this term for the outermost part of
81 the oak acorns (Book XVI 8 2): ‘*Distant inter se magnitudine, et cutis tenuitate*’ (they differ
82 between them according to their size and the fineness of their skin). Columella [Latin-French
83 translation of *De re rustica* by Du Bois (1844), Lib. III], from that period too, also used this
84 term for the outermost part of a variety of grapes: ‘...*nam quia minuti acini, et durae cutis*
85 *uvas habent...*’ (because they have grapes with small grains and a thick skin). The oldest
86 Latin lexicons (Furno 1997) from the Roman period or a little later [Varron, from 1 BC (see
87 the edition of Goetz and Schoell 1910); Festus and Festo, from the IInd and VIIIth century
88 taking in account the text of Flaccus ‘*De uerborum significatu*’ from 1 BC (see Lindsay

89 edition of 1913); Marcellus, from the IVth century (see the edition of Lindsay 1903); Isidore
90 of Seville, from the VIth century (see the edition of Lindsay 1911)], do not cite ‘*cuticula*’. For
91 instance, the text of Isidore of Seville contains a whole section on plants, but only mentions
92 ‘*cutis*’ (Liber XVII–VIII 7 and IX 104, *De rebus rusticis – De aromaticis arboribus* and *De*
93 *herbis aromaticiis*).

94

95 From the XIth century, with a great increase from the end of the XVth to the XVIth century,
96 ‘*cuticula*’ appeared increasingly in the first general dictionaries of various languages in
97 western Europe (or glossaries, lexicons, or equivalents), but is mostly linked with ‘*cutis*’ and
98 human and/or animal skin, whereas it was used more accurately for botanical purposes in
99 more specialised texts. In the ‘*Elementarium*’ of Papias from the XIth century (see the edition
100 of Mombritius 1485 or older manual versions), ‘*cuticula*’ was noted as a diminutive of ‘*cutis*’,
101 with a human and animal skin meaning. In 1286 (see an edition of 1460, by Gutenberg?),
102 Balbus used the same meaning. In one of the most widespread dictionaries of that period,
103 ‘*cuticula*’ was cited by Calepino (1502) as a diminutive of ‘*cutis pellis*’ [among numerous
104 uses of this text through almost three centuries, for instance in 1588 an English translation of
105 ‘*cuticula*’ was provided in eight languages (‘a little or thinne skinne’; p. 176); in the ninth and
106 last edition of this text (Facciolatti 1778), ‘*cuticula*’ was still cited (p. 197) with a human
107 meaning]. In the *Thesaurus linguae latinae* of Estienne (1532, also edited in 1573), ‘*cuticula*’
108 was also noted (p. 707) for a diminutive of ‘*cutis*’, *cutis* referring in one case to plants (‘*cutis*
109 *arboris*’). The Latin word ‘*cutis*’ was cited once in the index of the Greek-Latin translation of
110 Theophrastus *Historia plantarum* by Theodorus Gaza (1552 edition, from the end of the XVth
111 to the beginning of the XVIth century). Scaliger (1644, p. 878) in the Greek-Latin commentary
112 on Theophrastus *Historia plantarum*, in the text corresponding to the fern cited above for its
113 impermeability, cited Theodorus Gaza's translation (similar sentence in Gaza 1552 p. 153):

114 “*Adianti, id est, capilli Veneris, folium numquam madescit, nec quicquam adhaesisse humoris*
115 *constat* (= *Adiantus*, i.e. *Capillus veneris*, never gets wet, and it is well-known that no liquid
116 is adhering to it”; Luccioni, pers. com). In the early XVIIth century, the diminutive *cuticula*
117 appeared in other western Europe dictionaries as a synonym of ‘coticola’ in the Italian
118 ‘vocabolario degli accademici della crusca’ [(1612), p. 150 of the ‘indice delle voci e
119 locuzione latine’ part (index of the Latin voices and locutions)]. ‘*Cuticula*’ was used several
120 times by Scaliger (1644), but as a wide definition of the outer part of plants, particularly for
121 the outer part of the fruit of *Paliurus spina-christi* (= garland thorn, Rhamnaceae; p. 266,
122 comm. on Theophrastus Book III 17; Luccioni pers. com). ‘Cuticle’ in English appeared in
123 one of the first English dictionaries (1658, ‘The New World of English Words, or, a general
124 dictionary’) but as an anatomical term for the human (or animal?) skin outerpart, and
125 appeared more than 10 times in Grew (1672), apparently referring to different external parts
126 of plants. ‘*Cuticula*’ was cited about 20 times in Malpighi (1675; also, *cuticulam*, *cuticulae*,
127 *cuticulâ*) apparently connected with different outer parts of plants, but specifically with
128 ‘*epidermide*’ (epidermis) and “*folii*” (leaf) in some parts of the book. In other western
129 European languages, the French dictionary of Furetière (1690) cited ‘cuticule’, but as a
130 synonym of the epidermis of animals. In the Portuguese dictionary of Bluteau (1712; p. 648),
131 although it was partly devoted to botany, ‘cuticula’ was a synonym of ‘cutícula’ and described
132 anatomically as human epidermis. Similarly, in 1729 ‘cuticula’ and ‘cuticular’ appeared in the
133 in the Spanish ‘Diccionario de Autoridades’ with the same meaning.

134

135 The plant cuticle has been described increasingly precisely and in detail through time; in
136 Ludwig (botanist and doctor, 1742), the plant cuticle was cited several times and described in
137 Part II *Consideratio vegetabilium physica* (Chapter II paragraph 315: ‘*est lamina densa,*
138 *tenuis, pellucida... et totam plantae superficiem obtegit... haec cuticula plantarum dicitur*; it

139 is a dense, thin, transparent layer... and it covers the whole plant surface... it is called plant
140 cuticle'; partly Luccioni (pers. com). It was compared with animal cuticles (paragraph 316),
141 and also quoted in the index. In other paragraphs (431 and 432), maceration experiments on
142 various taxa were described, separating the cuticle from parenchyma tissue. Some years later,
143 Ludwig (1757), described the cuticle slightly differently and also included maceration, which
144 possibly represents one of the first experiments on plant cuticles, but the term was still
145 apparently used in a wide sense ("*Omnem plantae superficiem cingit lamina tenuis et*
146 *pellucida, quae cuticula dicitur, haec densa est et in maceratione plantarum non*
147 *dissoluitur...*"; paragraph 327; "the whole plant surface is surrounded with a thin and
148 transparent layer, called cuticle, it is dense and not soluble by plant maceration..."; partly
149 Luccioni pers. com), further in this paragraph the cuticle was precisely restricted to some
150 parts of plants. It appears several times in this 1757 text, also compared with human skin in
151 the paragraph 328 ("*cuticula plantarum 327. Huius et humanae comparatio 328*" as written in
152 the index).

153

154 Linked to more precise techniques and experiments, the early XIXth century was the starting
155 point for various steps recognizing the plant cuticle as it is currently defined. Treviranus
156 (1821) provided transversal sections ('durshnitt') of various extant plants, but unfortunately
157 with low magnifications where the cuticle was difficult to distinguish from the cell wall of the
158 epidermis (his Tab. I F. 1, 4, 13; his Tab. II F. 14 and 21). However, this was an important step
159 for observing details of the different parts of the outer part of the epidermis, and the term
160 'cuticula' was used, but not in its modern sense. In 1827, De Candolle used the French term
161 'cuticule' in Chapter V of his book ('de la cuticule et de l'épiderme' p. 66–77; = about cuticle
162 and epidermis), where he referred to transversal and longitudinal sections of *Tritomia varia*
163 (his Planche 2 Figs. 3 and 4). In Figure 3, he clearly called cuticule (named 'c' on the figure)

164 the epidermal cells surrounded with a thick cell wall, the cuticle thickening not visible. In the
165 text, he included epidermal cells in the ‘cuticule’, but cited other authors suggesting that the
166 cuticle seems to depend on the type of organ and taxon observed. Brongniart (1830) used the
167 same technique and provided, at a much higher magnification, very precise light microscope
168 figures of extant plant leaves with transversal sections (also called perpendicular sections:
169 ‘coupes transversales’, ‘coupes perpendiculaires à la surface... des feuilles’) obtained with
170 very sharp and delicate instruments (‘...avec des instrumens très-tranchants et très
171 délicats...’), where the cuticle seems to be drawn; however it is not clear for some cases if he
172 drew the cuticle or cell wall. Although the term cuticle was not used, it was drawn quite
173 precisely in the plates with extant *Lilium album* (Pl. 8 Fig. 3) and *Iris germanica* (Pl. 9 Fig. 2),
174 showing a thicker thickening over the upper epidermis, whereas it was thinner in the lower
175 epidermis. As other palaeobotanists of that period, Brongniart was very sensible to the quality
176 of the illustrations (Cleal et al. 2005), which were accurately observed and illustrated, as he
177 also researched extant immersed plants, such as extant *Potamogeton perfoliatum* and
178 *Ranunculus aquatilis* that have almost no thickenings above the epidermal cells, which is
179 well-known for plants in these environments. Brongniart referred to a continuous membrane
180 (‘membrane continue’), except in the middle part of the stomata, which was obtained with
181 extant *Brassica* leaves, after maceration in water for a few months.

182

183 Henslow (1831) published some clear cuticle preparations (without using this term) in extant
184 *Digitalis* (Scrophulariaceae) hybrid organs (his Plate XVII 6–7; transversal sections in Plates
185 XVII–XVIII also show cuticles over the epidermis) using nitric acid (an efficient technique
186 still currently used for preparations of cuticles), and wrote: “I am however quite positive upon
187 another point which has been a subject of dispute among physiologists; I mean the existence
188 of a delicate homogeneous membrane investing this epidermis. Such a membrane may be

189 distinctly separated by the action of nitric acid, from the epidermis of the corolla, filament,
190 and style. It is faintly marked by parallel longitudinal stria... and appears to coat over the
191 whole surface of these organs..." Brongniart (1834) cited Henslow in his text related to the
192 *Brassica* experiment, but more accurately described the possibility of chemical maceration to
193 detach a thin 'pellicule sus-épidermique' (= film over the epidermis) from the epidermis (= "cuticle"
194 in his text); according to his text he observed this in many other taxa. Lindley
195 (1835), published a whole part (Chapter II Section I 1; see also Plate III Figs. 3–6 and 9–11)
196 in the second edition of a botany book dedicated to 'cuticle', and summarised the research he
197 questioned about the cuticle as follows: "There is some reason to suppose that there is
198 occasionally present, on the outside of the cuticle, a transparent, very delicate membrane,
199 having no organic structure, as far as can be discovered with the most powerful microscopes".
200 Something similar was noticed by Brongniart in the cabbage leaf; an analogous structure was
201 commented on by Henslow in the *Digitalis* leaves; "and I have found it very conspicuous on
202 the upper side of the leaves of *Dionaea muscipula*... Brongniart has paid some attention to
203 this subject... and finds the pellicle by no means uncommon; he even thinks it is present in
204 submersed leaves, and imagines that it covers the stigma in some plants. I, however, find
205 nothing very definite in regard to this, except that the pellicle often exists, and that it does not
206 cover the stomates". The different editions of this book are of interest as they represent an
207 accurate reflection of the research regarding cuticles of that time. The first edition also
208 contained a 'cuticle' part (Lindley 1832), which was not very detailed. In the third edition
209 (Lindley 1839) the 'cuticle' part was removed and replaced by an 'epidermis' part, where the
210 cuticle was described to be 'organic mucus'. His Plates (I Fig. 2 and II Fig. 1) contained some
211 transversal sections but with the cuticle very simply drawn on the outer part of the epidermis.
212
213 Von Mohl (1842, 1847a) made a considerably more precise new finding, showing the

214 different parts of the cuticle (especially the presence of a ‘cuticle proper’, as cited by
215 Holloway 1982) and simultaneously used the German term ‘cuticula’ (= kutikula) in its
216 modern sense. The translation of the 1842 article in French (Von Mohl 1843) seems to be the
217 first appearance of the French term ‘cuticule’ in its modern sense, and contained a detailed
218 illustration (Plate 6) with various coloured sections of cuticles, two of them being clearly
219 bilayered (his Fig. 7 of *Viscum album*, his Fig. 10 of *Agave lurida*), other figures being from
220 mono- to multi-layered; interestingly for Coniferales, which are now among the well studied
221 fossil plant groups using transmission electron microscopy (TEM), the cuticle of extant
222 gymnosperm *Taxus baccata* was mentioned (as similar to his Figure 8). Hartig (1843)
223 published extant cuticles schemes of sections with 1 and 2–3 zones (his Pl. Figs. 1 and 2), and
224 a whole chapter entitled ‘cuticule’ in the translation in French of this article (Hartig 1844). In
225 the French translation (Von Mohl 1847b) of the article of Von Mohl (1847a), there was a
226 description of the use of transversal sections to determine the chemical reactions of the
227 cuticle. However, these new cuticle findings (several parts for the cuticle) seemed to have a
228 variable impact according to the authors. From Lindley’s botany book, the fourth edition
229 (1848) contained ‘back’ the ‘old wide sense’ of cuticle in much more detail than the first and
230 second editions, including the current research of this time, and the cuticle was compared with
231 the ‘skin or shell of Crustaceae’. With Henfrey’s English translation (1852) of von Mohl
232 (1851) the English term cuticle in its modern sense (‘... mass secreted on the outside of the
233 cells, the true *cuticle*... in which no cellular has been yet shewn to exist’) appeared seven
234 times. Transversal sections were provided (Figs. 17, 37, 40–42, 48) where the cuticle was
235 indicated in the legends by ‘a’ (Figs. 37, 41, and 48 of extant *Helleborus*, *Hoya*, and *Cereus*).
236 Moreover, his Fig. 41 was particularly informative, as the cuticle was very clearly shown as
237 detached from underlying layers, and corresponded to the cuticle proper in its modern sense,
238 the cuticular layer in its modern sense just underneath being named ‘b’ (‘wollen, laminated

239 layers of the epidermis cells’); the cell wall was present all over the cell and very precisely
240 drawn.

241

242 Bornemann (1856) published some transversal sections (‘Querschnitt’) of various extant
243 cycads (his Tafel XI 18–19; his Tafel XII 15–18 with two parts in Section 17 above the
244 epidermis cells, one for the cell wall, and one for the cuticle with a clear anticlinal wall
245 between the cells). In the same year Schacht (1856) provided coloured transversal sections
246 (his Tafel III) of extant plants with cuticles showing various thicknesses, distinct from the cell
247 wall just beneath in another colour; in one drawing the cuticle had two parts, an outermost
248 dark-brown and an inner yellow part. In addition, also in the same year Prillieux (1856)
249 published (his Pl. 2 and 3) some sections of various Oleaceae and Jasmineae, where the
250 distinction between the cell wall of the epidermis and cuticle was not very clear. Unger (1858)
251 provided a transversal section of extant *Richardia aethiopica* (his Taf. I 4) with a cuticle
252 apparently drawn in a darker colour than the cell wall, but in a very low magnification, not
253 showing any details of the cuticle. Besides, much earlier, this author had published the same
254 results for extant *Victoria regia* leaf (Unger 1853, his Figs. 2–3). Wolf (1865) provided
255 transversal sections of the epidermis of *Orchis mascula* and *Trichopilia suavis* (his Tafel XVI
256 3 with ‘cuticula’ mentioned in the same page of the text, and Tafel XVIII 5–6, respectively)
257 with the cuticle drawn but not mentioned in the captions. Similarly, Kraus (1865) observed
258 the cuticle of *Cycas revoluta*, however provided more precise observations of delicately
259 ornamented cuticles (his Tafel XIX 5–6 and Tafel XX 11), even showing the differences in
260 thickness between the upper and lower cuticles; the term ‘cuticle’ was used about 20 times in
261 this paper. However, Sachs (1873) showed a transversal section of the epidermis of extant *Ilex*
262 *aquifolium* (his Fig. 37) with a thick cuticle made of two parts [outer part labelled ‘a’, devoid
263 of colour according to his text (‘farblos’); inner part labelled ‘b’, with yellow colour (‘gelb’)].

264 Other authors very rarely figured transversal sections, except for extant reproductive organs
265 (De Saporta 1873–1891); however, De Saporta and Marion (1881–1885) published figures of
266 transversal sections of extant stems (dicotyledons *Nelumbium*, *Tradescantia*, and *Dracaena*)
267 and leaflets (Cycadales *Encephalartos* and *Bowenia* (their Fig. 49–50, 108–109) with clear
268 cuticle differences of thickness between them. Schimper (1874) provided some *Equisetum*
269 transversal sections, with limited details of the outermost part, whereas 10 years later De Bary
270 (1884) used largely transversal sections of cuticles in his figures, some of which were
271 extremely precisely drawn (Figs. 25–27 *Aloe*, *Ilex*, *Pinus*; Fig. 222 *Betula*, Fig. 220 *Ribes*).

272

273 Applying the above technique to fossil plants are apparently overlapped in time with extant
274 plant observations, sections of fossil plant cuticles have been documented using light
275 microscopy, and usually with limited details. A few years later after Brongniart's work on
276 extant plants in 1830 and 1834 containing clear descriptions of the cuticle, in the second
277 volume of his fossil plants book, Brongniart (1837) published some transversal sections of
278 permineralized material (petrological thin sections, Pl. 10 Fig. 2 and 3 on lycopods; Pl. 11 Fig.
279 1 on *Psilotum*), which were apparently influenced by his previous extant plant research: he
280 showed clear epidermis more or less thickened in their outermost parts (as cuticles...),
281 whereas the first volume of this fossil plants book (Brongniart 1828), before his description of
282 cuticles in extant plants, did not contain these details. Renault (1879) followed the same
283 approach with carboniferous material, similarly to 'silicified magma' as he described in the
284 introduction, where *Cordaites* leaves (petrological thin sections, his Plate 16 Figs. 2–7) with
285 epidermis cells were very precisely drawn (using a camera lucida of a light microscope) and
286 clearly different from other types of cells, where the upper epidermis was clearly thicker than
287 the lower epidermis; the cuticle also seemed different in thickness. These very delicate and
288 precise drawings of *Cordaites* were partly reproduced later in several Palaeobotany books

289 (Potonié 1921, Scott 1923). Some years later, Renault (1893–1896) provided some transversal
290 sections of silicified *Poroxylon incertae sedis* (included in ‘genres à place indéterminée’,
291 *incertae sedis* genera) with clear thickenings over the epidermis (Planche LXXV Figs. 5–6),
292 but without naming ‘cuticle’ in the captions.

293

294 These light microscopy section techniques also overlapped with thin sections of organic
295 remnants containing cuticles, according to drawings or descriptions provided, but they were
296 quite difficult to obtain because of their fragility. According to Stace (1965), Brodie (1842) is
297 apparently the first scientist who mentioned the fossil cuticle by distinguishing it from
298 impressions, but did not use the term ‘cuticle’ (‘the epidermis of the fossil frequently peels
299 off... these remains... have been deposited tranquilly between the waters’), which might
300 suggest organic remnants. Among other authors known so far, the term ‘cuticule’ (= cuticle)
301 was used in French by Zeiller in 1882, associated with an extraction method from the
302 sediment to obtain clean material observable with the microscope. He also provided seven
303 precise transversal cuticle sections of different thicknesses and shapes, some of them showing
304 various stomata (his Plate 9 Fig. 8 with *Callitris*, Plate 10 Fig. 14 with *Bothrodendron*, Plate
305 11 Figs. 7–10 with *Frenelopsis*). De Saporta, in spite of having studied cuticles of extant
306 plants, among his numerous publications, published surprisingly few transversal sections of
307 fossil plant cuticles. However, in 1894 his Plates 5 and 11 contained transversal sections of
308 *Rhizocaulon* roots with a clear outer dark layer of cells, but unfortunately did not include
309 detailed captions, and five sections of *Rhizocaulon* leaves, also with a darker outermost layer
310 of cells like an epidermis (where cuticle is not visible at all). Later on, Zeiller (1900)
311 published a transversal section of *Cordaites* (his Fig. 141), possibly from permineralized
312 material but where the thickening over the epidermis was clear. In 1912, Thompson provided
313 some light microscope photos and drawings of transversal sections of fossil cuticles (his

314 plates V–VI). In an article with both extant and fossil cycadean plants, Thomas and Bancroft
315 (1913) published some transversal sections of extant cycadean cuticles, which were very
316 clearly drawn in a darker colour than the cell wall (his plates 17–18). Halle (1915) provided
317 transversal sections of the fossils, *Pseudocycas* and *Sciatopitytes* (his Pl. 12 4–6 and 27–29),
318 including a whole leaf section. Seward’s book on fossil plants (1919) contained a transversal
319 section of *Nilssonia brevis* (his Fig. 620 B after Nathorst), which was unfortunately not
320 detailed, whereas that of *Ctenis* sp. (his Fig. 625 B) had a clear darkened cuticle above the
321 epidermis. Potonié (1921) published some transversal sections of a *Nilssonia* leaf (his Fig.
322 234 c), with a very homogeneous cuticle and devoid of cell remnants. Florin (1933, 1936)
323 provided fossil transversal sections (‘Querschnitte’) of cuticles of Ginkgoales (his Tab. 8 and
324 20) and Cycadales (from Tab. 2 to Tab. 13).

325

326 Following this period of sections in light microscopy through the XXth century (see Stace
327 1965 for a detailed chronology of cuticle findings), the first fossil cuticle sections of material
328 embedded in resin and observed using ultrastructural TEM began in 1986 with the genera
329 *Tarphyderma* and *Ticoa* (Archangelsky and Taylor 1986; Archangelsky et al. 1986), after
330 TEM images of Miocene leaves (angiosperm *Zelkova*, Niklas et al. 1978; see also Fig. 1. 24
331 of Taylor et al. 2009) were published, where the ‘cuticular membrane’ appeared to be
332 illustrated (their Fig. I, K), but was not described in detail.

333

334 1.2. Various aspects of plant cuticle ultrastructure

335

336 These fossil studies contained several important topics and contents quite classical in
337 palaeobotany that emerged from the beginning of this research and are still developing. The
338 first topic discussed in papers from 1986 concerned taxonomy. Although Holloway (1982)

339 proposed six types of cuticles for living plants, grouping very different taxa in each type
340 [from *Selaginella* to angiosperms] from different parts of plants (leaves, fruits, ovules, stems,
341 trichomes, stigma...), he was very possibly conscious of the importance of cuticles in
342 taxonomy as he concluded: ‘Because the CM of plants is so heterogeneous in structure it is
343 dangerous to oversimplify and generalize about its morphology and construction – there is no
344 typical CM. Consequently, each species must be considered individually...’ Although in
345 living plants, until recently, TEM details of cuticles have seldom been provided, they are
346 largely provided in fossil plant taxonomical studies, and still prove that 1) one cuticle may
347 refer to several types of those described by Holloway (1982) and many overlaps occur, and
348 therefore the six described types are not enough; 2) in one single type each species has its own
349 identity with specificities in thicknesses and/or proportions of layers and/or sub-layers. In a
350 study on *Ticoa*, Archangelsky et al. (1986) stated that ‘the structural and perhaps
351 histochemical complexities of fossil plant cuticles will require some further division of these
352 layers’, enhancing all the future detailed taxonomical articles developed.

353

354 Palaeoenvironment is the second topic discussed in term of number of papers. This topic has
355 been developed regularly widely in various fossil plant groups (Kerp and Barthel 1993,
356 Krings and Kerp 1997). More precisely for TEM, in a study on *Tarphyderma*, Archangelsky
357 and Taylor (1986) suggested ‘...additional insights into ecological parameters...’, ‘...response
358 to the habitat...’, and ‘...various structural features were evolving in other major groups of
359 plants in response to the habitat...’ Additionally, Archangelsky et al. (1986) pointed out that
360 “... the presence or absence of specific cuticle layers in the fossils may be directly associated
361 with the environment in which the plant was living...”

362

363 The third topic that has been up to now very rarely documented, concerns evolutionary
364 aspects. In the *Ticoa* study (Archangelsky et al. 1986), when emphasized the importance
365 comparisons between fossil and living plants cuticles, these authors pointed out comparisons
366 between fossil and living plants cuticles ('subsequent work with fossil plant cuticle
367 ultrastructure will consider both the taxonomic application and evolutionary significance of
368 these features'. In a study on *Tarphyderma*, Archangelsky and Taylor (1986), suggested
369 'adaptations' and more precisely 'structural adaptations in fossil plants'.

370

371 Two other points have not been included in this review, as they have not been documented in
372 detail. The first concerns ontogeny, which is classical if working on features of organisms,
373 evocated also by Archangelsky et al. (1986), who suggested that "...the presence or absence
374 of specific cuticle layers in the fossils... may reflect a particular level of organ development at
375 the time of fossilisation". This has been studied in living plants (*Sphagnum*, moss, Jeffree
376 2006; algae, Jeffree 2006; *Picea*, gymnosperm, Jeffree 2006; *Hedera helix* L., angiosperm,
377 Viougeas et al. 1995; *Utricularia*, angiosperm, Jeffree 2006; *Solanum lycopersicum*,
378 angiosperm, Dominguez et al. 2011b and 2012, Segado et al. 2016; *Arabidopsis*, angiosperm,
379 Nawrath 2006, Fabre et al. 2016; see also Fernandez et al. 2016; see also the reviews of Yeats
380 and Rose 2013 and Ingram and Nawrath 2017); however, until now, no fossil studies have
381 been carried out to the best of our knowledge in this regard, owing to the extreme rarity of
382 different stages of the same material found in the fossil record. The second concerns
383 diagenesis and TEM, carried out from 1986 (Archangelsky et al. 1986). Although this is
384 fundamental for the study of fossil plants, the analysis is difficult to carry out. At least within
385 one genus, TEM data comparing fossil (170 millions of years) and living ginkgos, Guignard
386 and Zhou (2005) found very few differences in the cuticles within this genus, although there
387 was a considerably minimised effect of diagenesis.

388

389

390 **2 Methods**

391

392 The details of the methods used to obtain the sections of cuticles are somewhat variable,
393 related to different chemicals used, also depending on the material (preservation and others...)
394 and taxa, the technique of the laboratories.. However, the methods for this especially difficult
395 preparation (Taylor 1999, in a special chapter on the ultrastructure of fossil cuticles) followed
396 a similar general scheme as summarised here. According to all the studies selected in Table 1
397 where the main differences in methods are listed, the material could be extracted from the
398 sediment treatments (or not) with several chemicals during variable times. Moreover, the
399 removal of minerals and sediments if necessary (in order to not destroy the glass or diamond
400 knife when cutting the sections) is also summarised: hydrofluoric acid, followed by then
401 Schulze's reagent (nitric acid and potassium chlorate; Gray 1965, Traverse 1988), and finally
402 dilute ammonia. Although the same method should be used for effective comparisons between
403 cuticles, the use of these treatments produced very damaged areas in some cuticles (holes,
404 breakings, and missing parts), not allowing detailed observations, especially collecting of
405 enough statistic data possible. In some cases, untreated cuticles provided much longer areas of
406 entire cuticles, which were very comparable with treated material, and are more suitable for
407 observations and photography. Apparently no (or very few) changes are noted in all taxa
408 where it is used (Guignard and Zhou 2005, Del Fueyo et al. 2013, Guignard et al. 2016, Yang
409 et al. 2018, Carrizo et al. 2019a, this issue; Guignard et al. 2019, this issue).

410

411 TEM methods (Appendix A of MethodX article) begin (or not) with the fixation of cuticles (in
412 glutaraldehyde or paraformaldehyde), then a postfixation (or not) in Osmium tetroxide,

413 followed by dehydration in degraded solutions of alcohol allied with graded percentages of
414 resin for its impregnation in the cuticle (Spurr, Epon, Araldite). Finally, a polymerisation of
415 the blocks of resin (at 37, 56, 60 or 70 °C) is achieved depending on the type of resin, during
416 variable times. The majority of research referred to only one kind of resin for each paper, this
417 homogeneity allows comparisons, since some differences are found with various uses of resin
418 for the same taxon (extant *Eucalyptus*, *Populus*, *Pyrus*; Guzman et al. 2014).

419 From this step, two kinds of sections, using an ultramicrotome, can be obtained from the
420 blocks of resin embedding the cuticles (Appendix B of MethodsX article), usually cut with a
421 diamond knife, but more rarely with a glass knife: 1) rarely mentioned but very useful are thin
422 sections (1 µm) mounted on glass slides and stained (in blue colour) using the methods of
423 Richardson et al. (1960), observed with a light microscope, enabling to the quality of the
424 cuticle to be analysed and determine the best sections with the maximum data (containing not
425 only ordinary epidermal cell cuticle, but also potentially stomata and the base of trichomes
426 and papillae); 2) ultrathin sections of 60–70 nm (silver to pale gold), observed using TEM,
427 deposited on uncoated or Formvar-coated 200–300 Mesh copper grids (or other meshes, other
428 metals, even with one slot grids in rare cases). The majority of sections are transversal to the
429 cuticle (i.e. perpendicular to the leaf length), but some are longitudinal (i.e. parallel to the leaf
430 length) to illustrate the homogeneity of the material through these two orientations.

431 The last step is staining, to obtain high contrasts of the different materials comprising the
432 cuticles. This step shows high variations of techniques, not only in the chemicals (potassium
433 permanganate, uranyl acetate, lead citrate), but especially in combinations of durations and
434 dilutions. Although most papers use only one method, some articles refer to several attempts
435 for the same material: in fossil gymnosperms, Taylor et al. (1989) provided TEM photos of
436 cuticle with different staining times (60-20-10 minutes, or 25-25-17, or 20-20-10) used for the
437 three chemicals, respectively. In Umkomasiales (Labe and Barale 1996), the staining was

438 adjusted (in times and dilutions) according to the material. In Czekanowskiales (Zhou and
439 Guignard 1998), five methods of staining were used with the three chemicals (or two or even
440 just one), with various times and percentages of solutions. Their best staining is indicated with
441 uranyl acetate (20 min) and lead citrate (20 min). As the type of molecules stained in TEM is
442 questionable by some authors, through the photos provided in the papers for one taxon (=
443 same type of material), apparently it is difficult to state if different molecules are stained with
444 different stains; only lower or higher contrasts seem to be relevant, but exercising caution is
445 recommended.

446 As an example, the technique of Lugardon (1971) is provided here (Appendix A of
447 MethodsX article), enabling very clean and highly contrasted details of cuticle sections. This
448 technique was formerly used for spores (living and fossil plants) with Spurr and/or Epon resin
449 (as in *Marattia* spores with Lugardon as a co-author, Wang et al. 2001), and adapted for
450 cuticles with Epon resin. Compared with other embedding techniques of 1–2 days very
451 common for TEM studies of living organisms, this one is particularly long (one week of
452 procedure, from day 1 to day 8), which is considered very useful for a low risk of breakings of
453 the cuticle when obtaining thin or ultra-thin sections. Osmium tetroxide postfixation and
454 staining with both uranyl acetate and lead citrate (Appendix B of MethodsX article) allow a
455 very satisfying contrast of the contents of cuticles, enabling high magnifications > 100,000 ×.

456
457 In this review, the methods of the three first aspects (taxonomy, palaeoenvironment,
458 evolution) were carried out, and multifactorial analyses were applied, using XLSTAT 2019.1
459 and XLSTAT3D Plot (Addinsoft (2019); XLSTAT statistical and data analysis solution. Long
460 Island, NY, USA. <https://www.xlstat.com>.), for chi-square automatic interaction detector
461 (CHAID), factorial component analysis (FCA), and hierarchical components (HCA).

462

463

464

465 **3 Major results and discussion**

466

467 3.1 Significances and application in fossil plant taxonomy

468

469 3.1.1. Detailed description of different parts of fossil plant cuticles.

470 Although in living plants mechanical, physical and chemical aspects are searched extensively

471 (Dominguez et al. 2015, and 2017 with more than ten articles of a special issue entitled “The

472 plant cuticle, old challenges, new perspectives”), allowing Cohen et al. (2017) to write that

473 “A search in Web of Science... shows that the number of published articles associated with

474 the cuticle gradually increased each decade starting in the 1950s up until the late 1990s...”,

475 detailed description of the different layers of the cuticle is seldom given. However they do

476 exist as in living *Ficus elastica* (Guzmán-Delgado et al. 2015), in *Arabidopsis thaliana*

477 (Mazurek et al. 2017, Berhin et al. 2019), in orchids (*Bulbophyllum weberi* and *B. cumingii*;

478 Kowalkowska et al. 2017), in tea (*Camellia sinensis* cv Fuyun; Zhu et al. 2018 and *Opuntia*

479 (Agüero et al. 2018). In fossil cuticles, mainly leaf cuticles being reviewed here [although

480 cuticles are also present in fruits, stems, flowers and embryos as revealed in living plants

481 (Heredia 2003, Heredia-Guerrero et al. 2017, Ingram and Nawrath 2017), all these organs

482 being very fragile for preservation], descriptions are very frequent, possibly because cuticles

483 are often the only remains of fossil plants. The description of the fossil cuticle (= cuticular

484 membrane CM) was first proposed by Archangelsky et al. (1986), following the scheme of

485 Holloway (1982) for living plant cuticles, who summarised detailed cuticle sections from von

486 Mohl’s description (1847; see particularly the distinct Figure 4 in this 1847 volume, showing

487 two clearly distinguished parts of the cuticle, above the cell wall; see also von Mohl 1842,

488 Geneau de Lamarlière 1906, Roelofsen 1952 for his photos and schemes, Roelofsen 1959 for

489 his Fig. 167). This scheme (Fig. 1 and Plate I for illustrations) with two parts in a fossil
490 cuticle is still used, completed by sublayers of A1 and A2, following the studies of Guignard
491 and Zhou (2005) and Nosova et al. (2016). This scheme is not as definite as that of Holloway
492 (1982), for extant plants, "...it is always inadvisable to present a single generalised scheme
493 for the construction of the plant CM". The cuticular membrane (appendix A) is divided into
494 two regions: an outer cuticle proper (= CP = A) directly in contact with the atmosphere in its
495 outermost part, and an inner cuticular layer (= CL = B) in contact with the cell wall of the
496 subjacent cell (mostly absent in fossil material) in its innermost part. The outer cuticle proper
497 A can be divided in two layers: an outermost A1 polylamellate layer subdivided in some cases
498 into A1U (= upper zone or sublayer, which can be straight or wavy) and A1L (= lower zone
499 or sublayer), then an inner A2 granular layer, divided in some cases into A2U (= upper zone
500 or sublayer) and A2L (lower zone or sublayer). The inner cuticular B can be divided in two
501 layers: fibrilous or spongy B1 in its outer part and granular B2 in its innermost part. This
502 scheme is very synthetic and generalized, and some taxa may have only part of these layers
503 and /or sublayers.

504

505 3.1.2. At the level of order and/or family: qualitative descriptions

506 According to the presence-absence of layers and/or sublayers (zones) of cuticles, which are
507 variable in fossil plants studied to date (mainly leaf cuticles), for the ordinary epidermal
508 cuticle cell a simple formula can be proposed, from the simplest one (A2) to complex ones
509 (for instance A1U + A1L + A2U + A2L + B1). Two syntheses are proposed here, one with the
510 presence or absence of A1, A2, B1, B2, selected successively (Fig. 2), one with an HCA
511 developed by software using the combination of all these characters (Fig. 3). In the potential
512 ultrastructural dichotomous key developed 'manually' (Fig. 2), in some cases, the words
513 'wavy, spongy, fibrilous' were added to this formula to enable the distinction from other

514 cuticles. Moreover, it is noticeable that although the number of combinations of layers and
515 sublayers is relatively high, the actual number of cuticle formulae is relatively low (11) and
516 corresponds to taxonomic groups, where two kinds of groups occur: homogeneous and
517 heterogeneous groups, making this formula an excellent tool for taxonomy.

518

519 As a result of Fig. 2, in homogeneous groups (5), each order (or family) contains genera with
520 only one cuticle formula: Coniferales Cheirolepidiaceae, comprising *Pseudofrenelopsis-*
521 *Suturovagina-Hirmeriella*, all with a wavy A1-A2-B1-B2 formula; Coniferales Miroviaceae,
522 comprising *Mirovia*, with a A1U-(A1L)-A2U-(A2L)-B1 formula; Coniferales Araucariaceae,
523 comprising *Nothopheuen-Araucaria-Brachyphyllum*, all with a A2-spongy B1 formula;
524 Caytoniales, comprising *Ruflorinia*, with a A2-B1-B2 formula different between upper and
525 lower cuticles; Czekanowskiales comprising *Arctobaiera-Phoenicopsis*, also with A2-B1-B2
526 formula different between upper and lower cuticles.

527

528 In heterogeneous groups (3), each order contains two groups of formulae (making a total of
529 six): Ginkgoales, comprising *Baiera-Sphenobaiera* with A2-B1 (type I) and *Ginkgo-*
530 *Ginkgoites-Pseudotorellia* (type II) with A1U-A1L-A2-B1; Pteridospermales, comprising
531 *Dichopteris-Komlopteris* with A2 (type I), and *Pachypteris* with A1-A2-B1 (type II) showing
532 differences between upper and lower cuticles; Cycadales comprising *Pseudoctenis-Ticoa*
533 (type I) with A1-A2-B1 and *Restrepophyllum* with A1-B2 (type II). If the ultrastructural unity
534 of cuticles of one order (or family) is reinforced if they have just one cuticle formula, for the
535 separation of an order in two ultrastructural cuticle groups the relationship between cuticle
536 ultrastructure and gross morphology is unclear. Actually, even with two groups it makes such
537 heterogeneity taxonomically inconclusive and it would be effective to increase the number of
538 taxa to accurately determine these formulae. For instance, in Ginkgoales (Guignard et al.

539 2019, this issue), the authors relate that *Ginkgo* and *Ginkgoites* are relatively close in gross
540 morphology (which suits their ultrastructural affinities), while *Pseudotorellia* is different in
541 gross morphology, however the three taxa have the same cuticle ultrastructural formula. It is
542 also the case for *Baiera* which is different from *Sphenobaiera* in gross morphology, but they
543 have the same cuticle ultrastructural formula.

544

545 Using the HCA of the same data and calculated by using data 0–1 (Fig. 3; Appendix A of data
546 in brief article), 52 ordinary epidermal cell cuticles from more than 25 taxa have been
547 considered. As these cuticles are considered individually when lower and upper cuticles are
548 available for one taxon, if their cuticle formula is different in one single taxon, it makes some
549 separations in these taxa. However, the identification of orders-families is similar to Fig. 2.
550 This combination of the presence-absence of layers and sublayers by HCA resulted in 9
551 groups (instead of 11 in Fig. 2; the two ‘missing’ groups corresponded to 2 orders allied with
552 5 other cuticles from other orders), namely: Cycadales type II, Coniferales
553 (Cheirolepidiaceae, Miroviaceae, Araucariaceae), Ginkgoales type I and II, Pteridospermales
554 type I and II, Czekanowskiales and Cycadales type I, plus some other cuticles of the former
555 groups.

556

557 So far as the former studies revealed where the stomatal apparatus could be observed,
558 subsidiary cell cuticles are usually similar to ordinary epidermal cell cuticles in ultrastructure
559 and give the same formula classification. It is not the case for guard cell cuticles, which are
560 usually different from the cuticle of ordinary epidermal cells and subsidiary cells. Although
561 they are rarely observed, owing to their fragility, the guard cell cuticle is of great taxonomic
562 importance as a multidimensional CHAID classification of the taxa could be also proposed.
563 (Fig. 4; Appendix B), using another order of characters than for ordinary epidermal cell

564 cuticles formerly developed in this paper (Fig. 2 with the following order: A1 or not A1, A2 or
565 not A2, B1 or not B1, B2 or not B2). For guard cell cuticles, firstly the cuticle proper A is
566 involved in the presence/absence of the A1U sublayer, then two possibilities occur
567 (presence/or not of the A2 layer, or presence/or not of the A1L sublayer). The cuticular layer
568 B is then involved, with the presence/or not of B1 and B2 layers. In the few taxa where cuticle
569 guard cell details could be observed, Coniferales (Araucariaceae, Cheirolepidiaceae; Carrizo
570 et al. 2019a this issue, Mairot et al. 2014, Yang et al. 2018, Yang et al. 2009, Guignard et al.
571 2017), and Pteridospermales (Guignard et al. 2004, Thévenard et al. 2005) only one type of
572 guard cell cuticle formula can be distinguished. Ginkgoales (Guignard and Zhou 2005,
573 Guignard et al. 2016, Del Fueyo et al. 2013, Wang et al. 2005, Guignard et al. 2019 this issue)
574 showed two possibilities (group 1 with A2 and B1 layers, group 2 with A1U and A1L, A2,
575 B1), and it is noticeable that two groups could be divided as those with ordinary epidermal
576 cell cuticles OEC (*Baiera* and *Sphenobaiera*, *Ginkgo-Ginkgoites-Pseudotorellia*) (Guignard et
577 al. 2019, this issue). As only one taxon (*Ginkgoites skottsbergii* which is referred to a form
578 genus of Ginkgoales) does not follow this scheme (it is grouped with *Baiera* and
579 *Sphenobaiera*), attention should be given to guard cell cuticles in the future.

580

581 3.1.3. At the level of the genus and species: quantitative description and statistical values

582 Cuticle ultrastructures and measurements are related to the creation of a taxon, its description,
583 and distinction from related taxa where, generally, values are largely involved. According to
584 the International Code of Nomenclature for Algae, Fungi, and Plants [2018 Shenzhen; see also
585 the IOP International organization of Palaeobotany:
586 <https://www.palaeobotany.org/page/nomenclature/nomenclature-typification-and-correct->
587 [description-of-fossil-plants](https://www.palaeobotany.org/page/nomenclature/nomenclature-typification-and-correct-description-of-fossil-plants), points 2. Correct description of new (fossil) plant taxa (genera,
588 species) and new combinations 2.1 General rules], a new taxon name must be accompanied

589 by a description or diagnosis (or by a reference to a previously published description or
590 diagnosis) (Art. 32.1d), a diagnosis of a taxon being ‘a statement of that which in the opinion
591 of its author distinguishes the taxon from other taxa’ (Art. 32.2). In the ‘recommendations’,
592 three points are of great interest as they state the level of importance attributed to the
593 characters used for a new taxon: 1) the diagnosis should be concise and based on the most
594 important characters only; 2) the description should include all additional data of secondary
595 importance (i.e. not included in the diagnosis), such as measurements; 3) a discussion with
596 comparison to other species; this should provide the differential characters in which the new
597 species differs from other species, at least in the same genus. Furthermore, a fourth point
598 should be added to these three formal points: in Palaeobotany the use of diagnosis emendation
599 also occurs, corresponding (in the case of cuticle ultrastructure) to a change of definition
600 thanks to newly observed characters compared with the original ‘older’ diagnosis, related to
601 the use of new methods of study.

602 Although there are different opinions as regard to the taxonomic value of fossil plant cuticle
603 ultrastructural features, which may include simple evaluations of layer and sublayer thickness,
604 their percentages, to more elaborate measurements with statistical evaluations (confidence
605 interval and other tests) based on a high number of values (up to 30 so far known in this
606 review), recent studies have become more and more clearly shown that the ultrastructural
607 features of fossil cuticles may be considered as significant in taxonomy, as any other
608 character.

609

610 First, cuticle ultrastructure characters were included in the diagnosis with the creation of the
611 genus *Squamastrobis* by Archangelsky and Del Fueyo (1989). After that, such important
612 characters have been given in the diagnosis of two conifers (*Morenoa fertilis*, Del Fueyo et al.
613 1990; *Glenrosa nanjinensis*, Zhou et al. 2000), a new Araucariaceae taxon (*Nothopehuen*

614 *brevis*; Del Fueyo 1991) or with araucarian affinity (*Brachyphyllum garciarum*, Carrizo et al,
615 2019 this issue), three new cycads (*Pseudecten ornata*, Archangelsky et al. 1995;
616 *Mesosingeria oblonga* and *Ticoa lanceolata*, Villar de Seoane 2005), three new Bennettiales
617 (*Otozamites ornatus*, Villar de Seoane 1999; *Otozamites patagonicus*, Villar de Seoane 2001;
618 *Ptilophyllum eminelidarum* Carrizo et al. 2019b), and one Pteridospermatophyta (*Ruflorinia*
619 *orlandoi*; Carrizo et al. 2014). Not only cuticle ultrastructure characters of vegetative organs,
620 but also those of the reproductive material (megaspore membranes) have also been
621 incorporated into the diagnosis (*Karkeniania* of Ginkgoales, Zhou et al. 2002; Del Fueyo and
622 Archangelsky, 2001).

623

624 Second, the description of a new taxon: a new genus (*Tarphyderma*; Archangelsky and Taylor
625 1986) was created, including after the diagnosis a description of the ‘cuticle ultrastructure’.
626 Artabe et al. (1991) included the cuticle ultrastructure of *Kurtziana brandmayri* in their
627 description; the description was used in 1992 for ‘complementary details’ of *Squamastrobis*
628 *tigrensis* (Barale et al. 1992); for a new combination (*Pachypteris bagualensis*) a cuticle
629 ultrastructural description was included (Baldoni and Barale 1996); Passalia et al. (2010),
630 created a new genus with a new species (*Restrepophyllum chiguoides*), and the cuticle
631 ultrastructure was included in their ‘description of the leaf cuticular membrane ultrastructure’.
632 Description was also detailed with three species of Miroviaceae (Coniferales; Nosova et al.
633 2016). Connected with the diagnosis of *Ptilophyllum eminelidarum* (Carrizo et al. 2019b), the
634 authors also provided a precise description of the layers of the cuticles.

635

636 Although many other papers do not include fine ultrastructural details in the definition of a
637 taxon, these papers enhance their importance for taxonomy in the ‘results’ or ‘discussion’
638 sections. This was the case for two Czekanowskiales (Zhou and Guignard 1998), with details

639 of their affinities and specificities. For *Pachypteris indica* and *Komlopteris indica* (Bajpai and
640 Maheshwari, 2000), the ultrastructure was described in ‘observations’ and ‘comparisons’
641 sections. For *Dichopteris* (Pteridosperm; Thévenard et al. 2005), the ‘cuticle microscopy’
642 occurred in the ‘descriptions’ section, whereas it was placed in ‘results’ section for
643 *Sphenobaiera huangii* (Ginkgoales; Wang et al. 2005). This was also the case for *Pachypteris*
644 (Guignard et al., 2004), *Pseudofrenelopsis*, and *Hirmeriella* (Cheirolepidiaceae, Guignard et
645 al. 1998, Yang et al. 2009), and *Ginkgoites* (Ginkgoales, Del Fueyo et al. 2013). Reproductive
646 material is also relevant in this field (fertile Podocarpaceae, Archangelsky and Del Fueyo
647 1987; corystospermaceous ovules, Barale et al. 2009).

648

649 Third, the use of ultrastructural comparisons between several taxa of the same group also exist
650 in single papers, with specificities and/or affinities enhanced, at various taxonomic levels.
651 Barale and Baldoni (1993) compared the ultrastructure of five species of Bennettitales; for
652 four Umkomasiales (Labe and Barale 1996), two species belonged to the same genus
653 (*Pachypteris*); taxonomy was then developed in their discussions, and the homogeneity at the
654 order level was enhanced, as well as specificities. Specificities within one genus were also
655 developed between two species of *Dicroidium* (Corystospermaceae) (Maheshwari and Bajpai,
656 1996a). In 2003, descriptions of six Bennettitales taxa were published, including TEM details,
657 and three ultrastructural groups were proposed (Villar de Seoane 2003).

658

659 In some studies, comparisons with living taxa were developed. Early in 1986, the *Ticoa*
660 *harrisii* cuticle ultrastructure characters were included in a description of this taxon and
661 compared with those of extant plants, mainly following the six types of Holloway (1982)
662 (Archangelsky et al. 1986). In 1989, two fossil cuticles (*Ginkgoites tigrensis* and
663 *Karinopteris*) were described and compared with the cuticle of living *Ginkgo biloba* (Taylor

664 et al. 1989). This living taxon was also compared with *Ginkgoites tigrensis* (Villar de Seoane
665 1997) and fossil *Ginkgo yimaensis* (Guignard and Zhou 2005). Similarly, *Mesodescolea*
666 *plicata* (fossil Cycadales) was compared with the living *Stangeria paradoxa* (Artabe and
667 Archangelsky 1992), and *Restrepophyllum chiguoides* (fossil Cycadales) was compared with
668 living *Zamia* (Passalia et al. 2010).

669

670 Fourth, an emendation of the diagnosis of *Ginkgoites tigrensis* was published, immediately
671 before the description, with the cuticle ultrastructure (Villar de Seoane 1997). The following
672 year, *Tomaxellia biforme* (Cheirolepidiaceae) and *Athrotaxis ungeri* (Taxodiaceae) were
673 compared with two living Taxodiaceae, and an emended diagnosis including ultrastructure
674 was proposed (Villar de Seoane 1998). This was also the case for *Araucaria grandifolia*
675 (Araucariaceae; Del Fueyo and Archangelsky 2002), *Ptilophyllum valvatum* and *Cycadolepis*
676 *coriacea* (Bennettitales; Villar de Seoane 2001), and the new combination of *Ginkgoites*
677 *patagonica* with an ‘emended species diagnosis’ (Villar de seoane et al. 2015).

678

679 Based on data of 41 fossil cuticles studied with TEM, of which absolute values and
680 percentage of characters (23 in total) could be analyzed [selected from the 52 cuticles used in
681 the 0-1 data matrix (Appendices A and B of data in brief article), and their curve tendencies
682 (Appendix C), the correlations between some of them (26) are simple and linear ($y = ax + b$)
683 for about 33% (8 of them: CM mean – A2 mean, A1U straight mean – A2L mean, A2L mean
684 – B1 fibrilous mean, A2U mean – B2 mean, OL mean - TL mean, A% - B%, A2U % - B1
685 fibrilous %, A2L % - B1 fibrilous %), and the remaining cuticles (18) having complex and
686 polynomial correlations (even at the order 6 for A1L % - A2U %, CM mean – A1U straight
687 mean). This is a demonstration of the complexity of the relations between cuticle characters as
688 shown by their fine details. In only 33% of the 41 cuticles, one character can be deduced

689 easily from another one, so although a part of the details are very simply (in mean and %)
690 correlated, most of them have very complex relations. The FCA developed with these 41
691 cuticles and 23 characters (Figs. 5 and 6; Appendix B of data in brief article) showed that,
692 with a very satisfying 93.54% of information on the three first axes (1, 2, 3), six main
693 characters are involved in the distinction between the taxa, i.e. firstly the cuticular layer B
694 (Fig. 5; B %, fibrilous B1%, B2 mean), then the cuticle proper (A % and A2U %), and finally
695 the total mean of the cuticular membrane (characters numbers in Fig. 5: 17, 21–22, 3, 13, 1).
696 At a much lower degree of importance, some other characters are relevant: A mean, A2U
697 mean, A2L mean and % of the cuticle proper; B mean, fibrilous B1 mean, B2% of the
698 cuticular layer (characters numbers in Fig. 5: 2, 23, 14–15, 16, 12, 20). As compared with the
699 former characters, none of the eight A1 characters of the cuticle proper (numbered 4–11 in the
700 figure) showed importance for the distinction between cuticles in this FCA. Concerning the
701 distinction between cuticles (disposed according the absolute values and % of characters; Fig.
702 6), all groups of the HCA (made with qualitative 0-1 presence-absence; Fig. 3) are also
703 relevant (except Cycadales type II, which could not be considered in this FCA): Coniferales
704 (Cheirolepidiaceae, Miroviaceae, Araucariaceae), Ginkgoales type I and II, Pteridospermales
705 type I and II, and Czekanowskiales. For some groups, the distinction is still clear (Coniferales
706 Araucariaceae, Pteridospermales type I, Ginkgoales type I) and explained by the homogeneity
707 and small variability of the absolute values and % of thickness of the constituents in each
708 group. However, this FCA is fundamental as the values and % of the cuticles of the other
709 groups are much more heterogeneous, demonstrating a higher variability, and the positions of
710 the cuticles are finer and inter-linked (especially Ginkgoales type II and Coniferales
711 Cheirolepidiaceae, at a lesser degree Coniferales Miroviaceae, Czekanowskiales,
712 Pteridospermales type II), revealing some possible affinities between these groups.

713

714 The cuticles of Cheirolepidiaceae have been studied in detail with TEM [showing 4 types of
715 cuticles: two *Suturovagina intermedia*, one from less and the other from more xerothermic
716 palaeoenvironments (Mairot et al. 2014, Yang et al. 2018); and two *Pseudofrenelopsis* species
717 also from Cretaceous of China (Yang et al. 2009, Guignard et al. 2017)], multidimensional
718 analyses revealed to be a good taxonomic tool and various points could be enhanced
719 (Appendix D). First, the correlations between the curve tendencies of all Cheirolepidiaceae
720 cuticle characters (Appendix E), are different and more particular to this family than those of
721 all 41 cuticles from various families: for these Cheirolepidiaceae cuticles, among 17
722 correlations, they were simple and linear ($y = ax + b$) only for 3 of them (A2 mean - B2 mean,
723 A% - B%, A2% - B1%), most of them having complex and polynomial correlations (even at
724 the order 6 for A1% - A2%, OL mean - TL mean, A1 mean - A2 mean, CM mean - A1
725 mean). This is a demonstration of the peculiarities of this family (in comparison, the curve
726 tendencies made with all four types of cuticles show eight linear correlations; Appendix C) in
727 which one cuticle character cannot be deduced very easily from another one, except for very
728 few of them (16.7%), and though each cuticle has its own equilibrium of details (in mean and
729 %), enhancing the interest of very delicate observations in this family. Second, from the FCA
730 developed with nine cheirolepidiaceous cuticles (ordinary epidermal cell and subsidiary cell
731 cuticles; 99.44% of information on the first 3 axes; Fig. 7; Appendix F), the importance of 15
732 characters (Fig. 7a) was ordered as follows: percentages are more important for the
733 construction of the first and main axis 1 (especially A% and A2% of the cuticle proper, B%
734 and B1% of the cuticular layer, all with more than 0.2 of contribution on axis 1), whereas
735 means (except CM mean for axis 2) and other percentages are less or not important. Third, the
736 thickness mean of opaque or translucent lamellae of the A1 wavy layer is not important at all
737 for the construction of the axes (contribution of 0 on axis 1). Concerning the relative positions
738 of the nine cuticles, except one (subsidiary cell cuticle of less xerothermic *Suturovagina*

739 *intermedia*), each of the four types was identified relatively clearly by HAC (Fig. 8).
740 Similarly, for the FCA drawn with the ellipse confidences (Fig. 7b), which was more subtle in
741 the relations between the cuticles as some of them had a higher variability in their absolute
742 values and % than did the others. It is interesting to note that confirming the originality of
743 guard cell cuticles GC discussed formerly, the addition in these multidimensional analyses of
744 three guard cell cuticles (HCA with 12 cuticles; Fig. 8b) increased the heterogeneity of the
745 scheme slightly as guard and subsidiary cell cuticles of less xerothermic *S. intermedia* are
746 more clearly separated from the ordinary epidermal cell cuticles of the same type of cuticles
747 and make then a stronger group. This was also true for the FCA with 12 cuticles (with added
748 GC) with slightly less information than the FCA with 9 cuticles (95.22% of information on
749 the 3 first axes; Appendix G), where the weight of the characters inducing the axes was also
750 different: the cuticle proper was the most informative for axis 1 (50.5% of information) with
751 B and B1 means, B% and B1%; the cuticular layer contributed to axis 2 (34.46% of
752 information) with A and A2 means, A1%.

753

754 Recently, Energy Dispersive Spectroscopy (EDS) element analyses were added to
755 ultrastructural features and included in the taxonomic value: *Ginkgoites* (Ginkgoales,
756 Guignard et al. 2016, Del Fueyo et al. 2013), *Suturovagina* and *Pseudofrenelopsis*
757 (Cheirolepidiaceae, Guignard et al. 2017, Yang et al. 2018), *Baiera* and *Sphenobaiera* of
758 Ginkgoales (Guignard et al. 2019 this issue), *Brachyphyllum* of Araucariaceae affinity
759 (Carrizo et al. 2019a, this issue).

760

761

762 3.2 Palaeoenvironmental significances

763 Cuticles have been increasingly intensively studied for various relations with the environment

764 in living plants [Dominguez et al. 2011a and b, 2017; Martin and rose 2014; Khanal and
765 Knoche 2017; Schuster et al. 2017; He et al. 2018; Heredia-Guerrero et al. 2018; particularly
766 in *Arabidopsis*, Kosma et al. 2009 (cited in Heredia-Guerrero et al. 2017); in pear fruit *Pyrus*
767 *communis* L., Serra et al. 2018; in eggplant (*Solanum melongena* L.), Valerga et al. 2019; in
768 sunflower (*Helianthus annuus*), Li et al. 2019; in two *Leucospermum* cultivars (Proteaceae),
769 Suarez et al. 2019; also including (Aragón et al. 2017) in their review of many papers,
770 external and internal environmental factors like fungi, bacteria, viruses, yeasts and insects
771 (Gorb and Gorb 2017) and herbivorous]. From the beginning of TEM fossil cuticle studies,
772 the putative influence of the environment on cuticles has been envisaged; however, at
773 different levels and various degrees of precision. In their TEM study of *Tarphyderma*,
774 Archangelsky and Taylor (1986) discussed ‘xeromorphic features’; however, unfortunately
775 did not include high enlargements. In the same year, Archangelsky et al. (1986), in *Ticoa*,
776 considered the ‘A2 sublayer’ could have ‘some ecological significance’. Taylor et al. (1989)
777 suggested that ‘the overall cuticle thickness differences could be attributed to a number of
778 factors, the most probable of which are ecological...’ in their *Ginkgo* study. Moreover, they
779 began to enhance fine details to determine the possible significance of the lucent lamellae
780 thickness of the A1 layer, evocating ‘ecological’ significance among other potential
781 interactions. Archangelsky (1991) discussed the interest of ultrastructural studies of fossil
782 cuticles in strata of different ages and varied ‘palaeoecological settings’, but unfortunately did
783 not provide details.

784

785 For the first time, Barale and Baldoni (1993), reported in their TEM study on the cuticle
786 ultrastructure of a bennettitalean fossil, a possible relation between cuticle details (their Fig.
787 8) and xerophytism; however, it did not directly concern the whole cuticle but a zone between
788 the polylamellate layer of the cuticle and outermost wax material. Several authors have

789 carried out similar studies: Maheswari and Bajpai (1996b) reported the biochemical
790 degradation of the cuticle of *Thinnfeldia* owing to fungous contamination using TEM
791 analysis. In the TEM study of *Cycas circinalis* (Bajpai, 2000), the possible influence of the
792 environment on the ‘outer polylamellate and the inner reticulate zones’ of the material was
793 discussed. For *Pachypteris indica*, Bajpai and Maheswari (2000) suggested the possible
794 ‘definite’ influence of ‘ecological conditions’. Villar de Seoane (2001) noted that the TEM
795 cuticle details of some Bennettitales (with ‘a compact and uniform layer, and a thick lower
796 layer that may be further divided in two sub-layers’) could be related to the volcanic activity).
797 Passalia et al. (2010) stated that “regardless the cuticular membranes thickness, most of them
798 were adapted to low rates of transpiration because they have an outer lamellate layer...” in
799 studying a fossil cycad (*Restrepophyllum*).

800

801 With the increasing number of studies, comparisons among genera of the same taxa (family or
802 orders) began to emerge and provided higher degrees of precision about the influence of the
803 environment on the cuticle details. These kinds of comparisons had been clearly enhanced
804 early (*Tarphyderma* study; Archangelsky and Taylor, 1986), where the authors suggested to
805 “...compare epidermal and cuticular features from similar taxa that grew in different
806 environments...” The studies of two groups can be evocated here. The first group is
807 Cheirolepidiaceae (Coniferales). All the following cheirolepidiaceans studied in ultrastructure:
808 *Hirmeriella muensteri* (Guignard et al. 1998); *Pseudofrenelopsis dalatzensis* (Yang et al.
809 2009); *Suturovagina intermedia* (Mairot et al. 2014) and Yang et al. (2018); *Pseudofrenelopsis*
810 *gansuensis* (Guignard et al. 2017) exhibited a number of xeromorphic characters, not only a
811 thick cuticle, the presence of a cutinized hypodermis, and other ‘morphological characters that
812 prevent excessive evaporation from the leaves’; but also fine details, including a thick and
813 wavy polylamellate zone (A1 layer) with measurements of opaque and translucent lamellae at

814 the nanometer scale. The second group of taxa with studied cuticles concerns Ginkgoales,
815 where several species and genera were compared. *Ginkgoites ticoensis* (Del Fueyo et al. 2013;
816 their Fig. 4A–4C), showed fibrils of the B1 cuticular layer becoming parallel to the cell
817 surface, interpreted as the volcanic environment effect, as a result of comparisons with living
818 *Pinus* cuticles in the same environment. This was also the case for electron-transparent areas
819 (their Figs. 4E, 4F, 7H) in the cuticle, related with oxalate calcium crystal deposits of
820 volcanic area in the same living *Pinus* material. A last aspect does not directly consider
821 cuticle details, but ‘epicuticular and epistomatal waxes’, which seem to ‘undergo fusion as
822 they are visible in *G. tigrensis* (Villar de Seoane 1997; their Pl. 1, Fig. 5, Pl. 2, Fig. 1)’, also
823 from volcanic environments. In *Ginkgo*, conversely to *Ginkgoites*, the potential effect of the
824 environment on the leaf cuticle ultrastructure seems to be not sensitive, as comparisons
825 between living *G. biloba* and the oldest species *G. yimaensis* in China (Lower Middle
826 Jurassic) concluded that ‘the consistent leaf cuticle ultrastructure of both fossil and living
827 ginkgos indicates that environmental conditions have also little effect’ (Guignard and Zhou
828 2005). In *Baiera* and *Sphenobaiera* (Guignard et al. 2019, this issue; see also Wang et al.
829 2005), although palaeoenvironments were warm and humid for both taxa, *Baiera* (with a
830 thinner cuticle) possibly vegetated under a slightly cooler climate than *Sphenobaiera* (with a
831 more than twice thicker cuticle); this is in congruence with associated flora and EDS elements
832 of the cuticle. The occurrence of a warm and humid, palaeoenvironment (Santa Cruz
833 province, Argentina), inhabited by the parental plants of *Brachyphyllum garciarum* was
834 suggested by Carrizo et al. (2019, this issue) based on the cuticle foliar features. This region
835 was probably quite diverse as for other Argentinian material (San Juan Province), high
836 temperatures and low humidity was suggested for *Zuberia papillata* (Martinez et al.; this
837 issue).

838

839 Theoretically, the most accurate comparisons occur within the same species from different
840 palaeoenvironments, representing extremely rare fossil material; however the characteristics
841 of the palaeoenvironment could be determined through two different studies. These studies
842 could answer at least partly to the questions of Bajpai and Maheshwari (2000), wondering
843 whether species differences (between *Pachypteris indica* and *Komlopteris indica*) were
844 actually species or genus differences, as in their study only one species in each genus was
845 reported.

846

847 The first example is the comparative studies made between specimens of the same species
848 (*Suturovagina intermedia*), from two different localities, and referring to two different macro-
849 paleoenvironments (= palaeoenvironments of the localities sensu lato), versus micro-
850 palaeoenvironment which is the environment just around a plant or a leaf (e.g. differences of
851 sun and wind and rain between upper part and lower part of leaf; differences between the top
852 and the bottom of the plant, between the center and the outer part of a plant especially for
853 plants with many branches like trees... One is more xerothermic (Mairou et al. 2014) and the
854 other is less xerothermic (Yang et al. 2018), as evidenced by the associated floristic elements
855 and the EDS data of the matrix. Although the cuticles are generally similar in microstructure,
856 significant differences in ultrastructure (their Table 3) were observed between them. Those of
857 the leaves from the less xerothermic locality (Yang et al; 2018, their Fig. 12), show not only a
858 reduction of the thickness, but also different proportions of the layers (wavy A1, A2, B1, and
859 B2) of ordinary epidermal and subsidiary cell cuticles. Based on statistics, the authors
860 proposed 12 ultrastructural characters of ecological significance for this species. Although
861 data are obtained from very limited observations, a potential palaeoenvironment significance
862 of FCA axes (from Fig. 7; Appendix G) was proposed here (Fig. 9), taking account of the
863 cuticle ultrastructure of *Suturovagina* from two localities. Among the 15 characters of these

864 analyses, the main characters responsible in construction of the FCA axes are A% and A2% of
865 the cuticle proper, B% and B1% of the cuticular layer. An increase in thickness of the
866 cuticular membrane also indicates a more xerothermic environment (South East China), and it
867 is further related to the thickness proportion of A2 and A1 and also the thickness of B2 layers,
868 whereas B1% decreased as compared with that of less xerothermic (North East China)
869 cuticles (Fig. 9). Furthermore, although they are not important for the construction of the FCA
870 axes, more xerothermic (South East China) palaeoenvironments are related to thicker opaque
871 lamellae of the A1 layer and thinner translucent lamellae. This is a demonstration of the
872 signature of environment, moreover it is also of interest for the probable role(s) and
873 equilibrium played by each layer for the elaboration of the cuticular membrane. It would be
874 very enlightening to obtain more material from the same species in other types of
875 palaeoenvironments.

876

877

878 The second study is different as it compared two kinds of leaves of the same species of
879 *Komlopteris nordenskiöldii* (Nathorst) Barbacka (Guignard et al. 2001) from one single
880 locality. Compared with some living leaf plants related to this fossil taxon, fossil sun and
881 shade leaves (different in their macro-morphologies) were selected, possibly referring to
882 potentially high sensibility to micro-palaeoenvironmental influences within each sun and
883 shade leaf two types (in macro- and microscopy). The sun leaf cuticles of ordinary epidermal
884 cells (more exposed to wind, rain...) were thicker than those of the shade leaf cuticles (less
885 exposed to wind, rain...). Moreover, the sun leaf upper cuticle was more exposed than the
886 lower cuticle. Similar is the case with the shadow leaf. In both types of leaves, therefore, the
887 upper cuticle was thicker than the lower cuticle. In total, there are four types of leaf cuticles
888 with different thickness (sun upper and lower leaf cuticles, shadow upper and lower leaf

889 cuticles; Fig. 10). Under TEM, these cuticles were considerably more elaborate in their
890 ultrastructure. Fibrils, pillar-shape structures made of condensed fibrils, granules, and
891 amorphous material are the constituent parts (their Fig. 1). The proportions of the four types
892 of material show distinct changes in the four types of cuticles, demonstrating the great
893 sensibility of this taxon to micro-palaeoenvironment differences. From shaded lower cuticles
894 (less exposed) to sun upper cuticles (most exposed), a gradual decrease of fibrils (and pillar-
895 shape structure), and gradual increase of amorphous material can be observed. The
896 intermediate cuticles (sun lower and shade upper cuticles) contain more granules, which are
897 fewer or absent in the extreme cuticles (sun upper and shade lower cuticles).

898

899 Finally, ultrastructural studies of cuticles of living plants (Coniferales, genus *Pinus*,
900 Bartiromo et al. 2012; Angiosperms, genus *Erica*, Bartiromo et al. 2013), also from the same
901 species living in two different environments (volcanic/non-volcanic areas) could be
902 significant for comparisons with fossil materials, as the precise data of these environments
903 can be analysed (soil and atmospheric CO₂ concentrations, wind direction...). In *Pinus*
904 *halepensis*, the cuticular membrane CM thickness with different zones of B1 increased in the
905 volcanic area, and the different zones of B1 also varied. However, the cell wall (CW) could be
906 measured for this living material (it is rarely the case with fossil material as it is more or less
907 damaged or absent in most cases). The CM was balanced by a thinner CW, making the whole
908 thickness of CM + CW significantly equal between the two environments. In *Erica arborea*
909 from the same localities, significant differences of CM + CW were found between the two
910 environments, also between the two CW and the two A2 layers. With these two types of
911 living plants, it is clear that the CW also participates in the isolation of the plant. As the CW
912 is rarely observed or entirely preserved in fossils, most often the rare parts with CW do not
913 allow for statistical measurements, so extant cuticle studies provide a great advance in the

914 knowledge of cuticles. In studying cuticle ultrastructure of these living plants, Bartiromo et al.
915 (2012, 2013) mentioned that ‘a tendency to a reduction in the fibrillar structure followed by
916 an increase of the granular component has been noted between non-fumigated and fumigated
917 leaves’. Such relation between granules-fibrils and the environment was cited and discussed
918 in the paper of fossil *Ginkgoites skottsbergii* (Guignard et al. 2016) (having a thicker granular
919 A2 layer and thinner B1 fibrilous layer), which shows a different equilibrium of the layers,
920 compared with the more xerothermic *G. ticoensis* (having a thinner A2 granular layer and a
921 thicker B1 fibrilous layer).

922

923

924 3.3 Evolution implications of fossil plant cuticles

925

926 Plant cuticle evolution is more and more enhanced for various aspects (Niklas et al. 2017,
927 Petit et al. 2017, Renault et al. 2017 and 2018; especially in tomato, Yeats et al. 2012 and
928 Yeats and Rose 2013), and as written by Cohen et al. (2017) “Certainly, the development of
929 plant lipophilic barriers had an immense impact on life on earth and thereby is often
930 considered as one of the most significant evolutionary events” (Kenrick and Crane, 1997;
931 Bateman et al., 1998). However, fossil remains are still scarcely taken in account. When
932 working on plant cuticle ultrastructural features, evolution has been a very attractive topic,
933 enhanced by authors studying fossil plants, from the beginning of TEM papers in fossil
934 cuticles: in their last paragraph of their *Tarphyderma* study, Archangelsky and Taylor (1986)
935 suggested that ‘various structural features were evolving in other major groups of plants in
936 response to the habitat’. The understanding of evolution was also enhanced through the
937 analysis of chemical compounds (Taylor 1999), for the ‘colonization of the terrestrial
938 environment’ (Edwards et al. 1982), and conservation of ‘biosynthetic pathways’ for the

939 'evolutionary radiation' of 'basal land plants' (Jeffree, 2006), and more particularly related to
940 a thickened cuticle in Cactaceae evolution (Ogburn and Edwards 2009). This topic is
941 potentially promising as in living plants recent molecular biology studies in very various taxa
942 enhanced more and more genes related with cuticles, in agronomic plants (fleshy fruits and
943 cereals; Taketa et al. 2008; Alkio et al. 2012; Lashbrooke, et al. 2015; D'Angeli et al. 2016;
944 Wang et al. 2016; Bi et al. 2017; Xu et al. 2018; Zhang et al. 2018; Shi et al. 2018; Belge et al.
945 2019; Cohen et al. 2019; Fasoli et al. 2019; Wang et al. 2019), and model plants (Xu et al.
946 2019, Zhao et al. 2019). Until recently, although cuticles have been known from very old
947 periods and in various taxa [from terrestrial algae (Good and Chapman 1978) to
948 angiosperms], and suggested to have evolutionary significance (Edwards et al. 1982, Riederer
949 2006, Niklas et al. 2017), there are still limited evolutionary data available in fossil plants
950 using fine cuticle details, mainly owing to limited taxa studied; however, some points can be
951 developed.

952

953 Through all orders, considering all the data available for comparisons in the cuticle formulae
954 (Figs. 2 and 3), from the most simple formula to the most complex one, a potential evolution
955 of formula through the orders is not clear at all. For instance, Cycadales (basal Gymnosperms
956 compared with Coniferales) have a simple formula, Ginkgoales (also basal Gymnosperms)
957 have a quite complex formula, while Coniferales have different types of formulae.

958

959 However, there are some interesting clues recently revealed by cuticle ultrastructure studies of
960 fossil cuticles as, at the level of the order, Coniferales can be interestingly evocated. Extant
961 Coniferales are studied more and more (Leslie et al. 2015; Rothwell et al. 2018) in phylogeny
962 through leaf anatomical characters and chloroplast genes (Taxaceae, Elpe et al. 2018;
963 Pinaceae including fossils, Gernandt et al. 2016 and 2018a and b, Saladin et al. 2017;

964 Cupressaceae and other families including fossils, Leslie et al. 2018), but none of these
965 approaches used fine details of the cuticle ultrastructure. Although fossils Coniferales are up
966 to now only represented by four Families in TEM (Araucariaceae, Cheirolepidiaceae,
967 Miroviaceae, Podocarpaceae; Figs. 2 and 3; appendix C), some remarks can be done.
968 Araucariaceae have the simplest formula (A2 and spongy B1) and they emerged in Palaeozoic
969 (Taylor et al. 2009), Triassic (Kunzmann 2007; Stockey 1982 and 1994) putatively in Late
970 Triassic (Axsmith and Ash 2006) while unambiguously in Early Jurassic (Leslie et al. 2018).
971 Cheirolepidiaceae have a more complex cuticle (wavy A1, A2, B1, B2) and they emerged
972 widely speaking later (in Mesozoic, Taylor et al. 2009), more precisely in Late Triassic to Late
973 Cretaceous (Yang et al. 2018). Miroviaceae are the most complex in their cuticle formula
974 (with A1U (+ A1L for some cuticles), A2U (+ A2L for some cuticles), B1 for *Mirovia*
975 *sibirica*) and this species is the youngest, dated to the Early Cretaceous. It seems that,
976 comparing the cuticles at the same age (Early Cretaceous, the age of the localities studied for
977 these three Families), although we have to be very cautious a potential relation between the
978 degree of simplification and emergence period exists, the oldest family having the most
979 simple cuticle and the youngest family having the most complex cuticle. The three-
980 dimensional FCA (Fig. 11), with the combination of 3 axes, made with the absolute values
981 and % of characters (also their absence as evaluated with values "0"), shows clearly with
982 almost 83% data characteristic that the three families are in accord with their emergence
983 period (Fig. 11b; the oldest is on one side of the 3D scheme, the youngest on the opposite
984 side), while Cheirolepidaceae and Miroviaceae are partly merged for some cuticles because of
985 similar values and % of layers along the three axes of the FCA. Although the number of
986 cuticles studied for their fine ultrastructural details is different in each family, they show great
987 differences in their variability of cuticle characters (means and % of characters; appendix B of
988 data in brief article). Araucariaceae are the most stable in their characters values, moreover

989 this stability is reinforced by the other Araucariaceae studied in TEM [Del Fueyo 1991, Del
990 Fueyo and Archangelsky 2002) are from the same age (Lower Cretaceous)], which show the
991 same cuticle formula. Cheirolepidiaceae cuticles show some variations in their cuticular
992 ultrastructural characters values, they are from the same age but quite related with
993 palaeoenvironment at least for some of them. Miroviaceae cuticles show the greatest
994 variations in their character values, they are from different ages and show different cuticle
995 formulae enabling some remarks detailed further for the potential evolution among this
996 family.

997

998 In constructing the preliminary tentative evolutionary scheme of the cuticle ultrastructure in
999 Coniferales, 8 characters (among a total of 23) are much more informative than the others
1000 (Fig. 11a). Firstly, spongy B1 layer of the cuticular layer (its mean and %, numbered 18-19 in
1001 the figure) is the main distinguishing character among taxa, making mainly the F1 Axis (the
1002 most important in significance, 39.9%). Secondly, A1L (its mean, numbered 8) and A2L (its
1003 mean and %, numbered 14-15) of the cuticle proper, making mainly the Axis 2 (second in
1004 importance, 26%) are also of evolutionary significance. Finally, A2U (its mean, numbered 12)
1005 and B2 (its mean and %, named 22-23) of the cuticle proper make mainly the Axis 3 (the third
1006 in importance, 16.6 %).

1007

1008 In this study, the 15 remaining characters (for instance the total cuticular membrane thickness)
1009 are located around the axes and not so important to the evolution scheme tentative of cuticle
1010 ultrastructure. Till now very few studies have been carried out on the evolution of the cuticle
1011 ultrastructure. The present study on the Coniferales is the first attempt to response to Taylor's
1012 (1999) early assertion, writing that "...the cuticle ultrastructure in palaeobotany may also
1013 provide an indirect method of determining how cuticles have evolved.". Similar evolutionary

1014 trend in cuticle structure was observed in another Order, Umkomasiales (Labe and Barale,
1015 1996), where the authors noticed an increase in the complexity of cuticle ultrastructure of 4
1016 taxa from Early to Late Jurassic, but without statistical data.

1017
1018 At the level of the family, the cuticle ultrastructure is variable among genera and species.
1019 However, potential evolutionary trends are also visible. In Miroviaceae (Nosova et al. 2016),
1020 three species of the genus *Mirovia* from different ages were studied by using TEM: *M.*
1021 *kazachstanica* from Early Jurassic (Toarcian), *M. macrophylla* from Middle Jurassic
1022 (Bajocian) and *M. sibirica* from Early Cretaceous (Berriasian-Valanginian). Although it is not
1023 at all the remarks of these authors, as for the three Families of Coniferales just evocated, the
1024 complexity of the cuticle ultrastructure of these three Miroviaceae species is increasing
1025 through time: A1, A2 and B layers visible in the oldest species; A1, A2U and A2L, B in the
1026 younger Middle Jurassic species; A1U and A1L, A2U and A2L, B in the youngest species.

1027
1028
1029
1030 Secondly, another very different Family case concerns Ginkgos, where macromorphological
1031 (gross morphology...) characters were studied in phylogeny (Hasebe 1997; De Franceschi and
1032 Vozenin-Serra 2000), more precisely for TEM ultrastructure of reproductive organs (Rohr
1033 1997). In 2003, allied with some studies (Zheng and Zhou 2004) comparing reproductive
1034 characters (ovules) and vegetative characters (leaves) between several ginkgos from different
1035 ages (from 170 million years ago to living *Ginkgo biloba*), Zhou and Zheng related a possible
1036 case of peramorphosis, which is a case of heterochrony. Using the same material than Zhou
1037 and Zheng (2003), cuticle ultrastructural details (the different layers, their values and
1038 percentages), allied with macromorphological characters of ginkgos, give some fruitful details
1039 within *Ginkgo* (Guignard and Zhou 2005). As result of comparisons between the oldest

1040 *Ginkgo* in China (*G. yimaensis*, 170 millions of years) and living *G. biloba*, male and female),
1041 if the cuticle ultrastructure of these leaves show the same layers of cuticles (A1U + A1L, A2,
1042 B1) allowing a characteristic of this genus, between the two taxa separated by 170 millions of
1043 years, only few differences in the thicknesses and % of each layer were revealed by statistical
1044 measurements, showing a high stability and though a very small degree of potential Evolution
1045 compared with the changes of macromorphology of the leaves in the same period. It is
1046 interesting to note that, for the same type of organ, i.e. leaves, macromorphological characters
1047 (as surfaces and lobes of the leaves) did not follow the same scheme than some
1048 micromorphological characters (cuticle ultrastructural characters). Further works are,
1049 therefore, needed to study the cuticles of other fossil species of Ginkgos from the Cretaceous
1050 and younger strata, such as *G. apodes*, *G. jiayinensis*, *G. adiantoides* and *G. cranei* (Zhou and
1051 Zheng 2003; Zhou et al., 2012; Zhou, 2009; Quan et al., 2010; Quan and Zhou, 2010), to
1052 check if there are any changes in ultrastructure of the layers.

1053

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1055

1056 Last point concerns the “adaptation” topics, frequently used in fossil cuticle fine detail studies
1057 and a component of evolution. Generally speaking, cuticles are known to be related with the
1058 arrival of plants on lands: in the introduction of the book on biology of the plant cuticle,
1059 Riederer (2006) enhanced in the “evolution of the plant cuticle” part “... the simultaneous
1060 appearance of cuticles and stomata as evidence for the physiological adaptations to the
1061 colonization of the land...”. In extant plants (Mesembryanthemaceae), Ihlenfeldt and
1062 Hartmann (1982) considered “fine structure of the epidermal wall” (actually including cuticle
1063 according to their figures) as one of the characters to “describe variation in the xeromorphic
1064 type of epidermis”. In fossil Bennettitales TEM cuticle study (Barale and Baldoni 1993),
1065 “adaptation” in a very wide sense is cited.

1066

1067 According to Taylor et al. (2009) in their fossil plants book's glossary, adaptation is "a
1068 structure or feature that performs a particular function and which reveals in increased survival
1069 or reproduction". In extant organisms the definition is quite similar, enhancing the non-
1070 reversibility of adapted features and the transmission to next generations. Adaptation is
1071 usually opposed to accommodation, which is also a change but related with the phenotypic
1072 plasticity, reversible and not transmitted to the next generations. It is clear that in fossil plants
1073 studies these two aspects (adaptation – accommodation) cannot be distinguished as conversely
1074 to extant plants neither changes of environment for one single plant nor reversibility and
1075 transmission to the next generations can be experimented. For instance in living plants where
1076 two different environments for one taxon were studied among two species (Bartirromo et al.
1077 2012, 2013), although the authors observed significant differences between the cuticles of
1078 volcanic and non-volcanic environments, very cautiously they did not use at all "adaptation",
1079 as neither non-reversibility of the changes nor transmission to the next generations was
1080 experimented for an eventual adaptation and/or accommodation case.

1081

1082 In TEM studies of fossil cuticles "adaptation" or "adapted" is actually used particularly in a
1083 very wide sense of unusual features, especially for extreme palaeoenvironments like
1084 xeromorphic or volcanic ones, where unusual cuticle fine details are observed. Haworth and
1085 McElwain (2008, 2009) concentrated on the critics of "ecological functions of "xeromorphic"
1086 adaptations..." including of course cuticles but unfortunately no ultrastructural fine details.
1087 Villar de Seoane (1999), in a Bennettitales TEM study, enhanced "...strategies of adaptation
1088 to the extreme environment..." (volcanic), not especially for the cuticle details but for the
1089 stomatal apparatus characters. Interestingly, ultrastructural cuticle fine details are included in
1090 the remark of Thevenard et al. (2005) about a Pteridospermales taxon (*Dichopteris*), for non-

1091 xeromorphic characters considering this cuticle with no unusual details. It is clear that several
1092 “evolutionary” terms are used in the papers, more or less mixed and apparently in various
1093 meanings, also very linked with palaeoenvironmental considerations. With the increase of
1094 data, these evolutionary aspects seem to become clearer, however as a synthesis of the use of
1095 these terms seems irrelevant, following papers are cited just to illustrate these remarks.

1096

1097 Particularly for volcanic palaeoenvironment, in Cycadales *Ticoa* study Archangelsky et al.
1098 (1986) enhanced for the cuticle ultrastructure to consider “...evolutionary significance of
1099 these features”. In Cycadales too, *Pseudoctenis* TEM cuticle details (Archangelsky et al.
1100 1995) is related potentially with the stress in volcanic areas. In Cycadales (Villar de Seoane
1101 2005), xeromorphy is used for volcanic palaeoenvironment. In Coniferales, *Tarphyderma*
1102 study (Archangelsky and Taylor, 1986) showed a very special stomatal apparatus and
1103 “adaptations” and “structural adaptations in fossil plants” are mentioned. In Bennettitales
1104 (Villar de Seoane 2001), «...xeromorphic characters...» are used to discuss in seven species
1105 study including other features than TEM details. Carrizo et al. (2014) wrote about the “great
1106 success at adapting to the different stressed environments” for a new *Ruflorinia*
1107 (Pteridospermales), through various features including TEM. Recently (Passalia et al. 2010)
1108 used the term “clade” for the study of a new Cycadales, and suggest a “Chigua clade” in
1109 “plant evolution”.

1110

1111 In Cheirolepidiaceae where special characters are usually observed (wavy A1 layer and other
1112 fine details...), xeromorphic features and adaptations are largely cited. Villar de Seoane
1113 (1998), comparing extant taxodiacean and fossil cheirolepidiacean taxa, asking in the
1114 discussion for the “interpretations of the phylogenetic relationships of the Cheirolepidiaceae
1115 with other conifer families...”, suggested in the “relationship” conclusion about “a close

1116 relationship between leaves of the Cheirolepidiaceae and Taxodiaceae”. *Suturovagina* and
1117 *Pseudofrenelopsis* studies (Yang et al. 2009, Mairot et al. 2014, Guignard et al. 2017, Yang et
1118 al. 2018) also cite these words.

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1122 **4 Conclusions and perspectives**

1123 Besides a brief review of the research history and methods of the plant cuticle, the
1124 achievements made in recent 33 years, of cuticle ultrastructure in the study of fossil plants, are
1125 summarized below. Since then, a number of contributions were made to explore the
1126 importance of cuticle ultrastructure in studying the taxonomy, palaeoenvironment and
1127 evolution of fossil plant cuticles (mainly leaf cuticles). TEM cuticle researches are most
1128 commonly applied in taxonomic interpretation of fossil plants. Including in the diagnosis and
1129 description of taxa, characteristic cuticle ultrastructure features are useful in comparison
1130 between different taxa. As it shows in the analyses of the 52 selected cuticles of fossil plant,
1131 simple formulae of ultrastructure characters showing the qualitative differences, i.e. the
1132 presence or absence of different layers and sublayers, could be efficiently applied in
1133 taxonomy at the level of order and/or family. More complex formulae showing the
1134 quantitative differences among 41 selected cuticles (i.e. measurements and statistic methods
1135 and two- and three-dimensional multifactorial analyses; HAC, FCA, CHIAS) could be
1136 efficiently applied in taxonomy at lower taxonomic levels. It should be noted that the
1137 taxonomic importance of ultrastructure characters may be different among orders and families
1138 or within one family, such as the case in the Cheirolepidiaceae. In recent years,
1139 palaeoenvironmental interpretation has become the second topic for the cuticle ultrastructure
1140 studies of taxa and even the same taxon from different localities. In some taxonomical groups,

1141 e.g. Cheirolepidiaceae where cuticle ultrastructural data are rather numerous, the signature of
1142 xeromorphy appears to emerge among taxa at various degrees. It depends obviously on the
1143 nature of the environments (micro- or macro-palaeoenvironment), and also the influences on
1144 different components of the cuticles (or even for instance cell walls, not belonging to cuticle
1145 but of course directly connected to it) are not impartial. In analyses of the cuticle
1146 ultrastructure data of selected taxa, it is of interest to note that some evolutionary trends have
1147 been detected among fossil coniferous group of different geological ages. The oldest family
1148 Araucariaceae yield the simplest ultrastructure, and the younger the more complex. In the
1149 youngest family Miroviaceae, the cuticle ultrastructures of the three species of the genus
1150 *Mirovia*, ranging from Early Jurassic to Early Cretaceous, show progressive complexity. In
1151 Ginkgoaceae, however, the contrary is the case: cuticle ultrastructure of the extant species of
1152 *Ginkgo* shows little difference as compared with that of the Jurassic species. As material
1153 suitable for cuticle ultrastructural study with the available methods is limited, exclusive of
1154 many other plant groups than gymnosperms with thick cuticles, it is still a very young and
1155 developing field in palaeobotany and till now accumulated data, especially those of statistic
1156 analyses, are meager. Nevertheless, the progress so far made in this field is interesting, as it
1157 opens a new window allowing an insight into the subtleties of cuticle ultrastructure, and their
1158 bearing on the taxonomy, palaeoenvironment and evolution of the fossil plant fine details in
1159 the geological ages. Further studies and more sophisticated methods in this field are urgently
1160 awaited.

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1163

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1171

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Caption of plate, tables, figures, appendices

Plate I. Illustrations of the Transmission electron microscopy different types of layers and sublayers from Figure 1. OEC = ordinary epidermal cell cuticle, GC = guard cell cuticle.

1-2. A1 straight polylamellate layer. Note the A2 granular layer below, and extracuticular material over the A1 layer. 1. rare case in *Ginkgoites ticoensis*, OEC. 2. rare case in *Pseudofrenelopsis gansuensis*, OEC. 3-5. A1 wavy polylamellate layer. Note the A2 granular layer below, and extracuticular material over the A1 layer. 3. *Pseudofrenelopsis gansuensis*, OEC. 4. *Suturovagina intermedia*, OEC. 5. *Pseudofrenelopsis gansuensis*, OEC. 6-7. A1U and A1L polylamellate sublayers. Note that the A1 layer is divided in two parts, A1U (U = upper) sublayer (or zone) with straight polylamellae and A1L (L = lower) sublayer (or zone), with fewer lamellae oriented more or less oblique or even rather transversal to the outermost part of the cuticle. 6. *Ginkgoites ticoensis*, OEC. 7. *Ginkgoites ticoensis*, OEC. 8-9. A2 granular layer, homogeneous or heterogeneous. Note the granules of this layer, very homogeneous for A2U (U = upper) or much more heterogeneous for A2L (L = lower). 8. *Pseudofrenelopsis gansuensis*, OEC. 9. *Mirovia sibirica*, OEC, Courtesy Nosova. 10-11. B1 layer, fibrilous or spongy. Note that the fibrils are more or less mixed with delicate granules, they can make a fibrilous scheme or spongy if the concentrations of fibrils is variable and then more or less visible. 10. *Brachyphyllum garciarum*, GC. 11. *Ginkgoites ticoensis*, OEC. 12-14. B2 granular layer. Note the granules of this thin layer in the inner most part of cuticles, directly connected with B1 fibrilous layer just above, above blackish cell remnants as in photo 13. 12-14. *Suturovagina intermedia*, OEC. For other photos of these cuticle taxa, see Del Fueyo et al. 2013 (for *Ginkgoites ticoensis*), Guignard et al. 2017 (*Pseudofrenelopsis gansuensis*), Nosova et al. 2016 (*Mirovia sibirica*), Mairot et al. 2014 and Yang et al. 2018 (*Suturovagina intermedia*), Carrizo et al. 2019 this issue (*Brachyphyllum garciarum*).

Table 1 selected criteria for various methods

1988 Note. Percentages and duration values are indicated when they are provided in the relevant
1989 papers.

1990

1991

1992

1993 Figure 1. Synthetic construction of a fossil cuticle.

1994

1995 Note. This scheme is based on Archangelsky et al. (1986) following Holloway (1982), and
1996 taking account of new data from Guignard and Zhou (2005) and Nosova et al. (2016).

1997

1998 Figure 2. Potential ultrastructural dichotomous key for fossil cuticles.

1999 To appear in colour

2000

2001 Note. a. cuticles with A1, A2 /or not, B1 /or not, B2 /or not

2002 b. cuticles with no A1, A2, B1 /or not, B2 /or not

2003

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2005 Figure 3. HCA made with cuticles formulae

2006 To appear in colour

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2010 Figure 4. CHAID made with guard cell cuticles features.

2011 To appear in colour

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2013 Note. The guard cell cuticle can be made with A1L (upper zone) and A1L (lower zone), A2,
2014 B1 and B2 layers and sub-layers. The present CHAID enables to distinguish each type of the
2015 11 guard cell cuticles according the presence-absence of firstly A1U, then A1L or A2, then B1
2016 or B2.

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2020 Figure 5. FCA three-dimensional representation of 23 cuticle characters, linked with figure 6.

2021 To appear in colour

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2023 Note. Two visualisations of the 3D representation are given, showing the relations between
2024 characters according 2 orientations of the three axes of the FCA. Because of their rarity
2025 among the cuticles, 6 characters are supplementary data: A2L (mean and %), B1 spongy layer
2026 (mean and %), B2 (mean and %). Data are from appendix B of data in brief article.

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2030 Figure 6. FCA three-dimensional representation of 41 cuticles, linked with Figure 5.

2031 To appear in colour

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2033 Note. Two visualisations of the 3D representation are given, showing the relations between
2034 cuticles according 2 orientations of the three axes of the FCA. UC and LC = upper and lower
2035 cuticles. Coniferales are represented in purple colour by : Cheirolepidiaceae (*Suturovagina*
2036 *intermedia* more xerothermic LC, less xerothermic thick cuticle LC, less xerothermic thin
2037 cuticle LC; *Pseudofrenelopsis dalatzensis* LC; *Pseudofrenelopsis gansuensis* LC);

2038 Miroviaceae (*Mirovia kazachtanica* UC and LC; *Mirovia macrophylla* UC and LC; *Mirovia*
2039 *sibirica* UC and LC); Araucariaceae affinity (*Brachyphyllum garciarum* UC and LC).
2040 Ginkgoales type I are represented in light green colour by: *Sphenobaiera huangii* UC and LC;
2041 *Baiera furcata* UC and LC. Ginkgoales type II are represented in dark green colour by:
2042 *Ginkgo biloba* male UC and LC, female UC and LC; *Ginkgo yimaensis* UC and LC;
2043 *Ginkgoites ticoensis* LC; *Ginkgoites skottsbergii* LC; *Pseudotorellia asiatica* UC and LC;
2044 *Pseudotorellia samylinae* UC and LC. Pteridospermales type I are represented in red colour
2045 by *Komlopteris nordenskiöldii* UC and LC sun leaf, *Komlopteris nordenskiöldii* UC and
2046 LC shade leaf; *Dichopteris visianica* UC and LC. Pteridospermale type II are represented in
2047 red colour by; *Pachypteris gradinarui* UC and LC. Czekanowskiales are represented in dark
2048 blue colour by: *Arctobaiera renbaoi* UC and LC; *Phoenicopsis eutyphylla* UC and LC. Data
2049 are from appendix B of data in brief article

2050

2051

2052 Figure 7. Two-dimensional FCA with 15 characters (a) and 9 cuticles from 4 taxa (b),
2053 belonging to 2 types: epidermal and subsidiary cells. Axes 1 and 2.

2054 To appear in colour

2055

2056 Note. mean = mean thickness in μm ; CM = total cuticular membrane thickness mean; Ol and
2057 TL = opaque and translucent lamellae thickness. OEC, SC, GC = ordinary epidermal,
2058 subsidiary, guard cell cuticles. *P. gansuensis* = *Pseudofrenelopsis gansuensis*; *P. dalatzensis*
2059 = *Pseudofrenelopsis dalatzensis*. *S. intermedia* = *Suturovagina intermedia*. more xe. = more
2060 xerothermic, less xe. = less xerothermic, thick c. = thick cuticle, thin c. = thin cuticle

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2064 Figure 8. 2 HCA with 9 (a) and 12 (b) cuticles from 4 taxa, 15 characters.

2065 To appear in colour

2066

2067 Note. OEC = ordinary epidermal cell cuticle; SC = subsidiary cell cuticle, GC = guard cell

2068 cuticle. *P. gansuensis* = *Pseudofrenelopsis gansuensis*; *P. dalatzensis* = *Pseudofrenelopsis*

2069 *dalatzensis*. *S. intermedia* = *Suturovagina intermedia*. more xe. = more xerothermic, less xe. =

2070 less xerothermic, thick c. = thick cuticle, thin c. = thin cuticle

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2073 Figure 9. Potential synthetic palaeoenvironmental scheme for *Suturovagina* cuticles of

2074 Cheirolepidiaceae in China.

2075 Note. Data are from Yang et al. 2018, especially their Fig. 12. The cuticle membrane CM is

2076 made of A1 and A2 layers of the cuticle proper, and B1 and B2 layers of the cuticular layer.

2077 Although of less statistical interest, opaque lamellae OL and translucent lamellae TL of the A1

2078 layer are also represented in these two schemes.

2079 To appear in colour

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2083 Figure 10. Diagram of reconstruction of the four types of cuticles of *Komlopteris* according to

2084 their ultrastructures.

2085 To appear in colour

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2087 Note. The data from Guignard et al. 2001 were used.

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2091 Figure 11. Three-dimensional FCA, potential evolution scheme among Coniferales cuticles.

2092 To appear in colour

2093

2094 Note. a. Representation of the 23 characters. b. 13 cuticles of three Coniferales families.

2095 UC =upper cuticle, LC = lower cuticle. *Brachyphyllum* 1 and 2 = *B. garciarum* UC and LC,

2096 Araucariaceae affinity. *Suturovagina* 1 = *S. intermedia* more xerothermic LC. *Suturovagina* 2

2097 and 3 = *S. intermedia* less xerothermic thick cuticle LC and thin cuticle LC.

2098 *Pseudofrenelopsis* 1 = *P. dalatzensis* LC. *Pseudofrenelopsis* 2 = *P. gansuensis* LC. *Mirovia*

2099 1 and 2 = *M. kazachstanica* UC and LC. *Mirovia* 3 and 4 = *M. macrophylla* UC and LC.

2100 *Mirovia* 5 and 6 = *M. sibirica* UC and LC. For original values and % data of each cuticle, see

2101 appendix C.

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2105 Appendix A. schemes of epidermis

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2108 Appendix B. original data for CHAID guard cell cuticles

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2111 Appendix C. curve tendencies for 41 cuticles

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Appendix D. Cheirolepidiaceae original values and % for 12 cuticles

Appendix E. curve tendencies for 12 Cheirolepidiaceae cuticles

To appear in colour

Appendix F. Cheirolepidiaceae, other axes of FCA with 9 cuticles from 2 types (axes 1-3, 2-3)

To appear in colour

Appendix G. Cheirolepidiaceae, FCA with 12 cuticles from 3 types (axes 1-2, 1-3, 2-3)

Appendices of MethodsX article

Appendix A. embedding technique of plant cuticles, for transmission electron microscopy

To appear in colour

Appendix B. some steps of the sectioning, staining and observation of the plant cuticles, for

transmission electron microscopy

To appear in colour

Appendices of data in brief article

2138 Appendix A. 0-1 data for 52 cuticle formulae

2139 to appear in colour

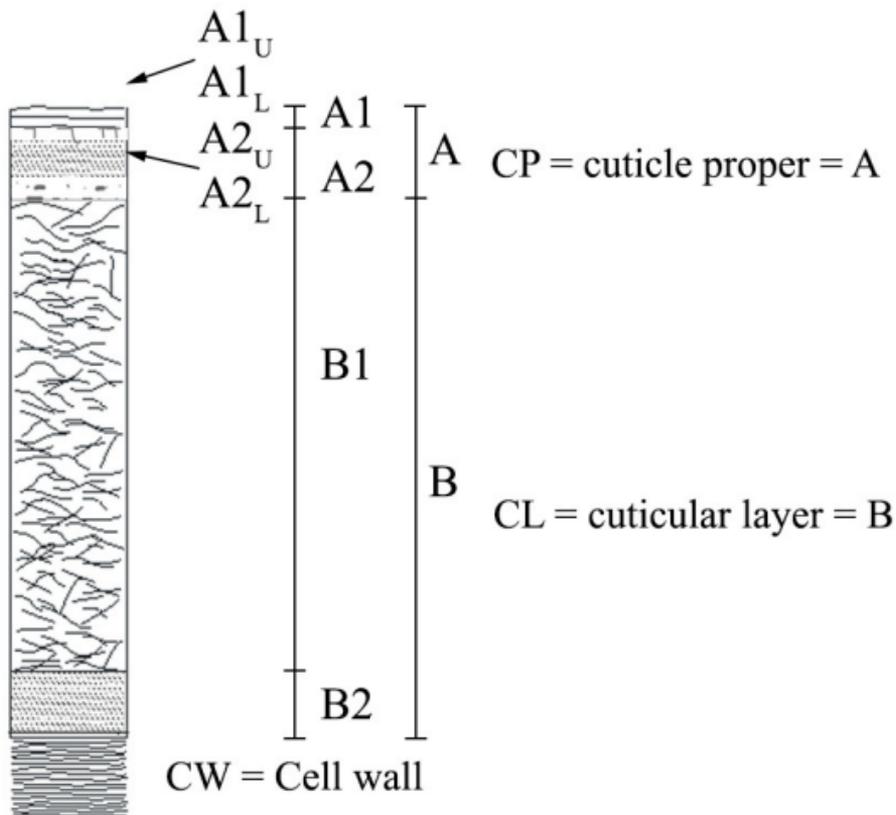
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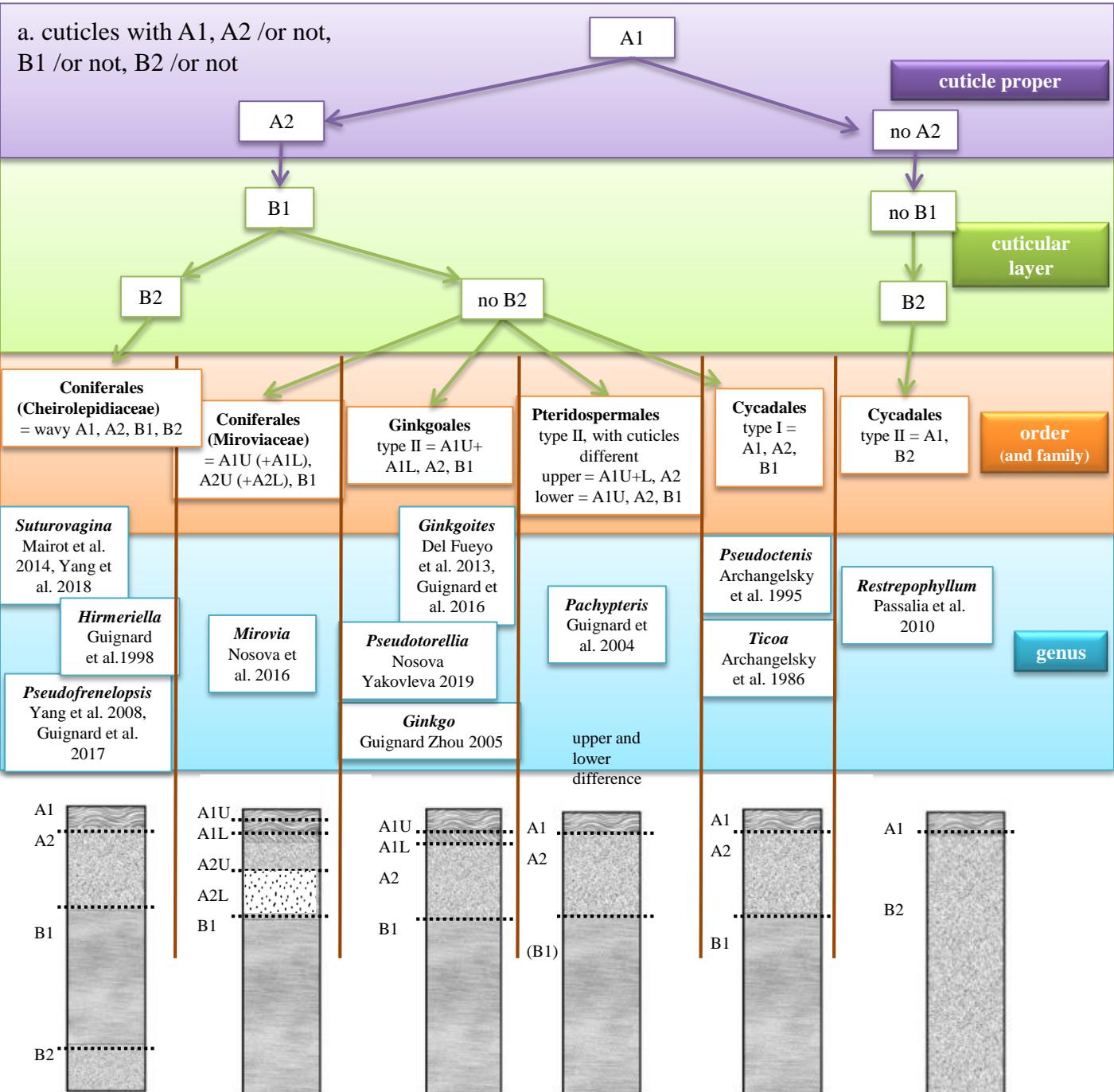
2141 Appendix B. values and % data for the 41 cuticles selected from 0-1 data

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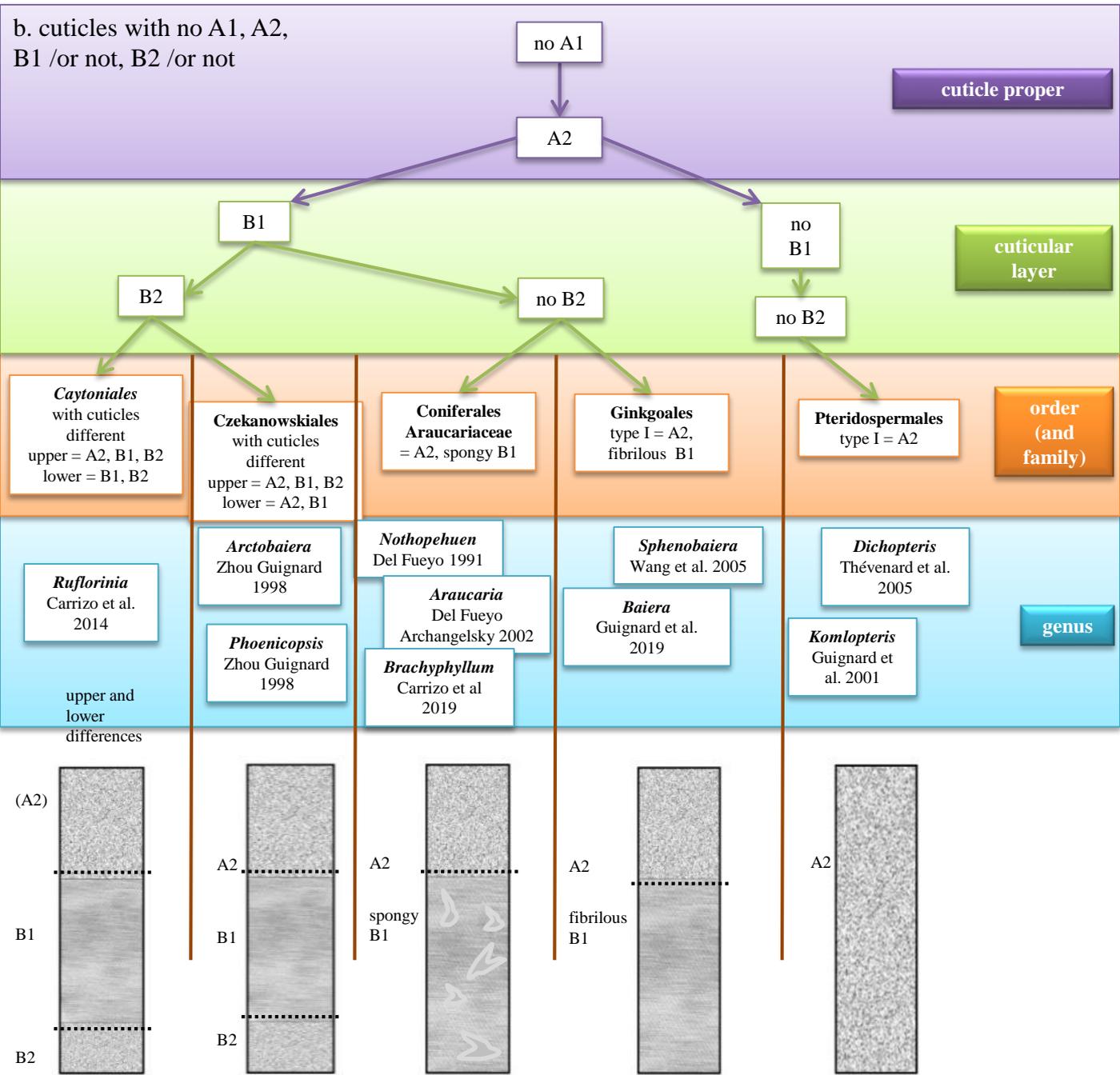
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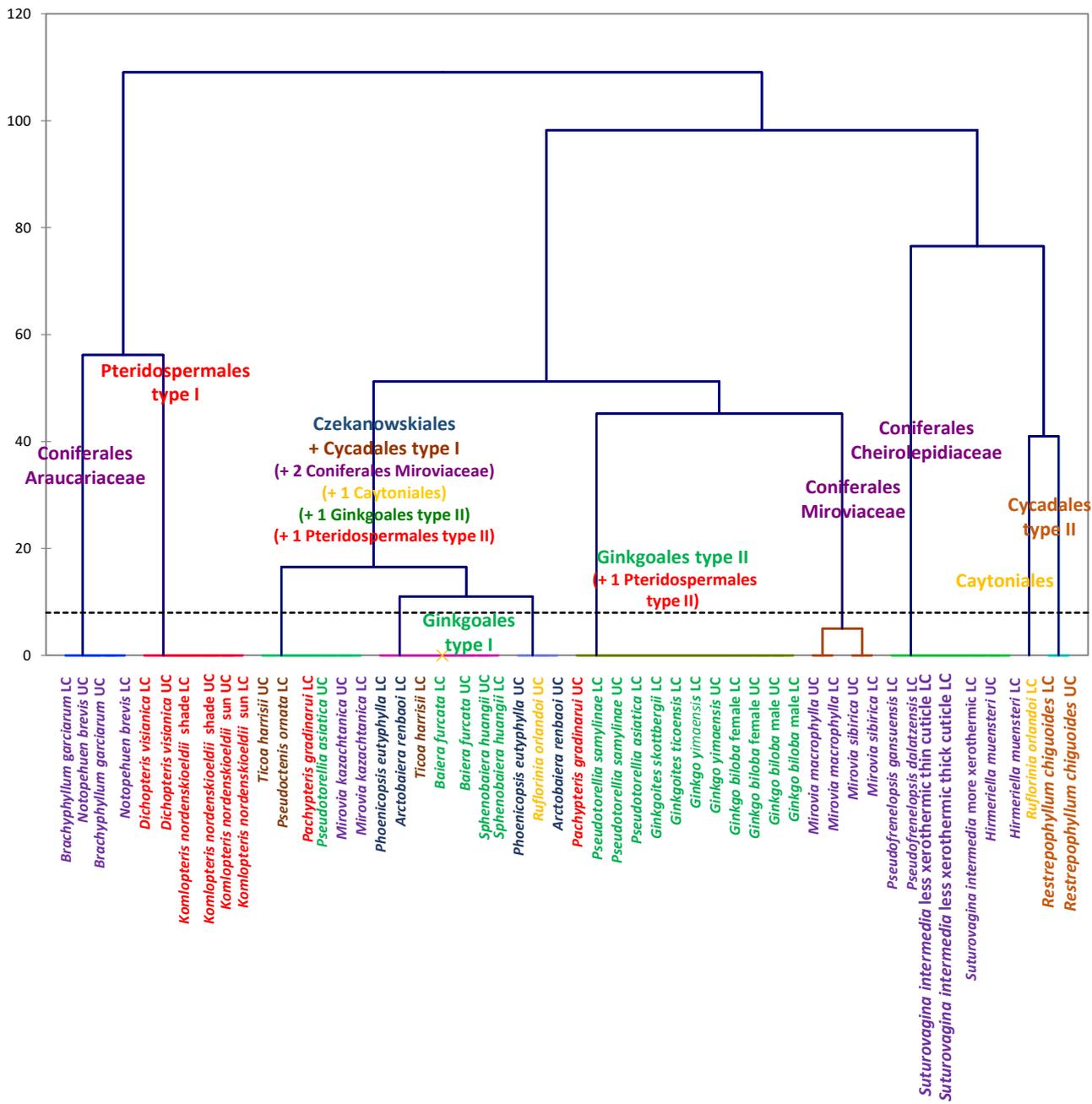
CM = cuticle membrane

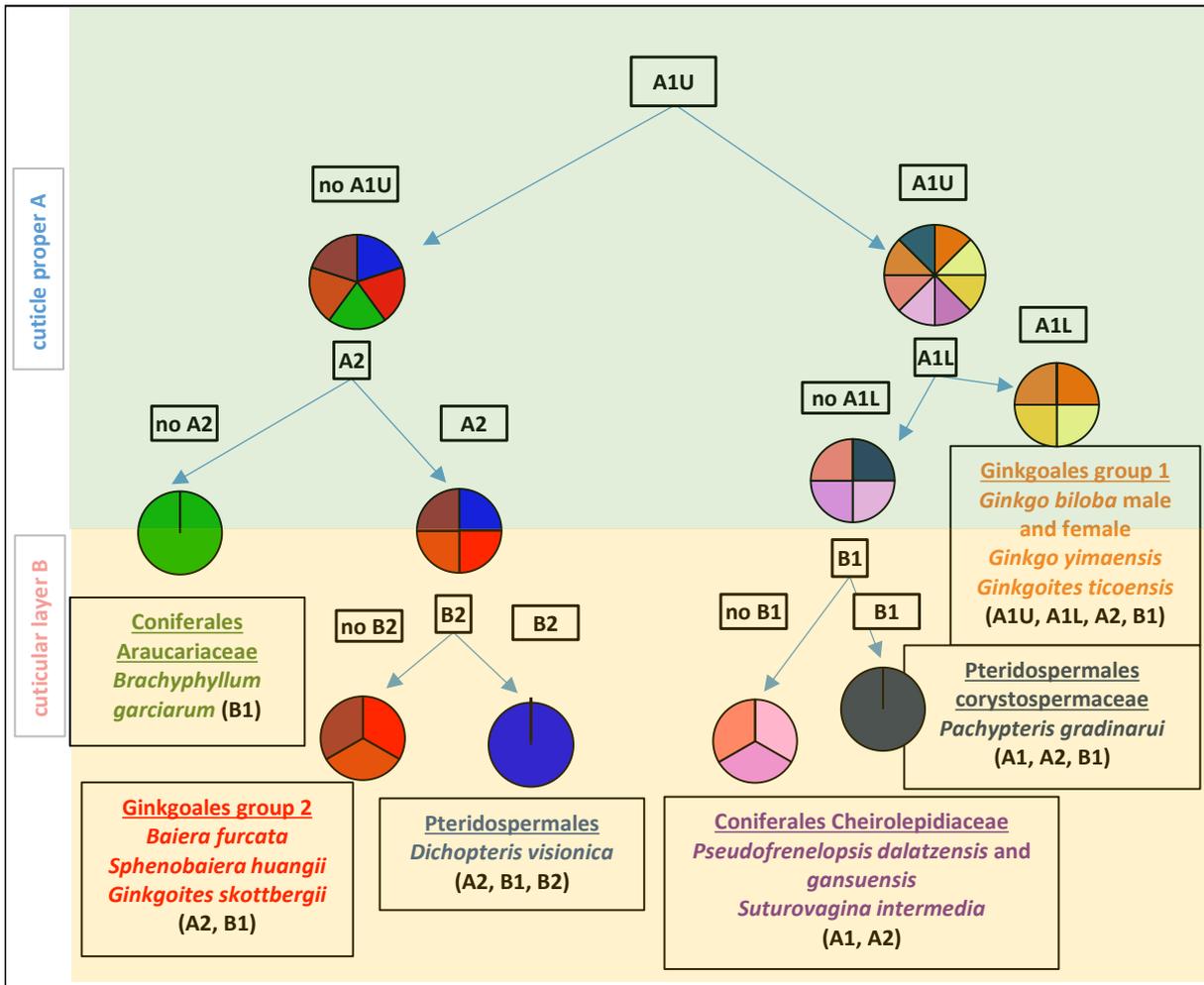


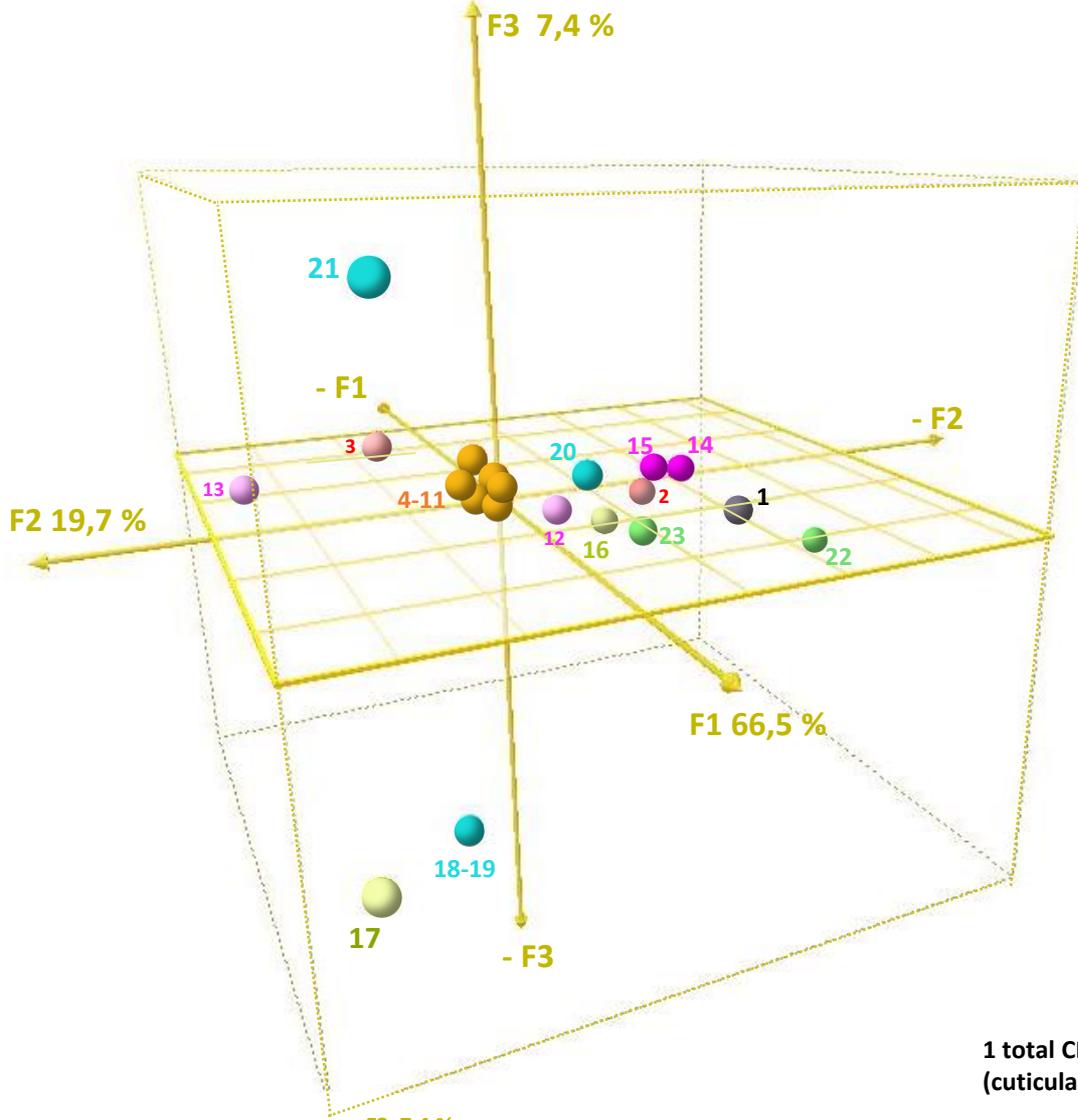


b. cuticles with no A1, A2,
B1 /or not, B2 /or not









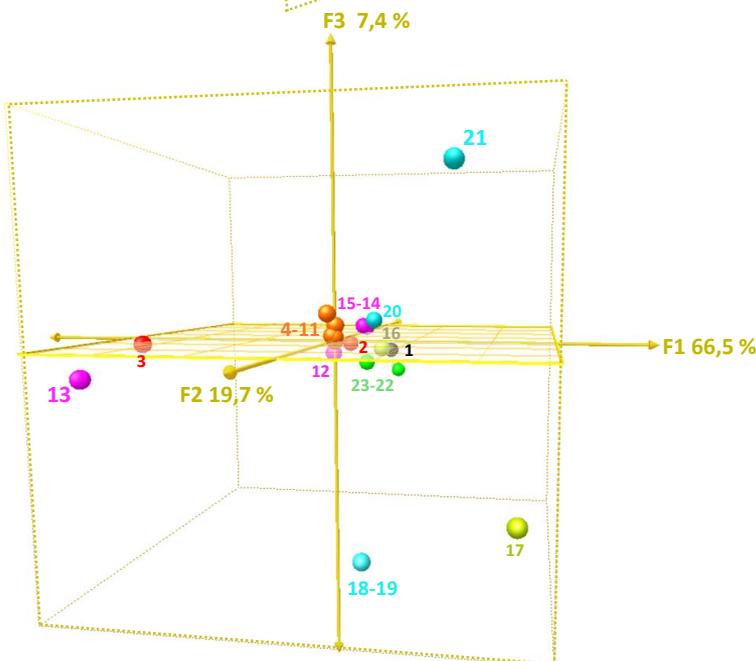
**1 total CM mean
(cuticular membrane)**

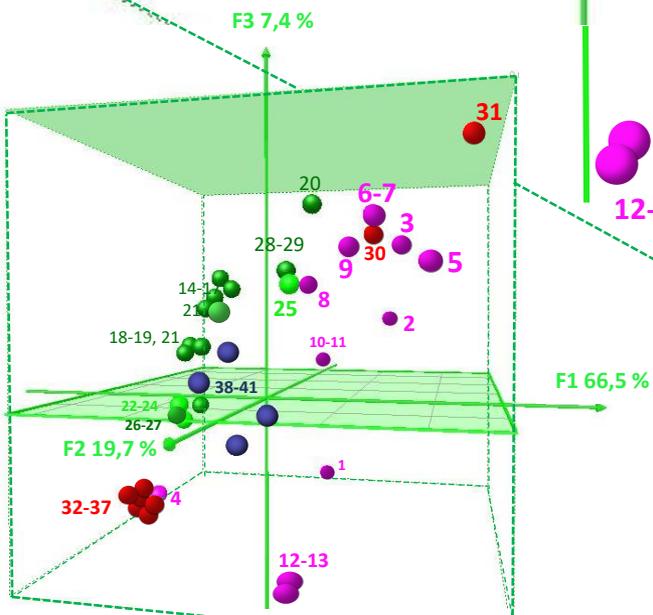
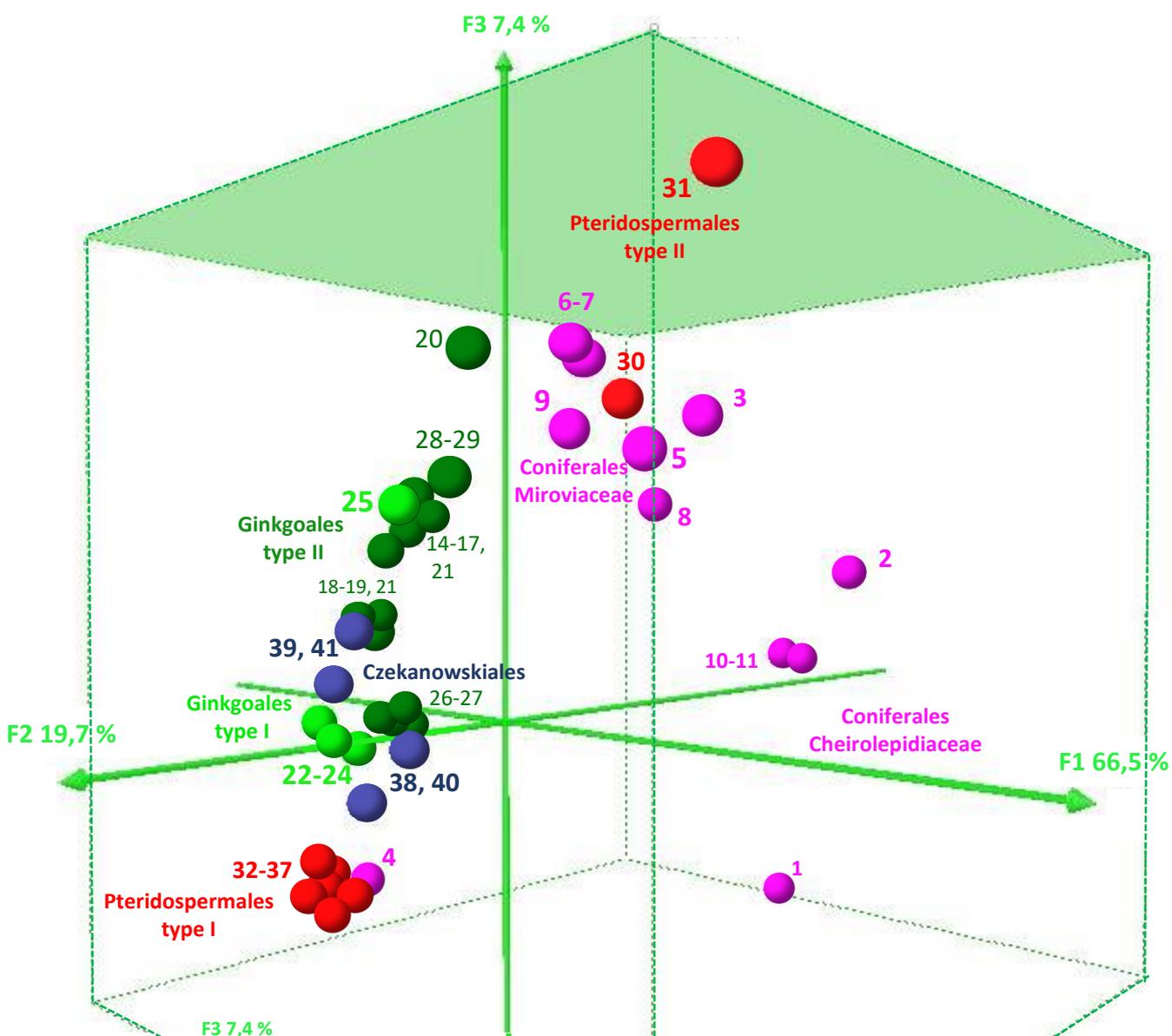
cuticle proper A

- 2, 3 A mean and A %**
- 4, 5 wavy A1U mean and %**
- 6, 7 straight A1U mean and %**
- 8, 9 A1L mean and %**
- 10 OL opaque lamellae of A1 mean in nm**
- 11 TL translucent lamellae of A1 mean in nm**
- 12, 13 A2U mean and %**
- 14, 15 A2L mean and %**

cuticular layer B

- 16, 17 B mean and %**
- 18, 19 spongy B1 mean and %**
- 20, 21 fibrilous B1 mean and %**
- 22, 23 B2 mean and %**





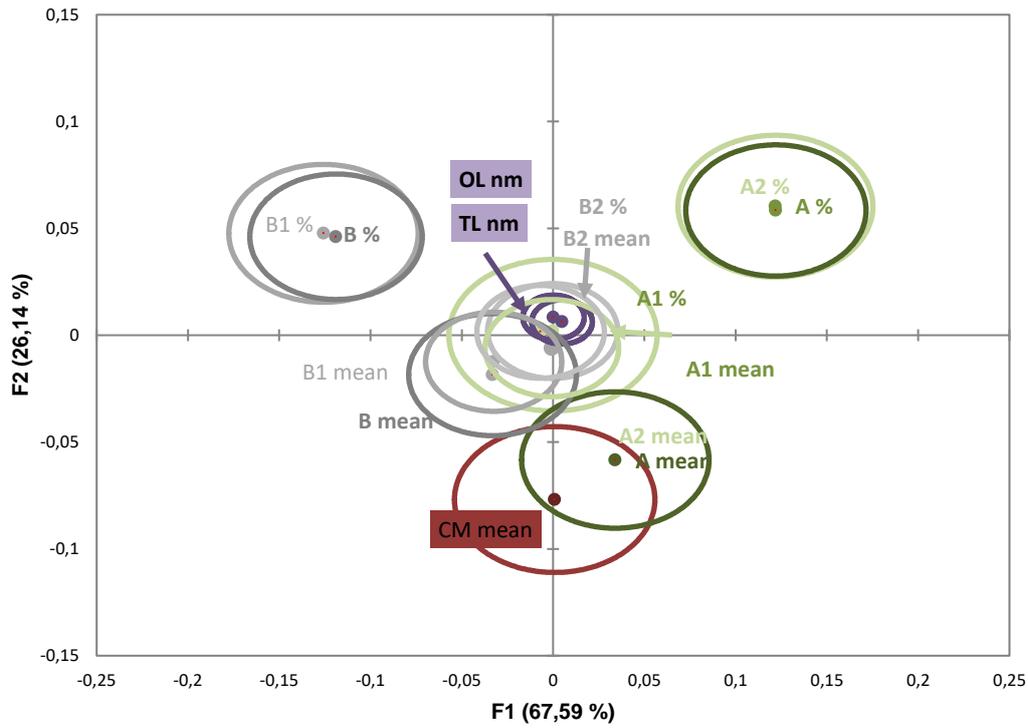
Coniferales
Cheirolepidiaceae 5 cuticles
Miroviaceae 6 cuticles
Araucariaceae 2 cuticles

Ginkgoales
Type I 4 cuticles
Type II 12 cuticles

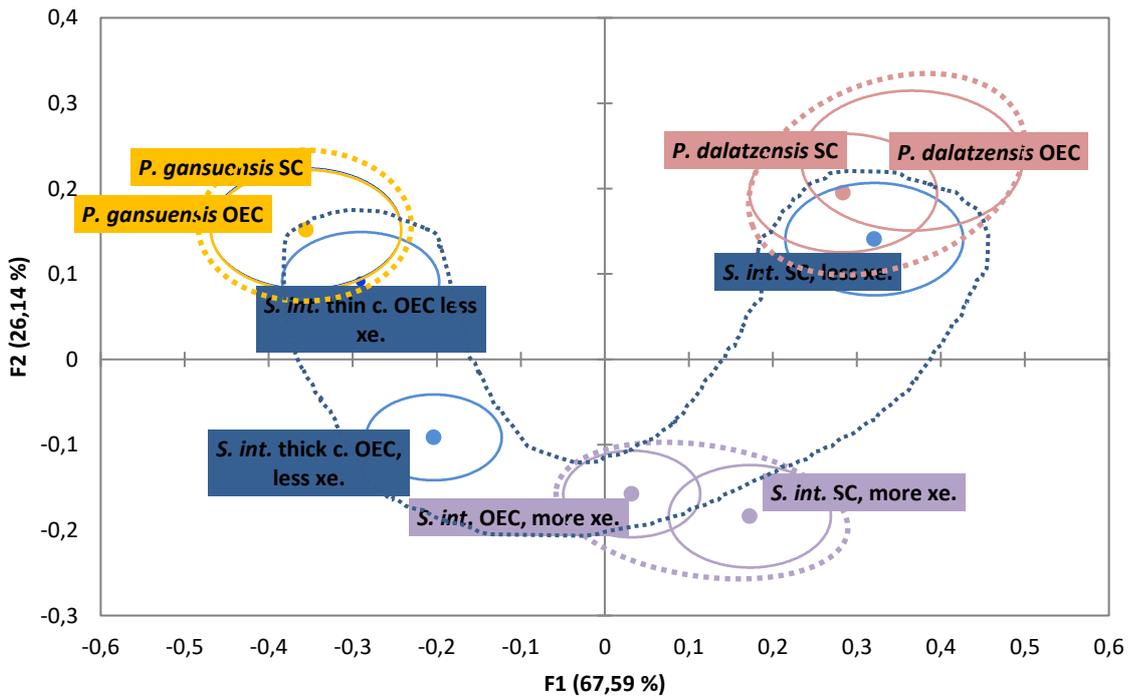
Pteridospermales
Type I 6 cuticles
Type II 2 cuticles

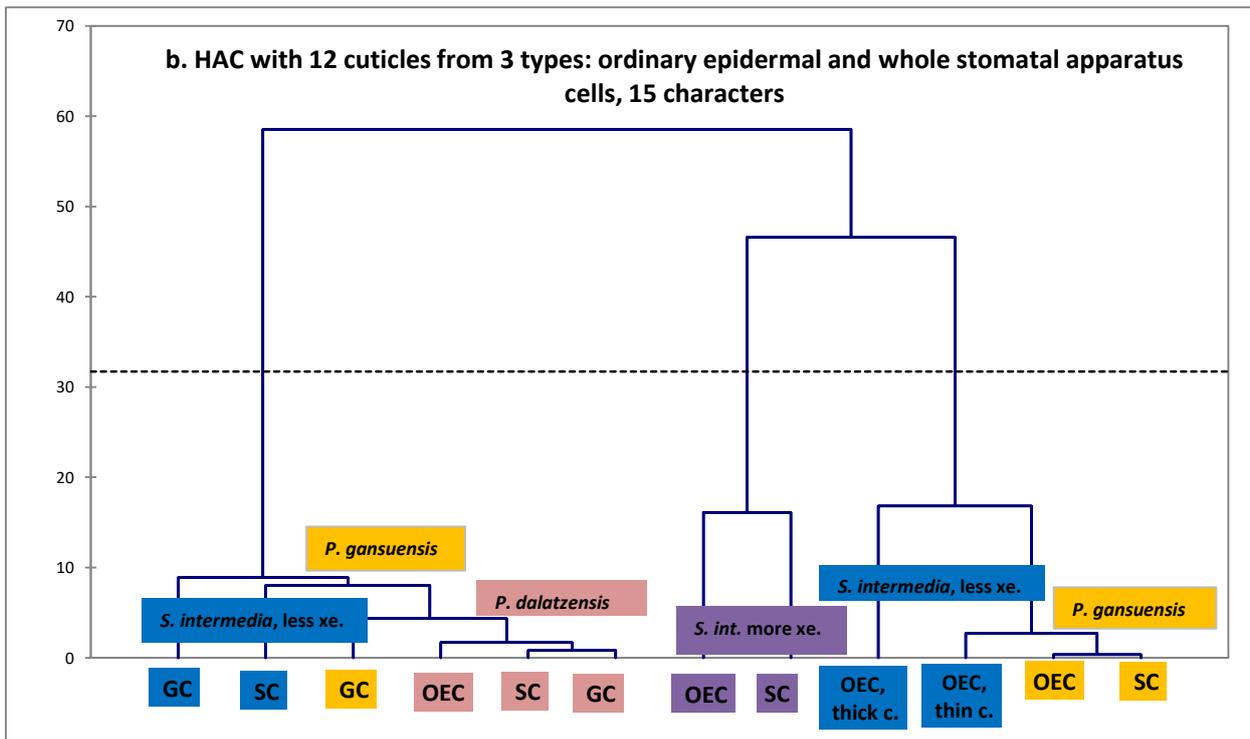
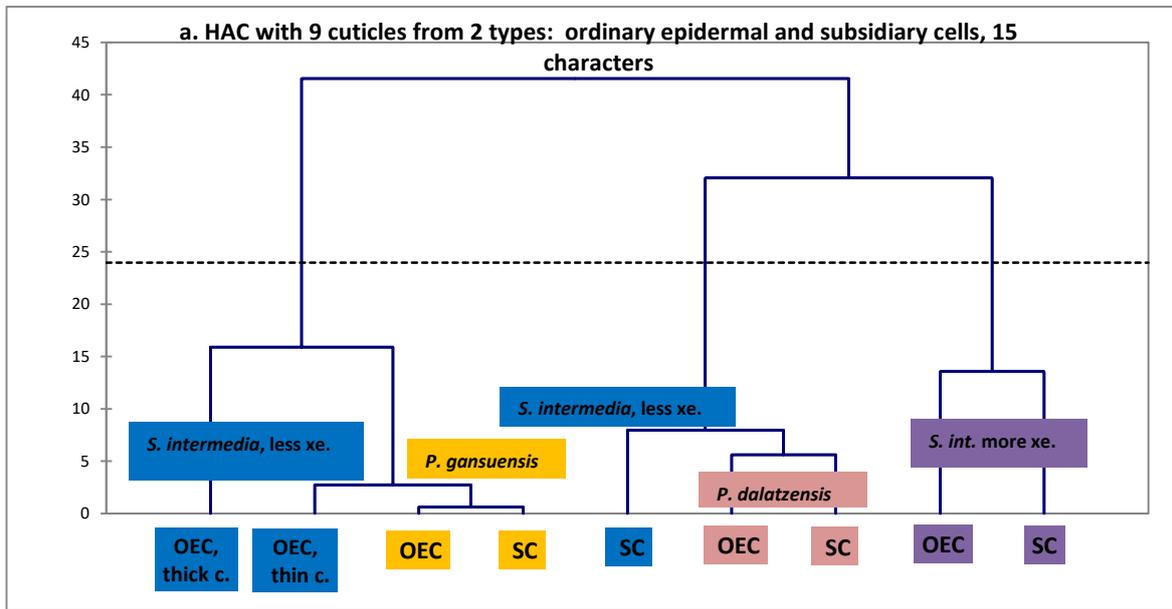
Czekanowskiales
4 cuticles

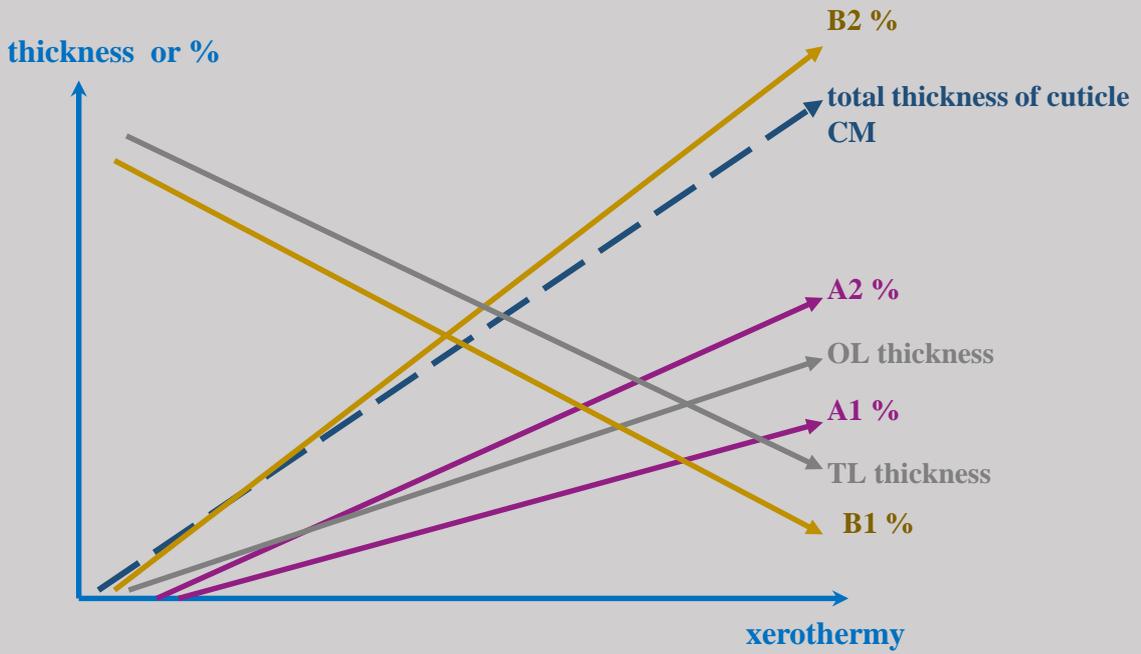
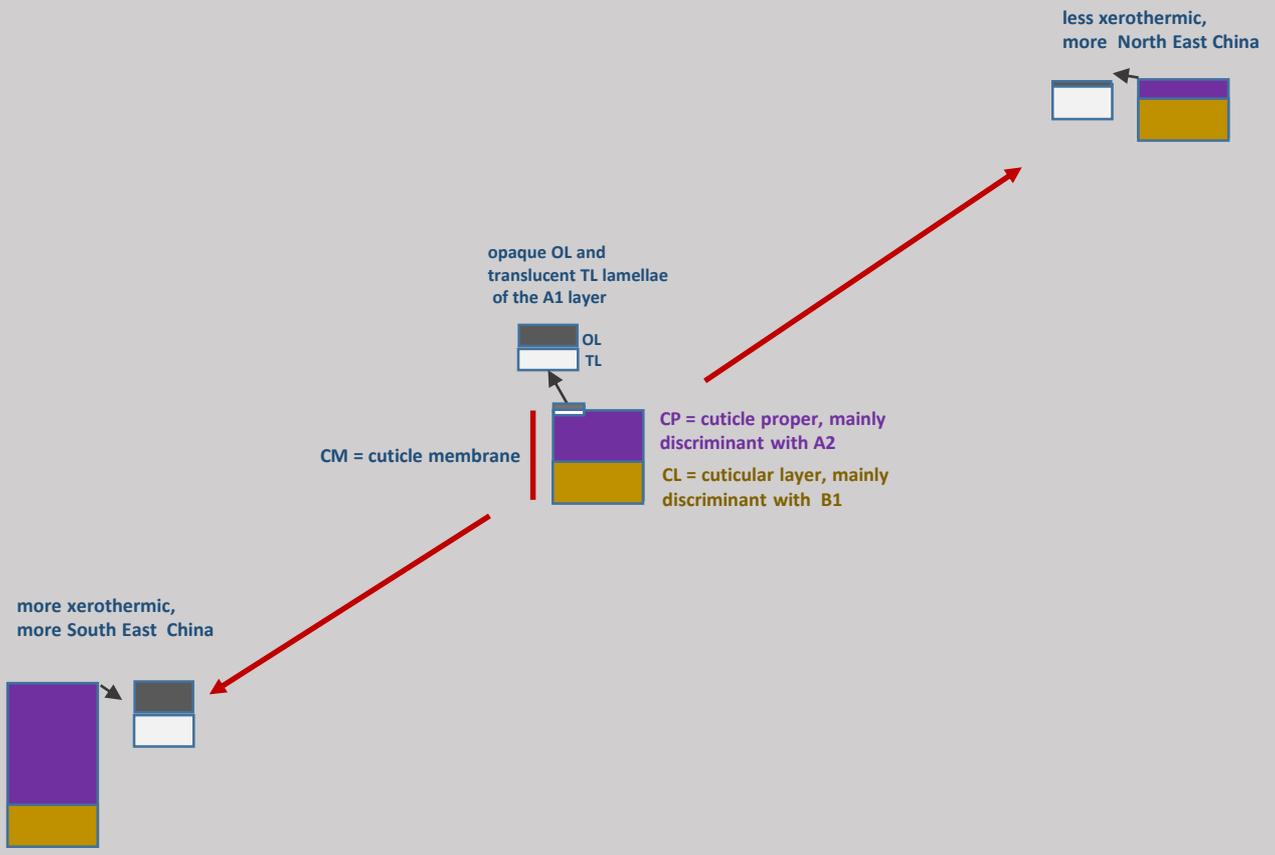
a. Contribution of the 15 characters, 93,72 % of information



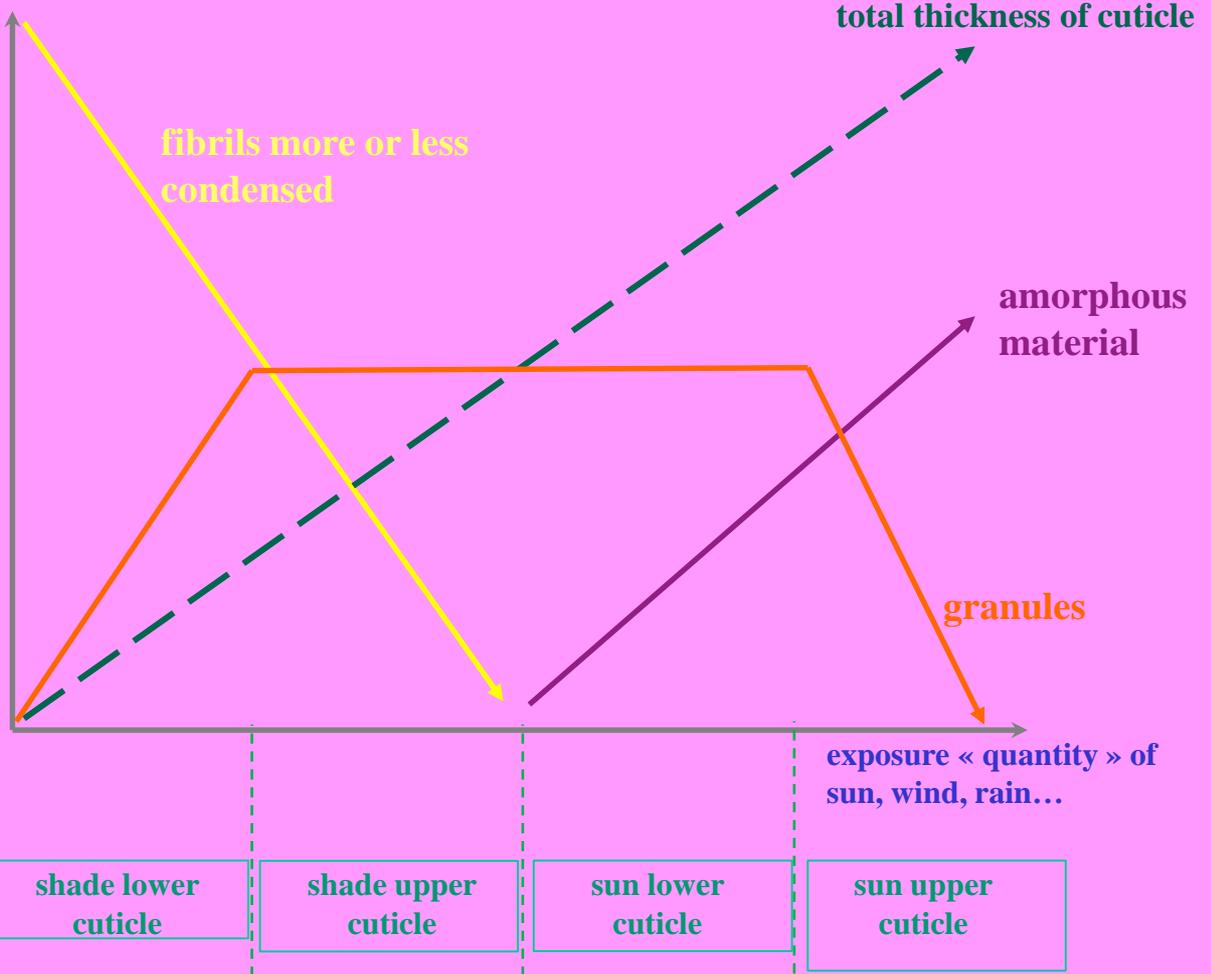
b. The 9 cuticles from 4 taxa belonging to 2 types: ordinary epidermal and subsidiary cells, 93,72 % of information



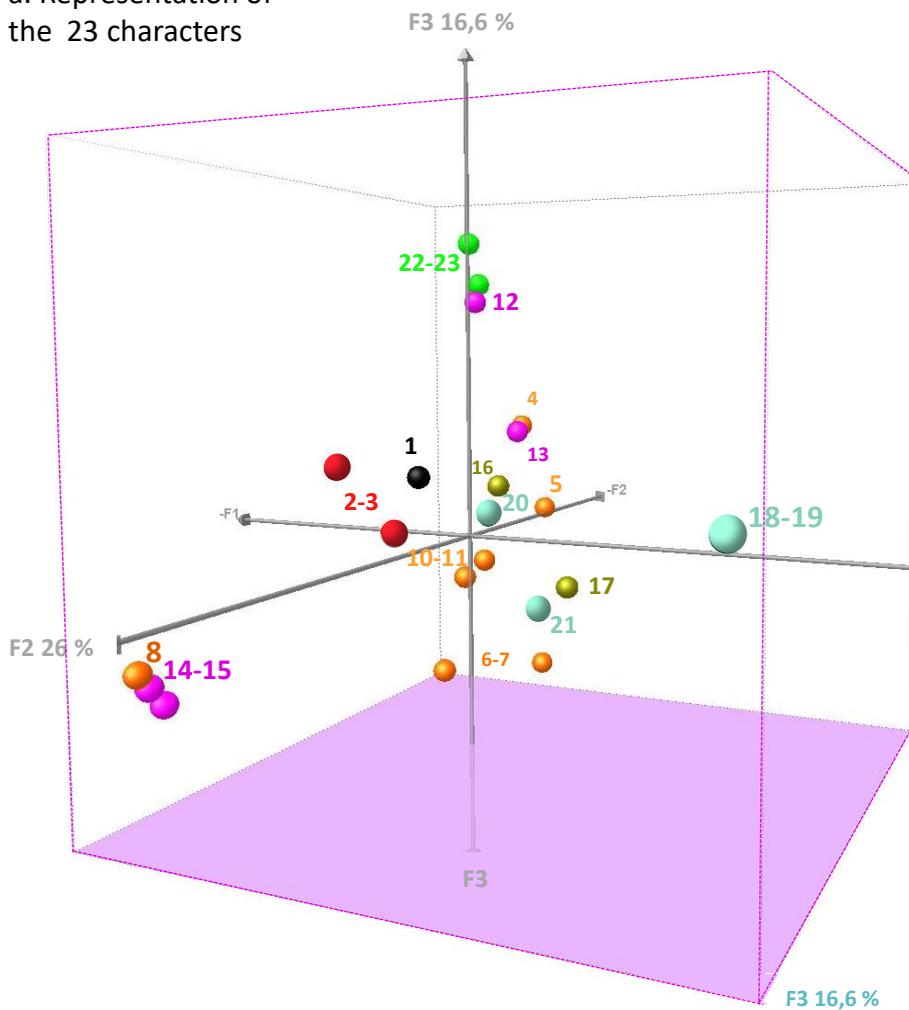




quantity of cuticle



a. Representation of the 23 characters



1 total CM (cuticular membrane) mean

cuticle proper A

- 2, 3 A mean and A %
- 4, 5 wavy A1U mean and %
- 6, 7 straight A1U mean and %
- 8, 9 A1L mean and %
- 10 OL opaque lamellae of A1 mean in nm
- 11 TL translucent lamellae of A1 mean in nm
- 12, 13 A2U mean and %
- 14, 15 A2L mean and %

cuticular layer B

- 16, 17 B mean and %
- 18, 19 spongy B1 mean and %
- 20, 21 fibrilous B1 mean and %
- 22, 23 B2 mean and %

b. 13 cuticles of three Coniferales families

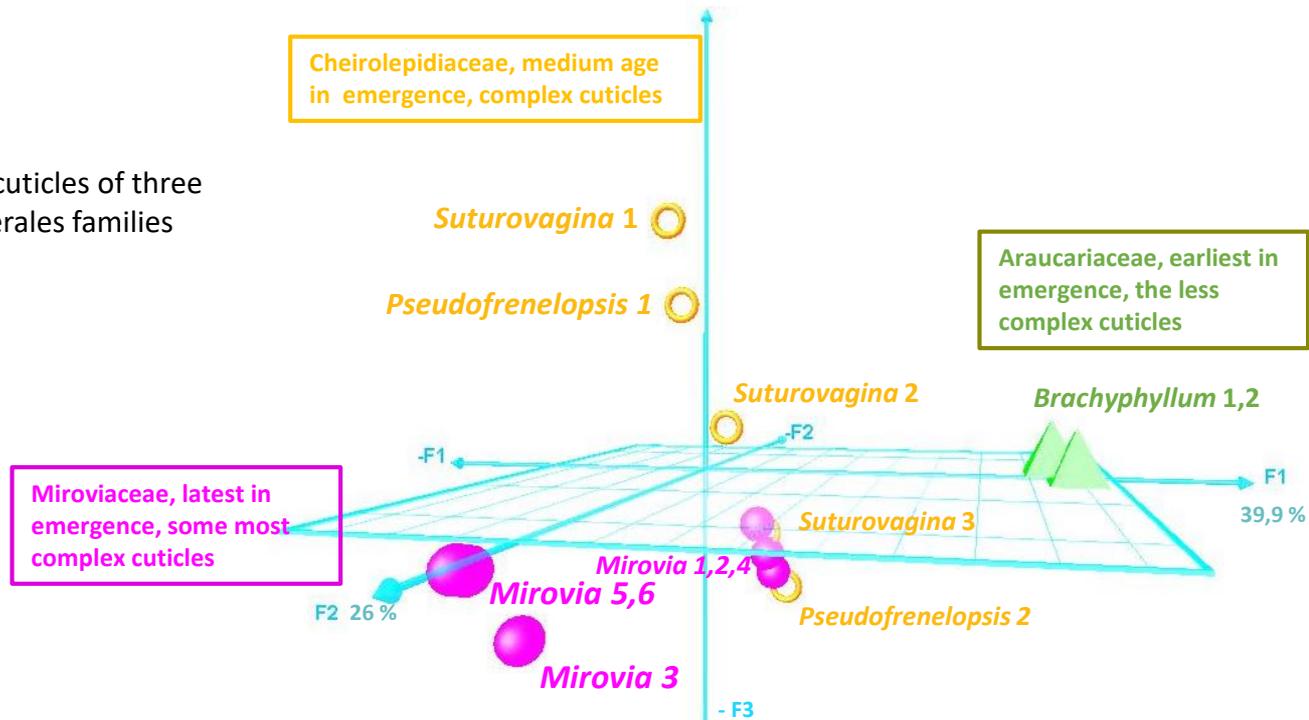


Table 1 selected criteria for various methods

	fixation	postfixation	resin type	staining
Coniferales Cheirolepidiaceae <i>Hirmeriella</i> (Guignard et al. 1998)	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 20 min and lead citrate 20 min
Coniferales Cheirolepidiaceae <i>Pseudofrenelopsis</i> (Yang et al. 2009)	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 min and lead citrate 20 min
Coniferales Cheirolepidiaceae <i>Suturovagina</i> (Mairot et al. 2014)	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 min and lead citrate 20 min
Coniferales Cheirolepidiaceae <i>Pseudofrenelopsis</i> (Guignard et al. 2017)	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 min and lead citrate 20 min
Coniferales Cheirolepidiaceae <i>Suturovagina</i> (Yang et al. 2018)	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 min and lead citrate 20 min
Coniferales <i>Glenrosa</i> Zhou et al. 2000			Spurr and Epon	
Coniferales Podocarpaceae <i>Squamastrobus</i> (Archangelsky and Del Fueyo 1989)		osmium tetraoxide 1%	Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s
Coniferales Podocarpaceae <i>Morenoa</i> (Del Fueyo et al. 1990)		osmium tetraoxide 1%	Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s
Coniferales Podocarpaceae <i>Squamastrobus</i> (Barale et al. 1992)			Spurr	potassium permanganate 1,5% 15 min, uranyl acetate 2% 5 min and lead citrate 2-3 min
Coniferales Taxodiaceae <i>Athrotaxis</i> (Villar de Seoane 1998)			Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s
Coniferales Cheirolepidiaceae <i>Tomaxellia</i> (Villar de Seoane 1998)			Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s
Coniferales Miroviaceae <i>Mirovia</i> (Nosova et al. 2016)		osmium tetraoxide 2%	Epon- araldite	lead citrate 5 min
Coniferales Araucariaceae <i>Araucaria</i> (Del Fueyo and Archangelsky 2002)		osmium tetraoxide 2%	Spurr	lead citrate 1 min and uranyl acetate 10 min
Coniferales Araucariaceae affinity <i>Brachyphyllum</i> (Carrizo et al. 2019)	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 min and lead citrate 20 min
Cycadales <i>Ticoa</i> (Archangelsky et al. 1986)		osmium tetraoxide 1%	Spurr	potassium permanganate 5% 10-30 min, uranyl acetate 2% 5-10min or lead citrate 1-5min
Cycadales <i>Pseudoctenis</i>			Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s

(Archangelsky et al. 1995)				
Cycadales <i>Cycas</i> (Bajpai 2000)	glutaraldehyde 4%	osmium tetraoxide 2%	Spurr	uranyl acetate and lead citrate
Cycadales <i>Mesosingeria, Ticoa</i> (Villar de Seoane 2005)		osmium tetraoxide	Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s
Cycadales <i>Restrepophyllum</i> (Passalia et al. 2010)	glutaraldehyde	osmium tetraoxide 2%	Spurr	lead citrate 1 min and uranyl acetate 10 min
Pteridospermales <i>Pachypteris</i> (Baldoni and Barale 1996)			Spurr	potassium permanganate 1% 10 min, uranyl acetate 2% 5 min, lead citrate 2-3 min
Corystospermaceous taxa <i>Dicroidium</i> (Maheshwari and Bajpai 1996a)	glutaraldehyde 4%	osmium tetraoxide 2%	Spurr	uranyl acetate 15 min and lead citrate 5 min
Pteridospermales <i>Pachypteris, Komlopteris</i> (Bajpai and Maheshwari 2000)	glutaraldehyde 4%	osmium tetraoxide 4%	Spurr	uranyl acetate and lead citrate
Pteridospermales <i>Komlopteris</i> (Guignard et al. 2001)	paraformaldehyde 4%	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 or 20 min and lead citrate 20 min
Corystospermales <i>Pachypteris</i> (Guignard et al. 2004)	paraformaldehyde	osmium tetraoxide	Epon	
Corystospermales <i>Zuberia</i> (Martinez et al. 2019)		osmium tetraoxide 2%	Spurr	potassium permanganate 5-10 min, uranyl acetate 30 sec
Bennettitales <i>Zamites, dictyozamites, Ptilophyllum, Otozamites, Cycadolepis</i> (Barale and Baldoni 1993)		potassium permanganate 1%	Spurr	potassium permanganate 1% 5 min, uranyl acetate 5% 10 min and lead citrate 2 min
Bennettitales <i>Otozamites</i> (Villar de Seoane 1999)			Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s
Bennettitales <i>Otozamites, Pterophyllum, Ptilophyllum, Cycadolepis</i> (Villar de Seoane 2001)			Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s
Bennettitales <i>Dictyozamites, Otozamites, Zamites, Ptilophyllum, Pterophyllum, Cycadolepis, Williamsonia</i> (Villar de Seoane 2003)		osmium tetraoxide	Spurr	potassium permanganate 5-10 min and uranyl acetate 30 s
Bennettitales <i>Ptilophyllum</i> (Carrizo et al. 2019a)	technique of Del Fueyo and Archangelsky 2005	Osmium tetraoxide 2%	Spurr	lead citrate 1 min, uranyl acetate 10 min
Ginkgoales <i>Ginkgoites</i> (Villar de Seoane 1997)			Spurr	
Ginkgoales <i>Ginkgoites</i> (Del Fueyo et al. 2013)	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 min and lead citrate 20 min
Ginkgoales <i>Ginkgoites</i> (Guignard et al. 2016)	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 min and lead citrate 20 min
Ginkgoales <i>Pseudotorellia</i> (Nosova and Yakovleva 2019)	paraformaldehyde - glutaraldehyde	osmium tetraoxide 2%	Epon-araldite	lead citrate 5 min
Ginkgoales <i>Baiera</i>	paraformaldehyde	osmium tetraoxide 1%	Epon	uranyl acetate 7% 15 min and lead citrate 20 min

(Guignard et al. 2019)				
Ginkgoales <i>Sphenobaiera</i> (Wang et al. 2005)		osmium tetraoxide	Epon	uranyl acetate and lead citrate

