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Tiny pollen grains: first evidence of Saururaceae from the Late Cretaceous of western North America

Fridgeir Grimsson Corresp. 1 , **Guido W Grimm** 1,2 , **Reinhard Zetter** 1

1 Department of Palaeontology, University of Vienna, Wien, Austria

2 Unaffiliated, None, Orléans, France

Corresponding Author: Fridgeir Grimsson
Email address: fridgeir.grimsson@univie.ac.at

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Background. The Saururaceae, a very small family of Piperales comprising only six species in four genera, have a relatively scanty fossil record outside of Europe. The phylogenetic relationships of the four genera to each other are resolved, with the type genus *Saururus* occurring in both eastern North America and East Asia. No extant species occurs in western Eurasia. Recent dated trees indicate an Eocene or younger crown age for the family. The most exceptional find so far has been an inflorescence with *in-situ* pollen, *Saururus tuckerae* S.Y.Sm. & Stockey from Eocene of North America with strong affinities to extant species of *Saururus*.

Methods. Dispersed fossil pollen grains from the Campanian (82–81 Ma) of North America are compared to dispersed pollen grains from the Eocene strata comprising *S. tuckerae* [containing that originally produced], the Miocene of Europe, and extant members of the family using combined LM and SEM imaging.

Results. The unambiguous fossil record of the Saururaceae can be [is] pushed back into the Campanian (82–81 Ma). Comparison with re-investigated pollen from the Eocene of North America, the Miocene of Europe, and of the [~~of the~~] modern species of the family shows that pollen morphology in Saururaceae is highly conservative, and remained largely unchanged for the last 80 myrs.

Discussion. The [Campanian] pollen rejects [of Saururaceae precludes/rules out] young (Eocene or younger) estimates for the Saururaceae root and crown age, but is in-line with maximum age scenarios. *Saururus*-type pollen appear to represent the primitive pollen morphology of the family. Often overlooked because of its small size, dispersed Saururaceae pollen may provide a unique opportunity to map the geographic history of a small but old group of Piperales, and should be sought out in Paleogene and Cretaceous sediment samples.

1   **Tiny pollen grains: first evidence of Saururaceae from the Late Cretaceous of**

2   **western North America**

3   Friðgeir Grímsson[1], Guido W. Grimm[1,2], Reinhard Zetter[1]

4

5   [1] University of Vienna, Department of Palaeontology, Vienna

6   [2] Unaffiliated, Orléans, France

7

8   **Corresponding author:**

9   Friðgeir Grímsson

10 Althanstraße 14, UZA II, 1090 Vienna

11 Email: fridgeir.grimsson@univie.ac.at

13   Abstract [500 words, 3000 characters]

14   **Background.** The Saururaceae, a very small family of Piperales comprising only six species in
15   four genera, have a relatively scanty fossil record outside of Europe. The phylogenetic
16   relationships of the four genera to each other are resolved, with the type genus *Saururus* occurring
17   in both eastern North America and East Asia. No extant species occurs in western Eurasia. Recent
     suggest   *transition needed*
18   dated trees indicate an Eocene or younger crown age for the family. The most exceptional find so
19   far has been an inflorescence with *in-situ* pollen, *Saururus tuckerae* S.Y.Sm. & Stockey from
20   Eocene of North America with strong affinities to extant species of *Saururus*.
21   **Methods.** Dispersed fossil pollen grains from the Campanian (82–81 Ma) of North America are
22   compared to dispersed pollen grains from the Eocene strata comprising *S. tuckerae*, the Miocene
23   of Europe, and extant members of the family using combined LM and SEM imaging.
24   **Results.** The unambiguous fossil record of the Saururaceae can be pushed back into the Campanian
25   (82–81 Ma). Comparison with re-investigated pollen from the Eocene of North America, the
26   Miocene of Europe, and of the modern species of the family shows that pollen morphology in
27   Saururaceae is highly conservative, and remained largely unchanged for the last 80 myrs.
28   **Discussion.** The pollen rejects young (Eocene or younger) estimates for the Saururaceae root and
29   crown age, but is in-line with maximum age scenarios. *Saururus*-type pollen appear to represent
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30   the primitive pollen morphology of the family. Often overlooked because of its size, dispersed
31   Saururaceae pollen may provide a unique opportunity to map the geographic history of a small but
32   old group of Piperales, and should be sought out in Paleogene and Cretaceous sediment samples.
33
34   **Keywords.** Angiosperm evolution; conservative traits; magnoliids; Molecular dating;
35   *Saururus*; paleophytogeography; Piperales
36

Introduction

38   Smith & Stockey (2007a) described inflorescences and flowers with *in-situ* pollen from the
                                                        ed them
39   Eocene of North America that they could assign to the modern genus *Saururus* (*S. tuckerae*
40   S.Y.Sm. & Stockey). Saururaceae are a very small magnoliid family included in the Piperales
41   (APG III 2009), with six currently accepted species in four genera. In addition to *Saururus cernuus*
42   L. and *S. chinensis* (Lour.) Baill., these are: *Anemopsis californica* Hook. & Arn., *Gymnotheca*

43 *chinensis* Decne., *Gymnotheca involucrata* Pei, and *Houttuynia cordata* Thunb. An interesting

44 pattern is the modern disjunct distribution of both of the two mutually monophyletic lineages in

45 the Saururaceae (*Anemopsis* + *Houttuynia* vs. *Gymnotheca* + *Saururus*; (Massoni, Forest &

46 Sauquet 2014) in North America and South/East Asia, indicating that the family probably had a

47 much wider distribution in the past (Table 1). The fossil record of Saururaceae is scanty (Table 2).

48 Most of the fossils, fruits/seeds are from the Eocene to Pliocene of western Eurasia and have been

49 affiliated with *Saururus* (*S. bilobatus* [Nikitin] Mai). In addition Mai (1999) described fruits/seeds

50 from the lower Miocene of Germany as *Houttuynia bavarica* Mai. The oldest fossil record so far

51 is fossil wood from the Upper Cretaceous (no detailed stratigraphic information available) of

52 Hokkaido described as *Saururopsis niponensis* Stopes & Fujii (1910, p. 58ff); the authors discuss

53 carefully the affinity of the fossil and suggest that it could represent an ancestral member of the

54 Saururaceae combining wood features typical for either *Saururus* or *Houttuynia*. Likewise, the

55 Eocene *Saururus tuckerae* (Smith & Stockey 2007a) is so far the only fossil reported from North

56 America. Though scanty, the fossil record confirms that Saururaceae were widespread by the

57 Paleogene. The fossil record is also in line with the latest molecular dating estimates of a magnoliid

58 dataset. According to the dating analyses of Massoni, Couvreur & Sauquet (2015b), the divergence

59 between the two clades of the Saururaceae (*Anemopsis* + *Houttuynia* vs. *Gymnotheca* + *Saururus*)

60 was established at the latest by the Eocene (> 45 Ma), and the modern genera (and disjunctions)

61 by the late Miocene (> 10 Ma) or much earlier (Table 3). Two nodes in the phylogenetic

62 neighbourhood of the Saururaceae were constrained using fossil age priors: the *Saururus* (≥ 44.3

63 Ma; 'safe' minimum constraint with reference to *S. tuckerae*) and Winteraceae root ages (=

64 Canellales crown age; ≥ 126 Ma; Massoni, Doyle & Sauquet 2015). Here, we document fossil

65 pollen from the middle Upper Cretaceous Eagle Formation (Fm) of Wyoming, western North

66 America, that is very similar to those of extant *Saururus* and nearly identical to that of pollen

67 associated with *Saururus tuckerae* from the Eocene of British Columbia. Our find is discussed on

68 the backdrop of newly documented dispersed *Saururus* pollen from the Eocene of British

69 Columbia and Miocene of Central Europe (Austria), and the recent dating estimates for the family.

Material & Methods

70

Palaeopalynological samples

71

72 The sedimentary rock samples containing the dispersed fossil *Saururus* pollen grains presented
73 in this study originate from three different localities:

74 1) the Elk basin, Wyoming, north-western United States (44°59′N/108°52′W); the sediment
75 sample comes from the Campanian Eagle Fm and was provided by the late Leo Hickey
76 (1940–2013). For detailed chronometric (absolute dating of the overlying benthonite; Hicks
77 1993) and stratigraphic information and palaeobotanical background of this locality see
78 Hicks (1993), Van Boskirk (1998), Manchester, Grímsson & Zetter (2015), and Grímsson
79 et al. (2016a).

80 2) an outcrop of the Princeton Chert beds, Similkameen River, British Columbia, Canada
81 (49°22′N, 120°32′W). The Princeton Chert beds are part of the middle Eocene Allenby Fm
82 and comprise at least 49 rhythmically bedded cherts, interbedded by carbonaceous layers
83 (e.g. Read 2000; Smith & Stockey 2007a; Mustoe 2011). The sample originates from chert-
84 bed 43 (uppermost quarter of the Princeton Chert unit) and was provided by Ruth Stockey.
85 Overlaying and underlying beds have been chronometrically dated. According to Moss,
86 Greenwood & Archibald (2005, fig. 2) an age of ca 48 Ma can be assumed for this part of
87 the formation.

88 3) An opencast clay pit, Stoob-Warasdorf-Forest, Burgenland, Austria. No chronometric
89 datings are available; bio- and lithostratigraphy indicate a Miocene age (Klaus 1982).

Sample preparation and the single grain method

90

91 The palynological samples were processed according to the protocol outlined in Grímsson,
92 Denk & Zetter (2008). The fossil Saururaceae pollen grains were investigated both by light
93 microscopy (LM) and scanning electron microscopy (SEM) using the single grain method as
94 described in Zetter (1989).

Pollen descriptions and comparison to extant material

95

96 The description of the fossil pollen grains includes diagnostic features observed both in LM and
97 SEM. Some grains were deliberately broken to expose the pollen wall to measure the exine and

98 nexine thickness using SEM. TEM measurements for *Saururus tuckerae* are based on Smith &
99 Stockey (2007a, fig. 29) and Smith & Stockey (2007b, fig.12B). Pollen terminology follows Punt
100 et al. (2007) and Hesse et al. (2009). The fossil pollen grains were compared to all previously
101 published Saururaceae pollen that have been documented using LM and SEM (Xi 1980; Takahashi
102 1986; Pontieri & Sage 1999; Sampson 2000; Furness, Rudall & Sampson 2002; Smith & Stockey
103 2007a; Smith & Stockey 2007b; Lu et al. 2015). Additional material (Table S1) from the herbarium
104 of the University of Vienna (WU) was used for a more detailed comparison (pollen figured in File
105 S1).

106 *Preparation of extant material*
107   A single or a few anthers from each sample were placed into drops of acetolysis liquid (nine to
108 one mix of 99% acetic anhydride and 95–97% sulphuric acid) on microscopic glass slides to soften
109 up the anthers, release the pollen grains from anthers, dissolve extra organic material on pollen
110 grain surfaces, rehydrate pollen grains and release their cell contents, and finally, to stain the grains
111 for LM photography. The slides were heated over a candle flame to speed up the process. Pollen
112 grains were then transferred into fresh drops of glycerine and photographed under LM and then
113 transferred to SEM stubs using a micromanipulator and washed with drops of absolute ethanol.
114 Stubs were sputter-coated with gold and the pollen grains photographed under a JEOL 6400 SEM.

115 *Conservation of fossil and extant pollen material*
116   SEM stubs produced under this study are stored in the collection of the Department of
117 Palaeontology, University of Vienna, Austria, under accession numbers IPUW 7513/101–130.
118

Systematic palaeobotany
120   **Nomenclatural note.** We believe that a fossil name should reflect the biological affinity
121 indicated by the morphology of the fossil. Taken together all evidence, our Cretaceous pollen
122 grains either represent an ancestral lineage within the Saururaceae that shared the primitive pollen
123 morphology of extant and Cenozoic *Saururus* (hypothesis 1 below) or an early member of the
124 *Saururus*-lineage (hypothesis 2). A name best reflecting hypothesis 1 would be to erect a new
125 genus named e.g. "*Protosaururus*". However, this is impractical. The genus diagnosis could only

126   be based on the Cretaceous pollen grains, and would be non-exclusive regarding pollen of the
127   actual *Saururus*-lineage. If the currently prevalent cladistic-phylogenetic nomenclature that only
128   accepts taxa that have a (putative) inclusive common origin should be followed, i.e. are
129   'monophyletic' in a strict sense (Hennig 1950; Hennig & Schlee 1978), termed also 'holophyletic'
130   by (Ashlock 1971), the Cretaceous fossils would need to be addressed as "Saururaceae gen. et sp.
131   indet." (hypothesis 1) or *Saururus* (hypothesis 2). For consistency, our and future *Saururus*-type
132   pollen grains would need to be named based on the currently accepted divergence ages for the
133   Saururaceae (Fig. 1). An alternative solution that serves the requirements of the Botanical Code
134   for unambiguous diagnoses is to follow the concept of "evolutionary classification" (e.g. Mayr &
135   Bock 2002; Hörandl 2006; Hörandl 2007), which allows naming also 'paraphyletic' groups to
136   avoid that groups of directly related organisms with a non-inclusive common origin and similar or
137   identical morphology are addressed by different names (Fig. 1). In this case, one does not need to
138   decide which hypothesis (paraphyletic *Saururus* pollen vs. holophyletic *Saururus*) applies when
139   naming the pollen; and all *Saururus*-type pollen can be addressed as *Saururus* spp.

140   **Saururus aquilae *sp. nov. (Figures 2–4A–G)***

141   **Holotype.** IPUW 7513/101 (Figs 2A, 4E,F)

142   **Paratypes.** IPUW 7513/102–111 (Figs 2B–H, 3, 4A–D,G,H)

143   **Type locality.** Elk Basin, Wyoming, United States.

144   **Stratigraphy and age.** Lettered Sands Member, Upper Eagle beds, Eagle Fm, Upper
145   Cretaceous (Campanian); 82–81 Ma (Hicks 1993; Van Boskirk 1998).

146   **Species diagnosis.** Sculpture perforate, psilate to granulate; proximal face with about five –six
147   perforations per μm^2; perforations can have lobate outlines and up to six free-standing and/or
148   protruding columellae; exine ≤ 400 nm and nexine < 200 nm thick. All other pollen features that
149   can be observed under LM and SEM as in the two modern species of the genus.

150   **Description.** Pollen, monad, shape oblate, form boat-like to globose, outline elliptic in
151   equatorial and polar view; size very small, polar axis 3–5 μm long in SEM, equatorial diameter 6–
152   11 μm in SEM; sulcate, sulcus with rounded ends (SEM); tectate; exine c. 400 nm thick, nexine c.
153   140 nm thick, nexine thinner than sexine (SEM); sculpture psilate in LM, perforate, psilate to
154   granulate in SEM, 20–25 perforations per 4 μm^2, perforations tiny to small, circular, elliptic,
155   irregular, irregular elongated to lobate in outline, perforations fewer and smaller on distal polar

156 face (SEM), perforations are characterized by 1–6 free-standing and/or protruding columellae,
157 free-standing columellae at periphery of perforations or sometimes filling them completely (SEM);
158 sulcus membrane microechinate, microechini mostly with blunt apex, microechini densely packed
159 to segregated (SEM).

160 **Remarks.** The description is based on c. 50 individual dispersed pollen grains studied both in
161 LM and SEM. The Cretaceous *S. aquilae* pollen grains are very similar to or indistinguishable
162 from the Eocene pollen of *S. tuckerae* that has been found both *in situ* in inflorescences/flowers
163 (Smith & Stockey 2007a) and dispersed in the same sediments (Zetter 2006; this study). The only
164 differences are found in the sculpture of the sulcus membrane: in some grains of *S. aquila,* the
165 microechini can be densely packed (Fig. 2A,C; Fig. 4C,E,F), whereas they are widely spaced in *S.*
166 *tuckerae* and the two modern species of *Saururus* (Table 4; Supplementary File S1). The pollen
167 grains of both taxa are even smaller than pollen of Miocene (*S. stoobensis* sp. nov., below) and
168 extant Saururaceae except for *Gymnotheca.* They show the same basic SEM sculpture ranging
169 from perforate, psilate to granulate; a variation also seen in the Miocene pollen but not to the same
170 degree in extant members of the Saururaceae. The main diagnostic feature distinguishing *S.*
171 *aquilae* ~from the Cretaceous~ and *S. tuckerae* from the Miocene and modern species of the genus is their high density of
172 perforations ($\geq$ 20 per 4 μm^2 on the proximal pollen face compared to $\leq$ 10 per 4 μm^2 in *S.*
173 *stoobensis, S. cernuus,* and *S. chinensis*). Furthermore, they both show up to six free-
174 standing/protruding columellae at the periphery of perforations compared to a maximum of four
175 in extant species of the Saururaceae. Occasionally lobate perforations in addition to the more
176 common circular, elliptical and irregular perforations represent a feature seen only in the fossil
177 *Saururus* pollen and the extant *S. chinensis.* Exine and nexine in both taxa are slightly but
178 consistently thinner than in extant Sauruaceae. The *S. aquilae* pollen grains differ from those of
179 *Gymnotheca, Houttuynia,* ~and~ *Anemopsis.* Pollen grains of *Gymnotheca* differ from *S. aquilae* and
180 fossil and extant *Saururus* by their prominently striate and nanoechinate SEM sculpture; their
181 perforations are without free-standing/protruding columellae. *Houttuynia* pollen grains are
182 considerably larger than pollen of *S. aquilae,* and are unique within Saururaceae in having a
183 microverrucate sulcus membrane; their exine is much thicker than in *S. aquilae. Anemopsis* pollen
184 grains have sulcus membranes that are echinate to rugulate, a feature not seen in any other fossil
185 or extant Saururaceae.

186 **Derivation of name.** The species is named after the Eagle (lat. *aquila*) Fm.

187 **Saurus tuckerae *S.Y.Sm. & Stockey (Figures 4H, 5, 6, 7A–D)***

188   2007 "*Anemopsipollis* sp." (nomen nudum) – Ferguson et al., pl. 2, figs 5-8.

189   2007a *Saururus tuckerae* – Smith & Stockey, figs 21, 22, 26, 29.

190   2007b *Saururus tuckerae* – Smith & Stockey, figs 11A-E, 12A-C.

191   **Age.** Middle Eocene, c. 48 Ma (Moss, Greenwood & Archibald 2005).

192   **Description.** Pollen, monad, shape oblate, form boat-like, outline elliptic in equatorial and polar

193 view; size very small, polar axis 3–5 μm long in SEM, equatorial diameter 6–11 μm in SEM;

194 sulcate, sulci with rounded ends (SEM); tectate; exine c. 370 nm thick, nexine c. 150 nm thick,

195 nexine thinner than sexine (TEM); sculpture psilate in LM, perforate, psilate to granulate in SEM,

196 23–26 perforations per 4 μm², perforations tiny to small, circular, elliptic, irregular, irregular

197 elongated or lobate in outline, perforations fewer and smaller on distal polar face (SEM),

198 perforations are characterized by 2–6 freestanding and/or protruding columellae, freestanding-

199 columellae at periphery of perforations or sometimes filling it completely (SEM); sulcus

200 membrane microechinate, microechini mostly with blunt apex, microechini segregated (SEM).

201   **Remarks.** The description is based on c. 200 individual dispersed pollen grains studied under

202 LM and SEM, and compared with the *in-situ* grains figured in Smith & Stockey (2007a); Smith &

203 Stockey (2007b). For additional remarks see remarks for *Saururus aquilae*.

204 **Saururus stoobensis *sp. nov. (Figure 7E–G)***

205   2007 "*Saururipollis* sp." (nomen nudum) – Ferguson et al., Pl. 2, figs 1–4 (same grain).

206   **Holotype.** IPUW 7513/124 (Fig. 7E–G).

207   **Type locality.** Opencast clay pit, Stoob-Warasdorf-Forest, Burgenland, Austria.

208   **Age.** Miocene (Pannonian ?; = Tortonian to Messinian, c. 12–6 Ma; Klaus 1982)

209   **Species diagnosis.** Sculpture perforate, psilate to granulate; perforations occasionally with

210 lobate outlines. All other pollen features (size, form, sculpture of sulcus membrane, number of

211 perforations per μm²) that can be observed under LM and SEM as in the two modern species of

212 the genus.

213   **Description.** Pollen, monad, shape oblate, form boat-like, outline elliptic in equatorial view;

214 size very small, polar axis 4–5 μm long in SEM, equatorial diameter 10–11 μm in SEM; sulcate,

215 sulci with rounded ends (SEM); tectate; sculpture psilate in LM, perforate, psilate to granulate in

216 SEM, 7–10 perforations per 4 μm², perforations tiny to small, circular, elliptic or irregular in

217 outline, perforations fewer and smaller on distal polar face (SEM), perforations are characterized
218 by 2–4 freestanding and/or protruding columellae, freestanding columellae at periphery of
219 perforations (SEM).

220   **Remarks.** The Miocene *Saururus stoobensis* pollen is more similar to the pollen of extant
221 *Saururus* (Table 4; Supplementary File S1) than the Cretaceous and Eocene *Saururus* pollen. It is
222 of similar size, has the same density of perforations and the same number of free-standing
223 columellae. It slightly differs from both modern species in the variation of the sculpture seen in
224 SEM, and the occasional occurrence of perforations with lobate outline, which can be found in *S.*
225 *chinensis* and the older fossil taxa, but has so far not been observed in *S. cernuus* or other
226 Saururaceae genera.

227

Discussion

Fossil records of Saururaceae on the backdrop of latest molecular age estimates (Massoni, Couvreur & Sauquet 2015b)

231   Until now the fossil record of Saururaceae has been confined to the Cenozoic except for wood
232 remains from the Late Cretaceous of Japan described a century ago (Stopes & Fujii 1910). Most
233 specimens have been linked to the extant genus *Saururus* (Table 2). Figure 8 shows the fossil
234 record in comparison to the magnoliid subtree that includes the Saururaceae, extracted from the
235 dated trees provided by Massoni, Couvreur & Sauquet (2015a). The fossil pollen described here
236 from the middle Late Cretaceous (Campanian) of Wyoming, *S. aquilae* conflicts with the youngest
237 dating estimates, which inferred a Late Cretaceous to Paleocene root age for the Saururaceae.
238 Under the oldest age scenario (Massoni, Couvreur & Sauquet 2015a; Massoni, Couvreur &
239 Sauquet 2015b), the Wyoming pollen falls (time-wise) in the (arithmetic) middle between the
240 Saururaceae root and crown divergence ages. On the backdrop of the dating estimates, *S. aquilae*
241 could be the pollen produced by a potential precursor of all extant Saururaceae genera (hypothesis
242 1). Hypothesis 1 would fit also with the interpretation of the Cretaceous fossil wood described as
243 *Saururopsis nipponensis* from Japan (Stopes & Fujii 1910). Although being more similar to wood
244 of *Saururus*, Stopes and Fujii state that some features are reminiscent of *Houttuynia*, which
245 belongs to the second lineage of extant Saururaceae (e.g. Massoni, Forest & Sauquet 2014), and

246 discussed the possibility that the wood comes from an ancestral member of the family. On the
247 other hand, the tip ages are poorly constrained (likely too young) using Massoni et al.'s (2015b)
248 dataset, who focussed on (much) deeper nodes. Hence, *S. aquilae* could represent an early member
249 of the *Saururus*-lineage (hypothesis 2). Notably, the age of *S. aquilae* is close to the lower
250 boundary of the highest posterior density (HPD) intervals for the older age scenarios (Fig. 8;
251 Massoni, Couvreur & Sauquet 2015a). Being interested in large-scale magnoliid processes,
252 Massoni, Couvreur & Sauquet (2015b) did not use any age priors from within the Piperales subtree
253 (Massoni, Doyle & Sauquet 2015) and relied on relatively slow-evolving gene regions. It is a
254 common observation that divergence ages towards the leaves of a tree tend to be (severely)
255 underestimated in studies using large datasets when compared to focused studies that rely on
256 ingroup constraints. Typically, the latter are in better agreement with the fossil record, as e.g. in
257 the case of the Fagaceae (Hubert et al. 2014; Grimsson et al. 2015; Grimsson et al. 2016a; Renner
258 et al. 2016). A similar observation can be made in the sister group of the Piperales, the Canellales.
259 Figure 8 also shows the oldest records of the Winteraceae, which are much older than the age
260 estimates by Massoni, Couvreur & Sauquet (2015a); ~~Massoni, Couvreur & Sauquet (2015b)~~.
261 Studies focusing on either Canellaceae (Müller et al. 2015) or Winteraceae (Marquínez et al. 2009;
262 Thomas et al. 2014), using different sets of age priors including Canellaceae and Winteraceae
263 crown group fossils, obtained (much) older ages than the oldest age scenario (angiosperm root
264 fixed to max. 200 Ma) in the set of analyses performed by Massoni, Couvreur & Sauquet (2015b).
265 Differences range from at least 13 Ma for the Canellaceae and Winteraceae roots to more than 40
266 Ma for close-to-tips nodes.

267 Based on pollen morphology, neither hypotheses can be rejected. *Saururus aquilae* is
268 essentially indistinguishable from pollen linked to the c. 30 Ma younger *S. tuckerae*, and all
269 modern Saururaceae pollen types ($\geq$ 35 Ma younger) differ only in a few characters (Table 4; File
270 S1). The differences are often expressed as a range of variability (such as outlines of perforations
271 or sculpture of the pollen surface). This demonstrates that pollen morphology is a very
272 conservative trait in the lineage, and provides an argument for hypothesis 2 that *S. aquilae* was
273 produced by an early member of the genus *Saururus*. On the other hand, the extant six species are
274 clearly only the last survivors of a once more widespread group (Table 1), and may be
275 unrepresentative regarding the actual variation in each generic lineage and the family over time.
276 The pollen of *Saururus* may simply be primitive within the family. Ancestral members of the

277   Saururaceae including precursors of all modern genera may have produced *essentially* the same pollen

278   (hypothesis 1), whereas pollen morphologies of the extant species of the other genera are more

279   derived (Fig. 9).

280   ### *The importance of an in-depth analysis of the dispersed pollen record*

281   Pollen of Saururaceae is very small (literally, using the size categories of Hesse et al. 2009),

282   and has probably been overlooked or ignored in many palynological studies. A major problem that

283   directly affects the ~~capturing~~ *recovery* of Saururaceae pollen is that the standard (LM) paleopalynological

284   approach is to sieve the sediment with 10 μm sieves, which means that most if not all Saururaceae

285   pollen will be lost. Hence, the lack of Saururaceae pollen all over the Northern Hemisphere may

286   be in part a sieving artefact. The single grain method using a combination of LM and SEM imaging

287   (e.g. Zetter 1989) is a time-consuming approach, but as demonstrated here the information

288   obtained can be highly beneficial to other botanical disciplines such as *(i)* molecular dating by

289   providing new/alternative age priors (e.g. Hubert et al. 2014) and *(ii)* the study of historical

290   biogeography by providing actual evidence for the occurrence of a certain lineage at a certain time

291   in a certain place (e.g. Denk, Grímsson & Zetter 2010; Grímsson, Zetter & Hofmann 2011;

292   Grímsson et al. 2015; Grímsson et al. 2016a). A main advantage of pollen for assessing past

293   distribution is its high evolutionary conservatism across long periods of time. For the Saururaceae

294   the data *presented* showed here and the *in-situ* grains showed by Smith & Stockey (2007a) prove that the

295   main characteristics of Saururaceae pollen (Smith & Stockey 2007b; this study) have remained

296   essentially unchanged for over 80 Ma. This is not an exception; *Fagus,* castaneoid and cornalean

297   pollen can be traced back at least to the Danian of western Greenland (Manchester, Grímsson &

298   Zetter 2015; Grímsson et al. 2016a), and is part of a very rich pollen flora covering at least 32

299   families of angiosperms (Grímsson et al. 2016b); castanoid pollen of the sister clade of *Fagus* (all

300   other Fagaceae) has been found in the same sample as the Saururaceae pollen described here

301   (Grímsson et al. 2016a) in addition to asteroid families such as the Araliaceae and Oleaceae

302   (Manchester, Grímsson & Zetter 2015). Friis, Crane & Pedersen (2011) consistently and

303   repeatedly express their concern regarding the affiliation of many (macro)fossils of the Cretaceous

304   fossil record with angiosperm taxa. For instance, regarding *Saururopsis niponensis*, the Late

305   Cretaceous wood from Hokkaido (Stopes & Fujii 1910), they state that "the relationships of this

306   material require further study" (Friis, Crane & Pedersen 2011, p. 248). We ~~do~~ agree; and advocate

307 the use of comprehensive, in-depth studies of the dispersed and *in-situ* pollen record using the
308 combination of LM and SEM imaging on the same, single grain to fill the many gaps obscuring
309 the origin of the angiosperms, their Cretaceous diversity and spatial distribution, and the roots of
310 the modern lineages and their precursors (e.g. Doyle, Hotton & Ward 1990; Takahashi 1997;
311 Zetter, Hesse & Huber 2002; Hofmann & Zetter 2007; Hofmann & Zetter 2010; Grímsson, Zetter
312 & Hofmann 2011; Hofmann et al. 2011; Grímsson et al. 2014; Mendes et al. 2014; Manchester,
313 Grímsson & Zetter 2015; Grímsson et al. 2016a)

Author contributions

315   FG and GWG conceived the study. FG and RZ processed the sediment samples, and
316 documented and determined the pollen grains. All authors took part in drafting the manuscript. FG
317 and GWG compiled the supplementary files. Artwork by FG (Figs 2–7) and GWG (Figs 1, 8, 9).

Acknowledgements

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Text to Figures and Tables

Figure 1. Practical shortcoming of cladistic classification for naming fossil and extant members of phylogenetic lineages (clades) using binominals. Shown are schematic phenograms using the current systematic-phylogenetic framework for extant taxa of the family *Saururaceae* (Massoni, Couvreur & Sauquet 2015b). **A.** Cladistic classification of Saururaceae accepting only holophyletic (Ashlock 1971), i.e. inclusively monophyletic groups: *All* organisms descending from a certain common ancestor are addressed by the same genus name. All stem fossils must be named 'Saururaceae gen. et spec. indet.', unless there is conclusive evidence that they represent extinct sister lineages with no ancestor-descendant relationship to the extant genera (triggering the erection of a new genus) or belong to the stem or crown lineages of an extant genus. **B.** Evolutionary classification, accepting groups with inclusive (holophyla) and exclusive (paraphyla) common origins, i.e. are monophyletic according Haeckel (1866). All fossil taxa can be named using binominals, either by extending an extant holophyletic genus to include ancestral members of Saururaceae with equally primitive morphology, which then becomes paraphyletic by definition (e.g. *Saururus*), or by introducing genera to collect stem fossils ancestral to more than a single, extant and holophyletic genus (e.g. the tentative *Protosaururus* to collect fossils with *Saururus*-like morphology that are older than the presumed split between *Saururus* and *Gymnotheca*-lineages). Such extinct genera are also paraphyletic by definition. Shading signifies the extent of each (potential) genus, dark shading the modern circumscription based on molecular data (i.e. descendants of the MRCA of all extant species of the genus). Abbreviations: CA, common ancestor; MRCA, most recent common ancestor; EAS, East Asia; EAM, Eastern North America; SAS; South Asia (Indian Peninsula); WAM, Western North America.

Figure 2. SEM micrographs of *Saururus aquilae* sp. nov. from the Upper Cretaceous (Campanian, 82-81 Ma) of Wyoming, western USA. A. Holotype, IPUW 7513/101; pollen grain in distal polar view, showing sulcus, microechini densely packed. B. Paratype, IPUW 7513/102; pollen grain in distal polar view, showing sulcus, microechini segregated. C. Paratype, IPUW 7513/103; pollen grain in equatorial view, showing sulcus. D. Paratype, IPUW 7513/104; pollen grain in equatorial view, showing sulcus. E. Paratype, IPUW 7513/105; pollen grain in proximal polar view. F. Paratype, IPUW 7513/106, pollen grain in proximal polar view. G. Paratype, IPUW

516　7513/107, pollen grain in proximal polar view (H) Paratype, IPUW 7513/108; pollen grain in
517　proximal polar view, with eroded parts revealing the columellae. Scale bars: 1 μm.

518

519　**Figure 3.** SEM micrographs of *Saururus aquilae* sp. nov. from the Upper Cretaceous
520　(Campanian, 82–81 Ma) of Wyoming, western USA. (A) Paratype, IPUW 7513/109; pollen grain
521　in proximal polar view, large perforations. (B) Close-up of Figure 2H, showing densely packed
522　columellae in an area of surface erosion. (C) Close-up of Figure 2G, showing tiny perforations. (D)
523　Paratype, IPUW 7513/110; close-up showing small circular perforations filled with columellae. (E)
524　Close-up of Figure 2D, showing irregular and lobate perforations and free-standing columellae. (F)
525　Close-up of Figure 2E, showing small irregular perforations and free-standing columellae. (G)
526　Close-up of Figure 3A, showing large circular to elliptic perforations and free-standing columellae.
527　(H) Paratype, IPUW 7513/111; close-up showing large irregular perforations and free-standing
528　columellae. Scale bars: 1 μm.

529

530　**Figure 4.** SEM and LM micrographs of *Saururus aquilae* sp. nov. (A–G) from the Upper
531　Cretaceous (Campanian, 82–81 Ma) of Wyoming, western USA, and LM micrographs of *Saururus*
532　*tuckerae* from the middle Eocene (c. 48 Ma) of Princeton, B.C., western Canada (H). (A) Close-up
533　of Figure 2B, showing sulcus membrane, segregated microechini. (B) Close-up of Figure 2B,
534　showing sulcus membrane, segregated microechini. (C) Close-up of Figure 2C, showing sulcus
535　membrane, densely packed microechini. (D) Close-up of Figure 2D, showing sulcus membrane,
536　densely packed microechini. (E) Close-up of Figure 2A (holotype), showing sulcus membrane. (F)
537　Close-up of Figure 4E (holotype), showing densely packed microechini. (G) *Saururus aquilae* sp.
538　nov. pollen in LM. (H) *Saururus tuckerae* pollen in LM. Scale bars: ~~Scale bars:~~ 1 μm in A–F, 10
539　μm in G, H.

540

541　**Figure 5.** SEM micrographs of *Saururus tuckerae* pollen from the middle Eocene (c. 48 Ma)
542　of Princeton, B.C., western Canada. (A) Pollen grain (IPUW 7513/112) in distal polar view,
543　showing sulcus, microechini segregated. (B) Pollen grain (IPUW 7513/113) in distal polar view,
544　showing sulcus. (C) Pollen grain (IPUW 7513/114) in distal polar view, showing sulcus. (D) Pollen
545　grain (IPUW 7513/115) in oblique equatorial view, showing sulcus. (E) Pollen grain (IPUW
546　7513/116) in equatorial view, showing sulcus and sulcus membrane. (F) Pollen grain (IPUW

547   7513/117) in proximal polar view. (G) Pollen grain (IPUW 7513/118) in proximal polar view. (H)

548   Pollen grain (IPUW 7513/119) in proximal polar view. Scale bars: 1 µm.

549

550   **Figure 6.** SEM micrographs of *Saururus tuckerae* pollen from the middle Eocene (c. 48 Ma)

551   of Princeton, B.C., western Canada. (A) Pollen grains preserved in a clump (IPUW 7513/120). (B)

552   Pollen grains preserved in a clump (IPUW 7513/121). (C) Pollen grain, close-up of Figure 5B,

553   showing small circular to elliptic perforations filled with free-standing columellae. (D) Close-up of

554   Figure 5H, showing small irregular to lobate perforations. (E) Close-up of Figure 5D, showing small

555   irregular to lobate perforations, some with up to 6 free-standing columellae. (F) Pollen grain, IPUW

556   7513/122; close-up showing irregular to lobate perforations. (G) Close-up of Figure 6B, showing

557   irregular to lobate perforations with ~~up~~ *up to #* free-standing columellae. (H) Close-up of pollen (IPUW

558   7513/123) grain showing irregular to lobate perforations. Scale bars: 1 µm.

559

560   **Figure 7.** SEM micrographs of *Saururus tuckerae* pollen from the middle Eocene (c. 48 Ma)

561   of Princeton, B.C., western Canada (A–D) and *Saururus stoobensis* sp. nov. from the Miocene

562   Opencast clay pit, Stoob-Warasdorf-Forest, Burgenland, Austria (E–G). (A) Close-up of Figure 5A,

563   showing microechinate colpus membrane, microechini segregated. (B) Close-up of Figure 5A,

564   showing colpus membrane, microechini segregated. (C) Close-up of Figure 5B, showing

565   microechinate membrane. (D) Close-up of Figure 6A, showing colpus membrane. E. *Saururus*

566   *stoobensis* sp. nov. holotype, IPUW 7513/124; grain (arrow) attached to a pollen grain of Apiaceae

567   illustrating the size difference. (F) Close-up of Figure 7E, overview of pollen. (G) Close-up of Figure

568   7F, showing perforate sculpture with relatively few and tiny perforations. Scale bars: 1 µm in A–

569   D, F, G, 10 µm in E.

570   *showing* OK as is.

571   **Figure 8.** ~~Mapping of~~ *showing* the fossil record of Saururaceae (and oldest fossils of the Winteraceae,

572   for comparison) on dated phylogenies (Bayesian uncorrelated clock) provided in the

573   accompanying data (Massoni, Couvreur & Sauquet 2015a) to Massoni, Couvreur & Sauquet

574   (2015b). Pink: North American fossils; cyan: western Eurasian (Central Europe to western Siberia)

575   fossils; yellow: East Asian fossils. Blue bars represent the 95% highest posterior density (HPD)

576   intervals of the minimum age and maximum age scenarios; node heights are averages (medians

577   are indicated by deep blue bars in the HPD intervals). Branch labels show posterior probabilities

578   (PP) < 1.0 (all other branches have PP = 1.00), red branches highlight topological conflict between

579   the chronograms (probably due to incomprehensive Bayesian runs getting stuck in local

580   suboptima, since all analyses were based on the same data set).

581

582   **Figure 9.** Hypothetical evolution of Saururaceae pollen. The fossil and extant *Saururus* show

583   a morphology that may be primitive within the family: all other genera differ by one or two unique,

584   putatively derived traits. Abbreviations: PS = (normal) pollen surface; SM = sulcus membrane.

585   Scale bars = 1 μm.

Table 1(on next page)

Modern and past distribution of Saururaceae genera

1   Table 1. Modern and past distribution of Saururaceae genera

Time period	North America	Western Eurasia	East Asia
Recent	*Saururus, Anemopsis*	None	*Gymnotheca, Saururus, Houttuynia*
Neogene	None	*Saururus* (pollen, fruit/seed), *Houttuynia* (fruit/seed)	None
Paleogene	*Saururus* (inflorescence with *in-situ* pollen; and dispersed pollen)	*Saururus* (fruit/seed)	*Saururus* (fruit/seed)
Upper Cretaceous	*Saururus*-type pollen	None	*Saururopsis* (wood)

2

Table 2(on next page)

Fossil record of Saururaceae

1 Table 2. Fossil record of Saururaceae
2

Taxon	Organ	Period (epoch)	Age in Ma	State/region, country	Reference
North America					
Saururus aquilae	Pollen	Upper Cretaceous (Campanian)	82–81	Wyoming, United States	This study
Saururus tuckerae	Inflorescence, flowers, pollen	Middle Eocene	~48	British Columbia, Canada	Smith & Stockey (2007a); This study
Western Eurasia					
Saururus bilobatus	Fruits/seeds	Late Eocene to Pliocene	~40–2.5	Germany	Reid & Reid (1915); Mai (1965); Mai (1967); Mai & Walther (1978); Mai & Walther (1985); Mai (1995); Mai (1999)
Saururus bilobatus (incl. *Helitropium* sp. and *Carpolithus* sp.)	Fruits/seeds	Miocene	~23–5	Poland	Raniecka-Bobrowska (1959); Łancucka-Srodoniowa (1979); Stuchlik et al. (1990); Lesiak (1994)
Saururus bilobatus *Saururus stoobensis*	Fruits/seeds Pollen	Middle Miocene (Langhian) Middle Miocene (late Serravallian; Sarmatian)	~16–14 ~12	Denmark Austria	Friis (1985) Ferguson, Zetter & Paudayal (2007) as "*Saururipollis* sp." (nomen nudum); formalized in this study
Houttuynia bavarica	Fruits/seeds	Early Miocene	~23–16	Germany	Mai (1999)
East Asia					
Saururopsis niponensis	Wood	Upper Cretaceous	> 66	Hokkaido, Japan	Stopes & Fujii (1910)
Saururus bilobatus (as *Carpolithus bilobatus*)	Fruits/seeds	Oligocene	~34–23	Western Siberia, Russia	Dorofeyev (1963); Nikitin (1965)

3

Table 3(on next page)

Divergence age estimates for the Saururaceae subtree according to minimum and maximum age scenarios (angiosperm root fixed to max. 130 or 200 Ma; Massoni, Couvreur & Sauquet 2015a)

Abbreviations: MRCA = most recent common ancestor; Ma = Million years ago.

1 Table 3. Divergence age estimates for the Saururaceae subtree according to minimum and
2 maximum age scenarios (angiosperm root fixed to max. 130 or 200 Ma; Massoni, Couvreur &
3 Sauquet 2015a). Abbreviations: MRCA = most recent common ancestor; Ma = Million years ago.
4

Node	Angiosperm root fixed to	
	Max. 130 Ma	Max. 200 Ma
Saururaceae root	97.0–60.4 (median: 78.3)	117.3–80.3 (median: 99.8)
Saururaceae crown	75.3–46.7 (median: 58.9)	80.8–48.6 (median: 62.8)
MRCA of *Gymnotheca+Saururus*	62.1–44.3 (median: 49.4)	65.6–44.3 (median: 50.6)
MRCA of *Anemopsis+Houttuynia*	64.5–10.3 (median: 37.1)	72.5–26.8 (median: 49.5)

5

Table 4(on next page)

Pollen features of extinct and extant Saururaceae

Note: unique (genus- or species-level) features in bold

Table 4. Pollen features of extinct and extant Saururaceae; unique (genus- or species-level) features in bold.

Species	Distribution/provenance	E (μm; SEM)	P (μm; SEM)	Surface sculpture (SEM)	Sulcus membrane (SEM)	Perforations per 4 μm² proximal face	Size and outline of perforations	Free-standing or protruding columellae	Exine thickness, mean (μm)	Nexine thickness, mean (μm)
Anemopsis californica	SW U.S., NW Mexico	12–13	5–6	Perforate, granulate	**Echinate, rugulate**	7–9	Tiny; circular, elliptic, irregular	up to 6	0.47	0.20
Gymnotheca chinensis	SW and S China, Vietnam	9–10	4–5	Perforate, striate, nanoechinate	Microechinate	18–20	**Small; circular, elliptic**	**No**	0.55	0.18
Gymnotheca involucrata	S Sichuan (S China)	10–11	5–6	Perforate, striate, nanoechinate	Microechinate	17–19	**Small; circular, elliptic**	**No**	0.54	0.15
Houttuynia cordata	S and E Asia	13–14	8–9	Perforate, psilate	Microechinate	10–12	Tiny; circular, elliptic, irregular			
Saururus cernuus	E U.S.	10–13	5–6	Perforate, granulate	Microechinate	7–9	Tiny; circular, elliptic, irregular	2–4	0.49	0.20
Saururus chinensis	S and E Asia	11–12	4–5	Perforate, psilate, indistinctly rugulate	Microechinate	6–8	Tiny; circular, elliptic, irregular, lobate	3–4	0.47	0.24
Saururus aquilae †	NW USA	6–11	3–5	Perforate, psilate to granulate	Microechinate, echini can be densely packed	20–25	Tiny to small; circular, elliptic, irregular, **irregular-elongated, lobate**	1–6	0.40	0.14
Saururus tuckerae †	SW Canada	6–11	3–5	Perforate, psilate to granulate	Microechinate, **echini segregated**	23–26	Tiny to small; circular, elliptic, irregular, **irregular-elongated, lobate**	2–6	0.37	0.15
Saururus stoobensis †	Austria	10–11	4–5	Perforate, psilate to granulate	Not observed	7–10	Tiny; circular, elliptic, irregular, lobate	2–4	Not observed	Not observed

2 Note: Measurements and features from/based on Xi (1980), Takahashi (1986), Pontieri & Sage (1999), Sampson (2000), Furness et al.
3 (2002), Smith & Stockey (2007a, 2007b), Lu et al. (2015) and our own observations

Figure 1

Practical shortcoming of cladistic classification for naming fossil and extant members of phylogenetic lineages (clades) using binominals

Shown are schematic phenograms using the current systematic-phylogenetic framework for extant taxa of the family (Massoni, Couvreur & Sauquet 2015b) . A) Cladistic classification of Saururaceae accepting only holophyletic (Ashlock 1971), i.e. inclusively monophyletic groups: *All* organisms descending from a certain common ancestor are addressed by the same genus name. All stem fossils must be named 'Saururaceae gen. et spec. indet.', unless there is conclusive evidence that they represent extinct sister lineages with no ancestor-descendant relationship to the extant genera (triggering the erection of a new genus) or belong to the stem or crown lineages of an extant genus. **B**) Evolutionary classification, accepting groups with inclusive (holophyla) and exclusive (paraphyla) common origins, i.e. are monophyletic according Haeckel (1866). All fossil taxa can be named using binominals, either by extending a today holophyletic genus to include ancestral members of Saururaceae with equally primitive morphology, which then becomes paraphyletic by definition (e.g. *Saururus*), or by introducing genera to collect stem fossils ancestral to more than a single, extant and holophyletic genus (e.g. the tentative *Protosaururus* to collect fossils with *Saururus*-like morphology that are older than the presumed split between *Saururus* and *Gymnotheca*-lineages). Such extinct genera are also paraphyletic by definition. Shading signifies the extent of each (potential) genus, dark shading the modern circumscription based on molecular data (i.e. descendants of the MRCA of all extant species of the genus). Abbreviations: CA, common ancestor; MRCA, most recent common ancestor; EAS, East Asia; EAM, Eastern North America; SAS; South Asia (Indian Peninsula); WAM, Western North America.

A: Cladistic classification

Morphology
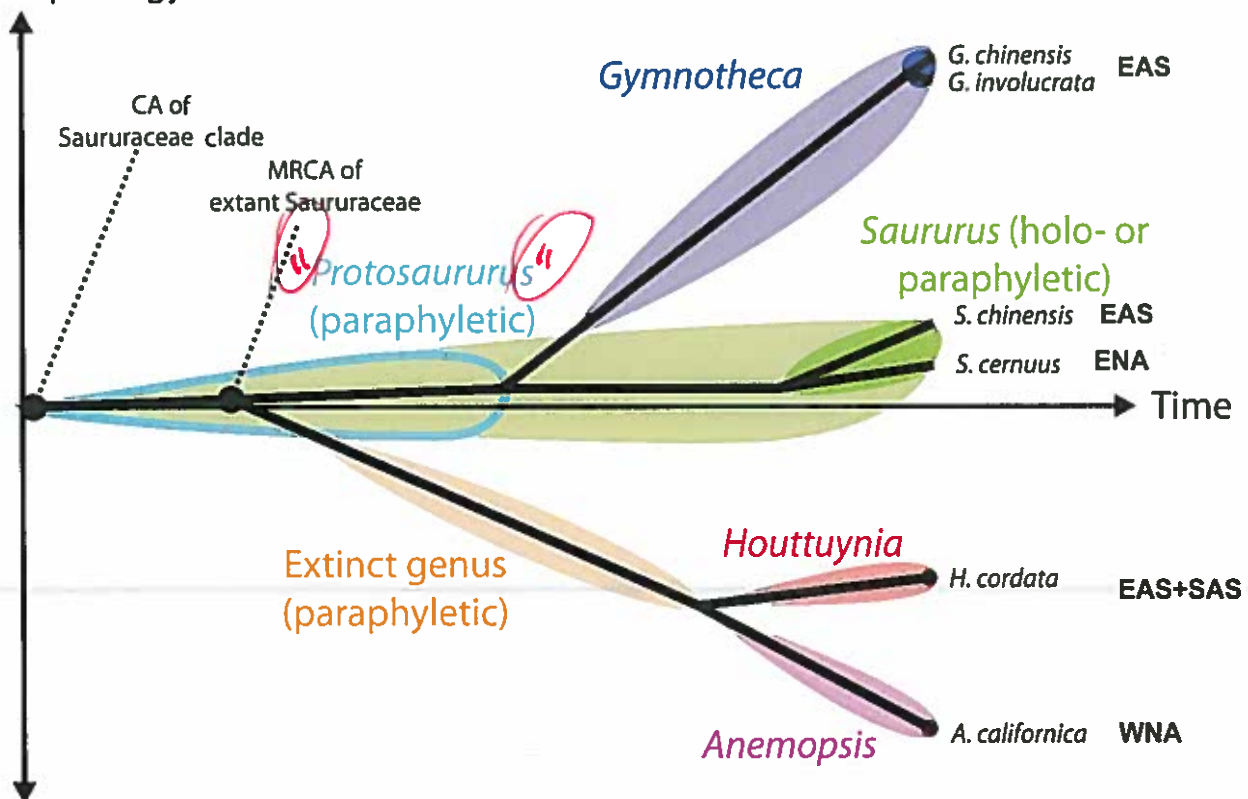
CA of
Saururaceae clade

MRCA of
extant Saururaceae

Gymnotheca

G. chinensis
G. involucrata   EAS

Saururus (holophyletic)

S. chinensis   EAS

S. cernuus   ENA

Time

ur
Sauraceae gen. indet.

Houttuynia

H. cordata   EAS+SAS

Anemopsis

A. californica   WNA

B: Evolutionary classification

Morphology

CA of
Saururaceae clade

MRCA of
extant Saururaceae

Gymnotheca

G. chinensis
G. involucrata   EAS

"*Protosaururus*"
(paraphyletic)

Saururus (holo- or
paraphyletic)

S. chinensis   EAS

S. cernuus   ENA

Time

Extinct genus
(paraphyletic)

Houttuynia

H. cordata   EAS+SAS

Anemopsis

A. californica   WNA

Figure 2

SEM micrographs of *Saururus aquilae* sp. nov. from the Upper Cretaceous (Campanian, 82-81 Ma) of Wyoming, western USA

A) Holotype, IPUW 7513/101; pollen grain in distal polar view, showing sulcus, microechini densely packed. B) Paratype, IPUW 7513/102; pollen grain in distal polar view, showing sulcus, microechini segregated. C) Paratype, IPUW 7513/103; pollen grain in equatorial view, showing sulcus. **D)** Paratype, IPUW 7513/104; pollen grain in equatorial view, showing sulcus. **E)** Paratype, IPUW 7513/105; pollen grain in proximal polar view. **F)** Paratype, IPUW 7513/106, pollen grain in proximal polar view. **G)** Paratype, IPUW 7513/107, pollen grain in proximal polar view. **H)** Paratype, IPUW 7513/108; pollen grain in proximal polar view, with eroded parts revealing the columellae. Scale bars: 1 μm.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.
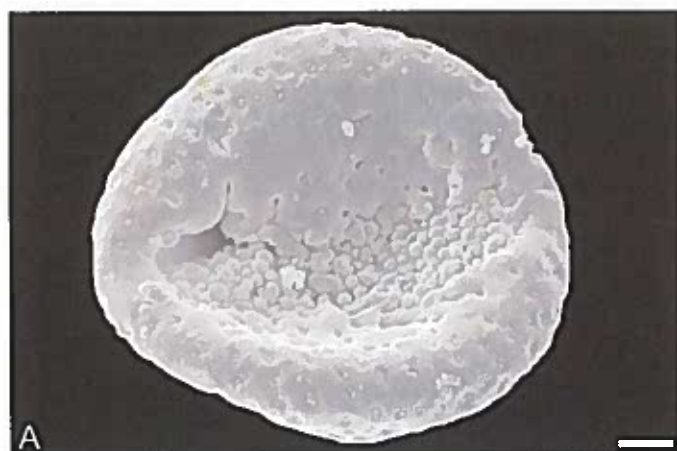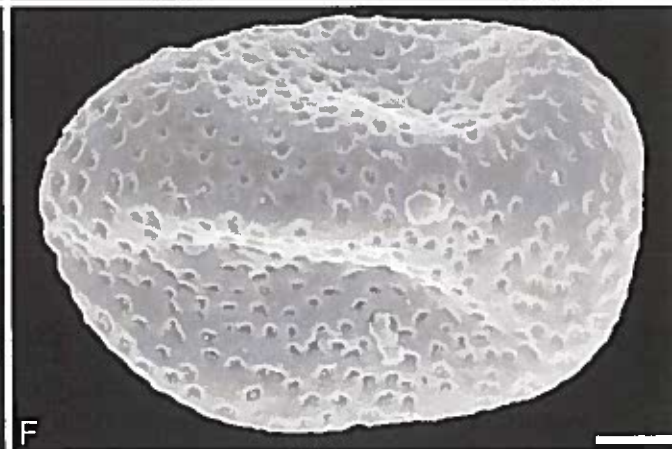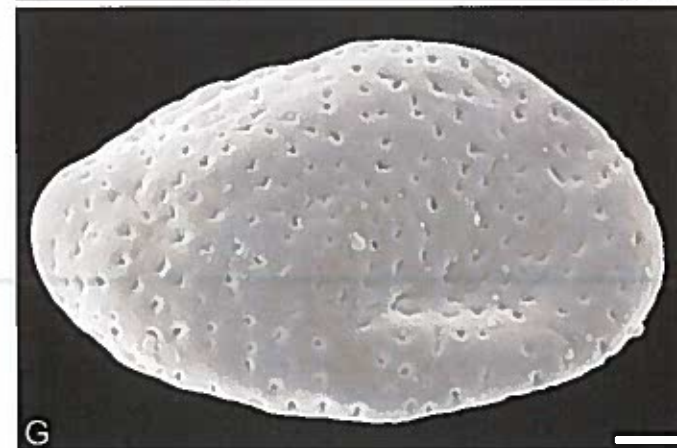
Figure 3

SEM micrographs of *Saururus aquilae* sp. nov. from the Upper Cretaceous (Campanian, 82–81 Ma) of Wyoming, western USA

A) Paratype, IPUW 7513/109; pollen grain in proximal polar view, large perforations. **B)** Close-up of Figure 2H, showing densely packed columellae in an area of surface erosion. **C)** Close-up of Figure 2G, showing tiny perforations. **D)** Paratype, IPUW 7513/110; close-up showing small circular perforations filled with columellae. **E)** Close-up of Figure 2D, showing irregular and lobate perforations and free-standing columellae. **F)** Close-up of Figure 2E, showing small irregular perforations and free-standing columellae. **G)** Close-up of Figure 3A, showing large circular to elliptic perforations and free-standing columellae. **H)** Paratype, IPUW 7513/111; close-up showing large irregular perforations and free-standing columellae. Scale bars: 1 μm.
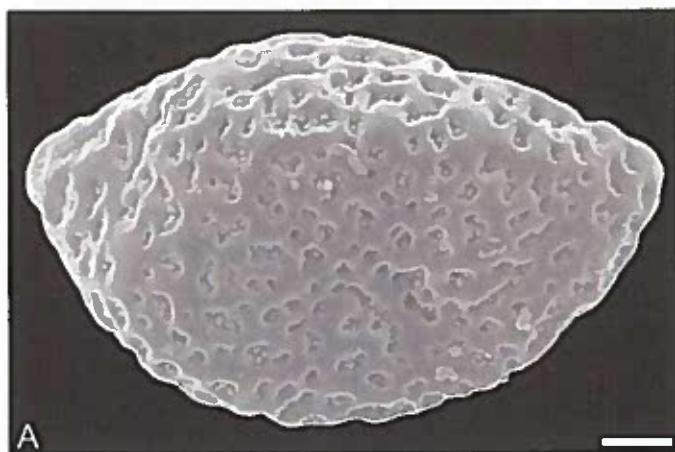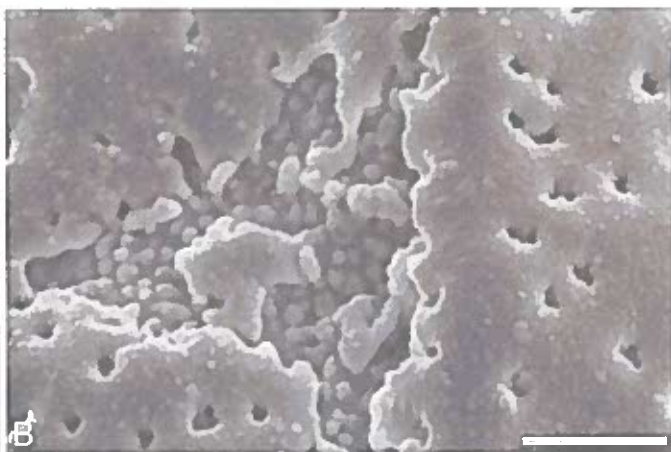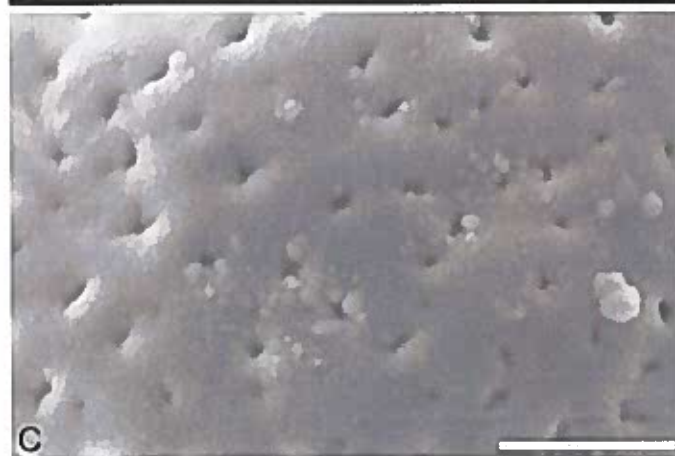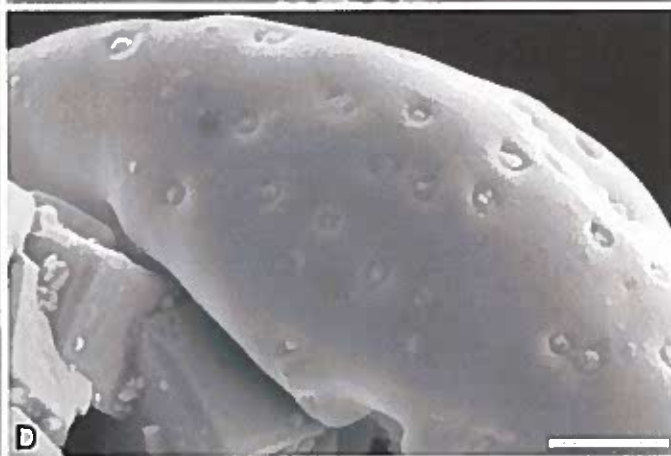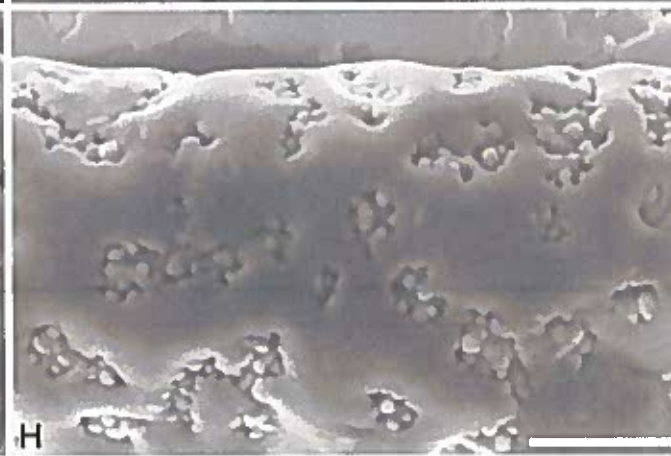
Figure 4

SEM and LM micrographs of *Saururus aquilae* sp. nov. (A–G; Campanian; Wyoming) and LM micrographs of *Saururus tuckerae* (H; middle Eocene; Princeton, B.C.)

A) Close-up of Figure 2B, showing sulcus membrane, segregated microechini. B) Close-up of Figure 2B, showing sulcus membrane, segregated microechini. **C)** Close-up of Figure 2C, showing sulcus membrane, densely packed microechini. **D)** Close-up of Figure 2D, showing sulcus membrane, densely packed microechini. **E)** Close-up of Figure 2A (holotype), showing sulcus membrane. **F)** Close-up of Figure 4E (holotype), showing densely packed microechini. **G)** *Saururus aquilae* sp. nov. pollen in LM. **H)** *Saururus tuckerae* pollen in LM. Scale bars: Scale bars: 1 µm in A–F, 10 µm in G, H.
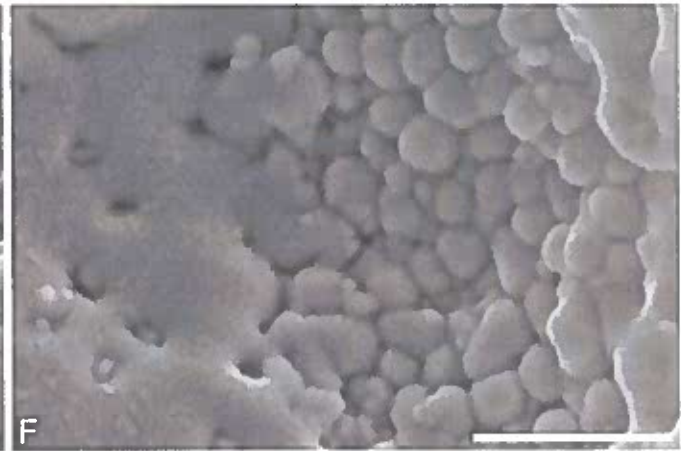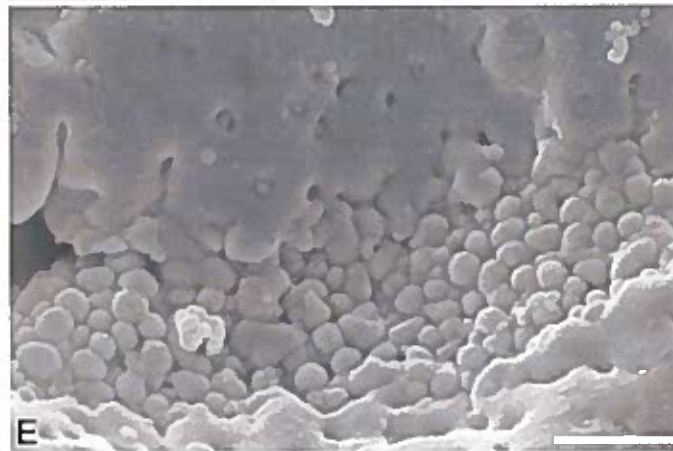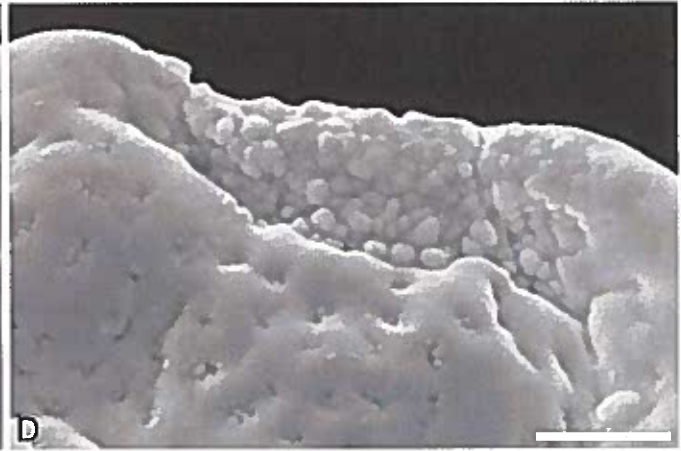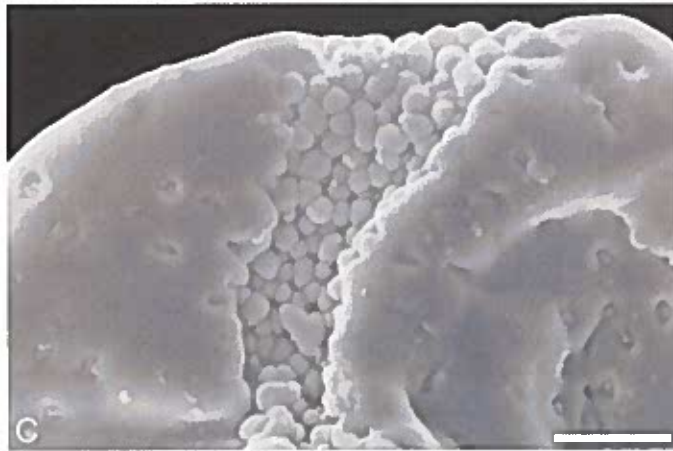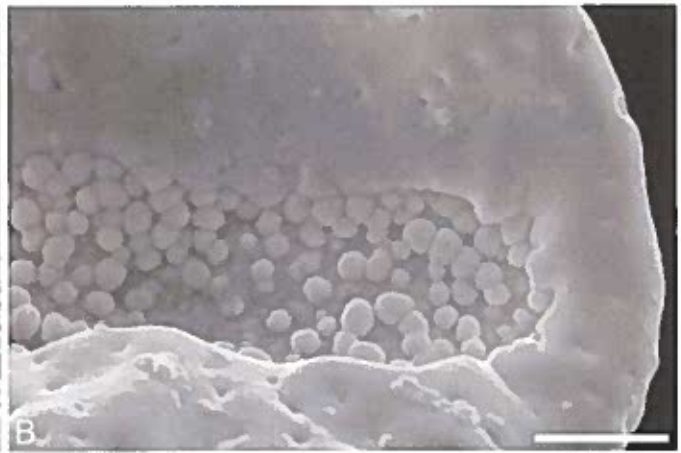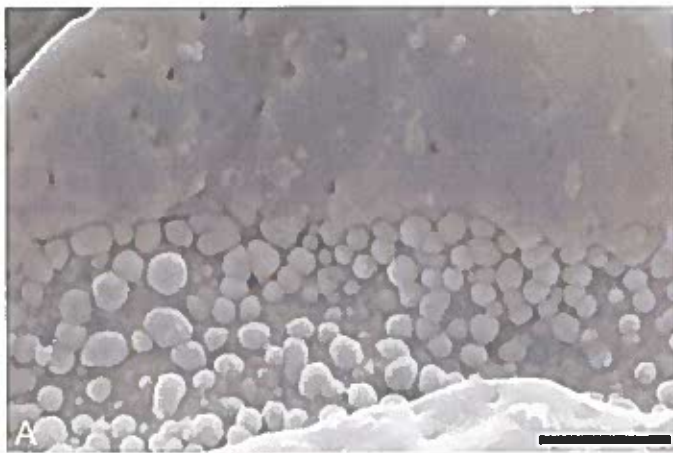
Figure 5

SEM micrographs of *Saururus tuckerae* pollen from the middle Eocene (c. 48 Ma) of Princeton, B.C., western Canada

A) Pollen grain (IPUW 7513/112) in distal polar view, showing sulcus, microechini segregated.
B) Pollen grain (IPUW 7513/113) in distal polar view, showing sulcus. **C)** Pollen grain (IPUW 7513/114) in distal polar view, showing sulcus. **D)** Pollen grain (IPUW 7513/115) in oblique equatorial view, showing sulcus. **E)** Pollen grain (IPUW 7513/116) in equatorial view, showing sulcus and sulcus membrane. **F)** Pollen grain (IPUW 7513/117) in proximal polar view. **G)** Pollen grain (IPUW 7513/118) in proximal polar view. **H)** Pollen grain (IPUW 7513/119) in proximal polar view. Scale bars: 1 μm.

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.
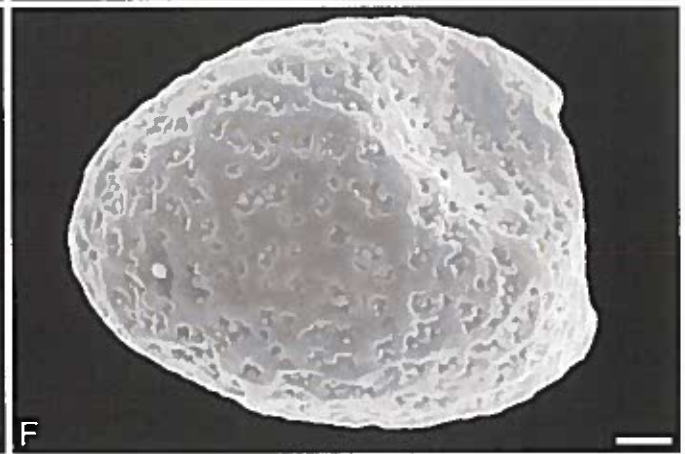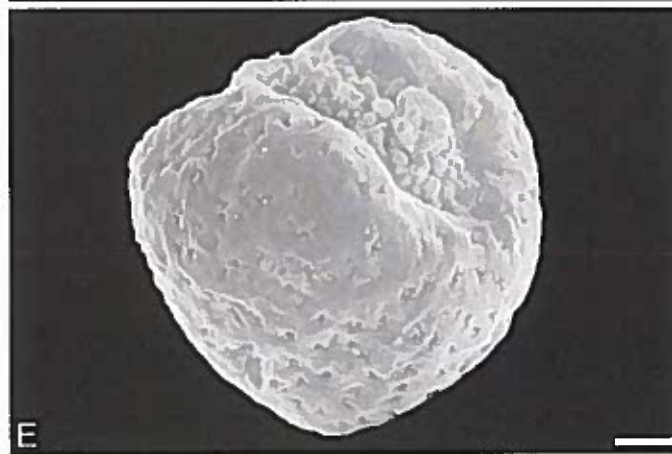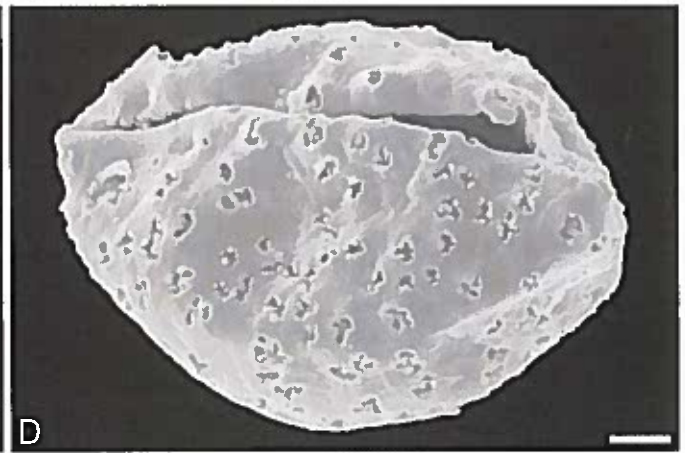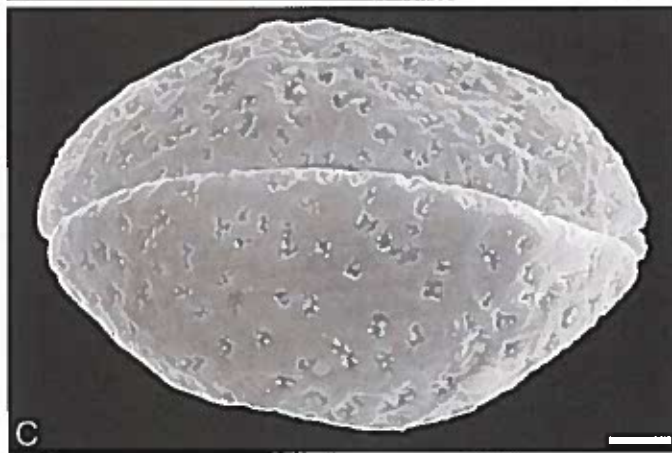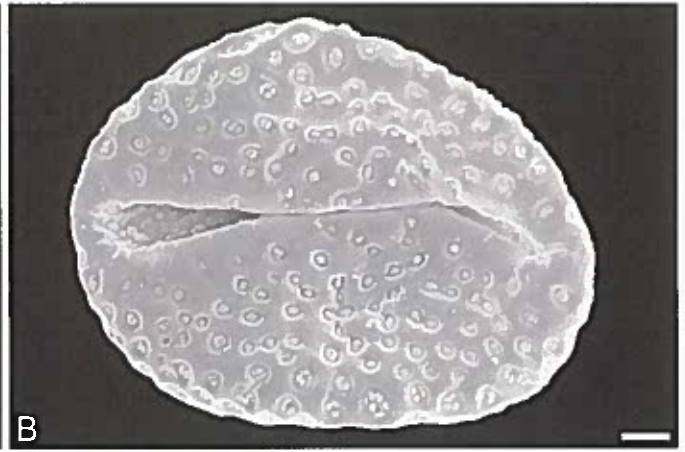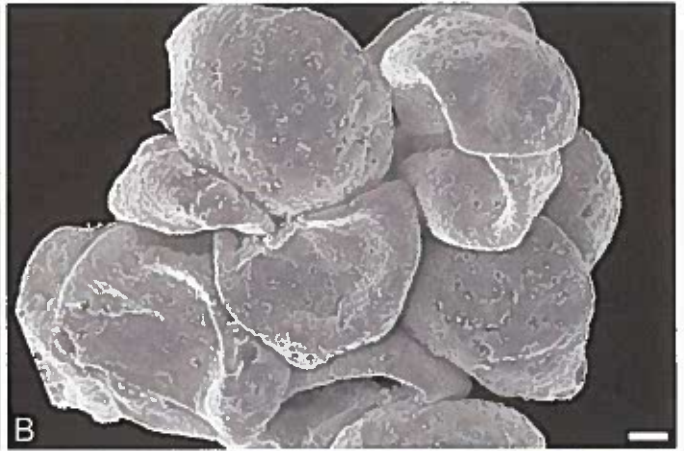
Figure 6

SEM micrographs of *Saururus tuckerae* pollen from the middle Eocene (c. 48 Ma) of Princeton, B.C., western Canada

A) Pollen grains preserved in a clump (IPUW 7513/120). **B)** Pollen grains preserved in a clump (IPUW 7513/121). **C)** Pollen grain, close-up of Figure 5B, showing small circular to elliptic perforations filled with free-standing columellae. **D)** Close-up of Figure 5H, showing small irregular to lobate perforations. **E)** Close-up of Figure 5D, showing small irregular to lobate perforations, some with up to 6 free-standing columellae. **F)** Pollen grain, IPUW 7513/122; close-up showing irregular to lobate perforations. **G)** Close-up of Figure 6B, showing irregular to lobate perforations with up free-standing columellae. **H)** Close-up of pollen (IPUW 7513/123) grain showing irregular to lobate perforations. Scale bars: 1 µm.
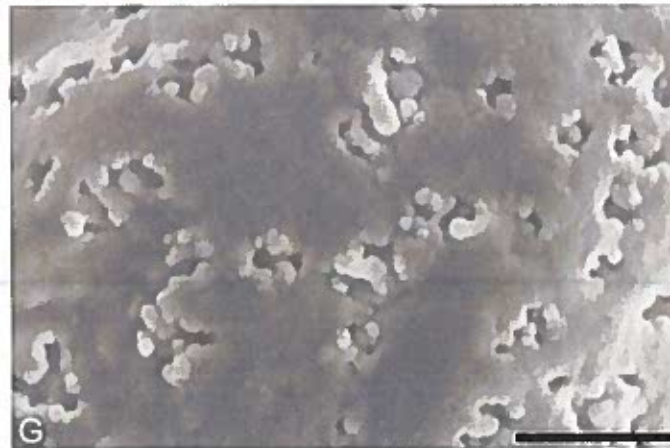
Figure 7

SEM micrographs of *Saururus tuckerae* (A–D; middle Eocene; Princeton, B.C.) and *Saururus stoobensis* sp. nov. from the Miocene Opencast clay pit, Stoob-Warasdorf-Forest, Burgenland, Austria (E–G)

A) Close-up of Figure 5A, showing microechinate colpus membrane, microechini segregated. **B)** Close-up of Figure 5A, showing colpus membrane, microechini segregated. **C)** Close-up of Figure 5B, showing microechinate membrane. **D)** Close-up of Figure 6A, showing colpus membrane. **E)** *Saururus stoobensis* sp. nov. holotype, IPUW 7513/124; grain (arrow) attached to a pollen grain of Apiaceae illustrating the size difference. **F)** Close-up of Figure 7E, overview of pollen. **G)** Close-up of Figure 7F, showing perforate sculpture with relatively few and tiny perforations. Scale bars: 1 μm in A–D, F, G, 10 μm in E.
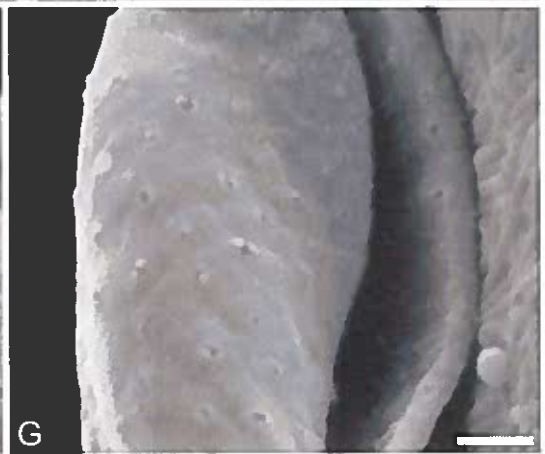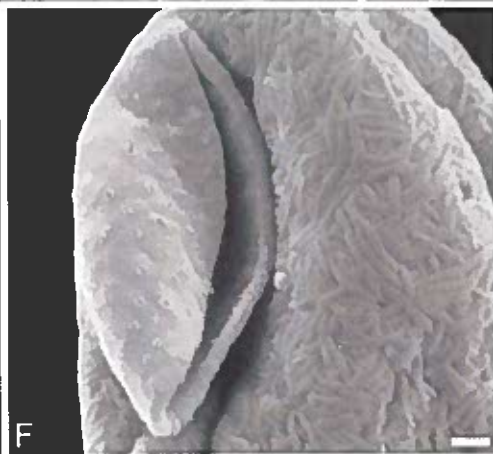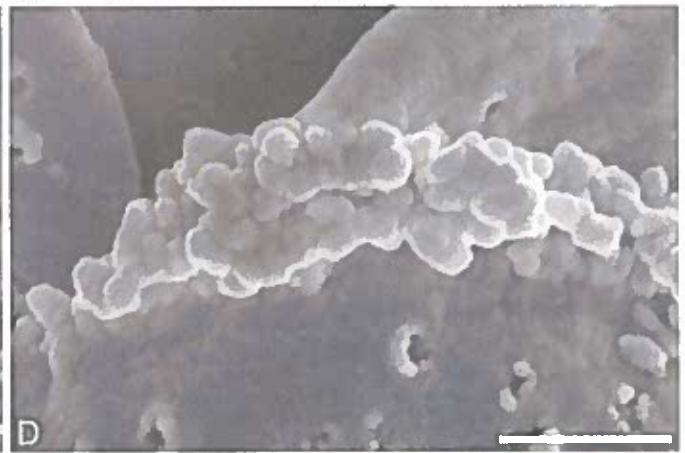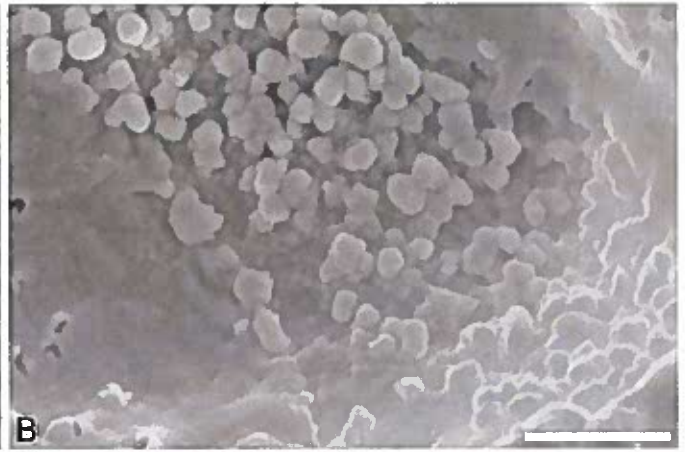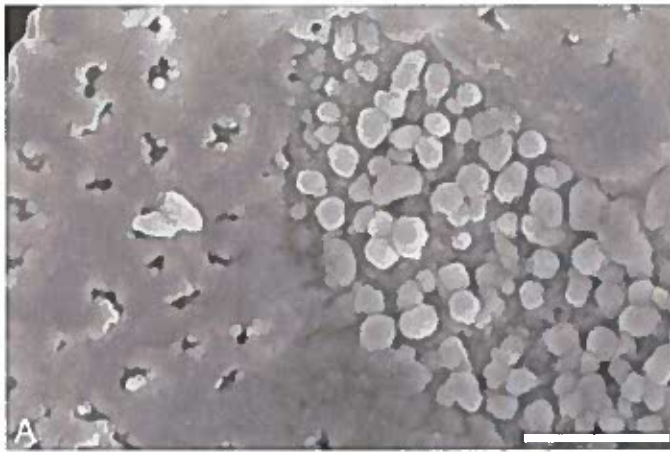
Figure 8

Mapping of the fossil record of Saururaceae on dated phylogenies (Bayesian uncorrelated clock; included in Massoni, Couvreur & Sauquet 2015a)

Oldest fossils of the Winteraceae are shown for comparison. Pink: North American fossils; cyan: western Eurasian (Central Europe to western Siberia) fossils; yellow: East Asian fossils. Blue bars represent the 95% highest posterior density (HPD) intervals of the minimum age and maximum age scenarios; node heights are averages (medians are indicated by deep blue bars in the HPD intervals). Branch labels show posterior probabilities (PP) < 1.0 (all other branches have PP = 1.00), red branches highlight topological conflict between the chronograms (probably due to incomprehensive Bayesian runs getting stuck in local suboptima, since all analyses were based on the same data set).

Angiosperm root age set to max. 130 Ma

Aristoloch-
iaceae
- Aristolochia macrophylla
- Thottea tomentosa
- Asarum canadense
- Lactoris fernandeziana
- Saruma henryi

Piperaceae
- Verhuellia elegans
- Peperomia obtusifolia
- Piper betle
- Manekia naranjoana
- Zippelia begonifolia

Saururaceae
- Anemopsis californica
- Houttuynia cordata
- Saururus cernuus
- Gymnotheca chinensis

Winteraceae
- Takhtajania perrieri
- Tasmannia insipida
- Drimys winteri
- Pseudowintera colorata

Canellaceae
- Warburgia ugandensis
- Capsicodendron dinisii
- Cinnamosma madagascariensis
- Pleodendron macranthum
- Cinnamodendron ekmanii
- Canella winterana

| 200 | 150 | 100 | 75 | 50 | 25 | 0 Ma |

| UJ | LC | UC | Pa | E | O | M | Pl | Q |

Angiosperm root age set to max. 200 Ma

Canellaceae
- Warburgia ugandensis
- Capsicodendron dinisii
- Cinnamosma madagascariensis
- Pleodendron macranthum
- Cinnamodendron ekmanii
- Canella winterana

Winteraceae
- Pseudowintera colorata
- Drimys winteri
- Tasmannia insipida
- Takhtajania perrieri

Aristoloch-
iaceae
- Saruma henryi
- Asarum canadense
- Aristolochia macrophylla
- Thottea tomentosa
- Lactoris fernandeziana

Saururaceae
- Gymnotheca chinensis
- Saururus cernuus
- Houttuynia cordata
- Anemopsis californica

Piperaceae
- Verhuellia elegans
- Peperomia obtusifolia
- Piper betle
- Zippelia begonifolia
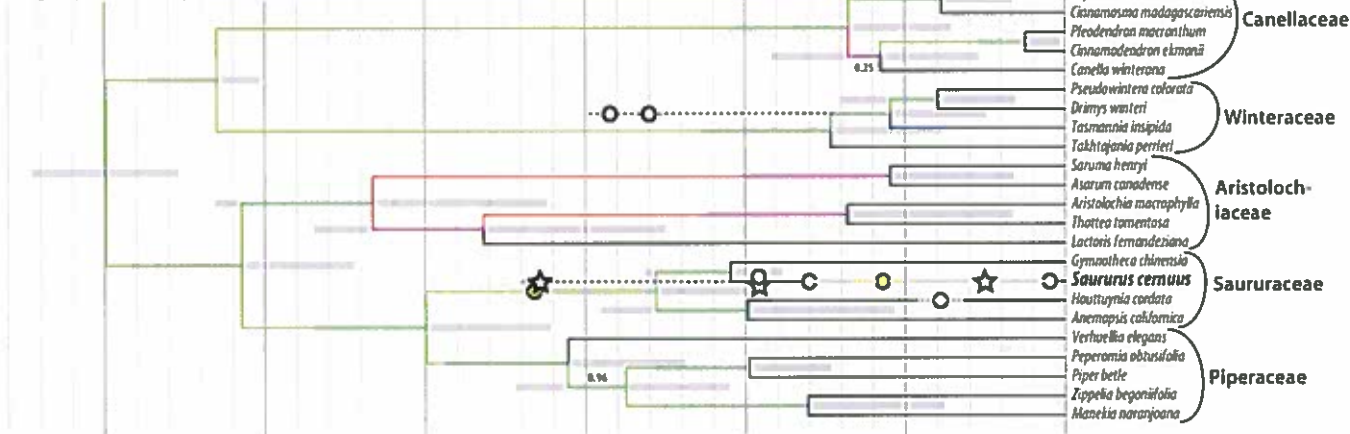- Manekia naranjoana

Figure 9

Hypothetical evolution of Saururaceae pollen

The fossil and extant *Saururus* show a morphology that may be primitive within the family: all other genera differ by one or two unique, putatively derived traits. Abbreviations: PS = (normal) pollen surface; SM = sulcus membrane. Scale bars = 1 μm.