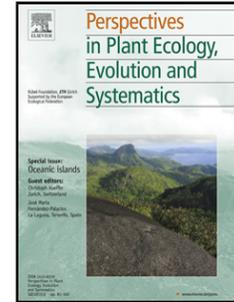


Accepted Manuscript

Title: Burmese amber fossils bridge the gap in the Cretaceous record of polypod ferns

Author: Harald Schneider Alexander R. Schmidt Jochen Heinrichs



PII: S1433-8319(16)30003-8
DOI: <http://dx.doi.org/doi:10.1016/j.ppees.2016.01.003>
Reference: PPEES 25299

To appear in:

Received date: 8-8-2015
Revised date: 15-11-2015
Accepted date: 7-1-2016

Please cite this article as: Schneider, H., Schmidt, A.R., Heinrichs, J., Burmese amber fossils bridge the gap in the Cretaceous record of polypod ferns, *Perspectives in Plant Ecology, Evolution and Systematics* (2016), <http://dx.doi.org/10.1016/j.ppees.2016.01.003>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Cretaceous fossils of derived leptosporangiate ferns are exceedingly rare.
We show that diverse polypod ferns existed in mid-Cretaceous woodlands of Myanmar.
New fossil evidence is consistent with DNA based divergence time estimates.
Hypothesis of a Cretaceous diversification of polypods is confirmed.

Burmese amber fossils bridge the gap in the Cretaceous record of polypod ferns

Harald Schneider^{a,b}, Alexander R. Schmidt^c, Jochen Heinrichs^{d*} jheinrichs@lmu.de

^aNatural History Museum, Department of Life Science, London, SW75BD, U.K.

^bSchool of Life Sciences, Sun Yatsen University, Guangzhou, 510275, Guangdong, China

^cGeorg August University, Department of Geobiology, Goldschmidtstraße 3, 37077 Göttingen, Germany

^dLudwig Maximilian University, Faculty of Biology, Department of Biology and Geobio-Center, Menzinger Str. 67, 80638 Munich, Germany

Abstract

Cretaceous fossils of derived leptosporangiate ferns (Polypodiales) are exceedingly rare yet they are needed to confirm the hypothesis of a Cretaceous diversification of polypod ferns as predicted by DNA-based divergence time estimates. Here we show that diverse polypod ferns existed in the mid-Cretaceous woodlands of Myanmar. We describe isolated sporangia with a vertical, broken annulus ring containing a differentiated stomium as well as distinguished pinnae fragments. *Krameropteris resinatus* gen. et sp. nov. is described based on a pinnae-fragment with free branched veins, exindusiate sori and polypod sporangia with trilete spores, and assigned to the early diverging polypod fern lineage Dennstaedtiaceae. Integration of the new fossil evidence in a phylogenetic framework provides support to the hypothesis of a Cretaceous diversification of polypod ferns.

Keywords

Cretaceous Terrestrial Revolution; Dennstaedtiaceae; divergence time estimates; *Krameropteris*; *Monachosorum*; Polypodiales

1. Introduction

Incongruences between divergence time estimates obtained from the fossil record and DNA sequence variation have been recognized for many lineages of organisms (Benton and Ayala, 2003). In particular, the age of the crown group diversification of angiosperms continues to be controversial because of conflicts between the age estimates based on DNA sequence data and fossil evidence (Bell et al., 2010; Zeng et al., 2014; Magallón et al., 2015). Less attention was given so far to the incongruence concerning the Cretaceous diversification of derived ferns (= core Polypodiales). The

hypothesis of a diversification of polypod ferns coinciding with the rise of the angiosperms has been established on the basis of DNA based divergence time estimates (Lehtonen et al., 2012; Schneider et al., 2004; Schuettpelz and Pryer, 2009). Precursors of this hypothesis were introduced before the advent of DNA based dating (Lovis, 1977; Rothwell, 1996; Smith, 1972), but were challenged by the rarity of Cretaceous fossils of Polypodiales (Collinson, 1996, 2001; Skog, 2001). This pattern is unlikely to be explained by rock bias against the preservation of ferns because Jurassic and Cretaceous fossils are known for many other fern lineages such as Cyatheaales, Gleicheniales, Osmundales, Salviniaceae, and Schizaeales (Hu & Taylor, 2014; Kvacek et al. 2006; Mohr et al., 2015; Skog 2001; Smith et al., 2003; Smith et al., 2015; Tidwell and Ash, 1994; Vera and Herbst, 2015). As pointed out in studies on the spore record of ferns (Nagalingum et al., 2002), the microfossil record suggests a decline of ferns in the late Mesozoic instead of the Cretaceous to Eocene radiation suggested by DNA-based divergence time estimates (Schneider et al., 2004). Thus, the paucity of fossil evidence supporting the occurrence of polypod ferns in the Early Cretaceous somewhat resembles the pattern observed in angiosperms (Magallón et al., 2015) and challenges the hypothesis of a Cretaceous radiation of derived ferns. However, the apparent conflict between fossil evidence and DNA based divergence time estimates may be solved by the discovery of new Cretaceous polypod fossils. In particular, fossil evidence is required to confirm the presence of representatives of early-diverging lineages of polypod ferns, such as Dennstaedtiaceae and Lindsaeaceae to confirm their occurrence in the Late Jurassic and Early Cretaceous as predicted by DNA based divergence time estimates (see Lehtonen et al., 2012; Schneider et al., 2004; Schuettpelz and Pryer, 2009). So far, only a single study provided unequivocal evidence for a Cretaceous occurrence of these ferns (Schneider and Kenrick, 2001) by verifying the apomorphic root cortex anatomy of lindsaeoid ferns (Lindsaeaceae sensu Christenhusz et al., 2011) in fossilized roots attached to Albian *Tmesipteris* stems. As shown by this study, polypod ferns can be identified unequivocally in the fossil record as long as apomorphic character states are preserved. The polypod clade can be recognized by the apomorphic sporangium type, i.e. a sporangium with a vertical, broken annulus ring containing a differentiated stomium (Schneider, 1996; Schneider et al., 2009). The largely poor preservation of many compression fossils disables the study of these structures. However, in the recent years, it has been shown that such sporangia are sometimes preserved as amber inclusions (Grimaldi et al., 2002; Lóriga et al., 2014).

Approximately 100 million-year-old Burmese amber (Grimaldi et al., 2002) provides a unique opportunity to study the composition of terrestrial vegetation during the Cretaceous-Tertiary Revolution (KTR). This period is marked by the rise of angiosperms, social insects, and early mammals (Benton, 2010; Lloyd et al., 2008; Meredith et al., 2011) but also by the fact that terrestrial diversity exceeded marine diversity for the first time (Vermeij and Grosberg, 2010). Discoveries based on Burmese amber fossils have provided important insights to the diversification of bryophytes (Feldberg et al., 2014; Hedenäs et al., 2014; Heinrichs et al., 2012, 2014a, b), the evolution of insect-mediated pollination (Cardinal and Danforth, 2013; Poinar and Danforth 2006; Ren et al. 2009), and the early diversification of ants (Barden and Grimaldi, 2014). However, little attention has been given to ferns in Burmese amber despite the occurrence of dispersed polypod sporangia (Grimaldi et al., 2002) and the polypod-like fossil *Cretacifilix fungiformis* (Poinar and Buckley, 2008).

Here, we aim to show that diverse polypod ferns occurred in the mid-Cretaceous forests of Myanmar. To achieve this we studied fragments of ferns preserved in

Burmese amber including isolated sporangia and leaf fragments. Special attention was given to sporangia showing the morphologically unique catapult mechanism representing the apomorphy of polypod ferns (Schneider, 1996; Schneider et al., 2009). This unique sporangium type is found in more than 95% of the Polypodiales but not in any other fern. The presence of these sporangia is therefore considered as convincing evidence for an occurrence of polypod ferns in the mid-Cretaceous and is thus comparable with the tricolpate pollen type widely recognized as significant evidence for the occurrence of eudicots (Magallón et al., 2015).

1. Material and methods

The Burmese amber collections of the American Museum of Natural History (New York), the Natural History Museum (London), and several private amber collections were screened for inclusions of ferns (Table 1). Burmese amber derives from the famous amber localities near the village of Tanai which is located on the Ledo Road about 105 km north of Myitkyina in Kachin State, Myanmar. Biostratigraphic studies suggested a late Albian age of the amber-bearing sediment (Cruickshank and Ko, 2003), hence the inclusions have a late Early Cretaceous age, with a minimum age of 98 million years (earliest Cenomanian, early Late Cretaceous) that is based on recent U-Pb dating of zircons (Shi et al., 2012).

Amber pieces with fern fragments were prepared and imaged as described by Lóriga et al. (2014). All images of Figs. 1-3 are digitally stacked photomicrographic composites of up to 65 individual focal planes, obtained by using the software package HeliconFocus 6.0 (HeliconSoft, <http://www.heliconsoft.com>) for an enhanced illustration of three-dimensional structures.

The phylogenetic relationships of the investigated fossils were interpreted using the phylogenetic framework corresponding to our current understanding of fern evolution (Lehtonen, 2011) and morphological evidence (Table S1) assembled in phylogenetic data-matrices used in phylogenetic studies (Schneider, 1996; Schneider et al., 2009). The evolution of characters was reconstructed using the maximum parsimony approach as implemented in Mesquite 3.03 (Maddison and Maddison, 2015). Structures observed in the fossils were compared to structures of extant species using the exhaustive fern herbarium of the Natural History Museum (London). The impact of the newly discovered fossils was tested using the most completely preserved pinnae fragments that included well-formed sporangia. A dated phylogenetic hypothesis was obtained for the putative extant relatives using DNA sequences of the plastid coding gene *rbcl* (Table S1) downloaded from Genbank (<http://www.ncbi.nlm.nih.gov>). The sequences were aligned manually using Mesquite whereas the chronograms were obtained using Bayesian divergence time estimates in BEAST 1.8.1 (Drummond et al., 2012) combined with jModeltest 2 (Darriba et al., 2012) and PhyML (Guindon and Gascuel, 2003) for model-selection. The results of the BEAST analyses were explored using TRACER 1.6 (Rambaut et al., 2014), summarized using Treeannotator (see Drummond et al., 2012), and visualized using FigTree 1.4.2 (Rambaut, 2014). Four alternative hypotheses were generated. Two hypotheses were based on the assumption of a constant clock with a rate of 5×10^{-4} (Villareal and Renner, 2014), whereas the other two hypotheses were generated using a lognormal relaxed clock calibrated with the amber fossil assigned to the split of the *Monachosorum* and *Hypolepis* lineage. The calibration was incorporated using lognormal distribution with the approximated age set to 98.79 (Shi et al., 2012) and a truncate range of 98.79 to 400 million years. Both constant clock and relaxed clock analyses were carried out either with a YULE tree parameter or Birth-Death tree

parameters with the latter considering incomplete sampling. Finally, we considered estimates published in Schuettpelez and Pryer (2009) as fifth hypothesis.

1. Results

1.1. Dispersed sporangia

Three amber fragments (AMNH-Bu-342, AMNH-Bu-731, and AMNH-Bu-1612, Table 1) contain numerous isolated, stalked sporangia that showed a vertically oriented annulus with a well-marked stomium including epi- and hypostomium cells (Fig. 1A-D). Fragment AMNH-Bu-1612 includes an aborted sporangium of ca 210 μm size and several mature sporangia of 220-260 μm size (Fig. 1B, D), of which one contained trilete spores 20 - 32 μm in diameter (Fig. 1D). Fragment AMNH-BU-342 contains mature polypod sporangia of ca 230-270 μm size (Fig. 1A, C) and isolated monoete spores of ca 20-35 μm diameter with a cristate perine that are sometimes still attached to the sporangia, whereas fragment AMNH-BU-731 comprised polypod sporangia of ca 230-240 μm size with trilete spores and an isolated trilete spore of 42 μm diameter possessing reticulate ridges and tubercles (Fig. 1E).

1.1. Pinnae-fragments

Five amber pieces contained leaf-fragments of which four were sterile (Table 1, Figs. 2 and 3). Fragment AMNH Bu-ASJH-3 (Fig. 2) includes pinnae with free veins, multicellular hairs of 30-310 μm length (Figs 2 F, G), and two well-developed round exindusiate sori at the end of veins (Fig. 2A, B). The sori contained ca. 20-25 sporangia of ca 140-200 μm size that were likely mature (Fig. 2C, D). Some sporangia were isolated (Fig. 2E). The sporangia had a distinct vertical annulus with a well-formed stomium (Fig. 2D). Trilete spores of 17-23 μm size were present within some sporangia (Fig. 2E). This combination of characters corresponds to the extant fern genus *Monachosorum* of the early diverging polypod family Dennstaedtiaceae (Figs. 4, 5). However, the pinnae showed a unique feature unknown to extant ferns. Ring to tuber shaped structures of ca 70-100 μm diameter are arranged irregularly at the lower leaf surface (Fig. 2A-C, see arrowhead in C). These structures appeared to be likely associated with stomata and resemble the remnants of fungal fruiting bodies found at the lower pinnae surface of extant *Monachosorum* specimens.

1.1. Taxonomic treatment of amber fossil AMNH Bu-ASJH-3

The pinnae-fragment preserved in amber piece AMNH Bu-ASJH-3 comprises a range of taxonomically useful characters. It does not match any previously described genus and thus we place it in a new fossil genus. The genus is classified as follows: Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Moniliformopses; Polypodiidae; Polypodiales; Denntstaedtiaceae.

Krameropteris H. Schneid, A.R. Schmidt & Heinrichs, gen. nov.

Diagnosis: Ferns with pinnae with free branched veins, multicellular hairs along veins, exindusiate sori at ends of veins, polypod sporangia, and trilete spores.

Type species: *Krameropteris resinatus* H. Schneid., A.R. Schmidt & Heinrichs, sp. nov., Fig. 2.

Holotype: AMNH Bu-ASJH-3.

Respository. American Museum of Natural History, New York.

Type locality. Amber mines near Tanai, about 105 km north of Myitkyina in Kachin State, Myanmar.

Etymology. The generic name was chosen in honour of the pteridologist Prof. Karl Ulrich Kramer (1928-1994), a distinguished fern taxonomist. The epitheton refers to the preservation of the holotype in amber, a fossil resin.

Description. The amber inclusion is composed of a single, 3.2 x 2.2 mm sized leaf fragment (Fig. 2A). The pinnae fragment has free branched veins, multicellular hairs of 30-310 μm length along some veins, and two exindusiate sori located at the ends of veins. Each sorus contains ca 20-25 mature polypod sporangia of ca 140-200 μm size with a vertical broken annulus and a distinct stomium. Some sporangia contain trilete spores of 17-23 μm diameter.

1.1. Divergence time estimates

The recovered divergence time estimates suggest a diversification of the dennstaedtioid crown group during the Early Cretaceous if the fossil is assigned as a calibration to the split of the *Monachosorum* clade and its sister lineage, the HYP-clade (Table 2). Slightly younger ages were found if the divergence time estimates were calculated using a standard molecular clock of 5×10^{-4} (see Table 2). Similarly, the tree prior Yule versus Death-Birth processes affected the age estimates with the Death-Birth prior providing the oldest estimates for most clades (Table 2).

1. Discussion

1.1. Amber fossils as a source to understand fern evolution

Until recently, amber inclusions have hardly been considered as a relevant source of information in studies on the evolutionary history of ferns. The lack of attention given to ferns in amber is likely caused by the rarity and small size of the preserved fragments including separated indusia and sporangia (Schmidt and Dörfelt, 2007), isolated hairs (Schmidt et al., 2010, Pérez-de la Fuente et al., 2012), or small leaf-fragments that do not show many characters of taxonomic value (Caspary and Klebs, 1907; Göppert and Berendt, 1845). Isolated indusia in Eocene Baltic amber provided evidence for the persistence of Matoniaceae in the European flora at least until the late Eocene (Schmidt and Dörfelt, 2007), while these ubiquitous Mesozoic ferns are now restricted to a few localities in the Malay Archipelago (Collinson, 2001; Tidwell and Ash, 1994). Thus, amber fossils showed that the extinction of these widespread Mesozoic ferns in the European flora did not happen before the late Eocene, providing evidence that this extinction is neither linked to the rise of the angiosperms nor to the Cretaceous-Paleogene mass extinction. Studies on fern fossils in Miocene Dominican amber did not only document the first fossil records of grammitid ferns (Gomez, 1982), and the genera *Elaphoglossum* (Lóriga et al., 2014) and *Pleopeltis* (Schneider et al., 2015), but also improved current estimates of the assembly of epiphytic fern diversity in the Neotropics.

The new fossils provide unambiguous evidence for the occurrence of polypod ferns in the Burmese amber forest because the sporangia possess the unique apomorphic catapult mechanism of polypod ferns. The presence of this sporangium type in several amber fragments may also indicate that polypod ferns were locally abundant in these forests, which challenges but not rejects the notion of an assumed rarity of polypod fossils throughout the Cretaceous (Collinson, 2001; Skog, 2001). Consistent with the frequency of polypod sporangia, the new fossil evidence suggests the occurrence of at least two lineages of polypod ferns. On one hand, polypod sporangia were found associated with trilete spores (AMNH-Bu-1612, AMNH Bu-ASJH-3). This combination is frequently found in basal polypod families such as Dennstaedtiaceae and Lindsaeaceae as well as the species rich family Pteridaceae. The second group of

sporangia (AMNH-Bu-342) combines the polypod sporangium type with the occurrence of monolete spores with a distinct perine. This character combination is found frequently in eupolypod ferns but monolete spores with distinct perines are also found in the *Pteridium-Hypolepis* clade of the Dennstaedtiaceae (Tryon & Lugardon, 1991). Monolete spores are also found in other orders of ferns but these spores lack the distinct perine (Tryon & Lugardon, 1991). All fossils documented in this study were distinct from *Cretacifilix fungiformis* (Poinar and Buckley, 2008). This fern possessed polypod sporangia producing monolete spores without an evident perine. However, it is not possible to accept the proposed relationship of this fossil to derived families of eupolypods because of the insufficient documentation of character states and the unavailability of this fossil.

1.1. Evidence for dennstaedtioid ferns in the Cretaceous

Divergence time estimates predicted the occurrence of early diverging polypod families in the Early Cretaceous (Lehtonen et al., 2012; Schneider et al., 2004; Schuettpelz and Pryer, 2009). However, so far only one unequivocally assigned fossil has been documented from this period, namely an Albian lindsaeoid root fossil (Schneider and Kenrick, 2001). The pinnae-fragment of the present study (Fig. 2) closes a gap in the fossil record of dennstaedtioid ferns. Sporangia of the polypod type in combination with trilete spores are found in the pteridoid ferns and in several families of the basal polypod grade including Cystodiaceae, Dennstaedtiaceae, Lindsaeaceae, Lonchitidaceae, and Saccolomataceae (Schneider, 1996; Schneider et al., 2009; Tryon and Lugardon, 1991). Of these families, only the Dennstaedtiaceae contain genera fitting with the remaining characters of the fossil such as the absence of indusiate structures (Figs. 4, 5). Sporangia arranged in sori with a distinct receptaculum and the absence of indusia characterize several fern genera, but only the extant genus *Monachosorum* possesses these character states in combination with polypod sporangia producing trilete spores. *Monachosorum* was considered to form an own family (Kramer, 1990) but nested in a clade comprising genera previously assigned to Dennstaedtiaceae and Hypolepidaceae in several molecular phylogenies (Hasebe et al., 1994; Lehtonen, 2011; Schuettpelz and Pryer, 2007). This clade is now considered as Dennstaedtiaceae (Christenhusz et al., 2011; Smith et al., 2006) although it comprises three distinct lineages. The three distinct Dennstaedtiaceae lineages arguably diverged in the Early Cretaceous (Fig. 5; Table 2). The first clade (DEN in Fig. 5) comprises genera with indusiate sori and trilete spores such as *Dennstaedtia*, *Leptolepia*, and *Microlepia*. The second clade (HYP in Fig. 5) comprises genera with different sporangia-arrangements, indusiate structures, and venation. Four out of the five genera, namely *Blotiella*, *Histopteris*, *Hypolepis*, and *Paesia*, possess monolete spores whereas *Pteridium* forms trilete spores (Fig. 5). However, only *Hypolepis* has its sporangia arranged in circular sori, whereas the sporangia are arranged in lines along the lamina margin in all other genera of this clade (Schneider et al., 2009). The third lineage—the putative sister to the HYP-clade—comprises the single genus *Monachosorum* (Fig. 5). *Monachosorum* species resemble the fossil in the formation of trilete spores, presence of exindusiate sori and a simultaneous maturity of sporangia. With the notable exception of *Monachosorum maximowiczii* (Kramer, 1990), the extant *Monachosorum* species have pinnulae resembling those of the fossil. Their current distribution range includes Japan, China, Indochina, the Indian subcontinent and parts of the Malay Archipelago (Kramer, 1990). The newly discovered fossil thus suggests the persistence of these ferns in Southeast Asia since the Early Cretaceous. Given the uncertainty of character state

evolution, the fossil may not be a member of the stem of *Monachosorum* as considered here but actually better placed at the branch leading to the split between *Monachosorum* and the HYP-clade (M&H in Fig. 5). However, this appears to be less likely given the character state reconstructions obtained. The unique ring-tubular structures surrounding some stomata of the fossil require further study. At the moment, we consider them as remnants of the fruiting bodies of phytoparasitic fungi because similar structures were found as remnants of fungal fruiting bodies at the lower surface of herbarium specimens of *Monachosorum*. These structures show similarities in size and irregular arrangement.

1.1. Divergence time estimates

DNA-based divergence time estimates reconstructing the split-times of clades over millions of years require to adjust the molecular clock using information provided by the fossil record (Parham et al., 2012). Application of the angiosperm pollen record as a maximum age constraint led to the hypothesis of a Cretaceous origin of the angiosperm crown group whereas a minimum age constraint allows to consider an initial diversification in the Triassic (Schneider et al., 2004; Bell et al., 2010; Smith et al., 2010; Magallón, 2014; Zeng et al., 2014; Magallón et al., 2015). However, node age calibrations are challenged by a range of issues and thus the impact of each fossil must be studied independently. This procedure helps to identify violations of assumptions taken in the assignment of fossils to lineages and nodes. For example, incorrect taxonomic assignments will result in inappropriate node calibrations and may subsequently cause incorrect divergence time estimates (Heinrichs et al., 2015). Another common issue is the usage of incorrect estimates of the age of fossils. However, the most important factor misleading divergence time estimates is the lack of fossils that can be unequivocally assigned to lineages. The impact of such fossils is further enhanced if their age dates back to the time period that is crucial to the investigated evolutionary hypothesis. Therefore, the impact of newly found fossil evidence should not only be published in form of a taxonomic description but it should be an imperative to explore the fossils' impact on our current understanding of the evolutionary history of the extant relatives using divergence time estimates. This cannot be achieved by the acceptance of published chronograms. Considering the limited number of assignable polypod fossils (see Schneider & Kenrick, 2001, 2004), *Krameropteris resinatus* fills a crucial gap in our knowledge on Cretaceous ferns, and assignment of the fossil to the split of *Monachosorum* and its sister lineage leads to divergence time estimates (Table 2) that were consistent with estimates obtained in the most comprehensive dating study of ferns available (Schuettpelz and Pryer, 2009). Most of the estimates of these authors overlapped with the confidence intervals obtained in this study (Table 2) with the notable exception of the split between the *Monachosorum* and the HYP-clade. This is caused by the rather conservative employment of the calibration log-model. We also explored a standard mutation rate proposed for chloroplast DNA of seed-free land plants (Villarreal & Renner, 2014) and arrived at somewhat younger ages than considering both fossil and molecular evidence.

1. Conclusions

The new fossil evidence from Burmese amber is consistent with DNA based divergence time estimates (see Schneider et al., 2004; Schuettpelz and Pryer, 2009), which predicted the occurrence of basal lineages of polypods in the mid-Cretaceous. Most importantly, the source forests of Burmese amber show a distinct fern diversity

which is so far exclusively formed by polypods, whereas all other previously reported Cretaceous fern floras were dominated by other fern orders including Cyatheaales, Gleicheniales, Marattiales, Osmundales, and Schizaeales (Coiffard et al., 2007; Hu and Taylor, 2014; Mays et al., 2015; Mendes et al., 2011; Mohr et al., 2015; Nagalingum and Cantrill, 2015; van Konijnenburg-van Cittert, 2002; Wing et al., 2012).

Acknowledgements

We thank David A. Grimaldi and Paul C. Nascimbene (AMNH New York) as well as Lee Davies, Hilary Ketchum, and Claire Mellish (NHM London) for providing access to museum collections. We are grateful to Beate Stolz (Bremen), Carsten Gröhn (Glinde) and James Zigras (Paramus) for their kind permission to illustrate the specimens shown in Fig. 3.

References

- Barden, P., Grimaldi, D., 2014. A diverse ant fauna from the Mid-Cretaceous of Myanmar (Hymenoptera: Formicidae). *PLoS ONE* 9, e93627.
- Bell, C.D., Soltis, D.E., Soltis, P.S., 2010. The age and diversification of the angiosperms re-visited. *Amer. J. Bot.* 97, 1296–1303.
- Benton, M.J., 2010. The origins of modern biodiversity on land. *Phil. Trans. R.Soc. B* 365, 3667–3679.
- Benton, M.J., Ayala, F.J., 2003. Dating the Tree of Life. *Science* 300, 1698–1700.
- Cardinal, S., Danforth, B.N., 2013. Bees diversified in the age of eudicots. *Proc. R. Soc. B* 280, 1–9.
- Caspary, R., Klebs, R., 1907. Die Flora des Bernsteins und anderer fossiler Harze des ostpreußischen Tertiärs. *Abh. kgl. preuss. geol. Landesanst. N. F.* 4, 1–181.
- Christenhusz, M., Zhang, X.C., Schneider, H., 2011. A linear sequence of extant families and genera of lycophytes and ferns. *Phytotaxa* 19, 7–54.
- Coiffard, C., Gomez, B., Thevenard, F., 2007. Early Cretaceous angiosperm invasion of Western Europe and major environmental changes. *Ann. Bot.* 100, 545–553.
- Collinson, M.E., 1996. “What use are fossil ferns?” - 20 years on: with a review of the fossil history of extant pteridophyte families and genera. In *Pteridology in Perspective* (eds JM Camus, RJ Johns, M Gibby), pp. 349–394. Kew: Royal Botanic Gardens.
- Collinson, M.E., 2001. Cainozoic ferns and their distribution. *Brittonia* 53, 173–235.
- Cruikshank, R.D., Ko, K., 2003. Geology of an amber locality in the Hukawng valley, Northern Myanmar. *J. Asian Earth Sci.* 21, 441–445.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Meth.* 9, 772.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A. 2012 Bayesian phylogenetics with BEAUTi and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973.

Feldberg, K., Schneider, H., Stadler, T., Schäfer-Verwimp, A., Schmidt, A.R., Heinrichs, J., 2014. Epiphytic leafy liverworts diversified in angiosperm-dominated forests. *Sci. Rep.* 4, 5974.

Goeppert, H.R., Berendt, G.C., 1845 *Der Bernstein und die in ihm befindlichen Pflanzenreste der Vorwelt*. Berlin: Nicolaische Buchhandlung.

Gómez, L.D., 1982. *Grammitis succinea*, the first new world fern found in amber. *Am. Fern J.* 72, 49–52.

Grimaldi, D.A., Engel, M.S., Nascimbene, P.C., 2002. Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. *Am. Mus. Nov.* 3361, 1–71.

Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Syst. Biol.* 52: 696–704.

Hasebe, M., Omori, T., Nakazawa, M., Sano, T., Kato, M., Iwatsuki, K., 1994. *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. *Proc. Natl. Acad. Sci. USA* 91, 5730–5734.

Hedenäs, L., Heinrichs, J., Schmidt, A.R., 2014 Bryophytes of the Burmese amber forest: amending and expanding the circumscription of the Cretaceous moss genus *Vetiplanaxis*. *Rev. Palaeobot. Palynol.* 209, 1–10.

Heinrichs, J., Reiner-Drehwald, M.E., Feldberg, K., von Konrat, M., Hentschel, J., Váňa, J., Grimaldi, D.A., Nascimbene, P.C., Schmidt, A.R., 2012. The leafy liverwort *Frullania* (Jungermanniopsida) in the Cretaceous amber forest of Myanmar. *Rev. Palaeobot. Palynol.* 160, 21–28.

Heinrichs, J., Schäfer-Verwimp, A., Feldberg, K., Schmidt, A.R., 2014a. The extant liverwort *Gackstroemia* (Lepidolaenaceae, Porellales) in Cretaceous amber from Myanmar. *Rev. Palaeobot. Palynol.* 203, 48–52.

Heinrichs, J., Schäfer-Verwimp, A., Hedenäs, L., Ignatov, M.S., Schmidt, A.R., 2014b. An acrocarpous moss in Cretaceous amber from Myanmar. *Cret. Res.* 51, 260–265.

Heinrichs, J., Scheben, A., Lee, G.E., Váňa, J., Schäfer-Verwimp, A., Krings, M., Schmidt, A.R. 2015. Molecular and morphological evidence challenges the records of the extant *Ptilidium pulcherrimum* in Eocene Baltic amber. *PLoS ONE* 10, e0140977.

Hu, S., Taylor, D.E., 2014. Floristics and paleoecology of an Early Cretaceous flora from Jordan. *Bull. Peabody Mus. Nat. Hist.* 55: 153–170.

Kramer, K.U., 1990. Monachosoraceae. In *Pteridophytes and gymnosperms* (ed. K. Kubitzki), pp. 187–188. Berlin, Springer Science & Business Media.

Kvaček, J., Dašková, J. & Renáta, P., 2006. A new schizaeaceous fern, *Schizaeopsis ekrtii* sp. nov., and its in situ spores from the Upper Cretaceous (Cenomanian) of the Czech Republic. *Rev. Palaeobot. Palynol.* 140, 51–60.

Lehtonen, S., 2011. Towards resolving the complete fern tree of life. *PLoS ONE* 6, e24851.

Lehtonen, S., Wahlberg, N., Christenhusz, M.J.M., 2012. Diversification of lindsaeoid ferns and phylogenetic uncertainty of early polypod relationships. *Bot. J. Linn. Soc.* 170, 489–503.

Lloyd, G.T., Davis, K.E., Pisani, D., Traver, J.E., Ruta, M., Sakamoto, M., Hone, D.W.E., Jennings, R., Benton, M.J., 2008. Dinosaurs and the Cretaceous Terrestrial Revolution. *Proc. R. Soc. B* 275, 2483–2490.

Lóriga, J., Schmidt, A.R., Moran, R.C., Feldberg, K., Schneider, H., Heinrichs, J., 2014. The first fossil of a bolbitidoid fern belongs to the early divergent lineages of *Elaphoglossum* (Dryopteridaceae). *Am. J. Bot.* 101, 1466–1475.

Lovis, J.D., 1977. Evolutionary patterns and processes in ferns. *Adv. Bot. Res.* 4: 229–415.

Maddison, W.P., Maddison, D.R., 2015. Mesquite: a modular system for evolutionary analysis. Version 3.03. <http://mesquiteproject.org>.

Magallón, S., 2014. A review of the effect of relaxed clock-method, long branches, genes, and calibrations in the estimation of angiosperm age. *Bot. Sci.* 92, 1–22.

Magallón, S., Gómez-Acevedo, S., Sánchez-Reyes, L.L., Hernández-Hernández, T., 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytol.* 207, 437–453.

Mays, C., Tosolini, A.-M.P., Cantril, D.J., Stilwell, J.D., 2015. Late Cretaceous (Cenomanian-Turonian) macroflora from the Chatham Islands, New Zealand: Bryophytes, lycophytes and pteridophytes. *Gondwana Res.* 27, 1042–1080.

Mendes, M.M., Dinis, J., Pais, J., Friis, E.M., 2011. Early Cretaceous flora from Vale Painho (Lusitanian basin, western Portugal): an integrated palynological and mesofossil study. *Rev. Palaeobot. Palynol.* 166, 152–162.

Meredith, R.W., Janečka, J.E., Gatesy, J., Ryder, O.A., Fisher, C.A., Teeling, E.C., Goodbla, A., Eizirk, E., Simao, T.L., Stadler, T., Rabosky, D.L., Honeycutt, R.L., Flynn, J.J., Ingram, C.M., Steiner, C., Williams, T.L., Robinson, T.J., Burk-Herrick, A., Westerman, M., Ayoub, N.A., Springer, M.S., Murphy, W.J., 2011. Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. *Science* 334, 521–524.

Mohr, B.A.R., Bernardes-de-Oliveira, M.E.C., Loveridge, R., Pons, D., Sucerquia, P.A., Castro-Fernandes, M.C., 2015. *Ruffordia goeppertii* (Schizaeales, Anemiaceae)

- a common fern from the Lower Cretaceous Crato Formation of northeast Brazil. *Cret. Res.* 54, 17–26.
- Nagalingum, N.S., Cantrill, D.J., 2015. The Albian fern flora of Alexander Island, Antarctica. *Cret. Res.* 55, 303–330.
- Nagalingum, N.S., Drinnan, A.N., Lupia, R., McLoughlin, S., 2002. Fern spore diversity and abundance in Australia during the Cretaceous. *Rev. Palaeobot. Palynol.* 119, 69–92.
- Parham, J.F., Donoghue, P.C., Bell, C.J., Calway, T.D., Head, J.J., Holroyd, P.A., Inoue, J.G., Irmis, R.B., Joyce, W.G., Ksepka, D.T., Patané, J.S.L., Smith, N.D., Tarver, J.E., van Tuinen, M., Yang, Z., Angielczyk, K.D., Greenwood, J.M., Hipsley, C.A., Jacobs, L., Makovicky, P.J., Müller, J., Smith, K.T., Theodor, J., Warnock, R.C.M., Benton, M.J., 2012. Best practices for justifying fossil calibrations. *Syst. Biol.* 61: 346–359.
- Pérez-de la Fuente, R., Delclòs, X., Peñalver, E., Speranza, M., Wierzchos, J., Ascaso, C, Engel MS., 2012. Early evolution and ecology of camouflage in insects. *Proc. Natl. Acad. Sci. USA* 109, 21414–21419.
- Poinar Jr, G.O., Buckley, R., 2008. *Cretacifilix fingiformis* gen. and sp. nov. an eupolypod fern (Polypodiales) in early Cretaceous Burmese amber. *J. Bot. Res. Inst. Texas* 2, 1175–1182.
- Poinar Jr, G.O., Danforth, B.N., 2006. A fossil bee from Early Cretaceous Burmese amber. *Science* 314, 614.
- Rambaut, A., 2014. FigTree v. 1.4.2. <http://tree.bio.ed.ac.uk>.
- Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6. <http://beast.bio.ed.ac.uk/Tracer>.
- Ren, D., Labandeira, C.C., Santiago-Blay, J.A., Rasnitsyn, A., Shih, C.K., Bashkuev, A., Logan, M.A.V., Hottón, C.L., Dilcher, D., 2009. A probable pollination mode before angiosperms: Eurasian, long-proboscid scorpionflies. *Science* 326, 840–847.
- Rothwell, G.W., 1996. Pteridophyte evolution: an often underappreciated phylogenetic success story. *Rev. Palaeobot. Palynol.* 90, 209–222.
- Schmidt, A.R., Dörfelt, H., 2007. Evidence of Cenozoic Matoniaceae from Baltic and Bitterfeld amber. *Rev. Palaeobot. Palynol.* 144, 145–156.
- Schmidt, A.R., Perrichot, V., Svojtka, M., Anderson, K.B., Belete, K.H., Bussert, R., Dörfelt, H., Jancke, S., Mohr, B., Mohrmann, E., Nascimbene, P.C., Nel, A., Nel, P., Ragazzi, E., Roghi, G., Saupe, E.E., Schmidt, K., Schneider, H., Selden, P.A., Vávra, N., 2010. Cretaceous African life captured in amber. *Proc. Natl. Acad. Sci. USA* 107, 7329–7334.
- Schneider, H. 1996. *Vergleichende Wurzelanatomie der Farne*. Aachen: Shaker.

- Schneider, H., Kenrick, P., 2001. An Early Cretaceous root-climbing epiphyte (Lindseaceae) and its significance for calibrating the diversification of polypodiaceous ferns. *Rev. Palaeobot. Palynol.* 115, 33–41.
- Schneider, H., Schmidt, A.R., Nascimbene, P.C., Heinrichs, J., 2015. A new Dominican amber fossil of the derived fern genus *Pleopeltis* confirms generic stasis in the epiphytic fern diversity of the West Indies. *Org. Div. Evol.* 15, 277–283.
- Schneider, H., Schuettpelz, E., Pryer, K.M., Cranfill, R., Magallón, S., Lupia, R., 2004. Ferns diversified in the shadow of angiosperms. *Nature* 428, 553–557.
- Schneider, H., Smith, A.R., Pryer, K.M., 2009. Is morphology really at odds with molecules in estimating fern phylogeny? *Syst. Bot.* 34, 455–475.
- Schuettpelz, E., Pryer, K.M., 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56, 1037–1050.
- Schuettpelz, E., Pryer, K.M., 2009. Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. *Proc. Natl. Acad. Sci. USA* 106, 11200–11205.
- Shi, G.H., Grimaldi, D.A., Harlow, G.E., Wang, J., Wang, J., Yang, M.C., Lei, W.Y., Li, Q.L., Li, X.H., 2012. Age constraint on Burmese amber based on U-Pb dating of zircons. *Cret. Res.* 37, 155–163.
- Skog, J.E., 2001. Biogeography of Mesozoic leptosporangiate ferns related to extant ferns. *Brittonia* 53, 236–269.
- Smith, A.R., 1972. Comparison of fern and flowering plant distribution with some evolutionary interpretations for ferns. *Biotropica* 4, 4–9.
- Smith, A.R., Pryer, K.M., Schuettpelz, E., Korall, P., Schneider, H., Wolf, P.G., 2006. A classification for extant ferns. *Taxon* 55, 705–731.
- Smith, M.K.A., Rothwell, G.W., Stockey, R.A., 2015. Mesozoic diversity of Osmundaceae: *Osmundacaulis whittlesii* sp. nov. in the Early Cretaceous of Western Canada. *Int. J. Plant Sci.* 176, 245–258.
- Smith, S.A., Beaulieu, J.M., Donoghue, M.J., 2010. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proc. Natl. Acad. Sci. USA* 107, 5897–5902.
- Smith, S.Y., Rothwell, G.W., Stockey, R.A., 2003. *Cyathea cranhamii* sp. nov. (Cyatheaceae), anatomically preserved tree fern sori from the Lower Cretaceous of Vancouver Island, British Columbia. *Am. J. Bot.* 90, 755–760.
- Tidwell, W.D., Ash, S.R., 1994. A review of selected Triassic to Early Cretaceous ferns. *J. Plant Res.* 107, 417–442.
- Tryon, A.F., Lugardon, B., 1991. *The spores of Pteridophyta*. Berlin: Springer.

Van Konijnenburg-van Cittert, J.H.A., 2002. Ecology of some Late Triassic to Early Cretaceous ferns in Eurasia. *Rev. Palaeobot. Palynol.* 119, 113–124.

Vera, E.I., Herbst, R., 2015. New cyathealean tree ferns from the Cretaceous of South Africa: *Natalipteris wildei* gen. et sp. nov. and *Kwazulupteris schaarschmidtii* gen. et sp. nov. *J. Afr. Earth Sci.* 101, 56–69.

Vermeij, G.J., Grosberg, R.K., 2010. The great divergence: when did diversity on land exceed that in the sea? *Integr. Comp. Biol.* 50, 675–682.

Villarreal, J.C., Renner, S.S., 2014. A review of molecular-clock calibrations and substitution rates in liverworts, mosses, and hornworts, and a timeframe for a taxonomically cleaned-up genus *Nothoceros*. *Mol. Phyl. Evol.* 78, 25–35.

Wing, S.L., Strömberg, C.A.E., Hickey, L.J., Tiver, F., Willis, B., Burnham, R.J., Behrensmeyer, A.K., 2012. Floral and environmental gradients on a Late Cretaceous landscape. *Ecol. Monogr.* 82, 23–47.

Zeng, L., Zhang, Q., Sun, R., Kong, H., Zhang, N., Ma, H., 2014. Resolution of deep angiosperm phylogeny using conserved nuclear genes and estimates of early divergence times. *Nature Com.* 5, 4956.

Fig. 1 Dispersed sporangia in Cretaceous Burmese amber. (A) Sporangium with four attached cristate, monolete spores (AMNH-Bu-342). (B, C) Empty sporangia with well-visible stomium (AMNH-Bu-1612 [B], and AMNH-Bu-342 [C]). (D) Sporangium containing trilete spores (AMNH-Bu-1612). (E) Isolated trilete spore possessing reticulate ridges and isolated tubercles (AMNH-Bu-731). Scale bars = 50 μm (A-D), and 10 μm (E).

Fig. 2 Holotype of *Krameropteris resinatus* sp. nov. (AMNH-Bu-ASJH-3). (A) Overview of the pinnae fragment. (B) Close-up showing two exindusiate sori. (C) Sorus with ca 25 sporangia. The arrowhead indicates what we interpret as remains of phytoparasitic fungi. (D) Sporangium containing trilete spores. (E) Remain of isolated sporangium containing two well-visible trilete spores (arrowheads). (F) Hairs at the lower leaf side. (G) Hairs (indicated by arrowheads) at the upper leaf side. Scale bars = 200 μm (A-C), and 30 μm (D-G).

Fig. 3 Burmese amber inclusions of pinnae fragments without sporangia further substantiating a diverse fern flora including polypods. (A) Leaf fragment with free vein with forked branches and hydathodes (Beate Stolz Amber Collection, sine numero). (B) Leaf fragment with free veins (Carsten Gröhn Amber Collection, no. 11039). (C) Leaf fragment with free venation (AMNH-Bu-158b). (D) Leaf fragment with free venation and possessing damage by invertebrate grazing (left) (AMNH-Bu-119). Scale bars = 1 mm.

Fig. 4 Fern phylogeny (based on Lehtonen 2011) showing the distribution of sporangia types among the main lineages of leptosporangiate ferns corresponding to

orders (white triangles) with the exception of Polypodiales which are shown as major groups (grey triangles: Eupolypods I, Eupolypods II, Pteridaceae) and basal polypod lineages (black triangles: Cystodiaceae, Dennstaedtiaceae, Lindsaeaceae, Lonchitidaceae, Saccolomataceae). Size of triangles reflects an approximation of the species diversity of each clade as approached by the number of species incorporated in the phylogeny to an estimate of the number of species included in phylogenetic studies. The black star marks the apomorphy of the polypods—the sporangium with a vertical annulus and a distinct stomium. Distribution of spore shapes is indicated for each main clade with the exception of the heterosporous Salviniales. Spore symbols: Triangle: trilete spores; Black ovals: monolete spores with the perine closely attached to the exine; Ovals with black border and grey center: monolete spores with distinct perine. The putative ancestral (and more common) type is shown on the left, whereas the derived (and sometime rare) type is shown on the right. Monolete spores with a distinct perine are the putative apomorphy of the clade comprising the eupolypod lineages.

Fig 5 Divergence time estimate of Dennstaedtiaceae based on *rbcL* DNA sequence data and analyses carried out in BEAST. The shown hypothesis is based on analyses using a relaxed molecular clock model with a YULE tree parameter and the calibration set to the M&H node. Major nodes are indicated by abbreviation given below branches (see also Table 2), whereas the time scale is given in million years in the past (Ma). The grey rectangle indicates the assumed duration of the Cretaceous Terrestrial Revolution (KTR). Grey horizontal lines indicate confidence intervals while stars indicate posterior confidence value $p \geq 0.95$. In parentheses, alternative generic names are given for species of the polyphyletic genus *Dennstaedtia*. Occurrences of three informative characters are shown for main clades using three squares organized from left to right character 1 to 3. Black filling = character state 0, white filling = character state 1, and grey filling = ambiguous. Character 1—Spore shape: trilete (0), monolete (1); Character 2—true indusia: present (0), absent (1); Character 3—arrangement of sporangia in: round to ovate sori (0), marginal coenosori or related arrangements (1).

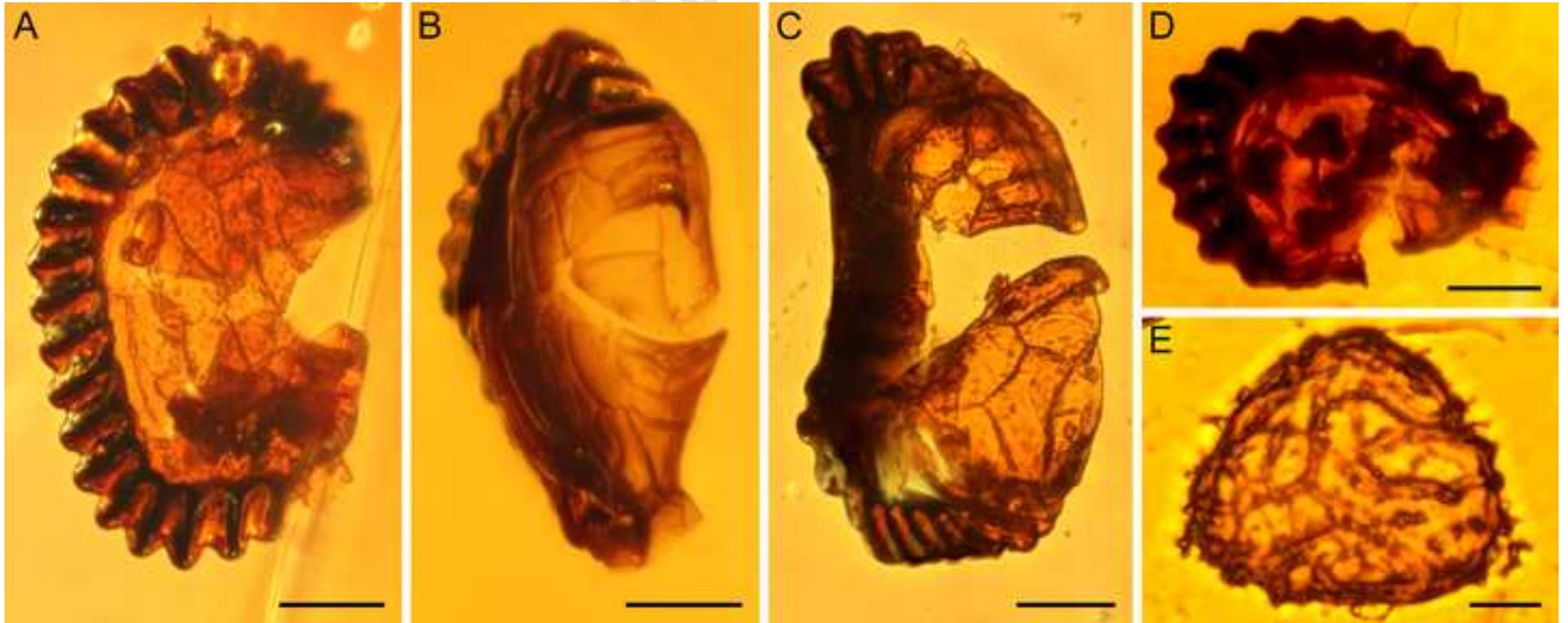
Table 1 Burmese Amber inclusions investigated in this study.

Collection	Illustration	Description
AMNH-Bu-119	Fig. 3D	Leaf fragment (partly damaged by invertebrate grazing) with free venation and without sporangia
AMNH-Bu-158b	Fig. 3C	Leaf fragment with free venation and without sporangia
AMNH-Bu-342	Fig. 1A, C	Several isolated polypod sporangia (with ca. 13 to 20 annulus cells) & isolated monolete spores with distinct cristate perine
AMNH-Bu-731	Fig. 1E	Several isolated polypod sporangia (ca 18 annulus cells) and one isolated trilete spore in the matrix. The isolated trilete spore shows some reticulate ridges and isolated

		tubercles
AMNH-Bu-1612	Fig. 1B, D	Several isolated polypod sporangia (ca 14-18 annulus cells), including one aborted sporangium, several empty mature sporangia, and one mature sporangium including trilete spores
AMNH Bu-ASJH-3	Fig. 2	Pinnae-fragment with exindusiate sori, polypod sporangia containing trilete spores
Carsten Gröhn Amber Collection (Glinde, Germany), no. 11039	Fig. 3B	Leaf fragment without sporangia and free veins
Beate Stolz Amber Collection (Bremen, Germany), sine numero	Fig. 3A	Leaf fragment without sporangia, free vein with forked branches (occasionally with 3-forkes) and hydathodes, indumentum not visible

Table 2 Divergence time estimates of main clades of Dennstaedtiaceae obtained from four analyses and compared with the results published in Schuettpelz & Pryer (2009). CalYU: estimates using relaxed clock plus the assignment of the *Krameropteris* fossil to the split between *Monachosorum*-clade and Hypolepidoideae clade using Yule parameter; CalBD: same as CalYU but with Birth-Death including incomplete sampling parameter; ClockYU: based on constant molecular clock with a rate of 5×10^{-4} and Yule process; ClockDB: same as ClockYU but with Birth-Death including incomplete sampling parameter; S&P: estimates published by Schuettpelz & Pryer 2009. Crown group age estimates are given as mean plus 95% confidence interval except for S&P because no confidence estimates were provided. The mean posterior value is given for each of the BEAST analyses in row PS. NA: not applicable because of the insufficient taxon sampling or different kind of analysis. Abbreviations for clades as in Fig. 4: MNH = Dennstaedtiaceae, DEN = *Dennstaedtia* clade, HYP = *Hypolepis* clade, MON = *Monachosorum* clade, M&H = *Monachosorum* clade & *Hypolepis* clade.

	CalYU	CalDB	ClockYU	clockDB	S&P
DMH	122.1 (99.7-158.8)	137.4 (99.2-214.7)	95.6 (82.2-111.0)	98.4 (83.2-114.6)	119.3
M&H	99.8 (98.8-103.9)	99.9 (99.0-103.7)	84.6 (70.9-101.0)	87.4 (16.6-34.3)	106.3
HYP	72.4 (51.1-90.3)	68.1 (40.2-93.1)	60.6 (42.5-62.7)	61.9 (50.9-74.8)	71.7
DEN	89.4 (60.3-132.1)	98.0 (44.8-153.7)	64.7 (52.4-77.9)	66.2 (53.7-80.5)	72.2
MON	34.8 (15.9-59.3)	29.9 (8.9-62.9)	24.9 (16.5-33.9)	24.8 (16.6-34.3)	NA
PS	-5178.9	-5166.7	-5211.7	-4973.9	NA



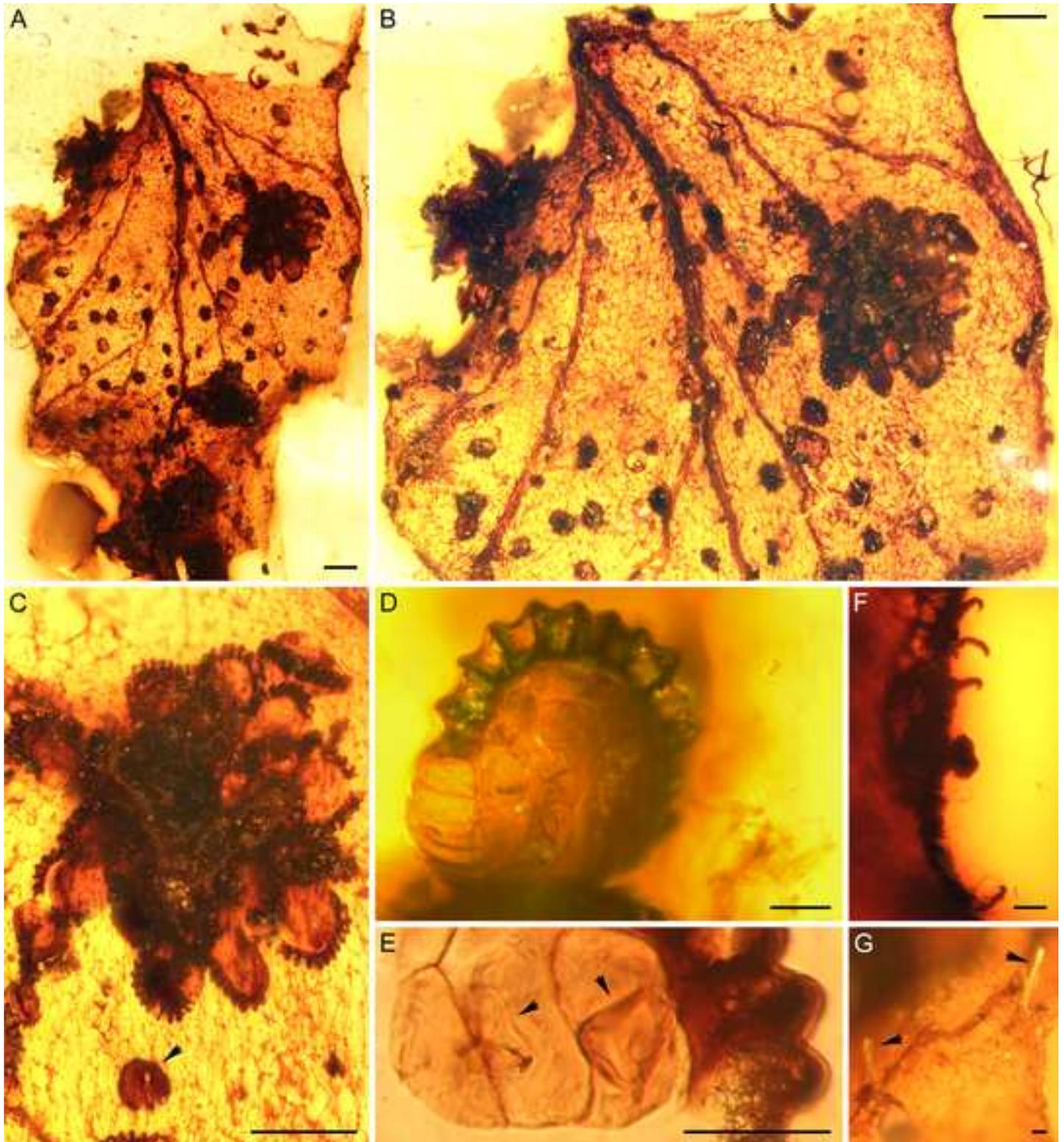


Figure 3
[Click here to download high resolution image](#)

