Cretaceous pollen cone with three-dimensional preservation sheds light on the morphological evolution of cycads in deep time

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Summary

- The Cycadales are an ancient and charismatic group of seed plants. However, their morphological evolution in deep time is poorly understood. While molecular divergence time analyses estimate a Cretaceous origin for most major living cycad clades, much of the extant diversity is inferred to be a result of Neogene diversifications. This leads to long branches throughout the cycadalean phylogeny that, with few exceptions, have yet to be rectified by unequivocal fossil cycads.
- We report a permineralized pollen cone from the Campanian Holz Shale located in Silverado Canyon, CA, USA (*c.* 80 million yr ago). This fossil was studied via serial sectioning, SEM, 3D reconstruction and phylogenetic analyses.
- Microsporophyll and pollen morphology indicate this cone is assignable to *Skyttegaardia*, a recently described genus based on disarticulated lignitized microsporophylls from the Early Cretaceous of Denmark. Data from this new species, including a simple cone architecture, anatomical details and vasculature organization, indicate cycadalean affinities for *Skyttegaardia*. Phylogenetic analyses support this assignment and recover *Skyttegaardia* as crown-group Cycadales, nested within Zamiaceae.
- Our findings support a Cretaceous diversification for crown-group Zamiaceae, which included the evolution of morphological divergent extinct taxa with unique traits that have yet to be widely identified in the fossil record.

Introduction

Recent studies characterizing exceptionally preserved gymnosperms from Cretaceous deposits (145–66 million yr ago (Ma)) are rapidly furthering our understanding on extinct seed plant diversity and evolution (e.g. Herrera *et al.*, 2017, 2020; Friis & Crane, 2019; Pattemore & Rozefelds, 2019; Friis *et al.*, 2021; Matsunaga *et al.*, 2021; Shi *et al.*, 2021; Klymiuk *et al.*, 2022). Permineralized fossils, with abundantly preserved characters, have allowed researchers to infer evolutionary relationships of enigmatic taxa within phylogenetic frameworks (Matsunaga *et al.*, 2021). While our understanding of seed plant evolution is steadily advancing from such studies, exceptionally preserved fossils assignable to Cycadales, one of the major groups of living gymnosperms, largely remain elusive (Rothwell *et al.*, 2022).

Cycadales (cycads) is a charismatic group of seed plants, comprising 25% of living gymnosperm species diversity (*c.* 300 species; Hill & Stevenson, 2004; Forest *et al.*, 2018) and is circumscribed into two families, Cycadaceae and Zamiaceae (Stevenson, 1990; Jones, 2002; Lindstrom, 2009; Salas-Leiva *et al.*, 2013; Coiro *et al.*, 2020a). The phylogenetic position of cycads within seed plants is still debated, being alternatively recovered as sister group to *Ginkgo* (Leebens-Mack *et al.*, 2019; Liu *et al.*, 2022), or as sister to all remaining groups of living seed plants (Hilton & Bateman, 2006; Li *et al.*, 2019). Members of this clade often receive the label of being 'living fossils' (Nagalingum *et al.*, 2011; Iwanycki Ahlstrand & Stevenson, 2021), stemming from crown-group cycads having ancient origins and the assumption that their morphology has been conserved throughout geologic time.

The reported cycad fossil record extends into the early Permian, as exemplified by *Crossozamia* (Gao & Thomas, 1989). While the vast majority of fossils of this group are vegetative organs (Watson & Cusack, 2005; Coiro & Pott, 2017), clear affinities of such organs can be problematic (Artabe & Archangelsky, 1992; Passalia *et al.*, 2010; Coiro *et al.*, 2020b), except for anatomically preserved stems (Artabe *et al.*, 2004; Martinez *et al.*, 2012). Conversely, recovered reproductive structures are rare, with only a handful of reports to date (e.g. Harris, 1964; Klavins *et al.*, 2003; Rothwell *et al.*, 2022). However, the phylogenetic positions of fossil cycads with known reproductive organs relative to stem or crown lineages of Cycadales are uncertain (Hermsen *et al.*, 2006; Coiro & Pott, 2017).

Despite their antiquity, molecular divergence time analyses estimate that modern cycad species richness is the result of diversification events that occurred during the Neogene (Condamine *et al*., 2015). The combination of a Permian origin and recent diversification events leads to exceptionally long branches across the cycadalean phylogeny (see Condamine *et al*., 2015). As seen in conifers (see Leslie *et al*., 2018), this may bias character distributions towards the Neogene, which limits our understanding of their phenotypic evolution in deep time. Unlike conifers, however, cycads have a relatively depauperate fossil record of systematically informative reproductive structures. This makes it difficult to reconstruct phenotypic evolutionary patterns of cycads throughout most of their evolutionary history. Therefore, the identification, characterization and phylogenetic integration of cycad fossils, especially well-preserved ones, can break up long branches, which has much potential for shedding light on cycad morphological evolution in deep time.

Here, we report a structurally preserved mature pollen cone with *in situ* pollen grains from the Late Cretaceous of western North America and assign it to a new species of the enigmatic genus *Skyttegaardia* E.M. Friis, P.R. Crane et K.R. Pedersen. Its exceptional preservation allowed us to analyse the phylogenetic position of *Skyttegaardia*, and results reveal affinities within crown-group Cycadales, more specifically Zamiaceae. The characterization of this new cycad cone contributes meaningful data on the early morphological evolution of this important gymnosperm group.

Materials and Methods

The studied cone was identified within an early diagenic calcium carbonate concretion collected from the Holz Shale Member of the Ladd Formation (Buck & Bottjer, 1985) exposed in the Silverado Canyon area, CA, USA. The Holz Shale is 250 m thick and is almost entirely exposed at the Silverado Canyon locality. Using biostratigraphy and magnetostratigraphy, the Holz Shale Member is estimated to be early Campanian in age (*c*. 80 Ma; Fry *et al*., 1985) and has been correlated with reversed magnetozones in lower Campanian strata of the Great Valley Sequence in central California (see Ward *et al*., 1983, 2012). Concretions from this locality preserve a diverse permineralized flora containing ferns, Cheirolepidiaceae, Pinaceae, Cupressaceae, a variety of gymnospermous seeds and angiosperm fruits. To date, no plants have been described from the Silverado Canyon locality.

Transverse serial sections of the pollen cone were made via the cellulose acetate peel technique (Joy *et al*., 1956). Peels were mounted onto microscope slides using Eukitt (O. Kindler, Freiberg, Germany). Photographs of the serial sections were captured with a DS-Fi3 Nikon camera mounted to a Nikon Eclipse LV100ND microscope (Nikon, Tokyo, Japan). Digital tracings (segmentations) of the pollen cone were made with ADOBE PHOTOSHOP CS6 (Adobe Systems, San Jose, CA, USA). Alignment of the traced sections and preliminary three-dimensional reconstructions were obtained in FIJI (Schindelin *et al*., 2012), final three-dimensional reconstructions and video renderings were obtained with AVIZO 9 Lite (Thermo Fisher Scientific, Waltham, MA, USA). Raw data and segmentations are archived in MORPHOSOURCE (Boyer *et al*., 2016) under the project title 'Late Cretaceous cycad pollen cone', ID: 000457842. One section of the cone (i.e. KUPB 18628 C top no. 139) was mounted with Colloidal Silver on a stub, coated with 5 µm gold with a Q150T Turbo-pumped Sputter Coater and examined using a Hitachi s-4700 scanning electron microscope on the University of Kansas Microscopy, Analytical and Imaging (MAI) Lab.

Higher-level relationships of *Skyttegaardia* E.M. Friis, P.R. Crane et K.R. Pedersen were evaluated by incorporating *Skyttegaardia nagalingumiae* Elgorriaga & Atkinson sp. nov. into the seed-plant wide morphological matrix of Hilton & Bateman (2006). Additionally, the position of *Skyttegaardia* within Cycadales was evaluated by incorporating characters from *S. nagalingumiae* to the combined matrix published by Coiro & Pott (2017), which was slightly modified by removing fossil taxa for which reproductive organs are not known (e.g. *Eobowenia*, *Ctenis*), removing taxa from the outgroup (e.g. Bennettitales) and rescoring characters 65 and 64 to the original scoring proposed by Hermsen *et al*. (2006), due to a possible frameshift error in the Martinez *et al*. (2012) and Coiro & Pott (2017) matrices (see also Marshall *et al*., 1989). Statistical data regarding each matrix can be found in Table 1. The analysed matrices can be found in Supporting Information Notes S1, GenBank accession numbers of the molecular partition of the matrix (analysed loci: CyAG, COS26, GroES, GTP, HTS; original data from Salas-Leiva *et al*., 2013) can be found in Table S1, and the morphological matrix can also be found in Morphobank (Project 4378). The character scoring for the fossil ingroup is based on the following taxa: *Crossozamia chinensis* + *Yuania striata* + *Tianbaolinia circinalis*, *Beania mamayi* + *Nilssonia tenuinervis* + *Androstrobus wonnacotti* + *Deltolepis* sp., *Antarcticycas schopfii* + *Delemaya spinulosa* + *Yelchophyllum omegapetiolaris* and *Skyttegaardia nagalingumiae*. For clarity, only the underscored generic names are displayed on the figures.

To test the influence of character coding for the limited number of characters preserved in *S. nagalingumiae*, we performed additional analyses with alternative coding schemes. Character 42 of the Hilton & Bateman (2006) matrix (microsporophyll morphology) was coded as '0: pinnate, multiveined or paddle-like' in the original analysis since that character state better captures our view of the multiveined *S. nagalingumiae* microsporophyll. In the tests, we alternatively coded this character as '?: missing'; '2: simple, one-

Table 1 Statistical information regarding matrices analysed in this study.

Matrices	Modif. Hilton & Bateman (2006)	Modif. Coiro & Pott (2017)[†]
Number of characters	102	89
Number of taxa	49	16
Matrix cells	4998	1335
Number of scored characters	3557 = 71.2%	1072 = 80.3%
Coded as missing or gap	1441 = 28.8%	263 = 19.7
Coded for *Skyttegaardia*	15 = 14.7%	12 = 13.4%

[†] Morphological partition.

(rarely three)-veined, with two pairs of longitudinal microsporangia'; and '3', a new state created for the purpose of this test. The other potentially contentious character that we tested is number 61 for the Coiro & Pott (2017) matrix, that is microsporangia clustering, which we coded as '1: free' in the main analysis. We alternatively coded this character as '?: missing' and '3' which is a new state solely created for the purpose of this test, since the two other character states of the matrix implied a degree of fusion of the microsporangia not present in *S. nagalingumiae*.

All phylogenetic analyses were performed with TNT v.1.5 (Goloboff *et al.*, 2008; Goloboff & Catalano, 2016) with the following parameters: heuristic search under equal weights based on 1000 random addition sequences of taxa (RAS), followed by tree-bisection and reconnection (TBR) and branch swapping (holding 10 trees per replication). The most parsimonious trees found were then used for a final round of TBR branch swapping, and a strict consensus was calculated for the resulting trees. Consistency index ('CI') and retention index ('RI') were calculated for the two matrices with 'STATS.run' macro on TNT v.1.5. Morphological matrices were coded with MESQUITE v.3.70 (Maddison & Maddison, 2021).

The strict-consensus tree of the Cycadales-specific analyses was plotted against geologic time with the use of R software (R Core Team, 2021), using the functions timePaleoPhy and geoscalePhylo in the paleotree and strap packages, respectively (Bapst, 2012; Bell & Lloyd, 2015). The scaling method used was 'minimum branch length ('mbl') with a value of three for internal nodes with unknown origination ages estimates. Ages for diversification of living species, origin of crown-group genera and possible stem members were obtained from Condamine *et al.* (2015), Coiro & Pott (2017) and Calonje *et al.* (2019).

Measurements of length and diameter of pollen cones of each species of cycads were compiled from Whitelock (2002), and complete list of measurements can be found in Table S2 (also containing other various measurements). A number of pollen sacs per microsporophyll (PSPM) were obtained from Chamberlain (Chamberlain, 1912, 1916, 1935) and Klavins *et al.*, (2003), and are based on the following species: *Bowenia serrulata* (W. Bull) Chamberlain, *Ceratozamia mexicana* Brongn., *Cycas media* R. Br., *Dioon edule* Lindley & *Dioon spinulosum* Dyer, *Encephalartos caffer* (Thunb.) Lehm, *Macrozamia miquelii* (F. Muell ex Miq.) A. DC., *Microcycas calocoma* (Miq.) A. DC., *Stangeria eriopus* (Kunze) Baillon, *Zamia integrifolia* L. fil. in Aiton.

For comparative purposes, we studied sporophyll tissue of living cycads. Apical and basal microsporophylls of *Zamia variegata* Warsz. and *Zamia pumila* L. were obtained from greenhouse facilities at the University of Kansas. With permission, megasporophylls of *Stangeria eriopus* were obtained from greenhouse facilities at California State Polytechnic University, Humboldt.

Results

Systematic palaeontology

Order Cycadales Pers. ex Brecht. et J.Presl.
Family Zamiaceae Miq.

Genus *Skyttegaardia* E.M. Friis, P.R. Crane et K.R. Pedersen emend

Emended generic diagnosis Pollen cone with helically arranged microsporophylls, each consisting of a proximal fertile stalk-like portion and a distal sterile extension. Fertile portion massive with two sporangia deeply sunken in two cavities arranged symmetrically on the abaxial side of the stalk-like portion with one sporangium in each cavity. Dehiscence of sporangium by a longitudinal slit facing the opening of the cavity. Sterile distal tip of microsporophyll elongate, conical, tapering distally to a slender point. Cuticle thick; stomata on distal tip haplocheilic, deeply sunken. Pollen boat-shaped, monocolpate, tectate–psilate to foveolate. Pith of cone surrounded by endarch cauline bundles, composed by loosely radially aligned metaxylem tracheids with scalariform secondary wall thickenings capped by phloem. Microsporophylls supplied by a bundle that bi- or trifurcates at the level of insertion to the stalk, with each lateral daughter bundle providing a trace for each pollen sac. Daughter bundles continue distally and are eventually replaced by transfusion tracheids with reticulate to pitted wall thickenings in the distal extension of the microsporophyll. Mucilage canals in cone axis and microsporophylls.

Species – *Skyttegaardia nagalingumiae* Elgorriaga & Atkinson sp. nov.

Etymology Named in honour of the late Dr. Nathalie Nagalingum (California Academy of Sciences) whose contributions have greatly improved our understanding on the evolution and diversification of cycads.

Holotype KUPB 18628 C top, slides 1–218, deposited on the Paleobotany collections of the University of Kansas.

Type locality Silverado Canyon, Santa Ana Mountains, CA, USA (lat. 33°44′43″N, long. 117°38′37″W).

Stratigraphy and age Holz Shale Member, Ladd Formation, Late Cretaceous. Early Campanian (*c.* 80 Ma; Buck & Bottjer, 1985; Fry *et al.*, 1985).

Diagnosis. Pollen cone with helically arranged microsporophylls, central axis of 1 mm diameter and total cone diameter of 2 mm. Microsporophyll stalk region short, up to 420 μm long, with a marked abaxial keel, width ranges from 40 up to 600 μm. Sterile distal extension measuring up to 100 μm in width and 1200–1800 μm in length. Pollen sacs with thick uniseriate walls, subcircular to elliptical in outline, measuring 200–300 μm wide and 400–450 μm long. Pollen measuring up to 14–20 μm long on its major axis, with foveolate exine surface. Mucilage canals measuring 50–150 μm in diameter. Transfusion tracheids from distal region of microsporophyll abundant, with reticulate to pitted secondary wall thickenings.

Description. *Skyttegaardia nagalingumiae* is described from a cylindrical pollen cone measuring *c.* 3–5 mm. tall and 2 mm. wide (Figs 1–3). The cone comprises a central axis 1 mm. in diameter, with up to five endarch cauline bundles and a

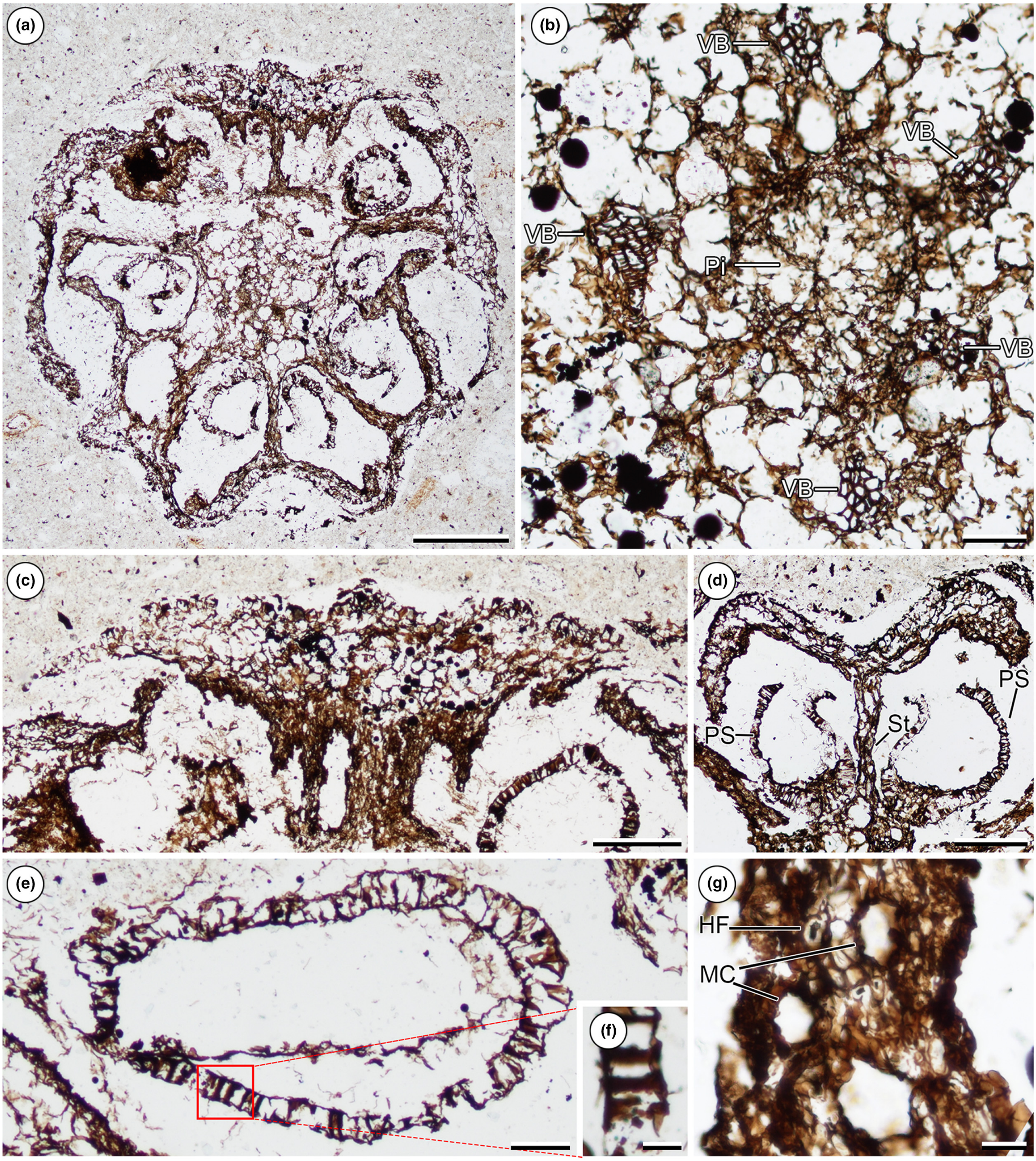

Fig. 1 *Skyttegaardia nagalingumiae* sp. nov. Elgorriaga et Atkinson. Specimen KUPB 18628, general anatomical features. (a) Transverse section of the cone displaying the central axis surrounded by helically arranged microsporophylls. Slide no. 100a. (b) Detail of main axis displaying five vascular bundles (VB) immersed in loosely organized parenchyma, note central pith (Pi). Slide no. 90a. (c) Transverse section of microsporophyll above the level of the pollen sacs. Slide no. 96a. (d) Transverse section of microsporophyll displaying abaxial keel of the stalk (St) flanked by dehisced pollen sacs (PS). Slide no. 110a. (e) Transverse to oblique section of pollen sac with ovoid outline. Slide #61a. (f) Detail of the uniseriate pollen sac wall displaying prominent thickenings. Slide no. 61a. (g) Detail of multiple mucilage canals (MC) and hypodermal fibres (HF) on the microsporophyll. Slide no. 72a. Bars: (a) 400 μm; (b, e) 50 μm; (c, d) 200 μm; (f, g) 15 μm.

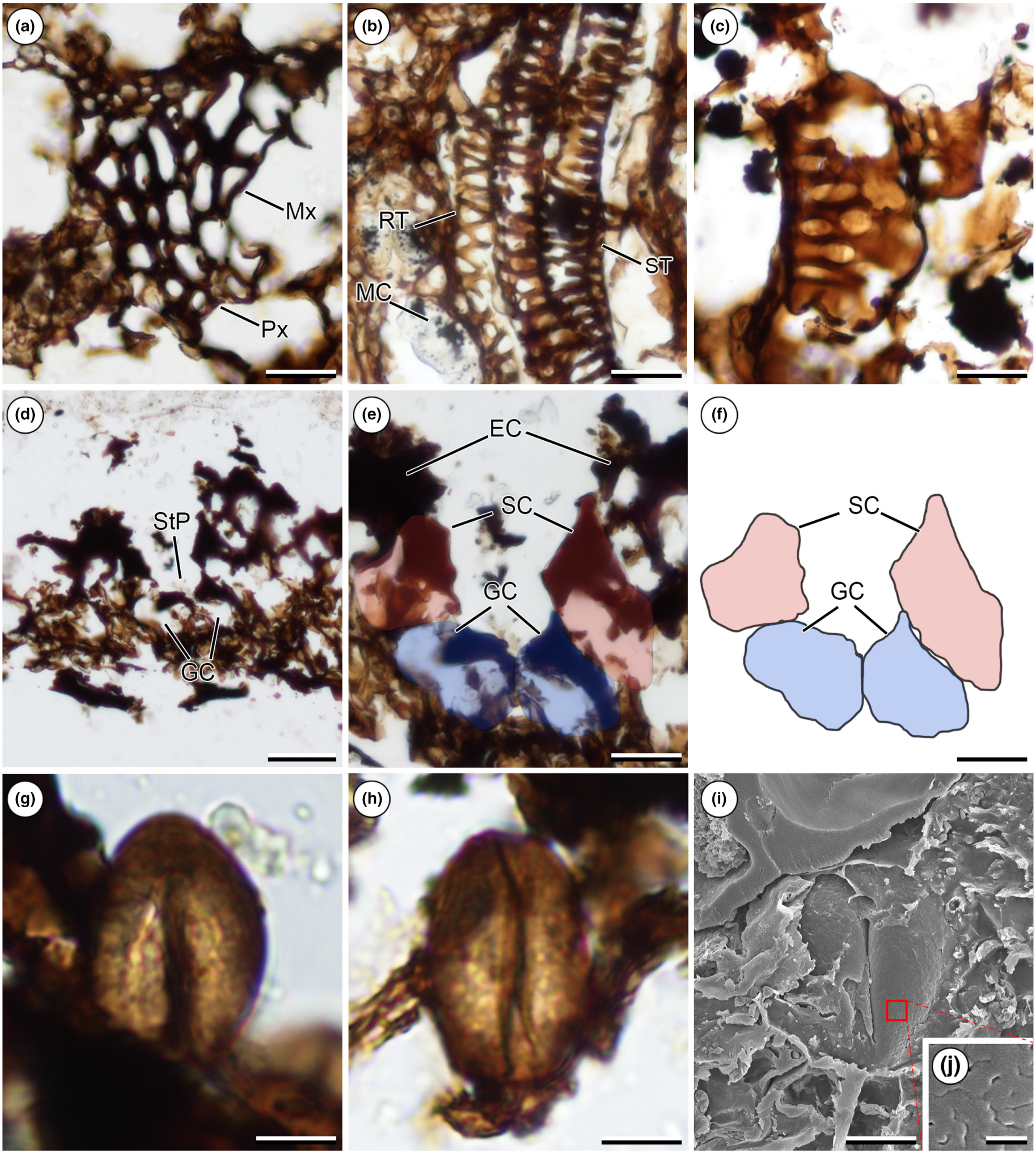

Fig. 2 *Skyttegaardia nagalingumiae* sp. nov. Elgorriaga et Atkinson. Specimen KUPB 18628, details of bundles, stomata and pollen grains. (a) Transverse section of vascular bundle displaying small protoxylem elements (Px) and larger metaxylem elements (Mx). Slide no. 96a. (b) Oblique section of vascular bundle displaying reticulate (RT) and scalariform wall thickenings (ST), and a possible mucilage canal towards the bottom left (MC). Slide no. 59a. (c) Detail of transfusion tracheid from the distal part of the microsporophyll displaying reticulate to pitted wall thickenings. Slide no. 96a. (d) Possible stomatal pore (StP) from the epidermis of the distal region of the microsporophyll, see possible guard cells (GC). Slide no. 68a. (e, f) Detail of possible stomata displaying superficial epidermal cells (EC), sunken guard cells (GC, blue) and subsidiary cells (SC, red). Slide no. 68a. (g, h) Detail of *in situ* monocolpate pollen grain with elliptical to oblong outline. Slide no. 139a. (i, j) SEM images of *in situ* monocolpate pollen grain displaying foveolate exine. Peel no. 139a. Bars: (a–c, e–i) 15 μm; (d) 30 μm; (j) 0.5 μm.

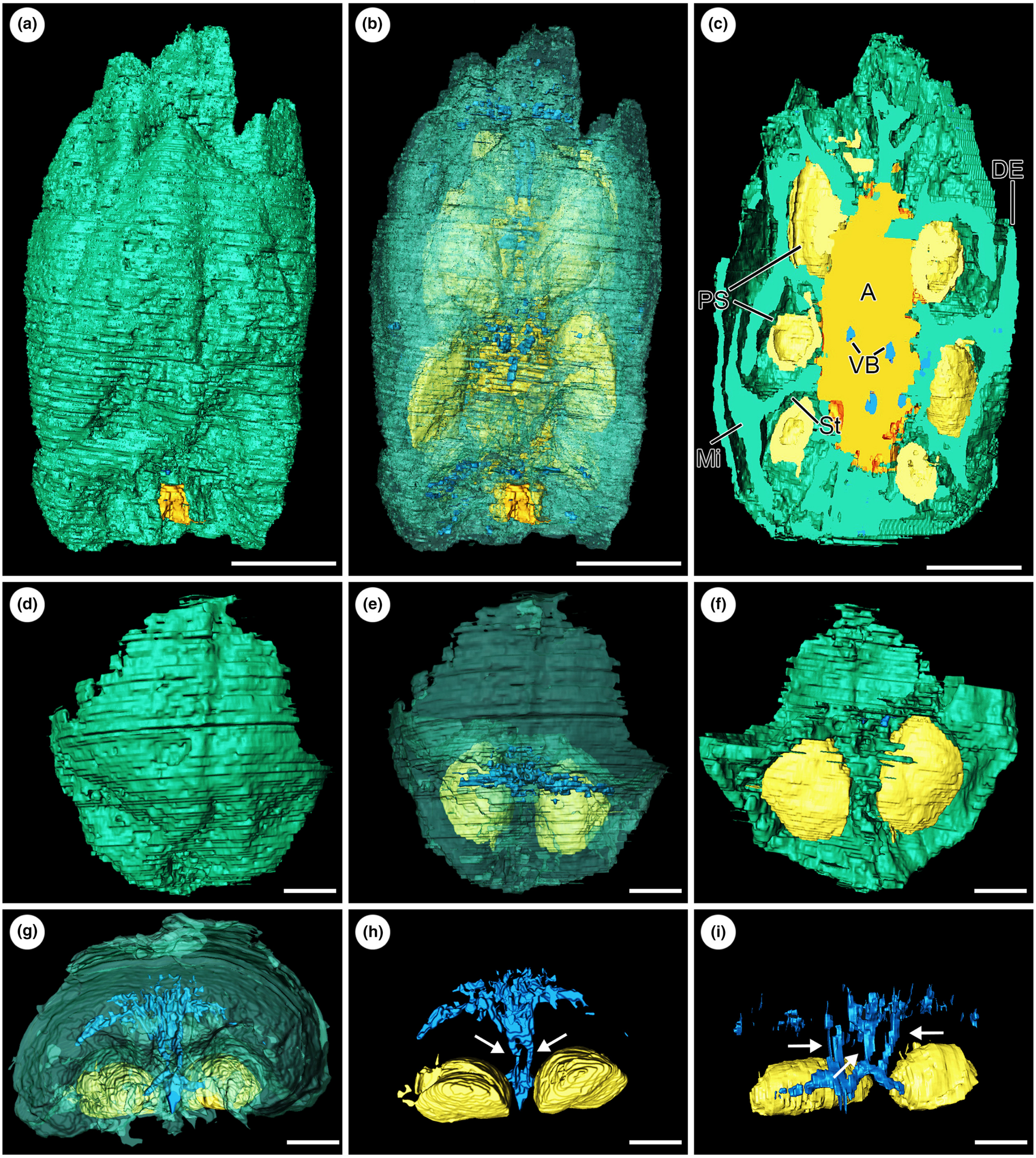

Fig. 3 Three-dimensional reconstruction of *Skyttegaardia nagalingumiae* sp. nov. Elgorriaga et Atkinson based on specimen KUPB 18628. (a) General view of the cone displaying the imbricate nature of the microsporophylls. (b) General view of the cone with transparent microsporophylls, showing the placement of pollen sacs and vasculature. (c) Longitudinal to slightly oblique orthoslice throughout the cone featuring the axis (A), vascular bundles (VB), pollen sacs (PS), distal extension (DE), microsporophyll (Mi) and stalk (St). (d–h) Isolated microsporophyll in different views; note the general morphology (d–f), the embedded nature of the pollen sacs within the microsporophyll tissues (e–g), and the architecture of the vascular tissues (g, h), with one small trace directed towards each pollen sac, and two bundles (arrows) directed towards the distal region, where they are replaced by an extended network of transfusion tracheids. (i) Detail of the vascular system of another microsporophyll, featuring three bundles (arrows) directed towards the distal region. Colour code: green, Microsporophylls; blue, vascular tissue; yellow, pollen sacs; orange, axis. Bars: (a–c) 500 μm; (d–i) 200 μm.

parenchymatous pith and cortex (Fig. 1a,b). Mucilage canals of the cone axis are present in cortex and pith and measure 50–100 μm in diameter and are lined by small epithelial cells.

Eight microsporophylls are helically arranged along the cone axis, each consisting of a proximal fertile stalk-like region, and a distal sterile expansion (Fig. 1a,c,d). Seven microsporophylls are fertile, while the basalmost one is sterile. The stalk region possesses a conspicuous abaxial keel < 100 μm wide and a flat adaxial surface reaching up to 600 μm at its widest point (Fig. 1c,d). Mucilage canals of up to 125 μm in diameter, as well as hypodermal fibres, occur in this region and extend up to the distal extension (Fig. 1g). Distally, the microsporophyll expands both basipetally and acropetally, with the latter expansion being longer, reaching up to 1600 μm long. In this way, the microsporophyll face shape is rhomboidal (see 'Three-dimensional reconstruction' in the Results section).

A subcircular to elliptic pollen sac is partially sunken, abaxially, on each side of the stalk keel near the distal extension of the microsporophyll, which also covers its lateral margins (Fig. 1a,c). The walls of the pollen sacs are uniseriate except near the point of attachment (suggesting ectokinetic type of dehiscence) and reach up to 60 μm thick (Fig. 1d–f). Their cells displaying prominent rectangular wall thickenings (Fig. 1e,f).

The pollen cone vascularization reveals that it is a simple cone. Axial bundles are composed of small centripetal protoxylem elements < 8 μm and centrifugal metaxylem tracheids with a loose radial arrangement, measuring 8–15 μm in diameter, featuring scalariform to reticulate secondary wall thickenings (Fig. 2a,b). A single trace diverges from each cauline bundle and bi- or trifurcates near the point of attachment of the microsporophyll. From each lateral daughter bundle, a small trace supplies a pollen sac, while the rest of the bundle expands towards the distal region, where they are replaced by an extensive network of wide transfusion tracheids that expands laterally, featuring reticulate to pitted secondary wall thickenings (Fig. 2c). Stomata are present on the epidermis of the distal expansion of various microsporophylls and comprise sunken guard cells overarched by subsidiaries (Fig. 2d–f).

Although the pollen sacs are dehisced, a few in situ pollen grains are present. Grains are boat-shaped, monocolpate and measure 14–20 μm long on its major axis and 9.5–12 on its minor axis (Fig. 2g–i). Pollen grain walls have a fine foveolate surface ornamentation that form a vermiculate pattern (Fig. 2i,j).

Three-dimensional reconstruction. The three-dimensional reconstruction of this pollen cone accurately illustrates its morphology. The cone is composed of highly imbricate rhomboidal microsporophylls that cover the entirety of the cone's external surface, obscuring the central axis and pollen sacs (Fig. 3a–d). The internal vascular system of both the cone and the microsporophylls is complex, showing helical divergences of traces. Upon entering the microsporophylls, each trace bi- or trifurcates with each lateral secondary trace supplying a small bundle that enters each pollen sac and continues distally into the laminar head (Fig. 3e–i; Videos S1, S2).

Remarks. The small size of the cone described in this contribution is striking. One could argue that it may be due to immaturity or incomplete fossilization via predepositional abrasion. However, all pollen sacs are dehisced, which obviously indicates the cone was mature before deposition. Moreover, the basal scales taper to a smaller size, with the lowest one being sterile. This pattern is seen in reproductive cones of many gymnosperms. Therefore, we interpret the cone described here as being mature and nearly complete.

Sporophyll morphology of selected living cycads

Apical and basal microsporophylls of *Zamia variegata* and *Z. pumila* are reduced in size compared with sporophylls from the rest of their respective pollen cones (Fig. 4a–f). These microsporophylls were found to have 2–7 pollen sacs on each side (Fig. 4a,c,d,f). Megasporophylls of *Stangeria eriopus* revealed two ovules abaxially sunken within ground tissue of the distal extension (Fig. 4g–i). Lateral margins of the distal extension envelope the lateral sides of each ovule (Fig. 4h,i).

Phylogenetic results

Phylogenetic analyses using a morphological matrix with a broad taxon sampling (i.e. Hilton & Bateman, 2006 matrix), including various groups of seed plants and the new information about *Skyttegaardia*, resulted in five most parsimonious trees ('MPT') of 315 steps, with a CI and RI of 0.453 and 0.8 respectively, and with 99% of the characters being informative. The strict consensus of these trees place *Skyttegaardia* in a polytomy together with 'Cycadaceae' and 'Zamiaceae' (Fig. 5), suggesting a cycadalean affinity for this enigmatic genus.

Phylogenetic analyses focused on relationships with Cycadales resulted in three MPT of 2561 steps, having a CI and RI of 0.86 and 0.57 respectively, with 78% of the characters being informative. In the strict consensus of these trees, *S. nagalingumiae* is recovered as member of the crown-group Zamiaceae, sister to *Stangeria* (Fig. 6). *Crossozamia* is recovered as sister to *Cycas*, whereas *Beania* (= 'NILANBE' in previous studies; Thomas & Harris, 1960; Harris, 1964) and the *Antarcticycas* plant (Klavins *et al.*, 2003; Hermsen *et al.*, 2009; Schwendemann *et al.*, 2009) are part of a polytomy due to their ambiguous placement, either as stem-group Cycadales or stem-group Zamiaceae (Fig. 6). Additional analyses with alternative character state schemes for the Hilton & Bateman (2006) and Coiro & Pott (2017) matrices (see the Materials and Methods section), resulted in trees that displayed the same topology as the trees in the main analyses.

Discussion

Phylogenetic position of *Skyttegaardia*

Skyttegaardia nagalingumiae is characterized from a minute compact cone comprising helically arranged microsporophylls with two abaxially attached pollen sacs that produce monocolpate

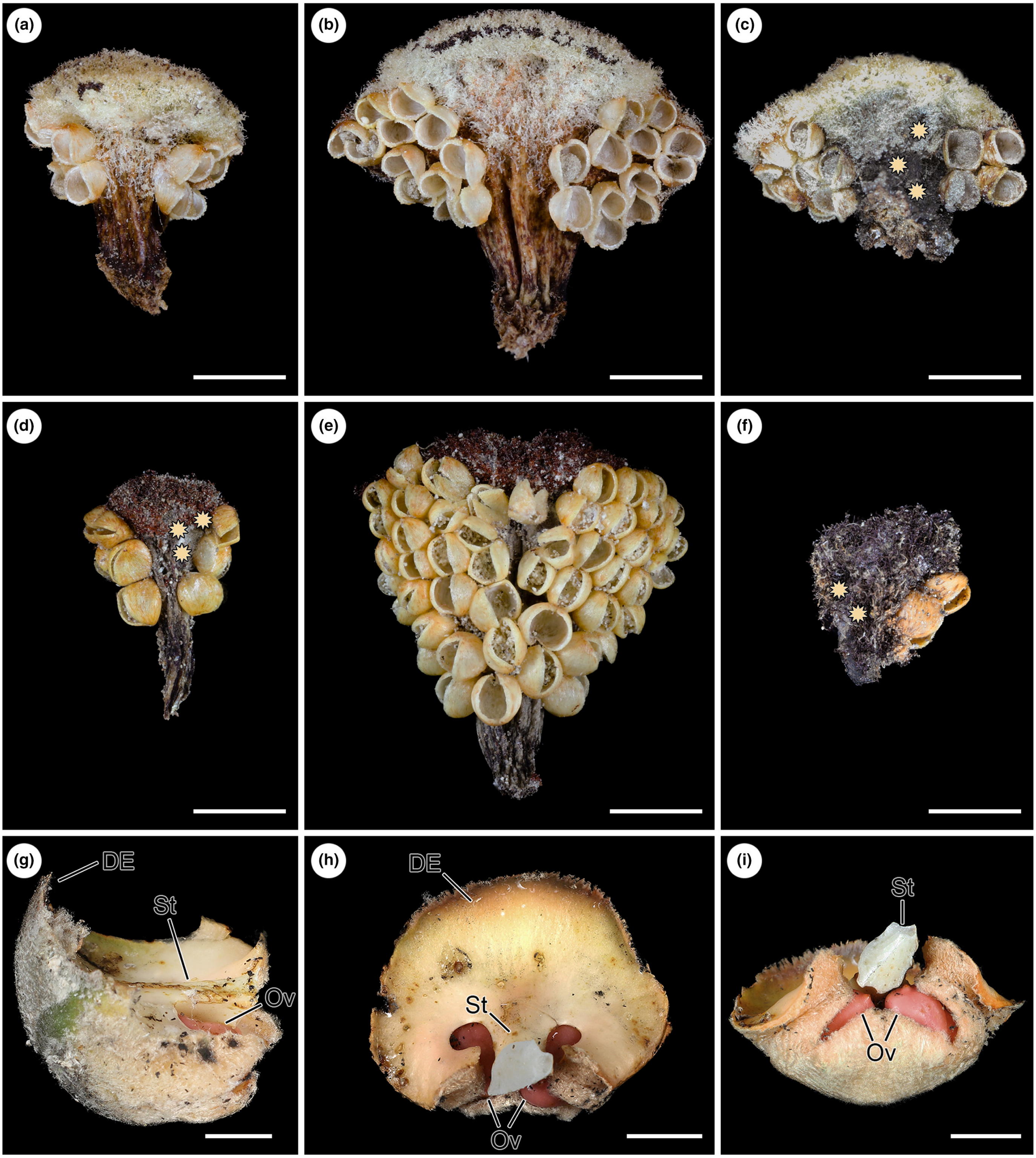

Fig. 4 Abaxial view of microsporophylls from different regions of the pollen cones of *Zamia variegata* (a–c) and *Zamia pumila* (d–f), and different views of a megasporophyll of *Stangeria eriopus* (g–i). (a). Microsporophyll from the upper portion of the cone with eight pollen sacs. (b). Microsporophyll from the *middle portion* of the cone with 24 pollen sacs. (c). Microsporophyll from the lower portion of the cone with 11 pollen sacs. (d). Microsporophyll from the upper portion of the cone with 8 pollen sacs. (e). Microsporophyll from the middle portion of the cone with 46 pollen sacs. (f). Microsporophyll from the lower portion of the cone with two pollen sacs. Yellow stars mark the position of pollen sacs probably lost during preparation of the material. (g–i). Lateral (g), proximal (g) and abaxial (i) views of *Stangeria eriopus* megasporophylls, note the ovules (Ov) sunken in tissue of the lateral regions of the distal extension (DE), and also partially covered by the stalk (St). Bars: (a–f) 2 mm; (g–i) 10 mm.

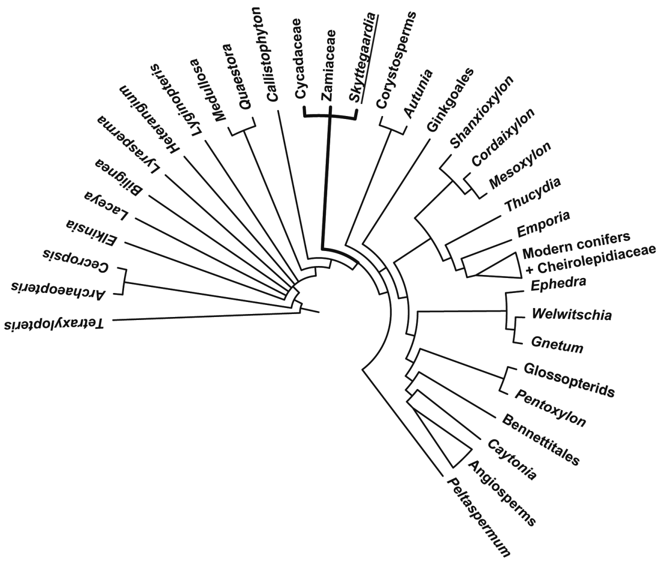

Fig. 5 Strict consensus of the five most parsimonious trees. Included are numerous representatives of completely extinct lineages of seed and nonseed plants, and all major living groups of seed plants; note the placement of *Skyttegaardia* (underlined) in the same clade as the 'Cycadaceae' and 'Zamiaceae' (thicker branch), reaffirming its cycadalean affinities.

pollen. The exceptionally preservation of this fossil allows us to infer its systematic and phylogenetic relationships through comparative and phylogenetic analyses. The general cone morphology stated above is comparable to several gymnosperm groups, most of which are distantly related, such as Erdtmanithecales, Glossopteridales, *Lasiostrobus*, Bennettitales, Coniferales, Ginkgoales and Cycadales.

Erdtmanithecales is an extinct order of seed plants with compact pollen cones that have been proposed to be closely related to Gnetales (reviewed in Friis *et al*., 2011). Erdtmanithecalean microsporophylls are peltate with several elongate pollen sacs attached to the underside of the distal expansion. In addition, Erdtmanithecales plants produced *Eucommiidites*-type pollen, which has three furrows that extend along the major axis of the grains (see Mendes *et al*., 2010; Friis *et al*., 2011). This pollen type clearly differs from that of *S. nagalingumiae*.

Late Paleozoic and Mesozoic 'seed ferns' typically lack compact pollen cones (Taylor & Taylor, 2009). Glossopterids, however, have structures similar to pollen cones with helically arranged multiveined microsporophylls, similar to *S. nagalingumiae* (Ryberg *et al*., 2012; Nishida *et al*., 2014; McLoughlin & Prevec, 2021). Pollen sacs in glossopterids are born on elongate branching axes that are attached to the adaxial surface of the

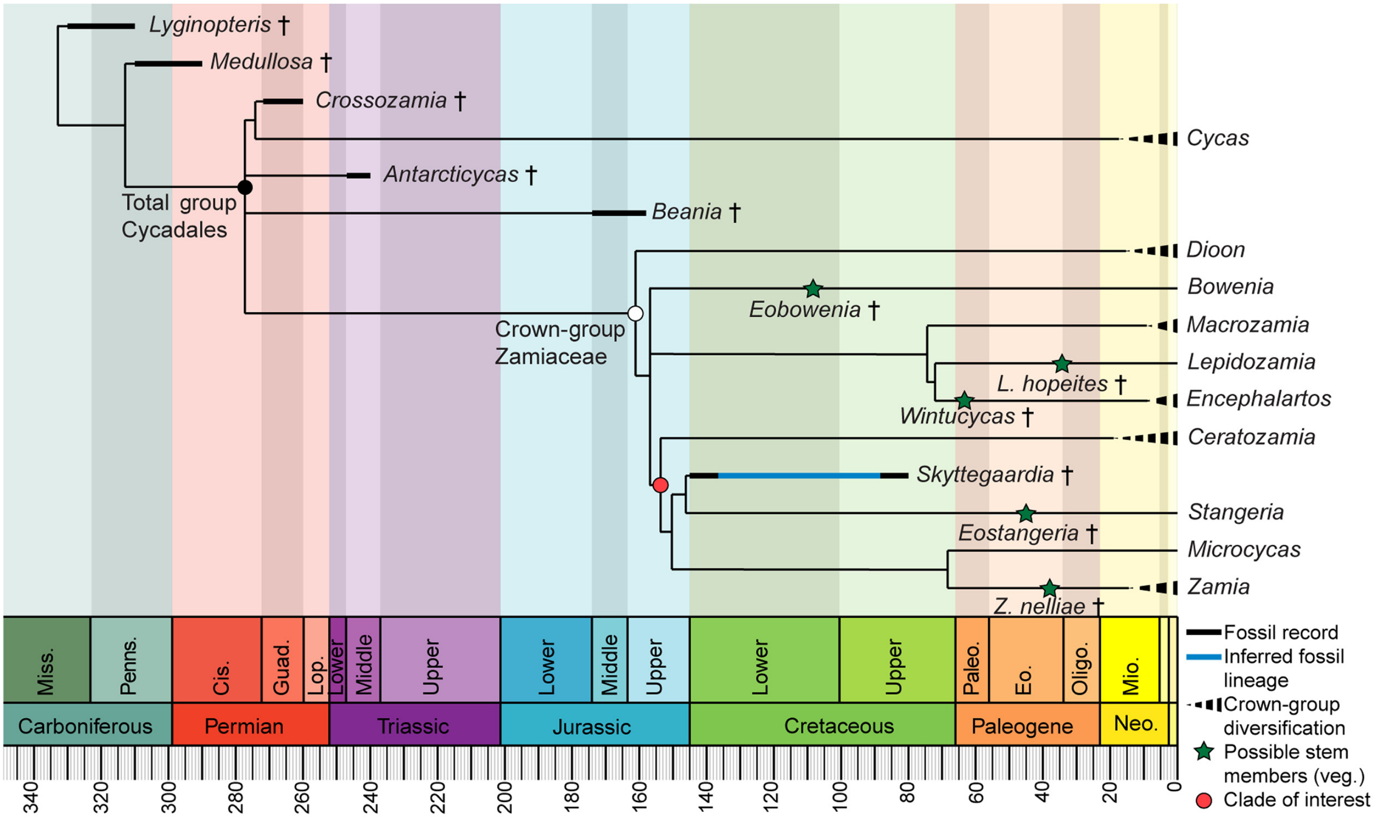

Fig. 6 Strict-consensus phylogenetic tree of cycads plotted against geologic time, incorporating *Skyttegaardia* and other fossil species (or species concepts) for which reproductive organs are known (i.e. *Crossozamia*, *Antarcticycas* and *Beania*) + outgroups (i.e. *Lyginopteris* and *Medullosa*). Note the possible phylogenetic and stratigraphic placement of putative stem-group members of various genera for which no reproductive organs are known (green stars).

microsporophylls, which differs from *S. nagalingumiae*. In addition, pollen grains of glossopterids are bisaccate and often striate (Ryberg *et al.*, 2012; Nishida *et al.*, 2014; McLoughlin & Prevec, 2021), while the pollen grains of *S. nagalingumiae* lack saccae and striae.

Lasiostrobus Taylor (1970) is an extinct genus based on permineralized compact pollen cones from the Carboniferous of North America. The pollen cone of this Carboniferous plant is similar to *S. nagalingumiae* by having helically arranged laminar microsporophylls with abaxially attached pollen sacs. However, the pollen sacs of *Lasiostrobus* produce polysaccate grains that are inaperturate (Taylor, 1970). In addition, the microsporophylls of *Lasiostrobus* are described as fleshy, which differs from those of *S. nagalingumiae*.

Similar to *S. nagalingumiae*, Bennettitales have cones that produce monocolpate grains. Pollen of Bennettitales can have a punctate, scabrate, verrucate or, less often, psilate surface (Osborn & Taylor, 1995; Zavialova *et al.*, 2009). However, bennettitalean cones are either bisporangiate with highly fused microsporangia (e.g. *Cycadeoidea*; Taylor *et al.*, 2009), or monosporangiate with a cup-shaped morphology bearing radiating pinnate microsporophylls divided in numerous lobes bearing partially fused, and adaxially located, pollen sacs (e.g. *Weltrichia*, *Lunzia*; Pott *et al.*, 2017; Popa, 2019). Both morphologies differ from the cone of *S. nagalingumiae*.

Like the pollen cone of *Skyttegaardia*, pollen cones of conifers often have numerously helically arranged microsporophylls that can have two abaxially attached pollen sacs (Bierhorst, 1971). However, the pollen grains of *Skyttegaardia* differ from those of conifers. Voltzialean, Pinaceae and Podocarpaceae (pre-)pollen grains are saccate (Ueno, 1960). Cupressaceae and Sciadopytiaceae pollen grains have a verrucate surface. Pollen grains of Araucariaceae, Taxaceae and Cephalotaxaceae have a psilate to granulate surface. Overall, conifer assacate pollen lack a germinal furrow, which differs from the monocolpate pollen grains of *Skyttegaardia*.

Pollen cones of *Ginkgo* have helically arranged microsporophylls, each with two pollen sacs that produce boat-shaped monocolpate pollen grains (Sprecher, 1907). In addition, pollen cones of *Ginkgo* contain mucilage/resin cavities (Ameele, 1980), somewhat similar to *S. nagalingumiae*. However, *Ginkgo* pollen cones are lax and microsporophylls have a reduced distal extension, which contrasts from the pollen cones of *Skyttegaardia*. Unlike *Skyttegaardia*, the pollen sacs of *Ginkgo* are particularly elongate. Moreover, a single vascular trace enters the microsporophylls of *Ginkgo* and then distally bifurcates with each daughter bundle entering and supplying the two distally attached pollen sacs (Thibout, 1896; Mundry & Stützel, 2004). This vascular pattern differs from *S. nagalingumiae* (see 'Description' in the Results section). In addition, the ornamentation of *Ginkgo* pollen grains is psilate to striate, which differs from the pollen grains of *Skyttegaardia*.

The only known extinct seed plant that has a similar microsporophyll morphology with a short stalk, only two pollen sacs deeply sunken within the microsporophyll, sunken stomata, boat-shaped monocolpate pollen with a foveolate and slightly rugulate surface, and a distal extension of the sporophyll is *Skyttegaardia galtieri* E.M. Friis, P.R. Crane et K.R. Pedersen (2021). Before this work, *S. galtieri* was the only species of the genus and was originally described from isolated sporophylls from the Berriasian of Bornholm, Denmark. While *S. galtieri* and *S. nagalingumiae* have a similar structure, they have some slight differences. Microsporophylls of *S. galtieri* lack regular symmetry while those of *S. nagalingumiae* are bilaterally symmetrical. The distal extension seen in *S. galtieri* is relatively thick and slightly angular with a more spinose morphology, which differs from the laminar and thin distal extension of *S. nagalingumiae*. In addition, they differ in measurements of microsporophyll stalks, distal extension and pollen sacs (Table 2). While these differences exist between the two species, we do not find them to be sufficient for separating these species at the generic level. Thus, we assign *S. nagalingumiae* as a new species of *Skyttegaardia* and we emend the diagnosis of the genus to reflect new information available from the permineralized cone of *S. nagalingumiae*.

Table 2 Comparison of morphological features of *Skyttegaardia nagalingumiae* and *Skyttegaardia galtieri*.

Characters	*S. nagalingumiae*	*S. galtieri*†
Stalk region length	0.35–0.6 mm	0.75–1 mm
Stalk region maximum width	0.6 mm	0.95 mm
Distal extension	Thin	Thick
Distal extension length	Up to 1.6 mm	Up to 2.9 mm
Distal extension maximum width	1.2 mm	1.3 mm
Pollen sacs length	0.4–0.6 mm	0.4–0.7 mm
Abaxial keel width	< 0.1 mm	0.12–0.14 mm

†Measured from published figures.

While cycadalean affinities were originally explored for Skyttegaardia, a confident assignment was understandably precluded by the incompleteness of the originally described specimens and its unusual features for a cycad (described below; Friis *et al.*, 2021). We argue that the permineralized articulated cone of *S. nagalingumiae* contains enough diagnostic characters to assign *Skyttegaardia* to Cycadales. Like *Skyttegaardia*, cycad pollen cones are compact with helically arranged microsporophylls with abaxially attached pollen sacs. While microsporophyll vasculature can vary in cycads, those with laminate distal extensions can have multiple bundles organized in a linear row (e.g. *Stangeria* T. Moore and *Cycas*), which is similar to the microsporophyll vasculature of *S. nagalingumiae* (see Stevenson, 1990; Klavins *et al.*, 2003). Like *S. nagalingumiae*, cycad cones have mucilage canals, and most genera have sunken stomata (Coiro *et al.*, 2021); this later trait may be an adaptation to xeric conditions. Cycads produce monocolpate pollen grains that are spheroidal and/or boat-shaped, which is similar to the pollen grains of *S. nagalingumiae*. Furthermore, Zamiaceae and *Skyttegaardia* both have pollen grains with a perforate and slightly rugulate surface (Dehgan & Dehgan, 1988; Marshall *et al.*, 1989). Thus, *Skyttegaardia* possess a combination of characters that is indicative of cycads and not present in any other known seed plant group.

On the contrary, the pollen cone of *S. nagalingumiae* may seem unusual for a cycad because some aspects of its morphology are not often regarded as typical cycadalean features. Living cycads often have exceptionally large pollen cones with many pollen sacs (sometimes hundreds) per microsporophyll, while *S. nagalingumiae* has a small pollen cone with a low number of pollen sacs sunken in each microsporophyll. This would lead one to preclude an assignment of the fossil to Cycadales (discussed in Friis *et al*., 2021) and potentially suggest that *Skyttegaardia* represents an extinct higher taxon (e.g. Order) that has yet to be recognized. However, below, we demonstrate that there is precedence for these features among living cycads, and therefore, they should not preclude cycadalean affinities for *Skyttegaardia*.

Small cone size One of the most striking features of *S. nagalingumiae* is the small size of its pollen cone relative to the pollen cones of most living cycads. However, there are four species of *Zamia* with relatively small pollen cones including: *Z. sinuensis* Calonje & J. Castro, *Z. imbricata* Calonje & J. Castro, *Zamia melanorrhachis* D.W. Stev. and *Z. hymenophyllidia* D.W.Stev (Stevenson, 2004; Calonje *et al*., 2021). The latter two species can have pollen cones that measure 1.0–3.0 cm in length (Stevenson, 2004); to our knowledge, these two species have the smallest pollen cones of any living cycad. While the pollen cone of *S. nagalingumiae* is estimated to be 3.0–5.0 mm in length (based on serial cross sections), it is not much smaller than the range of variation in pollen cone length of living cycads. Furthermore, while the pollen cone of *S. nagalingumiae* is permineralized, it certainly shrunk to some degree before or during fossilization, and during preparation via the acetate peel technique (Long *et al*., 2022). Moreover, many fossil cones of seed plants, such as conifers, are smaller than what is seen in today (see Leslie *et al*., 2009; Atkinson *et al*., 2014). Thus, the small cone size of *S. nagalingumiae* should not preclude an assignment to Cycadales.

Low number of pollen sacs As shown in our dissections of *Z. variegata* and *Z. pumila*, microsporophylls at the apex and base of the cone have fewer pollen sacs (2–7 on each side). This has also been documented by Chamberlain (1935); such a morphology is likely due to microsporophylls growth being terminated earlier in development. Low numbers of PSPM can be observed throughout the entire pollen cones of several additional species of *Zamia*, especially those with smaller cones, including *Z. melanorrhachis* and *Z. hymenophyllidia*, in which only a few microsporangia are abaxially attached on each side of the microsporophyll (Stevenson, 2004, figs 6D,E, 11E). These small-coned species can have microsporophylls with as few as 2–6 abaxial pollen sacs on each side (D.Wm. Stevenson, NY Botanical Garden pers. comm., 2022) The reduced microsporophyll morphology of *Skyttegaardia* can be explained as a case of heterochrony in which the growth of the pollen cones was short-lived, and by extension, development of microsporophylls was short, only giving enough space (and time) for two pollen sacs to develop.

It is worth mentioning that the number of pollen sacs per sporophyll can vary in different seed plant groups. For instance, Conifers can have 2 to >20 pollen sacs per sporophyll (Schulz *et al*., 2014). This trait can vary at the family level as well. Cupressaceae can have two to six pollen sacs per sporophyll. Thus, the number of pollen sacs does not seem like a useful trait for excluding a fossil such as *Skyttegaardia* from cycads.

Sunken pollen sacs The pollen sacs of *Skyttegaardia* are sunken within the microsporophylls. While this feature is not commonly seen in cycads, it has been documented in living species of *Zamia pseudoparasitica* and *Zamia fairchildiana*. Fig. 13.2a,b in Schutzman (2004) illustrates that removed or abscised pollen sacs leave pocket marks on the abaxial surface and margins of the microsporophylls.

As Friis *et al.* (2021) rightfully point out in their comparison of *Skyttegaardia* to cycads, extant cycads such as *Encephalartos successibus* Vorster have megasporophylls with sunken (‘embedded’) ovules (Vorster & Heibioem, 1995). Moreover, Stevenson (1990) and our own dissections illustrate ovules of *Stangeria* enclosed or sunken on each side of the abaxial surface of the megasporophyll (Fig. 4g–i). *Cycas media* has ovules sunken in megasporophyll tissue as well (Stevenson, 1990). Thus, it appears that enclosure of sporangial structures is a trait present in cycads that is shared with *S. nagalingumiae*.

The characters above are present in both *S. nagalingumiae* and living cycads. Thus, our comparative analysis indicates *Skyttegaardia* is a cycad. There are no data to suggest this fossil represents a previously unrecognized order or any other known group of seed plants outside of Cycadales.

The systematic assignment to Cycadales for *Skyttegaardia* is supported, by our phylogenetic analyses in which *Skyttegaardia* is recovered in a polytomy with ‘Cycadaceae’ and ‘Zamiaceae’ using the Hilton & Bateman matrix. This position is the result of numerous shared characters between these three terminals, such as the type of pollen grains, their ornamentation, microsporophyll and cone architecture, and details of their vascular system. Additionally, common to all MPT we recover as a synapomorphy of the clade formed by *Skyttegaardia*, Cycadaceae and Zamiaceae, the presence of secretory canals (Ch. 32). While secretory canals are also present in other groups (e.g. conifers), they are not necessarily synapomorphies in those groups. If *Skyttegaardia* is not included in the matrix, Cycadaceae and Zamiaceae are linked by five synapomorphies, relating to leaves and leaf traces (chars. 7 and 14), secretory structures (char. 32) and ovules (chars. 35 and 62). Due to their foliar and ovulate nature, characters 7, 14, 35 and 62 are understandably missing in *Skyttegaardia* and only additional fossils may help score these characters in the future. Nonetheless, the available information preserved in *Skyttegaardia* indicates that it shares more key features with cycads compared with any other known group of seed plants.

To obtain a more precise phylogenetic position of *Skyttegaardia* within the total group of cycads, we also included it in a matrix containing all extant genera of cycads as well as selected extinct representatives (i.e. matrix of Coiro & Pott, 2017). The version of the matrix that we used for this purpose lacks numerous outgroups because the cycad affiliation was already evaluated in the previous analyses using the Hilton & Bateman (2006) matrix, and it includes potential stem-group cycads such as *Antarcticycas* and *Beania* (referred as ‘NILAMBE’ in previous studies). In these analyses, *Skyttegaardia* is recovered as sister to

Stangeria, which is supported by the presence of free microsporangia (Ch. 61), and an elliptical pollen shape (Ch. 65). Considering this recovered relationship, it is striking that both *Stangeria* and *Skyttegaardia* have sporangial structures sunken within sporophyll tissue, which is a feature not sampled in the character matrix. In addition, *Skyttegaardia* is recovered in a clade comprising *Zamia*, *Microcycas*, *Ceratozamia* and *Stangeria*, which possess the smallest pollen cones and lowest number of PSPM in Cycadales (Fig. 7). These traits are not characters analysed in the matrix, thus providing independent support for *Skyttegaardia* as a member of crown-group Zamiaceae.

While the position of *S. nagalingumiae* is based on limited scorable characters, the available amount of information preserved in this fossil is informative enough to assign it to crown-group Zamiaceae. We acknowledge that this systematic assignment could potentially change if additional information from other organs and structures of *Skyttegaardia* is made known. However, at this time the data indicate that this extinct genus is a cycad and more specifically a member of Zamiaceae.

Cycad evolution in deep time

As characteristic of the Cycadales phylogeny, internal branches within Zamiaceae are exceptionally long (Condamine *et al.*, 2015). This is due to its Late Jurassic/Early Cretaceous crown node origins and Neogene crown node origins for extant genera (Condamine *et al.*, 2015). Due to superficial resemblances of living Zamiaceae (and cycads as a whole), it may appear the morphology of these plants has been highly conserved throughout time. However, the identification and integration of cycad fossils within a phylogenetic framework can provide a different perspective by breaking up some of these long branches in the cycadalean/Zamiaceae phylogeny (see Leslie *et al.*, 2018), thus having much potential for shedding light on cycad turnover and trait evolution in deep time.

Results from our phylogenetic and comparative morphological analyses identify *Skyttegaardia* as an extinct lineage nested within Zamiaceae. This extinct genus appears in the fossil record near the estimated crown age of Zamiaceae, supporting a Jurassic origin for the family (see Fig. 6). This highlights that the Mesozoic diversification of Zamiaceae may reflect, in part, a marked increase in morphological diversity or divergence (see Simões *et al.*, 2016), which resulted in extinct lineages with disparate morphologies relative to extant cycads. Thus, the morphology of crown-group cycads is probably not as conserved as previously thought when analysing extant taxa alone (Chamberlain, 1935; Nagalingum *et al.*, 2011), especially for reproductive structures. Below, we further explore this in light of cycad pollen cone morphology and diversity.

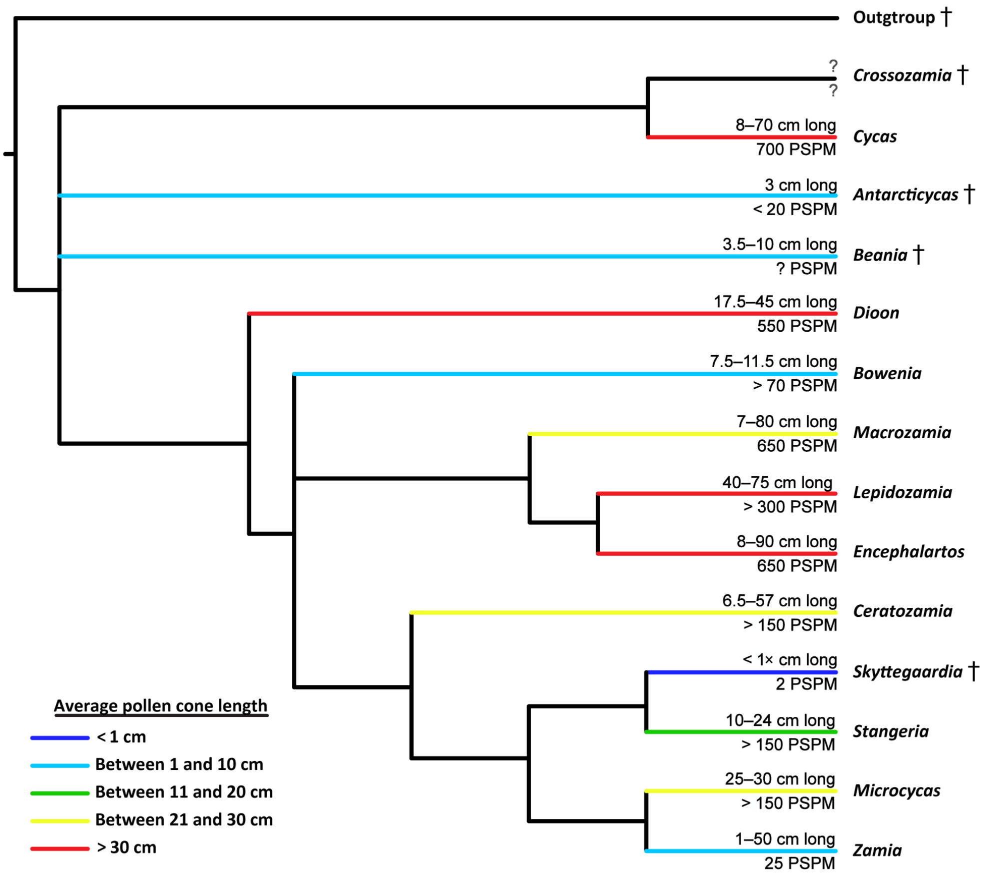

Fig. 7 Strict-consensus phylogenetic tree of cycads incorporating information about length of the pollen cones, displayed as range and number of pollen sacs per microsporophylls (PSPM). Branch colours indicate average pollen cone length.

Morphological diversity and evolution of pollen cones

The number of PSPM (pollen sacs per microsporophylls) in cycadaleans is variable at generic, species and individual level (Chamberlain, 1935; Bierhorst, 1971), and this number appears to be correlated with overall cone size (see Fig. 7). Regarding extant genera, *Cycas*, *Dioon*, *Macrozamia*, *Lepidozamia*, and *Encephalartos* produce the largest cones (*c.* 35 × 9 cm in average) and reach the highest number of PSPM (from > 300 to > 1000). On the contrary, the clade comprising *Zamia*, *Microcycas*, *Ceratozamia* and *Stangeria* (ZMCS clade) have the smallest pollen cones (*c.* 20 × 5 cm in average) and the lower number of PSPM of the order, with species of *Zamia* only reaching up to 25 PSPM, and the remaining genera bearing close to 150 PSPM (Fig. 7). While this clade displays smaller pollen cones relative to the remaining cycads, those of *Skyttegaardia* illustrate a case of size reduction that would be difficult to infer from analysing the evolution of this trait from extant only phylogenies.

Our observations highlight the number of PSPM and dimensions of the pollen cones could bear phylogenetic signal, and thus, their incorporation in morphological matrices should be considered in future phylogenetic analyses of the group. It is interesting to note that the smallest cycad pollen cones outside of the ZMCS clade (with the exception of *Bowenia* whose phylogenetic position remains ambiguous) are present in the fossil cycads included in the phylogenetic analysis (i.e. *Antarcticycas* and *Beania*; Fig. 7; Table 3), which may suggest that large pollen cones evolved in recent stages of the evolutionary history of cycads.

Pollination biology of *Skyttegaardia*

While seeds of most gymnosperms are wind-pollinated, insects, especially beetles, are the main pollination vectors for living cycads (Donaldson, 1997; Kono & Tobe, 2007; Procheş & Johnson, 2009; Terry *et al.*, 2012; Tang *et al.*, 2018). This biological interaction has also been observed in the fossil record, albeit indirectly, with cycad pollen grains on the surface of beetles preserved in Cretaceous amber (Cai *et al.*, 2018). Pollen sacs of the Triassic cycad *Delemaya spinulosa* (= *Antarcticycas*), were found containing coprolites exclusively comprising pollen grains, which implies that specialized pollinivory and possibly entomophily between arthropods and cycads predated insect pollination in angiosperms (Klavins *et al.*, 2005). While no coprolites were found in the pollen cones of *S. nagalingumiae*, we speculate arthropods were a pollination vector for this plant. For instance, as discussed in Klavins *et al.* (2005), most air-pollinated gymnosperms have pollen cones that elongate at maturity, exposing their pollen sacs to the wind. The mature pollen cones of *Delemaya* and *Skyttegaardia* are compact with short internodes and highly imbricate microsporophylls that would obscure their pollen sacs from air currents (see Klavins *et al.*, 2005). This suggests that arthropods would potentially be a more efficient pollination vector rather than air currents. In addition, as Friis *et al.* (2021) mention, pollen produced by *Skyttegaardia* have a foveolate surface, which in cycads is associated with insect pollination (Dehgan & Dehgan, 1988).

Conclusion

The permineralized cycad pollen cone of *S. nagalingumiae* sp. nov. reveals a previously unrecognized aspect of the morphological disparity of cycads. While Triassic shoots and Jurassic seeds of cycads display some novel characters (Smoot *et al.*, 1985; Rothwell *et al.*, 2022), their phylogenetic relationships relative to stem- or crown-group Cycadales remain uncertain. However, our findings suggest that crown-group cycads explored the

Table 3 Comparison of *Skyttegaardia* species with various types of fossil cycads pollen cones.

Taxon	Cone size (length × width)	Pollen sac position	Microsporophyll face shape	Microsporophyll spines	Pollen sacs per microsporophyll	Pollen exine	Stratigraphic occurrence
Skyttegaardia nagalingumiae[1]	3–5 × 2 mm	Abaxial	Rhomboidal (symmetrical to tall)	Absent	Very few (2)	Foveolate	Late Cretaceous
Skyttegaardia galtieri[2]	na	Abaxial	Rhomboidal (tall)	Absent	Very few (2)	Psilate, foveolate to fossulate	Early Cretaceous
Delemaya[3]	At least 30 × 8 mm	Abaxial	Rhomboidal (symmetrical)	Present	Few (< 20)	Psilate to fossulate	Middle Triassic
Androstrobus[4–7]	> 50 × 20 mm	Abaxial	Rhomboidal (wide)	Absent	Numerous (> 50)	Psilate, rugulose, fossulate and foveolate[†]	Early Jurassic to Early Cretaceous
Cycandra[8†]	> 150 × 85 mm	Abaxial	Rhomboidal to hexagonal (wide)	Absent	Numerous (> 50)	Psilate?	Late Jurassic
Semionandra[9†]	50 × 7 mm	Abaxial?	Flat	Absent	Few (4–8)	na	Early Cretaceous
Aegianthus[10†]	> 50 × 30 mm	Adaxial	Hexagonal	Absent	Numerous (> 50)	Psilate	Early Jurassic to Early Cretaceous

[1]This study; [2]Friis *et al.* (2021); [3]Klavins *et al.* (2003); [4]Harris (1964); [5]van Konijnenburg-van Cittert (1968); [6]Hill (1990); [7]Archangelsky & Villar de Seoane (2004); [8]Krassilov & Bugdaeva (1988); [9]Krassilov *et al.* (1996); [10]Deng *et al.* (2014). na, information not available.
[†]Affinities with Cycadales uncertain/doubtful.

phenotypic landscape of reproductive morphologies in a wider dimension than previously suspected. This work contributes towards a growing (yet overlooked) body of evidence indicating that while flowering plants phylogenetically and morphologically diversified rapidly during the Cretaceous, gymnosperms were also diversifying and exploring new areas of morphospace.

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Competing interests

None declared.

Author contributions

BAA collected the specimen. AE and BAA designed the research project and prepared the material. AE imaged the specimens, created the 3D reconstructions and conducted the phylogenetic analysis. AE and BAA wrote the manuscript.

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Data availability

All *S. nagalingumiae* fossils are deposited at the University of Kansas Paleobotanical Collections (Biodiversity Institute) in Lawrence, KS, USA. Raw image data and segmentations of *S. nagalingumiae* are archived in Morphosource (Boyer *et al*., 2016) under the project title 'Late Cretaceous cycad pollen cone'. The two morphological matrices (.nex files) can be found both in Supporting Information and Morphobank (Project 4378). Combined (morphology + DNA) matrix can be found in Supporting Information (.tnt file). Pollen cone measurements of extant cycads can be found in Supporting Information.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Notes S1 .zip compressed file containing matrices used in this study on .tnt and nex format (can be opened with either Tnt, Goloboff & Catalano, 2016, or Mesquite; Maddison & Maddison, 2021).

Table S1 GenBank accession numbers.

Table S2 Dimensions of pollen cones of extant species of cycads.

Video S1 Three-dimensional model of *Skyttegaardia nagalingumiae*.

Video S2 Three-dimensional model of a microsporophyll of *Skyttegaardia nagalingumiae*.