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Ancient noeggerathialean reveals the seed plant sister group diversified alongside the primary seed plant radiation

Jun Wang^{a,b,c,1}, Jason Hilton^{d,e}, Hermann W. Pfefferkorn^f, Shijun Wang^g, Yi Zhang^h, Jiri Bekⁱ, Josef Pšenička^j, Leyla J. Seyfullah^k, and David Dilcher^{l,m,1}

^aState Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China; ^bCenter for Excellence in Life and Paleoenvironment, Chinese Academy of Sciences, Nanjing 210008, China; ^cUniversity of Chinese Academy of Sciences, Shijingshan District, Beijing 100049, China; ^dSchool of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom; ^eBirmingham Institute of Forest Research, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom; ^fDepartment of Earth and Environmental Science, University of Pennsylvania, Philadelphia, PA 19104-6316; ^gState Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, China; ^hCollege of Paleontology, Shenyang Normal University, Key Laboratory for Evolution of Past Life in Northeast Asia, Ministry of Natural Resources, Shenyang 110034, China; ⁱDepartment of Palaeobiology and Palaeoecology, Institute of Geology v.v.i., Academy of Sciences of the Czech Republic, 165 00 Praha 6, Czech Republic; ^jCentre of Palaeobiodiversity, West Bohemian Museum in Plzeň, 301 36 Plzeň, Czech Republic; ^kDepartment of Paleontology, Geozentrum, University of Vienna, 1090 Vienna, Austria; ^lIndiana Geological and Water Survey, Bloomington, IN 47404; and ^mDepartment of Geology and Atmospheric Science, Indiana University, Bloomington, IN 47405

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Noeggerathiales are enigmatic plants that existed during Carboniferous and Permian times, ~323 to 252 Mya. Although their morphology, diversity, and distribution are well known, their systematic affinity remained enigmatic because their anatomy was unknown. Here, we report from a 298-My-old volcanic ash deposit, an in situ, complete, anatomically preserved noeggerathialean. The plant resolves the group's affinity and places it in a key evolutionary position within the seed plant sister group. *Paratingia wuhaia* sp. nov. is a small tree producing gymnospermous wood with a crown of pinnate, compound megaphyllous leaves and fertile shoots each with Ω -shaped vascular bundles. The heterosporous (containing both microspores and megaspores), bisporangiate fertile shoots appear cylindrical and cone-like, but their bilateral vasculature demonstrates that they are complex, three-dimensional sporophylls, representing leaf homologs that are unique to Noeggerathiales. The combination of heterospory and gymnospermous wood confirms that *Paratingia*, and thus the Noeggerathiales, are progymnosperms. Progymnosperms constitute the seed plant stem group, and *Paratingia* extends their range 60 My, to the end of the Permian. Cladistic analysis resolves the position of the Noeggerathiales as the most derived members of a heterosporous progymnosperm clade that are the seed plant sister group, altering our understanding of the relationships within the seed plant stem lineage and the transition from pteridophytic spore-based reproduction to the seed. Permian Noeggerathiales show that the heterosporous progymnosperm sister group to seed plants diversified alongside the primary radiation of seed plants for ~110 My, independently evolving sophisticated cone-like fertile organs from modified leaves.

Noeggerathiales | progymnosperm | seed plant | Permian | evolution

The origin of the seed in the Late Devonian, ~365 Mya, represents a key innovation in land plant evolution. Seeds provided a fundamentally new reproductive strategy that overcame the limitations of free-sporing, pteridophytic reproduction and enabled colonization of drier habitats (1–3). Progymnosperms, the evolutionary stem group leading to seed plants, display a mosaic of evolutionary characters combining free-sporing reproduction with production of secondary xylem (wood) through a bifacial vascular cambium characteristic of seed plants (2–4). Although not representing a monophyletic evolutionary group, progymnosperms are important for our present understanding of the origin of the seed and represent intermediates between pteridophytes and seed plants (1, 2, 4). Progymnosperms include

the basally divergent homosporous Aneurophytales, as well as the more derived heterosporous Archaeopteridales, Protopytales (1, 4), and the enigmatic Carboniferous cone *Cecropsis* (5). The transition to seed plants requires multiple character state transitions from known progymnosperm sister groups comprising either *Archaeopteris* (6, 7) or *Archaeopteris* + *Cecropsis* (e.g., ref. 8). For each of these cases, viable intermediates are absent from the fossil record.

In contrast, Noeggerathiales (9) have at times been proposed as progymnosperms (9–12), but this has been controversial. Comprising ~20 genera and 50 species, Noeggerathiales are known from the late Carboniferous–Permian (323 to 251 Ma) tropical floras in North America, Europe, and East Asia (10),

Significance

There were two heterosporous lignophyte lineages of which only one, the seed plants, survived the Permian–Triassic mass extinction. Based on exceptionally complete fossil trees from a 300-My-old volcanic ash, the enigmatic Noeggerathiales are now recognized as belonging to the other lineage. They diversified alongside the primary seed plant radiation and constitute seed plants' closest relatives. Noeggerathiales are reconstructed as members of a plexus of free-sporing woody plants called progymnosperms, extending their age range by 60 My. Following the origin of seed plants, progymnosperms were previously thought to have become gradually less abundant before dying out in Carboniferous. We show they diversified and evolved complex morphologies including cone-like structures from modified leaves before going extinct at the Permian–Triassic extinction.

Author contributions: J.W., J.H., H.W.P., and D.D. designed research; J.W., Y.Z., and J.P. performed research; J.W. contributed new reagents/analytic tools; J.B. collected the materials and macerated and described in situ spores; J.W., H.W.P., Y.Z., and J.P. collected the materials and prepared specimens; J.W., J.H., H.W.P., S.W., J.B., L.J.S., and D.D. analyzed data; J.H. and L.J.S. undertook the phylogenetic analysis; and J.W., J.H., H.W.P., S.W., and D.D. wrote the paper.

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The authors declare no competing interest.

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¹To whom correspondence may be addressed. Email: jun.wang@nigpas.ac.cn or dilcher@indiana.edu.

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where they are identified as a related group based on shared features of heterospory, adaxial sporangial attachment to “sporophylls,” longitudinal sporangial dehiscence, plagiotropic pinnule attachment to the rachis, and once-pinnate compound megaphylls (9–12). However, their systematic position remained uncertain because details of their stem anatomy were unknown. In the absence of this anatomical information, they have been postulated as close relatives of the progymnosperms, leptosporangiate ferns, sphenopsids, the extant fern *Tmesipteris*, or as a distinct class of their own (see ref. 9 for summary). Recently, leaves of *Plagiozamites oblongifolius* that were interpreted as noeggerathialean (9) have also been interpreted as cycads (13). Wang et al. (9) considered the Noeggerathiales to be putative progymnosperms, but as their specimens lacked wood, a more confident assignment was not possible. This uncertainty meant that even when considered as progymnosperms, their relationship to other progymnosperms and seed plants has remained speculative.

Here, we report a species that we name *Paratingia wuhaia* J. Wang et al. sp. nov. and ascribe to the Noeggerathiales. The fossils were collected from a single 66-cm-thick volcanic ash bed in the Chinese “vegetational Pompeii” from the Taiyuan Formation at Wuda open coalmine, Inner Mongolia (14, 15) (*SI Appendix, Geological Information, Materials*). The ash preserved in situ the morphology and anatomy of plants in exquisite detail (Fig. 1A–N) and has been dated to 298.34 ± 0.09 Ma during the Asselian stage of the early Permian (16).

Isolated noeggerathialean fertile shoots (*SI Appendix, Figs. S3–S8*) and megaphyllous leaves (*SI Appendix, Figs. S9–S14*) are common in the assemblage. The species *P. wuhaia* is based on >200 specimens, including >10 specimens that were preserved with intact crowns containing both leaves and fertile shoots in organic attachment to the stems, demonstrating that they belong to a single species (Fig. 1A and *SI Appendix, Figs. S2–S4*).

Systematics

Class—Noeggerathiopsida Kryshtofovich.

Order—Noeggerathiales Nemejc J. Wang et al. emend. nov.

Emended Order diagnosis—Heterosporous plants with compact, bisporangiate pseudostrobilus. Sporophylls laterally basally fused to form a semidisk or disk. Sporangia sessile, attached to sporophyll surface. Leaf once-pinnate, compound, with multiveined pinnule. Pseudostrobilus and leaf rachis with bilateral, Ω -shaped vascular bundle and secondary xylem.

Family—Tingiostachyaceae Gao and Thomas emend. S. J. Wang and Spencer (2017).

Genus—*Paratingia* Zhang.

Species—*Paratingia wuhaia* J. Wang et al. sp. nov.

Etymology—The specific epithet refers to Wuhai city to which the Wuda Coalfield belongs.

Species diagnosis—Monopodial stem with once-pinnate compound leaves and pseudostrobili forming a crown. Stem eustele with pith; cauline primary xylem strand adaxially curved; secondary xylem pycnoxylic; leaf traces tangentially elongated. Rachis of leaf and pseudostrobilus with a reversed Ω -shaped vascular bundle with protoxylem at lateral margin. Pinnules with radiate venation, arranged in four rows, with two rows of large, lanceolate pinnules on lower surface, two rows of small, oblong pinnules on upper surface of the axis. Pseudostrobilus heterosporous; sporophylls whorled, proximally disk-shaped or dissected into segments, distally upturned and dissected. Sporangia attached adaxially and proximally to

sporophyll in single ring. Megasporangia in vertical row bearing single functional megaspore. Microspores of the *Calamospora* type.

Holotype—PB22126 (Fig. 1A and *SI Appendix, Fig. S2*)

Paratypes—PB22127–22138 (*SI Appendix, Figs. S3–S14*).

Type locality—Wuda Coalfield, Wuhai, Inner Mongolia, China.

Horizon—Uppermost part, Taiyuan Formation.

Stratigraphic age— 298.34 ± 0.09 Ma, Asselian, Cisuralian, Permian.

Repository—All specimens are deposited at Nanjing Institute of Geology and Paleontology, Chinese Academy of Sciences.

Remarks—Following Wang et al. (9), we consider the noeggerathialean fertile shoot to be a pseudostrobilus as despite its radial exterior appearance, it is distinctly bilateral in organization and represents a complex, three-dimensional sporophyll. We use quotation marks to denote that what would traditionally be interpreted as sporophylls on the pseudostrobilus axis represent pinnule homologs and are not in fact sporophylls in the traditional sense. Detailed descriptions, measurement, and further illustrations are provided in the supporting information.

Description. The holotype is 160 cm long and 60 cm wide (Fig. 1A and *SI Appendix, Fig. S2*) and comprises a stem with apical crown of helically attached megaphyllous leaves and pseudostrobili. The crown has three parts: a 13-cm-long basal region bearing leaves, a middle 23-cm-long fertile zone, and apically a 6-cm-long region bearing more leaves. Below the crown, the stem is naked, extending for >32 cm, tapers little, and is ~5 cm wide with a smooth exterior, lacking evidence of abscission structures. More than 60 once-pinnate, megaphyllous leaves are attached to the stem above and below the fertile zone, with typical leaf rachises being 10.4 mm wide and <50 cm long. Most pinnules are not fully discernible and are either matted together, obscured by other parts of the plant overlying them, or remain embedded within the rock. A paratype (Fig. 1B and *SI Appendix, Fig. S12*) demonstrates clearly lanceolate, large pinnules on the lower surface of the leaf, and oval, small pinnules on the upper surface, both with radiate venation.

Anatomically, the stem has an eustele with a large pith (Fig. 1C and *SI Appendix, Fig. S15*); cauline primary xylem strands are adaxially curved (Fig. 1F and *SI Appendix, Fig. S16A–C*). Secondary xylem is pycnoxylic with uniseriate rays, which are mostly short/low and vary remarkably in size from one to seven cells high (Fig. 1H and *SI Appendix, Fig. S17C and D*). Secondary tracheid walls have scalariform thickenings (Fig. 1G and *SI Appendix, Fig. S17A and B*), and the cortex has collenchyma in its outer part. Leaf traces are tangentially elongated.

Approximately 8 to 10 pseudostrobili are attached to the circumference of the fertile zone and about 20 more longitudinally attached along the stem (Fig. 1A and *SI Appendix, Fig. S2*); we estimate an entire fertile zone would comprise 160 to 200 pseudostrobili. Pseudostrobili are elongated with parallel sides and bluntly rounded apices, and are attached more or less at right angles to the stem with one-third to one-quarter of their width without a stalk. They are 50 to 65 mm long and 13 mm wide (Fig. 1A and *SI Appendix, Figs. S3–S8*).

Both the leaf (Fig. 1B and *SI Appendix, Figs. S15 and S19A and B*) and pseudostrobili (Fig. 1D and *SI Appendix, Fig. S22A–C*) rachises have a reversed Ω -shaped vascular bundle. Protoxylem strands occur at the lateral margins of the bundle, while pinnule traces and sporophyll (i.e., pinnule homolog)

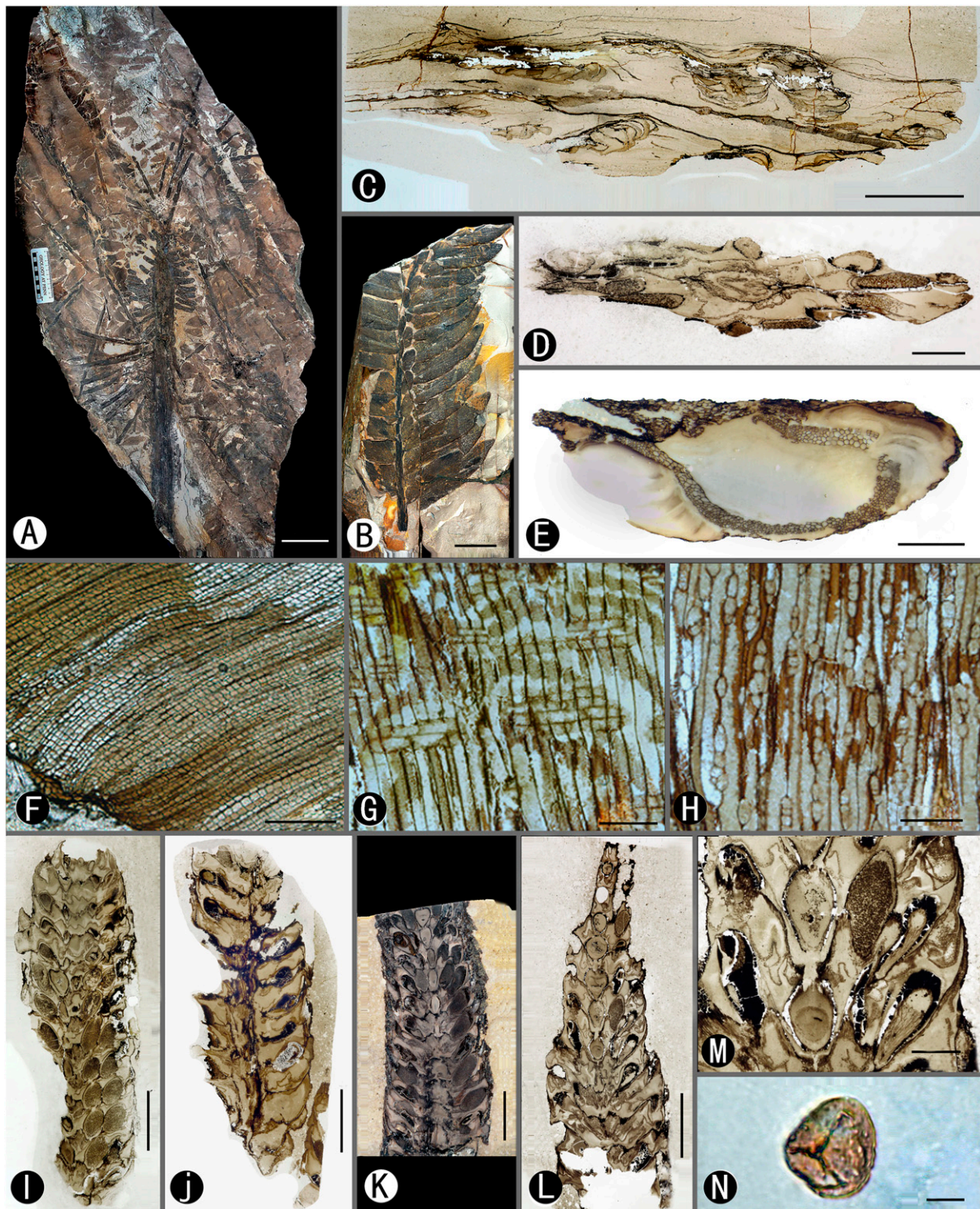


Fig. 1. *P. wuhaia* J. Wang et al. sp. nov. from the early Permian Taiyuan Formation of Wuda Coalfield, Inner Mongolia. (A) Holotype with an entire crown consisting of pseudostrobili and leaves. (B) Once-pinnate compound leaf with both large and small pinnules visible. Reprinted with permission from ref. 15. (C) Cross-section of a crown illustrating pseudostrobili around the stem. (D) Cross-section of pseudostrobilus with microsporangia around the axis with bilateral, inverted Ω -shaped vascular bundle. (E) Cross-section of a leaf rachis showing the same form of vascular bundle as that of pseudostrobili axes. (F–H) Partial cross, radial, and tangential sections of the stem showing the secondary xylem (wood). (I) Tangential section of pseudostrobilus showing sporangial arrangement with single line of megasporangia along with the axis (middle-right pseudostrobilus of the fragmental crown illustrated *SI Appendix, Fig. S8*; specimen PB22132; magnified in *SI Appendix, Fig. S21C*). (J) Radial section of pseudostrobilus showing adaxial sporangia and axis lacking nodes (upmost-left pseudostrobilus of the fragmental crown in *SI Appendix, Fig. S8*; magnified in *SI Appendix, Fig. S21A*). (K) Tangential section showing adaxial sporangia and a single line of megasporangia along with the axis (*SI Appendix, Fig. S7*; specimen PB22131, second up-right pseudostrobilus). (L) Tangential section through same specimen as *K* showing megasporangial arrangement (magnified in *SI Appendix, Figs. S21B and S23A*). (M) Detail of the middle part of *L* showing the megasporangia and microsporangia. (N) Single spore macerated from the holotype. (Scale bars: A, 10 cm; B, 3 cm; C–E, 1 cm; F, 100 μ m; G and H, 200 μ m; I–L, 5 mm; M, 2 mm; N, 10 μ m.)

traces are given off from the lateral ends of the vascular bundles of the rachis and “strobilus” axis, respectively (*SI Appendix*, Figs. S19E, S20 A–C, and S22C).

Many pseudostrobili show four to five rows of sporangia with rows arranged parallel to the rachis (Fig. 1 I and K and *SI Appendix*, Figs. S5–S8). As the specimens have been affected by postmortem compression (*SI Appendix*, Figs. S5–S8), we consider that there must have been a row of sporangia on either side that was laterally flattened; we estimate that a complete pseudostrobilus comprised 8 or 10 sporangia per whorl. Sporophylls are proximally fused laterally to adjacent sporophylls, while distally they are free with a small heel and upturned apex (*SI Appendix*, Figs. S21D and S23E). There are 32 to 35 sporangia per row in the pseudostrobili depending on the length of the pseudostrobili, producing five sporangia per centimeter’s length. Sporangia are sessile, attached to the sporophylls adaxial surface (Fig. 1 I–M). Megasporangia occur in a single vertical row on the pseudostrobilus axis (Fig. 1 K–M and *SI Appendix*, Figs. S21 B and F and S23A) and, although poorly preserved, have a single functional megaspore. As pseudostrobili are born horizontally from the stem, the vertical row of megaspores would have been organized horizontally in life. Due to preservation limitations, we cannot determine where in life the megasporangial row was positioned, but we suspect it was at the bottom of the pseudostrobilus and caused pooling of the hormone auxin under gravity to trigger megasporangial expression (9). Microspores are of the *Calamospora* type; trilete circular, subcircular to oval, 30 to 40 μm in diameter, with straight rays of the trilete mark forming a labrum extending 3/4 or the whole radius a laevigate exine that forms irregular folds usually parallel to the margin (Fig. 1N and *SI Appendix*, Fig. S23D).

Detailed descriptions, measurement, and further illustrations are provided in *SI Appendix, Detailed Description*.

P. wuhaia J. Wang et al. is reconstructed as a small, slender, unbranched palm-like tree in Fig. 2, two to many meters high. Its major characteristics are illustrated by a diagrammatic reconstruction as shown in Fig. 3. The details of its stem anatomy are consistent with that of progymnosperms and early seed plants (*SI Appendix*, Figs. S16–S18).

Comparisons. Following Wang et al. (9), we consider the noeggerathialean fertile shoot to be a pseudostrobilus because despite its radial exterior appearance, it is distinctly bilateral in organization and represents a complex, three-dimensional “sporophyll” (*SI Appendix, Terminology*). We use quotation marks to denote what would traditionally be interpreted as sporophylls on the pseudostrobilus axis that represent pinnule homologs and are not in fact sporophylls in the traditional sense.

P. wuhaia J. Wang et al. is most similar to the contemporaneous species *P. wudensis* (17) also from the Wuda Coalfield. Within the coalfield, the two species grew in different areas, with *P. wuhaia* dominating central and northern parts while *P. wudensis* occurs in low density only in the southern parts. *P. wudensis* is distinguished from *P. wuhaia* in forming smaller trees with comparatively larger pseudostrobili, and possessing needle-like small pinnules that are very different from the small, oval pinules in *P. wuhaia* (see *SI Appendix, Systematic Comparisons*, for further details). Furthermore *P. wudensis* has more than one megaspore per megasporangium (17) unlike the single megaspores in *P. wuhaia* megasporangia. The fertile shoots of *P. wuhaia* are similar to the late Permian heterosporous noeggerathialean pseudostrobilus *Dorsalistachya quadrisegmentorum* (9) that is associated with *Plagiozamites oblongifolius* megaphyllous leaves; its leaves and pseudostrobili also have Ω -shaped bundles but lack secondary xylem. *Dorsalistachya* is further distinguished from *Paratingia* in having winged monomegasporangia in short,

vertical rows (9) and possessing more complexly divided sporophylls. Adaxially curved and C-shaped primary xylem strands at the innermost part of the xylem cylinder in *Paratingia*’s stem differ from those in gymnosperms and *Archaeopteris* (progymnosperm) in which strands are isodiametric in cross-section. Broad, arc-shaped leaf traces in the innermost part of the xylem cylinder of the progymnosperm *Protopytis* (18) are similar to the c-shaped primary xylem strands in *Paratingia* stems, but *Protopytis* stems bear large, distichously arranged fronds with bilateral symmetry and plannated branching bearing terminal sporangia in fertile trusses that lack associated leaves or sporophylls (1, 5, 18) (see *SI Appendix, Tables S1 and S2* for detailed comparisons).

Discussion

The combination of free-sporing, pteridophytic reproduction and gymnospermous secondary xylem allows us to unambiguously resolve the systematic affinity of *Paratingia* and the Noeggerathiales as members of the progymnosperms (4), the seed plant evolutionary stem group (7, 8). Previous concepts of the progymnosperms suggested they ranged stratigraphically from the mid-Devonian (4) to the mid-Pennsylvanian (5), but the inclusion of Noeggerathiales extends their range by ~ 60 Ma to the end of the Permian (~ 251 Ma). Discovery of “gymnospermous” wood in a Permian aged progymnosperm means that similarly structured pycnoxylic, gymnospermous Carboniferous–Permian aged woods (e.g., ref. 19) can no longer, by default, be considered bona fide gymnosperms; reinvestigation of such species is now warranted.



Fig. 2. Reconstruction of the aerial parts of *P. wuhaia* J. Wang et al. sp. nov. from the early Permian of China (based on the holotype). Image credit: Yugao Ren and Sijia Tang (artists).

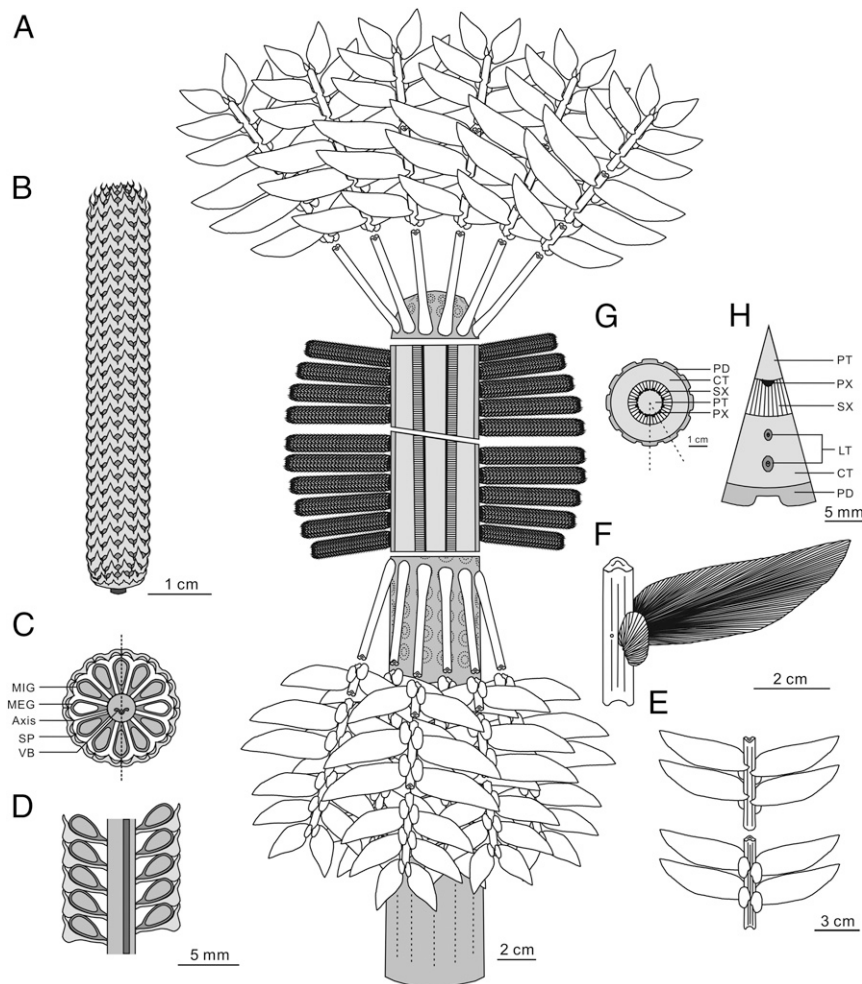


Fig. 3. Diagrammatic reconstruction of the key features of *P. wuhaia* J. Wang et al. (A) Stem bears pseudostrobili and once-pinnate compound leaves; pseudostrobili are attached to the fertile zone, above which the leaves are shown from the abaxial side, and below which the leaves are shown from the adaxial side. (B) A pseudostrobilus shown its longitudinal surface. (C) Cross-section of a pseudostrobilus showing both megasporangia and microsporangia, the bilateral symmetrical Ω -shaped vascular bundle and sporophylls; noting the relative position of the presumed two megasporangia and the orientation of the opening of the vascular bundle. (D) Partial longitudinal section through the central axis of the pseudostrobilus and the symmetric axis of the vascular bundle, approximately corresponding to the dashed line in C; (E) partial of the leaf showing the arrangement of the small and big pinnules, seen from the abaxial (Upper) and adaxial (Lower) sides, respectively; (F) a pair of small and big pinnules showing the radiate venation; (G and H) cross-section of the stem showing the anatomy; H is the magnification of a part (within the dashed line) of the stem in G. CT, cortex; LT, leaf trace; MEG, megasporangium; MIG, microsporangium; PD, periderm; PT, pith; PX, primary xylem; SP, sporophyll; SX, secondary xylem; VB, vascular bundle.

To evaluate the phylogenetic relationships of *Paratingia* and closely related taxa (*Dorsalistachya* and *Protopitys*), we included them in a cladistic analysis of Lignophytes (progymnosperms + seed plants) plus key Paleozoic ferns, using a suite of characters that encompass the range of morphological and anatomical variation within the dataset (see *SI Appendix, Cladistic Analysis*). *Protopitys* was not included in previous analyses (e.g., refs. 8 and 20). The analysis generated a single most parsimonious tree of 102 steps (Fig. 4) that resolves *Paratingia* as sister to *Dorsalistachya* within a Noeggerathiales clade that have Ω -shaped vascular traces in the rachis, heterosporous pseudostrobili, and helically arranged once-pinnate leaves, and places *Archaeopteris* that lacks pseudostrobili and has adaxial heterosporous sporangia (4, 21) as sister to Noeggerathiales. Noeggerathiales and *Archaeopteris* occur in a clade of heterosporous progymnosperms in which *Protopitys* that lacks pseudostrobili and has terminal heterosporous sporangia (1, 17) is basal-most, and with *Cecropsis* that has adaxial microsporangia and megasporangia indisposed on dissected leaves diverging from the next node and arising

from the node below *Archaeopteris* (Fig. 4). In this arrangement, Noeggerathiales represent most derived members of a heterosporous progymnosperm clade that is sister to seed plants. This differs from previous analyses in which *Archaeopteris* (6, 7) or (*Archaeopteris* + *Cecropsis*) (8, 20) were immediate sister to seed plants, but in our analysis the heterosporous progymnosperm clade resolves as sister to seed plants (Fig. 4). This significantly alters our understanding of the closest relatives of seed plants that now also include *Protopitys*, *Cecropsis*, *Archaeopteris*, and Noeggerathiales (see *SI Appendix, Cladistic Analysis, Cladistic results*, for further information).

Progymnosperms remain paraphyletic in our analysis and comprise the homosporous Aneurophytales (*Rellimia* + *Textaxlyopteris*) clade that represents the stem lineage leading to the more derived lignophytes including the seed plant and heterosporous progymnosperm clades (Fig. 4). While all progymnosperms have wood anatomy that is similar to seed plants in some respects (4, 8, 21) (see *SI Appendix, Table S1*), in our analysis reproductive characters are important in grouping the

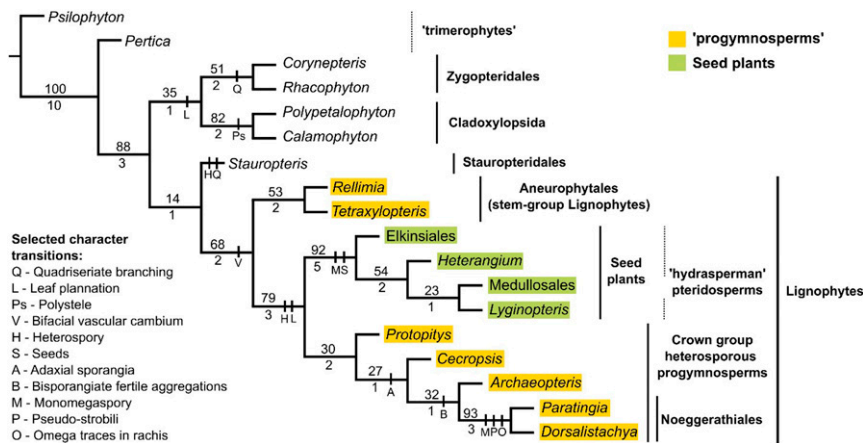


Fig. 4. Single most parsimonious 102 step tree resolving Noeggerthiales (*Paratingia* and *Dorsalistachya*) as the most derived members of a heterosporous progymsperm clade that is immediate sister group to seed plants. The heterosporous progymsperm clade and seed plants diverge from the stem after the homosporous Aneurophytales clade. Bootstrap values above branches, decay values below. Traditionally identified paraphyletic plant groups shown with dashed lines and their names in bounding commas.

reproductively sophisticated heterosporous progymsperms together as a clade, and grouping them as sister to seed plants. Despite the stems of Aneurophytales having similarity with seed plants (4, 22), their simple organization and homosporous reproduction contributes to their basal-most position within Lignophytes (6–8, 20). This supports seed plant ancestry from homosporous aneurophytalean progymsperms or closely related taxa based extensively on similarities in wood anatomy (23), and suggests that the heterosporous progymsperm clade that persisted through the Carboniferous and Permian evolved from aneurophytalean origins. Devonian *Stenokoleales* have been suspected of being progymsperms and putative seed plant ancestors (24, 25), but their reproductive organs are unknown precluding their inclusion in whole-plant cladistics analyses. Searching for transitional forms between homosporous aneurophytes and seed plants is imperative to better understand the evolutionary origin of the seed.

Although no other progymsperm order bore cone-like fertile aggregations (1, 9) (see *SI Appendix, Table S1*), an evolutionary relationship between *Archaeopteris* and the stratigraphically younger Noeggerthiales has been previously postulated (21, 26) but lacked cladistics support. *Archaeopteris* and *Paratingia* both possess eustelic stems, heterophylly, leaf organization in two orthostiches, and adaxially positioned sporangia, but in *Archaeopteris* sporangia are born on a modified leaf that arises from a radial structure and is very different from the sporophylls of Noeggerthiales in which disk or half-disk sporophylls arise from a bilateral pseudostrobilus. Nonetheless, the sister relationship between Noeggerthiales and *Archaeopteris* implies that the *Archaeopteris* fertile branch system is homologous with the noeggerthalian pseudostrobili (21, 26). In Noeggerthiales, the independent origin of bisporangiate (bisexual) cone-like pseudostrobili from modified leaves represents a unique adaptation that approaches the reproductive sophistication seen in cones of the most advanced Paleozoic lycophytes and sphenophytes (1, 9) and Paleozoic to recent gymnosperms. In these other plant groups, cones are independently derived from cauline (stem) rather than foliar (leaf) origins. Within seed plants, Cycadales, Cordaitales, Coniferales, Gnetales, Bennettitales, and *Pentoxylon* possess monosporangiate (unisexual) cones, while *Ginkgo* has male cones but has ovules born on peduncles in axils of leaves on short shoots. Bisexual cones are occasionally produced in conifers, but unlike Noeggerthiales where it appears to represent the normal condition, in conifers it results from developmental abnormalities (27). By contrast, lycophytes and sphenophytes can be either

monosporangiate or bisporangiate (1, 9), while the progymsperm *Protopytis* displays intrasporangial heterospory (anisospory) in which individual sporangia bear both microspores and megaspores (1, 18). Selective advantages to producing fertile organs in tightly packed cones or pseudostrobili include increased protection from herbivory (28), with multiple plant groups independently evolving the same strategy during the Paleozoic.

Within lignophytes, leaf axis vascularization with an Ω pattern occurs in Noeggerthiales and cycads (*SI Appendix, Fig. S25*; see *SI Appendix, Systematic Comparisons*). In cycads, this forms from numerous isolated, small vascular bundles that are circular in transverse section and it functions to provide the distal vegetative appendages along the length of the axis with a uniform water supply (29). This organization is very different to the single, long, and continuous bundle in Noeggerthiales (*SI Appendix, Fig. S25*) and suggests the Ω pattern arose independently in these two groups, presumably representing different functional approaches to irrigating the distal appendages and providing structural support.

Although Noeggerthiales exhibited advanced traits including cone-like pseudostrobili, production of a single functional megaspore per sporangium (i.e., monomegaspory; ref. 9) and wood, these adaptations proved insufficient to provide resilience to the profound environmental perturbations that occurred at the End Permian Mass Extinction where they were a notable terrestrial victim (10). This contrasts with seed plants that, while undoubtedly affected by the extinction mechanism(s) (e.g., ref. 30), included survivor lineages some of which persist to the present day (cycads, conifers, Ginkgoales). Within seed plants, the polyploidy (whole-genome duplication) event estimated from molecular data to have occurred at 319 Ma (31) during the mid-Carboniferous may have been key to the group's success compared with their heterosporous progymsperm sister group. Following polyploidy, gene loss and diploidization potentially offered selective advantage over other plant groups and also resilience to extinction mechanisms. The dependence on water for heterosporous reproduction appears to be a significant factor in the demise of Noeggerthiales (9), although certain progymsperms including *Archaeopteris* could have extended into aridlands (32). What is now abundantly clear is that this seed plant sister of advanced, heterosporous progymsperms diversified in stable, seasonally wet, and nutrient-rich environments (10) for ~110 My after the origin of the seed, alongside the primary seed plant radiation. It is similarly clear that, as an order of bona fide progymsperms, Noeggerthiales are the most derived members of a stratigraphically long-lived clade of late Paleozoic

heterosporous progymnosperms that constitute the seed plant sister group and can no longer be considered an obscure group within fern evolution.

Materials and Methods

Fossil Locality. Fossils were collected from the Taiyuan Formation in the Wuda Coalfield in Inner Mongolia, China (15). Fossil plants occur within a 66-cm-thick volcanic ash-fall (tuff) horizon previously termed the Chinese vegetational Pompeii (15). The ash horizon represents a T⁹ eruption assemblage with synchronous deposition, smothering in situ the plants growing in the swamp at the time of the ash fall. The flora was considered to be of late Early Permian age by Sun and Deng (33) based on plant fossil biostratigraphy. By contrast, we consider that the species in common between this and the Euramerican floral realm indicate a latest Carboniferous to earliest Permian age. The radiometric date for the volcanic tuff of 298.34 ± 0.09 Ma (16) provides a total age range of 298.43 to 298.25 Ma, placing the Wuda tuff in the lowermost part of the Permian Asselian Stage.

Fossil Preparation. Specimens were prepared by dégagement (34) to expose plant organs from the overlying sediment. Anatomically preserved specimens were prepared either as petrographic thin sections or by the acetate peel technique using a rock saw to cut specimens in specific orientations. Petrographic thin sections were typically used for the pseudostrobbili, initially embedding specimens in epoxy resin and then grinding to 40-μm thickness before mounting on a glass slide with coverslip using epoxy resin. For acetate peels (35), 5% HCl was used to etch surfaces for 30 s. Sporangia were macerated by 5% HCl for 10 s and 38% HF for 1 d prior to neutralizing with distilled water. Water and ethanol immersion was used for observation of

in situ spore morphology. Macerated sporangial remains were mounted onto slides for light microscopy, while others were selected to be mounted on stubs for scanning electron microscopy (SEM), using a LEO1530VP SEM without coating. Macrophotography was undertaken using a Nikon D800 DSLR camera using either a 50- or 100-mm macro lens. Microphotography was undertaken on a LEICAD300 stereomicroscope. Digital images were processed with Adobe Photoshop, version 7.0.

Phylogenetic Analysis. The cladistic matrix was compiled in Mesquite, version 3.31 (36), and analyses run in TNT (37) using traditional parsimony search and unordered, multistate characters. Our searches used TBR branch swapping and comprised 1,000 replicates, saving 100 trees per replicate, under equal implied weights. We provide bootstrap support using 10,000 replicates and Bremer support.

Data Availability. All study data are included in the article and/or supporting information.

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