Earliest record of megaphylls and leafy structures, and their initial diversification

HAO ShouGang[*] & XUE JinZhuang

Key Laboratory of Orogenic Belts and Crustal Evolution, School of Earth and Space Sciences, Peking University, Beijing 100871, China

Evolutionary changes in the structure of leaves have had far-reaching effects on the anatomy and physiology of vascular plants, resulting in morphological diversity and species expansion. People have long been interested in the question of the nature of the morphology of early leaves and how they were attained. At least five lineages of euphyllophytes can be recognized among the Early Devonian fossil plants (Pragian age, ca. 410 Ma ago) of South China. Their different leaf precursors or "branch-leaf complexes" are believed to foreshadow true megaphylls with different venation patterns and configurations, indicating that multiple origins of megaphylls had occurred by the Early Devonian, much earlier than has previously been recognized. In addition to megaphylls in euphyllophytes, the laminate leaf-like appendages (sporophylls or bracts) occurred independently in several distantly related Early Devonian plant lineages, probably as a response to ecological factors such as high atmospheric CO_2 concentrations. This is a typical example of convergent evolution in early plants.

The origin and evolution of leaves in vascular plants was one of the most important evolutionary events affecting the atmospheric environment and all terrestrial life on Earth [1,2]. The concept of two types of leaves, viz. microphylls and megaphylls, has been widely used in studies of particularly Paleozoic compression fossils [3,4]. The origination and spread of megaphyll leaves in crown groups of euphyllophytes, such as horsetails, ferns and seed plants, have been discussed in recent reviews [5–8]. When tracing the origins of megaphylls, researchers suggested the trimerophytes such as *Psilophyton* and *Pertica* of Early–Middle Devonian (mainly Emsian to early Eifelian; ca. 410–397 Ma ago) to be ancestral groups [5]. The three-dimensional (3-D) lateral branches of these plants were considered megaphyll precursors and the first widespread appearance of laminate megaphylls occurred in Late Devonian (Frasnian; 385 Ma ago) [9]. However, largely due to lack of fossil evidence from Upper Silurian–Lower Devonian deposits, the mor-

phology and evolutionary diversification of early leaves of basal euphyllophytes remain enigmatic.

During the past decades, many fairly large, complex megafossils from the Lower Devonian have been reported. These, particularly fossils from the Posongchong Formation (Pragian age) of Yunnan in southern China, suggest an early divergence of megaphylls, implying a need to re-evaluate the euphyllophyte diversification of the Early Devonian and the timing of the origin and evolution of various lineages [10]. This paper focuses on primitive leaves, leaf-like structures, and three-dimensional lateral branches of the morphologically well-preserved euphyllophytes *Psilophyton*, *Pauthecophyton*, *Estinnophyton*, *Celatheca*, and *Eophyllophyton* and on several plants with uncertain affinities, such as *Adoketophyton*, *Stachyophyton*, and *Dibracophyton*. All these genera have representative species reported from the Lower Devonian Posongchong Formation (Figure S1), which mainly outcrops in Wenshan, southeastern Yunnan, China [11,12]. The Posongchong Formation is Pragian, and most possibly, middle-late Pragian in age based on regional

*Corresponding author (email: sghao@pku.edu.cn)

stratigraphic correlation [11], dispersed spore assemblages [13], and fish assemblages [14]. These plants have been studied previously, but here we present a further examination (including dégagement) and an updated interpretation. This paper is a part of the summary of the Posongchong Flora [11,12]. Evidence from this indicates that by the Early Devonian megaphylls had evolved in several different groups within the euphyllophyte clade and already showed wide variance of morphologies [15–18].

1   Earliest megaphylls and their parent plants

1.1   *Psilophyton* and *Pauthecophyton*

Psilophyton and *Pauthecophyton* regarded as trimerophytes, previously interpreted as leafless plants, have distal axes with lateral, three-dimensional, synchronously dichotomous trusses (Figure 1(a), (b)) [19–21]. These trusses have been called "incipient fronds", "pinnules", "dichotomous pinnulelike appendages", and "proto-leaves" [2,22]. Here, we prefer the term branch-leaf complexes (*Psilophyton*-type BLC) because these branches are basically axial in nature, non-planated, and unwebbed. These lateral dichotomous trusses, however, have a reduced morphology, implying a determinate growth, and most probably performed a photosynthetic function similar to that performed by leaves. They lack apical meristems that have the capability of generating vegetative or reproductive organs. Distal divisions of the dichotomous trusses show a fundamentally leafy architecture, comprising a vascular bundle, thin-walled cells (corresponding to parenchymous mesophylls), and epidermis and lacking peripheral sterome [3,19]. However, the main axes and branches of *Psilophyton dawsonii* have an outer cortex comprising a continuous layer of thick-walled sterome, a structure common in axes of many primitive tracheophytes but absent in Rhynie plants [19,22]. It is debatable whether the whole branching system or only the dichotomous trusses were involved in the evolution of true leaves [20,23].

The three-dimensional lateral dichotomous trusses in *Psilophyton* might have been inherited by successive Middle–Late Devonian plants [10,24]. Lateral dichotomous branching trusses of the Middle Devonian iridopteridaleans, such as *Compsocradus* and *Ibyka*, reduced to some degree and borne in a regular way (whorled or helical), are generally called "ultimate appendages" or "ultimate branching systems" [25]. We consider these to be homologous to the BLC of *Psilophyton*. The pseudosporochnaleans including *Lorophyton*, *Pseudosporochnus*, and *Wattieza*, are a different case. Their axes bear whorled or irregularly arranged lateral branching systems (LBS) with ultimate dichotomous branching units [26]. The LBS of pseudosporochnaleans were described as "fronds" in early literature, and thus the ultimate branching units, were described as "pinnules". Ultimate appendages of more advanced cladoxylopsids and

Rhacophyton are similar in morphology to the BLC of *Psilophyton*, but show a more elaborate arrangement (subopposite or alternate) and some planation and lamination into a single plane, indicating that further evolution had taken place [27].

1.2   *Estinnophyton*

Stems of *Estinnophyton* bear simple leaves, which are lateral dichotomous branching systems. They are planated but unwebbed and once- or twice-bifurcated at the middle part with two or four straight, tapering segments [18]. These leaves are arranged nearly in pseudowhorls, three to six per gyre (Figure 1(c), (d)). Fertile leaves are morphologically identical to sterile ones, clustered on axes to form loose, strobilus-like structure, and the sporangia are attached at the adaxial surfaces of the leaves by recurved stalks (Figures 1(e), 5(f)).

Estinnophyton has been considered a precursor of the sphenopsids in that its leaves have a pseudowhorled arrangement and the fertile leaves have recurved sporangia and sterile filiform extensions [18,28]. Typical sphenopsids with whorled simple leaves appeared in the Late Devonian [29,30]. The Middle Devonian iridopteridaleans (e.g. *Ibyka*) and pseudosporochnaleans (e.g. *Calamophyton*), particularly the former, have long been regarded as possible ancestral groups of the sphenopsids [22,25,31,32]. However, they have terminal sporangia, like *Psilophyton*. The characteristics of the fertile leaves of *Estinnophyton*, such as recurved sporangia, sterile filiform extensions, and pseudowhorled arrangement, are similar to those of *Calamophyton* [33]. These two taxa may have a close phylogenetic relationship, considering that reproductive characters should outweigh those from anatomy in early vascular plants [18]. The ancient origin of the sphenopsids and simple leaves can be traced to the Early Devonian *Estinnophyton*.

1.3   *Celatheca*

Celatheca has main axes and three-dimensionally arranged lateral branches. The vegetative lateral branch has at least two to three orders of branching, finally forming a lateral branching system with ultimate dichotomous appendages. Through dégagement, and with reference to other *Celatheca* specimens, two reconstructions are shown in Figure 1(f), (g). Figure 1(f) shows that a lateral branch bifurcates once and the two unequal daughter branches bear ultimate dichotomous appendages with strongly recurved tips (cylindrical, tapering ends). The ultimate units are distributed distichously, at wide angles, showing a roughly pinnate arrangement. This is a characteristic morphology of *Celatheca*-type branch-leaf complexes (*Celatheca*-type BLC). The second reconstruction shows a complex vegetative branching system (Figure 1(g)). There are many closely spaced laterals along part of the axis, some of which further divide to produce higher-order

branches. Ultimate dichotomous units can be observed below the branching point on the axis and along the axes of different orders of lateral branches. This reconstruction shows that BLC could constitute different branching orders. In light of this explanation, the ultimate units, equivalent to pinnules, are borne along different orders of branches, probably representing a precursor of compound leaves (or "fronds"). The *Celatheca*-type BLC differ distinctly from the three-dimensional synchronously dichotomous trusses of *Psilophyton*-type BLC. Such ultimate units, which have strongly recurved tips helically or decussately arranged on monopodial branches, are common in the distal branching systems of aneurophytalean progymnosperms, such as *Tetraxylopteris* [5]. The megaphylls of early seed plants have dichotomous, pinnate branching patterns and, generally, the fronds are bifurcate [5,8]. These fronds are similar to those of *Celatheca*-type BLC to some degree. The bifurcate fronds of seed plants may have been derived from the bifurcate lateral branches.

Fertile structures of *Celatheca* are located on main axes and dichotomous lateral branches. Each fertile structure is ovoid in overall shape, generally composed of four elongate-oval, leaf-like appendages, with each surrounding an elongate-ovoid sporangium (Figures 1(h)–(j), 5(g)) [16]. These fertile structures superficially resemble a synangium and the sporangium/appendage organization is similar to that of primitive cupulate ovules. *Celatheca* may represent an ancestral type and have a close relationship with pteridosperms.

Figure 1 Early Devonian plants with branch-leaf complexes. (a) *Psilophyton dawsonii*. Lateral branching system [19], here called the *Psilophyton*-type branch-leaf complex. (b) *Pauthecophyton gracile*. Lateral dichotomous branching system with terminal sporangia. PKU-XH264b. (c)–(e) *Estinnophyton yunnanense*. (c) Partial reconstruction of a vegetative axis, with lateral, once or twice-bifurcated leaves arranged in low spirals or pseudowhorls. (d), (e) Lateral forked leaves, with sporangia in (e). BUH-Es.01. (f)–(g) *Celatheca beckii*. (f) Reconstruction of a branching system, based on Figure 3 of [16], as a *Celatheca*-type branch-leaf complex with more or less pinnate arrangement of ultimate dichotomous appendages. (g) Reconstruction of a vegetative specimen based on Figures 2 and 28 of [16], showing a branch-leaf complex with multi-ordered branching systems and ultimate appendages more or less pinnately arranged. Sometimes an ultimate appendage is borne below the branching point. (h)–(j) Fertile structures showing sporangia ((h) right arrow; (i) arrow) and, behind them, leaf-like appendages. (h) shows incomplete leaf-like appendages (left arrow) and (j) shows complete ones (arrows). (h), (i) From Figures 12 and 13 of [16]. (j) PUH.10-Cel. 01. (k)–(m) *Polythecophyton demissum*. (k), (l) Terminal portion of fertile branch showing umbrella-shaped fertile systems and pendulous sporangial clusters. From [34], Plate II, Figure 1, and Plate III, Figure 3. (m) Schematic reconstruction of branching of a fertile structure and reconstruction of attachment pattern of sporangia. From [34], Figure 2B. Bars: (a) 5 mm; (b) 2 mm; (c), (f), (h)–(j) 2 mm; (d), (e) 1 mm; (g) 4 mm; (k) (l) 5 mm; (m) 4 mm.

1.4 *Polythecophyton*

Polythecophyton exhibits a clear architecture of fertile structures but lacks anatomy and vegetative organs [34]. The axes branch dichotomously and helically in three dimensions. The fertile branches are terminated by pendulous umbrella-shaped fertile structures (Figures 1(k)–(m), 5(h)). Each fertile structure may initially bifurcate, after which each component bears three to four alternately arranged, short axes, which divide into branchlets terminated by numerous slender fusiform sporangia in pairs or in groups of three or four.

This plant is similar to *Pertica* [35] and the aneurophytalean progymnosperms such as *Aneurophyton* and *Rellimia* [36,37] in branching pattern and particularly in fertile structures, sporangial morphology, and attachments. Some *Pertica* plants have been suggested to have a close relationship to aneurophytalean progymnosperms [22]. We suggest that *Polythecophyton* may represent basal aneurophytaleans as ordinal level.

1.5 *Eophyllophyton*

Eophyllophyton has both laminar leaves and branch-leaf complexes (*Celatheca*-type BLC) [15,17,38]. Generally the two laminated leaves curve inward towards each other to form a leaf pair, borne laterally or terminally on the axis (Figure 2(a),(b)). Leaf pairs along each axis appear an acropetal developmental series. That is, more pairs occur at the basal parts than at the distal parts, and those on the distal parts are younger and have curved tips (Figure 2(b)). The dichotomous pattern of the leaf pairs constitutes isotomous or anisotomous venation. Each leaf of the pair is served by a branching vein system, and each segment of the leaf is supported by a single distal, tiny vein [17]. The leaves are very small (generally 2.2–6.0 mm long) and show great variability, from a three-dimensional branching pattern with weak lamination to more webbed, fan-shaped pattern with conspicuous lamination and dissected segments (Figure 2(e), (f)). They can be suggested reduced compound leaves with highly dissected laminar divisions (corresponding to
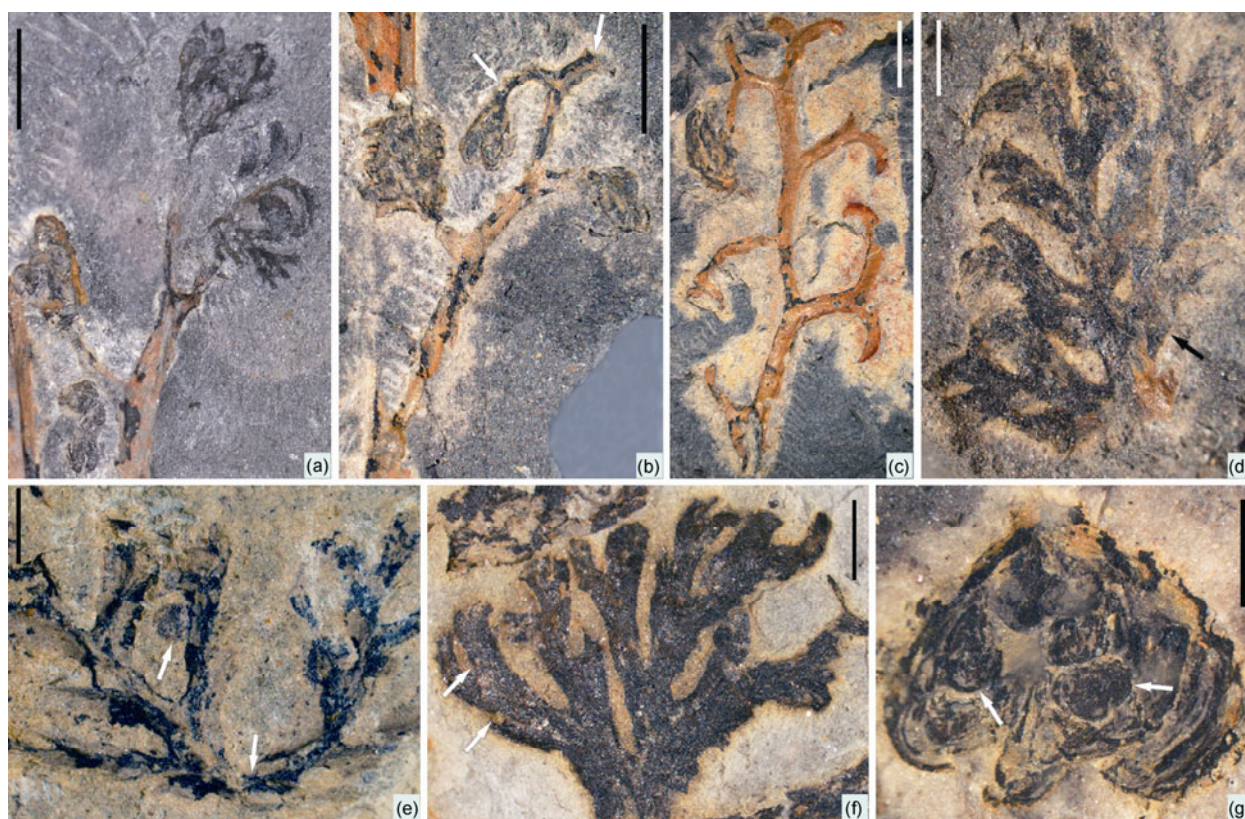

Figure 2   *Eophyllophyton bellum*. (a) Lateral branch with small laterals terminating in leaf pairs, either vegetative or fertile. BUPb137. (b) Lateral branch showing an acropetally developmental transformation: the basal part has mature leaf pairs, and the distal part has younger ones (left arrow) and recurved sterile tips (right arrow). BUPb102'. (a), (b) From [38], Plate III, 1 and Plate I, 4. (c) A branch-leaf complex with ultimate appendages (dichotomy with recurved tips) borne alternately along the axes, showing a roughly pinnate arrangement. PUH.10-Eop.01. (d) A leaf pair (two leaves, arrow indicates the faint appearance of bases of a leaf pair). The leaf margins of the laminate divisions are deeply incised and curved. A pinnately divided, laminar leaf reflects a branching system as shown in (c). PUH.10-Eop.02. (e) A fertile pair with two leaves shows a reduced, expanded branching system with weak lamination. The lower arrow points to common base of the two leaves, and upper arrow points to a sporangium. PUH.10-Eop.03. (f) A fan-shaped leaf with more conspicuous lamination. Note that the margins of the laminar divisions are deeply incised (arrows). PUH.10-Eop.03, 04. (g) Fertile leaf cluster with numerous sporangia. BUPb127. Bars: (a)–(c) 3 mm; (d)–(g) 1 mm.

pinnules) which are borne alternately along a presumed mid-vein (Figure 2(d)). The divisions of the leaf are not strictly held in a single plane (Figure 3(a)). Most leaf pairs are fertile (Figures 2(g), 5(d)). Vegetative leaves are far less common, although the fertile and vegetative leaves are identical in morphology. Random sampling, observations and statistics from over 300 leaves within the same horizons show that fertile leaves are ca. six times more common than vegetative ones, indicating that most of the laminate leaves of *Eophyllophyton* are fertile (Figure S4, Table S1).

Aside from laminate webbed leaves, sterile branching axes with ultimate dichotomous appendages (Figure 2(c)), i.e. branch-leaf complexes (*Celatheca*-type BLC), occur at the lower parts or basal regions of *Eophyllophyton*. The ultimate dichotomies, which have markedly recurved tips, are alternately attached. In morphology and disposition of divisions, the BLC is fully comparable to a leaf (Figure 2(c), (d)). Sometimes, one of the recurved tips of an ultimate dichotomy within a BLC terminates a leaf pair (vegetative or fertile) (Figure 2(b)). This implies that the tip of an ultimate dichotomy of the BLC corresponds to an arrested apex.

Anatomically, the leaf lamina is composed of veins, an outer single-layered epidermis, and a uniform mesophyll four to six cell layers thick. Mesophyll thin-walled cells, which appear to be randomly arranged, are polygonal or hexagonal in transverse view, elongated with oblique end walls in longitudinal view [15,17]. The mesophyll is structurally similar to that of a modern isobilateral leaf without differentiation into layers of palisade and spongy parenchyma. It shows an absence of any conspicuous system of intercellular air channels. The petiole is relatively thick and contains several tracheids in a centrarch primary xylem strand like that of the axis. Small veins, containing one to a

few tracheids, occur midway between the upper and lower surfaces of the division segment (Figure 3(a)–(e)).

In *Eophyllophyton*, multiple, nearly spherical sporangia are arranged in adaxial rows on laminate leaves. Anatomically, this plant has a columnar protostele, centrarch primary xylem [15]. The character combination, which distinguishes *Eophyllophyton* from zosterophylls and primitive lycopsids, trimerophytes, early ferns, fernlike plants, and progymnosperms, supports the assignment of *Eophyllophyton* to its own class and order.

The leaves of *Eophyllophyton* are distinct, but they exhibit more similarities to those of progymnosperms-seed plants than to those of early ferns or fernlike plants. The bifurcate morphology and broad laminar pinnules show strictly dichotomous venation, distinct characteristics of seed ferns, which differ from the fronds of ferns [5]. The early seed plants, like *Elkinsia*, exhibited highly dissected pinnules [8]. These were similar to the laminar divisions of *Eophyllophyton*'s leaves, although pinnule morphology can vary greatly. Anatomically, some axes of early seed plants have a small protostele and the departing leaf traces, like those of *Eophyllophyton*, have a single xylem bundle with centrarch or mesarch protoxylem abaxially situated [5,8].

2 Beyond megaphylls, plants with unusual leafy structures

Several Early Devonian plants such as *Adoketophyton*, *Stachyophyton* and *Dibracophyton* have fertile leaf-like appendages (sporophylls or bracts) to constitute a strobilar structure with different sterile organs, but they are neither microphyllous plants nor megaphyllous plants. The vegetative
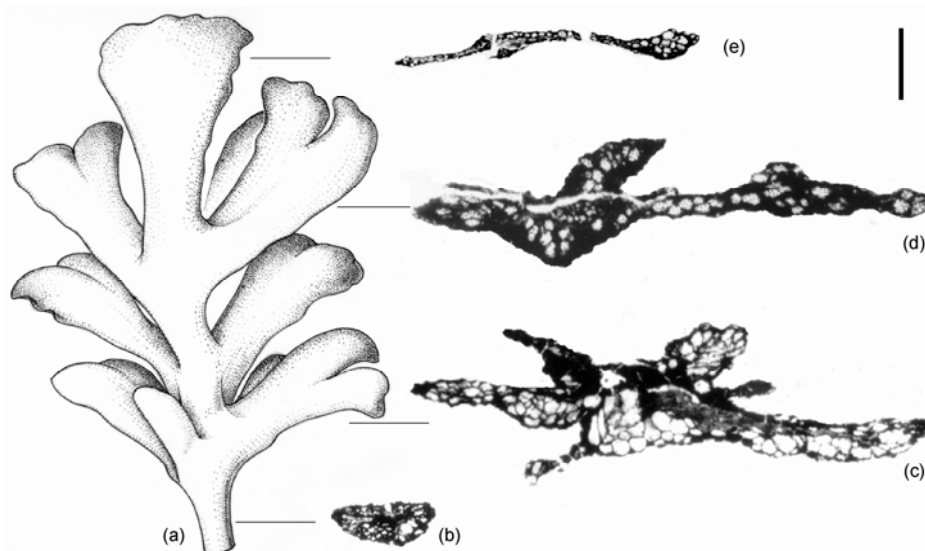

Figure 3 *Eophyllophyton bellum*. (a) Reconstruction of a leaf showing that laminar divisions are not held in one plane. (b)–(e) Transverse sections at different levels of the structurally preserved lamina, showing veins and mesophyll cells. (b) Through petiole of a leaf, note the main vein. Note main vein in (d) and observe that main vein in (c) and second-order veins in lateral divisions in (c) and (d) are missing. (e) Through distal region, note leaf vein. (b)–(e) From [17], Figure 3a–d. Bar: (a) 0.5 mm; (b)–(e) 250 μm.

axes of *Adoketophyton* dichotomously divide in three dimensions with some laterals ending in terminal, circinately coiled tips (Figure S3(a)) [39]. This vegetative axis represents another type of branch-leaf complex, *Adoketophyton*-type BLC. The strobilus is composed of fertile units, each of which consists of a fan-shaped sporophyll and a stalked sporangium (Figures 4(a), (b), 5(a)). The sporophyll is very regular in shape, with a parallel-sided stalk that expands into a triangular lamina with straight side margins and rounded or undulate distal margin. In the transverse view, the sporophyllous laminae appear crescent-shaped, and the abaxial surface of one or two subjacent layers seems to consist of heavily carbonized and thick-walled cells. The internal tissues are a combination of thin-walled cells and tracheids. The tracheids appear scattered in the transverse
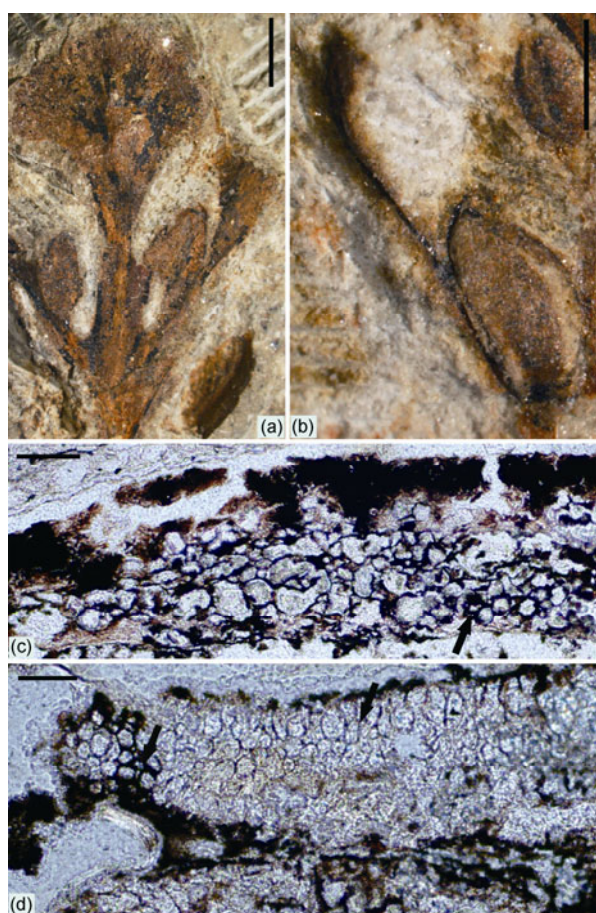

Figure 4 *Adoketophyton subverticillatum*. (a) Abaxial view of a sporophyll with a fan-shaped blade. (b) Lateral view of a fertile unit, with a sporangium adaxially attached on the sporophyll base. (a), (b) From [40], Plate I, Figures 3 and 2. (c) Transverse section of a sporophyllous lamina, showing variation in cells. Arrow points to probable tracheids. (d) Transverse section of distal part of a sporangium, showing structure of sporangial walls and marginal dehiscence grooves and thickenings (left arrow). Also note that the cells in the outermost layer are elliptical. Their long axes are perpendicular to the surface of the wall. Among them can be found prominent intercellular spaces (right arrow); dark material between two walls presumably represents a broken tapetal layer. (c), (d) From [40], Plate III, Figures 3 and 6. Bars: (a) 2 mm; (b) 1 mm; (c), (d) 60 μm.

section. The sporangial wall comprises six to nine rows of thin-walled, circular to polygonal cells, which form a single homogeneous layer, containing well-developed intercellular air channels (Figure 4(c), (d)).

In the original research, lacking detailed anatomical data, *Adoketophyton* was treated as Plantae *Incertae Sedis* [41], or had been placed in the barinophytopsids [42]. Considering the positional similarity of sporophylls in *Adoketophyton* and in lycopsids, Kenrick and Crane [22] suggested that the sporophylls of *Adoketophyton* and lycopsids are homologous, and *Adoketophyton* was placed in a basal position within the Lycophytina. Hao et al. [40] added anatomical data and described the vegetative terminal circinate tips of this plant. As to phylogenetic relationship, they suggested that *Adoketophyton* might represent an isolated group which evolved in parallel with lycopsids, and is far less related to the barinophytopsids. This plant is considered to be independent of the Zosterophyllopsida on the basis of the fertile unit composed of fan-shaped leaves and adaxially attached sporangia, and from Lycopsida in the absence of vegetative microphylls and presence of vegetative terminal circinate tips. Its centrarch primary xylem differs from the exarch order of maturation of both the zosterophyllopsids and lycopsids. Now *Adoketophyton* is tentatively treated as class and order *Incertae Sedis*, with an uncertain phylogenetic position, though it is well known based on detailed morphology and anatomy.

Stachyophyton has lateral leaf-like branches arranged helically along main axes. The leaf-like branches generally show a fan-shaped form (Figures S2, S3(b)) [43], and are here considered a distinct type of branch-leaf complex, *Stachyophyton*-type BLC. These BLCs show morphological similarities to leaves with many divisions expanding distally in one plane, but they are rigid and unreduced, departing from the main axes. They show great variability, from the branching in a plane to more "webbed", fan-shaped patterns with planation, and their distal segments are highly dissected divisions with rounded or cuneate tips [44]. We speculate that these BLC's retain an axial nature with a planate branching system but have distal foliar divisions and thus perform some photosynthetic functions. More intriguing, the strobili are located at the distal regions of the digitate branchlets of such fertile BLCs. The sporophylls are helically arranged, elongated, and laminate with broad bases and bifurcated tips. Each elongated elliptical sporangium is adaxially attached on a sporophyll (Figure 5(b)).

In *Stachyophyton*, the digitate branching of leaf-like branches, strobili consisting of sporophylls and sporangia, and the exarch primary xylem distinguish it from other known groups of early vascular plants [43,44]. Li [42] placed *Stachyophyton* into barinophytopsids and suggested that its vegetative branches are slightly planate but not related with megaphylls. In contrast, Wang and Cai [44] suggested that the vegetative branches as "megaphyll-like, leaf-like branches". The presence of strobili borne on the

multiple branchlets of digitate "leaf-like branches" effectively distinguishes *Stachyophyton* from the contemporaneous primitive lycopsids, sphenopsids and others. The Middle–Upper Devonian lycopsids generally have terminal strobili, but they differ from *Stachyophyton* in the presence of microphylls. *Stachyophyton* probably represents another independent group, mainly considering lack of detailed morphology and anatomy, and particularly precence of digitate leaf-like branching structures and strobili.

Dibracophyton possesses terminal strobili. Each fertile unit comprises a stalked long-elliptical sporangium, with dehiscence into two equal valves, and two discrete long-ovate bracts covering the sporangium from above and below (Figure 5(c)) [45]. The sterile axes of *Dibracophyton* bear helically dichotomous appendages with curved/round tips. Some dichotomous appendages are alternately borne at the basal areas of the fertile axes. *Dibracophyton* resembles *Barinophyton* and *Protobarinophyton* [46–48]. Their sporangia have two valves and distal dehiscence (sporangial characters were demonstrated in *B. norvegicum* and *Dibracophyton*), and possible unvascularized bracts. We suggest that these plants perhaps are close in affinity. Concerning other less well-known plants such as *Bracteophyton variatum*, *Krithodeophyton croftii* and *Enigmophyton superbum* [49,50], which were considered as putative members of barinophytopsids [41], their bract details and the exact relationship with sporangia need further examination. Moreover, the vegetative branches of most barinophytopsids have not been determined. These plants were regarded as leafless, due to the limited material. It is interesting in *Dibracophyton* that, besides the independent vegetative axes, a few vegetative appendages are distributed along the basal area of the fertile axes, and that the long upper region of the fertile axes lacks any appendages (Figure S3(c)).

3 Multiple origins of megaphylls in the Early Devonian

The monophyly of euphyllophytes has been widely supported [22,51]. However, as a foliar organ, the megaphylls of euphyllophytes have been shown to have evolved independently four, six, or even nine times in several different lineages [6,7,52]. Studies of leaf development and genetic expression have indicated that megaphylls are homoplastic across euphyllophytes [7,52]. The evolutionary scenario proposed by Zimmermann [53], in which three-dimensional lateral branches led to true leaves through overtopping, planation and webbing (as the Telome Theory), has been accepted by many neo- and paleobotanists [2]. Evidence from plant megafossils is anticipated to show intermediate examples of these morphological transformations.

It has been suggested that early vascular plants of the Late Silurian through Middle Devonian, with few exceptions, lack any true megaphyllous leaves. Lateral axes of a plant from the late Early Devonian show an elliptical trace departing from a lobbed protostele. This plant has been explained as the beginning of the evolution of megaphylls [54]. The branch-leaf complexes (three-dimensional lateral branching systems) of trimerophytes of Emsian to early Eifelian age have been long considered the earliest megaphyll precursors [19,20]. However, the evidence from Posongchong Formation of the Pragian age, prior to the known other trimerophytes (e.g. *Pertica* and most *Psilophyton* species), indicates multiple ancient types of megaphylls and a much earlier appearance of laminate megaphylls (in *Eophyllophyton*).

Combined with fertile features and anatomy (where present) and comparisons with Middle–Late Devonian plants, several lineages can be traced and recognized (see above and Figure 5): (1) *Psilophyton* (and *Pauthecophyton*)-iridopteridaleans and pseudosporochnaleans (cladoxylopsids), the latter groups being linked with early ferns or fern-like plants. They have paired elongate or fusiform sporangia borne at the ends of synchronous isotomies (Figure 5(e)) [19–23]; (2) *Estinnophyton*-sphenopsids. They share fertile structures with recurved sporangia and simple leaves in pseudowhorls (Figure 5(f)) [18,28]; (3) *Polythecophyton*-aneurophytaleans. Their fusiform sporangia are in clusters borne along the inner sides of the fertile structures (Figure 5(h)) [34]; (4) *Celatheca*-pteridosperms (seed ferns). *Celatheca* bears elongate-oval fertile structures with sporangia and surrounding leaf-like appendages, structurally resembling a synangium or cupule (Figure 5(g)) [16]; and (5) *Eophyllophyton*, which shows a unique combination of characteristics, different from those of any known group (truly extinct) [15,17]. These five lineages, with different types of branch-leaf complexes (BLC) and initial leaves, clearly demonstrate the multiple origins of megaphylls (Figure 5), given that the differences among these BLC can be hardly explained by ecological factors and these BLC have a "modular construction" that allows flexibility in organ production in response to changes in environmental conditions.

4 Implication of developmental mechanisms of leaves

The branch-leaf complexes of *Psilophyton*, *Celatheca*, and *Eophyllophyton* are all basically lateral organs with determinate growth and some degrees of reduction. The interactions between class 1 KNOX and ARP genes seem to be a shared mechanism responsible for determinacy of lateral appendages: KNOX genes are expressed in the shoot apical meristem (SAM) and ARP genes are expressed in leaf primordial tissues [2,52]. The BLC and leaves of these plants exhibit features of an axial branching system, reflecting a plesiomorphic state. The laminar leaves of *Eophyllophyton* appear as miniatures of axial branching systems. The mesophyll cells are elongate, extending parallel to the vein strand, like cortical tissues of a lateral branch system. It seems reasonable
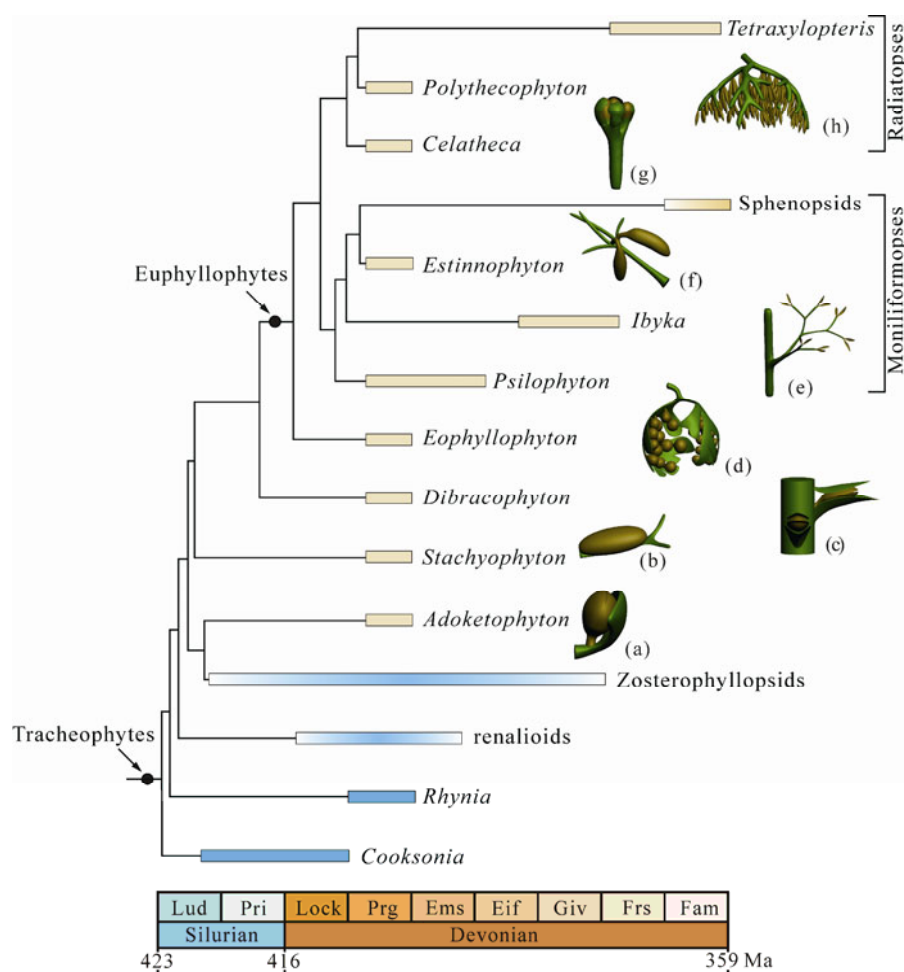
Figure 5 Phylogeny of basal tracheophytes (based on [12]) plotted against geological time (numerical ages from [55]), showing the appearance of major clades, including euphyllophytes in Late Silurian-Early Devonian. (a)–(h) Schematic reconstruction of fertile structures of selected plants from the Lower Devonian Posongchong Formation in Yunnan, southern China: (a) *Adoketophyton*; (b) *Stachyophyton*; (c) *Dibracophyton*; (d) *Eophyllophyton*; (e) *Psilophyton* (*Pauthecophyton*); (f) *Estinnophyton*; (g) *Celatheca*; (h) *Polythecophyton*. The phylogenetic framework is mainly based on the cladistic analyses of [12]. The dating of the oldest zosterophyllopsids and lycopsids is based on [56,57]. The sudden appearance of plants with unusual fertile features during the Early Devonian Pragian (ca. 410 Ma ago) is shown in (a)–(h).

to suggest that the genetic pathway controlling leaf development may have been built upon variation in the genetic mechanisms controlling the axial system.

As defined by Tomescu [52], a true leaf of vascular plants should have four features: vascularization, determinate growth, bilateral symmetry (adaxial-abaxial polarity), and definite arrangement (phyllotaxis). However, in the Early Devonian plants, these four features may have not evolved fully in any given type of ancient leaf (branch-leaf complexes, proto-leaves, or leaf precursors), and they show different acquisition sequences in different plant lineages. The branch-leaf complexes (BLC) of *Psilophyton* and *Celatheca* show different branching patterns and, in our opinion, foreshadow different types of true leaves with different venation and configuration. We use the term "branch-leaf complexes" because they are basically axial in nature, but their ultimate dichotomous appendages (or ultimate units) have undergone overtopping and reduction, showing erect

sterile tips (in *Psilophyton*) or recurved tips (in *Celatheca*). In this way, they seem to show a determinate growth pattern, indicating little or no possibility of further growth.

Megaphylls of *Eophyllophyton bellum* show a uniform, fundamentally leafy histology, consisting of a vascular bundle (vein), parenchymous mesophylls (thin-walled cells) and epidermis [15]. Similar structures occurred in at least ultimate units of the branch-leaf complexes (BLC) of *Psilophyton dawsonii* and *Triloboxylon ashlandicum* [19,58]. Although different in morphology, these early leaf-like structures have similar homogeneous thin-walled cells (mesophyll-like organization). That is, they lack adaxial-abaxial polarity and tissue differentiation between palisade and spongy cells. The subsequent acquisition of adaxial-abaxial polarity (and the associated genetic pathways) in euphyllophytes probably occurred independently. Various types of branch-leaf complexes and leaves in Early Devonian euphyllophytes (*Psilophyton*, *Estinnophyton*, *Celatheca*,

and *Eophyllophyton*) represent very different lineages, indicating a complex pattern. Class III HD-zip, KANADI, and YABBY genes, micro RNAs 165 and 166, and their interactions seem to contribute to the establishment of adaxial-abaxial polarity [2,8,59]. It is noteworthy that the leaves of *phan* mutants of the angiosperm plant *Antirrhinum majus* show radial symmetry [60]. They also show architecture similar to those ultimate units of the branch-leaf complexes (BLC) of *Psilophyton dawsonii* and *Triloboxylon ashlandicum*, and *Eophyllophyton*. Mutant analyses and gene expression studies have revealed epigenetic phenomena that regulate leaf laminar development. In *Eophyllophyton* the tips of ultimate dichotomy of a BLC could be transformed into the laminar leaves demonstrating this phenomenon in the early stage of leaf evolution.

5 Appearance of fertile laminar leaf-like appendage (or leaves) as an adaptation for protection of sporangia

Plants such as *Adoketophyton*, *Stachyophyton*, *Dibracophyton*, and *Celatheca* have laminated leaf-like appendages (sporophylls or bracts) subtending sporangia [16,39–45]. The leaf-like appendages of *Adoketophyton* are vascularized and probably have heavily carbonized cuticles. The associated sporangia have well-developed intercellular air channels in their sporangial walls. Anatomical details of leaf-like appendages in *Stachyophyton*, *Dibracophyton*, and *Celatheca* are unclear. These various leaf-like appendages have been considered a phenomenon of convergent evolution in that their parent plants show large differences in branching, sporangial features, and other characters. Based on biogeochemical models and studies of paleosol and plant stomatal indexes, the Early Devonian has been deduced to be a period with high concentrations of atmospheric CO_2 [1,9,61]. These laminar leaf-like structures, besides photosynthesis, may mainly have had a protective function, protecting the sporangia from solar heat in this harsh-atmosphere environment. Environmental factors and physiologic requisition, such as high atmospheric CO_2 concentrations and high temperatures, may have driven selection and adaptation. As a result, fertile laminate leaf structures enclosing sporangia may have evolved independently in different plant lineages and have been laminated before the formation of vegetative leaf structures (e.g. Figure S4, Table S1). Unlike several lineages with megaphylls, the aforementioned plants, with unusual fertile leaf-like appendages, were short-lived and quickly became extinct. They have been replaced by plants with true leaves and sporophylls, i.e. lycopsids, ferns, horsetails, and eventually by seed plants.

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Supporting Information

Figure S1   Stratigraphic column of the Posongchong Formation at the Zhichang section showing distribution of megafossil plants.

Figure S2   *Stachyophyton yunnanense.*

Figure S3   Partial restorations of *Adoketophyton parvulum* (a), *Stachyophyton yunnanense* (b), and *Dibracophyton acrovatum* (c).

Figure S4   *Eophyllophyton bellum.*

Table S1   Occurrences of fertile and sterile leaves in specimens of *Eophyllophyton bellum*

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