Plastome phylogenomic analysis reveals evolutionary divergences of Polypodiales suborder Dennstaedtiineae

Jin-Mei Lu1*,†, Xin-Yu Du1†, Li-Yaung Kuo2, Atsushi Ebihara3, Leon R. Perrie4, Zheng-Yu Zuo1, Hui Shang5, Yi-Han Chang6 and De-Zhu Li1*

Abstract

**Background:** Polypodiales suborder Dennstaedtiineae contain a single family Dennstaedtiaceae, eleven genera, and about 270 species, and include some groups that were previously placed in Dennstaedtiaceae, Hypolepidaceae, Monachosoraceae, and Pteridaceae. The classification and phylogenetic relationships among these eleven genera have been poorly understood. To explore the deep relationships within suborder Dennstaedtiineae and estimate the early diversification of this morphologically heterogeneous group, we analyzed complete plastomes of 57 samples representing all eleven genera of suborder Dennstaedtiineae using maximum likelihood and Bayesian inference.

**Results:** The phylogenetic relationships of all the lineages in the bracken fern family Dennstaedtiaceae were well resolved with strong support values. All six genera of Hypolepidoideae were recovered as forming a monophyletic group with full support, and Pteridium was fully supported as sister to all the other genera in Hypolepidoideae. Dennstaedtioidae (Dennstaedtia s.l.) fell into four clades with full support: the Microlepia clade, the northern Dennstaedtia clade, the Dennstaedtia globulifera clade, and the Dennstaedtia s.s. clade. Monachosorum was strongly resolved as sister to all the remaining genera of suborder Dennstaedtiineae. Based on the well resolved relationships among genera, the divergence between Monachosorum and other groups of suborder Dennstaedtiineae was estimated to have occurred in the Early Cretaceous, and all extant genera (and clades) in Dennstaedtiineae, were inferred to have diversified since the Late Oligocene.

**Conclusion:** This study supports reinstating a previously published family Monachosoraceae as a segregate from Dennstaedtiaceae, based on unique morphological evidence, the shady habitat, and the deep evolutionary divergence from its closest relatives.

**Keywords:** Dennstaedtiaceae, Plastid phylogenomics, Monachosoraceae, Monachosorum, Monophyly, Morphological character, Divergence time

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**Background**

The purpose of a phylogeny is the construction of a genealogical tree of organisms, its substantiation, and interpretation [1]. Classification can provide a framework to identify plants and to conduct evolutionary and physiological studies, and it can serve us better if we can name those groups that are readily recognizable and characterized by morphological diagnosability and homogeneity, at least at higher ranks (families and orders) [2].
comprehensive phylogeny-based classification of pteridophytes (including ferns and lycophytes) was established by the Pteridophyte Phylogeny Group in 2016 and has been widely accepted and cited in recent years [3]. Given the limitations of sampling and research methods, the PPG classification should not be the final decision on lycophytes and ferns classification, although it incorporated all phylogenetic data available at the time.

Numerous phylogenetic studies have been conducted since the publication of the PPG classification [3], especially on those globally distributed families, e.g., Lycopodiaceae [4], Polypodiaceae [5, 6], Pteridaceae [7], and Thelypteridaceae [8, 9]. The increase in the number of samples, especially in regions and groups with low sampling density, and the accumulation of molecular data, have improved the ability of taxon delimitation and identification and prompted the publication of some new taxa. One new family was published by Zhou et al. [10], and some new genera have continued to be recognized (e.g., [11, 12, 13, 14, 15, 16]). The systematic positions of several remaining enigmatic groups in the PPG classification [3] were resolved [5, 6, 17, 18, 19, 20]. In addition, some hybrid genera have been found (e.g., × Cyclobotrya, [21]; × Woodsimantium Li Bing Zhang, N.T. Lu & X.F. Gao, [22]; reviewed in [23]).

Polypodiaceae were first recognized as a distinct taxonomic group at the beginning of the nineteenth century, and its monophyly has been strongly supported by a unique morphological character (the interruption of the vertical annulus by a sporangium stalk) and molecular studies (e.g., [17, 18, 24, 25, 26, 27]). During the nineteenth century nearly all leptosporangiate ferns were placed in Polypodiaceae in the broadest sense (Polypodiaceae s.l.). Ching [28] was the first to recognize the heterogeneity of Polypodiaceae s.l., and divided it into 33 families. Smith et al. [2] recognized 15 families in Polypodiaceae, while Christenhusz et al. [29] included two newly described families and recognized 23 families in Polypodiaceae. In the following five years, four new families, Hemidictyaceae, Arthropteridaceae, Didymochlaenaceae, and Desmophlebiaceae, were described [30, 31, 32, 33]. PPG I [3] accepted Hemidictyaceae, Didymochlaenaceae, and Desmophlebiaceae, but not Arthropteridaceae, and recognized 26 families in six suborders for Polypodiaceae. Zhou et al. [10] established a new family of Pteridryaceae (comprising four genera) as a segregate from Tectariaceae based on the criteria of monophyly and the diagnosability, and they also advocated for the recognition of Arthropteridaceae. Hence ten new families have been published and/ or recognized in Polypodiaceae, specifically in suborder Aspleniineae (5 families), suborder Polypodiineae (3 families), suborder Lindsaeineae (1 family), and suborder Saccorrhizinae (1 family) in the last two decades. But the remaining two suborders Pteridinaceae and Dennstaedtiineae each contain one relatively heterogeneous family.

Polypodiales suborder Dennstaedtiineae were recently considered to contain one family Dennstaedtiaceae, eleven genera and about 270 species [2, 3, 34, 35, 36], and include some groups that were previously placed in Hypolepidaceae (e.g., Hypolepis Bernh.), Monachosoraceae (Monachosorum Kunze), and Pteridaceae (Pteridium Gled. ex Scop.) (Table 1). Ching [28] included Dennstaedtia Bernh., Leptolepia Prantl, Microlepsia C. Presl, Oenotrichia Copel., and four additional genera of tribe Saccolomeae in his Dennstaedtiaceae, and Hypolepis in Hypolepidaceae, and he placed Monachosorum and Ptilopteris Hance in Monachosoraceae. Pichi Sermolli [37] classified Monachosoraceae and Ptilopteris of Monachosoraceae Ching into Dennstaedtiaceae, and circumscribed about ten genera in Dennstaedtiaceae, while he assigned Blotiella R.M. Tryon, Histiopteris (L. Agardh) J. Sm., Hypolepis, Lonchitis L., Paeia A. St.-Hil., and Pteridium in Hypolepidaceae. Kramer [38] circumscribed Dennstaedtiaceae more broadly and included subfamily Saccolomatoideae (1 genus), subfamily Dennstae dioideae (10 genera), and subfamily Lindsaeoideae (5 genera). Early molecular studies showed that Monachosoraceae were nested within Dennstaedtiaceae while the lindsaeoid ferns were relatively distant from Dennstaedtiaceae [26, 39]. Further expanded phylogenetic analyses of Dennstaedtiaceae [40] showed that Coptodipteris Nakai & Momose; Leptolepia, Microlepsia, and Oenotrichia fell within Dennstaedtiaceae. Shang et al. [35] described a new genus Hiya H. Shang from Hypolepis based on combined evidence of morphology, cytology, and molecular phylogeny. The molecular phylogeny based on five plastid DNA markers further recovered three subfamilies in Dennstaedtiaceae: Dennstae dioideae, Hypolepidioideae, and Monachosorioideae [34], which is consistent with the study of the morphological anatomy of the rhizomes [41]. Most genera (except for Dennstaedtiaceae) in Dennstaedtiaceae have been supported as monophyletic; However, the phylogenetic relationships among these genera have not been resolved [34, 35, 40, 41].

In this study, we used a phylogenomic approach to resolve the phylogenetic relationships among all genera in Polypodiales suborder Dennstaedtiineae (i.e., Dennstaedtiaceae s.l., Dennstaedtiaceae sensu PPG I), and test the phylogenetic and taxonomic positions of Monachosorum. Special attention was paid to explore the deep divergences in suborder Dennstaedtiineae using the analyses of morphological characters and the estimation of the divergence time.
Results

Characteristics of plastomes and datasets

Forty-four complete or nearly complete plastomic sequences of Polypodiales suborder Dennstaedtiineae were generated in this study (Additional file 1). All sequenced plastomes showed a high similarity in genome structure and gene content to the published Dennstaedtiaceae plastomes. The GC content of novel plastomes ranged from 41.5% (\textit{Pteridium caudatum}) to 45.5% (\textit{Monachosorum maximowiczii}), and the size of the plastomes ranged from 147,263 bp (\textit{Leptolepia novae-zelandiae}) to 158,547 bp (\textit{Histiopteris herbacea}) (Additional file 1). \textit{Monachosorum} showed the highest sequence divergence between genera in suborder Dennstaedtiineae.

Phylogenetic relationships within Dennstaedtiaceae s.l.

All four analyses (Table 2) recovered identical three major lineages previously recognized as subfamilies of Dennstaedtiaceae: Dennstaedtiioideae, Hypolepidioideae, and Monachosoroideae of the currently defined Dennstaedtiaceae (Fig. 1). The topologies from different analyses were almost identical, only with minor differences in \textit{Pteridium} (Fig. 1; Additional files 2 and 3). All relationships among subfamilies, genera, and clades were fully or strongly supported (MLBS > 90%, BIPP = 1.0).

All subfamilies and genera were recovered as monophyletic with full support, except for \textit{Dennstaedtia} (Fig. 1). \textit{Monachosorum} was resolved as sister to all the remaining genera. Within the Hypolepidioideae, \textit{Pteridium} was fully supported as a sister of all the other genera, followed sequentially by \textit{Hypolepis}, \textit{Paesia}, \textit{Hiya}, \textit{Blotiella}, and \textit{Histiopteris}. The \textit{Dennstaedtia} s.l. clade (i.e., Dennstaedtiioideae sensu Schwartsburd et al.) contained four clades—the \textit{Microlepia} clade, the northern \textit{Dennstaedtia} clade, the \textit{D. globulifera} clade, and the \textit{Dennstaedtia} s.s. clade. \textit{Leptolepia} and \textit{Oenotrichia} were nested within the \textit{Dennstaedtia} s.s. group.

| Genus                  | PPG I (2016) [3]       | Smith et al. (2006) [2] | Kramer (1990a, b) [38, 42] | Pichi Sermolli (1970) [37] | Ching (1940) [43] |
|------------------------|------------------------|-------------------------|-----------------------------|----------------------------|-------------------|
| \textit{Blotiella} R.M.Tryon (1962) | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Hypolepidaceae           |                   |
| \textit{Dennstaedtia Bernh} | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Dennstaedtiaceae          | Dennstaedtiaceae |
| \textit{Histiopteris} (J.Agardh) J.Sm | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Hypolepidaceae           | Pteridiaceae      |
| \textit{Hypolepis Bernh} | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Hypolepidaceae           | Hypolepidaceae    |
| \textit{Leptolepia} Mett. ex Prantl | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Dennstaedtiaceae          | Dennstaedtiaceae |
| \textit{Microlepia} C. Presl | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Dennstaedtiaceae          | Dennstaedtiaceae |
| \textit{Oenotrichia} Copel | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Dennstaedtiaceae          | Dennstaedtiaceae |
| \textit{Paesia} St. Hilaire | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Hypolepidaceae           | Pteridiaceae      |
| \textit{Pteridium} Gled. ex Copel | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Dennstaedtiaceae | Hypolepidaceae           | Pteridiaceae      |
| \textit{Lonchitis} L | Lonchitidaceae          | Lindseaeaceae           | Dennstaedtiaceae subfamily Dennstaedtiaceae | Hypolepidaceae           | Pteridiaceae      |
| \textit{Monachosorum} Kunze | Dennstaedtiaceae        | Dennstaedtiaceae        | Monachosoroideae            | Dennstaedtiaceae          | Monachosoraceae   |
| \textit{Ptilopteris} Hance | Dennstaedtiaceae        | Dennstaedtiaceae        | Monachosoroideae            | Dennstaedtiaceae          | Monachosoraceae   |
| \textit{Hiya} H. Shang (2018) [35] | Dennstaedtiaceae        | Dennstaedtiaceae        | Monachosoroideae            | Dennstaedtiaceae          | Monachosoraceae   |
| \textit{Coptidipteris} Nakai & Monose (1937) | Dennstaedtiaceae        | \textit{Dennstaedtia} | Dennstaedtiaceae subfamily Dennstaedtiaceae | Dennstaedtiaceae          | Dennstaedtiaceae |
| \textit{Saccoloma} Kauffuss | Dennstaedtiaceae        | Dennstaedtiaceae        | Dennstaedtiaceae subfamily Saccolomatoideae | Dennstaedtiaceae          | Dennstaedtiaceae |
| \textit{Ormoloma} Maxon | Dennstaedtiaceae        | \textit{Saccoloma}      | Dennstaedtiaceae subfamily Saccolomatoideae | Dennstaedtiaceae          | Dennstaedtiaceae |
| \textit{Ithycaulon} Copel | Dennstaedtiaceae        | \textit{Saccoloma}      | Dennstaedtiaceae subfamily Saccolomatoideae | Dennstaedtiaceae          | Dennstaedtiaceae |
| \textit{Orthiopteris} Copel | Dennstaedtiaceae        | \textit{Saccoloma}      | Dennstaedtiaceae subfamily Saccolomatoideae | Dennstaedtiaceae          | Dennstaedtiaceae |
Divergence time estimation

Based on the dating results from the BEAST analysis using 191.62–219.87 Ma as the minimum–maximum secondary age constraint of the root (the stem of Polypodiales) combined with four fossil records, the currently defined Dennstaedtiaceae were estimated to have originated at 162.43 Ma (95% HPD: 141.29, 186.63 Ma), with its crown group dated to 113.19 (101.42, 129.04) Ma (Fig. 2). The early diversification of Dennstaedtiaceae mainly occurred during the Early Cretaceous period.
Table 2  Data characteristics with models selected and used in different analyses

| Data set | No. of taxa (ingroup) | No. of sites (bp) | Variable/Parsimony informative sites (%) | Methods | Models |
|----------|----------------------|-------------------|------------------------------------------|---------|--------|
| Pt       | 80 (57)              | 124,738           | 77,883 (62.4%)/63,703 (51.1%)            | ML      | GTR + H4 |
|          |                      |                   |                                          | BI      | GTR + I + G |
| CDS      | 80 (57)              | 72,828            | 42,547 (58.4%)/35,797 (49.2%)            | ML      | PartitionFinder2 defined |
|          |                      |                   |                                          | BI      | GTR + I + G |

Fig. 2  Chronogram of Polypodiales suborder Dennstaedtiineae. Blue horizontal bars on the nodes indicate 95% credible intervals of the divergence time estimates (95% HPD), and numbers on the bars indicate the median age (Ma). Geological timescale and subdivisions: E, Early; L, Late; M, Middle
giving rise to Dennstaedtiioideae and Hypolepidoideae (93.81 (75.04, 111.23) Ma), and Monachosoroideae.

The second diversification occurred mainly during the Paleogene, resulting in the radiation of Dennstaedtiioideae and Hypolepidoideae. The crown ages of Dennstaedtiioideae and Hypolepidoideae were estimated to be 60.88 (50.31, 73.6) Ma and 45.5 (33.2, 58.4) Ma, respectively. All genera (and clades) in Dennstaedtiaceae were inferred to have started to diversify in the Oligocene or later (29.5–1.07 Ma), and the diversification of extant Monachosorum was inferred to have begun only at about 13.31 (5.68, 23.81) Ma.

Discussion
The phylogenetic position of suborder Dennstaedtineae in Polypodiales
In his phylogenetic classification of the homosporous ferns, Nayar [44] proposed that Dennstaedtaceae had a close relationship with Dicksonioideae and Thyrsopteridioideae, and the family gave rise to Lindsaeaceae and Hypolepidoideae in order Cyatheales. Molecular phylogenetic studies suggested that Dennstaedtaceae were sister to a clade comprising Pteridioideae and the eupolyponds [26, 27, 45], or sister to the eupolyponds [25, 46, 47, 48], or sister to Pteridioideae [17, 18].

Our phylogeny based on 57 plastomes from Polypodiales suborder Dennstaedtineae and 23 plastomes from other families in Polypodiales supports the monophyly of the currently defined suborder Dennstaedtineae as in the PPG classification. All four analyses based on the plastomic and CDS data sets resolve suborder Dennstaedtineae to be sister to suborder Pteridioideae with full support values in the present study. A recent study showed that Dennstaedtioideae (Dennstaedtiaceae) share a 3-codon deletion in the ndhB gene with Pteridioideae (Pteridaceae) [17]. There is morphological evidence supporting the sister relationship between Dennstaedtiaceae and Pteridaceae, rather than that supporting the sister relationship between Dennstaedtiaceae and the eupolyponds. The marginal (false) sori protected by reflexed indusia formed by reflexed upper side of the margins of pinnae or leaflets are found in some genera of Dennstaedtiaceae (Paesia and Pteridium) and most genera of Pteridaceae (e.g., [49, 50]), but never found in members of the eupolypond clade. Secondly, a solenostele can be found in both Dennstaedtiaceae and Pteridaceae, but never in eupolyponds. Furthermore, eupolypond families are characterized by having a circumendodermal band (CB, a distinctive, second, innermost layer of the fundamental tissue adjacent to the innermost endodermis) that surrounds the leaf traces in the petioles [51], while there is no CB in the early-diverging members of Polypodiales (e.g., Dennstaedtiaceae, Lindsaeaceae, Pteridaceae, and Saccolomataceae).

Plastome phylogeny of Dennstaedtiaceae
Previous research on morphology, development, and ultrastructure was considered to provide additional phylogenetic insights [39]. There are many different morphological characteristics in sporophytes among the genera of Dennstaedtiaceae, making the systematic circumscription of the family difficult.

The previous molecular phylogenetic studies showed that Dennstaedtiaceae comprises three clades (subfamilies); however, the relationships among the three clades were not well supported [34]. The relationships among Dennstaedtiioideae and Hypolepidoideae, genera, and clades are strongly supported with full support values in our analyses. Monachosorum is strongly supported as sister to all the remaining genera in Dennstaedtiaceae. All six genera of Hypolepidoideae [34] are recovered as forming a monophyletic group with full support. Pteridium is fully supported as sister to the remaining Hypolepidoideae, which is consistent with most previous studies [35, 40, 52] and the morphological characteristics of spores. Hiya is strongly supported as sister to the assembly of Blotiella and Histiopteris by 93% ML bootstrap support values and 1.0 Bayesian confidence values (Fig. 1). The present result differs from previous studies [34, 35], in which Paesia was sister of the Blotiella-Histioperis clade although this topology was only weakly supported.

Species of the Dennstaedtia s.l. clade are distributed mainly in the tropics and sub-tropics and grow in open habitats. They are edge-colonizers, and have long-creeping rhizomes, marginal or submarginal sori protected by cup-shaped or half-cup-shaped indusia, and trilete spores. Our study supports a broadly defined Dennstaedtia s.l. and does not corroborate the monophyly of Dennstaedtia s.s., which is consistent with three previous studies based on a single or a few chloroplast markers [34, 35, 40]. Our study suggests that Dennstaedtia s.l. falls into four clades each with full support, and the newly identified D. globulifera clade is the sister group of the Microlepiia clade and the northern Dennstaedtia clade. Morpho-anatomical studies of the rhizomes of Dennstaedtioideae [41] showed only the outermost layer of the pith is occupied by sclerified parenchyma in Dennstaedtia globulifera (Poir.) Hieron., while in species of the northern Dennstaedtia clade sclerified parenchyma occupies almost the entire pith. More recently, a phylogenetic study of Dennstaedtioideae based on four chloroplast markers also confirmed that Dennstaedtioideae consist of four clades, and proposed a classification of four genera corresponding to the four clades in Dennstaedtioideae [53]. The type species of Leptolepia and Oenotrichia were sampled in our study; however, the samples of the type species of Microlepira (M. polypodioides (Sw.) C. Presl)
and *Dennstaedtia* (*Dennstaedtia flaccida* (G. Forst.) Bernh.) were not sampled in the present study. When the *rbcL* sequence of *D. flaccida* (GenBank accession: MT657694) was compared with those of our samples, *D. flaccida* is nested within the *Microlepis* clade and was closely related to *M. speluncae* (L.) T. Moore (not shown). Another study based on four chloroplast markers also proposed inclusion of *D. flaccida* in *Microlepis* [53]. We suggest that the specimens of the type species *M. polytopioides* need to be further sampled to test the circumscription of this genus. Until then, we prefer the retention of a more inclusive *Dennstaedtia*.

**On the isolated position of Monachosorum in suborder Dennstaedtiineae**

*Monachosorum* Kunze is a small genus in Asia comprising approximately six species, mainly distributed in the temperate regions. Morphologically, *Monachosorum* is somewhat related to the davallioid (in the relationship of the lamina of a leaflet to the rachis bearing it), thelypteroid ferns (in petiolar structure and vascular cylinder), dennstaedtioid ferns (in frond form, the absence of scales, and the absence of hair on the gametophytes), and anogrammoid ferns (in the forked and open veins, exindusiatesori, and trilete spores with perine).

Ching [28] recognized Monachosoraceae for the first time and included two Asian genera *Monachosorum* and *Ptilopteris* in it, and he proposed that Monachosoraceae was related to the thelypteroid and the athyrioid ferns. However, Ching [28] did not provide a Latin description and thus failed to validly publish this family in 1940. Ching [43] validated the family Monachosoraceae in his classification of Chinese fern families and genera. Some pteridologists [42, 54] treated *Monachosorum* as the type genus of the Monachosoraceae, and some others did not recognize the family and considered that *Monachosorum* had a close relationship with Dennstaedtiaceae [37, 44, 55, 56, 57, 58], Thelypteridaceae [59], Davalliaceae [60], Cyatheoids [61], or the aspidioid ferns [62]. The early molecular phylogenetic study by Wolf [39] implied including *Monachosorum* in Dennstaedtiaceae although there were low bootstrap value and the ambiguity in the phylogenetic tree.

There is a curious mixture of primitive and advanced characters in *Monachosorum* [54]. The most distinctive feature of *Monachosorum* is the production of the typical monachosorioid hair (one-, two-, or three-celled, club-shaped, or catenate unbranched hairs) on the frond and the sporangial stalk, and the lack of scales and true hairs on the rhizome (Table 3). Becari-Viana and Schwartsburd [41] carried out a detailed study of rhizome morphology and anatomy, and showed that the main rhizome type of Dennstaedtiaceae is long-creeping with alternate phylloaxy and a solenostele, and while *Blotiella* and *Monachosorum* have ascending to short-creeping rhizomes with radial phylloaxy and a dictyostele. Becari-Viana and Schwartsburd [41] proposed that the ancestor of Dennstaedtiaceae had a short-creeping to ascending

| Table 3 | Comparison of major morphological characters of three subfamilies of Dennstaedtiaceae |
|---------|------------------------------------------|
| **Hypolepidoideae** | **Dennstaedtiodeae** | **Monachosoroideae** |
| **Habitat** | edge-colonizer or thicket-forming of open habitats | edge-colonizer of open habitats | shade-tolerant |
| **Rhizomes** | long-creeping (ascending in *Blotiella*) | long-creeping | ascending to short-creeping |
| **Type of stel** | Solenostele (dictyostele in *Blotiella*) | solenostele | dictyostele |
| **Hairs** | multicellular hairs (bristles in *Paesia* and *Hypolepis*) on rhizome and fronds | multicellular hairs on rhizome and fronds | multicellular hairs on fronds, monachosorioid hairs (minute, few-celled, club-like, glandular hairs) on frond and sporangial stalk |
| **Phyllotaxy of fronds** | alternate (radial in *Blotiella*) | alternate | radial |
| **Vascular bundles** | one (*Paesia*) or several (*Pteridium*) vascular bundles; Omega-shaped or dissected C-shape (*Pteridium*), | Omega-shaped or C-shaped | two hypocampus bundles |
| **Type of development of archegonia** | common Leptosporangiate-type | common Leptosporangiate-type | almost simultaneous development of archegonia in the midst of several antheridia |
| **Sori** | marginal or submarginal | marginal or submarginal | terminal |
| **Indusia** | one (adaxial, outer) or two (abaxial and adaxial) indusia | one (adaxial) or two (abaxial and adaxial) indusia | absent |
| **Spores** | monolete (trilete in *Pteridium*) | trilete | trilete |
| **Chromosome basic number (x)** | 24, 26, 28, 29 | 30, 31, 33, 34, 40, 42, 43, 46, 47 | 56 |
rhizome. The solenostele evolved on the main lineage of Dennstaedtiaceae, while the dictyosteole was primitive in Monachosorum (and was secondarily derived in Blotiella). All the morphological evidence indicated that Monachosorum occupies an isolated position, which also increases the difficulty in conclusively determining the position of Monachosorum.

Although the growing ecological conditions variously affect the prothalli of ferns, the salient morphological characteristics, such as the gross form and structure of the adult thallus, the nature of trichomes borne on the thallus, and the morphology of sex organs, the sequence of cell divisions at spore germination, the sequence of developmental stages, the type of development, are little altered [63]. The almost simultaneous development of archegonia in the midst of several antheridia in Monachosorum is a unique feature among the ferns [54], whereas that of Hypolepidoideae and Dennstaedtioideae is the common leptosporangiate-type.

Monachosorum maximowiczii (Baker) Hayata is sometimes regarded as the independent genus Ptilopteris Hance based on its 1-pinnate and lanceolate fronds. The sister relationship between this species and members of Monachosorum was first confirmed by the chloroplast phylogeny [64]. In addition, nuclear gapCp phylogeny by Ebihara et al. [65] showed that *M. maximowiczii* is a diploid parental species of *Monachosorum × flagellare* and *M. nipponicum*. In our analyses, *M. maximowiczii* and other members of Monachosorum began to diverge in the Miocene, and the latter was further differentiated in the Quaternary.

Monachosorum maximowiczii is the only known diploid species in the genus [65]. Hybridization occurs easily in mixed populations of the two parental species *M. maximowiczii × M. nipponicum* in Japan, and the hybrid taxon *Monachosorum × flagellare* (Maxim. ex Makino) Hayata is common and can produce irregularly shaped spores [65]. There are many common morphological characteristics between Ptilopteris and Monachosorum—terrestrial shade plants, erect or decumbent rhizomes, two hypocampus vascular bundles, thinly herbaceous leaves, monachosorioid hairs, sori with few (10 ~ 20) sporangia, exindusiate, and trilete spores. Considering the reticulate relationship [65], we prefer to place *M. maximowiczii* in Monachosorum rather than recognizing it as a separate genus Ptilopteris.

The origin and diversification of suborder Dennstaedtiinae

The currently defined Dennstaedtiaceae (suborder Dennstaedtiinae) are estimated to have originated during the Jurassic and began to diversify during the Early Cretaceous, which is earlier than estimation from previous studies [34, 52, 66]. There is no doubt that a robust phylogenetic framework is foundationally important for molecular dating. However, intra-familial and intergeneric phylogenetic relationships in Dennstaedtiaceae have not been resolved or only weakly supported in these previous studies mentioned above. The lack of resolution has inhibited phylogenetic dating of Dennstaedtiaceae. Due to differences in phylogenetic relationships, the same fossil may be assigned as a calibration of different nodes. The fossils of *Krameropteris* (100.5 Ma) were used to calibrate the crown of Monachosorum-Hypolepidoideae by Schneider et al. [52], while they were calibrated at the crown of Dennstaedtiaceae in the study of Schwartzburd et al. [34].

Different interpretations of the strata and different placements of fossils in molecular dating can result in substantially different estimates of divergence times. The Late Cretaceous fossil genus Microlepiopsis Serbet & Rothwell was ascribed to Dennstaedtiaceae [67] based on solenostelic rhizomes, with sclerenchymatous pith and cortex and relatively simple frond trace anatomy. Eberth and Braman [68] revised the stratigraphy for the Horseshoe Canyon Formation of Canada, and described it as about 72 Ma. Both Schuettpezl and Pryer [66] and Testo and Sundue [27] used *Microlepiopsis* fossil with 70.6 Ma to calibrate the crown of Dennstaedtiaceae, and got different molecular ages—the former estimated Dennstaedtiaceae to have originated at about 166 Ma, while the latter estimated as about 240 Ma. Schwartzburd et al. [34] used the same fossil of *Microlepiopsis* with 80 Ma to calibrate the crown of Dennstaedtiodeae-Hypolepidoideae (excluding Monachosorum), and Dennstaedtiaceae to have originated in the Early Cretaceous (135.7 Ma). The fossils of *Microlepiopsis* have not been incorporated in the present study with the consideration of their complicated stele types and anatomical heterogeneity of the vascular bundle (two U-shaped bundles that unite distally to form a W-shaped trace at the base of the stipe in *Microlepiopsis bramanii*; a C-shaped trace that diverges as a single bundle in *Microlepiopsis aulenbackii*) [67].

The BEAST analysis reveals a Jurassic origin of suborder Dennstaedtiinae in Asia, and the divergence of Monachosorum and other lineages of Dennstaedtiaceae occurred in the Early Cretaceous. The Late Jurassic-Early Cretaceous climatic conditions of higher rainfall and temperatures [69] favored the diversification of early-divergent shade-tolerant ancestors of suborder Dennstaedtiinae. With the increase of the continental arid belt and the decrease in global rainfall and temperature, the mid–high latitude fossil sites (e.g., Albert and Saskatchewan, Canada) became warmer during the mid-Cretaceous (95–70 Ma), which may have accelerated to the diversification of evolved with a change in habit: from
shade-tolerant to edge-colonizing plants thriving in a warm climate in higher-latitude areas in this period.

*Microlepiopsis* fossils were found in the Horseshoe Canyon coal formation in Alberta, Canada, where the formation is unusually dry, producing no formation water in most parts, and the average water hydrostatic pressure is less than 30% [70]. After the mass extinction at the Cretaceous-Paleogene (K-Pg) boundary (66.0 Ma), Dennstaedtiaceae might have begun the second diversification in the Paleogene-Eocene to the Early Eocene. The second diversification of Dennstaedtiaceae occurred mainly during the Paleogene, and the divergence of Dennstaedtioidae and Hypolepidoideae was estimated to be at 60.88 and 45.5 Ma, respectively. The ancestors of Dennstaedtioidae-Hypolepidoideae may have become edge-colonizers with a long-creeping rhizome, and occupied new niches [34], which is very important to a disturbance-succession ecological regime associated with a Late Cretaceous wildfire regime, which possibly was responsible for its reorganization and higher diversification of Dennstaedtiaceae after the K-Pg event.

Some fern fossils, e.g., *Dennstaedtia americana* and *Dennstaedtia sorinarginata* of Dennstaedtiaceae, *Onoclea hesperia* of Onocleaceae, *Woodwardia gravida* of Blechnaceae, were found in the early Paleocene (Danian) Ravenscrag Formation (Canada and USA; [71]). The Ravenscrag flora was considered to have been in the period when terrestrial ecosystems were recovering from a major shock of the K-Pg extinction [71]. Paleo-climatic and paleo-ecological studies of the Ravenscrag Butte flora indicated that the early Paleocene climate for southwestern Saskatchewan was warm, humid, with mild winters, and wet with some indication of precipitation seasonality, but without a pronounced dry season [71, 72]. The reconstructed climate of the Ravenscrag Butte flora is similar to modern coastal climates that exist near inland seas. The Ravenscrag Butte flora is most similar to contemporaneous fossil macrofloras from throughout western and northern North America, suggesting a potential consequence of vegetation reorganization after the K-Pg event [72]. There are also more recent fossils, from the Eocene (*Dennstaedtiopsis*, Canada and USA; [73]), Neogene, and even Quaternary [74, 75, 76]. All extant genera (and lineages) in Dennstaedtioidae and Hypolepidoideae were inferred to begin to diversify after the Late Oligocene.

**Elevating Monachosorum to the family rank**

In the phylogenetic classification, monophyly is the primary principle and maximizing stability is the secondary principle for the recognition of taxa [3, 77]. A good example of this balance in the classification of ferns is the validation of Didymochlaenaceae. The phylogenetic analyses have resolved *Didymochlaena* Desv. as sister to the rest of the eupolypods I clade (the suborder Polypodiineae) [33, 45, 78] or sister to a clade comprising Dryopteridaceae and the DANLOPPT Clade [18]. Zhang and Zhang [33] evaluated the morphological characteristics that include sori, indusia, and spores of *Didymochlaena*, and found that *Didymochlaena* is distinct from the rest of eupolypods I in having elliptic-oblong sori, elongate indusia, and monolette spores with tuberculate and echinate on perispore. Zhang and Zhang [33] suggested the recognition of the family Didymochlaenaceae and validly described the family based on the principle of maximizing phylogenetic information—emphasizing distinct, deeply isolated lineages.

Differentiation time and diversity are other possible principles or criteria for taxon recognition in phylogenetic classifications. The divergence times of most families in polypods are estimated during the early Cretaceous (and even the late Jurassic) although a few families of eupolypods I clade seem to have differentiated slightly later and diverged from their closest sister group before the Paleogene [18]. *Monachosorum* (Monachosoroidae sensu Schwartzburd et al.) is a deeply isolated lineage from any other extant group of Dennstaedtiaceae. The divergence time estimates support the most recent common ancestor of Monachosoraceae and its closest relative dating back to the Early Cretaceous, which is consistent with the origin time of most families in polypod families.

*Monachosorum* is strongly supported as monophyletic with full support in our phylogenetic study. Based on the principle of monophyly for the recognition of taxa in phylogenetic classification, and the differentiation in genetic and morphological characteristics between Monachosoraceae and Dennstaedtiaceae, we argue for recognizing Monachosoraceae as a family in Polypodiales suborder Dennstaedtiinae. The remaining Dennstaedtiaceae become more homogeneous and easier to define (edge-colonizing or thicket-forming habit; long-creeping solenostelic rhizomes clothed with true hairs; marginal or submarginal sori; one or two indusia) (Table 3) when *Monachosorum* is segregated. The deep evolutionary divergence from its closest relatives and the shady habitat of *Monachosorum* also support that the genus belongs to a distinct family from Dennstaedtiaceae.

**Methods**

**Taxon sampling, sequencing, and assembly**

A total of 57 plastomes from suborder Dennstaedtiinae were sampled, consisting of 44 newly generated plastomes and the remaining 13 downloaded from GenBank (Table 4). Major lineages of Dennstaedtiaceae were represented by at least three species each (i.e., *Blotiella*, *Dennstaedtia*, *Histiopteris*, *Hiya*, *Hypolepis*, *Microlepis*,...
### Table 4  List of plastomes used in this study

| Family name                  | Taxon                                                                 | GenBank accession No |
|-----------------------------|-----------------------------------------------------------------------|----------------------|
| Dennstaedtiaceae            | Blotiella coursii (Tardieu) Rakotondr. ex J.P. Roux                   | MT130655             |
| Dennstaedtiaceae            | Blotiella lindeniana (Hook.) R.M. Tryon                              | OP081137             |
| Dennstaedtiaceae            | Blotiella madagascariensis (Hook.) R.M. Tryon                        | OP081099             |
| Dennstaedtiaceae            | Dennstaedthia ampla (Baker) Bedd.                                    | OP081098             |
| Dennstaedtiaceae            | Dennstaedthia appendiculata (Wall. ex Hook.) J. Sm.                   | OP081114             |
| Dennstaedtiaceae            | Dennstaedthia glabrata (Cesati) C.Chr.                               | OP081102             |
| Dennstaedtiaceae            | Dennstaedthia globulifera (Poir.) Hieron.                            | OP081133             |
| Dennstaedtiaceae            | Dennstaedthia pilosella (Hook.) Ching                                | MT130587             |
| Dennstaedtiaceae            | Dennstaedthia punctilobula (Michx.) T. Moore                         | OP081100             |
| Dennstaedtiaceae            | Dennstaedthia samoensis T. Moore                                     | OP081119             |
| Dennstaedtiaceae            | Dennstaedthia scabra (Wall. ex Hook.) T. Moore                       | MT130669             |
| Dennstaedtiaceae            | Dennstaedthia scandens (Blume) T. Moore                              | OP081103             |
| Dennstaedtiaceae            | Dennstaedthia tripinnatifida Copel.                                   | OP081120             |
| Dennstaedtiaceae            | Dennstaedthia wilfordii (T. Moore) Christ                            | OP081117             |
| Dennstaedtiaceae            | Histerotes herbacea Copel.                                            | OP081129             |
| Dennstaedtiaceae            | Histerotes incisa (Thunberg) J. Smith                                | NC040220             |
| Dennstaedtiaceae            | Histerotes incisa (Thunberg) J. Smith                                | MT130622             |
| Dennstaedtiaceae            | Histerotes stipulacea Copel.                                          | OP081108             |
| Dennstaedtiaceae            | Hiya brooksiae (Alderw.) H. Shang                                    | OP081135             |
| Dennstaedtiaceae            | Hiya brooksiae (Alderw.) H. Shang                                    | OP081126             |
| Dennstaedtiaceae            | Hypolepis alpina (Blume) Hook.                                       | OP081110             |
| Dennstaedtiaceae            | Hypolepis goetzei Reimers                                            | OP081125             |
| Dennstaedtiaceae            | Hypolepis punctata (Thunb) Mett. ex Kuhn                            | OP081123             |
| Dennstaedtiaceae            | Hypolepis punctata (Thunb) Mett. ex Kuhn                            | MT130616             |
| Dennstaedtiaceae            | Hypolepis repens (L.) C. Presl                                      | OP081118             |
| Dennstaedtiaceae            | Leptolepia novae-zelandiae (Colenso) Mett. ex Diels                   | OP081136             |
| Dennstaedtiaceae            | Microlepia herbacea Ching & C.Chr. ex C.Chr. & Tardieu               | OP081107             |
| Dennstaedtiaceae            | Microlepia hiasyana (Hook.) C. Presl                                | MT130653             |
| Dennstaedtiaceae            | Microlepia marginata (Panzer) C. Christensen                         | MT130649             |
| Dennstaedtiaceae            | Microlepia obtusiloba Hayata                                        | MT130570             |
| Dennstaedtiaceae            | Microlepia platyphylla (D. Don) J. Smith                             | MT130685             |
| Dennstaedtiaceae            | Microlepia speluncae (L.) T. Moore                                  | OP081121             |
| Dennstaedtiaceae            | Microlepia speluncae (L.) T. Moore                                  | OP081101             |
| Dennstaedtiaceae            | Microlepia speluncae (L.) T. Moore                                  | OP081106             |
| Dennstaedtiaceae            | Microlepia tenera Christ                                             | OP081122             |
| Dennstaedtiaceae            | Microlepia trapeziformis (Roxb.) Kuhn                               | OP081128             |
| Dennstaedtiaceae            | Microlepia yaoshanica Ching                                          | OP081097             |
| Dennstaedtiaceae            | Monachosorum arakii Tagawa                                           | OP081116             |
| Dennstaedtiaceae            | Monachosorum flagellare (Maxim. ex Makino) Hayata                    | OP081127             |
| Dennstaedtiaceae            | Monachosorum henryi Christ                                           | MT130593             |
| Dennstaedtiaceae            | Monachosorum henryi Christ                                           | OP081132             |
| Dennstaedtiaceae            | Monachosorum henryi Christ                                           | OP081138             |
| Dennstaedtiaceae            | Monachosorum henryi Christ                                           | OP081105             |
| Dennstaedtiaceae            | Monachosorum henryi Christ                                           | OP081112             |
| Dennstaedtiaceae            | Monachosorum maximowiczii (Baker) Hayata                             | OP081109             |
| Dennstaedtiaceae            | Monachosorum nipponicum Makino                                      | OP081111             |
| Dennstaedtiaceae            | Monachosorum nipponicum Makino                                      | OP081131             |
| Dennstaedtiaceae            | Monachosorum subdigitatum (Blume) Kuhn                              | OP081130             |
Monachosorum, Paesia, and Pteridium), and attention was paid to Dennstaedtia s.l. and Monachosorum to re-evaluate the phylogenetic status of these two genera based on plastomes. Additional 23 plastomes from Polypodiales suborder Pteridineae (10 taxa), suborder Aspleniineae (4 taxa), suborder Polypodiineae (4 taxa), suborder Lindsaeineae (4 taxa), and suborder Saccolomatineae (1 taxa) were employed as outgroups in the phylogenetic and dating analyses (Table 4).

Leaf material was collected from living plants in the field, and herbarium specimens (CSH, KUN, MO, TAIF, TNS, and WELT). DNA extraction, library preparation, and Illumina sequencing were conducted following the protocol of plastome sequencing from herbarium specimens [79]. The libraries were then sequenced on Illumina Hiseq 2500 or X-Ten sequencing system (Illumina Inc.) to generate 150 bp paired-end reads, with ca. 1–3 Gb raw data for each sample. DNA extraction and library preparation were conducted at the Molecular Biology Experiment Center, Germplasm Bank of Wild Species, Kunming Institute of Botany (CAS), and Illumina sequencing was conducted at BGI Genomics Co., Ltd (Shenzhen, China). De novo assemblies were constructed with GetOrganelle toolkits [80]. Reference-guided connection and gene

### Table 4 (continued)

| Family name        | Taxon                                      | GenBank accession No |
|--------------------|--------------------------------------------|----------------------|
| Dennstaedtiaceae   | Monachosorum subdigitatum (Blume) Kuhn     | OP081124             |
| Dennstaedtiaceae   | Oenotrichia maxima (E. Fourn.) Copel.      | OP081113             |
| Dennstaedtiaceae   | Paesia elmeri Copel.                       | OP081115             |
| Dennstaedtiaceae   | Paesia elmeri Copel.                       | OP081104             |
| Dennstaedtiaceae   | Paesia elmeri Copel.                       | OP081134             |
| Dennstaedtiaceae   | Paesia glandulosa (Sw.) Kuhn               | OP081139             |
| Dennstaedtiaceae   | Pteridium aquilinum (L.) Kuhn              | NC014348             |
| Dennstaedtiaceae   | Pteridium caudatum (L.) Maxon              | OP081096             |
| Dennstaedtiaceae   | Pteridium revolutum (Blume) Nakai          | MT130625             |
| OUTGROUPS          |                                            |                      |
| Cystodiaceae       | Cystodium sorbilium (Sm.) J. Sm.           | MT130630             |
| Cystopteridaceae   | Acystopteris japonica (Luerss.) Nakai      | MT130696             |
| Diplaziopsidaceae  | Diplaziopsis brunoniaria (Wall.) W.M. Chu  | MT130567             |
| Dryopteridaceae    | Dryopteris crassirhizoma Nakai             | MT130689             |
| Hypodematiaceae    | Hypodematum crenatum (Forssk.) Kuhn & Decken | MT130540         |
| Lindsaeaceae       | Lindsaea culttata (Willd.) Sw.             | MT130672             |
| Lindsaeaceae       | Osmolindsaea odorata (Rosxb.) Lehtonen & Christenh | MT130576 |
| Lonchitidaceae     | Lonchitis hirsute L.                       | MT130654             |
| Nephrolepidaceae   | Nephrolepis cordifolia (L.) C. Presl       | MT130673             |
| Oleandraceae       | Oleandra wallichii (Hook) C. Presl         | MT130650             |
| Pteridaceae        | Acrostichum aureum L.                      | MT130571             |
| Pteridaceae        | Adiantum hispidulum Sw.                    | LT615217             |
| Pteridaceae        | Adiantum shastense Huet & A.R. Sm.         | NC037478             |
| Pteridaceae        | Calciphilopteris ludens (Wall. ex Hook.) Yesilyurt & H. Schneid. | MT130590     |
| Pteridaceae        | Ceratopteris pteridoides (Hook.) Hieron.   | MT130583             |
| Pteridaceae        | Chelantes micropteris Sw.                  | MH173078             |
| Pteridaceae        | Cryptogramma acrostichoides R. Br.         | MH173081             |
| Pteridaceae        | Uleia cordifolia Lag.                      | MH173088             |
| Pteridaceae        | Onychium japonicum var. lucidum (D. Don) Christ | MT130597      |
| Pteridaceae        | Pteris vittata L.                          | MT130668             |
| Saccolomataceae    | Saccoloma elegans Kaulf.                   | MT130580             |
| Thelypteridaceae   | Macrothelypteris torresiana (Gaud.) Ching  | MT130591             |
| Woodsiaceae        | Woodsia manchuriensis Hook.                | MT130698             |
Data matrices and phylogenetic analyses

We utilized different data sets, including the complete plastid genome (plastome) sequences (pt) and the coding region of the 86 protein-coding genes (CDS) to conduct the phylogenetic reconstruction. All sampled plastomes were aligned using Mafft [83], and the unreliably aligned regions were filtered using Gblocks v0.91b [84] with default parameters except that all gap positions were allowed. The Pt data set (the main data set) has an aligned length of 124,738 bp and an average GC content of 42.7%. We also constructed a sub-dataset consisting of all 86 coding sequences (CDS data set), which has an aligned length of 72,828 bp and an average GC content of 43% (Table 2).

We performed Maximum Likelihood (ML) analyses and Bayesian Inferences (BI) on the main data set (pt) and the CDS data set. The heterogeneous GHOST [85] model GTR+H4 were used in ML analyses of the main data set, and the gene-partitioned model estimated by PartitionFinder2 [86] were used in ML analyses of the CDS data set. ML analyses were conducted using IQ-tree 1.6.12 [87] with support values estimated by 10,000 ultrafast bootstrap replicates. Bayesian inferences were conducted using MrBayes 3.2.6 [88], and with the GTR+I+G substitution model. Five-million-generation iterations with trees being sampled per 1,000 generations, two runs with four chains were performed in parallel. The MCMC (Markov chain Monte Carlo) output was examined to check convergence and to ensure that all the ESS (effective sample sizes) values were above 500.

Divergence time estimation

We estimated the divergence times of Polypodiales suborder Dennstaedtiineae based on the main pt data set. Bayesian estimations of divergence times were conducted with BEAST v.2.6.6 [89], using an un-partitioned GTR+I+G nucleotide substitution model, birth-death tree prior and lognormal uncorrelated relaxed clock model, and the phylogeny from our ML analysis as the starting tree, and four fossil calibrations (two from Dennstaedtiaceae and two from Pteridaceae) were adopted in the dating analyses.

The oldest fossils of Dennstaedtiaceae were found in the Lower Cretaceous of Kachin, Myanmar (100.5 Ma, Late Albian, early Late Cretaceous [52]). The fossils of Krameropteris resinitatus H. Schneid., A. R. Schmidt & Heinrichs were unequivocally assigned to the early diverging Dennstaedtiaceae based on its polypl sporangia with trilete spores, exindusiate sori, and free branched veins. The fossils of Krameropteris (100.5 Ma) were used to calibrate the crown of Dennstaedtiaceae (the split between Monachosorum and the aggregate of Dennstaedtiaceae-Hypolepidioideae). The early Eocene fossils of Dennstaedia christophelii (48.9 Ma) [75] were used to calibrate the crown of Dennstaedtiaceae (Dennstaedtia s.l.) based on its cuplike indusial, marginal and round sori, a vertical and interrupted annulus, and once-pinnate-pinnatifid to bipinnate lamina. The combination of characteristics of fossil Heinrichsia cheilanthis L. Regalado, A.R. Schmidt, M. Krings & H. Schneid. (100.5 Ma, Kachin, Myanmar; [90]), tetrahedral-globose trilete spores and marginal sori protected by reflexed pseudo-indusia, is strong support of the fossil’s affinities with Pteridaceae. This fossil was adopted to calibrate the crown of Pteridaceae. The Maastrichtian Acrostichum fossil (66 Ma, [91]) was used to constrain the divergence between Parkerioideae (including Acrostichum and Ceratopteris) and Pteridoideae. All fossil ages were used as an offset in the lognormal priors (mean: 3.0, SD: 1.0). Furthermore, the estimated divergence times of the Polypodiales crown (95% HPD: 191.62–219.87 Ma, [18]) were employed as the minimum–maximum age constraints (uniform prior) of secondary molecular root calibrations. One run of 10 billion generations was conducted, sampling every 2,000 generations. Convergence was attained within two to five billion generations, and the ESS values for all parameters were over 200. We removed the first five billion generations as burn-in, and used the remaining ca. 250 thousand trees to generate the maximum clade credibility tree (MCC) by TreeAnnotator with a posterior probability limit of 0.5 and median node heights.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12870-022-03886-1.

Acknowledgements
We thank the Missouri Botanical Garden Herbarium for providing samples and the Germplasm Bank of Wild Species at the Kuming Institute of Botany for laboratory assistance and computation. We are grateful to Cheng-Wei Chen (National Tsing Hua University) and Professor Li-Bing Zhang (Missouri Botanical Garden) for sample collection, Professor Yue-Hong Yan (the National Orchid Conservation Center of China) for suggestions on species identification, and Professor Jun Wen (Smithsonian Institution) for comments on the earlier versions of the manuscript.

Additional file 1. Information of Voucher specimens, the characteristics, and GenBank accession numbers of newly generated plastomes in this study.

Additional file 2. Maximum likelihood (ML) phylogeny of Polypodiales suborder Dennstaedtiineae based on CDS data set.

Additional file 3. Bayesian inference (BI) phylogeny of Polypodiales suborder Dennstaedtiineae based on CDS data set.
Authors' contributions
Jin-Mei Lu: Conceptualization, Methodology, Validation, Investigation, Writing, Visualization, Supervision, Project administration, Funding acquisition. Xing-Yu Du: Methodology, Software, Validation, Formal analysis, Data curation, Writing – review & editing. Li-Yang Kuo: Investigation, Writing – review & editing. Xi-Ying Li: Investigation, Writing – review & editing. Leon R. Perrie: Investigation, Writing – review & editing. Zheng-Yu Zuo: Investigation, Writing – review & editing. Hui Shang: Investigation, Species identification, Writing – review & editing. Yi-Han Chang: Investigation. De-Zhu Li: Writing – review & editing, Funding acquisition. All the authors read and approved the final version of the manuscript.

Funding
The study was supported by the National Natural Science Foundation of China (31970232) and the Large-scale Scientific Facilities of the Chinese Academy of Sciences (2017-LSF-GBDW5-02).

Availability of data and materials
The complete chloroplast genomes generated in this study were submitted to the NCBI database (https://www.ncbi.nlm.nih.gov/) with GenBank accession numbers from OP081096 to OP081139. All other data and material analyzed in the current study are included in the manuscript and the supplementary information files.

Declarations

Ethics approval and consent to participate
We complied with all relevant institutional, national and international ethics approval and consent to participate.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest.

Author details
1. Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences.

Received: 10 August 2022 Accepted: 19 October 2022
Published online: 02 November 2022

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