Research Article

A contribution to Gymnosphaera (Cyatheaceae) in mainland Asia: Two new species, reinstatement of Cyathea bonii, and their phylogenetic positions

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Abstract Gymnosphaera represents a minor lineage within the scaly tree-fern family Cyatheaceae. Tropical and subtropical mainland Asia is a main distribution area of Gymnosphaera. However, the species diversity of Gymnosphaera is currently incompletely known in mainland Asia due to lacking critical revision. Here we present new findings of species diversity and their relationships to mainland Asian Gymnosphaera based on field surveys, the examination of herbarium collections, and phylogenetic analyses of sequences of multiple chloroplast and nuclear regions. Two new species, G. saxicola from southwestern Yunnan and G. bachmaensis from central Vietnam, are established. Traditionally recognized G. podophylla is revealed to be a complex, from which G. bonii is reinstated as a distinct species. Our phylogenetic analyses identified four clades within Gymnosphaera in mainland Asia: G. denticulata clade, G. gigantea clade, G. podophylla clade, and G. sallietii clade. The newly discovered G. bachmaensis, which is characterized by the spathulate frond, was positioned in the G. podophylla clade. The mountainous region from south-central Vietnam northwards to western Yunnan is a diverse center of Gymnosphaera and more species of this group are probably to be discovered there.

Key words: chloroplast sequence, morphology, nuclear sequence, phylogeny, taxonomy, tree fern.

1 Introduction

The genus Gymnosphaera Blume represents one of four monophyletic lineages in the scaly tree-fern family Cyatheaceae (Korall et al., 2007; Korall & Pryer, 2014; Sosa et al., 2016; Dong & Zuo, 2018; Loiseau et al., 2020). It is a minor group of scaly tree ferns compared with three other genera (Alsophila R.Br., Cyathea Sm., and Sphaeropteris Bernh.). According to PPG I (2016), there are approximately 230 species in Alsophila s. str. (excluding Gymnosphaera group), 265 in Cyathea, and 103 in Sphaeropteris Bernh. There are only 43 taxa (39 species, one subspecies, and three varieties) currently known in Gymnosphaera, most of them growing in montane forest in the paleotropics from eastern Africa and Madagascar eastwards through SE Asia to New Guinea and the Pacific Islands, with only one species and one subspecies in the neotropics (Dong & Zuo, 2018). The Gymnosphaera group was frequently treated as a section or a subgenus under the comprehensive Cyathea or under Alsophila (e.g., Tindale, 1956; Holttum, 1963, 1965; Xia, 1989; Zhang, 2004; Zhang & Nishida, 2013). Morphologically, the bulk of Gymnosphaera is featured by the castaneous or blackish frond axes, the absence of hairs on abaxial surface of costae, and the exindusiate sori (Holttum, 1963). Additionally, the dimorphism of lamina and the skeletonized pinnae near bases of stipes frequently occur in this group (Tardieu, 1951; Tindale, 1956; Holttum, 1963). All species of Gymnosphaera can be clearly distinguished by the marginate stipe scales
from *Sphaeropteris*, by the apex-setiform scales from *Cyathea* (as shown in Korall et al., 2007), and by 64 spores per sporangium from *Alsophila*, which has 16 spores per sporangium.

Tropical and subtropical mainland Asia is a main distribution area of *Gymnosphaera*, although in this area the species diversity of scaly tree ferns as a whole is considerably low. According to Holttum (1963, 1965), there are as many as 191 species of scaly tree ferns in Malesia but only 25 in the rest of Asia. However, of the 25 species recorded by Holttum (1965), 36% species (nine) belong to the *Gymnosphaera* group. These *Gymnosphaera* species are generally restricted to mainland Asia ranging from northeastern India and nearby regions southwards to Indochina and southeasterwards to South China, with the exception of *C. ogurae* (Hayata Domin (= *G. ogurae* Hayata) Tagawa) which is endemic to Bonin Islands and three species extending to nearby regions (i.e., *G. gigantea* [Wall. ex Hook.] S.Y. Dong to Peninsular Malaysia and Java, and *G. metteniana* [Hance] Tagawa and *G. podophylla* [Hook.] Copel. to Taiwan and Ryuku islands) (Holttum, 1963, 1965). Since then two additional species have been reported from mainland Asia. One is *G. austroyunnanensis* (S.G. Lu) S.G. Lu & C.X. Li, first discovered in southern Yunnan (Lu, 1998) and later found in northern Vietnam (Zhang, 2015). The other is *Alsophila gammiei* R.D. Dixit (= *Gymnosphaera gammiei*).

![Fig. 1. Maximum likelihood tree of *Gymnosphaera* based on combined chloroplast regions (*rbcL*, *rbcL*-accD, *rbcL*-atpB, *trnG*-trnR, and *trnL-trnF*) (A), associated with two states of veins (B) and three states of sori arrangement (C). The sample names of two new species and *G. bonii*, the latter has long been mistaken for *G. podophylla*, are highlighted with different colors in the tree. (The three photographs showing sori were from Dong 5183 [ *G. gigantea* ], Dong 4216 [ *G. metteniana* ], and Dong 5017 [ *G. saxicola* ], respectively from top to bottom; and the two drawings of venation were based on Zeng 3470 [ *G. podophylla* ] and Dong 4987 [ *G. bonii* ].)
[R.D. Dixit] S.Y. Dong, **comb. nov.** described from Sikkim and nearby regions (Dixit, 1992). To date, these 10 are all known species of *Gymnosphaera* in mainland Asia, accounting for one-quarter of extant species of this group in the world. They are *G. andersonii* (J. Scott ex Bedd.) Ching & S. K. Wu, *G. austroyunnanensis*, *G. denticulata* (Baker) Copel., *G. gammiei*, *G. gigantea* (Baker) S.R. Ghosh, *G. khasyana* (T. Moore ex Kuhn) Ching, *G. metteniana*, *G. podophylla*, and *G. salletii* (Hook.) S.Y. Dong. All these were recorded under *Cyathea* or *Alsophila* instead of *Gymnosphaera* in modern checklists or floras of various regions within mainland Asia (e.g., Tagawa & Iwatsuki, 1979; Zhang, 2004; J. Syst. Evol. 60(2): 433–444, 2022).
Phylogenetic analyses of either chloroplast regions (Dong & Zuo, 2018) or multiple nuclear loci (Dong et al., 2019) strongly supported the Gymnosphaera from mainland Asia to be monophyletic in terms of current samplings which covered 90% species diversity of Gymnosphaera in mainland Asia but with Malesian species underrepresented. All known species of Gymnosphaera except G. gammiei from mainland Asia were sampled and were well resolved into four clades in the tree based on the nuclear loci (Dong et al., 2019: Fig. 1). Gymnosphaera metteniana and G. podophylla were revealed to be not monophyletic species. Of the four clades currently identified in scaly tree ferns, Gymnosphaera was estimated to have its crown group originated at around the Paleocene-Eocene boundary (57 Ma) (Sosa et al., 2016) to Oligocene (27.58 Ma) (Ramirez-Barahona et al., 2016). Gymnosphaera, as well as Alsophila, were thought to have invaded Madagascar during the Miocene (ca. 23–5 Ma) and subsequently were subjected to a rapid diversification of species, which resulted for high endemism of scaly tree ferns in Madagascar (Jansen et al., 2008). In mainland Asia there are also high proportion (nearly 70%) of endemic species in Gymnosphaera, which were estimated to have originated in the middle Miocene period (ca. 11.8 Ma) (Dong et al., 2019).

With further surveys on scaly tree ferns in mainland Asia, we discovered surprisingly new findings on Gymnosphaera from mainland Asia. The species G. henryi, which had long been mistaken for or subsumed under G. gigantea (e.g., Holtum, 1965; Xia, 1989; Zhang, 2004; Zhang & Nishida, 2013), was clarified to be a distinct species (Dong et al., 2020). More new findings are reported here, which include the re-circumscribed G. podophylla, the discovery of two special new species, and updated phylogenetics of Gymnosphaera in mainland Asia.

2 Material and Methods

2.1 Sampling and morphology study

We conducted extensive morphological observations of scaly tree ferns in the field of China, Bangladesh, and Vietnam since 2015; during these surveys we collected DNA samples with voucher specimens of a total of 212 populations. A complete set of our collections was deposited in the Herbarium of the South China Botanical Garden, Chinese Academy of Sciences (IBSC); those from Vietnam were, with duplicates, deposited in the Vietnam University of Science (HNU). The first author (Dong) examined specimens of all scaly tree ferns from mainland Asia in the herbaria BO, DACB, HN, HNU, IBSC, and TAIF. High-quality images of nomenclature types and those of hundreds of specimens from mainland Asia preserved in other herbaria were also checked by visiting online databases such as plants.jstor.org, science.mnhn.fr, apps.kew.org, collections.nmnh.si.edu, bioportal.naturalis.nl.

2.2 Molecular analyses

All known species of Gymnosphaera in mainland Asia were sampled in this study. We assembled two matrices of sequences for analysis (http://doi.org/10.5061/dryad.pg4f4qmg); one included combining five chloroplast regions (rbcl, rbcL-accD, rbcL-atpB, trnG-trnR, and trnL-trnF) of 54 accessions (Doc. S1) and the other was composed of combining 12 single-copy nuclear loci of 44 accessions (Doc. S2). In the chloroplast matrix, the ingroup (Gymnosphaera) included accessions of eight taxa from Africa-Madagascar which were available in GenBank, one from tropical America (G. salvinii [Hook.] S.Y. Dong), one from Borneo (G. ramispina [Hook.] Copel.), and 14 species from mainland Asia; the nuclear matrix consisted only of Asian accessions. Of these accessions, some were included in the study of Dong & Zuo (2018) to construct plastid-based tree and most were included in Dong et al. (2019) for the analysis of nuclear loci. The five plastid regions of 44 samples and the 12 nuclear loci of 11 samples are newly generated and analyzed in this study (Docs. S1, S2).

To obtain sequences of each target DNA region, firstly we used a modified CTAB method (Doyle & Doyle, 1987) to extract the genomic DNA from silica-dried leaves. For DNA amplification, the same primers used in previous studies (Dong & Zuo, 2018; Dong et al., 2019) were used here in polymerase chain reactions (PCR). All plastid regions and single-copy nuclear loci were amplified separately following standard protocols for PCR. The PCR products were sequenced using the BigDye Terminator Cycle Sequencing kit in accordance with the instructions of the manufacturer (Applied Biosystems, Foster City, CA, USA) on an ABI 3730XL automated sequencer.

We assembled and aligned the newly obtained sequences and those from GenBank using MAFFT (Katoh et al., 2005), then manually corrected the sequences and subsequently concatenated sequences of all five plastid regions into a matrix and those of all 12 nuclear loci into a separate matrix in BioEdit version 7.2.0 (Hall, 1999). We analyzed the two matrices independently using Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP). The software jModeltest2 (Posada, 2008) was used to determine the best-fitting substitution models and the results suggested GTR + G + I to be the best-fitting model for both datasets. BI analyses were conducted using MrBayes 3.2.6 software (Ronquist et al., 2012), using 10 million generations with one tree sampled every 1000 generations; four runs with four chains were discarded as burn-in. ML analyses were conducted using RAxML 7.2.8 (Stamatakis, 2006). A thorough tree search for the best ML tree was performed. ML Bootstrap analyses were performed with 1000 replications; bipartition information from the bootstrap trees was drawn on the best ML tree. The MP analyses were implemented in PAUP* version 4.0d00 (Swofford, 2002), with all characteristics weighted equally and gaps as missing data. In total 1000 heuristic replicated searches were carried out using random stepwise addition with branch swapping by tree bisection-reconnection, saving 100 trees per replicate. MP Bootstrap support for nodes was estimated with 1000 heuristic replicates and tree bisection-reconnection branch swapping.

3 Results

3.1 Morphological observations

Of the species of Gymnosphaera present in mainland Asia, none has the characteristic skeletonized pinnae which frequently occur in this group from all other phytogeographical regions (the neotropics, Africa and Madagascar,
Malesia, and Pacific Islands). The dimorphism of lamina, which also frequently presents in Gymnosphaera in other regions, occurs only in G. austroyunnanensis in our region. The morphological diversity of Gymnosphaera species from mainland Asia lies mainly in the variation of the following characteristics: arborescent habit, stipe scales, lamina shape, pinna-stalk, pinnule dissection, indument on abaxial surface of costae, venation, and sori arrangement. Here we just focused on the differences of the species that are intended to be newly established (G. bachmaensis and G. saxicola) or be reinstated (G. bonii) from their affinities.

At first glance at Gymnosphaera saxicola in the field of southwestern Yunnan, we were surprised about its saxiculous habitus, in which habitat no other scaly tree fern from mainland Asia was known to us or had been reported before. The individuals of the first population and subsequent three populations all grew on wet cliffs by small streams or at mouths of rocky caves in forests. In general morphology, G. saxicola looks somewhat like G. khasyana and G. denticulata; these three species share some characteristics such as two-pinnate-pinnatifid fronds with ultimate segments being dentate throughout the margin, stipes lacking two-rowed, regularly outspreading scales, and bullate scales present on abaxial surface of costules and midribs of segments. However, these three species are clearly different in the arborescent habit (the presence and height of trunks). Well developed plants of G. saxicola have short trunks that are up to 12 cm thick and 50 cm tall. In contrast, the trunk is entirely lacking in G. denticulata and is present, up to 4.5 m tall, in G. khasyana. The second obvious difference between them is the arrangement of sori on the ultimate segments (Fig. 1). In G. saxicola and G. khasyana the sori are in two close rows on the ultimate segments (Fig. 1C: state 2), whereas in two distinctly separate rows in G. denticulata (Fig. 1C: state 1). Furthermore, G. saxicola is differentiated by more sori borne on most veins of segments, leaving only two distal pairs of veins sterile (Fig. 3E); while in G. khasyana, the sori are restricted to the proximal half to two-third parts of segments, with distal three or four pairs of veins sterile.

Gymnosphaera bachmaensis was discovered in central Vietnam. It is apparently similar to G. podophylla in the characteristic imparipinnate fronds and the entire or subentire pinnules. However, G. bachmaensis is sharply different from the latter in the spathulate fronds, a unique shape of frond in scaly tree ferns, and the lower pinnae are gradually reduced toward the base of the stipe (Figs. 4B, 4C). A careful examination of the pinna-stalk and venation between them revealed that the traditional G. podophylla diagnosed by entire pinnules is complex and more differences emerge between G. bachmaensis and the true G. podophylla.

In G. bachmaensis, all pinnules are sessile and the venation is a kind of partial anastomosing (Fig. 1B: state 1), which form a striking comparison with the long-petiolulate pinnae and the wholly free venation (Fig. 1B: state 0) that frequently occur in G. podophylla. Previously, the venation of G. podophylla was considered variable, being wholly free or sometimes with the basal veins anastomosing, and the variation of petiolule’s length was seldom mentioned in the literature. Through a critical examination of herbarium specimens and our new collections, we found that the characteristics of sessile pinnae is associated with the partially anastomosing venation, while the distinctly petiolulate pinnae is always accompanied with the wholly free venation in broadly identified G. podophylla. Furthermore, the form of one-pinnate fronds is restricted to populations in which the fronds have sessile pinnae and partially anastomosing veins, and in the populations that have distinctly petiolulate pinnae and free veins the fronds are always two-pinnate. Therefore, the broadly defined G. podophylla are readily grouped into two entities: one is the true G. podophylla characterized by distinctly petiolulate pinnae and wholly free venation and the other is represented by Cyathea bonii Christ (1890) (=G. bonii) characterized by the sessile pinnae and partially anastomosing veins.

3.2 Molecular phylogeny
The concatenated alignment of five plastid regions counts 5355 base pairs (bp), of which 486 characters are variable and 356 are parsimony informative. The concatenated alignment of 12 nuclear loci is 8209 bp, of which 539 characters are variable and 393 are parsimony informative. The characteristics of the sequences of these two matrices are summarized in Table 1.

The analysis of the chloroplast sequences firstly split Gymnosphaera capensis (L. f.) S.Y. Dong subsp. capensis, which has long been thought to belong to Alsophila characterized by indusiate sori, from other Gymnosphaera species (Fig. 1A). All sampled species from Africa and Madagascar (G. schleienii [Reimers] S.Y. Dong to G. boivini [Mett. ex Ettingsh.] Tardei) formed a well supported clade, while all from Asia formed a separate one. The Asian clade was resolved to be sister to G. salvinii, a representative of Gymnosphaera from America. Within the Asian clade, two samples of G. ramsispersa from Borneo clustered together, forming a sister branch to all samples from mainland Asia. The samples of each species from mainland Asia clustered together except those of G. andersonii and G. henryi, which together with samples of G. gammiei and G. metteniana formed a polytomy in the tree. The new species G. saxicola was strongly supported to be sister to G. austroyunnanensis; these two together with G. salletii formed a well supported subclade in the Asian clade. The new species G. bachmaensis was resolved together with G. bonii, forming a sister relationship to G. podophylla with high supports. Gymnosphaera bonii is the species long subsumed under G. podophylla, which was proved monophyletic and was closely allied to G. bachmaensis.

The analysis of concatenated sequences of 12 nuclear loci was lacking samples from Africa and Madagascar, but recovered a much better resolved phylogeny. All samples of Gymnosphaera from mainland Asia, except those of an unidentified species (Tong 1978), were resolved into four highly supported subclades (Fig. 2). Subclade I includes five species (G. andersonii, G. gammiei, G. gigantea, G. henryi, and G. khasyana), of which G. khasyana was resolved as sister to the remaining species. Subclade II is composed of G. denticulata and G. metteniana, forming a sister relationship to Subclade I but with weak supports. Subclade III includes G. salletii, the new species G. saxicola, and G. austroyunnanensis; the latter two are sister to each other. Subclades IV comprises G. podophylla, the new species G. bachmaensis, and G. bonii; the latter two formed sister relationships.
Subclade IV (G. podophylla group) was resolved to be sister to the unidentified species (Tong 1078) with low supports.

4 Discussion

4.1 Four species groups of Gymnosphaera in mainland Asia and the position of G. denticulata group

The phylogeny of mainland Asian Gymnosphaera based on the nuclear loci in this study is completely consistent with the result documented in Dong et al. (2019), although the sampling is somewhat changed. In this study, two samples of G. gammiei and more samples of G. bonii and G. bachmaensis are added, and the total accessions in the clade of Gymnosphaera from mainland Asia are reduced from 49 to 40 compared with the sampling in Dong et al. (2019). The analyses of the nuclear loci identified four species groups in mainland Asia and a clade containing two samples of Tong 1078 whose identity remains uncertain. The four groups are: G. gigantea group, G. denticulata group, G. salletii group, and G. podophylla group. Each of these four is well supported to be monophyletic and generally supported by the morphological characteristics of sori arrangement. As shown in Fig. 2, the G. denticulata group has separate two-rowed sori, the G. salletii group has close two-rowed sori, while the G. gigantea group (except G. khasyana) and the G. podophylla group both have V-shaped sori.

There is a conflicting hypothesis on the position of G. khasyana as inferred from the analysis of the chloroplast or nuclear sequences. As the position of G. khasyana as sister to all other mainland Asian species was weakly supported in the plastid-based tree (Fig. 1), we prefer to take the result based on the nuclear sequences in which G. khasyana was strongly supported to be a member of the G. gigantea group (Fig. 2). In the nuclear loci-based tree, G. khasyana was positioned as
sister to the rest of the species in the *G. gigantea* group, but was apparently remotely allied to the rest, which was indicated by the long branches in the tree (Fig. 2). The relatively remote distance between *G. khasyana* and the rest of the *G. gigantea* group is also reflected by the difference in the arrangement of sori (V-shaped or in two close rows).

*Gymnosphaera metteniana* is suggested not to be a monophyletic species, as some samples of this species clustered with *G. gigantea* and others with *G. denticulata* in the nuclear loci-based tree (Dong et al., 2019). In this study, the same three samples of *G. metteniana* were placed in the *G. gigantea* clade in the plastid-based tree (Fig. 1A), but were resolved as sister to *G. denticulata* in nuclear loci-based tree (Fig. 2). Further analyses with large sampling proved *G. metteniana* to be a hybrid, deriving from *G. denticulata* and *G. henryi* (a member of *G. gigantea* group) (Wang et al., 2020). It

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**Fig. 4. Gymnosphaera bachmaensis** S.Y. Dong. A, One erect trunk with several stipes on its apex. B, A complete frond in type locality. C, Holotype (Dong 4880). D, Three scales of basal stipe. E, Basal part of a pinnule, showing V-shaped sori and partially anastomosing veins. (All from Dong 4880.)
appears that G. denticulata might have contributed more than G. henryi to the hybrid origin of G. metteniana, as G. metteniana has the same type of sori arrangement with G. denticulata (Fig. 2) and has nearly identical distribution ranges with the latter (mainly in tropical and subtropical East Asia).

The Gymnosphaera salletti group includes three relatively isolated species: G. austroynannanensis, G. saxicola, and G. salletti. There are considerable divergences between the three species in either the selected plastid or the nuclear sequences, as shown in Figs. 1 and 2 (each species clade presenting a long branch). They are also isolated in geographical distribution and are very different in some morphological characteristics such as frond dimorphism, plant habit, and stipe appendages. In contrast, the G. podophylla group comprises three very closely allied species: G. podophylla, G. bonii, and G. bachmaensis. More comments on these two groups are given in later paragraphs (Section 4.2).

The relationships between the four groups were not satisfactorily resolved in this study. In the nuclear loci-based tree, the sister relationships between the four groups were all weakly supported. A conflicting hypothesis exists on the position of the G. denticulata group, which was placed as sister to the G. podophylla group in the tree based on chloroplast regions (Fig. 1A), but as sister to the G. gigantea group in the nuclear loci-based tree (Fig. 2). Taking geographical distribution into account, the G. denticulata group appears to be more closely allied to the G. podophylla group, because these two groups have the similar pattern of distribution (both in warm area of East Asia to Indochina), while the G. gigantea group is mainly distributed in the East Himalayas to northern Indochina. For example, four out of total five species in the G. gigantea group (G. andersonii, G. gammiei, G. gigantea, and G. khashaya) were restricted to or mainly distributed in the East Himalayas (Fraser-Jenkins et al., 2017).

4.2 Molecular analyses provide strong support for the distinctness of Gymnosphaera saxicola and the separation of G. bonii from G. podophylla

The analyses of either the nuclear loci or chloroplast regions strongly supported the monophyly of Gymnosphaera saxicola, a newly discovered species in southwestern Yunnan. The samples representing three populations of G. saxicola were consistently resolved in a separate clade with high support. Gymnosphaera saxicola turned out to be a member of the G. salletti group and was resolved to be sister to G. austroynannanensis. In the tree based on either nuclear loci or chloroplast regions, each of the three terminal clades (G. saxicola, G. austroynannanensis, and G. salletti) has a long branch, indicating considerable divergence in DNA sequences between the three species. Our analyses of both nuclear and chloroplast regions, no matter which analysis approach (BI, ML, or MP) was adopted, clearly supported the distinctness of G. saxicola from all other species.

Gymnosphaera bonii was originally described by Christ (1890) under Cyathe, although he did not mention its similarity with G. podophylla nor the features of venation and pinna-stalk. It was reduced to synonymy with G. podophylla by Tardieu & Christensen (1939) and had not been mentioned in taxonomy since then. Our analyses of the nuclear and chloroplast sequences consistently resolved all samples of G. bonii and G. bachmaensis in a well supported clade, while the samples of G. podophylla were placed in a sister branch with high support values using all methods (BI, ML, and MP) (Figs. 1A and 2). This result lends strong support to separate G. bonii from G. podophylla as a different entity. The molecular evidence and the divergence of morphological characteristics clearly show that the G. podophylla documented by previous authors (e.g., Tardieu & Christensen, 1939; Holtum, 1965; Zhang, 2004; Lindsay & Middleton, 2012; Zhang & Nishida, 2013) is a complex, in which G. bonii was erroneously subsumed.

Gymnosphaera bachmaensis is certainly a member of the G. podophylla complex, as indicated by the analyses of both nuclear and chloroplast sequences and by the morphological similarity. In phylogenetic trees (Figs. 1A and 2), G. bachmaensis is consistently well resolved to be sister to G. bonii, but this pair of sister branches each has a very short branch length. This reflects that there exists only minor divergence between G. bachmaensis and G. bonii in genetics, although they are distinctly different in some morphological characters.

4.3 Species diversity of Gymnosphaera in mainland Asia

With the confirmation of Gymnosphaera saxicola, G. bachmaensis, and G. bonii as new or separate species, there are a
total of 13 species of Gymnosphaera now known in mainland Asia. It is possible that more species of Gymnosphaera could be discovered in the future from this area. As far as we know, the collection Tong 1078 (IBSC) from southern Vietnam doubtlessly represents an undescribed taxon of Gymnosphaera, because the phylogenetic analyses of both the chloroplast and the nuclear sequences clearly placed Tong 1078 in a separate clade from the four species groups. Morphologically, further surveys are needed to study this unknown taxon (Tong 1078), as we so far did not obtain even one fertile frond of it. In addition, it is credited that there is a fourth member in the G. podophylla complex in northern Myanmar, except for G. podophylla, G. bachmaensis, and G. bonii. We have seen several herbarium collections of this new member, but did not obtain any DNA materials for phylogenetic test. Further investigations, including field surveys and laboratory analyses of sequences, are needed for this group in mainland Asia. When comparing the species richness and endemicism of this group within mainland Asia, we found that there were three diverse centers for Gymnosphaera species, that is, south-central Vietnam, the border area between Vietnam and Yunnan, and western Yunnan to northern Myanmar. To better understand the species diversity of Gymnosphaera in mainland Asia, more efforts for field surveys and material collection should be made for scaly tree ferns in south-central Vietnam and northern Myanmar.

Incidentally, the first author thought it necessary to point out some misapplications of two species names in previous studies conducted by himself and his collaborators. The name G. gigantea used in Dong & Zuo (2018), Dong et al. (2019), and Wang et al. (2020) should be corrected to G. henryi, which is characterized by V-shaped sori on the ultimate segments and the stipe throughout, covered with two-rowed, outspreading scales. The name G. glabra used in Dong & Zuo (2018) should be corrected to G. gigantea, which is featured by V-shaped sori on the ultimate segments but without two-rowed scales on the stipe above the base.

5 Taxonomic Treatment

5.1 Gymnosphaera saxicola S.Y. Dong & Z.Y. Zuo, sp. nov. (Fig. 3)
岩生黑桫椤

Type: CHINA. Yunnan: Mengla Village, Mengma, Menglian County, 22°09′01″N, 99°24′33″E, 1550 m, 6 August 2018, S.Y. Dong 5017 (IBSC); Wenxin Village, Wenjing, Jingdong County, 24°06′11″N, 100°55′59″E, 2500 m, 18 October 2019, Z.Y. Zuo 2825 (KUN); Menghui Village, Mengdong, Cangyuan County, 23°10′35″N, 99°06′07″E, 1390 m, 28 December 2019, Z.Y. Zuo 2927 (KUN).

Habitat and distribution: Terrestrial in montane forest, on wet cliffs or the mouths of caves by stream, at 1390–2500 m. It is currently known only in southwestern Yunnan Province (Cangyuan, Jingdong, Menglian).

During the field survey in southwestern Yunnan, we found four populations of Gymnosphaera saxicola, one in Cangyuan, two in Menglian, and one in Jingdong at higher elevations (2500 m). The Jingdong population is small, with approximately 20 individuals living at the mouth of a stone cave and nearby crevices. Most individuals there are smaller than those of the two Menglian populations or those of the Cangyuan population, lacking massive caudices and with few plants having two-pinnate fronds. The other three populations are considerably larger, each containing more than 100 individuals. The mature, fertile individuals all have two-pinnate fronds.

5.2 Gymnosphaera bachmaensis S.Y. Dong, sp. nov. (Fig. 4)
白马黑桫椤

Type: VIETNAM. Thua Thien-Hue: Bach Ma National Park, 107°51′34″E, 16°12′31″N, 680 m, 24 November 2017, S.Y. Dong 4880 (holotype, IBSC; 10 sheets; isotypes: HNLU, IBSC).

Trunks erect, up to 1.5 m tall, 15 cm thick, with 8–10 fronds on the apex; stipe and rachis dark reddish, with copious scales on croziers and on bases of stipes; scales linear, 10–20 mm long and 1–1.6 mm wide at base, stiff, castaneous, lustrous, usually with a black band on either side, of marginate type, with very narrow erose edges and marginal setae present (or the setae usually deciduous), basifixed,
distally frilllose; each with a terminal seta, those upwards smaller and fewer; the stipe (the basal part of frond-axe lacking normal or reduced pinnae) very short, 8–10 cm long (rarely to 20 cm in smaller plants with one-pinnate fronds), 1–2 cm thick. **Smaller plants**: shrub-like, lacking erect trunks; fronds one-pinnate, oblancoate, ca. 150–200 × 35–40 cm; lateral pinnae 20–28 on either side of rachis, usually alternate, linear, sessile, entire or crenate or sometimes pinnatifid at base, lower 5–8 pairs gradually reduced in size, the lowest becoming auriculate, ca. 5–10 × 5 mm; the frond with normal pinnae like a lateral pinna of fronds in well developed plants. **Well developed plants**: fronds 1.8–2.3 × 1–1.2 m, two-pinnate, spathulate, broadest at upper part, with the lower seven or eight pairs of pinnae somewhat suddenly reduced in size, gradually tapering toward bases; lower reduced pinnae sessile, ranging from pinnatifid to entire and with one or a pair of basal lobes downwards, and to simply auricle-like near the base of stipe, the lowest ones as small as ca. 5–10 × 5 mm with a distance of 8–10 cm away from the base of stipe; pinnae upwards fully one-pinnate, 4–6 pairs, alternate or opposite, 10–20 cm apart, oblong or oblancoate, 35 × 18–70 × 25 cm, sessile, more or less narrowed and truncate at base, apex acute and pinnatifid; pinnaules linear, generally alternate, 10–13 pairs on lower pinnae, 15–20 pairs on upper pinnae, mostly 10–17 × 1.6–2 cm, sessile or very shortly petiolate (less than 1 mm), entire or undulate, cordate at base, distally acuminate; distal part of lamina like a lateral pinna but with a long petiole (8–15 cm), its basal pinnaules the longest and pinnatifid or pinnatisect at base; veins mostly free, simple, with a central vein and three or four pairs of lateral ones forming a group, the basal basiscopic one of each group arising from the central vein or often from costules, the basal veins of adjacent groups joining at the middle way from the base to margin, sometimes in one group the basal vein joining the second one near the margin of lamina, ends of free veins close to but not reaching the margin; the lamina chartaceous, with short hairs present on adaxial surface of frond axes (stipe, rachis, costae), two types of scales, that is, linear ones and much smaller furfuraceous ones, present on abaxial surface of rachis; sori spherical, three or four pairs per group, dorsal on veins, at middle or more often close to the base of veins, exindusiate; 64 spores per sporangia.

**Paratypes**: Thua Thien-Hue: Mt. Bach Ma, Bach Ma National Park, 150–200 m, 26 December 1996, K. Iwatsuki V-96088 (P-000784413[photo!]). Da Nang: Bana Hills, 600–800 m, May 1936, R.P. Cadiere 112 (P-01156883[photo!]).

**Habitat and distribution**: Terrestrial in rain forest with constant high humidity, on mountain slopes, at 100–800 m. Restricted to central Vietnam (Da Nang and Thua Thien-Hue).

During an exploration of scaly tree ferns in central and southern Vietnam, we met only one population of *Gymnosphaera bachmaensis* in Mt. Bach Ma, Thua Thien-Hue, which grew in a rainforest at 680 m above sea level, at a slope of ca. 30–40° to the mountain. We were able only to survey 50 × 50 m area there but found 30 mature individuals of the species. Two other collections of *G. bachmaensis* were found in herbarium P; one (Iwatsuki V-96088) was from the same mountain but at a lower elevation (150–200 m) and the other (Cadiere 112) was from Bana Hills, ca. 20 km southwards from Mt. Bach Ma. The distribution range of *G. bachmaensis* is very narrow but the population size, at least the Bach Ma population, seems not very small.

5.3 Gymnosphaera bonii (Christ) S.Y. Dong, comb. nov.

*Cyatheo* bonii Christ, J. Bot. (Morot) 4: 410. 1890. Type: VIETNAM. **Tonkin**: Ninh Thai, 22 December 1888, H. Bon 4073 (holotype, P-01556900[photo!]).

Alsophila podophylla var. procumbens Tutcher, J. Linn. Soc., Bot. 37: 68. 1905. Type: CHINA. **Hong Kong**, Feb 1904, Herb. Hong Kong Bot. Gard. n. 977 (syntype, not seen.). VIETNAM. **Hanoi**: Ouonbi Village, Nov 1885, B. Balansa 33 (syntypes: P-01556979[photo!], P-01556906[photo!]).

*Cyatheopsis podophylla* var. *oculata* C. Chr. & Tardieu in H. Lecomte, Fl. Gén. de l’Indo-Chine 7(6): 82. 1939. Type: VIETNAM. **Hanoi**: Mt. Bavi, 27 April 1887, B. Balansa 1797 (lectotype, P-00625642[photo!], designated here); i.c., 26 July 1886, B. Balansa 1866 (syntype, P-00625643[photo!]).

**Habitat and distribution**: Terrestrial in forest, frequently occurring in limestone area, 50–1500 m, very common. Now known in South China (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hong Kong, and southeastern Yunnan) and Vietnam.
Key to species of Gymnosphaera bonii group

1a. Well developed plants having erect trunks; fertile fronds two-pinnate; lateral pinnae (at least lower two pairs) distinctly petiolate; stipes bearing few scales above the base; veins all free, or basal veins of each group sometimes (but never regularly and frequently) anastomosing........................................... *G. podophylla*

1b. Erect trunks present or not; fertile fronds one- or two-pinnate; lateral pinnae all sessile; stipes and rachides more or less scaly; basal veins (except those on distal tips of pinnules or on intermediate fronds) regularly anastomosing........................................... 2

2a. Trunks erect, up to 1.5 m tall; lamina, if two-pinnate, pinnate, with four or five pairs of normal-sized pinnae; lower pinnae gradually reduced, the lowest one ca. 10 × 5 mm, with a distance of only 8–10 (20) cm to the base of stipe; restricted to central Vietnam...................................................... *G. bachmaensis*

2b. Trunks lacking or rarely present; lamina, if two-pinnate, elliptic and mostly with two or three pairs of pinnae; lower pinnae not or slightly reduced, the lowest one considerably far away (50 cm or more) to the base of stipe; widespread in Vietnam and S China...................................................... *G. bonii*
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**Supplementary Material**

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12679/suppinfo:

**Doc. S1.** List of accessions’ information about five chloroplast regions used in this study. For each accession, in order listed are species name, collector and collection number (herbarium), locality, and GenBank accession number (ref. Dong et al., 2019, Table 2). The asterisk (*) indicates the species with its sequences newly generated and the plus (+) means the species being firstly sampled in this study.

**Doc. S2.** List of accessions’ information about 12 sing-copy nuclear loci used in this study. For each accession, in order listed are species name, collector and collection number (herbarium), locality, and GenBank accession numbers for nuclear locus 5229/5243/5320/5521/5604/5770/5839/5870/5894/6132/6148/6318 (ref. Dong et al., 2019, Table 2). The asterisk (*) indicates the species with its sequences newly generated and the plus (+) means the species being firstly sampled in this study.