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ORIGINAL ARTICLE

Rediscovery of *Lepisorus cespitosus* supported the floristic affinities between western Yunnan and southeast Tibet

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Abstract

The northern parts of Yunnan belong to 'Mountains of SW China' biodiversity hot spot, one of the most species-rich areas on earth. Although some progress, relatively little attention has been given to explore the evolutionary history of the fern diversity of this hot spot. According to recent progress, Yunnan exhibits the heterogeneity of species diversity among regions. In this study, we focused on the Dehong Dai-Jingpo Autonomous Prefecture, an overlooked area in western Yunnan geographically and geologically, which is connected to SE Tibet and NE Myanmar; thus, the flora of this region will likely share several unique taxa, but this pattern may be obscured by the lack of reported distributions. To examine this, we conducted floristic investigation targeting rare species shared between Dehong and adjacent areas in *Lepisorus*. We performed morphological, phylogenetical, and biogeographical analyses with the accessions obtained. *Lepisorus cespitosus* was firstly found in Dehong, which was previously only known from the type location in SE Tibet. Our analyses revealed that *L. cespitosus* is a lineage morphologically and phylogenetically isolated from all other sections of *Lepisorus*. Divergence time estimates and ancestral area reconstruction supported that *L. cespitosus* is a relict species survived in this region since the Late Miocene. Our results showed the affinity in the flora between western Yunnan and SE Tibet and supported the hypothesis that this region has served as a climate refugia for relict species during the Cenozoic. Thus, we propose that more attention should be given to floristic investigation in this area and adjacent regions.

Keywords Climate refugia · Divergence time estimation · *Lepisorus* · Relict species · Species diversity · Yunnan

Introduction

Southwest China in particular Yunnan is well known for the high species diversity (Tang et al. 2006; López-Pujol et al. 2011). Ferns and lycophytes are not an exception with about 10% of the global diversity of ferns and lycophytes occurring alone in Yunnan (Schneider pers. comm.). Yunnan and adjacent regions have been proposed to be long stable refugia of plant diversity (López-Pujol et al. 2011; Tang et al. 2018) which is supported by studies reporting population persistence of relict plant species, e.g., *Tetracentron sinense* (Tang et al. 2013), *Taiwania cryptomerioides* (He et al. 2015), and *Craigia yunnanensis* (Yang et al. 2016). Supporting evidence was also recovered by the discovery of long-term survival of current rare fern species such as *Trichoneuron microlepioides* Ching (Liu et al. 2016) and *Christensenia aesculifolia* (Blume) Maxon (Liu et al. 2019a). The first species is the only representative of Dryopteridaceae subfam. Polybotryoideae occurring in the Paleotropics with the remaining extant members of this lineage occurring in the Neotropics (Liu et al. 2016). This taxon has been only known from a single location (Mt. Daweishan) in SE Yunnan until the more recent discovery in northern Vietnam (Liu et al. 2016). The second species is represented by one or two locations in Yunnan that are genetically distinct from the

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more widespread relatives occurring in the Malay Archipelago (Liu et al. 2019a; Liu pers. comm.). However, many species with small restricted occurrences in Yunnan have arguably not been discovered yet. For example, a recent study on the grammitioid fern genus *Scleroglossum* Alderw. not only clarified the taxonomy of this genus in China—by showing that two rather than one species occur—but also reported the occurrence of this species in the Dehong area, western part of Yunnan for the first time (Liu et al. 2019b). Thus, the exploration of the fern diversity of Yunnan may provide evidence for the occurrence of relict species as well as species occurrence unknown until now. Species new to science are still discovered in Yunnan such as *Asplenium simaoense* K.W.Xu, Li Bing Zhang & W.B.Liao which is only known from limestone mountains of southwestern Yunnan (Xu et al. 2019a, b). In contrast to the species as *Trichoneuron microlepioides*, this taxon is arguably young because it is nested within a recently diverging lineage of spleenworts with close relatives occurring throughout SE Asia (Xu et al. 2019a, b; Schneider pers. comm.). Thus, Yunnan is not only a museum but also a cradle of fern diversity.

Studies documenting the spatial distribution of angiosperm diversity in Yunnan showed evidence for a strong differentiation of species richness and phylogenetic diversity among the regions that may reflect climatic, topographic, and historical differentiation (Li and Yue 2019). For example, the southern tropical regions, such as Xishuangbanna Dai Autonomous Prefecture, the southern parts of Honghe Hani-Yi Autonomous Prefecture, and Wenshan Zhuang-Miao Autonomous Prefecture, showed a high species richness and phylogenetic diversity, whereas the regions in the western Yunnan especially Dehong Dai-Jingpo Autonomous Prefecture and Baoshan showed low species richness and phylogenetic diversity. However, the differences among these regions were less pronounced in considering a linear regression model of phylogenetic diversity against species richness (Li and Yue 2019). The main reasons for these differences have not been fully understood and further studies are required to confirm these patterns across the tree of life. So far, the distribution of ferns has not been taken into account and further studies are required to confirm these differences and their underlying mechanisms. In particular, the western areas of Yunnan arguably share many species with the adjacent regions in Myanmar and southeast Tibet because this region is located on a mountain arc constrained by the Hengduan Mountains in the east, the Tibetan Plateau in the north, the eastern Himalaya in the west, and the lowland of northwest India and Myanmar in the south. Thus, the flora of Dehong Dai-Jingpo Autonomous prefecture is arguably expected to be closely related to the floras of Kachin State in northern Myanmar and Nyingchi region of SE Tibet, given the topography of the region is shaped by mostly north–south directed canyons that are part of the water-shed systems of the Nujiang/Salween River, Irrawaddy River, and the Yarlung Tsangpo/Brahmaputra River. The uniqueness of this floristic region, however, has not been tested using the composition of plants in particular fern diversity. Based on the previous results on the diversity of the fern genus *Lepisorus* (J. Sm.) Ching (Wang et al. 2011, 2012 a, b), this region is expected to be home of relict species. In turn, the test will further enhance the argument that the biodiversity richness of Yunnan is partly caused by the different evolutionary histories among different regions including the western part of this province.

To explore this hypothesis, we focus here on a lineage of derived ferns that showed distinct diversification patterns in Southeast Asia (Wang et al. 2010a, b, 2011, 2012a, b). The genus *Lepisorus* is one of the most complicated and controversial lineages of Polypodiaceae in SE Asia and Yunnan in particular. As a consequence, comprehensively sampled phylogenetic studies made this genus one of the best sampled genera in SE Asia (Wang et al. 2010a, b, 2011, 2012a, b; Zhao et al. 2019). The circumscription of the genus has been recently expanded to include previously recognized segregates, such as *Lemmaphyllum* C.Presl, *Lepidomicrosorium* Ching & K.H.Shing, *Neocheiropteris* Christ, *Neolepisorus* Ching, *Paragramma* (Blume) T. Moore, and *Tricholepidium* Ching besides the core *Lepisorus* clade (Wang et al. 2010a, b; PPGI 2016; Zhao et al. 2019). A recent study for the first time revealed that *L. jakonensis* (Blanf.) Ching that previously considered to be part of the sect. *Hymenophyton* is one of the basal lineages of the whole genus, and thus demonstrated the need to focus on rare and often overlooked species to obtain a more comprehensive understanding of the diversification of this genus (Zhao et al. 2019). By combining divergence time estimates and ancestral distribution range reconstruction, it has been shown that the diversification of the genus, in particular the accumulation of the high species richness in Southwest China was correlated with the strengthening of the South East Asian monsoons (Wang et al. 2012b). The study found also evidence for two distinct lineages of *Lepisorus* occurring mainly in Japan and Taiwan that may present independent colonization and survival of *Lepisorus* species in oceanic climate zones (Wang et al. 2012b). However, some evidence was found for relict species occurring in Southwest China in sympatry with lineages dominating Southwest China today such as sect. *Hymenophyton*, sect. *Pachyphyllon*, and sect. *Pseudovittaria*. For example, evidence for a survival or at least relative recent survival of a member of the *Paragramma* clade was discovered (Wang et al. 2012a; Zhao et al. 2019) besides the occurrence of most of the basal lineage of *Lepisorus* in Southwest China. In this study, we aimed to explore evidence for overlooked species that represent isolated lineages in the genus *Lepisorus* (see Zhao et al. 2019*)*. Furthermore, we focused on Dehong and adjacent regions because these regions may be home of some hidden diversity as shown in the recent discovery of *Scleroglossum* in this region (Liu et al. 2019b). To test the hypothesis that the affinity of floras of Dehong and the Nyingchi region, the Medog region in particular, we aimed specifically on taxa that have been reported only in the Medog region, e.g., *L. cespitosus* Y.X.Lin and *L. medogensis* Ching & Y.X.Lin. The discovery of one of these taxa would increase the list of species known from both regions, such as *Deparia medogensis* (Ching & S.K.Wu) Z.R.Wang and *Dryopteris medogensis* (Ching & S.K.Wu) Li Bing Zhang.

Materials and methods

Fieldworks were carried out in Dehong region with focus on the Tongbiguan Provincial Nature Reserve and nearby forests toward the Gaoligong Mountain range in the North. We focused on collecting all ferns and lycophytes but targeted on *Lepisorus*. Using morphology-based identification and available floristic treatments (Lin 2000; Zhang et al. 2013), we separated accessions identified as species well-known to occur in Yunnan from accessions that did not match species described to occur in Yunnan. Specifically, we focused on one accession—collection number FRG-201901-170—collected at Tongbiguan Provincial Nature Reserve, Yingjiang County (24° 48′ 59.0″ N, 97° 44′ 05.1″ E). To improve the interpretation of this accession, we consulted the taxonomic and floristic literatures on *Lepisorus* (Lin 2000; Qi and Zhang 2009; Qi et al. 2010; Wang et al. 2010a; Zhao et al. 2019). This accession was further studied by comparing them with the holotype of *L. cespitosus* (*B.S.Li* and *S.Z.Cheng 04336*, Tibet, Medog, 27 Apr 1983 HT: PE!) and specimens of other related species of *Lepisorus*. The accession will be deposited at the herbarium of XTBG (HITBC).

Total DNA was extracted from silica dried sample of *Lepisorus cespitosus* using CTAB method (Doyle and Doyle 1987). The four plastid regions, *rbc*L gene, *rbc*L-*atp*B intergenic region, *rps*4-*trn*S intergenic region, and *trn*L-F intergenic region were amplified as following protocols provided in previous studies (Wang et al. 2010a, 2012b; Fujiwara et al. 2018; Zhao et al. 2019). Newly generated sequences were submitted to GenBank (see Online Resource 1).

For phylogenetic analysis, we downloaded sequences for each species from GenBank. Insufficiency of taxon coverage in phylogeny often causes inference error for divergence time estimation and biogeographical reconstruction used in subsequent analyses. Our taxon sampling succeeded in covering of 100% for section in the genus and 96% for species recognized in core *Lepisorus*, because *Lepisorus* is one of the best phylogenetically studied fern genera in SE Asia (Wang et al. 2010a, 2012b; Zhao et al. 2019; Chen et al. 2020). Accessions for all sequences used in this study including newly generated sequences are listed in Online Resource 1. Sequences of each plastid region were separately aligned using MUSCLE (Edgar 2004) and manually edited in Aliview (Larsson 2014). Substitution models for each region were determined using JModelTest 2 (Darriba et al. 2012) with AIC criteria. Tree reconstruction was performed with two different methods: maximum likelihood (ML) and Bayesian inference (BI) methods. For the ML method, we used RAxML-HPC2 8.2.626 (Stamatakis 2014) on the CIPRES Science Gateway portal (Miller et al. 2010) (http://www.phylo.org/) with GTR GAMMA as substitution model and performed 1000 bootstrap (BS) replicates. For BI method, MrBayes 3.2.6 (Ronquist et al. 2012) on the CIPRES Science Gateway portal was used where two runs of four MCMC chains for 100,000,000 generations with samples taken every 1000 generations were conducted. Tracer 1.6 (Rambaut and Drummond 2013) was used to evaluate convergence. The first 0.25 were discarded as burn-in.

We conducted divergence time estimations with BEAST 2.6.1 (Bouckaert et al. 2014) on the CIPRES Science Gateway portal. BEAST analyses were carried out with a log-normal relaxed clock and the sequence matrix partitioned by marker based on the result of substitution model selection in JModelTest. To reduce computational time, starting tree was fixed using the tree topology from the best tree generated in RAxML. A birth–death tree model was used as tree prior. We used one secondary calibration point corresponding to the split between *Lepisorus* (*Paragamma*) *longifolius* Ching and the remaining *Lepisorus* clade with an age estimate of 29.83 Ma as estimated in large-scale dating analysis of ferns (Testo and Sundue 2016). The value was entered using a normal distribution prior with sigma 1.0. Two runs of 100,000,000 generations with samples taken every 4000 generations were performed for both runs. Tracer 1.6 was used to evaluate convergence and define burn-in periods. LogCombiner 2.6.1 and TreeAnnotator 2.6.1 were used to combine log and tree file for each two runs and to compute the maximum clade credibility tree.

The biogeographical history of the *Lepisorus* was reconstructed with the R package 'BioGeoBEARS' (Matzke 2013). Two analyses with different settings namely DEC model and DEC model with jump parameter (*j*) were carried out. The best fitted model was selected by comparing the two models based on AIC criteria. The distribution information for each species was collected using the previous study (Wang et al. 2012b), Flora of China (Zhang et al. 2013), and GBIF. The information on species distributions was separated into eight areas: South Asia, Africa, Japan, Taiwan, Southwest China, Hainan, East, Central, and North China and remaining South China. This scoring scheme was also used in Wang et al. (2012b).

Throughout the study, our classification follows the recently introduced expansion of *Lepisorus* (Zhao et al.

2019) instead of the narrower classification favored in PPG I (2016).

Results

Morphology-based observations

The specimen collected in Yingjiang County, Dehong Prefecture, Yunnan Province, China (voucher number FRG-201901-170) exhibited following morphological characteristics (Fig. 1): (a) short rhizome (Fig. 1a), (b) clustered frond (Fig. 1a and 1e), (c) clathrate rhizome scale with denticulate margin (Fig. 1b), (4) sorus attached near margin of lamina (Fig. 1a and 1d), and (5) stellate paraphyses with thick lumina (Fig. 1c). Based on these characters, this sample was recognized as the first record of *Lepisorus cespitosus* to occur in Yunnan and also only the second collection of this species since the collection of the type specimen from Medog, Tibet in 1983.

Phylogenetic inference

In all phylogenetic analyses, *Lepisorus cespitosus* was nested in the core *Lepisorus* clade but not closely related to *L. medogensis* which is nested in the sect. *Pseudovittaria*. In the hypothesis obtained in the maximum likelihood analyses (Fig. 2), *L. cespitosus* was discovered as sister species of the sect. *Obtusi* containing *L. onoei* (Franch. & Sav.) Ching, *L. hachijoensis* Sa.Kurata, *L. monilisorus* (Hayata) Tagawa, and *L. boninensis* (Christ) Ching with a low bootstrap support values (54%). Sect. *Drymotaenium* was sister to this clade with low bootstrap support values (51%). In the majority consensus hypothesis obtained from the Bayesian analyses, *L. cespitosus* and *L. miyoshianus* (Makino) Fraser-Jenk. & Subh.Chandra did not form a clade together with the sect. *Obtusi*. Plotting 15 morphological characters onto the phylogenetic hypothesis (Fig. 2) did not reveal any morphological evidence supporting a close relationship of *L. cespitosus* and the species of the sect. *Obtusi*. Instead the unique combination of morphological characters supported the phylogenetic isolation of this species, which was consistent with the relative long branches recovered in the phylogenetic analyses of the DNA sequence data (Fig. 2a). It was noted that the monotypic sect. *Drymotaenium* has also a rather unique combination of characters.

Divergence time estimates

The divergence time estimates obtained under the log-normal related clock model suggested a crown age of the core *Lepisorus* clade between 29.14 and 22.31 Ma (Fig. 3, Table 1). The recognized sections showed stem group age confidence intervals ranging from the Early Miocene to Early Oligocene (sect. *Pachyphyllon*), Early Miocene to Late Oligocene (sect. *Neolepisorus*, sect. *Hymenophyton*,

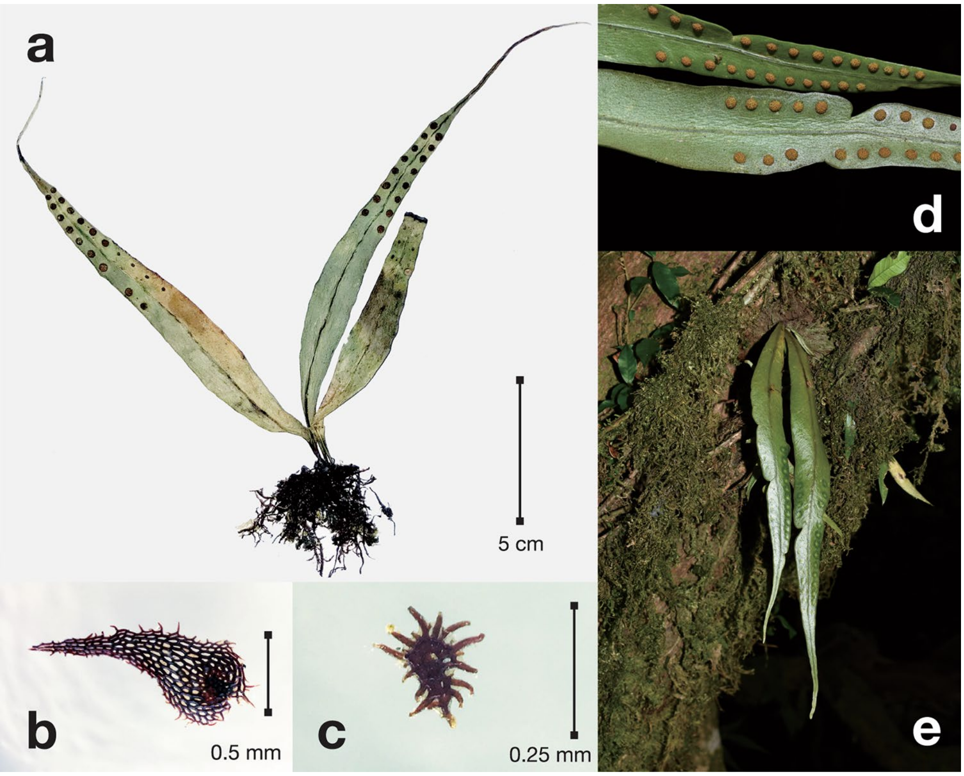

Fig. 1 *Lepisorus cespitosus* Y. X. Lin accession (FRG-201901-170, 3 Jan 2020) collected in the Yingjiang County, Dehong Prefecture, Yunnan Province, China (24°48′59.0″N, 97°44′05.1″E). **a** Habit. **b** Rhizome scale. **c** Paraphyses. **d** Abaxial part of lamina to show sorus. **e** Plant growing on tree trunk

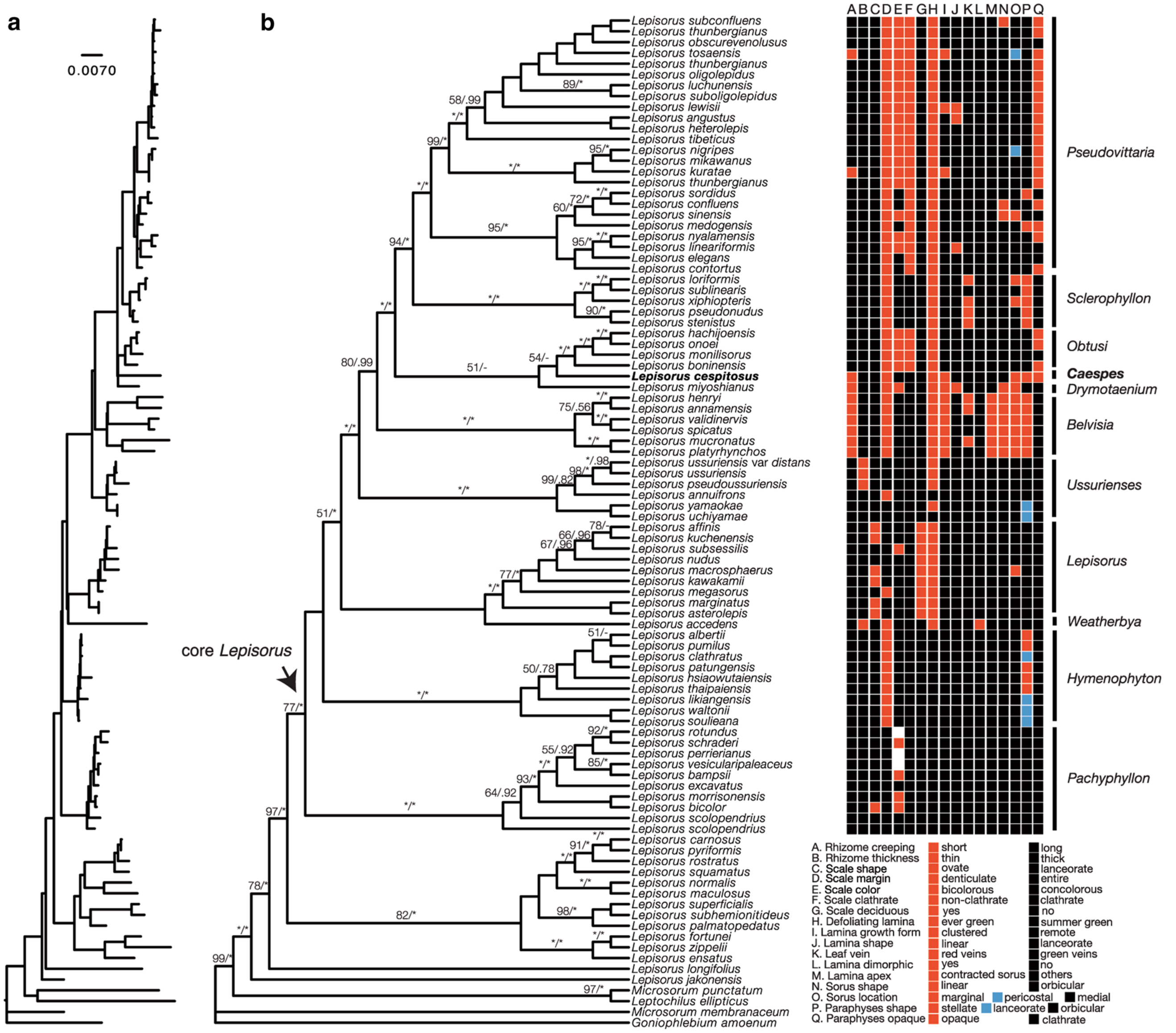

Fig. 2 Phylogeny of *Lepisorus* depicted as phylogram obtained in the maximum likelihood analyses (**a**) and the consensus cladogram obtained by the Bayesian inference of phylogeny (**b**). In **b**, numbers on each branch are support values as the order ML bootstrap value/BI posterior probability. The asterisk '*' and dash '-' indicate supports of 100% for ML and 1.0 for BI, and supports of < 50% for ML and < 0.5 for BI, respectively. Columns on left side in **b** show 15 morphological characteristics for each species of core *Lepisorus* as shown below. Each color indicates different state according to legends below columns and white color means 'no information'

sect. *Lepisorus*, and sect. *Weatherbya*), Mid Miocene to Late Oligocene (sect. *Lepidomicrosorium*), Late Miocene to Early Miocene (sect. *Tricholepidium*, sect. *Lemmaphyllum*), Mid Miocene to Early Miocene (sect. *Caespes*, sect. *Drymotaenium*, sect. *Obtusi*, sect. *Sclerophyllon*, and sect. *Pseudovittaria*). Similarly, the crown group age of each of sections (Table 1) including more than one extant species showed confidential intervals ranging from the Mid Miocene to Early Miocene (sect. *Neolepisorus*, sect. *Belvisia*) or Pliocene to Mid Miocene (sect. *Lepidomicrosorium*, sect. *Tricholepidium*, sect. *Lemmaphyllum*, sect. *Pachyphyllon*, sect. *Hymenophyton*, sect. *Lepisorus*, sect. *Ussurienses*, sect. *Obtusi*, sect. *Sclerophyllon*, and sect. *Pseudovittaria*).

Ancestral areas reconstruction

DEC was selected as the best model based on AIC criteria by comparing independent analyses conducted with different settings of the DEC and DEC + *j* models as implemented in BioGeoBEARS (Fig. 3). Southwest China was reconstructed as the most probable area of origin of core *Lepisorus* (Fig. 3). Although most of the sections also have

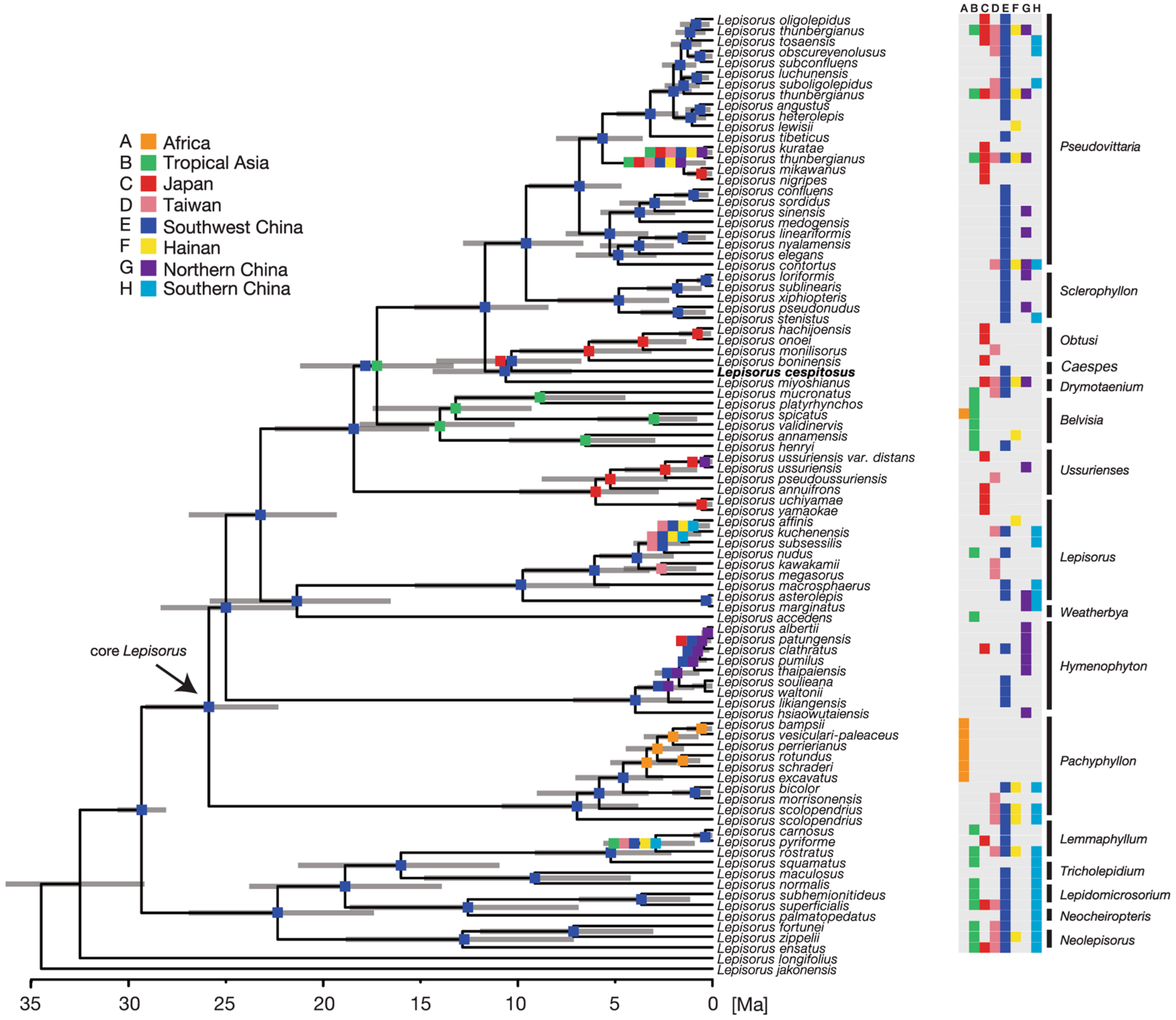

Fig. 3 Consensus chronogram obtained by Bayesian divergence time estimation under a log-normal relaxed molecular clock and reconstruction of ancestral biogeographical pattern of *Lepisorus* inferred with DEC model in 'BioGeoBEARS.' Colored squares on right side of the chronogram and on internal nodes indicate current and inferred historical distribution of each species and each node, respectively. Gray bar on each node is 95% HPD confidence intervals of the estimated divergence times. Red arrow indicates the calibration point, 29.83 Ma

the origin in southwest China, we observed range expansions/colonization in several lineages. Sect. *Pachyphyllon* colonized Africa, whereas sect. *Ussurienses* and sect. *Obtusi* colonized Japan and Taiwan. Sect. *Belvisia* expanded its range to colonize major parts of tropical Asia and some parts of tropical Africa. Evidence for a further range expansion was found in sect. *Pseudovittaria* with one clade containing species occurring in Japan namely *L. kuratae* T.Fujiw. & Seriz., *L. mikawanus* Sa Kurata, *L. nigripes* T.Fujiw. & Seriz., and *L. thunbergianus* (Kaulf.) Ching s.s. Most specie of core *Lepisorus* occurring in Southwest China belong to the following sections, sect. *Hymenophyton*, sect. *Lepisorus*, sect. *Pachyphyllon*, sect. *Pseudovittaria*, and sect. *Sclerophyllon*. *Lepisorus cespitosus* was one of the few species occurring exclusively in this region but did not belong to these sections, meanwhile its possible closest living relative sect. *Obtusi* occurred only in Japan and Taiwan.

Discussion

In our study, we discovered *Lepisorus cespitosus* in Dehong area (western Yunnan) for the first time. This species was previously only known from the type location in the Medog

Table 1 Summary of the divergence time estimates obtained using the DNA sequence data analyses using a log-normal relaxed molecular clock calibrated with 29.83 Ma as the age of the separation between *Lepisorus longifolius* and the remaining *Lepisorus* lineages (Fig. 3)

Clade	Ma-SG	Mi-SG	Ma-CG	Mi-CG
core *Lepisorus*	30.57	28.07	29.14	22.31
sect. *Neolepisorus* (Ching) C.F.Zhao, R.Wei & X.C.Zhang	26.91	17.49	18.84	7.13
sect. *Neocheiropteris* (Christ) C.F.Zhao, R.Wei & X.C.Zhang	23.80	13.91	NA	NA
sect. *Lepidomicrosorium* (Ching & K.H.Shing) C.F.Zhao, R.Wei & X.C.Zhang	23.80	13.91	6.86	1.14
sect. *Tricholepidium* (Ching) C.F.Zhao, R.Wei & X.C.Zhang	21.29	10.94	14.81	4.20
sect. *Lemmaphyllum* (C.Presl) C.F.Zhao, R.Wei & X.C.Zhang	21.29	10.94	9.12	2.11
sect. *Pachyphyllon* S.L.Yu	29.14	22.31	10.83	3.82
sect. *Hymenophyton* Ching ex S.L.Yu & Y.X.Lin	28.35	21.26	7.16	1.56
sect. *Lepisorus*	25.83	16.54	15.29	5.29
sect. *Weatherbya* (Copel.) C.F.Zhao, R.Wei & X.C.Zhang	25.83	16.54	NA	NA
sect. *Ussurienses* X.P.Qi ex C.F.Zhao, R.Wei & X.C.Zhang	22.48	14.56	9.93	2.77
sect. *Belvisia* (Mirb.) C.F.Zhao, R.Wei & X.C.Zhang	21.18	13.30	18.13	10.17
sect. *Drymotaenium* (Makino) C.F.Zhao, R.Wei & X.C.Zhang	14.37	7.24	NA	NA
sect. *Caespes* T. Fujiw.	14.19	6.74	NA	NA
sect. *Obtusi* (S.L.Yu) C.F.Zhao, R.Wei & X.C.Zhang	14.19	6.74	9.92	3.01
sect. *Sclerophyllon* S.L.Yu	12.81	6.64	7.96	2.24
sect. *Pseudovittaria* (C.Chr.) Ching	12.81	6.64	9.44	4.67

The crown and stem group age estimates were given for the sections of *Lepisorus* with the exception of the two basal sections namely sect. *Paragramma* and sect. *Pseudoclathrati*. Besides the sections, the crown and stem group age were also given for the core *Lepisorus* clade (Fig. 3). Instead of mean ages, we provide the minimum and maximum age of the 95% confidence interval. *Ma-SG* Maximum age of stem group, *MI-SG* Minimum age of stem group, *Ma-CG* Maximum age of crown group, *Mi-CG* Minimum age of crown group. All age estimates were given in million year to the present (Ma). *NA* section included by a single species in our sampling

area, SE Tibet (Lin 2000; Qi et al. 2010; Zhang et al. 2013). The rediscovery of this species out of Tibet supports the hypothesis of a shared flora among western Yunnan, SE Tibet, and potentially also northern Myanmar in particular Kachin State. Taking advantage of the rediscovered species, we recovered *L. cespitosus* as a phylogenetically isolated species that diverged from other lineages of *Lepisorus* during Mid to Early Miocene—a time of notable diversification of *Lepisorus* particularly in SW China. By studying the type specimen, we are able to confirm the taxonomic interpretation that the Yunnan accession represents this species and we introduce a new section for *Lepisorus* to enable the classification of this species.

Taxonomic interpretation

Our interpretation is based on a careful comparison of our new accession with not only the description provided in the literatures (Lin 2000; Qi et al. 2010; Zhang et al. 2013) but also the type specimen. All critical characters of our accession we observed in this study are highly congruent with those of the type specimen. Given the rather unique combination of characters, a confusion of this taxon with other *Lepisorus* species is rather unlikely. However, we want to stress out the need to re-determine specimens held in herbaria that have not been studied by taxonomists with expertise in *Lepisorus*. Such a study is needed to confirm the range and to assess the threat status of this taxon. Additionally, particular attention needs to be given to the occurrence of this species in northern Myanmar and northeastern India besides Tibet and Yunnan. The most comprehensive study of ferns in northern Myanmar (Khine et al. 2017) did not report evidence for this species although they recorded eleven species belonging to the core *Lepisorus* clade in two transects obtained from Kachin State, namely Hponyinrazi and Hponkanrazi. Ten out of the eleven species reported have been included in our phylogenetic analyses and placed in the sect. *Belvisia* (3 spp.), sect. *Lepisorus* (2 spp.), sect. *Pachyphyllon* (3 spp.), sect. *Pseudovittaria* (2 spp.), and sect. *Sclerophyllon* (2 spp.). All of these species have occurrences in Yunnan and four are recorded in Tibet. The only species reported in northern Myanmar which was not included in the phylogenetic analyses, *L. mehrae* Fraser-Jenk., is known to occur in Bhutan and Nepal but has not been recorded in China. This species resembles the widespread *L. scolopendrium* (Ching) Mehra & Bir and further studies are needed to resolve the taxonomy of these ferns in Yunnan, Myanmar, and the Himalaya.

Phylogenetic relationships

Similar to previously published phylogenetic analyses of *Lepisorus* (Wang et al. 2010a, b; Zhao et al. 2019), several nodes of the backbone of the phylogenetic hypothesis have been recovered with high bootstrap values and Bayesian confidence values; however, more sampling of DNA sequence variation is required to resolve the deep time history of this lineage to solve the placement of some sections at the base of the core *Lepisorus* clade such as sect. *Hymenophyton* and sect. *Pachyphyllon* that were recovered as part of a grade (see Fig. 2) instead of a sister clade relationship (Zhao et al. 2019). However, our current phylogeny as well as previous studies (Wang et al. 2010a, b; Zhao et al. 2019) recovered all other sections as part of a grade with the same sequence leading from sect. *Lepisorus* toward sect. *Pseudovittaria*. The newly included *L. cespitosus* was found to be isolated by several unique base-pair combinations and a relative long branch compared to the close relatives (Fig. 2a). This species was recovered as the putative sister to the species-poor sect. *Obtusi* and together grouped with the monotypic sect. *Drymotaenium*. The latter was found to be sister to the sect. *Obtusi* in previous studies (Wang et al. 2012a, b; Zhao et al. 2019). Thus, *L. cespitosus* was recovered as the third isolated species nested in the core *Lepisorus* clade besides *L. miyoshianus* (sect. *Drymotaenium*) and *L. accedens* (sect. *Weatherbya*), the latter two species have been recognized as separate genera, namely *Drymotaenium* and *Weatherbya*, in the past. All other sections have been recovered as in previous studies (see Wang et al. 2010a, b; Zhao et al. 2019) which is not surprising given the usage of the same dataset. The result of *L. cespitosus* as a separate and isolated lineage was supported by the unique character combinations that have not been found in any other sections of *Lepisorus*. The arguments based on morphological disparity need to take into account that only some sections show highly conserved character combinations such as sect. *Hymenophyton* and homoplasy was found to be rather common among the characters investigated (Fig. 2b). Future studies may want to explore the temporal assembly of phenotypic disparity in this lineage to reconstruct the occupation of the morphospace through time as the study on the Neotropical derived fern genus *Pleopeltis* (Schneider 2016). In summary, our analyses supported the distinctiveness of this species and rejected speculations about other relationships inferred from morphological similarities to *L. pseudonudus* Ching and *L. xiphiopteris* (Baker) W.M. Chu ex Y.X. Lin that have been considered based on some characters such as clathrate scale and stellate paraphyses (Lin 2000). Instead, the result of the phylogenetic analyses emphasized the uniqueness of the morphological character combinations including clathrate scales, stellate paraphyses, short rhizome, and clustered fronds (Lin 2000). Based on all these evidences, we introduce a new section to enable the placement of *L. cespitosus* into the classification introduced in Zhao et al. (2019).

Biogeographical implications

As pointed out above, the discovery of *Lepiosrus cespitosus* in western Yunnan supports the proposed link between the fern floras of the Nyingchi region of SE Tibet and those of western Yunnan. Unfortunately, this taxon has not been discovered in Myanmar until now but this may be caused by the relatively little collecting efforts in the Kachin region of Myanmar (Khine pers. comm.). The importance of dense collecting efforts is highly appreciated because this taxon has not been discovered before in western Yunnan nor has it been rediscovered in the Medog region since the first record (Lin 2000; Qi et al. 2010). This pattern suggests that this species may be rare but further collecting efforts are needed to confirm this interpretation, because several other species show a similar range such as *Deparia medogensis* and *Dryopteris medogensis*. In this context, it is crucial to emphasize that the high similarity among many species of *Lepisorus* may result in misidentification and likely under-recording of less common species.

Divergence time estimates combined with ancestral area reconstruction support the hypothesis that *Lepisorus cespitosus* is a relict taxon that survived in this region since the Late Miocene (Fig. 3). The occurring range of the next closest extant relatives is restricted to the island chain including Japan and Taiwan, whereas the other putative close relative *L. miyoshianus* has a wider distribution including several provinces of China namely Guangdong, Guizhou, Hubei, Hunan, Shaanxi, Sichuan, Tibet, Yunnan, and Zhejiang besides Japan and Taiwan Island (Zhang et al. 2013). This species is also known to occur in NE India. These observations may support a scenario in which the current occurrences represent the survival areas of the sect. *Obtusi* and sect. *Caespes* during the climatic fluctuation and other environmental changes since the origin of these lineages in the Miocene. The current range of the sect. *Obtusi* may have been evolved by a range expansion toward Japan and Taiwan, whereas the current range of sect. *Caespes* was either part of the ancestral range or the consequence of range expansion toward the West (Fig. 3). Previously discovered relict ferns in Yunnan occur preferable in the S and SE Yunnan such as *Christensenia (*Liu et al. 2019a) and *Trichoneuron* (Liu et al. 2016), but the evidence reported here indicates western Yunnan to be a refugia of ferns too.

The obtained divergence time scenario has to be considered in the context of the limited fossil evidences available. Only two fossils may be assigned to *Lepisorus,* namely *Neolepisorus chingii* described from the Upper Bangmai Formation considered to be of Late Miocene age (Xie et al. 2016) and *Palaeosorum ellipticum* described from the Dajie Formation considered to be of Middle Miocene age (Jacques et al. 2013). Both fossils are assigned to the crown group of sect. *Neolepisorus* according to Zhao et al. (2019) because the described morphological evidence supports the assignment of this fossil to the crown clade of sect. *Neolepisorus*. The estimated crown age is consistent with the age assignment for the clade provided by this fossil. Besides supporting the obtained age estimates based on the log-normal relaxed clock, the fossil also provides support for the hypothesis of long-term survival of *Lepisorus* species in Yunnan. Other fern fossils from SW China especially Yunnan support the continuity of the generic composition of fern floras in this region since the Mid Miocene (Table 2). Besides the two fossils assigned to sect. *Neolepisorus*, the following fossils comprise mainly genera belonging to the derived fern family Polypodiaceae such as *Drynaria* (Su et al. 2011; Wu et al. 2012; Wen et al. 2013; Huang et al. 2016) and *Goniophlebium* (Xu et al. 2017). Only three non-Polypodiaceae genera have been recorded in SW China (Table 2), namely *Christella* (Xu et al. 2019a), *Davallia* as *Humata*, (Wen et al. 2013) and *Oleandra* (Xie et al. 2018). The phylogenetic association of these fossils was considered in the context of our current understanding of the diversification of these lineages (PPGI 2016; Tsutsumi et al. 2016; Chen et al.

Table 2 Summary of the Miocene–Pliocene fossil record of derived ferns in Southwest China

Fossil Taxon	Stratigraphic Age	Geological Formation	Location	Fossil Publication	Affinities	Phylogeny
Christella nervosa (J.R.Tao) C.L.Xu, T.Su & Z.K.Zhou	Paleocene	Liuqu formation	Liuqu, Lhatse County, Tibet	Xu et al. (2019a, b)	*Christella*	PPGI (2016)
Christella sp.	Middle Miocene	Sanhaogua Formation	Jinggu Basin, Jinggu County, Yunnan	Xu et al. (2019a, b)	*Christella*	PPGI (2016)
Drynaria callispora Su, Zhou & Liu	Late Pliocene	Sanying Formation	Yangjie, Yongping, Yunnan	Su et al. 2011	Sino-Drynaria clade	Schneider et al. in prep.
Drynaria dimorpha J.Y.Wu & B.N.Sun	Late Pliocene	Mangbang Formation	Tuantian, Tengchong, Yunnan	Wu et al. 2012	Sino-Drynaria clade	Schneider et al. in prep.
Drynaria lanpingensis Huang, Su & Su	Late Pleiocene	Sanying Formation	Fudong, Lanping County, Yunnan	Huang et al. 2016	Sino-Drynaria clade	Schneider et al. in prep.
Drynaria propinqua (Wall. ex Mett.) J.Smith ex Bedd.	Late Miocene	Bangmai Formation	Zhongzhai, Lincang City, Yunnan	Wen et al. 2013	Sino-Drynaria clade	Schneider et al. in prep.
Goniophlebium macrosorum C.L.Xu & Z.K.Zhou	Middle Miocene	Xiaolongtan Formation	Dashidong, Wenshan, Yunnan	Xu et al. 2017b	*Goniophlebium* (*Polypodioides*) *amoenum* lineage	Chen et al. 2020
Humata henryana (Baker) Ching	Middle Miocene	Bangmai Formation	Zhongzhai, Lincang City, Yunnan	Wen et al. 2013	*Davallia* sect. *Humata*	Chie et al. 2016
Neolepisorus chingii Xie et al.	Late Miocene	Bangmai Formation	Zhongzhai, Lincang City, Yunnan	Xie et al. 2016	*Lepisorus* sect. *Neolepisorus*	Zhao et al. 2019
Oleandra bangmaii San-Ping Xie & Xian-CHing Zhang	Late Miocene	Bangmai Formation	Zhongzhai, Lincang City, Yunnan	Xie et al. 2018	*Oleandra*	PPGI (2016)
Palaeosorum ellipticum F.M.B.Jacques & Z.K.Zhou	Middle Miocene	Dajie Formation	Shanzhangtian Basin, Zhenyuan, Yunnan	Jacques et al. 2013	*Lepisorus* sect. *Neolepisorus*	Zhao et al. 2019

Name of fossil species (alphabetical order), stratigraphic age, geological formation, location, fossil publication, phylogeny used to interpret the fossil

2020; Schneider et al. pers. comm.). The western parts of Yunnan are strongly influenced by the Indian summer monsoons, whereas the eastern parts are impacted by the East Asian summer monsoons. Thus, *L. cespitosus* may represent a taxon that survived in climatic conditions shaped by the Indian summer monsoons, whereas several sections of core *Lepisorus* diversified under the impact of the South East Asian monsoons (Wang et al. 2012b). This scenario is consistent with our current understanding of the intensified monsoon regimes during the Pliocene and Late Miocene (Farnsworth et al. 2019).

Conservation status

Our newly discovered locality is a disturbed forest patch along a paved road close to a river. At the location, we observed only four individuals in which one was collected. Thus, it is suggested that this species may be very rare in this area. With only two available collections obtained from rather distinct regions, The IUCN Red List status of this taxon is best designed as data deficient. Both known occurrences were collected in regions under natural protection namely the Medog Nature Reserve in Nyingchi, Tibet and the Tongbiguan Provincial Nature Reserve, Dehong, Yunnan. Thus, both occurrences are within the vicinity of areas identified as biodiversity conservation priority areas in China (López-Pujol et al. 2011). This pattern is quite encouraging because established protection areas will support the conservation of the species during the current biodiversity crisis. Despite the putative protection, further studies are required to confirm the distribution range and local abundance of this species with emphases on the occurrence in less disturbed forests that are under protection.

Perspectives

The rediscovery of *Lepisorus cespitosus* not only expanded its range from SE Tibet toward western Yunnan in southwest China but also provided new insights into the phylogenetic history of a taxonomic complex genus *Lepisorus*. Furthermore, this rediscovery added support to the hypothesis that western Yunnan shares a fern flora with SE Tibet and perhaps northern Myanmar. This flora may contain a large number of relict ferns that require special attention to preserve the unique genetic diversity that only survived in this region. In summary, the reported evidence suggests the need of a large-scale and comprehensive research program to explore the hypothesis that this region acted as a refugia of land plant diversity during the climatic fluctuations in the Neogene and its role during the Anthropocene.

Taxonomic treatment

Lepisorus* sect. *Caespes T.Fujiw., **sect. nov.**—TYPE: *Lepisorus cespitosus* Y.X.Lin

Description: Rhizome short-creeping or erect; scales lanceolate, clathrate with small lumina, margin dentate. Fronds clustered; Lamina lanceolate, apex elongated. Sori close to margin; Paraphyses stellate, opaque with thick lumina.

Species and distribution: One known species, *Lepisorus cespitosus*, distributes in southern Tibet and western Yunnan close border to Myanmar.

Section *Caespes* was used to be treated as belonging to the sect. *Sclerophyllon* based on morphological observation in Qi et al. (2010). These two sections share several morphological characteristics including concolorous clathrate scales, coriaceous laminae, and stellate paraphyses. However, *L. cespitosus* does not exhibit red veins in fresh lamina and large lumina in scales that is synapomorphies in the sect. *Sclerophyllon*. The former is easily distinguished from the latter by shortly creeping rhizome and clustered leaves.

Key to the sections of the core *Lepisorus*

1a Lamina dimorphic .... **sect. *Weatherbya*** (Copel.) C.F.Zhao, R.Wei & X.C.Zhang
1b Lamina monomorphic 2
2a Rhizome scale clathrate 3
2b Rhizome scale with opaque band 10
3a Rhizome scale margin dentate 4
3b Rhizome scale margin entire 9
4a Paraphyses stellate 5
4b Paraphyses circular or lanceolate 8
5a Plants summer green; Lamina herbaceous .... **sect. *Hymenophyton*** Ching ex S.L.Yu & Y.X.Lin
5b Plants evergreen; Lamina coriaceous 6
6a Lamina apex contracted into fertile tip .... **sect. *Belvisia*** (Mirb.) C.F.Zhao, R.Wei & X.C.Zhang
6b Lamina apex obtuse, acute to attenuate, not contracted fertile into tip 7
7a Rhizome long-creeping; Fronds remote .... **sect. *Sclerophyllon*** S.L.Yu
7b Rhizome short-creeping; Fronds clustered .... **sect. *Caespes*** T.Fujiw
8a Lamina narrowly linear; Sori linear, continuous; Paraphyses circular .... **sect. *Drymotaenium*** (Makino) C.F.Zhao & X.C.Zhang
8b Lamina lanceolate; Sori circular; Paraphyses circular or lanceolate .... **sect. *Ussurienses*** X.P.Qi ex C.F.Zhao, R.Wei & X.C.Zhang

9a Plants evergreen; Rhizome scale ovate or orbicular with or without long hairs on the base .... **sect. *Lepisorus***
9b Plants summer green; Rhizome scale lanceolate or broad lanceolate .... **sect. *Pachyphyllon*** S.L.Yu
10a Lamina lanceolate, apex obtuse or subobtuse...... .... **sect. *Obtusi*** C.F.Zhao, R.Wei & X.C.Zhang
10b Lamina lanceolate or linear, apex acute, acuminate or cordate but obtuse when lamina is linear (*Lepisorus lewisii*) .... **sect. *Pseudovittaria*** (C.Chr.) Ching

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Authors' contributions TF, HL, and HS planned and designed research; TF, PKK, AZ, SZ, and DX collected specimens. TF conducted experiments and performed analyses. TF, HL, PKK, and HS prepared manuscript.

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Availability of data and material Voucher specimen used in this study was deposited in HIBIC. All sequences generated in this study were uploaded in GenBank.Code availability Nexus file used for phylogenetic analysis can be downloaded as Supplementary Data.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Information on Electronic Supplementary Material

Online Resource 1. Information about vouchers and GenBank accession numbers of all accessions used in this study.
Online Resource 2. Nexus File including the alignment used to generate the phylogenetic hypotheses and divergence time estimates.

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