•研究报告•

利用整合分类学方法进行蕨类植物复合体的物种划分：以线裂铁角蕨复合体为例

梁思琪^{1,2} 张宪春¹ 卫 然^{1*}

1 (中国科学院植物研究所系统与进化植物学国家重点实验室, 北京 100093)

2 (中国科学院大学, 北京 100049)

摘要：广泛的杂交和多倍化使得铁角蕨属(*Asplenium*)下存在着许多分类困难的物种复合体，针对这些类群进行整合分类学的研究，有助于我们更加全面和深入地理解物种的界限以及形成机制。线裂铁角蕨复合体(*Asplenium coenobiale* complex)是铁角蕨属下一个形态多样性较高的类群，由于缺乏全面取样和系统研究，该复合体的物种划分长期存在争议。本研究选取线裂铁角蕨复合体中形态变异和地理分布具有代表性的个体，通过孢粉学研究确定该类群的生殖特性，运用流式细胞分析获取倍性信息，同时结合叶绿体和核基因组片段系统发生分析的证据，对该类群的系统演化关系和起源方式进行了探讨。结果表明：(1)虽然部分孢子囊败育的情况在线裂铁角蕨复合体中十分普遍，但正常孢子囊内形成的64个孢子说明该类群植物仍能进行正常的有性生殖；(2)该复合体中存在着倍性变异，其中多角铁角蕨(*A. cornutissimum*)是二倍体，而其他成员均为四倍体；(3)依据母系遗传的叶绿体序列所构建的系统发生关系将该类群划为4个分支，与基于核基因序列构建的系统树存在冲突，这暗示杂交可能在该复合体的形成过程中起到了重要的推动作用。综上所述，我们建议将线裂铁角蕨复合体划分为4个物种，即同源四倍体新种马关铁角蕨(*A. maguanense* sp. nov.)，二倍体多角铁角蕨，以及两个由同一对亲本正反交产生的异源四倍体线裂铁角蕨(*A. coenobiale*)和叶基宽铁角蕨(*A. pulcherrimum*)。

关键词：叶绿体基因组序列；*pgiC*；线裂铁角蕨复合体；杂交；多倍体

Integrative taxonomy resolved species delimitation in a fern complex: A case study of the *Asplenium coenobiale* complex

Siqi Liang^{1,2}, Xianchun Zhang¹, Ran Wei^{1*}

1 State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093

2 University of Chinese Academy of Sciences, Beijing 100049

Abstract: Due to wide hybridization and polyploidization, there are numerous species complexes with taxonomic challenges in the fern genus *Asplenium*. Integrative taxonomy using evidence of morphology, cytology and molecular phylogeny provides one of the best ways for the discovery and delimitation of species. The *Asplenium coenobiale* complex represents one of the spleenwort complexes, which are morphologically variable and difficult in species delimitation. Owing to the lack of comprehensive sampling and systematic study, the taxonomy of this complex remains unresolved. In this study, we selected representative individuals of this complex covering differences in morphology and geography. We conducted a palynological study to explore reproductive characteristics, and inferred the ploidy level through flow cytometry. Furthermore, based on the phylogenetic evidence from chloroplast and nuclear genomes, we discussed the evolutionary relationship and origin of this complex. Our results showed that: (1) The development of 64 spores within a single normal sporangium is indicative of the ability of sexual reproduction, although abortive sporangia are common in the *Asplenium coenobiale* complex. (2) Ploidy variation is found in this complex, i.e. *A. cornutissimum* is diploid, whereas other members are all tetraploid. (3) The maternally inherited chloroplast

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* 通讯作者 Author for correspondence. E-mail: weiran@ibcas.ac.cn

phylogeny supported four clades within this complex, and this was incongruent with the nuclear phylogeny; therefore, it was inferred that hybridization could be an important driving force during the formation of the complex. Based on our analyses, we conduct a revision to the *A. coenobiale* complex, i.e. one newly discovered autotetraploid species (*A. maguanense* sp. nov.), one diploid species (*A. cornutissimum*), and two allotetraploids with reciprocal origins (*A. coenobiale* and *A. pulcherrimum*).

Key words: chloroplast sequences; *pgiC*; *Asplenium coenobiale* complex; hybridization; polyploidy

自然杂交和多倍化事件在植物的演化历史中较为常见,对物种多样性的形成具有重要的意义(Rieseberg & Willis, 2007; Soltis et al, 2014; 商辉和严岳鸿, 2017; 王玉国, 2017)。蕨类植物中的多倍体比例显著高于植物界其他类群(Wood et al, 2009),而铁角蕨科铁角蕨属(*Asplenium*)作为蕨类植物中种类较多、形态变异较大且多倍体水平较高的类群,是研究蕨类植物物种形成和演化机制的经典模式类群(PPG I 2016; 常艳芬, 2017; Schneider et al, 2017)。线裂铁角蕨复合体(*Asplenium coenobiale* complex)是铁角蕨属的一个自然类群,它以略带紫色光泽的黑褐色叶柄区别于同属变异叶系(*Ser. Variantia*, 吴兆洪, 1999)的其他类群,其单系性在基于多个叶绿体片段的系统发生研究中也得到了支持(Xu et al, 2019)。

由于叶片分裂程度存在很大幅度的连续变异,线裂铁角蕨复合体内部的物种划分长期以来存在着较大争议(DeVol, 1975; 王培善和王筱英, 2001; 李建宗, 2004; 吴兆洪, 2006; 张光飞, 2006)。在该复合体中,目前只有广西靖西特有种多角铁角蕨(*A. cornutissimum*, Jiang et al, 2011)不存在分类上的争议和鉴定上的困难。而剩余的类群在《中国植物志》(吴兆洪, 1999)中被处理为4种:线裂铁角蕨(*A. coenobiale*)、乌木铁角蕨(*A. fuscipes*)、都匀铁角蕨(*A. toramanum*)和黑柄铁角蕨(*A. subtoramanum*);但*Flora of China* (Lin & Viane, 2013)则以末回裂片中的叶脉数(2–6条,或仅1条)为主要分类依据,仅承认了线裂铁角蕨和叶基宽铁角蕨(*A. pulcherrimum*)两种。然而,近期的叶绿体系统发生研究支持了将剩余类群划分为4支的处理方式,并显示该复合体中可能还存在一个形态上与线裂铁角蕨较为相似的隐种(Xu et al, 2019)。

近年来对多个铁角蕨属下物种复合体的研究表明,整合分类学的方法,即综合考虑形态学、细胞学以及来自核基因组和叶绿体基因组的分子系

统学证据可以很好地帮助解析复合体内部的关系,并为物种划分提供参考依据(Dyer et al, 2012; Ohlssen et al, 2015; Chang et al, 2018; Liang et al, 2019)。在蕨类植物中,杂交起源的物种形态通常表现为介于其两亲本间的中间过渡状态(Wagner, 1954; Lovis, 1977; Viane & Reichstein, 2003),而杂交和通常与之相伴的多倍化现象是否是造成线裂铁角蕨复合体物种划分困难的原因,目前还有待论证。现有的分子系统学证据仅来自于叶绿体基因组(Xu et al, 2019),但由于铁角蕨的叶绿体是单亲母系遗传(Vogel et al, 1998),缺乏双亲遗传的分子证据,所以无法对杂交问题进行探究。在细胞学方面,线裂铁角蕨和叶基宽铁角蕨均为四倍体(Lin & Viane, 2013),而多角铁角蕨的倍性仍属未知。此外,由于部分败育的孢子在这群植物中经常被观察到(Lin & Viane, 2013),其生殖模式(是否为无融合生殖)同样值得深入挖掘。

综合以上论述,本研究取样覆盖了线裂铁角蕨复合体中形态变异最具代表性的个体,整合形态学、孢粉学、细胞学的研究方法,以及叶绿体基因组片段和核基因组单低拷贝基因分子系统学的比较分析,旨在回答以下问题:(1)该类群中是否存在无融合生殖的现象?(2)该类群中是否存在倍性变异?(3)该类群植物连续性形态变异的形成是否与杂交有关?(4)如何对该类群进行较为合理的物种划分?隐种在该类群中是否存在?

1 材料与方法

1.1 样品采集

在查阅该复合体中正式发表过的名称对应的原始文献和模式标本,以及中国科学院植物研究所标本馆(PE)的所有相关标本后,我们认为*Flora of China* (Lin & Viane, 2013)中的形态划分标准是相对明确且广泛适用的,因而对所有样品的初步鉴定都据此进行。

本研究中收集到的样品覆盖了线裂铁角蕨复合体中不同程度的形态变异类型(图1), 共27份取自不同个体的硅胶干燥叶片, 用于总DNA的提取及后

续实验, 并运用流式细胞分析对其中部分保留有新鲜叶片的个体进行了核DNA含量测定。样品对应的分布情况见图2。同时, 选取变异叶系中, 与该复合

图1 线裂铁角蕨复合体的叶片分裂程度变异(比例尺均为2 cm)。(A)叶基宽铁角蕨(8573)全株, 二回羽状至羽状全裂; (B)叶基宽铁角蕨(9525)叶片, 二回羽状至三回羽状-羽状全裂; (C)线裂铁角蕨(9439-1)全株, 二回羽状至羽状半裂; (D)线裂铁角蕨(9524-6)叶, 二回羽状至羽状全裂; (E)线裂铁角蕨(9524-10)全株, 二回羽状至三回羽状-羽状半裂; (F)叶基宽铁角蕨(9443)基部羽片, 三回羽状至四回羽状-羽状全裂; (G)叶基宽铁角蕨(9524-1)基部羽片, 三回羽状至四回羽状-羽状全裂; (H)线裂铁角蕨(9524-17)叶片, 二回羽状至三回羽状-羽状半裂; (I)线裂铁角蕨(9524-4)基部羽片, 三回羽状至羽状全裂; (J)线裂铁角蕨(9524-16)叶片, 二回羽状。

Fig. 1 Variation of the frond division within the *Asplenium coenobiale* complex (Scale bar = 2 cm). (A) Habit of *A. pulcherrimum* (8573), bipinnate-pinnatisect; (B) Lamina of *A. pulcherrimum* (9525), bipinnate to tripinnate-pinnatisect; (C) Habit of *A. coenobiale* (9439-1), bipinnate-pinnatifid; (D) Frond of *A. coenobiale* (9524-6), bipinnate-pinnatisect; (E) Habit of *A. coenobiale* (9524-10), bipinnate to tripinnate-pinnatifid; (F) Basal pinnae *A. pulcherrimum* (9443), tripinnate to quadripinnatisect; (G) Basal pinnae *A. pulcherrimum* (9524-4), tripinnate to quadripinnatisect; (H) Lamina of *A. coenobiale* (9524-17), bipinnate to tripinnate-pinnatifid; (I) Basal pinnae *A. coenobiale* (9443), tripinnate-pinnatisect; (J) Lamina of *A. coenobiale* (9524-16), bipinnate.

叶片形态特征(末回裂片中所包含的叶脉数) Lamina morphology (number of veins per ultimate segment):

◇ 仅一条叶脉 Only a single vein ◇ 一至多条叶脉 One to several veins

在叶绿体基因组片段系统发生树中所处位置 Chloroplast phylogeny:

○ 分支I Clade I ○ 分支II Clade II ○ 分支III Clade III ○ 分支IV Clade IV

在核基因片段 $pgiC$ 系统发生树中所处位置 $pgiC$ nuclear phylogeny:

● 分支I Clade I ● 分支II Clade II ● 分支III Clade III ● 分支IV Clade IV

举例 For example:

◆ 线裂铁角蕨 *A. coenobiale* 9524-17

- 末回裂片中包含一至多条叶脉 One to several veins per ultimate segment
- 位于叶绿体基因组片段系统发生树中的分支I Located in Clade I within the chloroplast phylogeny
- 多个核基因拷贝分别位于核基因片段 $pgiC$ 系统发生树中的分支I和分支IV Multiple copies from Clade I and Clade IV within the nuclear phylogeny

图2 线裂铁角蕨复合体的采样分布图。DZ、LD、JL、NP、DY、DQK、ML、PA、CL、HL、BZ、GLQ、PJ含义同表1。
Fig. 2 Map of localities of the examined accessions of the *Asplenium coenobiale* complex. DZ, LD, JL, NP, DY, DQK, ML, PA, CL, HL, BZ, GLQ, PJ are the same as Table 1.

体中的叶基宽铁角蕨在形态上易混淆且在产地上有重合的细裂铁角蕨(*A. tenuifolium*)、线柄铁角蕨(*A. capillipes*)和易变铁角蕨(*A. fugax*)加入研究。所有凭证标本保存在PE, 详细信息见表1。

1.2 孢粉学研究

使用Leica DM-4000光学显微镜(Wetzlar, Germany)和Hitachi S-4800扫描电子显微镜(Tokyo, Japan)对孢子形态、发育情况、每孢子囊内孢子数以及孢子外壁长度(length of exospore, Lexo)进行观察统计和测量。收集干燥叶片上未经处理的孢子置于载玻片上, 使用中性树胶封片后用光镜下的形态观察和长度测量(每个个体至少测量30个孢子)。由于在蕨类植物近缘类群中, 物种的倍性与孢子大小存在一定的正相关性(Lovis & Reichstein, 1964; Barrington et al, 1986; Lin & Viane, 2013; Liang et al, 2019), 因此与该属变异叶系其他已有记录的类群相比较, 可对相应个体的倍性进行初步的推测。挑

取发育正常的完整孢子囊置于载玻片上, 破坏孢子囊释放出所有孢子后封片, 用以每孢子囊内孢子数的统计(每个个体选取2-10个孢子囊)。将未经处理的孢子用双面胶固定在样品台上, 喷铂金后置扫描电镜下观察。

1.3 分子系统发生分析

用植物基因组DNA提取试剂盒(TIANGEN Biotech, 北京)从硅胶干燥的组织样品中提取总DNA。用4对引物(表2)对叶绿体基因组中的3个片段(*rbcL*、*trnL-trnF*、*rpl32-trnP*)和核基因 $pgiC$ 片段进行PCR扩增, 扩增反应体积为25 μ L。反应程序为: 94 $^{\circ}$ C预变性5 min, 94 $^{\circ}$ C变性30 s, 退火30 s (退火温度: *rbcL*, 52 $^{\circ}$ C; *trnL-trnF*, 56 $^{\circ}$ C; *rpl32-trnP*, 56 $^{\circ}$ C; *pgiC*, 58 $^{\circ}$ C), 72 $^{\circ}$ C延伸75 s, 循环反应35次后72 $^{\circ}$ C延伸5 min。PCR产物经1%琼脂糖凝胶电泳检测后送至测序公司纯化并测序。对于具有多个不同核基因 $pgiC$ 拷贝的个体, 使用pEASY-T1克隆试剂盒

表1 本研究中的铁角蕨属样品采集和序列信息。所有凭证标本均保存在PE。**Table 1** Information of locality and sequences of *Asplenium* involved in this study. All specimens are deposited in PE.

类群 Taxon	采集地 Locality	凭证标本, 采集人 Voucher, collector (s)	基因库序列号 GenBank accession number			
			<i>rbcL</i>	<i>rpl32-trnP</i>	<i>trnL-trnF</i>	<i>pgc</i>
多角铁角蕨 <i>A. cornutissimum</i>	广西靖西地州	Dizhou, Jingxi, Guangxi (DZ)	MN688474	MN688505	MN688535	MN688445, MN688446
叶基宽铁角蕨 <i>A. pulcherrimum</i>	广西靖西地州	Dizhou, Jingxi, Guangxi (DZ)	MN688470	MN688500	MN688530	MN688434, MN688435
叶基宽铁角蕨 <i>A. pulcherrimum</i>	广西靖西禄峒	Ludong, Jingxi, Guangxi (LD)	—	MN688499	MN688529	MN688431, MN688432, MN688433
叶基宽铁角蕨 <i>A. pulcherrimum</i>	广西龙州金龙	Jinlong, Longzhou, Guangxi (JL)	—	MN688502	MN688532	MN688438, MN688439
线裂铁角蕨 <i>A. coenobiale</i>	广西那坡	Napo, Guangxi (NP)	MN688452	MN688481	MN688511	MN688381, MN688382, MN688383
(似)线裂铁角蕨 <i>A. aff. coenobiale</i>	广西那坡	Napo, Guangxi (NP)	MN688473	MN688504	MN688534	MN688442, MN688443, MN688444
叶基宽铁角蕨 <i>A. pulcherrimum</i>	广西那坡	Napo, Guangxi (NP)	MN688460	MN688489	MN688519	MN688403, MN688404, MN688405
线裂铁角蕨 <i>A. coenobiale</i>	贵州都匀	Duyun, Guizhou (DY)	MN688453	MN688482	MN688512	MN688384, MN688385
叶基宽铁角蕨 <i>A. pulcherrimum</i>	贵州都匀	Duyun, Guizhou (DY)	MN688469	MN688498	MN688528	MN688429, MN688430
线裂铁角蕨 <i>A. coenobiale</i>	贵州都匀	Duyun, Guizhou (DY)	MN688451	MN688480	MN688510	MN688378, MN688379, MN688380
叶基宽铁角蕨 <i>A. pulcherrimum</i>	贵州荔波大七孔	Daqikong, Libo, Guizhou (DQK)	MN688465	MN688494	MN688524	MN688417, MN688418
线裂铁角蕨 <i>A. coenobiale</i>	贵州荔波大七孔	Daqikong, Libo, Guizhou (DQK)	MN688459	MN688488	MN688518	MN688400, MN688401, MN688402
线裂铁角蕨 <i>A. coenobiale</i>	贵州荔波大七孔	Daqikong, Libo, Guizhou (DQK)	MN688464	MN688493	MN688523	MN688415, MN688416
线裂铁角蕨 <i>A. coenobiale</i>	贵州荔波大七孔	Daqikong, Libo, Guizhou (DQK)	MN688458	MN688487	MN688517	MN688396, MN688397, MN688398, MN688399
线裂铁角蕨 <i>A. coenobiale</i>	贵州荔波大七孔	Daqikong, Libo, Guizhou (DQK)	MN688457	MN688486	MN688516	MN688394, MN688395
线裂铁角蕨 <i>A. coenobiale</i>	贵州荔波大七孔	Daqikong, Libo, Guizhou (DQK)	MN688455	MN688484	MN688514	MN688388, MN688389, MN688390
叶基宽铁角蕨 <i>A. pulcherrimum</i>	贵州荔波大七孔	Daqikong, Libo, Guizhou (DQK)	MN688467	MN688496	MN688526	MN688422, MN688423, MN688424, MN688425
线裂铁角蕨 <i>A. coenobiale</i>	贵州荔波茂兰	Maolan, Libo, Guizhou (ML)	MN688454	MN688483	MN688513	MN688386, MN688387
线裂铁角蕨 <i>A. coenobiale</i>	贵州荔波茂兰	Maolan, Libo, Guizhou (ML)	MN688456	MN688485	MN688515	MN688391, MN688392, MN688393
线裂铁角蕨 <i>A. coenobiale</i>	贵州三都普安	Pu'an, Sandu, Guizhou (PA)	MN688450	MN688479	MN688509	MN688376, MN688377
叶基宽铁角蕨 <i>A. pulcherrimum</i>	贵州三都普安	Pu'an, Sandu, Guizhou (PA)	MN688468	MN688497	MN688527	MN688426, MN688427, MN688428
叶基宽铁角蕨 <i>A. pulcherrimum</i>	湖南张家界慈利	Cili, Zhangjiajie, Hunan (CL)	MN688462	MN688491	MN688521	MN688409, MN688410, MN688411
叶基宽铁角蕨 <i>A. pulcherrimum</i>	台湾花蓮	Hualian, Taiwan (HL)	MN688461	MN688490	MN688520	MN688406, MN688407, MN688408
(似)线裂铁角蕨 <i>A. aff. coenobiale</i>	云南马关八寨	Bazhai, Maguan, Yunnan (BZ)	MN688472	MN688503	MN688533	MN688440, MN688441
叶基宽铁角蕨 <i>A. pulcherrimum</i>	云南马关八寨	Bazhai, Maguan, Yunnan (BZ)	MN688471	MN688501	MN688531	MN688436, MN688437
叶基宽铁角蕨 <i>A. pulcherrimum</i>	云南马关古林箐	Gulinqing, Maguan, Yunnan (GLQ)	MN688466	MN688495	MN688525	MN688419, MN688420, MN688421
叶基宽铁角蕨 <i>A. pulcherrimum</i>	云南马关坡脚	Pojiao, Maguan, Yunnan (PJ)	MN688463	MN688492	MN688522	MN688412, MN688413, MN688414
线柄铁角蕨 <i>A. capillipes</i>	甘肃文县铁楼	Tielou, Wenxian, Gansu	MN688475	MN688506	MN688536	MN688448
易变铁角蕨 <i>A. fugax</i>	四川盐边百灵山	Mt. Bailingshan, Yanbian, Sichuan	MN688476	MN688507	MN688537	MN688449
细裂铁角蕨 <i>A. tenuifolium</i>	云南贡山独龙江	Dulongjiang, Gongshan, Yunnan	MN688477	MN688508	MN688539	MN688447
细裂铁角蕨 <i>A. tenuifolium</i>	云南马关八寨	Bazhai, Maguan, Yunnan	MN688478	MK828791	MN688538	MK828922

表2 本研究所采用的分子标记信息

Table 2 Information of molecular markers in this study

片段 Fragment	引物 Primer	引物序列(5'-3') Primer sequence (5'-3')	最佳碱基替代模型 Best substitution model	长度 Length (bp)	参考文献 Reference
<i>trnL-trnF</i>	Fern-1	GGCAGCCCCCARATTCAGGGRAACC	K81UF+G	693	Trewick et al, 2002
	f	ATTTGAACTGGTGACACGAG			Taberlet et al, 1991
<i>rbcL</i>	1F	ATGTCACCACAAACAGA(G/A)ACTAAAGC	GTR+I+G	1,197	Gastony & Rollo, 1995
	1351R	CTTCACAAGCAGCAGCTAGTTCAGGACTCC			Gastony & Rollo, 1995
<i>rpl32-trnP</i>	112F	TCCATCTTAACCGGTCGTCGTTCA	TVM+G	644	Liang et al, 2019
	858R	AGTTTGGTAGCGCGTCATCT			Liang et al, 2019
<i>pgiC</i>	14F	GTGCTTCTGGGTCTTTTGAAGT	HKY+F+G4	648	Ishikawa et al, 2002
	16R	GTTGTCCATTAGTTCAGGTTCCCC			Ishikawa et al, 2002

(Transgen Biotech, 北京)对纯化后的PCR扩增产物进行克隆, 每个个体随机选取10–20个阳性克隆进行测序。

所得叶绿体序列使用ContigExpress v.6.0 (Informax, Inc., USA)拼接, 通过Clustal X (Thompson et al, 1997)进行比对, 之后在BioEdit v.7.0.1 (Hall, 1999)中进行人工校正。从GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>)下载以下类群的*rbcL*序列加入分析: 线裂铁角蕨、变异叶系内的其他类群代表、铁角蕨属变异叶系外的代表和膜叶铁角属(*Hymenasplenium*)的代表。用PhyloSuite (Zhang et al, 2018)对3个叶绿体片段进行拼接组合, 以AIC (Akaike, 1973)标准在PartitionFinder2 (Lanfear et al, 2016)中选择3个片段最佳的碱基替代模型。

对于核基因*pgiC*序列, 先对每个个体的所有克隆测序结果进行拼接和比对, 并去除其中相同和嵌套的序列。然后将所有个体的剩余序列合并为一个序列矩阵, 使用MEGA7 (Kumar et al, 2016)进行邻接(neighbor-joining, NJ)和最大似然(maximum likelihood, ML)分析, 从所得系统发生树中筛选出不同的基因型, 并手工修正其中由PCR错误造成的可疑位点, 获得最终的一致序列矩阵。从GenBank下载铁角蕨属下变异叶系以内和以外的代表类群的*pgiC*序列加入分析, 以AIC标准在ModelFinder2 (Kalyaanamoorthy et al, 2017)中筛选出最佳的碱基替代模型。

依据上面所选定的碱基替代模型, 使用最大似然分析和贝叶斯推断(Bayesian inference, BI)两种方法分别构建叶绿体基因组片段和*pgiC*序列矩阵的系统发生关系。最大似然法分析采用IQ-TREE (Nguyen et al, 2015)实现, 选用默认参数, 重复检验

5,000次以获得自展支持率。贝叶斯推断法则采用MrBayes 3.2.6 (Ronquist et al, 2012)实现, 运行2,000,000代, 起始的25%样本作为老化样本(burn-in samples)丢弃。

1.4 核基因组含量测定

使用BD LSRFortessa流式细胞仪(San Jose, CA, USA)对所收集到的新鲜材料进行核基因组含量测量。选取玉米(*Zea mays* ssp. *mays* L. “B73”, 5.64pg/2C, Díez et al, 2013)作为内部参照, 通过Otto (1990)的两步法进行细胞核提取, 经荧光染料碘化丙啶(propidium iodide, PI)染色后进行上机检测。对每个样品的分析尽可能重复3次以上, 并计算平均值和标准差。参考*Flora of China* (Lin & Viane, 2013)中对线裂铁角蕨和多角铁角蕨的倍性记录, 对所有样品的倍性进行推断。

2 结果

2.1 孢粉学研究

大量的孢子取样和观察结果表明, 线裂铁角蕨复合体的多数类群(除多角铁角蕨外)中都存在正常孢子和败育孢子共存的情况(图3D–K), 且所有类群中正常发育的孢子囊内的孢子数均为64个。同时, 孢子大小测量结果(表3)显示, 多角铁角蕨孢子外壁长度的平均值(27 μm), 小于该复合体中其他类群的平均值(32–35 μm), 因而多角铁角蕨在倍性上很有可能与其他类群存在差异。通过扫描电镜观察正常孢子(图3A–C, L–M), 我们发现该复合体内不同类群的周壁纹饰存在差异: 多角铁角蕨孢子周壁具明显的狭翅, 而其余类群则主要为脊状–鸡冠状隆起。

2.2 分子系统发生分析

经比对照正后, 最终用于系统发生分析的单个

图3 线裂铁角蕨复合体的孢子。(A) (似)线裂铁角蕨(8214), 正常孢子, 孢子外壁具脊(鸡冠状-翅状); (B) (似)线裂铁角蕨(9439-2), 正常孢子, 孢子外壁具脊(鸡冠状-翅状); (C)多角铁角蕨(9444), 正常孢子, 孢子外壁具翅; (D) (似)线裂铁角蕨(8214), 正常孢子(肾形, 饱满)和败育孢子(干瘪); (E) (似)线裂铁角蕨(9439-2), 正常孢子和畸形且发黑的孢子; (F)多角铁角蕨(9444), 正常孢子, 明显小于该复合体中其他类群的孢子; (G)线裂铁角蕨(9524-10), 正常孢子和干瘪、畸形的孢子; (H)线裂铁角蕨(9524-16), 正常孢子和畸形孢子; (I)叶基宽铁角蕨(9524-1), 正常孢子和发黑的孢子; (J)叶基宽铁角蕨(9443), 正常孢子和畸形、发黑的孢子; (K)线裂铁角蕨(8057), 干瘪的败育孢子; (L)线裂铁角蕨(9524-17), 正常孢子, 孢子外壁具脊(鸡冠状-翅状); (M)叶基宽铁角蕨(8059B), 正常孢子, 孢子外壁具脊(鸡冠状-翅状)。A-C, K-M中比例尺为10 μm ; D-J中比例尺为50 μm 。

Fig. 3 Spores of the *Asplenium coenobiale* complex. (A) *A. aff. coenobiale* (8214), a normal spore, perispore lophate (cristate-alate); (B) *A. aff. coenobiale* (9439-2), a normal spore, perispore lophate (cristate-alate); (C) *A. cornutissimum* (9444), a normal spore, perispore alate; (D) *A. aff. coenobiale* (8214), a normal spore (kidney-shaped, well-filled) and abortive spores (shrivelled); (E) *A. aff. coenobiale* (9439-2), normal spores and several misshapen, blackened spores; (F) *A. cornutissimum* (9444), normal spores, and the size of which is relatively small compared with other accessions' spores; (G) *A. coenobiale* (9524-10), normal spores and several misshapen, shrivelled spores; (H) *A. coenobiale* (9524-16), normal spores and several misshapen spores; (I) *A. pulcherrimum* (9524-1), normal spores and several blackened spores; (J) *A. pulcherrimum* (9443), normal spores and several misshapen, blackened spores; (K) *A. coenobiale* (8057), shrivelled, abortive spores; (L) *A. coenobiale* (9524-17), a normal spore, perispore lophate (cristate-alate); (M) *A. pulcherrimum* (8059B), a normal spore, perispore lophate (cristate-alate). Scale bars in A-C and K-M are 10 μm ; scale bars in D-J are 50 μm .

表3 线裂铁角蕨复合体细胞学和孢粉学研究结果。-: 数据缺失。**Table 3** Results from cytological and palynological examinations of the *Asplenium coenobiale* complex. -, Data missing.

凭证标本 Voucher specimen	核DNA含量 Nuclear DNA content (pg)		孢子外壁长度 Length of exospore (μm)	推定倍性 Inferred ploidy level	孢子囊内孢子数(统计的孢子囊个数) Spore number per sporangium (Number of checked sporangia)
	2C	1Cx			
<i>A. cornutissimum</i> 9444	11.3 ± 0.5	5.7 ± 0.2	(22-) 25-27-29 (-30)	2x	64 (10)
<i>A. pulcherrimum</i> 9443	15.8 ± 0.1	4.0 ± 0.0	(29-) 32-35-37 (-39)	4x	64 (4)
<i>A. pulcherrimum</i> 9525	16.1 ± 0.5	4.0 ± 0.1	(26-) 30-33-36 (-37)	4x	64 (2)
<i>A. pulcherrimum</i> 8059B	16.7	4.2	—	4x	64 (2)
<i>A. pulcherrimum</i> 9524-1	17.3 ± 0.2	4.3 ± 0.0	(28-) 31-34-38 (-41)	4x	64 (6)
<i>A. coenobiale</i> 9524-13	17.4 ± 0.0	4.3 ± 0.0	—	4x	64 (6)
<i>A. pulcherrimum</i> 9440	17.5 ± 0.2	4.4 ± 0.1	—	4x	—
<i>A. coenobiale</i> 9524-10	17.6 ± 0.4	4.4 ± 0.1	(28-) 29-33-36 (-41)	4x	64 (4)
<i>A. coenobiale</i> 8059A	17.9	4.5	—	4x	—
<i>A. coenobiale</i> 8057	18.3 ± 0.1	4.6 ± 0.0	—	4x	64 (4)
<i>A. coenobiale</i> 9505	18.3 ± 0.1	4.6 ± 0.0	—	4x	64 (3)
<i>A. coenobiale</i> 9524-17	18.3 ± 0.2	4.6 ± 0.0	—	4x	64 (2)
<i>A. coenobiale</i> 9524-16	18.3 ± 0.3	4.6 ± 0.1	(30-) 31-33-35 (-37)	4x	64 (4)
<i>A. coenobiale</i> 9439-1	18.7 ± 0.2	4.7 ± 0.1	—	4x	64 (2)
<i>A. coenobiale</i> 9524-4	18.7 ± 0.4	4.7 ± 0.1	—	4x	64 (4)
<i>A. aff. coenobiale</i> 9439-2	21.2 ± 0.2	5.3 ± 0.1	(28-) 30-33-35 (-39)	4x	64 (1)
<i>A. aff. coenobiale</i> 8214	21.2 ± 0.2	5.3 ± 0.1	(29-) 31-33-35 (-36)	4x	64 (8)
<i>A. pulcherrimum</i> 8245	—	—	(27-) 30-32-35 (-39)	4x	64 (3)
<i>A. pulcherrimum</i> 2016002	—	—	—	—	64 (4)
<i>A. coenobiale</i> 2016017	—	—	—	—	64 (2)

分子标记序列矩阵的长度和对应的最佳碱基替代模型见表2。

2.2.1 叶绿体基因组片段系统发生分析

对叶绿体基因组3个DNA序列的联合矩阵进行最大似然分析和贝叶斯分析所得出的拓扑结构一致(图4): 线裂铁角蕨、叶基宽铁角蕨以及多角铁角蕨共同形成一个分支,并具有很高的支持率($\text{BS}_{\text{ML}} = 100$, $\text{PP}_{\text{BI}} = 1$),而细裂铁角蕨、线柄铁角蕨和易变铁角蕨则均位于该分支之外。该结果支持线裂铁角蕨复合体是一个由几个物种组成的单系群。

在线裂铁角蕨复合体内部存在4个小分支,其中,分支I和II,分支III和IV各自构成两对姐妹群(图4)。分支I和II基本对应于依据形态所划分出的细裂铁角蕨和叶基宽铁角蕨,但两支中均存在一个与鉴定结果有冲突的例外(9440和9524-10);形态上与细裂铁角蕨十分相似的两个个体(8214和9439-2)形成独立的分支III;多角铁角蕨则构成独立的分支IV。分支I除包括分布于中国的个体外,还包含了采自

日本的细裂铁角蕨(AB574855.1,对应凭证标本TNS763925, Ebihara et al, 2010)。

2.2.2 核基因片段 $pgiC$ 系统发生分析

核基因片段 $pgiC$ 在线裂铁角蕨复合体内所有类群中均为多拷贝:多角铁角蕨有2个拷贝,线裂铁角蕨和叶基宽铁角蕨则有2-4个拷贝;细裂铁角蕨、线柄铁角蕨和易变铁角蕨中只存在单拷贝。对 $pgiC$ 序列矩阵进行最大似然分析和贝叶斯分析所得出的拓扑结构一致(图5):线裂铁角蕨、叶基宽铁角蕨以及多角铁角蕨的所有核拷贝共同形成一个分支,并具有很高的支持率($\text{BS}_{\text{ML}} = 100$, $\text{PP}_{\text{BI}} = 1$),细裂铁角蕨、线柄铁角蕨和易变铁角蕨均位于该分支之外。

在线裂铁角蕨复合体中,来自不同个体的核拷贝同样形成了4个小分支:分支I、II、III关系较近,与分支IV构成姐妹类群(图5)。线裂铁角蕨和叶基宽铁角蕨的所有个体均有1-2个核拷贝分别位于分支I和分支IV中;多角铁角蕨的两个核拷贝则分别位于

图4 基于叶绿体基因组3个DNA片段进行最大似然分析获得的系统发生树。系统发生分析的支持率表示顺序为: 最大似然法的自展支持率(BS_{ML})和贝叶斯分析的后验概率(PP_{BI})。图形含义同图2。

Fig. 4 The phylogenetic tree inferred from the maximum likelihood (ML) analysis based on three chloroplast DNA sequences. Numbers above the branches indicate bootstrap values or posterior probability by ML (BS_{ML})/Bayesian inference (PP_{BI}). Meanings of symbols are the same as Fig. 2.

分支III (由其自身独立形成)和分支IV中; 两个疑似线裂铁角蕨的个体(8214和9439-2)分别贡献出两个相似的拷贝构成分支III, 此外, 个体9439-2还有一个位于分支I的核拷贝。

2.3 核基因组含量测定

通过流式细胞分析测算出的核DNA含量见表3 (表中显示个体顺序按2C值进行从小到大排列)。多角铁角蕨的核DNA含量明显小于其他类群, 为11.3 pg/2C; 叶基宽铁角蕨和线裂铁角蕨的核DNA含量

均具有较大幅度的变异, 分别为15.8–17.5 pg/2C和17.4–21.2 pg/2C。

3 讨论

3.1 杂交和多倍化起源假说

孢子囊中32个未减数孢子的产生是蕨类植物进行无融合生殖的前提条件之一(Lovis, 1977), 但此次研究中在线裂铁角蕨复合体观察到的正常孢子囊均含有64个孢子(表3), 由此我们认为该类群在

图5 基于核基因 $pgiC$ 序列进行最大似然分析获得的系统发生树。系统发生分析的支持率表示顺序为：最大似然法的自展支持率(BS_{ML})和贝叶斯分析的后验概率(PP_{BI})。图形含义同图2。

Fig. 5 The phylogenetic tree inferred from the maximum likelihood analysis based on $pgiC$ sequence. Numbers above the branches indicate bootstrap values or posterior probability by ML (BS_{ML})/Bayesian inference (PP_{BI}). Meanings of symbols are the same as Fig. 2.

一般情况下不产生未减数孢子, 不进行无融合生殖。因而有性生殖作为该类群的生殖方式, 为该复合体发生种间杂交提供了前提条件。

本研究中, 所有受试个体均有大部分孢子能正常发育, 因此奇数倍性在线裂铁角蕨复合体中存在的可能性首先被排除; 线裂铁角蕨和叶基宽铁角蕨的染色体数是 $2n = 144$, 均为四倍体(Lin & Viane, 2013), 而多角铁角蕨在孢子大小和核DNA含量上均显著小于这两种(表3), 且与近缘的北京铁角蕨(*Asplenium pekinense*)和变异铁角蕨(*A. varians*)复合体中的二倍体物种相近(Liang et al, 2019), 因此我们推测多角铁角蕨是一个二倍体, 也就是说二倍体和四倍体共存于线裂铁角蕨复合体中。从分子系统发生上看, 基于3个叶绿体DNA片段和核基因构建的系统树(图4, 5)均强烈支持了线裂铁角蕨复合体的单系性(Xu et al, 2019), 然而, 二者的拓扑结构存在着明显的冲突。基于以上两点原因, 我们认为杂交和多倍化机制很可能在该复合体的多样化过程中起到了重要作用。

线裂铁角蕨和叶基宽铁角蕨的种间界限是对该复合体进行物种划分的重点和难点。依据母系遗传和双亲遗传的分子标记各自构建的系统发生关系(图4, 5), 我们推测二者均为异源四倍体, 是同一对亲本(核系统树中的分支I和分支IV)正反交产生的不同结果: 当母本来源于叶绿体系统树分支I时, 异源四倍体为叶片分裂较粗的线裂铁角蕨; 当母本来源于叶绿体系统树分支II时, 异源四倍体为叶片分裂极细的叶基宽铁角蕨。此外, 我们在线裂铁角蕨和叶基宽铁角蕨的所有个体中都观察到了部分败育孢子的产生(图3), 与*Flora of China* (Lin & Viane, 2013)的记录相吻合。已有研究表明, 蕨类植物孢子的败育一般出现在种间杂交后代中, 即来自双亲的染色体在减数分裂期间联会失败, 孢子母细胞无法进行正常的分裂产生孢子(Lovis, 1977; Lin & Sleep, 1989; Viane & Reichstein, 2003; Sigel, 2016); 但在本次研究过程中, 我们并没有在线裂铁角蕨复合体中观察到完全败育的个体。考虑到二者在核基因系统树上的位置难以区分, 我们在这里对线裂铁角蕨和叶基宽铁角蕨中时常产生的败育孢子提出一种可能的解释: 作为同一对亲本正反交的产物, 在杂交及多倍化物种形成初期, 两者的基因组分别经历了各自的快速重组(Leitch & Bennett, 2004;

Soltis et al, 2015), 产生了一定的遗传分化, 然而由于它们的核基因组本质上具有相同的起源, 因而生殖隔离没有完全形成; 当两者杂交时, 正常和败育孢子得以共存, 这使得较弱的基因流在这两个物种间始终存在; 现存多数个体的亲本可能在历史上都或多或少地经历过这样的杂交, 因而部分败育成为这群植物的普遍特性, 同时这也导致了该类群在形态和核基因组含量上较大幅度的连续性变异。两个叶绿体分子系统结果和形态划分相冲突的个体(9440, 9524-10)则很可能是二者近期杂交的例证。

多角铁角蕨是一个很好的二倍体物种(表3), 它在形态上很好辨认, 且不存在孢子败育的现象(图3)。从分子系统学方面来看, 多角铁角蕨很可能是经两个不同二倍体祖先杂交形成的: 其自身在叶绿体系统树中形成一独立的分支IV (图4), 其两个核拷贝之一在核系统树中形成一独立的分支III (图5), 这说明多角铁角蕨具有一个较为独特的母本; 另一个核拷贝则位于主要由线裂铁角蕨和叶基宽铁角蕨构成的核系统树分支IV中(图5), 因此我们推测其父本的来源与线裂铁角蕨和叶基宽铁角蕨相同。一般认为, 由于同倍体杂种在形成初期减数分裂异常, 很难产生能够正常行使功能的配子, 且具有与其两亲本产生回交的风险, 因此相较于多倍化杂交物种形成(产生异源多倍体), 同倍体杂交物种形成事件在自然界中较为罕见且难于被人所发现(Mallet, 2007; Rieseberg & Willis, 2007; Soltis & Soltis, 2009; Yakimowski & Rieseberg, 2014), 在蕨类植物中更是少有记录(Sigel, 2016)。如果能收集到多角铁角蕨的亲本, 并运用多学科的手段进行研究, 将会有助于人们理解蕨类植物中的同倍体杂交物种形成机制。

本研究还发现了一个形态上近似线裂铁角蕨(图6), 但在系统位置上相对独立的四倍体类群(8214), 由于其两个核拷贝差异较小且位于同一分支II中(图5), 我们认为它是同源四倍体, 并将其处理为一个新种——马关铁角蕨(*A. maguanense* S. Q. Liang, R. Wei & X. C. Zhang, sp. nov.)。另有一个异源四倍个体(9439-2)在与马关铁角蕨共享母本起源的同时, 还具有一部分与细裂铁角蕨相同的遗传成分(核系统树分支I); 但该个体较幼小, 仅有一株, 且孢子部分败育似乎是该类群的特性, 因此不能很充分地断定它是一个独立的异源四倍体种, 还是偶

图6 马关铁角蕨(8214)。(A)全株;(B)叶片远轴面;(C)叶片近轴面;(D)叶片基部羽片近轴面;(E)叶片中部羽片远轴面;(F)孢子囊群;(G)末回裂片顶端,示齿和排水器;(H)鳞片。疑似杂交种(9439-2):(I)生境;(J)全株;(K)末回裂片远轴面。A-C, J-K中比例尺为2 cm; D-H中比例尺为1 mm。

Fig. 6 *A. maguanense* sp. nov. (8214). (A) Habit; (B) Abaxial side of lamina; (C) Adaxial side of lamina; (D) Adaxial side of basal pinna; (E) Abaxial side of middle pinna; (F) Sorus; (G) Abaxial side of ultimate segment, apex with teeth and hydathodes; (H) Scales. *A. aff. coenobiale* (9439-2), a suspected hybrid shared half genome with *A. maguanense*: (I) Habitat; (J) Habit; (K) Abaxial side of ultimate segment. Scale bars in A-C and J-K are 2 cm; scale bars in D-H are 1 mm.

然形成的杂交个体, 所以这里暂时不将它作为新种处理, 需对其采集地(广西那坡)进行更充分的考察后再做判断。

综上所述, 我们认为杂交和多倍化过程各自的单独作用(同倍体杂交和同源多倍化)和共同作用(异源多倍化), 在线裂铁角蕨复合体的物种形成及多样化过程中起到了十分关键的作用。但由于物种形成后可能发生复杂的基因组重组事件造成基因拷贝的部分丢失(Soltis et al, 2015), 且此次研究并未收集到杂交和多倍化起源类群对应的二倍体亲本, 因而所得推论还有待进一步验证。

3.2 物种划分

综合考虑多个层面的研究结果, 目前我们支持将线裂铁角蕨复合体划分为4个种: 多角铁角蕨、线裂铁角蕨、叶基宽铁角蕨和马关铁角蕨。其中, 多角铁角蕨不仅在分布范围上较为局限, 形态上较为特殊(叶较多、平展且质地极薄, 叶柄极短, 叶片基部羽片回缩, 鳞片线形, 孢子具翅状纹饰), 其倍性和分子系统位置相较于复合体中的其他成员也是非常独特的。此外, 本次研究结果基本支持了 *Flora of China* (Lin & Viane, 2013) 对线裂铁角蕨复合体中的四倍体类群的物种划分方式, 即仅承认分裂极细、末回裂片中仅包含一条叶脉、能育裂片仅包含一个

孢子囊群的叶基宽铁角蕨为独立的种, 而将在其他植物志书(如吴兆洪, 1999, 2006; 王培善和王筱英, 2001; 张光飞, 2006)中出现过的乌木铁角蕨、都匀铁角蕨和黑柄铁角蕨等全部处理为线裂铁角蕨的异名。同时, 在这种划分的基础上, 我们的分子系统学证据还强烈支持马关铁角蕨作为一个独立的同源四倍体种的存在, 虽然它与线裂铁角蕨中二回羽裂且末回裂片较大的个体十分相似(图6), 但仍有一些细微差异有助于将二者从形态上区分开来。

依据以上物种划分方案, 通过对PE馆藏标本的重新鉴定, 并结合 *Flora of China* (Lin & Viane, 2013) 中的记录, 我们对线裂铁角蕨复合体内的4个物种的分布情况进行了统计。结果表明, 多角铁角蕨和马关铁角蕨目前确定的分布都还局限于其模式采集地(广西靖西和云南马关)。而线裂铁角蕨和叶基宽铁角蕨在中国西南地区(包括重庆、福建、广东、广西、贵州、湖南、四川和云南)及邻国越南都有较广泛的分布, 但在大陆邻近岛屿上的分布存在着差异: 纬度较高的日本仅有线裂铁角蕨的记录, 而纬度较低的中国台湾和马来西亚则仅有叶基宽铁角蕨的记录。未来结合亚欧大陆与周边岛屿在地理上的分隔历史可以对这一对正反交起源的物种各自的物种形成和扩散过程进行更为深入的探讨。

以下检索表可用以区分线裂铁角蕨复合体的4个成员:

1. 末回小羽片或裂片线性, 仅包含单条叶脉; 孢子囊群可达叶边。
 2. 叶片质地较厚, 草质, 基部最宽; 鳞片似披针形或线状钻形, 细胞内壁平滑; 孢子外壁长度均值大于 30 μm ; 孢子周壁具脊状纹饰; 植株四倍体 1. 叶基宽铁角蕨 *A. pulcherrimum*
 2. 叶片质地较薄, 膜质, 基部回缩; 鳞片线形, 细胞内壁具疣凸; 孢子外壁长度均值小于 30 μm ; 孢子周壁具翅状纹饰; 植株二倍体 2. 多角铁角蕨 *A. cornutissimum*
1. 末回小羽片或裂片非线性, 包含多条叶脉; 孢子囊群一般不达叶边。
 3. 叶片二回羽状半裂至三回羽状全裂; 末回裂片顶端的齿较长, 长度大于宽度; 鳞片具长尾, 尾长可达 2 mm 以上 3. 线裂铁角蕨 *A. coenobiale*
 3. 叶片二回羽状半裂至全裂; 末回裂片顶端的齿较短, 长度一般不大于宽度; 鳞片具短尾, 尾长不超过 0.6 mm 4. 马关铁角蕨 *A. maguanense* sp. nov.
1. Ultimate pinnules or segments linear, with a single vein; sorus reaching frond margin.
 2. Lamina more firm, herbaceous, widest at base; scales lanceate to linear-subulate, with inner part of cell walls smooth; mean exospore length more than 30 μm ; perispore lophate; plants tetraploid 1. *A. pulcherrimum*
 2. Lamina thin, membranous, not widest at base; scales linear, with inner part of cell walls warty; mean exospore length less than 30 μm ; perispore alate; plants diploid 2. *A. cornutissimum*
1. Ultimate pinnules or segments not linear, with several veins; sorus rarely reaching frond margin.
 3. Lamina bipinnate-pinnatifid to tripinnate-pinnatisect; ultimate segment apex with long teeth, length longer

- than width; scale apex ending in a long apical tail sometimes longer than 2 mm3. *A. coenobiale*
 3. Lamina bipinnate-pinnatifid to bipinnate-pinnatisect; ultimate segment apex with short teeth, length rarely longer than width; scale apex ending in a short apical tail shorter than 0.6 mm4. *A. maguanense* sp. nov.

Asplenium maguanense S. Q. Liang, R. Wei & X. C. Zhang, **sp. nov.** TYPE: China. Yunnan Province: Maguan County, Bazhai Town, Valley behind Langqiao Village, 7 Apr. 2017, X. C. Zhang *et al.* 8214 (holotype, PE). Figure 6A-H.

Description. Plants epilithic, ca. 10–25 cm tall. Rhizomes erect, short, scaly; scales (1.7–4.0) mm × (0.4–0.7) mm wide at their base, narrowly triangular to linear-subulate, clathrate, margin fimbriate to subentire, apex acuminate, ending in an short apical tail 0.2–0.6 mm; anticlinal cell walls dark brown to black, thickened, often with a central zone of cells nearly occluded, periclinal cell walls translucent, yellow to light brown. Fronds caespitose; stipes 5–11 cm, generally longer than laminae, 0.6–1 mm wide at the base, shiny, purplish black, terete, rigid and threadlike, subglabrous, adaxially sulcate; rachis similar to that of distal part of stipe, and becoming green in upper 3/4–4/5 toward apex. Laminae firmly herbaceous, green, (5–10) cm × (2–8) cm wide at their base, triangular, tripinnate–pinnatisect to bipinnate–pinnatisect at the base, becoming bipinnate–pinnatifid to pinnate toward the apex, apex caudate; pinnae 10 to 13 pairs, subopposite to alternate, lower pinnae stalked, upper almost sessile, stalk green, basal 1 or 2 pair usually the longest and falcate to oblong, (1.2–4.5) cm × (0.8–1.5) cm, base asymmetrical, on acroscopic side auriculate-truncate, basiscopic side cuneate, up to bipinnate–pinnatisect, apex caudate to obtuse; pinnules 4–6 pairs, anadromous, shortly stalked to sessile, basal acroscopic pinnule largest, ovate, (4–8) mm × (3–5) mm, base asymmetrical, acroscopic side truncate, basiscopic cuneate, apex obtuse to truncate; ultimate segments ovate-oblong, apex with 2–6 obtuse to submucronate teeth. Costa sulcate adaxially, green, veins often raised adaxially when dry, anadromously branching, 2–6 veins per ultimate segment, terminal hydathode obvious, and not reaching margin. Sori 1–5 per pinnule or segment, oblong to linear, 1–3 mm long, medial to subterminal on acroscopic vinlets; indusia grayish green, oblong to linear, membranous, transparent, margin slightly repand or entire, persistent. Spores brown to dark brown, exospore (29–) 31–35 (–36) μm long, perispore lophate (cristate–alate).

Habitat and distribution. *Asplenium maguanense* is known from Yunnan, China, growing on limestone under evergreen forest, at the elevation of 1,150–1,800 m.

Etymology. Maguan, a county of Yunnan Province, is the type locality of the new species.

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