•研究报告•

祖先性状重建法揭示铁线蕨属植物孢子表面纹饰的形态多样性及其演化

赵国华^{1,2} 王莹² 商辉² 周喜乐³ 王爱华⁴ 李玉峰⁵
王晖¹ 刘保东⁶ 严岳鸿^{2*}

1 (深圳市中国科学院仙湖植物园, 广东深圳 518004)

2 (上海辰山植物园, 中国科学院上海辰山植物科学研究中心, 上海 201602)

3 (湘西土家族苗族自治州森林资源监测中心, 湖南吉首 416000)

4 (南宁师范大学北部湾环境演变与资源利用教育部重点实验室, 南宁 530001)

5 (湖南科技大学生命科学学院, 湖南湘潭 411201)

6 (哈尔滨师范大学植物生物学黑龙江省高校重点实验室, 哈尔滨 150025)

摘要: 蕨类植物孢子表面的纹饰形态复杂多样, 在分类学和古生物学研究中具有重要意义, 目前探索祖先性状演化常用形态学与系统发育学结合的方法。本文选取一回羽叶铁线蕨属植物为材料, 用扫描电子显微镜观察其孢子形态, 用5个叶绿体基因序列构建系统树, 用最大似然法和最大简约法来分析孢子性状的演化。结果表明: 一回羽叶铁线蕨属的孢子纹饰有5种类型, 即光滑、粗糙、颗粒、疣状及瘤状; 这一类群的祖先孢子纹饰有很大的可能是瘤状纹饰; 疣状纹饰可能是由瘤状纹饰演化而来; 孢子纹饰由简单向复杂演化, 越进化的物种其孢子纹饰越复杂。

关键词: 蕨类; 孢子纹饰; 系统发育; 性状演化

Ancestral state reconstruction reveals the diversity and evolution of spore ornamentation in *Adiantum* (Pteridaceae)

Guohua Zhao^{1,2}, Ying Wang², Hui Shang², Xile Zhou³, Aihua Wang⁴, Yufeng Li⁵, Hui Wang¹, Baodong Liu⁶, Yuehong Yan^{2*}

1 FairyLake Botanical Garden, Shenzhen & Chinese Academy of Sciences, Shenzhen, Guangdong 518004

2 Shanghai Chenshan Botanical Garden, Shanghai Chenshan Plant Science Research Center, Chinese Academy of Sciences, Shanghai 201602

3 Xiangxi Tujia and Miao Autonomous Prefecture Forest Resources Monitoring Center, Jishou, Hunan 416000

4 Key Laboratory of Beibu Gulf Environment Change and Resources Use of Ministry of Education, Nanning Normal University, Nanning 530001

5 School of Life Science, Hunan University of Science and Technology, Xiangtan, Hunan 411201

6 Harbin Normal University, Key Laboratory of Plant Biology, College of Heilongjiang Province, Harbin 150025

Abstract: Spore morphology is diverse and complex in ferns, thus serving as an important indicator of taxonomy. In conjunction, morphological traits and phylogeny are used to study taxonomy and recent character evolution. This study analyzes the evolution of spore ornamentation in once-pinnate maidenhair ferns (*Adiantum*) using scanning electron microscopy and ancestral state reconstruction. Our results show that there are five ornamental types in once-pinnate maidenhair ferns: psilate, scabrate, granulate, verrucate and tuberculate. The ancestral state of spore ornamentation in *Adiantum* is probably tuberculate, with verrucate ornamentation possibly deriving from tuberculate. We suggest that spore ornamentation evolved from simple to complex in *Adiantum*. In other words, the more evolutionary species, will with a more complex spore ornamentation.

Key words: fern; spore ornamentation; phylogenetic; character evolution

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* 通讯作者 Author for correspondence. E-mail: yan.yh@126.com

孢粉学在地球科学、生命科学、环境科学等很多领域都具有重要的研究意义,还广泛应用于农学、医学、药学及司法破案等方面(Patricia, 1992; Caroline & Patrica, 2001; Luo & Sun, 2001; 杨振京和徐建明, 2002; 王伟铭, 2009)。蕨类植物是一类配子体世代与孢子体世代交替的起源较早的植物类群,孢子在世代交替中扮演着纽带的重要角色。蕨类植物孢子的形态和纹饰类型复杂多样,有高度的稳定性,是分类的重要依据。孢子的壁分为内壁、外壁和周壁,孢子的表面纹饰大多是指周壁或外壁的纹饰,是由孢粉素和纤维素沉积而成,沉积的程度决定孢子纹饰的复杂程度(Tryon & Lugardon, 1991)。表面纹饰具有保护和传播细胞遗传物质的功能。蕨类植物的孢子和种子植物的花粉是同源的(Blackmore & Barnes, 1987)。在种子植物中,花粉的外壁结构可被分为三大类型:柱状、泡状和颗粒状。Campo和Lugardon (1973)的研究表明,颗粒状结构是早期被子植物的祖先性状,是从柱状衍生而来的。在蕨类植物的孢子表面,也有与花粉颗粒状结构类似的纹饰,凤尾蕨科、骨碎补科和水龙骨科等植物的孢子表面纹饰大多是颗粒状类型的纹饰,如粗糙纹饰、粒状纹饰、疣状纹饰和瘤状纹饰等。长期以来,蕨类植物孢子和花粉的颗粒状类型的纹饰之间的演化关系一直不甚清楚。

王全喜和戴锡玲(2001)在《中国水龙骨科(真蕨目)植物孢子形态的研究》中描述了我国蕨类47科156属近700种孢子的形态,总结了29种孢子纹饰的基本类型,认为表面纹饰非常复杂,其变化是多方向的,很难推断纹饰之间的进化程度。以往关于孢子形态演化的假说主要是基于形态特征而提出的,缺少分子数据作为支撑(Devi, 1979; Tryon & Lugardon, 1991; 王全喜和戴锡玲, 2001)。用形态学结合系统发育学的研究逐渐成为趋势(Liu et al, 2007; Co-David et al, 2009; Wang et al, 2015b)。Wang等(2015b)基于系统发育分析了骨碎补科孢子形态的演化,结果表明疣状纹饰是骨碎补科的祖征性状。Tao等(2018)基于叶绿体基因组的系统发育,对蔷薇分支的一个支系的21个花粉形态进行演化分析,发现花粉的部分性状存在明显的演化趋势,纹饰由简单演化到复杂。形态学与系统发育学的研究结果可以达到互补,形态学在一定程度上可以解决分子系统上未解决的问题,系统发育学又可以帮助阐述性

状的发生和演化(Wei et al, 2013; Tao et al, 2018)。

凤尾蕨科铁线蕨属(*Adiantum*)的孢子纹饰多样,特别是有多种颗粒状类型的纹饰。本文选取铁线蕨属的一个单系即一回羽叶铁线蕨类作为材料,用电子显微镜观察孢子形态,用5个叶绿体基因序列构建系统树,结合最大似然法(maximum parsimony, MP)、最大简约法(maximum likelihood, ML)和贝叶斯法(Bayesian inference, BI),探讨了孢子纹饰的演化。

1 材料与方法

1.1 材料来源

材料来源见表1,凭证标本存放于上海辰山植物园标本馆(CSH)。扇叶铁线蕨(*A. flabellulatum*)、*A. latifolium*、荷叶铁线蕨(*A. reniforme* var. *sinense*)及*A. reniforme* var. *reniforme*为外类群。

1.2 孢子形态观察

在粘有导电双面胶的载物台上均匀的洒满干燥的孢子,喷金后,用扫描电镜(JSM-6380LV, JEOL, 北京), 15 kV电压下观察并照相,用ImageJ软件测量每种20个孢子的大小,取平均值。孢子纹饰性状的描述参照王全喜和戴锡玲(2001)及Lellinger (2007)所使用的术语。

1.3 用最大似然法、最大简约法和贝叶斯法分析孢子性状演化

本实验分别测定了团羽铁线蕨(*A. capillus-junonis*)、*A. zolligeri*、翅柄铁线蕨(*A. soboliferum*)、半月形铁线蕨(*A. philippense*)、*A. latifolium* 5个物种7个样本的叶绿体DNA序列(*rbcL*, *atpB*, *atpA*, *trnL-F*, *rps4-trnS*)。在Eppendorf梯度PCR扩增仪上进行扩增反应,ABI 3730XL测序仪上完成PCR产物的测序。叶绿体DNA序列引物设计参照Lu等(2012)的方法,PCR反应条件、体系及DNA序列分析同王莹等(2015)。在GenBank中下载一回羽叶铁线蕨类5个叶绿体DNA序列(附录1),以扇叶铁线蕨、*A. latifolium*、荷叶铁线蕨及*A. reniforme* var. *reniforme*为外类群,采用最大简约法、最大似然法和贝叶斯法构建分子系统树。用PAUP4.0b10软件构建最大似然树;用Mega 5.05软件构建最大简约树;用MrBayes v.3.1.2软件构建贝叶斯树。

将本实验观察到的孢子纹饰和已报道的荷叶铁线蕨及*Adiantum reniforme* var. *reniforme* (Wang et al, 2015a)的孢子纹饰整合到分子系统树上。用

表1 铁线蕨属植物采集信息及孢子形态

Table 1 The locality information and spore morphology in *Adiantum*

物种 Species	标本号及采集地 Vouchers and locality	赤道轴 × 极轴 Equatorial diam × polar diam (μm)	孢子纹饰 Spore ornamentation	图1中位置 Position in Fig. 1
<i>A. latifolium</i>	Zx19882. 泰国曼谷植物标本馆 Bangkok Herbarium, Bangkok, Thailand	33.5 × 27.0	粗糙 Scabrate	1A
扇叶铁线蕨 <i>A. flabellulatum</i>	Zx110018. 中国广东南岭国家森林公园 Nanling National Forest Park, Guangdong, China	45.5 × 31.2	颗粒 Granulate	1B
团羽铁线蕨 <i>A. capillus-junonis</i>	Zx19959. 中国广东南岭国家森林公园 Nanling National Forest Park, Guangdong, China	46.5 × 43.0	光滑 Psilate	1C
半月形铁线蕨 <i>A. philippense</i>	Yan12405, Yan12086. 中国云南元江; Zx19839. 泰国彭世洛府电萨朗峦国家公园 Yan12405, Yan12086. Yuanjiang, Yunnan, China; Zx19839. Thung Salaeng Luang National Park, Phitsanulok, Thailand	33.4 × 25.7	粗糙 Scabrate	1D
孟连铁线蕨 <i>A. menglianense</i>	Yan12415, Yan12409. 中国云南元江 Yuanjiang, Yunnan, China	31.5 × 26.6	颗粒 Granulate	1E
翅柄铁线蕨 <i>A. soboliferum</i>	Yan11393. 中国海南昌江 Changjiang, Hainan, China	34.0 × 30.5	颗粒 Granulate	1F
<i>A. zollingeri</i>	Zx19812, Zx19911, ZXL9851. 泰国碧武里府康卡沾国家公园 Kaeng Krachan National Park, Phetchaburi, Thailand	34.0 × 27.3	瘤状 Tuberculate	1G
鞭叶铁线蕨 <i>A. caudatum</i>	Yan12408. 中国云南元江 Yuanjiang, Yunnan, China	40.6 × 33.5	瘤状 Tuberculate	1H
白垩铁线蕨 <i>A. gravesii</i>	WYG393. 中国贵州贞丰 Zhenfeng, Guizhou, China	51.5 × 36.1	瘤状 Tuberculate	1I
小铁线蕨 <i>A. mariesii</i>	Zx19685. 中国湖南桑植 Sangzhi, Hunan, China	36.8 × 30.1	疣状 Verrucate	1J
仙霞铁线蕨 <i>A. juxtapositum</i>	Yan1205071. 中国广东仁化 Renhua, Guangdong, China	60.4 × 45.7	疣状 Verrucate	1K
普通铁线蕨 <i>A. edgeworthii</i>	Yan12412. 中国云南元江 Yuanjiang, Yunnan, China	30.0 × 25.6	瘤状 Tuberculate	1L
假鞭叶铁线蕨 <i>A. malesianum</i>	Yan12416, Yan12404. 中国云南元江 Yuanjiang, Yunnan, China	38.8 × 32.8	瘤状 Tuberculate	1M
梅山铁线蕨 <i>A. × meishanianum</i>	Yan12411. 中国云南元江 Yuanjiang, Yunnan, China	—	瘤状 Tuberculate	1N
苍山铁线蕨 <i>A. sinicum</i>	Yan112601, Yan12414. 中国云南元江 Yuanjiang, Yunnan, China	31.7 × 29.1	瘤状 Tuberculate	1O
哀牢山铁线蕨 <i>A. × ailaoshanense</i>	Yan12413, Yan12410. 中国云南元江 Yuanjiang, Yunnan, China	—	瘤状 Tuberculate	1P

Mesquite v. 2.7.5软件进行最大似然法和最大简约法分析,以Markovk-state 1参数模型重建孢子纹饰性状的演化,用Trace-charactersover-trees来计算每个节点的祖先状态和概率。

2 结果

2.1 孢子形态

铁线蕨属的孢子辐射对称,呈三裂缝四面体,极面观三角至三角圆形,赤道面观半圆形至超半圆形,裂缝长度为孢子半径的1/2至几达赤道面,少数裂缝不明显。孢子的表面纹饰由周壁形成,周壁外层薄,易破裂脱落。本研究所观察到的铁线蕨属孢子纹饰多样,有5种类型(表1,图1)。(1)光滑(psilate):

孢子表面光滑,无突出物与凹陷。鞭叶铁线蕨系(*A. Ser. Caudata*)的团羽铁线蕨为本类型。(2)粗糙(scabrate):孢子表面有小突出物,粗糙。鞭叶铁线蕨系的半月形铁线蕨和外类群的*A. latifolium*为本类型。(3)颗粒(granulate):孢子表面有颗粒状的突起,直径较小,大小不一。鞭叶铁线蕨系的孟连铁线蕨(*A. menglianense*)、翅柄铁线蕨及外类群的扇叶铁线蕨为本类型。(4)疣状(verrucate):孢子表面有扁而大的突起,顶部扁圆形,较整齐。白垩铁线蕨系(*A. Ser. Gravesiana*)的小铁线蕨(*A. mariesii*)及仙霞铁线蕨(*A. juxtapositum*)为本类型。(5)瘤状(tuberculate):孢子表面有近球形的突起,顶端半圆形,较整齐。白垩铁线蕨系的白垩铁线蕨(*A. gravesii*)和鞭叶铁线

图1 一回羽叶铁线蕨类孢子形态。(A) *A. latifolium*; (B)扇叶铁线蕨; (C)团羽铁线蕨; (D)半月形铁线蕨; (E)孟连铁线蕨; (F)翅柄铁线蕨; (G) *A. zollingeri*; (H)鞭叶铁线蕨; (I)白垩铁线蕨; (J)小铁线蕨; (K)仙霞铁线蕨; (L)普通铁线蕨; (M)假鞭叶铁线蕨; (N)梅山铁线蕨; (O)苍山铁线蕨; (P)哀牢山铁线蕨。

Fig. 1 Spore morphology in once-pinnate maidenhair ferns (*Adiantum*). (A) *A. latifolium*; (B) *A. flabellulatum*; (C) *A. capillus-junonis*; (D) *A. philippense*; (E) *A. menglianense*; (F) *A. soboliferum*; (G) *A. zollingeri*; (H) *A. caudatum*; (I) *A. gravesii*; (J) *A. mariesii*; (K) *A. juxtapositum*; (L) *A. edgeworthii*; (M) *A. malesianum*; (N) *A. × meishanianum*; (O) *A. sinicum*; (P) *A. × ailaoshanense*.

图2 铁线蕨属孢子大小。黄色代表白垩铁线蕨系, 蓝色代表鞭叶铁线蕨系, 黑色代表外类群。

Fig. 2 Spore size of *Adiantum*. Yellow shapes indicate *A. Ser. Gravesiana*. Blue shapes indicate *A. Ser. Caudata*. Black shapes indicate outgroup.

蕨系的*A. zollingeri*、鞭叶铁线蕨(*A. caudatum*)、普通铁线蕨(*A. edgeworthii*)、假鞭叶铁线蕨(*A. malesianum*)、梅山铁线蕨(*A. × meishanianum*)、苍山铁线蕨(*A. sinicum*)及哀牢山铁线蕨(*A. × ailaoshanense*)为本类型。

本研究统计了铁线蕨属14个种的孢子大小(梅山铁线蕨与苍山铁线蕨孢子畸形不做统计), 图2表明铁线蕨属孢子的赤道轴长度和极轴长度呈线性相关, 该属的孢子大小差异较大。

从孢子大小上看, 除团羽铁线蕨外的鞭叶铁线蕨系孢子大小差别不大, 白垩铁线蕨系的孢子大小差别很大。小铁线蕨和大部分鞭叶铁线蕨系的孢子

大小更接近, 仙霞铁线蕨和白垩铁线蕨的孢子明显大于鞭叶铁线蕨系的大部分种。

2.2 一回羽叶铁线蕨系统发育

联合5个叶绿体DNA序列构建的最大简约树、最大似然树和贝叶斯树拓扑结构一致(图3)。团羽铁线蕨为一回羽叶铁线蕨类的基部类群; 半月形铁线蕨与孟连铁线蕨互为姐妹群, 是两个独立的种; 翅

柄铁线蕨与孟连铁线蕨和半月形铁线蕨亲缘关系最近, 比二者更进化; 产于泰国的*A. zollingeri*与产于中国的鞭叶铁线蕨互为姐妹类群; 白垩铁线蕨系与普通铁线蕨亲缘关系最近。

2.3 孢子纹饰演化

孢子纹饰演化的重建: 用贝叶斯法推理得到900棵最大似然树, 圆形表示每个节点出现的性状

图3 5个叶绿体序列*rbcL*、*atpB*、*atpA*、*trnL-F*、*rps4-trnS*联合构建系统发生树, 数字代表支持率(ML/MP/Bi)。*: ML和MP = 100%, BI = 1.0。

Fig. 3 The phylogenetic tree constructed based on five chloroplast markers (*rbcL*, *atpB*, *atpA*, *trnL-F*, *rps4-trnS*). Numbers on branches are support values (ML/MP/Bi). *, ML, MP = 100%, and BI = 1.0.

图4 一回羽叶铁线蕨类孢子纹饰的溯祖分析

Fig. 4 Evolution of spore ornamentation in once-pinnate maidenhair ferns (*Aidantium*)

的平均可能性(图4)。溯祖分析结果表明:一回羽叶铁线蕨类的基部类群为光滑纹饰,进化类群中瘤状纹饰多次出现,而疣状纹饰只在白垩铁线蕨系中有表现;一回羽叶铁线蕨类群的祖先孢子形态有多种可能性,但瘤状纹饰是祖征的可能性最大;白垩铁线蕨系的祖先孢子形态为瘤状纹饰。

3 讨论

一回羽叶铁线蕨类种间孢子纹饰的差异与该类群的系统发育表现出一致性。基部类群为简单的光滑纹饰,越进化的类群其孢子纹饰越复杂,亲缘关系越近的物种,它们的孢子纹饰差异越小。从这一结果可以推测,孢子的纹饰是由简单向复杂的方向演化,支持Tao等(2018)的研究结果。

溯祖分析结果显示,疣状纹饰只出现在白垩铁线蕨系,而该系的祖征是更复杂的瘤状纹饰,推测

疣状纹饰是由瘤状纹饰演化而来。白垩铁线蕨系的系统位置完全嵌入在鞭叶铁线蕨系中,可形态上白垩铁线蕨系的顶端并不能伸长成鞭状并着地生根,明显有别于鞭叶铁线蕨系。Lu等(2012)认为,这一性状的差异有可能是与生境有关,白垩铁线蕨系生长在石灰岩和丹霞地貌地区,这类地形土壤含水量较低,无法为鞭叶着地提供充足的水分,从而导致“鞭叶”这一性状退化消失。本文推测,疣状纹饰可能也是瘤状纹饰退化的表现。

在一回羽叶铁线蕨中,有2个自然杂交种:梅山铁线蕨(假鞭叶铁线蕨为母本、半月形铁线蕨为父本)和哀牢山铁线蕨(苍山铁线蕨为母本、孟连铁线蕨为父本)(Zhang et al, 2014; Wang et al, 2015c),其孢子纹饰都是瘤状纹饰,母本也均为瘤状纹饰,与各自的父本的纹饰差异较大。本文推测,自然杂交种的孢子纹饰的性状表达可能来自于母本,为母系遗传,但这一推测还需更多的样本及数据支持。

蕨类植物的孢子为单细胞,有学者认为孢子的大小与倍性之间是存在相关性的,通过孢子的大小能反映细胞的倍性(Barrington et al, 1986)。这一理论被应用于细胞学和孢粉学的相互联系中。一回羽叶铁线蕨的孢子大小有较大差异,孢子纹饰多样,该系的倍性也呈多样化,如普通铁线蕨、*A. zollingeri*及孟连铁线蕨是二倍体,假鞭叶铁线蕨、翅柄铁线蕨及鞭叶铁线蕨是四倍体,半月铁线蕨既有三倍体也有二倍体(CCDB, 2014; Zhang et al, 2014; Shang et al, 2016)。白垩铁线蕨系虽没有细胞倍性的研究,但可以推测该系的倍性也是多样的,且仙霞铁线蕨应是多倍体,可能是其为丹霞地貌特有物种的特性造成的。目前,仅在一回羽叶铁线蕨内就已经发现有2个自然杂交种,铁线蕨属物种孢子大小和纹饰及细胞倍性的多样化,预示着该类群可能存在更多的杂交种或隐性种。

在对种子植物花粉的研究中,平滑的花粉被认为是风媒植物的特性,而精细复杂的花粉是虫媒植物的特性(Walker, 1974)。Tanaka等(2004)提出花粉的形态与授粉机制密切相关。Tao等(2018)认为花粉的萌发孔及外壁覆盖上层的形态与花粉的传播方式有显著的相关性。传统上科学家们认为蕨类孢子的传播并不依靠昆虫为媒介,而主要依靠孢子囊自身的弹射机制和非生物因素完成(Midgley et al, 2002; Mehltrreter et al, 2010)。事实上,蕨类植物比昆

虫及被子植物的起源更早,也有很多研究及化石证据表明蕨类植物是部分昆虫的重要食物(Smart & Hughes, 1973; Ottosson & Anderson, 1983; Hummel et al, 2008)。但只有极少研究发现昆虫可以传播孢子(Tryon, 1985; Walker, 1985; 商辉和严岳鸿, 2014)。蕨类植物的孢子形态与其传播方式之间的关系仍有待于进一步研究。

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附录 Supplementary Material

附录1 GenBank下载的参比材料(*rbcL*, *atpB*, *atpA*, *trnL-F*和*rps4-trnS*)

Appendix 1 Accession numbers of DNA sequences downloaded from GenBank in this study (*rbcL*, *atpB*, *atpA*, *trnL-F* and *rps4-trnS*)

<http://www.biodiversity-science.net/fileup/PDF/2019111-1.pdf>

附录 1 GenBank 下载的参比材料(*rbcL*, *atpB*, *atpA*, *trnL-F* 和 *rps4-trnS*)

2 Appendix 1 Accession numbers of DNA sequences downloaded from GenBank in this study (*rbcL*, *atpB*, *atpA*,
trnL-F and *rps4-trnS*)

4

A. caudatum Lu209: JF935296; JF935373; JF937249; JF980641; JF980558.

6 *A. chienii* Lu568: JF935303; JF935381; JF937256; JF980649; JF980565.

A. menglianense Lu114: JF935311; JF935392; JF937266; JF980660; JF980575.

8 *A. gravesii* Lu129: JF935312; JF935393; JF937267; JF980664; JF980576.

A. gravesii Lu451: JF935317; JF935398; JF937272; JF980664; JF980581.

10 *A. juxtapositum* Lu575: JF935305; JF935383; JF937258; JF980651; JF980567.

A. leveillei Lu163: JF935313; JF935394; JF937268; JF980661; JF980577.

12 *A. lianxianense* Lu441: JF935306; JF935385; JF937259; JF980653; JF980569.

A. caudatum Lu050: JF935297; JF935374; JF937250; JF980642; JF980559.

14 *A. mariesii* Lu120: JF935302; JF935380; JF937255; JF980648; JF980564.

A. philippense Wen8257: JF935330; JF935412; JF937285; JF980675; JF980595.

16 *A. philippense* Wen10206: JF935340; JF935422; JF937295; JF980685; JF980606.

A. sinicum Lu269: JF935300; JF935378; JF937253; JF980646; JF980562.

18 *A. philippense* Zhang009: JF935299; JF935376; JF937252; JF980644; JF980561.

Adiantum sp. Wen8295: JF935329; JF935411; JF937284; JF980674; JF980594.

20 *Adiantum* sp. Wen9616: JF935326; JF935407; JF937280; JF980671; JF980590.

Adiantum sp. Wen9648: JF935327; JF935408; JF937281; JF980672; JF980591.

22 *A. capillus junonis* Lu111: JF935314; JF935395; JF937269; JF980662; JF980578.

A. flabellulatum Lu450: JF935295; JF935372; JF937248; JF980640; JF980557.

24 *A. malesianum* Yan12404: KP637230; KP637194; KP637179; KP637260; KP637245.

A. philippense Yan12405: KP637236; KP637200; KP637185; KP637266; KP637251.

26 *A. sinicum* Yan112601: KP637240; KP637204; KP637189; KP637270; KP637255.

A. philippense Yan12086: KP637235; KP637199; KP637184; KP637265; KP637250.

28 *A. sinicum* Yan112601: KP637240; KP637204; KP637189; KP637270; KP637255.

A. philippense Yan12086: KP637235; KP637199; KP637184; KP637265; KP637250.

30 *A. sinicum* Yan12406: KP637237; KP637201; KP637186; KP63726; KP637252.

A. sinicum 12407: KP637238; KP637202; KP637187; KP637268; KP637253.

32 *A. caudatum* Yan12408: KP637228; KP637192; KP637177; KP637258; KP637243.

A. menglianense Yan12409: KP637233KP637197KP637182KP637263KP637248.

34 *A. × ailaoshanense* Yan12410: KP637241KP637205KP637190KP637271KP637256.

A. × meishanianum Yan12411: KP637232; KP637196; KP637181; KP637262; KP637247.

36 *A. malesianum* Yan12416: KP637231; KP637195; KP637180; KP637261; KP637246.

A. edgeworthii Yan12412: KP637229; KP637193; KP637178; KP637259; KP637244.

38 *A. × ailaoshanense* Yan12413: KP637242; KP637206; KP637191; KP637272; KP637257.

A. sinicum Yan12414: KP637239; KP637203; KP637188; KP637269; KP637254.

40 *A. menglianense* Yan12415: KP637234; KP637198; KP637183; KP637264; KP637249.

A. reniforme var. *sinense*: JF935287; 1JF935364; 1JF937240; 1JF980633; 1JF980549.1.

42 *A. reniforme* var. *reniforme*: KJ742759.1; KJ742741.1; KJ742784.1; KJ779978.1; KJ780003.1.