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Author(s): Wan-Yu Zhang, Li-Yaung Kuo, Fay-Wei Li, Chun-Neng Wang, and Wen-Liang Chiou
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The Hybrid Origin of *Adiantum meishanianum* (Pteridaceae): A Rare and Endemic Species in Taiwan

Wan-Yu Zhang,[1,4] Li-Yaung Kuo,[1,4] Fay-Wei Li,[2] Chun-Neng Wang,[1,5] and Wen-Liang Chiou[3,5]

[1]Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei 10617, Taiwan.
[2]Department of Biology, Duke University, Durham, North Carolina 27708, U. S. A.
[3]Division of Botanical Garden, Taiwan Forestry Research Institute, Taipei 10066, Taiwan.
[4]These authors contributed equally.
[5]Author for correspondence (CNW: leafy@ntu.edu.tw; WLC: chiou@tfri.gov.tw)

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Abstract*—Adiantum meishanianum* is an endemic species distributed only in Meishan village, Kaohsiung, Taiwan. Because its sporangia contain only abortive spores, *A. meishanianum* has been regarded as having a hybrid origin, presumably with *A. caudatum, A. malesianum*, and *A. philippense* as the putative parents. The aim of this study is to confirm the hybrid origin and determine the parental lineages of *A. meishanianum* by examining cytology, reproductive modes, and using molecular phylogenetics. We found that the sequences of two chloroplast regions, *rps16-matK* intergenic spacer and the *matK* gene, are identical between *A. meishanianum* and *A. malesianum*, suggesting *A. malesianum* is the maternal parent. The nuclear phylogeny reconstructed based on the low-copy marker, *CRY2* first intron, reveals that *A. meishanianum* has three types of sequences: one type groups with sequences of sexual diploid individuals of *A. philippense* and the other two group with sequences of the sexual tetraploid *A. malesianum*, indicating that *A. philippense* is the paternal species. Our data further imply that the extant *A. meishanianum* probably originated from a single hybridization event, and its rarity is likely due to the limited distribution of the paternal parent.

Keywords*—Adiantum malesianum*, *Adiantum philippense*, *CRY2* first intron, cryptic species, hybridization.

Adiantum meishanianum F. S. Hsu ex Y. C. Liu & W. L. Chiou (Pteridaceae), a rare species endemic to Taiwan (Liu et al. 2009), has only been found in Meishan village, Kaohsiung City, Taiwan, with a single population, and is considered as critically endangered (Wang et al. 2012). Hsu (1993) first reported that *A. meishanianum* is a triploid species based on a somatic chromosome count ($2n = 90$). Lee et al. (2007) later found that *A. meishanianum* produces only aborted spores, which either fail to germinate or develop into abnormal gametophytes, suggesting that this species is a sterile triploid hybrid. The persistence and expansion of the population of *A. meishanianum* seems to rely solely on vegetative propagation via adventitious buds borne on the elongated rachis tips. Among the once-pinnate species of *Adiantum* in Taiwan, *A. caudatum* L., *A. malesianum* Ghatak. and *A. philippense* L. have been hypothesized as the parental candidates due to their morphological similarities, including the long pinna stalk (3–4 mm), the semi-orbiculate basal pinnae, and the adventitious bud on the elongated rachis (Hsu 1993; Lee et al. 2007; Liu et al. 2009).

This study aims to resolve the hybrid origin of *A. meishanianum* by reconstructing the phylogenies based on both chloroplast and nuclear DNA sequences of all once-pinnate *Adiantum* species in Taiwan. The ploidy and reproductive modes of the candidate parental species, as determined by the spore number per sporangium and cytological evidence, were further examined to confirm the hybrid relationships.

Materials and Methods

***Chloroplast dataset*—**We sampled 17 *Adiantum* species, representing all major clades in the genus (Lu et al. 2012) and including all of the once-pinnate species in Taiwan (Knapp 2011). DNA was extracted using a modified CTAB procedure (Wang et al. 2004). We adopted the strategy of Kuo et al. (2011) to design specific primers for the chloroplast DNA (cpDNA) regions, *matK* and *rps16-matK* intergenic spacer (IGS). First, we applied universal primers ("FERN chlB fYAA", "FERN rps16 fQCGR", "FERN rps16 fSRQE", "FERmatK fEDR", and "FERmatK rAGK") to generate sequences of *matK*, *rps16* second exon and *rps16-matK* IGS regions of selected *Adiantum* species. Based on the sequences obtained, we designed three specific primers: "Adn rps16 fEET", "Adn matK rRLF", and "Adn matK fHIS". The PCR reactions were performed in 15 μL reaction volumes, including 20 ng genomic DNA, 1 × PCR buffer, 200 μM dNTP, 15 pmol of each primer, and 0.5 U polymerase (GENET BIO ExPrime Taq DNA Polymerase, Korea). The detailed information of these primers and PCR conditions can be found in Table 1.

***Nuclear dataset*—**Phylogenies based on biparentally inherited DNA markers, particularly low-copy nuclear genes, provide an efficient way to disentangle the reticulate relationships caused by hybridization and/or polyploidization (Ebihara et al. 2005; Adjie et al. 2007; Li et al. 2012; Sessa et al. 2012). Only recently have such molecular markers become readily available to fern biologists (Rothfels et al. 2013). However, the universality of these regions and developed primers for these nuclear genes usually vary among fern lineages (Chen at al. 2012; Rothfels et al. 2013).

As a case study, we developed a novel low-copy nuclear marker: the first intron of the cryptochrome gene (*CRY*), which encodes a blue light photoreceptor. It is an ideal nuclear marker for *Adiantum* species because the copy number and exon/intron positions are known from *A. capillus-veneris* L. (Kanegae and Wada 1998; Imaizumi et al. 2000).

Among the non-coding regions of the five *A. capillus-veneris CRY* genes, the first intron is most stable in its exon/intron boundary. Thus, based on the published *A. capillus-veneris CRY1–5* coding sequences, we first designed one degenerate primer set to target the first intron ("Adn CRY fPEE" and "Adn CRY rDLL"). By electrophoresis in 1 × TBE 0.8% agarose gel, we verified, isolated, and sequenced the PCR products of ~400 bp, which contains only the amplicons from *CRY2*. After we obtained partial *CRY2* first exon and second exon sequences from *A. caudatum*, *A. meishanianum*, and *A. philippense*, a specific primer set to amplify once-pinnate *Adiantum CRY2* first intron was designed: "Adn CRY2 fHLN" and "Adn CRY2 rVKQ". All PCR reactions were carried out as described above. Other details of primer information and PCR conditions can be found in Table 1.

To distinguish different sequence types after PCR (i.e. alleles or homeologs), single-strand conformation polymorphism (SSCP) was used when the direct sequencing of amplicons of "Adn CRY2 fHLN + Adn CRY2 rVKQ" failed. The protocol of SSCP electrophoresis followed Ebihara et al. (2005). Before SSCP electrophoresis, the PCR products were purified using a Gel/PCR purification kit (Genomics, Taipei, Taiwan) and eluted with ddH_2O. After SSCP electrophoresis, all gel slices containing the separated single-strand DNA products (i.e. bands) were purified by the same purification kit, and were re-amplified and sequenced.

Voucher information of the materials used in chloroplast or nuclear DNA analyses of this study is summarized in the Appendices.

***Phylogenetic analyses*—**DNA sequences were aligned using ClustalW implemented in BioEdit (Hall 1999) and the alignments were edited

TABLE 1. The sequences and PCR condition of primers used in this study. *Primer for sequencing. #Primer for sequencing *Adiantum caudatum rps16-matK* IGS.

Primer names	Primer sequence 5′-3′	Target region	Target taxon	PCR Tm / extension time	Reference
Adn CRY fPEE*	CCWGADGARGARGGNCARTT	*CRY1–5* intron1	*Adiantum*	49°C / 90s	this study
Adn CRY rDLL*	TTYAATGSMGAYYTBCTGTATGAGCC	*CRY -5* intron1	*Adiantum*	49°C / 90s	this study
Adn CRY2 fHLN*	CACCTCAATGAATCTCTCACAA	*CRY2* intron1	once-pinnate *Adiantum*	65°C / 30s	this study
Adn CRY2 rVKQ*	GCCTGRGAGAGCCCCTGTTTCAC	*CRY2* intron1	once-pinnate *Adiantum*	65°C / 30s	this study
FERN ch1B fYAA#	GATGTRAYGTATGCRGCYAAAGA	*rps16-matK* IGS	for all ferns	55°C / 90s	Kuo et al. 2011
FERN rpsl6 fQCGR	CRMTRTGGTAGRAAGCAAC	*rps16-matK* IGS	for all ferns	55°C / 90s	Kuo et al. 2011
FERN rpsl6 fSRQE*	CCCGRMRAGAAGGGARAG	*rps16-matK* IGS	for all ferns	55°C / 90s	Kuo et al. 2011
Adn rps16 fEET*	GAGGAAACYCAGCTAGATMTTT	*rps16-matK* IGS	*Adiantum*	49°C / 90s	this study
Adn rps16 rRLF*	ACATCTYTAATTTGTCGTCGAAAYAATCG	*rps16-matK* IGS and *matK*	*Adiantum*	49°C / 90s	this study
FERmatK fEDR*	ATTCATTCRATRTTTTTATTTHTGGARGAYAGATT	*matK*	for all ferns	53°C / 60s	Kuo et al. 2011
FERmatK rAGK	CGTRTTGTACTYYTRTGTTTRCVAGC	*matK*	for all ferns	53°C / 60s	Kuo et al. 2011
Adn matK fIHS*	ATTCATTCRATATTTYYATTTYTGGAAGATAGATT	*matK*	*Adiantum*	53°C / 60s	this study

manually. To infer the appropriate nucleotide substitution model for the following phylogenetic analyses, jModelTest (Posada 2008) was employed, and the model was selected based on Akaike information criterion (Akaike 1974). Garli 2.0 (Zwickl 2006) was used to reconstruct the maximum likelihood (ML) phylogeny. The proportion of invariant sites and state frequencies were estimated by the program. The "genthreshfortopoterm" option was set to 20,000. To calculate ML bootstrap support (MLBS) values, 500 replicates were run under the same criteria. Bayesian phylogenetic inference was performed by MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Two simultaneous runs were carried out with four chains (10^6 generations each), in which each chain was sampled every 1,000 generations. The first 25% of the sample was discarded as burn-in, and the rest were used to calculate the 50% majority-rule consensus tree.

Ploidy and reproductive mode inference—Ploidy analyses were conducted on *A. caudatum*, *A. malesianum, A. meishanianum,* and *A. philippense*, and were carried out by either somatic chromosome squashing or flow cytometry.

Root tip squashes were used to count somatic chromosomes. Root tips were collected between 6–8 AM and pretreated with 70 ppm cycloheximide and 250 ppm 8-hydroxyquinoline (1:1) at 18–20°C for 8 h. They were fixed sequentially in 100% acetic acid and absolute ethanol (1:3) overnight and preserved in 70% ethanol at 4°C. Then they were macerated in 1 N HCl at 60°C for 10 s, and digested with 4% pectinase (SIGMA, St. Louis, Missouri) for 1–2 h. Finally, mitotic chromosomes were stained by modified carbol fuchsin stain and examined under a microscope (Sharma 1982; Huang et al. 2006).

By using the individuals with known somatic chromosome number as the standards, flow cytometry analyses were applied to confirm the ploidy of other living collections. The pretreatment of materials for flow cytometry analyses followed Ebihara et al. (2005), and the BD FACSCan system (BD Biosciences, Franklin Lake, New Jersey) was used.

Reproductive mode was assessed by counting spore number per sporangium. In *Adiantum*, 64 and 32 spores per sporangium suggest sexual and apomictic individuals, respectively (Ko 2011). In this study, five sporangia per individual were selected to examine their spore numbers per sporangium.

RESULTS

Phylogenetic analyses—The cpDNA alignment matrix of *rps16-matK* IGS + *matK* contained a total of 2,364 characters with 1,404 variable sites. The log-likelihood score for the most likely ML tree was -1709.230276 (Fig. 1). In the cpDNA phylogeny, all the once-pinnate *Adiantum* species formed a highly supported monophyletic group (Fig. 1). Within this clade, the monophyly of *A. malesianum* and *A. meishanianum* received MLBS of 100. All the *matK* + *rps16-matK* IGS sequences of *A. meishanianum* were identical with those of *A. malesianum*, except for the *A. malesianum* sample Ma2 collected from Nantou County.

The nuclear DNA (nrDNA) matrix of *CRY2* first intron contained a total of 471 characters, 157 of them were variable. The log-likelihood score was -16,723.999137 for the most likely ML tree. Among three sequence types of *A. meishanianum*, two of them were identical to those of *A. malesianum*, and the other identical to those of the sexual diploid *A. philippense* (Fig. 2).

Cytotype and reproductive mode—By somatic chromosome counting, one *A. caudatum*, one *A. philippense*, and one *A. meishanianum* were shown with $2n = 90$ (or ca. 90) (Table 2; Fig. 3). As a result, they were all designated as triploids since the known chromosome basic number is 29 or 30 in *Adiantum* (Löve et al. 1977; Tryon et al. 1990). Using these individuals as the standards, the ploidy of other living collections was examined by flow cytometry (Fig. 3D). For *A. malesianum* and *A. meishanianum*, only sexual tetraploids and sterile triploids were found, respectively (Table 2). In *A. caudatum*, sexual diploids and apomictic triploids were found (Table 2). In *A. philippense*, sexual diploids, apomictic diploids, and apomictic triploids were found (Table 2).

DISCUSSION

Hybrid origin of A. meishanianum—The monophyly of *A. meishanianum* + *A. malesianum* in the cpDNA phylogeny indicates that these two species share the same maternal progenitor (Fig. 1), assuming that the chloroplast is maternally inherited as in many ferns (Gastony and Yatskievich 1992; Vogel et al. 1998; Adjie et al. 2007). The phylogeny reconstructed using low-copy nuclear marker reveals that *A. meishanianum* contains identical sequence types of *CRY2* first intron with *A. malesianum* and with the sexual diploid *A. philippense* (Fig. 1; Table 2). Taken together, triploid *A. meishanianum* is likely the hybrid of *A. malesianum* (as maternal progenitor providing diploid egg) and *A. philippense* (as paternal progenitor providing haploid sperm).

Lee et al. (2007) proposed that the *A. caudatum* complex in Taiwan, which includes *A. caudatum* and *A. malesianum*, and *A. philippense* are the parental candidates of *A. meishanianum*. *Adiantum caudatum* should be excluded from the ancestors of *A. meishanianum* because these two species are not closely related in the cpDNA and nrDNA phylogenies (Figs. 1, 2). However, there are several additional species

FIG. 1. The ML phylogeny based on *rps16-matK* + *matK* of 17 *Adiantum* species and *Cheilanthes lindheimeri*. ML bootstrap support (MLBS) values and posterior probabilities of Bayesian phylogenetic inference (PP) are indicated on each branch, as MLBS/PP. The plus (+) sign represents MLBS = 100 or PP = 1.00. The thickened branch indicates MLBS ≧70 and PP ≧0.95. The gray arrow indicates the lineage with *A. meishanianum*.

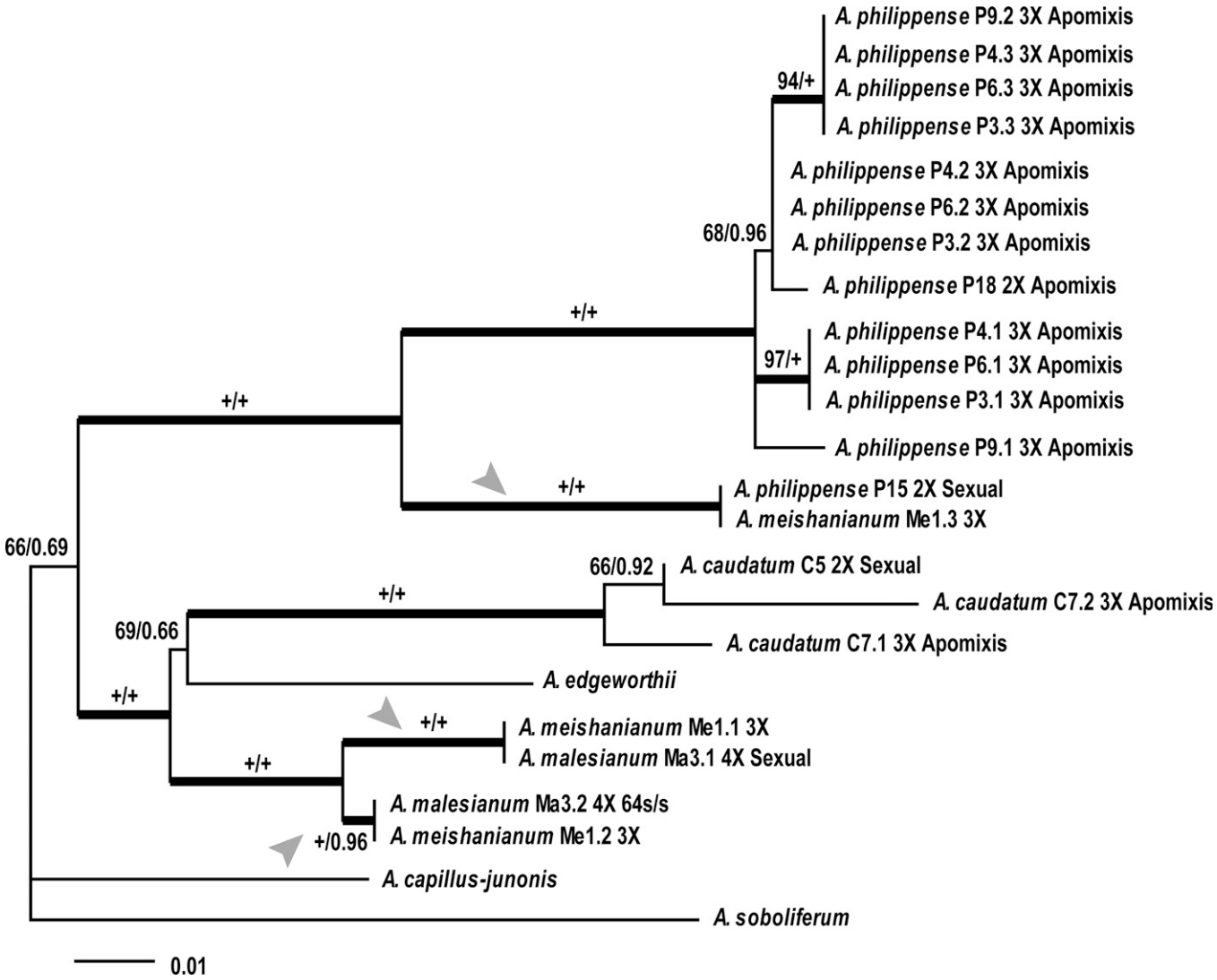

FIG. 2. The ML phylogeny based on *CRY2* first intron of only once-pinnate *Adiantum* species in Taiwan. ML bootstrap support (MLBS) values and posterior probabilities of Bayesian phylogenetic inference (PP) are indicated on each branch, as MLBS/ PP. The plus (+) sign represents MLBS = 100 or PP = 1.00. The thickened branch indicates MLBS ≧70 and PP ≧0.95. The gray arrows indicate the lineages with *A. meishanianum*.

in the *A. caudatum* complex that occur outside of Taiwan. Some of them are morphologically similar but cytologically appear as different species, including *A. incisum* Forssk., *A. indicum* Ghatak [or *A. recurvatum* (D. Don) Fraser-Jenk.], and *A. Adiantum zollingeri* Mett. ex Kuhn (Manton et al. 1967, 1970; Sinha and Manton 1970; Fraser-Jenkins 2008). Further phylogenetic investigation including these species is necessary to clarify their status and relationships.

As suggested by the SSCP banding pattern, the *CRY2* first intron sequence and its composition seem consistent across the sampled individuals (data not shown). Thus, the ploidy, cpDNA, and nrDNA sequences are completely identical across all the *A. meishanianum* individuals we sampled. These data do not provide any evidence supporting the multiple origins of *A. meishanianum*. Instead, a single hybrid origin followed by subsequent population expansion via vegetative propagation (i.e. adventitious buds at the rachis tip) is a more likely scenario.

Cryptic species Under A. philippense—The sexual diploid *A. philippense* in Taiwan (Fig. 4B), the paternal parent of *A. meishanianum*, is morphologically indistinguishable with the conspecific apomictic triploid (Table 3), which belongs to subsp. *philippense* (Verma and Fraser-Jenkins 2008). However, genetically, they diverge greatly, as revealed by the cpDNA and nrDNA phylogenies (Figs. 1, 2). Although diploids of subsp. *philippense* were also reported outside of Taiwan, all of them are known as apomictic, (i.e. producing 32-spored sporangia; Cheng and Zhang 2010), including the individual from Thailand sampled here. Interestingly, in our cpDNA and nrDNA phylogenies, the apomictic diploid from Thailand (sample P18) grouped with apomictic triploids instead of with other sexual diploids (Figs. 1, 2). In addition, based on our flow cytometry results, this apomictic diploid contains a larger nuclear genome size compared to the sexual ones (data not shown). These data suggest the possibility of one or more cryptic species under *A. philippense*, and that the sexual diploid members of *A. philippense* in Taiwan are different from their apomictic counterparts. To further clarify the species boundaries, studies including another sexual diploid subspecies, subsp. *teestae* S. C. Verma & Fraser-Jenkins, are needed.

TABLE 2. The information of *Adiantum* materials examined with ploidy level, reproductive mode, and molecular phylogeny. All the vouchers are kept in TAIF. KBCC = Dr. Cecilia Koo Botanic Conservation Center. [a]The ploidy confirmed by flow cytometry analyses.

Taxa	Sample No.	Locality	Ploidy	Reproduction	Voucher	Living Collections in KBCC
Adiantum caudatum L.	C1	Dakeng, Taichung City, Taiwan	3X[a]	Apomixis	20081109–21MO	-
	C2	Qingshuiyan, Changhua County, Taiwan	3X[a]	Apomixis	20100502–034MO	-
	C3	Zengwen Reservoir, Chiayi County, Taiwan	3X[a]	Apomixis	Liu6808-e	K017626
	C4	Mt. Dagang, Kaohsiung City, Taiwan	3X[a]	Apomixis	20081125–16bMO	-
	C5	Liangshan, Pingtung County, Taiwan	2X[a]	Sexual	-	K013900
	C6	Mt. Laofo, Pingtung County, Taiwan	3X[a]	Apomixis	-	K017393
	C7	Jinfeng, Taitung City, Taiwan	3X[a]	Apomixis	ZWY310	-
	C8	Gushan, Kaohsiung City, Taiwan	3X ($2n$ = ca. 90)	Apomixis	Kuo3924	K022992
Adiantum malesianum Ghatak	Ma1	Mt. Shitou, Miaoli County, Taiwan	4X[a]	Sexual	Kuo3933	K019612
	Ma2	Zhongliao, Nantou County, Taiwan	4X[a]	Sexual	20080907–05MO	-
	Ma3	Guanyin waterfall, Chiayi County, Taiwan	4X[a]	Sexual	Kuo3932	K013775
	Ma4	Longci, Tainan County, Taiwan	4X[a]	Sexual	20070903–113MO	-
	Ma5	Meishankou, Kaohsiung City, Taiwan	4X[a]	Sexual	Kuo3934	K022539
	Ma6	Jiayi, Pingtung County, Taiwan	4X[a]	Sexual	20070730-MO	-
	Ma7	Dali, Haulian County, Taiwan	4X[a]	Sexual	Liu9530	-
Adiantum meishanianum	Me1	Meishankou, Kaohsiung City, Taiwan	3X[a]	Sterile	Liu5002	-
F. S. Hsu ex Y. C. Liu &	Me2	Meishankou, Kaohsiung City, Taiwan	3X ($2n$ = ca. 90)	Sterile	Kuo3917-M02	-
W. L. Chiou	Me3	Meishankou, Kaohsiung City, Taiwan	3X[a]	Sterile	Kuo3917-M03	-
	Me4	Meishankou, Kaohsiung City, Taiwan	3X[a]	Sterile	Kuo3917-M05	-
	Me5	Meishankou, Kaohsiung City, Taiwan	3X[a]	Sterile	Kuo3917-M07	-
	Me6	Meishankou, Kaohsiung City, Taiwan	3X[a]	Sterile	Kuo3917-M08	-
Adiantum philippense L.	P1	Fuxing, Taoyuan County, Taiwan	3X[a]	Apomixis	-	-
	P2	Jianshih, Hsinchu County, Taiwan	3X[a]	Apomixis	-	K022991
	P3	Chunyang, Nantou County, Taiwan	3X[a]	Apomixis	-	-
	P4	Caohu, Taichung City, Taiwan	3X[a]	Apomixis	-	K022990
	P5	Wanda Reservoir, Nantou County, Taiwan	3X ($2n$ = 90)	Apomixis	-	K022545
	P6	Mt. Duli, Chiayi County, Taiwan	3X[a]	Apomixis	-	K022993
	P7	Zengwen Reservoir, Chiayi County, Taiwan	3X[a]	Apomixis	-	K022542
	P8	Zengwen Reservoir, Chiayi County, Taiwan	3X[a]	Apomixis	Liu6810	-
	P9	Nanhua Reservoir, Tainan County, Taiwan	3X[a]	Apomixis	Kuo3935	K022543
	P10	Nanhua Reservoir, Tainan County, Taiwan	3X[a]	Apomixis	-	K022544
	P11	Shanping, Kaohsiung City, Taiwan	3X[a]	Apomixis	-	K022546
	P12	Neishi, Pingtung County, Taiwan	3X[a]	Apomixis	MO2390	-
	P13	Shanping, Kaohsiung City, Taiwan	2X[a]	Sexual	Kuo3936	K018606
	P14	Meishankou, Kaohsiung City, Taiwan	2X[a]	Sexual	MO Awan-1	-
	P15	Meishankou, Kaohsiung City, Taiwan	2X[a]	Sexual	-	K022540
	P16	Mt. Kuolen, Cambodia	-	-	MO1635	K018416
	P17	Cat Tien, Vietnam	-	-	Wade1406	-
	P18	Kan-chanaburi, Thailand	2X[a]	Apomixis	Kuo3937	K019788
	P19	Hainan County, China	-	-	Kuo1679	K032799
Adiantum caudatum L.	C1	Dakeng, Taichung City, Taiwan	3X[a]	Apomixis	20081109–21MO	-
	C2	Qingshuiyan, Changhua County, Taiwan	3X[a]	Apomixis	20100502–034MO	-

TABLE 3. The morphological comparison of the *Adiantum* taxa examined in this study.

Species	*A. caudatum*	*A. caudatum*	*A. malesianum*	*A. meishanianum*	*A. philippense*	*A. philippense*
Ploidy ($2n$)	2X	3X	4X	3X	2X	3X
Reproductive mode	sexual	apomixis	sexual	sterile	sexual	apomixis
Number of pinnae	20–30	20–40	20–30	10–20	5–20	5–15
Shape of basal pinna	flabellate	flabellate	semi-orbiculate	semi-orbiculate	flabellate	flabellate
Shrink basal pinnae	no	yes	no	no	no	no
Length of pinnae stalk (mm)	0.5–1	0.5–1	1–3	3–5	5–15	3–25
Rachis adxial	densely hairy	densely hairy	densely hairy	glabrous	glabrous	glabrous
Rachis abxial	densely hairy	sparsely hairy	densely hairy	sparsely hairy	glabrous	glabrous
Lamina adxial	glabrous or sparsely hairy	sparsely hairy	densely hairy	glabrous	glabrous	glabrous
Lamina abxial	glabrous or sparsely hairy	sparsely or densely hairy	densely hairy	glabrous	glabrous	glabrous
False indusium meddle	densely hairy	densely hairy	glabrous or sparsely hairy	glabrous	glabrous	glabrous
False indusium margin	sparsely hairy	densely hairy	densely hairy	glabrous	glabrous	glabrous
Sterile pinna dissection	deep	shallow - middle	shallow	shallow-middle	deep	deep
Fertile pinna dissection	deep	shallow - middle	shallow	shallow-middle	deep	shallow

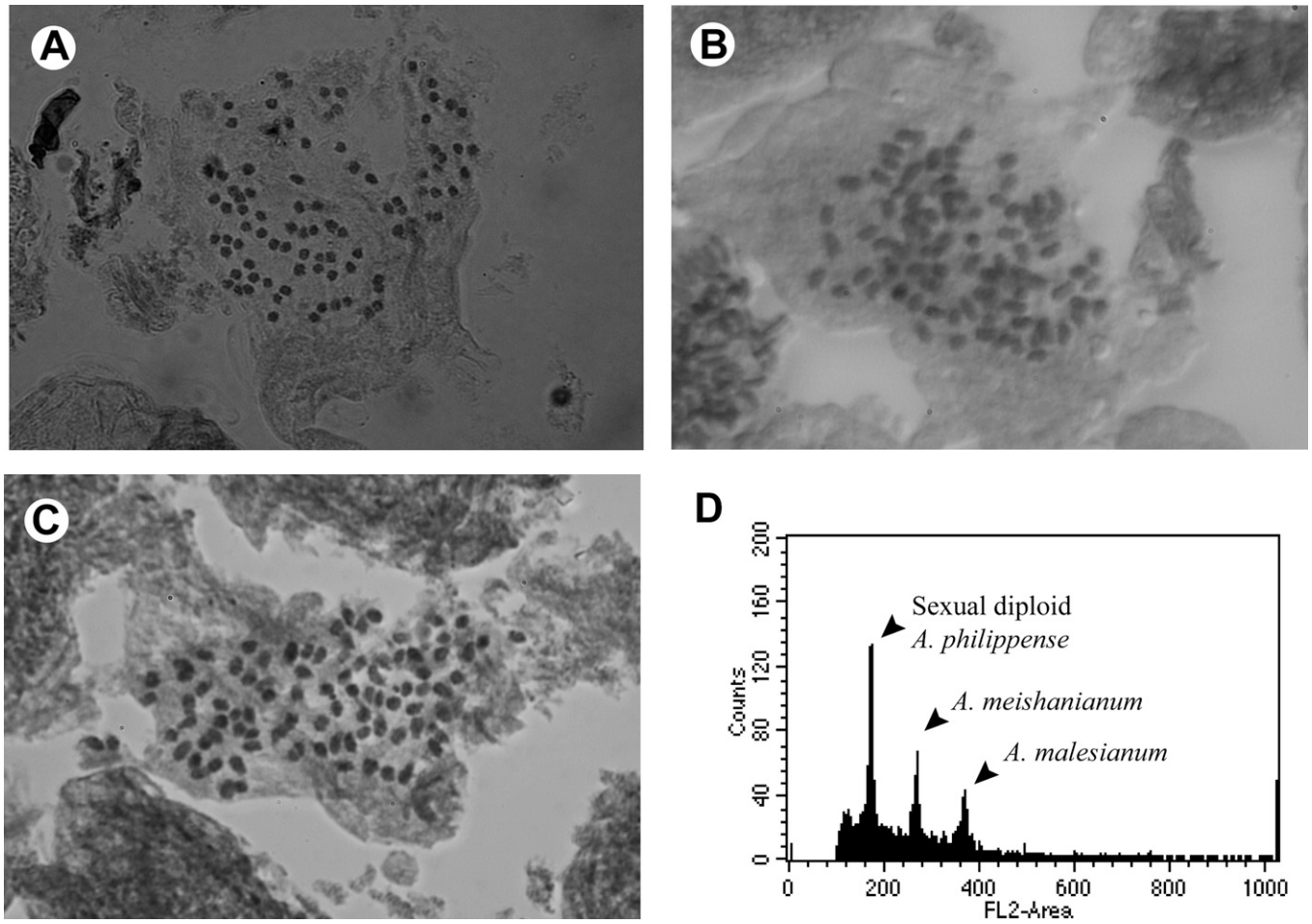

FIG. 3. Somatic chromosome photograph of *Adiantum caudatum* (A, 2*n* = ca. 90), *A. philippense* (B, 2*n* = 90), and *A. meishanianum* (C, 2*n* = ca. 90). Flow cytometry results (D) of sexual diploid *A. philippense*, *A. meishanianum*, and *A. malesianum*.

In conclusion, our study resolved the hybrid origin of *Adiantum meishanianum* through the analyses of ploidy, sequences of chloroplast and nuclear DNA, and reproductive modes. We show that *A. meishanianum* (Fig. 4A) is the sterile triploid hybrid between sexual diploid *A. philippense* (as the paternal parent; Fig. 4B) and sexual tetraploid *A. malesianum* (as the maternal parent; Fig. 4C). The extant population of *A. meishanianum* probably originated from a single hybridization event. The rarity and endemism of *A. meishanianum* are likely due to the limited distribution of the sexual diploid populations of *A. philippense*, which probably represent a cryptic species under subsp. *philippense* and have only been recorded from Meishan village and Shanping in Southern Taiwan.

TAXONOMIC TREATMENT

Adiantum ×meishanianum F. S. Hsu ex Y. C. Liu & W. L. Chiou (pro sp.) Description: A hybrid species between *Adiantum malesianum* Ghatak (as the maternal parent) and sexual diploid *Adiantum philippense* L. (as the paternal parent).

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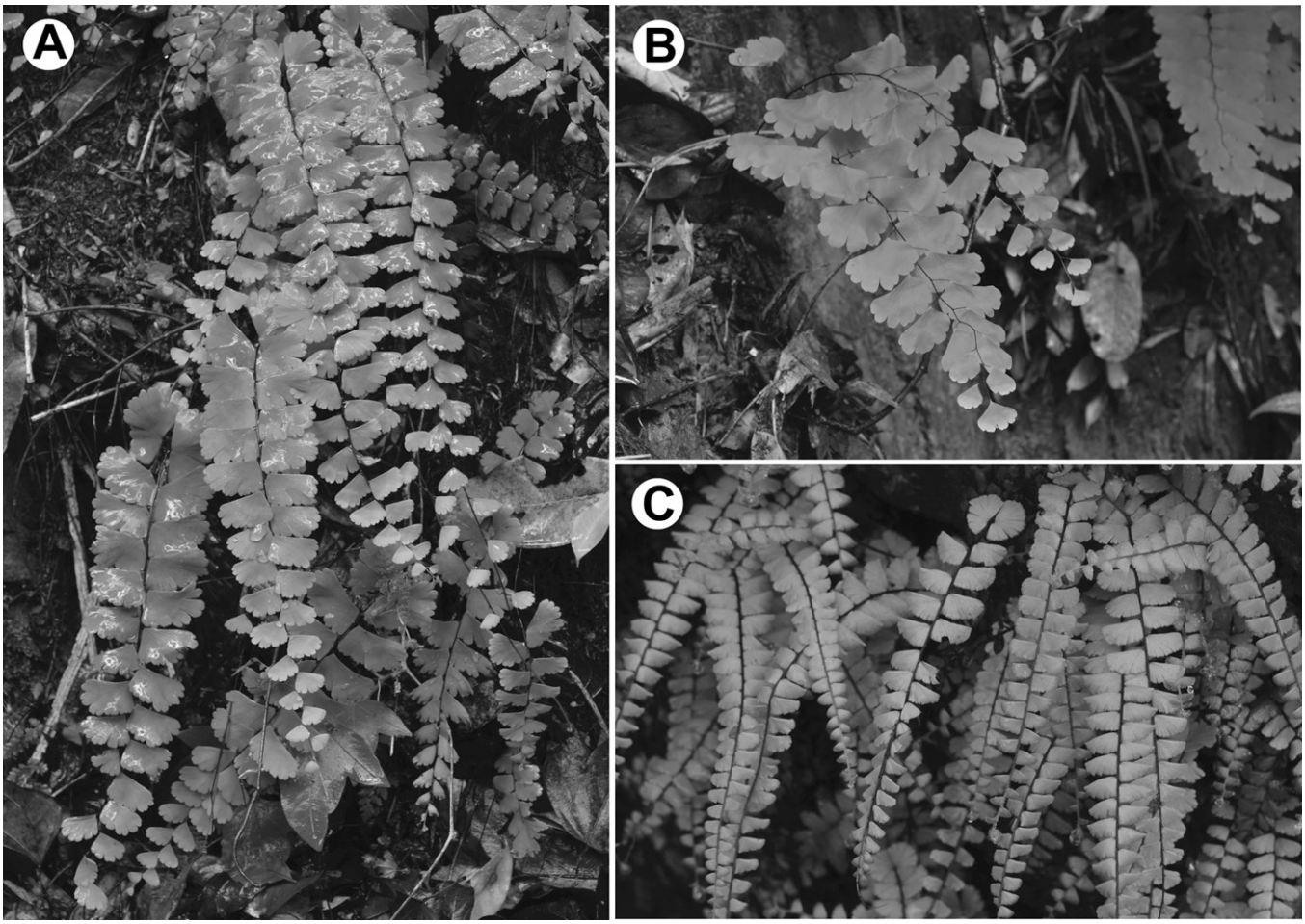

FIG. 4. Habitats and living forms of *Adiantum meishanianum* (A, photo provided by Wei-Hsiu Wu), sexual diploid *A. philippense* (B, photo provided by Wei-Hsiu Wu), and *A. malesianum* (C, photo provided by Pi-Fong Lu).

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Appendix 1. The materials used in cpDNA phylogeny. Information is presented as taxon name followed by locality, collection number (its herbarium and/or Dr. Cecilia Koo Botanic Conservation Center), rps16-matK IGS, and matK GenBank accession.

Adiantum capillus-junonis Rupr. TAIWAN. Nantou, *K012967* (KBCC), KJ605554, KJ605503. ***Adiantum capillus-veneris*** L. -, -, AY178864, AY178864. ***Adiantum caudatum*** L. TAIWAN. Changhua, *20100502-034MO* (TAIF), KJ605555, KJ605504; Chiayi, *Liu6808-e* (TAIF) & *K017626* (KBCC), KJ605556, KJ605505; Kaohsiung, *20081125-16MO* (TAIF), KJ605557, KJ605506; Pingtung, *K013900* (KBCC), KJ605558, KJ605507; Pingtung, *K017393* (KBCC), KJ605559, KJ605508; Taichung, *20081109-21MO* (TAIF), KJ605560, KJ605509; Taitung, *ZWY310* (TAIF), KJ605561, KJ605510. ***Adiantum edentulum*** Christ. TAIWAN. Taitung, *Kuo430* (TAIF), KJ605562, KJ605511. ***Adiantum edgeworthii*** Hook. CHINA. Yunnan, *K015018* (KBCC), KJ605563, KJ605512. ***Adiantum flabellulatum*** L. TAIWAN. Ilan, *K013833* (KBCC), KJ605564, KJ605513. ***Adiantum hispidulum*** Sw. TAIWAN. Chiayi, *Kuo3921* (TAIF) & *K017619* (KBCC), KJ605565, KJ605514. ***Adiantum macrophyllum*** Sw. cultivated, *Kuo3922* (TAIF) & *K023105* (KBCC), KJ605566, KJ605515. ***Adiantum malesianum*** J. Ghatak. TAIWAN. Chiayi, *Kuo3932* (TAIF) & *K013775* (KBCC), KJ605567, KJ605516; Haulian, *Liu9530* (TAIF), KJ605568, KJ605517; Kaohsiung, *Kuo3934* (TAIF) & *K022539* (KBCC), KJ605569, KJ605518; Miaoli, *Kuo3933* (TAIF) & *K019612* (KBCC), KJ605570, KJ605519; Nantou, *20080907-05MO* (TAIF), KJ605571, KJ605520; Pingtung, *20070730-MO* (TAIF), KJ605572, KJ605521; Tainan, *20070903-113MO* (TAIF), KJ605573, KJ605522. ***Adiantum meishanianum*** F. S. Hsu ex Y. C. Liu & W. L. Chiou. TAIWAN. Kaohsiung, *Liu5002* (TAIF), KJ605574, KJ605523; Kaohsiung, *Kuo3917-M02* (TAIF), KJ605575, KJ605524; Kaohsiung, *Kuo3917-M03* (TAIF), KJ605576, KJ605525; Kaohsiung, *Kuo3917-M05* (TAIF), KJ605577, KJ605526; Kaohsiung, *Kuo3917-M07* (TAIF), KJ605578, KJ605527; Kaohsiung, *Kuo3917-M08* (TAIF), KJ605579, KJ605528. ***Adiantum pedatum*** L. JAPAN. Aomori, *Kuo1224* (TAIF), KJ605580, KJ605529. ***Adiantum peruvianum*** Klotzsch. cultivated, *Kuo3931* (TAIF) & *K026690* (KBCC), KJ605581, KJ605530. ***Adiantum philippense*** L. VIETNAM. Cat Tien, *Wade1406* (TAIF), KJ605582, KJ605531; TAIWAN. Chiayi, *K022991* (KBCC), KJ605583, KJ605532; Chiayi, *K022542* (KBCC), KJ605584, KJ605533; Chiayi, *Liu6810* (TAIF), KJ605585, KJ605534; CHINA. Hainan, *Kuo1679* (TAIF) & *K013279* (KBCC), KJ605586, KJ605535; TAIWAN. Hsinchu, *K022993* (KBCC), KJ605587, KJ605536; THAILAND. Kan-chanaburi, *Kuo3937* (TAIF) & *K019788* (KBCC), KJ605588, KJ605537; TAIWAN. Kaohsiung, *K022546* (KBCC), KJ605589, KJ605538; Kaohsiung, *Kuo3936* (TAIF) & *K018606* (KBCC), KJ605590, KJ605539; Kaohsiung, *MO Awan-1* (TAIF), KJ605591, KJ605540; Kaohsiung, *K022540* (KBCC), KJ605592, KJ605541; CAMBODIA. Mt. Kuolen, *MO1635* (TAIF) & *K018416* (KBCC), KJ605593, KJ605542; TAIWAN. Nantou, -, KJ605594, KJ605543; Nantou, *K022545* (KBCC), KJ605595, KJ605544; Pingtung, *MO2390* (TAIF), KJ605596, KJ605545; Taichung, *K022990* (KBCC), KJ605597, KJ605546; Tainan, *Kuo3935* (TAIF) & *K022543* (KBCC), KJ605598, KJ605547; Tainan, *K022544* (KBCC), KJ605599, KJ605548; Taoyuan, -, KJ605600, KJ605549. ***Adiantum raddianum*** C. Presl. cultivated, *Kuo3923* (TAIF) & *K013914* (KBCC), KJ605601, KJ605550. ***Adiantum reniforme*** L. cultivated, *Kuo3925* (TAIF) & *K012975* (KBCC), KJ605602, KJ605551. ***Adiantum soboliferum*** Wall. TAIWAN. Tainan, *Kuo3926* (TAIF) & *K017623* (KBCC), KJ605603, KJ605552. ***Adiantum tenerum*** Sw. cultivated, *Kuo3930* (TAIF) & *K013712* (KBCC), KJ605604, KJ605553. ***Cheilanthes lindheimeri*** Hook. -, -, HM778032, HM778032.

Appendix 2. The materials used in nrDNA phylogeny. Information is presented as taxon name followed by the locality, collection number (its herbarium and/or Dr. Cecilia Koo Botanic Conservation Center), and CRY2 1st intron GenBank accession. The sequences obtained from SSCP separation are noted with "*".

Adiantum capillus-junonis Rupr. TAIWAN. Nantou, *K012967* (KBCC), KJ605605. ***Adiantum caudatum*** L. TAIWAN. Pingtung, *K013900* (KBCC), KJ605606; Taitung, *ZWY310* (TAIF), *KJ605607-08. ***Adiantum edgeworthii*** Hook. CHINA. Yunnan, *K015018* (KBCC), KJ605609. ***Adiantum malesianum*** J. Ghatak. TAIWAN. Chiayi, *K013775* (KBCC), *KJ605610-11. ***Adiantum meishanianum*** F. S. Hsu ex Y. C. Liu & W. L. Chiou. TAIWAN. Kaohsiung, *Liu5002* (TAIF), *KJ605612-14. ***Adiantum philippense*** L. THAILAND. Kan-chanaburi, *Kuo3937* (TAIF) & *K019788* (KBCC), KJ605615; TAIWAN. Kaohsiung, *K022540* (KBCC), KJ605616; Nantou, -, *KJ605617-19; Taichung, *K022990* (KBCC), *KJ605620-22; Tainan, *Kuo3935* (TAIF) & *K022543* (KBCC), *KJ605623-24; Chiayi, *K022993* (KBCC), *KJ605625-27. ***Adiantum soboliferum*** Wall. TAIWAN. Tainan, *Kuo3926* (TAIF) & *K017623* (KBCC), KJ605628.