



## Systematics of the *Adiantum philippense* complex (Pteridaceae, Polypodiales) in Taiwan

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### Abstract

*Adiantum philippense* s.l. is a member of once-pinnate *Adiantum* but comprise several cytotypes with different ploidies and reproductive modes. According to the differences of cytotypes, the up-to-date revision had identified three subspecies under the *A. philippense* complex. In Taiwan, different ploidies and reproductive modes had also been found in this species complex, and existence of (a) cryptic species among these cytotypes had been suggested. The current study focuses on taxonomical revision of Taiwanese *A. philippense* complex. We sampled not only different Taiwanese cytotypes/populations but also all subspecies under *A. philippense* and the closely related species of them. By re-examining their phylogeny, ploidies, and reproductive modes, we confirmed that the sexual diploids in Taiwan belong to *Adiantum menglianense*, and this species is phylogenetically separated from *A. philippense*. We further approved that *A. menglianense* can be morphologically distinguished from *A. philippense* by their frond features and spore numbers in sporangia. Another taxon of Taiwanese *A. philippense* complex is *A. philippense* subsp. *philippense*, which is an apomictic and triploidy subspecies. Additionally, we assigned lectotypes of *A. menglianense*, and revised the hybrid combination of *A. × meishanianum*. We also provided a key in order to identify two taxa under Taiwanese *A. philippense* complex and the other once-pinnate *Adiantum* species in Taiwan.

**Key words:** cryptic species, *meishanianum*, *menglianense*, species complex

### Introduction

Species complex is a group of closely related taxa with unclear species boundaries. In ferns, species complexes are usually involved with hybridization, apomixis, and polyploidization [e.g. *Vandenboschia radicans* (Sw.) Copel., *Pteris cadieri* Christ, *Pteris cretica* L., *Davallia repens* Desv., and *Dryopteris varia* (L.) Kuntze; Ebihara *et al.* 2005, 2009, Chao *et al.* 2012, 2015, Jaruwattanaphan *et al.* 2013, Chen *et al.* 2014, Hori *et al.* 2014]. These evolutionary events result in continuous morphological variations and/or similar appearance among them. Such situation also seems to occur in the *Adiantum philippense* L. complex, from which apomixis and polyploids have been reported (Mehra & Khullar 1977, Hsu 1993, Cheng & Zhang 2010, Zhang *et al.* 2014), and their morphological boundaries between species or subspecies are still vague (Verma & Fraser-Jenkins 2008, Zhang *et al.* 2014). Despite this, there are still several taxa treated as either synonym or subspecies under *A. philippense*.

“*Adiantum philippense*” first appeared in Petiver’s drawing in 1702 (p. 8, tab. IV, fig. 4.) based on one collection from Luzon, the Philippines. This species was formally published with Latin diagnosis by C. Linnaeus in his publications “Species Plantarum” (tomus 2, p. 1094) in 1753, and Pichi Sermolli (1957) then assigned Petiver’s drawing as the lectotype. Nonetheless, because of limited morphological information based on Petiver’s drawing, some authors (e.g. Verma 1962, Morton 1974, Sledge 1982, Verdcourt 2002, Mickel & Smith 2004) had preferred the name of *A. lunulatum* Burm. f., a species published in 1768, over *A. philippense*. In order to advocate the name of *A. philippense*, Verma & Fraser-Jenkins (2008) proved the identity of the Petiver’s drawing is *A. philippense*, and further

provided an epitype using a triploid apomictic collection from India. At the same time, they regarded *A. lunulatum* and other seven taxa as synonyms under *A. philippense*, and identified other two subspecies, subsp. *teestae* S.C.Verma & Fraser-Jenkins (2008: 83) and *intermedium* S.C.Verma & Fraser-Jenkins (2008: 82) according to their differences in cytology and morphology.

In Taiwan, the *Adiantum philippense* complex had been found with apomictic triploids and sexual diploids (Hsu 1993, Zhang *et al.* 2014). Zhang *et al.* (2014) implied that the sexual diploids belong to a cryptic species because of its distinctive cytotype, reproductive mode, and phylogenetic position from the type of *A. philippense* subsp. *philippense*, which is regarded as an apomictic triploid (Verma & Fraser-Jenkins 2008). However, other two subspecies of *A. philippense* and *A. menglianense* Y.Y.Qian, the close relatives of *A. philippense* (Verma & Fraser-Jenkins 2008, Wang *et al.* 2014, Wang *et al.* 2015), had not been taken into consideration in Zhang *et al.* (2014). Current study focused on the systematics of Taiwanese *Adiantum philippense* complex. First, in addition to the data from Zhang *et al.* (2014), we increased our sampling from Asia, especially for *A. philippense* and its close relatives. By examining their cytology, reproductive mode, phylogenetic relationships, and morphological characters, we revised the taxa under Taiwanese *Adiantum philippense* complex and provided their morphological comparisons.

## Materials & Methods

### Taxa sampling

*Adiantum capillus-veneris* and *A. reniforme* were selected as outgroup for once-pinnate *Adiantum* species. A total 21 samples of the *A. philippense* complex (including *A. menglianense*) was included. In addition to the *A. philippense* complex, 9 Asian species of once-pinnate *Adiantum* species were also sampled. Voucher information of the samples used in this study is summarized in the Table 1 and 2.

### Chloroplast sequences

The *matK* and *rps16-matK* intergenic spacer (IGS) were included in the chloroplast DNA (cpDNA) dataset; and primer “Adn rps16 fEET”, “Adn matK rRLF”, “Adn matK fHIS”, and “FERmatK rAGK” were used. DNA extraction, PCR condition, and primer information were detailed in Zhang *et al.* (2014). For Taiwanese *Adiantum menglianense* sample, we additionally generated *rbcL* and *trnL-L-F* (*trnL* gene + *trnL-F* IGS) sequences for comparing the sequence variation with the Chinese populations; the primers information and PCR condition were detailed in Li *et al.* (2010, 2011).

### Phylogeny analyses

CpDNA dataset of *matK* + *rps16-matK* IGS were aligned using ClustalW implemented in BioEdit (Hall 1999). 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 infer most likely ML tree, ten independent replicates were performed, and the tree with highest likelihood was selected. 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 (Ronquist & 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. Log likelihoods of MCMC runs were inspected into Tracer v1.6 (Rambaut & Drummond 2013) to determine convergence. The first 25% of the sample was discarded as burn-in, and the rest were used to calculate the 50% majority-rule consensus tree. For maximum parsimony (MP) phylogeny, we used PAUP\* 4.0 (Swofford 2003) with the setting of random-taxon-addition, TBR swapping, gaps as missing data and equal weighting. Heuristic bootstrap analysis of the MP phylogeny was performed with 500 bootstrap replicates, ten random addition cycles per bootstrap replicate, TBR swapping, and equal weighting.

### Reproductive mode and ploidy determination

The methods to determine reproductive mode and ploidy in *Adiantum philippense* complex followed Zhang *et al.* (2014). 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). Sporophyte ploidies were determined by flow cytometry analyses using the standards with known cytotype (Zhang *et al.* 2014).

**TABLE 1.** The sample information of *Adiantum philippense* complex examined with ploidy level, reproductive mode, and molecular phylogeny.

Taxa	Locality	Ploidy	Reproductive mode	Voucher <sup>b</sup>	Living collection in KBCC <sup>c</sup>	<i>rps16-matK</i> IGS GenBank no.	<i>matK</i> GenBank no.
<i>Adiantum menglianense</i>	Shanping, Kaohsiung City, Taiwan	2X <sup>a</sup>	Sexual <sup>a</sup>	<i>Kuo3936</i>	<i>K018606</i>	KJ605590	KJ605539
	Meishankou, Kaohsiung City, Taiwan	2X <sup>a</sup>	Sexual <sup>a</sup>	<i>MO Awan-1</i>	-	KJ605591	KJ605540
	Fuxing, Taoyuan County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4254</i>	-	KJ605600	KJ605549
	Jianshih, Hsinchu County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4202</i>	<i>K022991</i>	KJ605583	KJ605532
	Chunyang, Nantou County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4255</i>	-	KJ605594	KJ605543
	Caohu, Taichung City, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4200</i>	<i>K022990</i>	KJ605597	KJ605546
	Wanda Reservoir, Nantou County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4201</i>	<i>K022545</i>	KJ605595	KJ605544
	Mt. Duli, Chiayi County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4203</i>	<i>K022993</i>	KJ605587	KJ605536
	Zengwen Reservoir 1, Chiayi County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4203</i>	<i>K022542</i>	KJ605584	KJ605533
	Zengwen Reservoir 2, Chiayi County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Liu6810</i>	-	KJ605585	KJ605534
<i>A. philippense</i> subsp. <i>philippense</i>	Nanhua Reservoir 1, Tainan County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo3935</i>	<i>K022543</i>	KJ605598	KJ605547
	Nanhua Reservoir 2, Tainan County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4256</i>	<i>K022544</i>	KJ605599	KJ605548
	Shanping, Kaohsiung City, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo4253</i>	<i>K022546</i>	KJ605589	KJ605538
	Neishi, Pingtung County, Taiwan	3X <sup>a</sup>	Apomixis <sup>a</sup>	<i>MO2390</i>	-	KJ605595	KJ605544
	Hainan County, China	3X	Apomixis <sup>a</sup>	<i>Kuo1679</i>	<i>K032799</i>	KJ605586	KJ605535
	Yunnan County, China	-	Apomixis	<i>Kuo3176</i>	-	KU945414	KU945407
	Sichuan County, China	-	Apomixis	<i>Kuo1564</i>	-	KU945413	KU945406
	Cat Tien, Vietnam	-	-	<i>Wade1406</i>	-	KJ605582	KJ605531
	Mt. Kuolen, Cambodia	-	-	<i>MO1635</i>	<i>K018416</i>	KJ605593	KJ605542
	Kan-chanaburi, Thailand	2X <sup>a</sup>	Apomixis <sup>a</sup>	<i>Kuo3937</i>	<i>K019788</i>	KJ605588	KJ605537
<i>A. philippense</i> subsp. <i>intermedium</i>							
<i>A. philippense</i> subsp. <i>teestae</i>	Pokhara, Nepal	-	Apomixis	<i>TAIF357240</i>	-	KU945412	KU945405

<sup>a</sup> Information from Zhang *et al.* 2014; <sup>b</sup> the specimen kept in TAIF; <sup>c</sup> the collection from Dr. Cecilia Koo Botanic Conservation Center.

**TABLE 2.** The sample information of other *Adiantum* species applied in phylogenetic analyses.

Taxa	Locality	Voucher and/or KBCC living collection	<i>rps16-matK</i> IGS GenBank no.	<i>matK</i> GenBank no.
<i>Adiantum capillus-junonis</i> Rupr.	Nantou (Taiwan)	<i>K013775</i> (KBCC)	KJ605567	KJ605516
<i>Adiantum capillus-veneris</i> L.	-	-	AY178864	AY178864
<i>Adiantum caudatum</i> L.	Taichung (Taiwan)	<i>20081109-21MO</i> (TAIF)	KJ605560	KJ605509
<i>Adiantum edgeworthii</i> Hook.	Yunnan (China)	<i>K015018</i> (KBCC)	KJ605563	KJ605512
<i>Adiantum gravesii</i> Hance	Guangxi (China)	<i>Wade2494</i> (TAIF)	-	KU945408
<i>Adiantum incisum</i> Forssk.	Nepal	<i>Kuo3919</i> (TAIF) & <i>K023103</i> (KBCC)	KU945415	KU945410
<i>Adiantum juxtapositum</i> Ching	Hunan (China)	<i>Kuo726</i> (TAIF)	KU945416	KU945411
<i>Adiantum malesianum</i> J.Ghatak	Chiayi (Taiwan)	<i>Kuo3932</i> (TAIF) & <i>K013775</i> (KBCC)	KJ605567	KJ605516
<i>Adiantum mariesii</i> Baker	Guangxi (China)	<i>Wade2493</i> (TAIF)	-	KU945409
<i>Adiantum meishanianum</i> F.S.Hsu ex Y.C.Liu & W.L.Chiou	Kaohsiung (Taiwan)	<i>Liu5002</i> (TAIF)	KJ605574	KJ605523
<i>Adiantum reniforme</i> L.	cultivated	<i>Kuo3925</i> (TAIF) & <i>K012975</i> (KBCC)	KJ605602	KJ605551
<i>Adiantum soboliferum</i> Wall.	Tainan (Taiwan)	<i>Kuo3926</i> (TAIF) & <i>K017623</i> (KBCC)	KJ605603	KJ605552

## Results

### *Phylogeny and cpDNA differences*

The cpDNA alignment matrix of *rps16-matK* IGS + *matK* contained a total of 2,273 characters with 909 variable sites. The log-likelihood score for the most likely ML tree was -9,073.223478 (Fig. 1). Within once-pinnate *Adiantum*, the *A. philippense* complex consists of two highly supported monophyletic groups: *A. menglianense* and *A. philippense* s.s. The latter was further consisted by three subspecies: *philippense*, *intermedium*, and *teestae*. Subsp. *philippense* was not formed a monophyletic group, and subsp. *intermedium* was sister to subsp. *philippense* + *teestae*. For *A. menglianense* in Taiwan, the *rbcL* and *trnL-L-F* sequences (GenBank accession no. KU945417 and KU945404, respectively) have 2–7 substitutions (among 1,275bp alignment) and 2–3 substitutions (among 764 bp alignment), respectively, slightly different from those of Yunnan and Guangdong populations in China (Wang *et al.* 2014, Wang *et al.* 2015)

### *Reproductive mode and ploidy*

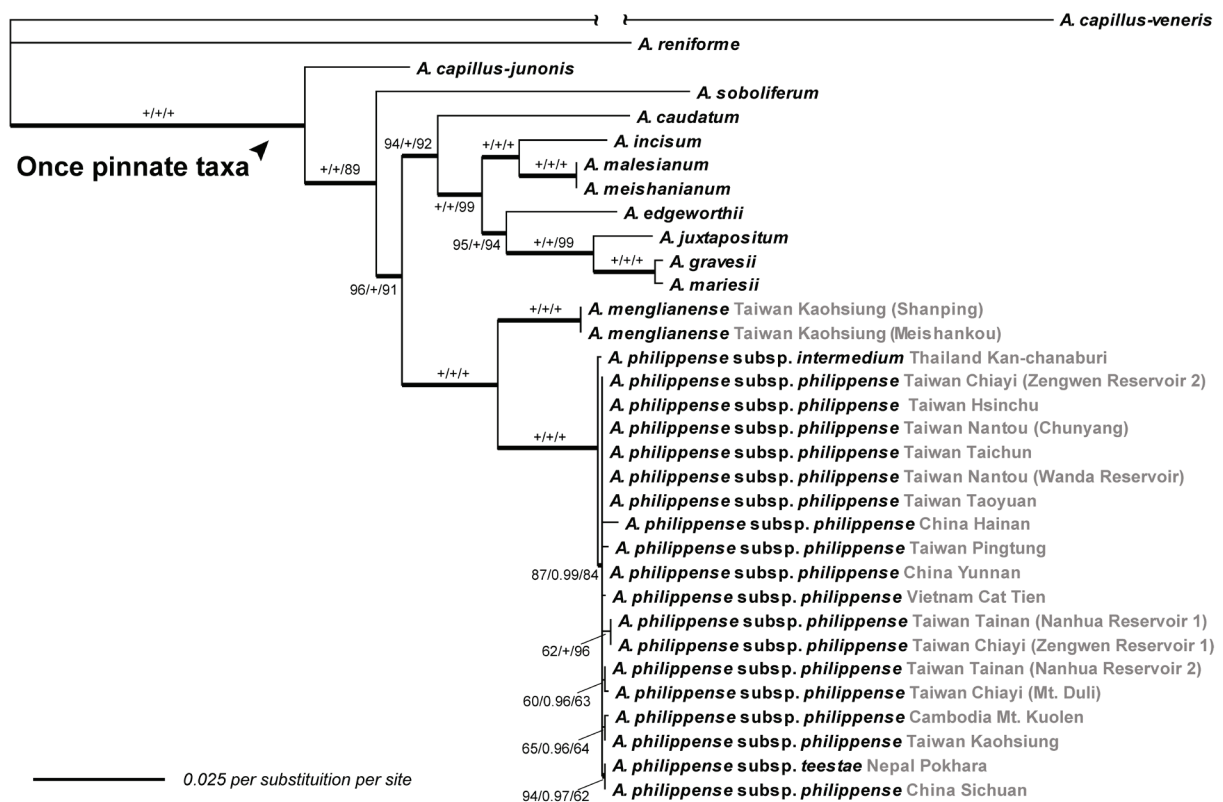
Among the taxa of the *Adiantum philippense* complex, *A. menglianense* was found with only sexual diploids, and *A. philippense* subsp. *philippense* was found with only apomictic triploids (Table 1 and Appendix 1). Apomictic diploid was found in *A. philippense* subsp. *intermedium* (Table 1). For *A. philippense* subsp. *teestae*, only apomictic individuals were found but their ploidies were unknown because their living materials were unavailable (Table 1 and Appendix 1). In Taiwan, only *A. philippense* subsp. *philippense* and *A. menglianense* were found.

## Discussion

### *Adiantum menglianense—a newly recorded species in Taiwan*

In this study, we confirmed that the sexual diploids of *Adiantum philippense* in Taiwan are *A. menglianense*, and that *A. menglianense* is a distinct species from *A. philippense*. Morphologically, these sexual diploids in Taiwan are similar to type description of *A. menglianense* having more dissected fertile pinnules than *A. philippense* (Qian 1992). They also tend to have narrower false indusia (2–6 mm) than that in *A. philippense* subsp. *philippense* (2–12mm),

which apparently agree with the morphological differences revealed by Wang *et al.* (2014). In addition, same as the type description of *A. menglianense*, they have 64-spored sporangia (i.e. the indication of sexual reproduction; Table 1). 64-spored sporangium is a very useful character to distinguish from *A. philippense* subsp. *philippense*, which is referred to apomictic triploid (i.e. with 32-spored sporangia) and most widespread distributed among the three subspecies. Comparing to *A. philippense* subsp. *teestae*, a sexual diploid subspecies (Verma & Fraser-Jenkins 2008), *A. menglianense* has much larger plant sizes and the lowest pinna with basal angles  $>180^\circ$  (vs.  $\leq 150^\circ$ ). Phylogenetically, *Adiantum menglianense* and *A. philippense*, including three subspecies, belong to two separate and sister monophyletic groups (Fig. 1), and such relationship is congruent with previous phylogenies (Wang *et al.* 2014; Wang *et al.* 2015). Taiwanese and Chinese *A. menglianense* populations share a very high sequence identity in *trnL-L-F* and *rbcL* (as detailed in result section), and both phylogenies based on these two genetic regions also support the monophyly of *A. menglianense* sister to *A. philippense* (data not shown). In addition, phylogeny of low-copy nuclear gene, *CRY2* intron 1, further implies that *A. menglianense* is unlikely involved in the reticulation (i.e. polyploidization and hybridization) within *A. philippense* (Zhang *et al.* 2014). Based on above evidences, *A. menglianense* should be regarded as a species biologically independent from *A. philippense* although two species are very similar in morphology.



**FIGURE 1.** Plastid phylogeny of the *Adiantum philippense* complex in Taiwan. ML bootstrap support (MLBS) values, the posterior probabilities of Bayesian phylogenetic inference (PP), and MP bootstrap support (MPBS) are indicated on each branch, as MLBS/PP/MPBS. The plus (+) sign represents MLBS = 100, PP = 1.00, or MPBS = 100. The thickened branch indicates MLBS  $\geq 70$  and PP  $\geq 0.95$ .

To reveal distribution of *Adiantum menglianense* in Taiwan, the herbarium collections were examined. Based on frond morphology and spore number in sporangia, we report three populations in Taiwan. All the known distribution records of *A. menglianense* in Taiwan are mapped on Fig. 2, and their voucher specimens are cited in Appendix 2.

### Lectotypification of *Adiantum menglianense*

*Adiantum menglianense* Y.Y.Qian, Acta. Bot. Austro Sin. 8: 37. 1992.

Type:—CHINA. Yunnan: Menglian, 900–1400 m, 23 December 1984, *Qian 801* (lectotype: HITBC-81381!, **here designated**; isolectotypes: HITBC-81380!, HITBC-109908!).

**Chinese name:**—孟連鐵線蕨

In the protologue of *Adiantum menglianense* (Qian 1992), the *Qian 801* collection conserved in Simao Forest Bureau (SMAO) was designated as holotype. The type material should now be stored in the herbarium of Xishuangbanna Tropical Botanical Garden (HITBC) since all collections of SMAO were transferred to HITBC in 2005 (<http://www.cvh.ac.cn/her/HITBC>, accessed 8 Nov 2015). At HITBC, however, we found altogether three sheets of *Qian 801*, including two (HITBC-81380 and HITBC-81381) marked presumably by Qian as “holotypus” and one more duplicate (HITBC-109908) without any type label. Since there is no clear indication that HITBC-81380 and HITBC-81381 sheets belong to a single gathering, both sheets should be corrected as syntypes in accordance with Art 8.3, Art 9.5 and Art 40 Note 1 of the Melbourne Code (McNeill *et al.* 2011), and the HITBC-109908 sheet is then treated as another syntype. A lectotype is subsequently designated based on Art 9.2 and Art 9.12, and HITBC-81381 is here chosen since the gathering on this sheet contains more complete sterile and fertile fronds among the syntypes.

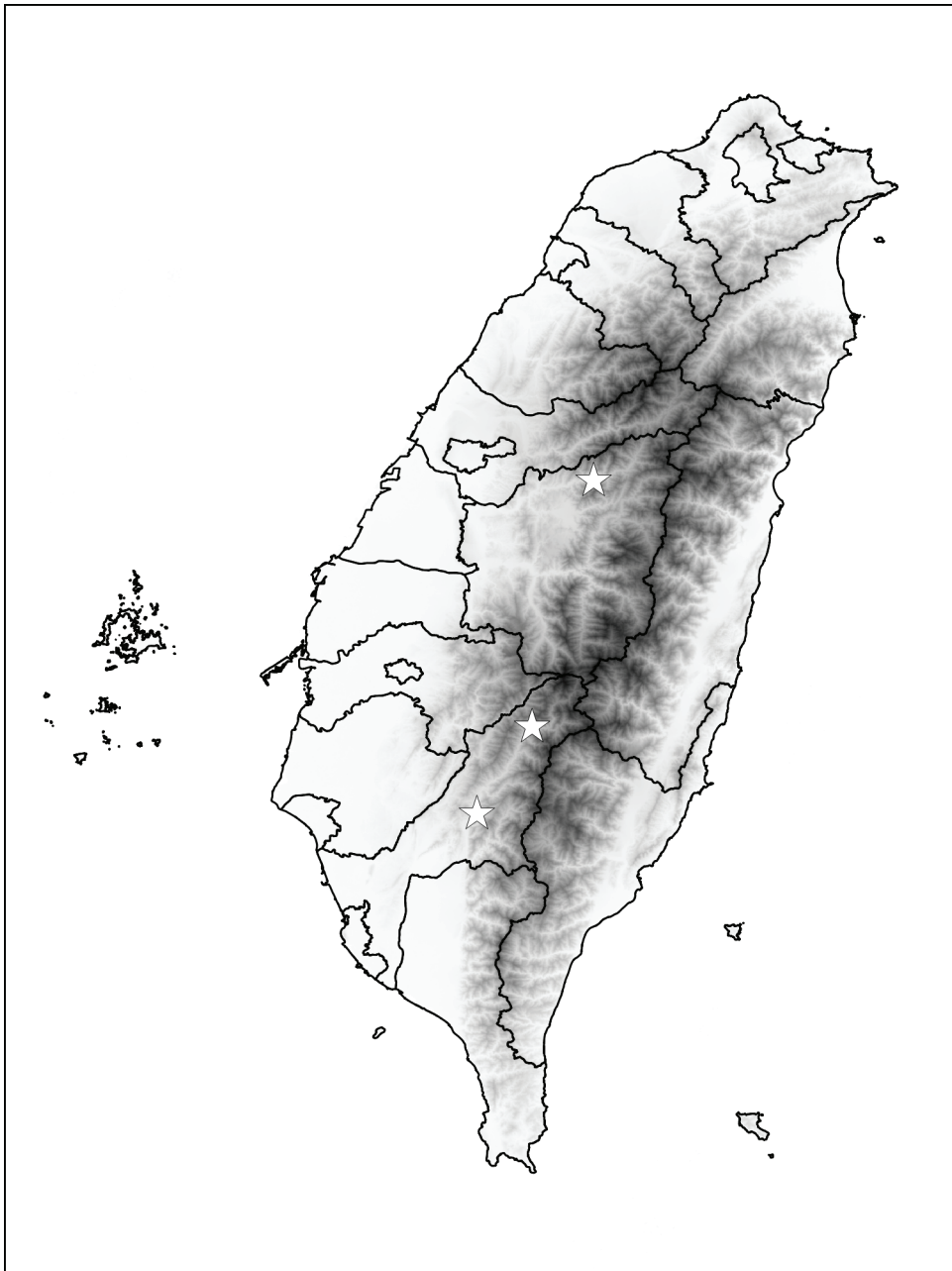


FIGURE 2. Distribution of *Adiantum menglianense* (stars) in Taiwan.

#### *Differences and taxonomy among subspecies under Adiantum philippense*

Verma & Fraser-Jenkins (2008) provided an updated revision of the *Adiantum philippense* complex, and identified three subspecies within a single species *A. philippense*. Based on this revision, types of these three subspecies are referred to different cytotaxa: subsp. *philippense*  $n=90$ , subsp. *teestae*  $n=30$ , subsp. *intermedium*  $n=60$  (meiotic chromosome counts). Also, combing with records of sporophyte chromosome numbers (mitotic counts; reviewed

in Mehra & Khullar 1977, Hsu 1993, Cheng & Zhang 2010, Zhang *et al.* 2014), these records suggested subsp. *philippense* is an apomictic triploid, subsp. *teestae* is a sexual diploid, and subsp. *intermedium* is an apomictic diploid or sexual tetraploid. Morphologically, subsp. *teestae* tends to have smaller frond sizes (8–12cm mostly) among three subspecies, and has lowest pinna with basal angles less than 150° (Verma & Fraser-Jenkins 2008). Subsp. *intermedium* has intermediate frond sizes among three subspecies, and has pinna with basal angles around 100° (Verma & Fraser-Jenkins 2008). So far, in Taiwan, only apomictic triploids of *A. philippense* are found, and they morphologically fit subsp. *philippense*.

However, based on such cytotaxon concepts, morphological boundaries between subspecies seem still unclear. For example, a sexual diploid was revealed similar to either subsp. *philippense* or *intermedium* by Mehra & Khullar (1977), and, in this study, all individuals morphologically fitting subsp. *teestae* were found to be apomictic. Besides, current phylogenetic evidences are inadequate to disentangle reticulation within the *Adiantum philippense* complex. Thus, more sampling with cytotype information, including materials from America and Africa, should be applied into future investigation of nuclear phylogeny. Only relying on well resolved reticulate relationships can biological species or subspecies appropriately assign to the *A. philippense* complex, such as the taxonomic treatments for the *Vendenboschia radicans* complex (Ebihara *et al.* 2009).

### Hybridization origin of *Adiantum* × *meishanianum*

Previously, Zhang *et al.* (2014) revealed that *Adiantum* × *meishanianum* is a sterile hybrid between the sexual diploid *A. philippense* and *A. malesianum*, which are parental and maternal parents, respectively. In this study, we confirmed that this sexual diploid *A. philippense* is actually *A. menglianense* (see in the first section of discussion). Therefore, the parental parent of *A.* × *meishanianum* should change to *A. menglianense*. *A.* × *meishanianum* was also reported in China recently (Yunnan; Wang *et al.* 2014). However, the data of reproductive mode and nuclear DNA sequences for Chinese population are still lacked, and its parentages require further confirmation to compare that in the type population/locality.

### Key to the once-pinnate *Adiantum* species in Taiwan

1. Stipes, rachis and stalks winged ..... *A. soboliferum* 2
- Stipes, rachis and stalks terete ..... 2
2. Rachis densely hirsute adaxially, sparse to densely hairy abaxially; laminae glabrous to densely hirsute ..... 3
- Rachis glabrous adaxially, glabrous to very sparsely hairy abaxially; laminae glabrous ..... 4
3. Basal pinnae semiorbicular; stalks 1–3 mm ..... *A. malesianum*
- Basal pinnae flabellate; stalks 0.5–1 mm ..... *A. caudatum*
4. Stalks of basal pinnae less than 3 mm long ..... 5
- Stalks of basal pinnae 3–25 mm long ..... 6
5. Pinnae opposite or subopposite; stalks 1–3 mm long; pinnules nearly orbicular ..... *A. capillus-junonis*
- Pinnae alternate or only basalmost pair subopposite; stalks less than 1 mm long; pinnules semidimidiolate ..... *A. edgeworthii*
6. Rachis sparsely hairy abaxially; basal pinnae semiorbicular ..... *A.* × *meishanianum*
- Rachis glabrous; basal pinnae flabellate ..... 7
7. Spores 64 per sporangia; sterile pinnules with upper margin lobed for 1/3–2/3 of length; fertile pinnules with upper margin lobed for 1/3–1/2 of length; false indusia 2–6 mm wide ..... *A. menglianense*
- Spores 32 per sporangia; sterile pinnules with upper margin crenate or lobed for less than 1/3 of length; fertile pinnules with upper margin subentire, undulate or lobed for 1/3(–1/2) length; false indusia 2–12 mm wide ..... *A. philippense* subsp. *philippense*

### Acknowledgments

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<http://dx.doi.org/10.1109/TAC.1974.1100705>

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**APPENDIX 1.** Additional specimens in TAIF and TAI herbarium examined with spore numbers per sporangium (s/s).

*Adiantum philippense* subsp. *philippense*: 32 s/s, TAIF no. 296814, 301087, 301097, 301118, 301130, 301092, 301149, 301185, 334965, 301123, 301146, 454520, TAI no. 3216, 153141, 264095. *Adiantum philippense* subsp. *teestae*: 32s/s, TAIF no.301003, 301128, 320281, 390445. *Adiantum menglianense*: 64s/s, TAI no. 162293, 157285, 153122, 156374.

**APPENDIX 2.** Additional examined specimens of *Adiantum menglianense* in Taiwan.

Nantou Co., Kuan-tao-chi, 9 Sep 1972, *H. C. Lin* 673 (TAI 162293), *C. M. Kuo* 2171 (TAI 153122); Nantou Co., Nen-Kao Forest Experiment Station, 9 Sep 1972, *T. C. Huang* & *M. T. Kao* 6176 (TAI 156374); Kaohsiung City, Meishankou, 15 May 2015 *P. F. Lu* 10542 (TAIF), 3 Nov 2015, *L. Y. Kuo* 4251; Kaohsiung City, Shanping, 22 Aug 1972, *T. Y. Yang* 45 (TAI 157285), 5 Feb 2014, *L. Y. Kuo* & *C. M. Chen* 3936 (TAIF 447311).