Taiwania 65(2): 253–260, **2020**
DOI: 10.6165/tai.2020.65.253

NOTE

New addition to the *Asplenium normale* complex (Aspleniaceae): an endemic forma in Taiwan

Zhi-Xiang CHANG[1,#], Li-Yaung KUO[2,3,#], Pi-Fong LU[4], Yao-Moan HUANG[5,*]

1. School of Forestry and Resource Conservation, National Taiwan University, 1, Sec.4, Roosevelt Rd., Taipei 10617, Taiwan.
2. Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu 30013, Taiwan.
3. Bioresource Conservation Research Center, College of Life Science, National Tsing Hua University, Hsinchu 30013, Taiwan.
4. Taiwan Society of Plant Systematics, No. 1, Sec. 4, Roosevelt Rd., Taipei 10617, Taiwan.
5. Taiwan Forestry Research Institute, No. 53, Nanhai Rd., Taipei 10066, Taiwan.
[#]The authors contributed equally. [*]Corresponding author's email: huangym@tfri.gov.tw

(Manuscript received 25 September 2019; Accepted 2 March 2020; Online published 20 April 2020)

ABSTRACT: Evolutionary histories of species complexes in ferns are usually complicated with hybridization and polyploidization. In the *Asplenium normale* D.Don complex, we here identify a new taxon - *Asplenium normale* f. *scythiforme* Z.X. Chang, *f. nov*., by clarifying its position in the reticulated tree of the species complex. Our phylogenetic and flow cytometric results surprisingly support that this new taxon is a non-hybridized diploid and conspecific with *A. normale.* Nonetheless, the forma *scythiforme* can be separated from the *normale* by having more dissected and falcate pinnae with apices acuminate to fishbone-like tails. Currently, *A. normale* f. *scythiforme* was found to be endemic to Taiwan with only one northern and one southern populations.

KEY WORDS: *Asplenium normale* f. *scythiforme*, *Asplenium normale* complex, flow cytometry, morphology, phylogenetic.

INTRODUCTION

Asplenium L. is one of the most species-rich genera among ferns and comprises of more than 700 species (Schneider *et al*., 2004; Rothfels *et al.*, 2012; PPG I, 2016), and also known with a great number of species complexes (see citation below). These species complexes are usually revealed with hybridization and polyploidization behind their evolutionary histories (e.g. Ekrt and Štech, 2008; Shepherd *et al.*, 2008; Dyer *et al*., 2012; Chang *et al*., 2013, 2018; Fujiwara *et al*., 2017; Sessa *et al.*, 2018). One of the most widespread *Asplenium* complexes in East Asia is *A. normale* D.Don. In total, ten species have been recognized in the *A. normale* complex (*sensu* Chang *et al*., 2018), including diploid species - *A. pifongiae* L.Y.Kuo, F.W.Li & Y.H.Chang,, *A. normaloides* Y.Fen Chang & H.Schneid., *A. oligophlebium* Baker, *A. pseudonormale* W.M.Chu & X.C.Zhang, *A. guangdongense* Y.Fen Chang & H.Schneid., polyploid species - *A. boreale* (Ohwi ex Sa.Kurata) Nakaike, *A. kiangsuense* Ching & Y.X.Jing, *A. shimurae* (H.Ito) Nakaike, *A. hobdyi* W.H.Wagner, and *A. normale* which is known with both diploids and polyploids (Fujiwara *et al*., 2017; Chang *et al*., 2018). Except for *A. boreale*, the polyploids in this species complex have been revealed with hybrid origins, and thus, are allopolyploids (Fujiwara *et al*., 2017; Chang *et al*., 2018). Diploid species *A. pifongiae* is sister to the rest species of *A. normale* complex that include all polyploidy members (Li *et al.*, 2016; Chang *et al*., 2018).

The *Asplenium normale* complex likely includes cryptic species and needs further studies of cytology and nuclear phylogeny (Fujiwara *et al*., 2017; Chang *et al*., 2018). Since 2007, one uncertain *Asplenium* fern was found with two populations respectively in northern and southern Taiwan. This *Asplenium* is morphologically similar to members in the *A. normale* complex, particularly *A. oligophlebium* because both produce lobbed pinnae (Fig. 1). In comparison, *A. oligophlebium* has a smaller plant size and has been found only in Japan.

In this study, we provide phylogenetic evidence to clarify reticulation and flow cytometric evidence to infer ploidy, and confirmed that this new taxon belongs to the species *Asplenium normale* and is endemic to Taiwan. Nonetheless, it can be morphologically distinguished from *A. normale* (f. *normale*) by its pinnae shape. We therefore descript a new forma of *A. normale* for this new taxon - *Asplenium normale* f. *scythiforme* Z.X. Chang. We also compare the morphology (Table 1) and provide a key to all related species in Taiwan.

MATERIALS AND METHODS

(I). Plant materials

For the new taxon of the *A. normale* complex, only two populations were found in Taiwan - one northern (Mingchi Forest Recreation Area, Yilan County) and one southern (Mt. Guzilun, Pingtung County) population. In each population, one individual was collected (collection no. *Kuo4231* and *Kuo4283*, respectively) as our phylogenetic and cytometric sampling. Additional collections (collection no. *P.-F. Lu 20525*, *P.-F. Lu 20857*

Table 1. Morphological comparisons used to distinguish *A. oligophlebium* and *Asplenium normale* complex in Taiwan.

Character	***A. boreale***	***A. normale* f. *normale***	***A. normale* f. *scythiforme***	***A. oligophlebium***	***A. pifongiae***
Pinna shape	Oblong, margin serrate	Oblong, margin serrate	Falcate and dissected	Linear and dissected with irregular crenulate	Oblong to quadrangular, margin serrate
Apex of pinna	Obtuse to rounded	Acute	Acuminate	Acuminate	Obtuse
Frond vegetative bud	Absent	Bearing on the rachis apex and do not elongate to whip.	Bearing on the rachis apex and do not elongate to whip.	Bearing on the rachis apex and elongate to whip.	Absent
The pinna ratio of length to width	1:0.6	1:0.5	1:0.3	1:0.3	1:0.4
Number of sori per pinna	Usually more than 5	Usually more than 5	NA	Usually more than 4	1 or 2, rarely to 4

Note: *Asplenium boreale* is treated as a different species here instead of a subspecies under *A. normale* as the treatment in TPG (2019).

Fig. 1. *Asplenium normale* f. *scythiforme* Z-X Chang. **A**. Habitat. **B**. Buds. **C**. Adaxial side of leaf. **D**. Abaxial side of leaf. Scale bar: A = 10 cm; B = 5 cm; C = 1 cm; D = 1 cm. (Photos A, B provided by Pi-Fong Lu; Photos C, D provided by Zhi-Xiang Chang)

and *ZXC 001515*) from these two populations were examined to confirm their morphological variation. For our phylogenetic analyses, taxon sampling in the *Asplenium normale* complex and outgroups followed Li *et al*. (2016), Fujiwara *et al*. (2017), and Chang *et al*. (2018). Voucher information and GenBank accession numbers are provided in the Appendix.

(II). Genome size estimation

We used the modified Beckman protocol following Kuo and Huang (2017), and the leaf tissue of *Nicotiana tabacum* L. 'Xanthi' was used as the internal standard

(genome size = 5.02 pg/C; Johnston *et al.*, 1999). The BD FACScan System (BD Biosciences, Franklin Lake, New Jersey) was used, and all samples were run with three independent replicates to calculate the mean genome size of each sample. In each replication, the criteria of over 1,000 nucleus particles per peak and lower than 5% CV (coefficient of variation) were set for both sample and internal standard.

(III). DNA extraction and sequencing chloroplast and nuclear regions

DNA was extracted using the modified CTAB protocol following Kuo (2015). For chloroplast (cp) DNA regions, we sequenced *rbcL*, *trnL-F* [*trnL* gene + *trnL-F* intergenic spacer (IGS)], *trnG-R* (*trnG* gene + *trnG-R* IGS) and *rps4-trnS* (*rps4* gene + *rps4-trnS* IGS). Their PCR primers, PCR conditions, and sequencing was followed the protocols of Schuettpelz and Pryer (2007), Li *et al*. (2010), Nagalingum *et al.* (2007), and Korall *et al*. (2006), respectively.

To examine whether or not this new taxon has a hybrid origin, we sequenced a single-copy nuclear gene - *pgiC*. The PCR primers, PCR conditions, and sequencing followed Ishikawa *et al*. (2002). We obtained these *pgiC* sequences directly by Sanger's sequencing because from these sequencing results, no single nucleotide polymorphism was found.

(IV). Sequence alignment and phylogenetic analyses

Our cpDNA alignment was modified from Li *et al*. (2016) with eight sequences newly generated in this study and those from Chang *et al*. (2018) (Appendix). For the nuclear (nu) DNA alignment, we mainly used those from Chang *et al*. (2013, 2018) and Fujiwara *et al*. (2017) (Appendix), and added three newly generated sequences in this study. These sequences were first aligned by ClustalW (Thompson *et al*., 1994), and then edited manually in BioEdit v. 7 (Hall, 1999).

For phylogenetic analyses of cpDNA matrix, we initially specified eleven partitions in the alignment, including each of three codon positions in *rbcL*, each of three codon positions in *rps4*, *rps4-trnS* IGS, *trnL* gene, *trnL-F* IGS, *trnG* gene, and *trnG-R* IGS. PartitionFinder v. 2.1.1 (Lanfear *et al*., 2017) was used to infer the partition scheme and nucleotide substitution models (Table 2) based on AICc criteria.

IQ-TREE v. 1.6.10 (Nguyen *et al*., 2015) in CIPRES (Miller *et al.*, 2011) was used to reconstruct maximum likelihood (ML) phylogeny. For the ML bootstrap (MLBS) trees, 1000 replicates were run at the same criteria. Bayesian inference (BI) was conducted by MrBayes v .3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) in CIPRES (Miller *et al*., 2011). Two runs were put into practice, and each contains four Markov chain Monte Carlo (MCMC) chains. In each chain, 20 million generations were run, and MCMC status was sampled every 1000 generations. Tracer v. 1.7.1 (Rambaut and Drummond, 2013) was used to determine convergence through generations among chains. The first 25% of the generations were discarded as burn-in.

Table 2. The partition scheme and substitution models for the chloroplast DNA matrix.

Subset	Models for ML analysis	Models for BI analysis
trnG gene + *trnL* gene	TVM + G	GTR+G
trnL-F IGS + *trnG-R* IGS	TVM + G	GTR+G
rps4-trnS IGS	TVM + G	GTR+G
rps4 2nd codon position	TVM + G	GTR+G
rps4 1st codon position	TRN + I	GTR+I
rps4 3rd codon position	K80 + G	K2P+G
rbcL 1st codon position	TRN + G	GTR+G
rbcL 2nd codon position	TVM + I	GTR+I
rbcL 3rd codon position	TVM + I + G	GTR+ I +G

RESLUTS AND DISCUSSION

Ploid estimate and phylogenetic position of *scythiforme*

For the new taxon, we name it *scythiforme* as a new forma under *Asplenium normale* (see discussion below and also **TAXONOMIC TREATMENT**). The genome sizes of *scythiforme* were estimated to be 11.2 pg/C (± 0.009 for 1SD; for *Kuo4231* in average) and 10.9 (± 0.004 for 1SD; for *Kuo4283* in average) (Fig. 2). In Chang *et al*. (2013), the diploid and tetraploid in *A. normale* were respectively detected with genome sizes of 9.8 and 18.2 pg/C. Hence, the *scythiforme* is more likely a diploid rather than a polyploid.

Our cpDNA phylogenies reveal species relationships congruent with previous ones (Chang *et al*., 2013, 2018; Li *et al*., 2016; Fujiwara *et al.*, 2017), and no strongly supported conflict between our ML and BI trees was

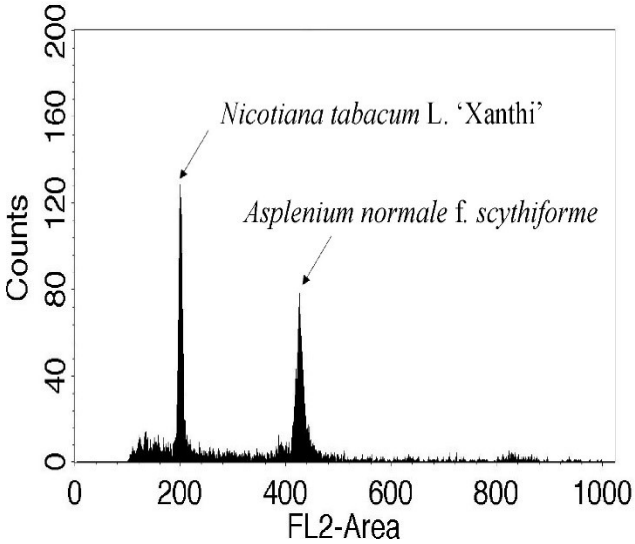

Fig. 2. Genome size of *Asplenium normale* f. *scythiforme* inferred by flow cytometry. *Nicotiana tabacum* 'Xanthi' (5.02 pg/C) was used as the internal standard.

found. *Scythiforme* is phylogenetically placed in *A. normale*. Meanwhile, *A. oligophlebium* is distantly related to this new taxon and belongs to a different clade (Fig. 3). Our nuDNA phylogenies—*pgiC* gene trees (Fig. 4)—are congruent with the previous ones, and, generally, the diploid species are each found in a single clade (Fujiwara *et al.*, 2017; Chang *et al*., 2018). *Scythiforme* is here found to consist a single sequence type (i.e. no single nucleotide polymorphism were found), which is nested within one diploid *A. normale* clade (i.e. NORMALE 2 in Fig. 4).

Reticulation under the *Asplenium normal*e complex and origin of *scythiforme*

Among the *Asplenium normale* complex, the tetraploid *A. normale* is most "problematic", polyphyletic in the nuDNA trees, and comes from multiple allopolyploid origins (Fujiwara *et al.*, 2017) although, in cpDNA trees (Fig. 3; Chang *et al*., 2013, 2018; Li *et al*., 2016; Fujiwara *et al.*, 2017), these tetraploids all form in a single clade together with conspecific diploids which are suggested to be maternal progenitors of these allotetraploids (because cpDNA is maternally inherited in *Asplenium* ferns like all other studied fern cases; Vogel *et al.*, 1997; Kuo *et al.*, 2018). For instance, the Japanese allotetraploids have been found with at least two independent hybrid origins, and either the diploid relative of *A. boreale* or *A. oligophlebium* is their paternal progenitors (i.e. with a corresponding *pgiC* sequence respectively belonging to Japan O-a or Japan O-b, d, e; Fig. 4 and Fujiwara *et al.*, 2017). Such a similar phylogenetic pattern of allopolyploid is also found in *A. shimurae* which is revealed with different nuDNA sequence types locating in two distinctive lineages (Fig. 4), and thus this tetraploid species presumably has a hybrid origin too (Fujiwara *et al.*, 2017).

The topological differences between species trees by *pgiC* and by cpDNA likely result from incomplete lineage sorting and/or poor phylogenetic resolution of analyzed regions (Fujiwara *et al.*, 2017). For example, in the case of *A. boreale* + *A. oligophlebium*, the two species are mixed in the *pgiC* tree in a single clade (Fig. 4; Fujiwara *et al.*, 2017), while they both are resolved as distinctive lineages in other nuclear gene trees and in cpDNA trees (Fujiwara *et al.*, 2017). Nonetheless, except for "tetraploid *A. normale*", no phylogenetic evidence so far implies a continuously interbreeding between different species in the *A. normale* complex (sensu Fujiwara *et al*., 2017 and Chang *et al*., 2018). Even for the tetraploid *A. normale*, there was no confirmed case of a fertile hybrid with other species (Ebihara, 2016). In other words, these morphologically distinctive species under the *A. normale* complex are suggested to be reproductively isolated in their natural habitats. Many of them have different ploidies which inherently form reproductive barriers among them.

For the case of *scythiforme*, sporing individual is, however, unavailable in order to examine the normality of spore formation and the possibility of sterile hybrid. Even we had continuously visited the habitats over ten years, and also cultivated two individuals in Dr. Cecilia Koo Botanic Conservation Center (KBCC) greenhouse over three years, only sterile individuals had been observed. It is also unlikely that these individuals are still immature to produce spores because their maximum frond sizes are close to or even exceed those of mature individuals of the other *A. normale* complex taxa (Table 1; Ebihara, 2016; Li *et al.*, 2016; Chang *et al.*, 2018). Alternatively, we used a nuDNA phylogeny to examine whether or not it has a hybrid origin. Unlike that found in those allotetraploids in *A. normale* and *A. shimurae*, f. *scythiforme* is, in our nuDNA phylogeny, found in a single clade with a single sequence type. Hence, *scythiforme* unlikely has a hybrid origin with nuDNA sequences from two parental species. Results from another single-copy nuclear marker—*LEAFY* second intron, also support the non-hybridized nature of *scythiforme* (data not shown).

Unfortunately, because the DNA sequences we generated from the two *scythiforme* populations are all identical, and also identical to those found in some *A. normale* (f. *normale*), our current data are still unable to verify whether *scythiforme* phylogenetically came from a single origin or not. One plausible scenario can be that *scythiforme* consists of two or more morphologically convergent "mutants", and the local populations have sustained and reproduced solely by asexually budding, rather than forming spores to disperse and establish geographically distant populations. Such a scenario can explain why there are only two, but distant populations found so far and no oversea-established population found outside of Taiwan.

The appropriate taxonomic rank for *scythiforme*

Based on our cytometric and phylogenetic analyses, *scythiforme* is supported as a non-hybrid diploid and nested within diploids of *Asplenium normale*. Morphologically, *scythiforme* agrees with the *Asplenium normale* complex in having one-pinnate fronds with black rachis and bud (Fig. 1). *Scythiforme* has deeper lobbed and falcate pinnae with long-tail at apices, compared with *A. normale* (f. *normale*). To distinguish from *A. oligophlebium*, another diploid taxon also with lobed pinnae, *scythiforme* produces pinna auricles paralleled to and appended on rachises, and, most importantly, its vegetative buds are never elongated to whip (other details see Table 1). Because *scythiforme* has at least two populations and unique frond morphology distinctive from the other taxa in the *A. normale* complex, we believe this taxon is worthy of having a formal name and to be identified taxonomically. Here, we provide a

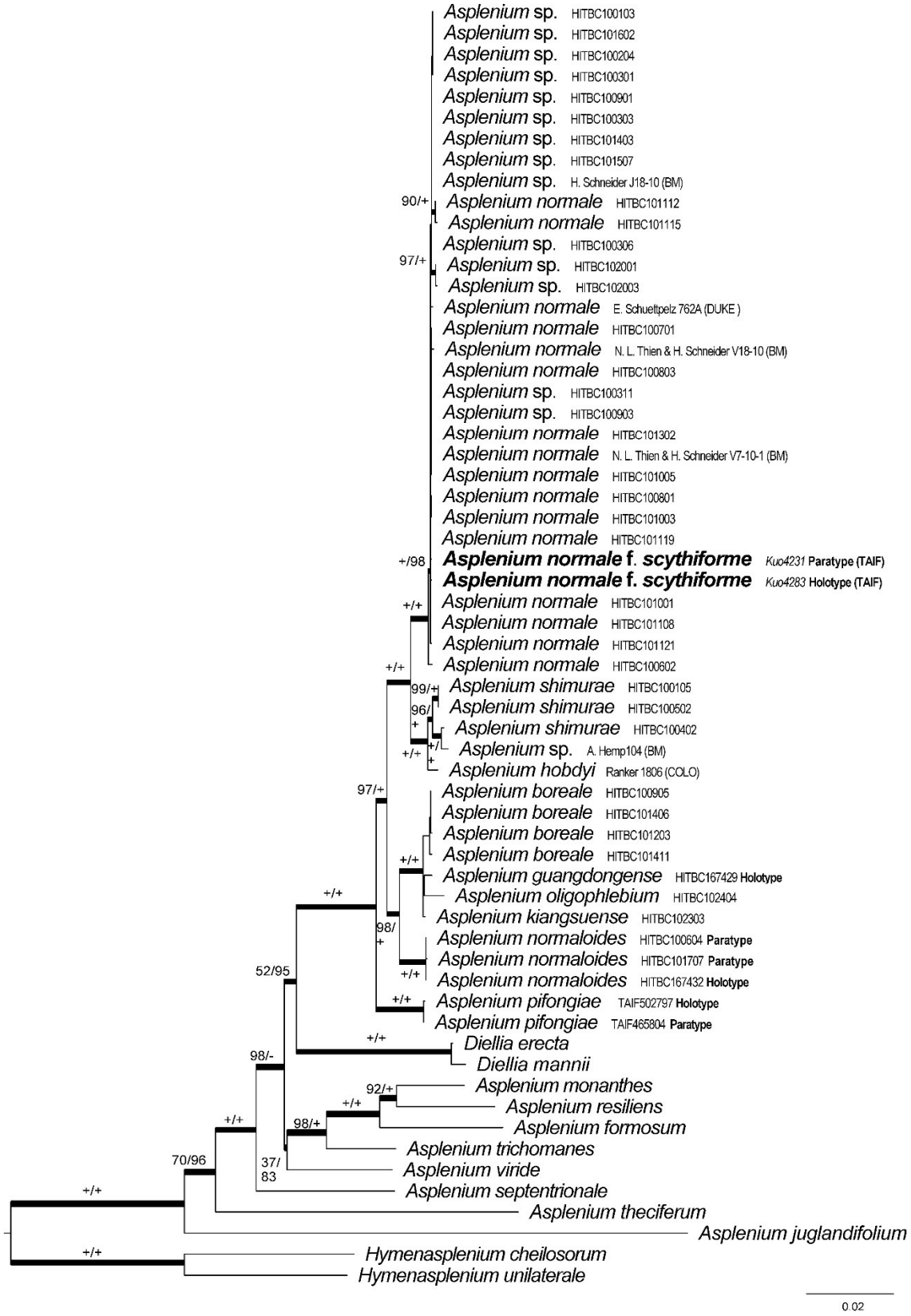

Fig. 3. Maximum likelihood (ML) phylogeny based on the chloroplast DNA dataset. ML bootstrap (MLBS) and Bayesian posterior probability (PP) are shown on each branch as MLBS/PP. MLBS =100 or PP=1.0 are represented as "+".

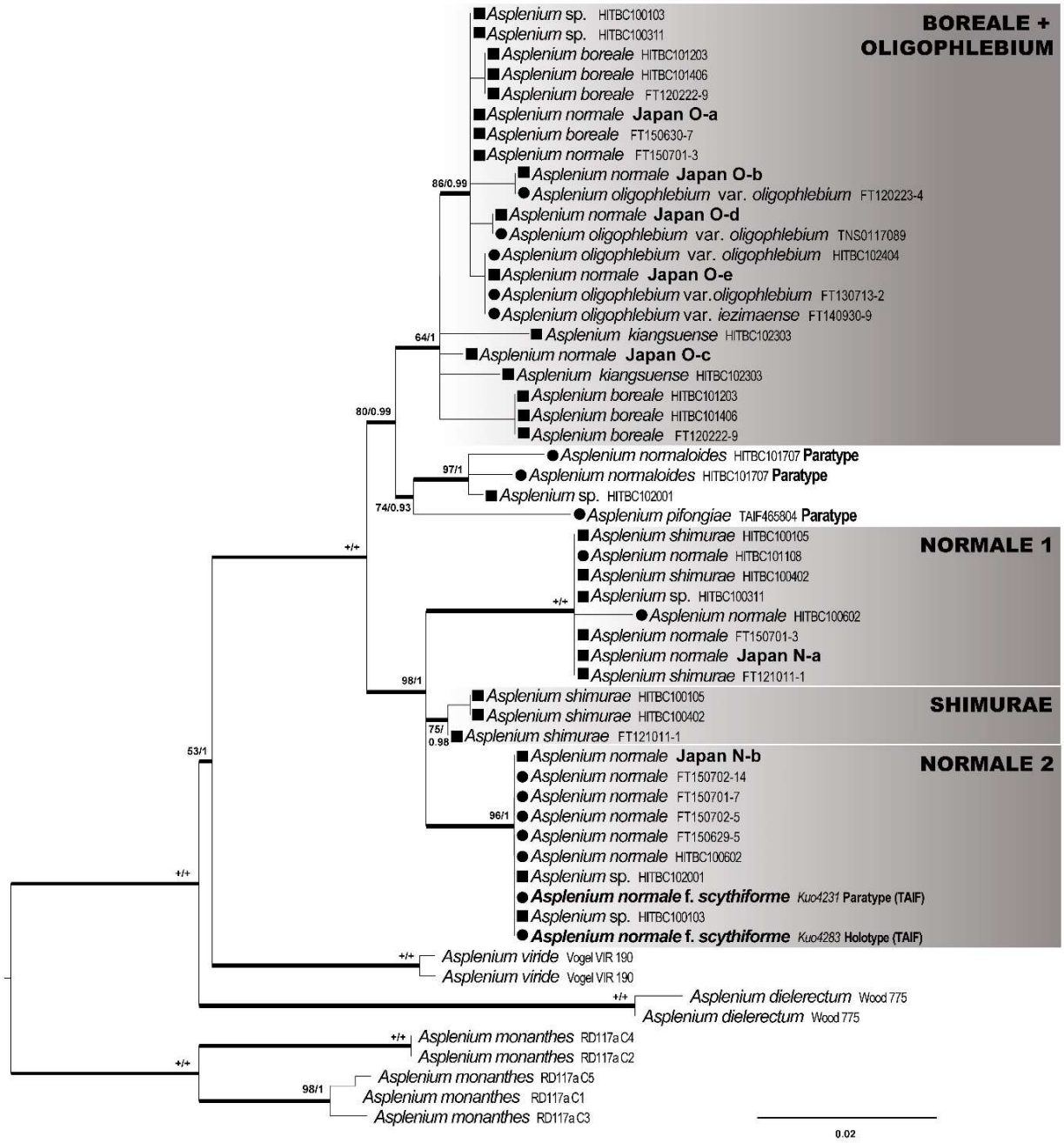

Fig. 4. ML phylogenetic tree based on the nuclear dataset. ML bootstrap (MLBS) and Bayesian posterior probability (PP) are shown on each branch as MLBS/PP. MLBS =100 or PP=1.0 are represented as "+". In *Asplenium normale* complex clade, the shape of symbols indicates cytotypes: circle = diploid, square = tetraploid. (Fujiwara *et al*., 2017; Chang *et al*., 2018). For loci of tetraploid *Asplenium normale* from Japan that close to diploid *Asplenium normale* genome was labeled as "Japan N" and that close to other species was labeled as "Japan O" (Fujiwara *et al*., 2017). Different clades inferred by Fujiwara *et al*. (2017) are also indicated behind the tips.

taxonomic description for *scythiforme*, and assign it as a new forma under *A. normale* because, despite morphological differences, other biological features, i.e. phylogenetic and cytological ones, all suggest the two taxa to be undifferentiated and still conspecific. However, we also like to note that more cryptic and undescribed taxa under the *A. normale* complex are very likely, particular for those putative diploid progenitors of polyploid lineages, such as that found in *A. boreale* and *A. normale*. Current taxonomic ranks and statuses of these described taxa under the *A. normale* complex will be possibly revised after reticulation pathway under the *A. normale* complex being better resolved, and important type materials of the described names being analyzed phylogenetically and cytologically, like the case of *Vandenboschia radicans* complex (Ebihara *et al.*, 2009).

TAXONOMIC TREATMENT

Asplenium normale* f. *scythiforme Z.X. Chang, ***f. nov.*** **鐮羽鐵角蕨 Fig.1**

***TYPE*:** TAIWAN: Pingtung County, Chunri Township, Mt. Guzilun, 1450–1500m, May 28, 2016, *Kuo 4283* (holotype, TAIF).

***Description*: Plants** terrestrial or growing on the fallen tree, 40–50 cm tall. **Rhizome** shortly erect, covered by scales. **Scales** lanceolate with filiform apices, ca. 1.5 × 0.5mm, clathrate, bicolor with a dark brown portion in the central. **Leaves** caespitose, monomorphic, 40–50 × 3–5 cm, linear lanceolate. **Petioles** dark brown to dark purple brown, lustrous, wingless, 8–10 cm long, 1–1.5 mm in diameter, tetragonous, with a broad sulcation adaxially. **Blades** 1-pinnate, linear lanceolate, 25–45 cm × 3–5 cm, glabrous; rachises dark brown to dark purple brown, lustrous, wingless, glabrous, with a narrow sulcation adaxially, usually gemmiferous near apex; apices acuminate. **Pinnae** falcate, dissected, 30–40 pairs, 1.5–2.5 × 0.4–0.8 cm, short petiolule, ca. 0.5 mm; acroscopic and basioscopic margins incised or deeply crenulate, with 4–6 serrations; bases truncate slightly curved, acroscopic side auriculate; pinnae apices acuminate with a fishbone-like tail. (Fig. 1)

***Distribution*:** This forma is currently known with only two small populations that grow in *Chamaecyparis* montane mixed cloud forest and *Pasania–Elaeocarpus* montane evergreen broad-leaved cloud forest (Li *et al*., 2013) in northern and southern Taiwan respectively, and coexists with *Asplenium normale* f. *normale*. We speculate that the local populations have propagated asexually with vegetative buds.

***Etymology*:** The ‘‘scythiforme’’ means the pinnae shape like the scythe, which was used to mow the grass.

***Note*:** Since 2007, we have never seen any individuals that producing fertile leaves in wild as well as three-year planted individuals in the Dr. Cecilia Koo Botanic Conservation Center (KBCC).

***Additional specimen examined*:** TAIWAN: Pingtung County, Chunri Township, Mt. Guzilun, Aug. 28, 2010, *P.-F. Lu 20857* (TAIF); Feb. 14, 2019, *ZXC 001515* (TAI, TAIF); Yilan County, Datong Township, Mt. Babo Kulu Trail, Dec. 01, 2007, *C.-H. Liao 355* (TNM *P013196*) ; Mingchi Forest Recreation Area, 1100–1200m, Jun. 27, 2010, *P.-F. Lu 20525* (TAIF), Nov. 01, 2015, *Kuo 4231* (TAIF)

Key to Taiwanese taxa of *A. normales*

1a. Fronds without vegetative buds ……..…..…… 2
1b. Fronds with vegetative buds ……..……3
2a. Sori only 1 or 2, rarely to 4, restrict and parallel to the basiscopic side of pinnae ……. ***Asplenium pifongiae* L.Y. Kuo *et al.***
2b. Sori more than five and on both side of pinnae ……..…… …… ***Asplenium boreale* (Ohwi *ex* Sa. Kurata) Nakaike**
3a. Pinna falcate, dissected and apices acuminate with a fishbone-like tail …… ***Asplenium normale* D. Don f. *scythiforme* Z.X. Chang**
3b. Pinna oblong, margin serrate, apices obtuse ……..…… ……. ***Asplenium normale* D. Don f. *normale***

ACKNOWLEDGMENTS

The authors thank staffs in Dr. Cecilia Koo Botanic Conservation Center (KBCC) for maintaining the living collection of *Asplenium normale* f. *scythiforme*; Chia-Hung Liao and Wen-Liang Chiou for assistance on field collections; and three anonymous reviewers and two editors for providing comments on the manuscript. This work was supported by the Bioresource Conservation Research Center in College of Life Science from the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

LITERATURE CITED

Chang, Y., A. Ebihara, S. Lu, H. Liu and H. Schneider. 2018. Integrated taxonomy of the *Asplenium normale* complex (Aspleniaceae) in China and adjacent areas. J. Plant Res. **131(4)**:573–587.

Chang, Y., J. Li, S. Lu and H. Schneider. 2013. Species diversity and reticulate evolution in the *Asplenium normale* complex (Aspleniaceae) in China and adjacent areas. Taxon **62(4)**:673–687.

Dyer, R.J., V. Savolainen and H. Schneider. 2012. Apomixis and reticulate evolution in the *Asplenium monanthes* fern complex. Ann. Bot. **110(8)**:1515–1529.

Ekrt, L. and Štech, M. 2008. A morphometric study and revision of the *Asplenium trichomanes* group in the Czech Republic. Preslia **80(3)**:325–347.

Ebihara, A. 2016. The Standard of Ferns and Lycophytes in Japan. Gakken Plus, Tokyo, Japan. **1**: pp. 190–215, and 409–420.

Ebihara, A., S. Matsumoto and M. Ito. 2009. Taxonomy of the reticulate *Vandenboschia radicans* complex (Hymenophyllaceae) in Japan. Acta Phytotax. Geobot. **60(1)**:26–40.

Fujiwara, T., A. Uehara, T. Iwashina, S. Matsumoto, Y.-H. Chang, Y.-S. Chao and Y. Watano. 2017. Allotetraploid cryptic species in *Asplenium normale* in the Japanese Archipelago, detected by chemotaxonomic and multi-locus genotype approaches. Am. J. Bot. **104(9)**:1390–1406.

Hall, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser **41**:95–98.

Huelsenbeck, J.P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics **17(8)**:754–755.

Ishikawa, H., Y. Watano, K. Kano, M. Ito and S. Kurita. 2002. Development of primer sets for PCR amplification of the *PgiC* gene in ferns. J. Plant Res. **115(1)**:65–70.

Johnston, J.S., M.D. Bennett, A.L. Rayburn, D.W. Galbraith and H.J. Price. 1999. Reference standards for determination of DNA content of plant nuclei. Am. J. Bot. **86(5)**:609–613.

Korall, P., K.M. Pryer, J.S. Metzgar, H. Schneider and D.S. Conant. 2006. Tree ferns: monophyletic groups and their relationships as revealed by four protein-coding plastid loci. Mol. Phylogenet. Evol. **39(3)**:830–845.

Kuo, L.-Y. and Y.-M. Huang. 2017. Determining genome size from spores of seedless vascular plants. Bioprotocol **7**:e2322.

Kuo, L.-Y., T.-Y. Tang, F.-W. Li, H.-J. Su, W.-L. Chiou, Y.-M. Huang, and C.-N. Wang. 2018. Organelle genome

inheritance in *Deparia* ferns (Athyriaceae, Aspleniineae, Polypodiales). Front. Plant Sci. **9**:486.

Kuo, L-Y. 2015. Polyploidy and Biogeography in Genus *Deparia* and Phylogeography in *Deparia lancea*. Ph.D. dissertation, NTU, Taipei, Taiwan.

Lanfear, R., P.B. Frandsen, A.M. Wright, T. Senfeld and B. Calcott. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. **34(3)**:772–773.

Li, C.-F., M. Chytrý, D. Zelený, M.-Y. Chen, T.-Y. Chen, C.-R. Chiou, Y-J Hsia, H-Y Liu, S-Z Yang, C-L Yeh, J-C Wang, C-F Yu, Y-J Lai, W-C Chao and C-F Hsieh. 2013. Classification of Taiwan forest vegetation. Appl. Veg. Sci. **16(4)**: 698–719.

Li, F.-W., L.-Y. Kuo, Y.-H. Chang, T.-C. Hsu, H.-C. Hung, W.-L. Chiou, Carl J. Rothfels and Y.-M. Huang. 2016. *Asplenium pifongiae* (Aspleniaceae: Polypodiales), a new species from Taiwan. Syst. Bot. **41(1)**:24–31.

Li, F.-W., L.-Y. Kuo, Y.-M. Huang, W.-L. Chiou and C.-N. Wang. 2010. Tissue-direct PCR, a rapid and extraction-free method for barcoding of ferns. Mol. Ecol. Resour. **10(1)**:92–95.

Miller, M.A., W. Pfeiffer and T. Schwartz. 2011. The CIPRES science gateway: a community resource for phylogenetic analyses. In "Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery" ACM, New Orleans. LA. pp. 1–8.

Nagalingum, N.S., H. Schneider and K.M. Pryer. 2007. Molecular phylogenetic relationships and morphological evolution in the heterosporous fern genus *Marsilea*. Syst. Bot. **32(1)**:16–25.

Nguyen, L.T., H.A. Schmidt, A. von Haeseler and B.Q. Minh. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. **32(1)**:268–274.

PPG I. 2016. A community-derived classification for extant lycophytes and ferns. J. Syst. Evol. **54(6)**:563–603.

Rambaut, A. and A.J. Drummond. 2013. Tracer v1.6. Available from http://tree.bio.ed.ac.uk/software/tracer/.

Ronquist, F. and J.P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19(12)**:1572–1574.

Rothfels, C.J., A. Larsson, L.-Y. Kuo, P. Korall, W.-L. Chiou and K. M. Pryer. 2012. Overcoming deep roots, fast rates, and short internodes to resolve the ancient rapid radiation of eupolypod II ferns. Syst. Biol. **61(3)**:490–509.

Schneider, H., S.J. Russell, C.J. Cox, F. Bakker, S. Henderson, F. Rumsey, J. Barrett, M. Gibby and J.C. Vogel. 2004. Chloroplast phylogeny of asplenioid ferns based on *rbcL* and *trnL-F* spacer sequences (Polypodiidae, Aspleniaceae) and its implications for biogeography. Syst. Bot. **29(2)**:260–274.

Schuettpelz, E. and K.M. Pryer. 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. Taxon **56(4)**:1037–1050

Sessa, E.B., Vicent, M., Chambers, S.M. and y Galán, J.M. G. 2018. Evolution and reciprocal origins in Mediterranean ferns: the *Asplenium obovatum* and *A. adiantumnigrum* complexes. Ann. Mo. Bot. Gard. **103(2)**:175–188.

Shepherd, L.D., L.R. Perrie and P.J. Brownsey. 2008. Low-copy nuclear DNA sequences reveal a predominance of allopolyploids in a New Zealand *Asplenium* fern complex. Mol. Phylogenet. Evol. **49(1)**: 240–248.

Thompson, J.D., D.G. Higgins and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22(22)**:4673–4680.

Taiwan Pteridophyte Group (TPG). 2019. Updating Taiwanese pteridophyte checklist: a new phylogenetic classification. Taiwania **64(4)**:367–395.

Vogel, J.C., S.J. Russell, F.J. Rumsey, J.A. Barrett, and M. Gibby. 1998. Evidence for maternal transmission of chloroplast DNA in the genus *Asplenium* (Aspleniaceae, Pteridophyta). Bot. Acta **111(3)**:247–249.

Supplementary materials are available from Journal Website.