

A Study of Structural Peculiarities and Reproductive Traits of *Amorphophallus* (Araceae) in Taiwan

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Abstract. Besides taxonomic information, scant literature is available regarding the growth, productivity, and natural resources of konjac or elephant foot yam and related *Amorphophallus* species in Taiwan. The distribution, reproductive phenology, structural peculiarities of growth patterns, and propagation of seeds/tubers/subsidiary buds of four species of *Amorphophallus* in Taiwan were studied, and a maximum-likelihood analysis of the *matK* gene was conducted. All flowering habits after seasonal dormancy first produced inflorescences, followed by leaves and fruits. The earliest blooming species each year usually were *A. kiusianus* and *A. henryi*, and both were earlier than *A. hirtus* and *A. paeoniifolius*. However, all were delayed in the most recent 2 years. Leaf traits of the shade-loving *A. henryi* showed that the flattest adaxial lamina with thin and loose palisade mesophylls might be related to the production of large daughter tubers in low light intensities. The largest midrib in *A. paeoniifolius* with a great protrusion that formed several hollow canal-like spaces was not only for mechanical support of laminae but also produced high theoretical axial resistivity of the midrib. This species produced huge tubers and favored vegetative propagation with many subsidiary buds. Our molecular data on the affinity of *Amorphophallus* species in Taiwan provide sustaining documented records for future accurate identification and further conservation development. We suggest developing commercial crop projects for nutrition, health, and medicinal benefits. Understanding the functional morphology, including anatomical variations, is not only important for taxonomic identification but also for conservation of natural resources.

Key words: leaf traits, phenology, reproduction, plant functional morphology.

INTRODUCTION

The genus *Amorphophallus* of the family Araceae is estimated to encompass some 170 species (Hettterscheid 1994; Mayo *et al.* 1997), and a few species have been domesticated and have edible parts. Recognized edible *Amorphophallus* species such as *A. paeoniifolius*, *A. campanulatus*, *A. konjac*, *A. mulleri*, *A. riveiri*, *A. onchophyllus*, *A. yunnanensis*, *A. yuloensis*, *A. nanus*, and *A. krausei*, commonly known as elephant foot yam, are a group of tropical, perennial, tuberous crops. Konjac (*A. konjac* K.

Koch ex N. E. Br.) native to China is among the major vegetable (tuber) crops grown in Asian countries (Ravi *et al.* 2011; Behera and Ray 2017). Since it has long been used as a traditional medicine and food source (Arvill and Bodin 1995; Chua *et al.* 2010), wild relatives of domesticated plants are important gene reservoirs for improving commercial varieties (Oyama *et al.* 2006).

Currently, only a few studies have been published regarding the genetic diversity of *A. konjac* (Zhang *et al.* 2001; Teng *et al.* 2006a, b; Xuan 2010; Ren and Pan 2013; Zheng *et al.* 2013). Most studies attempted to identify species taxonomically and resolve phylogenetic

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relationships of germplasm resources of *Amorphophallus* species (Pan *et al.* 2015). To date, no evaluation of the conservation of wild *Amorphophallus* resources in Taiwan has been undertaken to reveal the essential and sustainable development of crop materials.

Beyond the juvenile stage, mature plants of *Amorphophallus* exhibit seasonal dormancy and produce solitary leaves in the growing season. The leaf has a trisected blade, with primary divisions of pinnatisect, bipinnatisect, or dichotomously further divided laminae which are huge compared to the supporting petioles. Analyses of growth and tuber production of *Amorphophallus* at different shading levels in Indonesia suggested that certain species are shade-loving plants (Santosan *et al.* 2006). However, none of the leaf functional traits of Taiwanese *Amorphophallus* species has been investigated in terms of adaptations to diverse environmental conditions.

The aims of the present study were to provide: (1) the distributions of four species of *Amorphophallus* in Taiwan; (2) the phenology and the leaf structure and growth patterns; (3) the establishment of a maximum-likelihood (ML) analysis of the *matK* gene for *Amorphophallus* species; and (4) recommendations for their propagation and conservation.

MATERIALS AND METHODS

Both living plants and herbarium specimens were examined in this study. Four species of *Amorphophallus* in Taiwan were investigated and evaluated, including the endemic *A. henryi* N. E. Br. and *A. hirtus* N. E. Br. (Hettterscheid and Peng. 1995; Huang, 2000). Another species, *A. kiusianus* (Makino) Makino, native to southeastern China and Japan, has been used in floristic arrangements because of its colorful fruits. The fourth species, *A. paeoniifolius* (Dennst.) Nicolson, also known as elephant foot yam, is naturalized in Taiwan, is widely distributed from Madagascar to the Polynesian islands and is often cultivated as a tuberous crop in Southeast Asia. All living plants were authenticated by Shau-Ting Chiu and confirmed by Tzu-Huan Hung with chloroplast *matK* sequencing.

To record the blooming and fruiting seasons, field work and conservative propagation were

carried out from 2013. The growth rate of the inflorescence was measured by time-lapse photography using a Nikon D80 camera (Nikon, Tokyo, Japan) with an erect 2-m ruler. Occurrence data of *Amorphophallus* were collected from databases, including metadata of 133 specimens deposited in four herbaria, TAI, TAIF, HAST, and TNM, and 35 field observations from two national biological resource inventory projects (National Vegetation Mapping in 2003~2008, and the Survey of Invasive Alien Plants in 2009~2012). Metadata of each specimen were georeferenced according to coordinates noted by the collector. Specimens with a collection locality but without coordinates were assigned by consulting archival place name databases or online maps. Finally, 168 occurrences in total were compiled into a dataset to illustrate the geographical distributions of *Amorphophallus* species using R 3.5.1 software.

In total, 20 samples of terminal leaf segments (hereafter called leaflets) of the four species were collected, with at least three samples per species, fixed in formalin: propionic acid: ethanol: glycerol: distilled water = 1:1:7:3:8 (v/v), embedded in paraffin through tertiary-butanol dehydration, sectioned to a 10- μ m thickness, and stained with 0.5% safranin O and 0.1% fast green through an ethanol dehydration protocol.

Total genomic DNA was isolated from leaf tissues using the hexadecetyl trimethylammonium bromide (CTAB) (H9151, Sigma-Aldrich) method following the protocol of Doyle and Doyle (1987). The *matK* gene was amplified with the *matK*-2F (AATTCCTCGAATGTACCAACAG) and *matK*-3R primers (GATCCTACAGGGTTGAGACCA). Double-stranded DNAs were amplified with 35 cycles of amplification (30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C) with an additional 7 min at 72 °C. Sequences were manually aligned using Bioedit (Hall 1999). An ML phylogenetic analysis was conducted with MEGA-X software (Kumar *et al.* 2018). The optional substitution model of the ML analysis was the set model T92 + G (Tamura, 1992) with the lowest Akaike information criteria (AIC) value. A bootstrap analysis was performed using 1000 replicates. *Typhonium venosum* and *Arisaema tortuosum* were chosen as outgroups.

For comparison of micro-scale leaf traits of the lamina and midrib, the thickness and width in ratios were determined. Leaf traits of the lamina and midrib were evaluated as the thickness ratio =

lamina/midrib and midrib index = thickness/width of the midrib. The included angle between the two adaxial laminae along the veins was measured.

RESULTS

Distribution and phenology

With the exception of naturalized and cultivated *A. paeoniifolius*, the distributions of *Amorphophallus* species in Taiwan reflect habitat conditions of each wild species (Fig. 1, Table 1). *Amorphophallus henryi* is distributed in lowlands and hills from north to south, but major populations are found in southern Taiwan, such as at Tienliao and Chungliao. *Amorphophallus hirtus* grows mostly in low elevations of southern Taiwan, contrary to the northern distribution of *A. kiusianus*, a species that mainly inhabits higher elevations in northern Taiwan. Major populations of *A. hirtus* are confined to the Shoushan and Takangshan areas of Kaohsiung. A large population of *A. kiusianus* at Wufeng is mixed with litchi orchards. Two closely related populations of *A. paeoniifolius* are located at Hsinpi of Pingtung County.

All four *Amorphophallus* species were monitored for 6 years (Fig. 2). *Amorphophallus henryi*, and *A. kiusianus* produced fewer inflorescences and showed a marked delay in the time of flowering in 2019. The productivity of the reproductive season has declined since 2018. The Luye population of *A. hirtus* seems

to have diminished over the past 3 years. The Hsinpi population of *A. paeoniifolius* also became smaller and smaller during the years it was monitored.

The flowering habit of the four species of *Amorphophallus* in Taiwan is to mostly produce inflorescences first, then leaves and fruits (Fig. 2). Based on our observations and time-lapse photography, the elongation period of the inflorescence stalk of *A. henryi* was about 28.5 h. Most elongation of appendages at the top of the inflorescence subsequently occurred for another 20 h. The average elongation rate was about 9 cm/day, but the rate was not even. However, the fastest rate during elongation reached 1.1 cm/h. When the spathe was expanding and the stinky odor was being disseminated, elongation stagnated, and this was sustained for about 75 h. The shedding of pollen grains, however, occurred only in a few hours. Fruiting usually began in 10 days, and the fruits matured within a month.

The inflorescence stalks of *A. hirtus* often exceeded 70 cm and sometimes exceeded 100 cm, so its elongation took more time than other Taiwanese *Amorphophallus* species. The optimal elongation rate of about 2.3 cm/h occurred when the inflorescence height exceeded 110 cm, which was about 2/3 the length of the mature inflorescence.

Among the four species, *A. paeoniifolius*, with the widest and perhaps the heaviest inflorescence but an almost-sessile spadix, elongated the least. Therefore, its elongation rate, measured 0.71

Table 1. A summary of the geographical distributions of the four *Amorphophallus* species in Taiwan

Species	<i>A. henryi</i>	<i>A. hirtus</i>	<i>A. kiusianus</i>	<i>A. paeoniifolius</i>
Distribution	Endemic to Taiwan, in lowlands and hills from north to south, but major populations in southern Taiwan	Endemic to Taiwan, mostly at low elevations of southern Taiwan	Native to southeastern China, Japan, and Taiwan, mainly in northern Taiwan	Widespread from Madagascar to the Polynesian islands, naturalized mainly in Pingtung County in Taiwan
Occurrence data (no.)	70	38	34	26
90% quantile elevation (m)	645	1084	1168	215
Mean elevation ¹ (m)	315 ^b	312 ^b	673 ^c	70 ^a
10% quantile elevation (m)	25	18	195	11

1) a, b, and c, indicate groups divided by Tukey's honest significant difference test (at a 0.95 confidence level). Means in the same group do not significantly differ by Tukey's test.

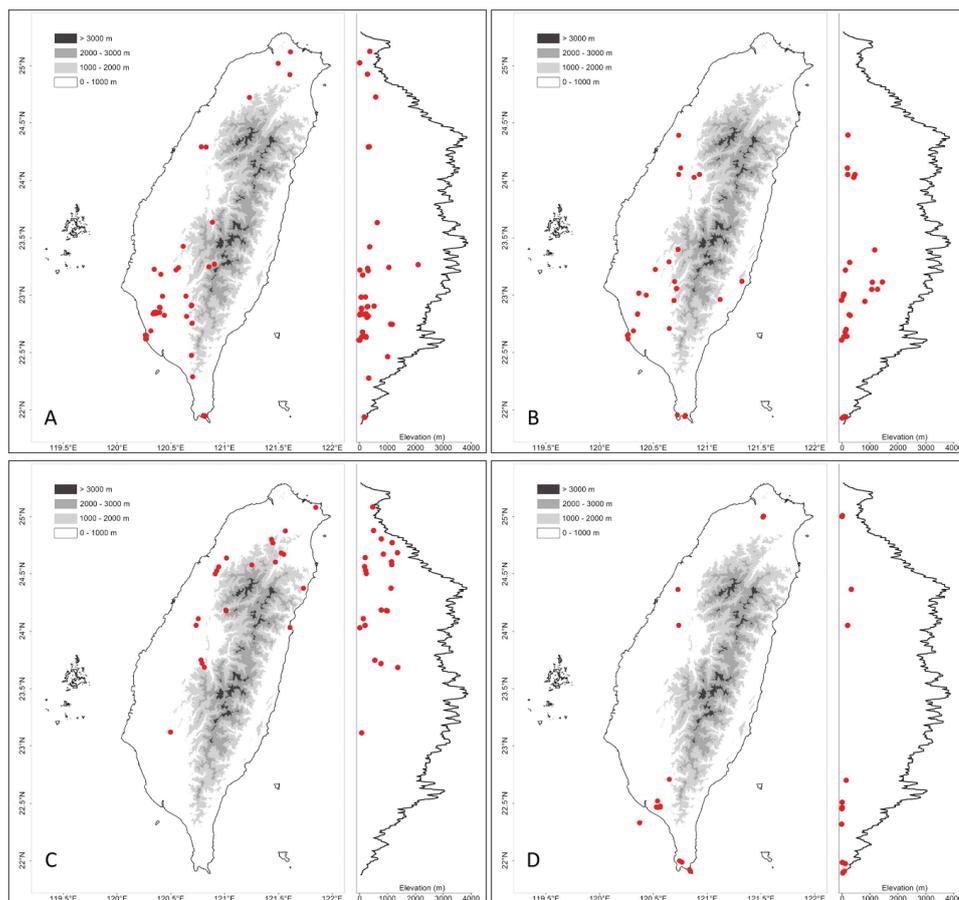


Fig. 1. Distributions of four species of *Amorphophallus* in Taiwan. A. *A. henryi*; B. *A. hirtus*; C. *A. kiusianus*; D. *A. paeoniifolius*.

and 0.65 cm/day from initial 10 to 15 days, was mainly optimized during the initial 2 weeks. Its expansion from 2 cm in diameter at the opening of the floral bud scale cylinder to 14.2 cm in diameter with a mature spathe accelerated from 0.035 to 0.8 cm/day. At the maximal size of the spadix, maturation of pistils and stamens occurred within a few days, accompanied by the opening of the spathe. Regardless of whether or not pollination was successful, the inflorescence wilted in the following weeks. By the end of this study, we had observed no fruits.

Leaf traits and structural peculiarities

The development of the leaf morphology from the juvenile to adult stage greatly varied (Fig. 3A). It was difficult to differentiate these species in the juvenile stage. Most diagnostic vegetative characteristics depend on the petiole length, coloration, and pattern of spots. We attempted to collect leaf lamina traits and functional traits for identification purposes. The huge, highly dissected lamina was up to tripinnatisect. Small

leaf segments are hereafter called leaflets. The flatness of the expanded leaflets manifested the degree of overlap of the large and highly dissected construction (Fig. 3). Terminal leaflets of *A. henryi* were the flattest among the four species. Although *A. hirtus* exhibited the least overlap of the leaflets, the waviness of the margins and the degree to which the midrib was bent backwards were the greatest (Fig. 3B). The petiole color of dirty olive green extended even to the midribs of *A. kiusianus* leaflets (Fig. 3C). The lamina of *A. paeoniifolius* was not only huge in size but also in the degree of overlap, which resulted in a large dense net to capture sunlight for photosynthesis (Fig. 3D).

An anatomical examination of the transected leaf segments, herein called leaflets, revealed an arrangement of palisade mesophylls that were identical in all species (Fig. 4). *Amorphophallus henryi* with thin and loose palisade mesophylls showed the flattest adaxial lamina compared to the other three species. *Amorphophallus hirtus*, *A. kiusianus*, and *A. paeoniifolius* had compact

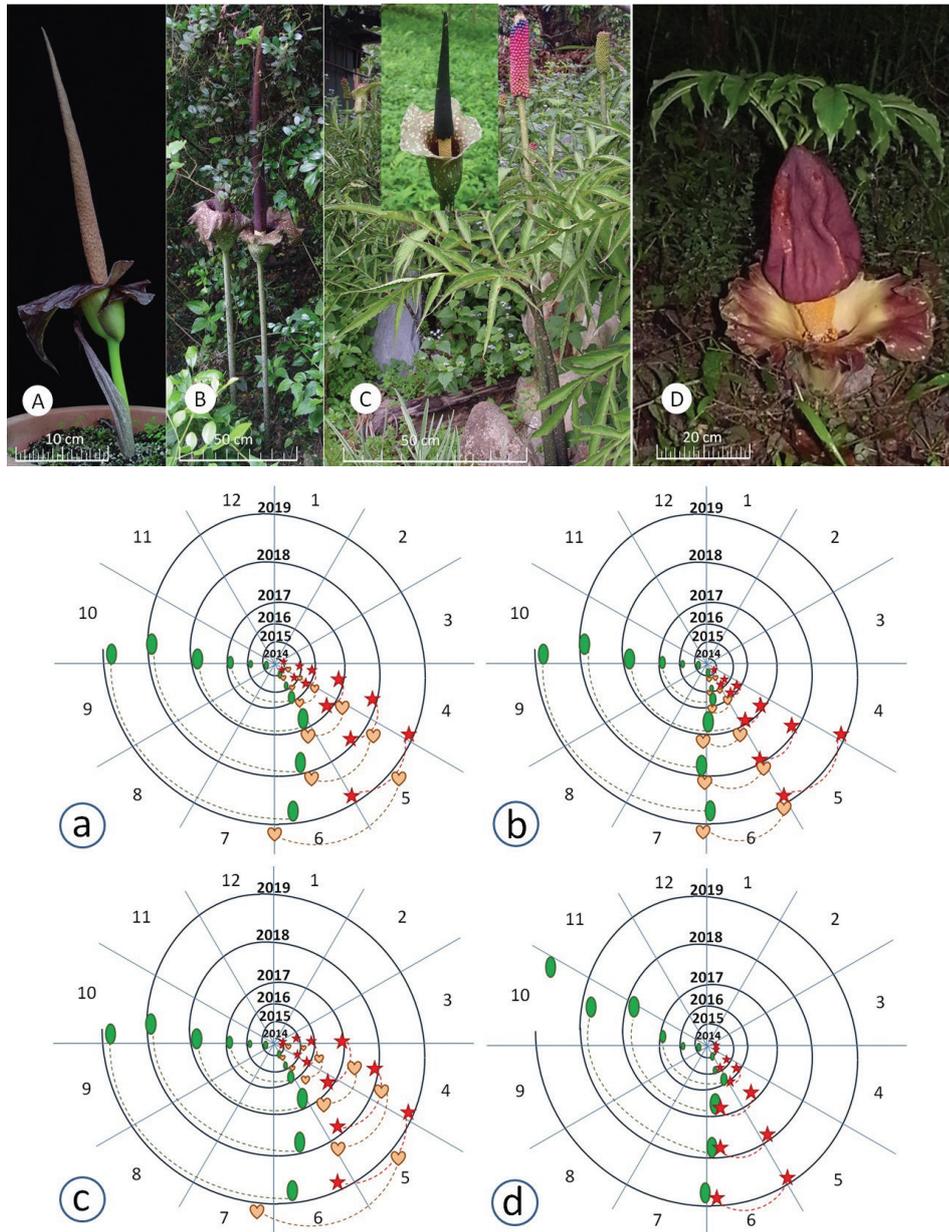


Fig. 2. Inflorescences and habitat (A-D), and phenology of flowering and fruiting months (a-d) of Aa. *Amorphophallus henryi* at Tienliao. Bb. *A. hirtus* at Shoushan. Cc. *A. kiusianus* at Wufeng. Dd. *A. paeoniifolius* at Hsinpi in Taiwan from 2014 to 2019. The star (★) indicates the flowering period; the heart-shaped symbol (♥) indicates the fruiting period; and the oval/circle (○) indicates the leafy stage.

palisade mesophylls the ratios of which varied by 30%, 40%, and 25%, and they were comparable to each species' own leaflet thickness (Fig. 4). Almost no large xylem lacuna occurred in the midrib of *A. henryi*. However, several xylem lacunae in the midrib of *A. paeoniifolius*, labeled with black stars (★) in Fig. 4D, formed a number of hollow canal-like spaces. Some of these expanded to adjacent lamina mesophylls and intermingled with canal spaces of minor veins.

Two to three protrusions on the midribs of

leaf segments comprised the mechanical support of the relatively huge lamina with a structure of collenchyma strands in their interior. The level of protrusion was great in *A. henryi* and *A. paeoniifolius*, with only a slight change in *A. hirtus* and with no obvious bump in *A. kiusianus* (Fig. 4). Two collenchyma strands (the trapezoid-labeled area in Fig. 4) inside both lateral protrusions were usually larger than or equal to the middle one. The extent of the protrusion reflected the same comparison. Those on both



Fig. 3. Leaf habit. A. *Amorphophallus henryi* after fruiting. B. *A. hirtus*. C. *A. kiusianus*. D. *A. paeoniifolius*.

lateral sides were usually greater than or equal to the middle one. The groove between the lamina and midrib protruding on the abaxial surface reflected the strength and compressive pressure. From a transectional view, the groove including the angle was measured as an acute angle in *A. henryi* and *A. paeoniifolius*, almost a right angle in *A. hirtus*, and an obtuse angle in *A. kiusianus* (Fig. 4).

In a comparison of laminae and midribs, micro-scale leaf traits (Table 2) were evaluated as a lamina/midrib thickness ratio and a midrib index. The greatest and smallest lamina/midrib thickness ratios were 0.67 in *A. hirtus* the lamina

of which at 0.3 mm was the thinnest and 0.32 in *A. paeoniifolius* the midrib of which at 0.7 mm was the thickest. Only the measured length, width, and diameter of the midrib of *A. paeoniifolius* appeared nearly equal in shown in Fig. 4, and the ratio 1.01 was used as an index for the lamina mechanical support.

The included angle between the two adaxial laminae along the veins was 113° in *A. henryi*. It was the widest among the four species and also reflected the flatness (Table 2). In comparison, the included angles of the other three species with long petioles and large laminae were $<90^\circ$; they were 84° in *A. hirtus*, 61° in *A. kiusianus*, and 73°

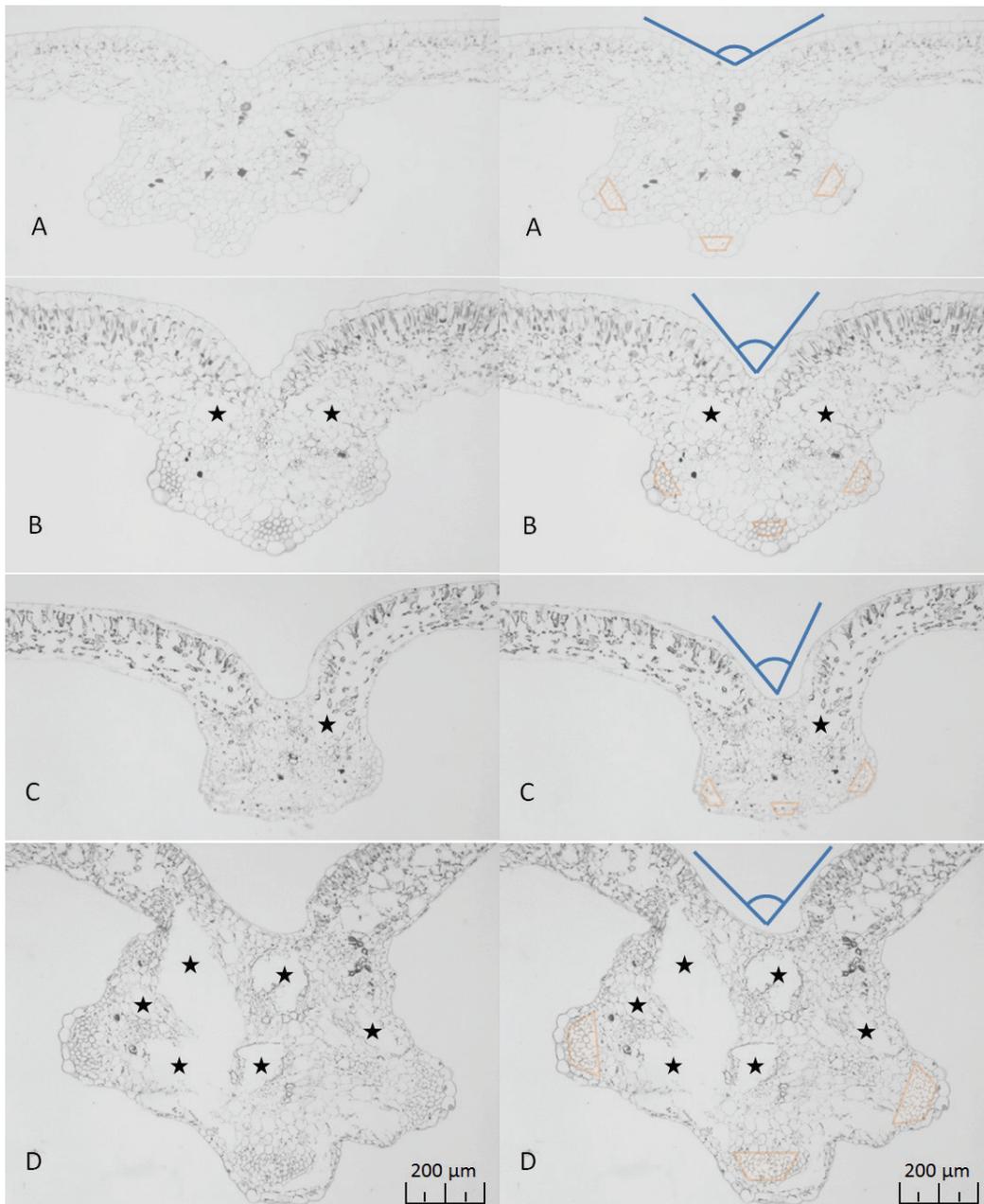


Fig. 4. Micrographs of leaf transects. A. *Amorphophallus henryi* with thin and loose palisade mesophylls of about 20% of the leaf thickness. B. *A. hirtus* with compact palisade mesophylls of about 30% of the leaf thickness. C. *A. kiusianus* with compact palisade mesophylls of about 40% of the leaf thickness. D. *A. paeoniifolius* with compact palisade mesophylls of about 25% of the leaf thickness. The star (★) indicates the large xylem lacuna, the trapezoid (Δ) indicates the collenchyma, and the “v” symbol (v) is the measure of the included angle.

in *A. paeoniifolius*. The outline of the midrib was nearly a flat pentagon in *A. henryi*, *A. hirtus*, and *A. kiusianus* (Table 2).

Maximum likelihood analysis and conserved propagation data

A study of the affinity of the four *Amorphophallus* species in Taiwan based on an ML analysis of *matK* gene sequences indicated

that these four species were clearly identical (Fig. 5). *Amorphophallus hirtus*, *A. henryi*, and *A. kiusianus* formed a clade which was related to other species of *Amorphophallus* of continental Asia. On the other hand, *A. paeoniifolius* was closer to species of Southeast Asia. Our molecular study confirmed that the four *Amorphophallus* species in Taiwan were distinct. Genetic variations were found among different

Table 2. Petiole and lamina traits of four *Amorphophallus* species in Taiwan

Species	<i>A. henryi</i>	<i>A. hirtus</i>	<i>A. kiusianus</i>	<i>A. paeoniifolius</i>
Lamina (L) thickness (μm)	197±3	305±6	161±6	228±17
Midrib (M) thickness (μm)	478±85	456±48	311±9	711±92
Thickness ratio (L/M)	0.41	0.67	0.51	0.32
Midrib index (M/width)	0.64	0.72	0.62	1.01
Included angle [#]	113°±2°	84°±2°	61°±2°	73°±2°
Petiole length (cm)	25~60	20~100	30~80	55~200
Petiole surface	Pale green or dark green	Dark green	Dirty olive green or grayish green	Pale green or dark green, scabrate or verrucae

The included angle between two adaxial laminae along the veins.

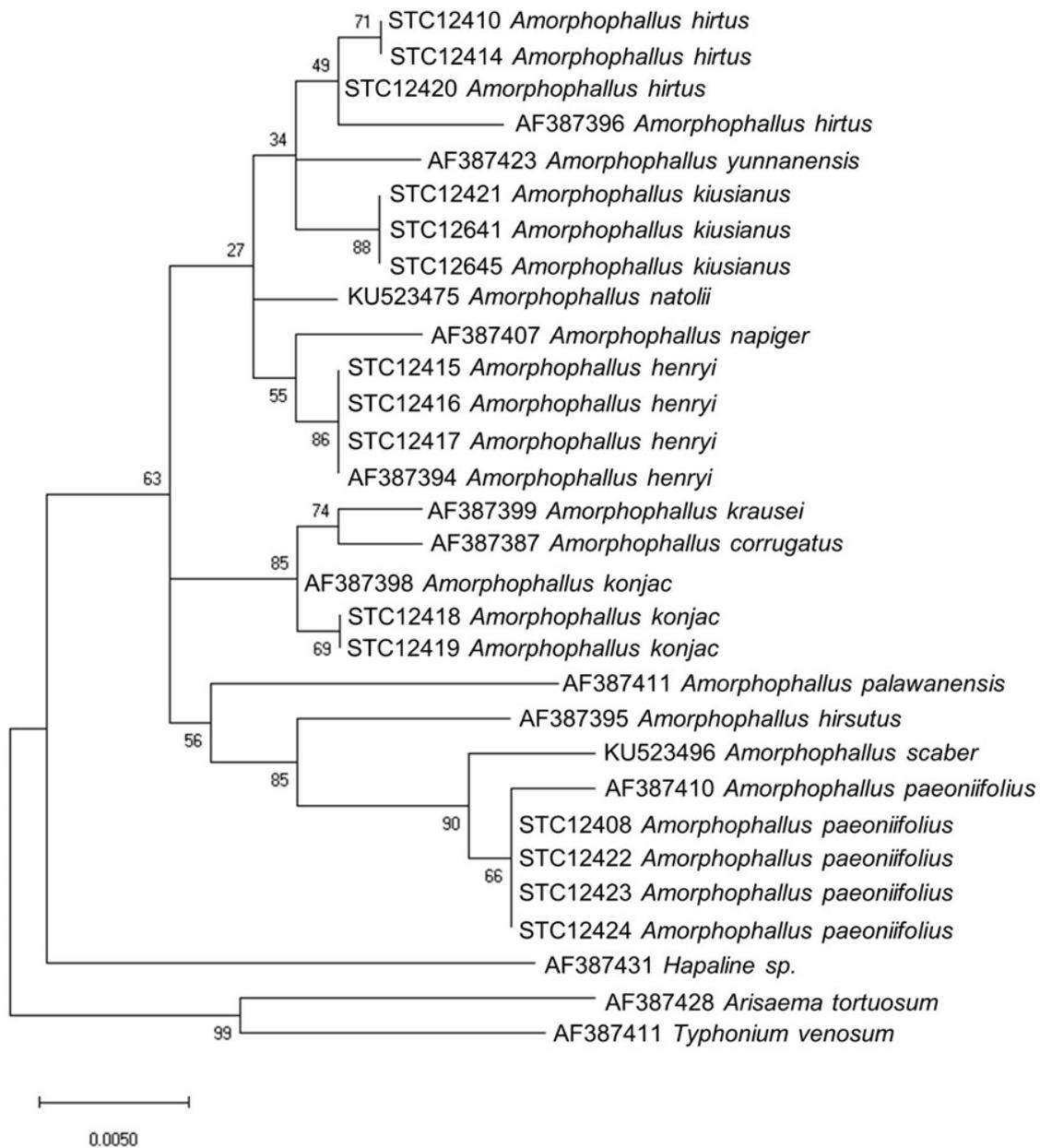


Fig. 5. Maximum-likelihood analysis of the *matK* gene for *Amorphophallus* species and three outgroups by MEGA-X software. Numbers indicate bootstrap values.



Fig. 6. Seed germination and sprouting from tubers of A. *Amorphophallus henryi*; B. *A. hirtus*; C. *A. kiusianus*; D. *A. paeoniifolius*. The arrow (→) indicates the hypocotyl; the arrowhead (>) indicates adventitious roots from the base of the epicotyl.

populations of *A. hirtus*.

The fruiting of each species was also distinct, except that no fruiting was recorded for *A. paeoniifolius*. As mature fruits dropped to the ground, some seeds of *A. henryi* and *A. kiusianus* were able to rapidly absorb nutrients before germination (Fig. 6). When the ovate, one-side-flattened seeds grew or swelled to about the original berry size, most of the seed germ became teardrop-shaped and tumbler-like before germination to prevent becoming upside down or to maintain a miniature form of the adult. When the pointed tip sprouted, stolon-like adventitious roots emerged from the base of the epicotyl (Fig. 6A, C). The bottom of the hypocotyl continued swelling until it looked like a miniature adult tuber.

After dormancy, the terminal or major buds emerged from the root corms (Fig. 6). The newly emerged adventitious roots developed very fast and extended to reach a water source as quickly as possible, even over a long distance. The number of subsidiary buds on the tuber was few (0 or 1) in *A. henryi*, and several (0~5) in *A. hirtus*. However, subsidiary buds on the tubers were very common and not few in *A. kiusianus* and *A. paeoniifolius* (Fig. 6C, D). The subsidiary buds usually developed and were recorded on large or injured tubers (Fig. 6C). Certain tiny subsidiary buds were even produced beside the main ones as a means to conserve totipotency (Fig. 6D), but only the main bud developed. The largest tuber of about 50 cm in diameter and 11.5 kg in fresh weight was recorded in *A. paeoniifolius* at Wufeng. Based on our field observations, diameters of tubers capable of blooming ranged 6~13 cm in *A. henryi*, 7~19 cm in *A. hirtus*, 7~18 cm in *A. kiusianus*, 17~50 cm in *A. paeoniifolius*.

DISCUSSION

Previous studies of *Amorphophallus* species mainly focused on cultivation for crops, pesticide impacts, pollination ecology, and conservation of wild populations, but later switched to nutritional or potential health benefits and pharmaceutical sciences (Purwal *et al.* 2011). This study on population distributions, phenology, and fundamental reproduction information provides future perspectives in conservation and applications of natural resources of *Amorphophallus* in Taiwan.

A study of inflorescence odors and pollinators of *Amorphophallus* indicated that the carrion smells consist mainly of dimethyl oligosulphides and varied with one or two dominant compounds (Kite *et al.* 1998). Osmophores situated at the top part of the spadix secrete a strong fragrance during the period of blooming (Weryszko-chmielewska and Stpicznaska 1995). Although this study focused on phenology, we noted that the odors of the four species slightly differed. The reason for the smell may be related to chemotaxonomy and chemo-identification regardless of what pollinators visit. It may be related to the appendage morphology, that is, globoseconic or elongated-cone type and the bright or dark mimicry color. We assume that the globoseconic and folded appendage of *A. paeoniifolius* emits odors for horizontal expansion, while the elongated-cone type of the other three species produces a chimney effect to attract pollinators from high above.

Based on the leaf morphology and ML analysis of molecular data, the petiole length, coloration pattern, the smooth, verrucae, or scabrate surface, and lamina traits were useful in identifying these species, in addition to the spadix morphology of the flowering and fruiting characteristics. However, three forms of *A. paeoniifolius* are cultivated by farmers: (1) a long petiole with many scabrate protrusions, that mainly produces large tubers; (2) a long petiole with a few very tiny protrusions, which produces many small tubers; and (3) a short petiole with many scabrate protrusions in a high density, that produces many small tubers. The first form, which is similar to the naturalized *A. paeoniifolius*, was analyzed, and it was confirmed to be close to the southeastern clade. A study of the affinity of undescribed taxa of *Amorphophallus* with *A. paeoniifolius* indicated that wild and cultivated Javanese forms were confirmed to be two distinct but closely related taxa (Widjaja and Lester 1987). The smooth-petioled *Amorphophallus* EAW 1177 which was collected on Timor Island as a cultivated plant in a kitchen garden was very similar to the cultivated *A. paeoniifolius* in morphological and tuber electrophoretic characteristics (Widjaja and Lester 1987). The second form with a comparably smooth petiole may be a similar case with no other distinguishable characters to separate it as a distinct taxon. However, conserving different strains of *A. paeoniifolius* may benefit future

research in health nutrition and pharmaceutical sciences. A review of *A. paeoniifolius* indicated that its popularity with complementary medicine has increased in recent years. Over 50% of all modern drugs originate from natural products, and they play important roles in drug development programs of the pharmaceutical industry (Madhurima *et al.* 2012). Our molecular data on the affinity of current *Amorphophallus* species in Taiwan provide valid identity records for future identification and further conservation development.

In our propagation evaluation, naturalized *A. paeoniifolius* produces large tubers and many subsidiary buds but no fruit. Its fruits in Southeast Asia are ellipsoid to globular, orange to red. The other three species produce comparable small tubers, and their fruits mature besipetally by changing color from green to red and then blue. All data suggested that the two groups are distinct. A combined phylogenetic analysis suggested that blue, purple, green, and yellow berries evolved from red/orange/white ones (Sedayu *et al.*, 2010). It implies that *A. paeoniifolius* originating from Southeast Asia has not totally adapted to the local environment and has survived only by vegetative propagation.

The three major clades identified in all analyses were comparable to the biogeographical distribution pattern of *Amorphophallus* in Asia (Sedayu *et al.* 2010). *Amorphophallus henryi* and *A. hirtus* are endemic and grouped together. *Amorphophallus kiusianus* has a closer relationship with *A. konjac* than with the other three species, but it has not been commercially evaluated. However, the blooming seasons have been postponed and productivity has been reduced in recent years, which may have been influenced by global climate change (Fig. 2). Fluctuations in temperature, humidity, and precipitation have become more severe. All environmental problems, such as air pollution, invasive pests, and urban development, increase the severity of those impacts. Specimens of *A. henryi* in several locales with field records disappeared in a short time, and the distribution of this species seemed to have expanded northwards. Greater attention and increased monitoring of *Amorphophallus* species are becoming ever more urgent.

The peculiar habit of a solitary petiole supporting a relatively huge leaf blade resembles a tree trunk bearing an umbrella-like crown, and the growth cycle is accomplished in a short period

of time. A study of the structural and mechanical peculiarities of the petioles of the giant leaves of the titanic arum support the postulate that the aerenchyma has a mechanical function (Hejnowicz and Barthlott 2005). The space that acts like an aerenchyma which develops from the xylem lacuna in the midribs contracts to provide mechanical stability of the petioles, which is a shell composed of compact parenchyma with embedded collenchyma strands, while the core of the shell is empty space or filled with the aerenchyma. The mechanical stability can be manipulated by turgor in the parenchyma of the shell and core. This implies that the anatomical basis of the mechanical stability of the huge petiole relies entirely on turgor pressure extended to the minor midribs. This reveals that water is not a limiting factor in its habitat, and the leaves are relatively lightweight despite their size in the growing season, which often emerge after a light spring thunderstorm. As the climate changes, the trigger precipitation may become a limiting factor, and fluctuations in sustainable water resources could impact the survival of *Amorphophallus*.

Whole-plant performance depends on the integrated function of a complex of leaf traits, such as carbon economy traits associated with the leaf mass per area (LMA) (Ackerly 2004, Wright *et al.* 2004, Niinemets and Sack 2005) and the hydraulic resistance of the leaf (R_l), a major bottleneck in the entire plant's water transport pathway, which may thus be linked to enormous variations in leaf structure and function (Sack and Frole 2006). The hydraulic resistance of the leaf (R_l), which is related to both the venation architecture and mesophyll structure, is determined by the numbers and dimensions of the xylem conduits. It was positively related to the theoretical axial resistivity of the midrib and negatively related to the palisade mesophyll thickness (Sack and Frole 2006). Those containing enlarged xylary canal-like spaces and a thin palisade mesophyll (Fig. 4D) revealed high theoretical axial resistivity of the midrib. This study provides certain aspects of the functional morphology for further research.

What do the variations in leaf structure and function mean? *Amorphophallus henryi* with thin and loose palisade mesophylls exhibited the flattest adaxial lamina and most-acute abaxial groove including the angle in transectional view of the midrib, and these structures indicate shade-loving characters. A comparison of two

shade-loving species of *Amorphophallus* at four shading levels suggested that leaf size increased with an increase in the shading level, resulting in the production of larger daughter corms at low light intensities (Santosan *et al.* 2006). The light intensity affects the time of flowering and tuber productivity, but these results might not be applicable to wild species.

Sun-favoring *A. hirtus*, with compact palisade mesophylls of ca. 30% of the lamina thickness, the thickest lamina at 0.3 mm, and an almost right angle of the groove-included angle, appears to be well adapted to a coral edaphic condition. Its amazing hairy spadix is the tallest in Taiwan, as it was recorded to be more than 2.1 m long. *Amorphophallus kiusianus*, with compact palisade mesophylls of ca. 40% of the lamina thickness and with the thinnest lamina at 0.16 mm thick, possesses an obtuse groove that includes an angle on the adaxial surface and 61° of the included angle between two adaxial laminae along the veins. Those leaflets often face inward adaxially. Both species possess long petioles that elevate the blade height to prevent shading by other adjacent ground plants. The angle of the leaflets facing the sunlight may also be adapted to rapid changes in sun exposure as the leaf sprouts and the petiole elongates. For sun-favoring *Amorphophallus* species, it is suggested that the longer the petiole is elongated, the more acute is the included angle of the leaflets.

Most evidence supports the general idea that intraspecific variations are generally much smaller than interspecific differences, but intraspecific variability in leaf traits may imply enormous effects on the leaves which play a central role in the exchange of energy and nutrients between plants and their environment (Lecerf and Chauvet 2008). *Amorphophallus paeoniifolius* has compact palisade mesophylls of ca. 25% of the lamina thickness and a thick midrib that has a great protrusion which forms several hollow canal-like spaces. The greatest midrib index was 1.01 in *A. paeoniifolius*, which provides mechanical support of the lamina. It produces huge tubers and is prone to vegetative propagation with many subsidiary buds. Another aspect of farming using plant growth regulators or fungicides resulted in impacts on the leaf's anatomical characteristics of *Amorphophallus* crops (Gopi *et al.* 2008) and influenced crop productivity. We suggest a project to develop commercial crops with nutritional, health, and

medicinal benefits.

In conclusion, functional morphological traits of the leaf imply that shade-loving species might be related to the production of large daughter tubers at low light intensities and minimum light densities become of the limiting factor of a reduced time of flowering. All of the above factors can affect the production of tubers and survival strategies.

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REFERENCES

- Ackerly, D.D. 2004. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecol. Monogr.* 75: 25-44.
- Arvill, A. and L. Bodin. 1995. Effect of short-term ingestion of konjac glucomannan on serum cholesterol in healthy men. *Am. J. Clin. Nutr.* 61: 585-589.
- Behera, S.S. and R.C. Ray. 2017. Nutritional and potential health benefits of konjac glucomannan, a promising polysaccharide of elephant foot yam, *Amorphophallus konjac* K. Koch: review. *Food Rev. Int.* 33: 22-43.
- Chua, M., T. C. Baldwin, T. J. Hocking, and K. Chan. 2010. Traditional uses and potential health benefits of *Amorphophallus konjac* K. Koch ex NE Br. *J. Ethnopharmacol.* 128: 268-278.
- Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.
- Gopi, R., C.A. Jaleel, and R. Panneerselvam.

2008. Leaf anatomical responses of *Amorphophallus campanulatus* to triazoles fungicides. *EurAsia J. BioSci.* 2: 46-52.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *In* Nucleic acids symposium series. Information Retrieval, London. pp. 1979-2000.
- Hejnowicz, Z. and W. Barthlott. 2005. Structural and mechanical peculiarities of the petioles of giant leaves of *Amorphophallus* (Araceae). *Am. J. Bot.* 92(3): 391-403.
- Hettterscheid, W.L.A. 1994. Notes on the genus *Amorphophallus* (Araceae): 2. New species from tropical Asia. *Blumea* 39(1-2): 237-281.
- Hettterscheid, W.L.A. and C.-I. Peng. 1995. Notes on the genus *Amorphophallus* (Araceae) IV. Revision of the species in Taiwan. *Bot. Bull. Acad. Sin.* 36: 101-112.
- Huang, T.-C. 2000. 29. Araceae. *In* Flora of Taiwan, 2nd edition (台灣植物誌第二版). 5: 669.
- Lecerf, A. and E. Chauvet. 2008 Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. *Basic Appl. Ecol.* (9): 598-605.
- Kite, G.C., W.L.A. Hettterscheid, M.J. Lewis, P.C. Boyce, J. Ollerton, E. Cocklin, A. Diaz, and M.S.J. Simmonds. 1998. Inflorescence odours & pollinators of *Arum* and *Amoprphophallus* (Araceae). *In* S.J. Owens and P.J. Rudall (eds.). Reproductive biology. Royal Botanic Garden, Kew, UK. pp. 295-315.
- Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35(6): 1547-1549.
- Mayo, S.J., J. Bogner, and P.C. Boyce. 1997. The genera of Araceae. Royal Botanic Gardens, Kew, UK.
- Madhurima P., I.J. Kuppast, and K.L. Mankani. 2012. A review on *Amorphophallus paeoniifolius*. *Int. J. Adv. Sci. Technol.* 2(2): 99-111.
- Oyama, K., S. Hernandez-Verdugo, C. Sanchez, and A. Gonzalez-Rodriguez. 2006. Genetic structure of wild and domesticated populations of *Capsicum annuum* (Solanaceae) from northwestern Mexico analyzed by RAPDs. *Genet. Resour. Crop Evol.* 53: 553-562.
- Pan, C., A.W. Gichira, and J.M. Chen. 2015. Genetic variation in wild populations of the tuber crop *Amorphophallus konjac* (Araceae) in central China as revealed by AFLP markers. *Genet. Mol. Res.* 14(4): 18753-18763.
- Purwal, L., V. Shrivastava, and U.K. Jain. 2011. Studies on antidiarrhoeal activity of leaves of *Amorphophallus paeoniifolius* in experimental animals. *Int. J. Plant Sci. Res.* 2(2): 468-471.
- Ravi, V., C.S. Ravindran, G. Suja, J. George, M. Nedunzhiyan, G. Byju, and S.K. Naskar. 2011. Crop physiology of elephant foot yam (*Amorphophallus paeoniifolius* (Dennst. Nicolson)). *Adv. Horticult. Sci.* 25: 51-63.
- Ren, P.Y. and M.Q. Pan. 2013. Population genetic structure of five *Amorphophallus* species from the south of Yunnan Province by inter-simple sequences (ISSR) markers. *J. Wuhan Univ. (Nat. Sci. Ed.)* 1: 99-104.
- Sack, L. and K. Frole. 2006. Leaf structural diversity is related to hydraulic capacity in tropical rainforest trees. *Ecology* 87(2): 483-491.
- Santosan, E., N. Sugiyama, M. Nakata, and O.N. Lee. 2006. Growth and corm production of *Amorphophallus* at different shading levels in Indonesia. *Jpn. J. Trop. Agric.* 50(2): 87-91.
- Sedayu, A., M.C.M. Eurlings, B. Gravendeel, and W. Hettterscheid. 2010. Morphological character evolution of *Amorphophallus* (Araceae) based on a combined phylogenetic analysis of *trnL*, *rbcL* and *LEAFY* second intron sequences. *Bot. Stud.* 51: 473-490.
- Tamura, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Mol. Biol. Evol.* 9: 678-687.
- Teng, C.-Z., Y. Diao, F.-H. Chang, S.-Q. Xie, Y.-H. Han, and Z.-L. Hu. 2006a. ISSR analyses of relative relationships among germplasm resources of *Amorphophallus* Blume from Yunnan Province. *Anhui Agric. Sci. Bull.* 12: 54-56.
- Teng, C.-Z., Y. Diao, J.-B. Yi, G.-Q. Zong, Y.-B. Chen, Y.-H. Han, and Z.-L. Hu. 2006b. AFLP analysis of relative relationships among germplasm resources of *Amorphophallus*. *Amino Acids Biotic. Res.* 28: 33-35.
- Weryszko-chmelewska E. and M. Stpicznaska, 1995. Osmophores of *Amorphophallus riveri* Durieu (Araceae). *Acta Soc. Bot. Poloniae* 64(2): 121-129.
- Widjaja, E.A. and R.N. Lester. 1987.

- Morphological, anatomical and chemical analyses of *Amorphophallus paeoniifolius* and related taxa. *Reinwardtia* 10(3): 271-280.
- Wright, I., P. B. Reich, M. Westoby, and GLOPNET researchers. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821-827.
- Xuan M. 2010. Diversity of morphology and ISSR analysis of *Amorphophallus* germplasm. Master's thesis, Southwest University, Chongqing, China.
- Zhang Y.J., X.G. Zhang, P.Y. Liu, and C.J. Feng. 2001. RAPD analysis of *Amorphophallus* germplasms. *J. Southwest Agric. Univ.* 23: 418-421.
- Zheng X.F., C. Pan, Y. Diao, Y.N. You, C.Z. Yang, and Z.L. Hu. 2013. Development of microsatellite markers by transcriptome sequencing in two species of *Amorphophallus* (Araceae). *BMC Genomics* 14: 490.

台灣魔芋屬的構造特點與繁殖特性研究

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除分類學外，台灣鮮有有關魔芋或象腳山藥相關物種的生長、生產力和自然資源的文獻。本文探討台灣四種魔芋的分佈、生植物候、生長方式的結構特點，並對種子/塊莖/輔助芽的繁殖以及matK的最大似然度進行了研究。季節性休眠後所有的開花習性是首先產生花序，然後是葉子和果實。通常一年中最早開花的物種是東亞魔芋(*A. kiusianus*)和台灣魔芋(*A. henryi*)，兩者均比密毛魔芋(*A. hirtus*)和疣柄魔芋(*A. paeoniifolius*)早。但是最近兩年花期都往後延遲。喜陰的台灣魔芋的葉特性顯示，最平坦的近軸葉片具有較薄且較疏的柵狀組織，可能與弱光下大塊莖的產生有關。疣柄魔芋具有最大的中脈，中脈側突出較大，葉片內含幾個空腔，形成類似內通道的空間，不僅用於薄葉的機械性支撐，也呈現出較高的理論性中脈的阻力，同時能產生相當大的塊莖與許多副芽，有助於營養繁殖。目前台灣魔芋屬近似性的分子數據可提供紀錄作為永續鑑別，及深入的保育發展。本研究所獲結果對於魔芋屬植物在作物生產、營養、健康及藥用等領域具有參考價值；此外，本研究之解剖變異及功能形態成果，亦可用於分類學的識別及自然資源的保護。

關鍵詞：葉特性, 物候, 繁殖, 植物功能形態學, 魔芋屬