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Morphological Evidences for Hybrid of *Dumasia* (Fabaceae) in Taiwan

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ABSTRACT: Plants intermediate in appearance between *Dumasia miaoliensis* and *D. villosa* subsp. *bicolor* are assumed to be the result of hybridization. *Dumasia miaoliensis* has the leaf base truncate, leaves glabrous or pubescent, style and gynophore long and glabrous, pods falcate, black and glabrous and seeds yellowish brown. *Dumasia villosa* subsp. *bicolor* has the leaf base acute, leaves soft hairy, style and gynophore short and villose, pods terete, yellowish brown and villose and seeds blue. The putative hybrid is more similar to *D. miaoliensis*, but has terete pods and short gynophore similar to *D. villosa* subsp. *bicolor*. Pollen stainability for *D. miaoliensis*, the putative hybrid and *D. villosa* subsp. *bicolor* is 92.5%, 55.3% and 87.4%, respectively. *Dumasia villosa* subsp. *bicolor* occurs in the mountains at 500-2500 m. *Dumasia miaoliensis* occurs only at Chingchieng and Erpenshong between 1100 and 1500 m. The putative hybrid was found intermixed with or growing near the *D. villosa* subsp. *bicolor* and *D. miaoliensis*.

KEY WORDS : Morphology, Hybridization, *Dumasia*, Fabaceae, Taiwan.

INTRODUCTION

Dumasia DC. (Fabaceae, Faboideae, Phaseoleae) consists of about 8 species mainly distributed in Asia and Africa and particularly in southwest China (Lackey, 1981). Two species and a putative natural hybrid occur in Taiwan (Huang and Ohashi, 1993). *Dumasia miaoliensis* Y. C. Liu & F. Y. Lu, endemic to Taiwan, occurs only in Chingchieng and Erpenshong between 1100 and 1500 m (Lu, 1977). *Duamsia villosa* DC. subsp. *bicolor* (Hayata) H. Ohashi & Y. Tateishi, also endemic to Taiwan, occur throughout the mountains between 500 and 2500 m. It was first described as a distinct species, *D. bicolor* by Hayata (1908), then made a subspecies of *D. villosa* by Ohashi and Tateishi (1984). Some plants collected at Erpenshong, Tahu, Miaoli Hsien, by T. C. Huang and S. F. Huang in 1987, are morphologically intermediate between *D. miaoliensis* and *D. villosa* subsp. *bicolor* and are suspected of being hybrids between the two. The brief introduction of these three taxa is presented as follows:

Dumasia miaoliensis Y. C. Liu & F. Y. Lu is characterized by its leaves glabrous or pubescent, terminal leaflet 3-6 cm long, 1.5-3.5 cm wide, apex truncate, stipules 2-2.5 cm long; flowers remotely spaced, calyx 2-6 mm long, mouth oblique, truncate, gynophore 2-3 mm long; pod falcate, glabrous, 2-3 cm long, black; seeds yellowish brown. *Dumasia villosa* DC. subsp. *bicolor* (Hayata) H. Ohashi & Y. Tateishi is characterized by its leaves soft hairy, terminal leaflet 3-6 cm long, 1.5-3.5 cm wide, apex acute, stipules 5 cm long; flowers densely

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spaced, calyx 9 mm long, mouth oblique, subtruncate, gynophore 1 mm long; pod terete, villose, 1-2 cm long yellowish brown; seeds blue. *D. miaoliensis* Y. C. Liu & F. Y. Lu x *D. villosa* DC. subsp. *bicolor* (Hayata) H. Ohashi & Y. Tateishi is characterized by its leaves pubescent, terminal leaflet 3-5 cm long, 1.5-3.5 cm wide, apex truncate, stipules 2-2.5 cm long; flowers remotely spaced, calyx 2-6 mm long, mouth oblique, truncate, gynophore 1 mm long; pod terete, glabrous, 2-3 cm long; seeds yellowish brown.

Natural hybrids has been reported infrequently in Taiwan (Hu and Chang, 1975; Kuo, 1988, 1990; Peng, 1983,1990; Lin and Wang, 1991; Wang and Huang, 1992; Peng and Su, 2000). The objective of our research was to use morphological, cytological, embryological, isozyme and RAPD data to verify the occurrence of natural hybridization in *Dumasia*. This paper presents the results of morphological evidences.

MATERIALS AND METHODS

Plants were collected in the field and cultivated in the greenhouse of the Botany Department, National Taiwan University. To examine the pollen, fresh anthers were prefixed in 2.5% glutaraldehyde in 0.1 m phosphate buffer (pH 7.3) for 4 h, after three buffer rinses they were postfixed in 1% OsO_4 for 2 h. They were then dehydrated in a graded ethonal-acetone series and embedded in Spurr resin (Spurr, 1969). Thick sections (1 μm) were stained with 1% toludine blue in 1% borax at 60℃, thin sections (70-90 nm) were double stained with uranyl acetate and lead citrate. The materials were examined and photographed using light microscopy (LM, Leica) and transmission electron microscope (TEM, Hitachi-600). Pollen was dehydrated in an ethanol series, dried by critical point drying, then coated with gold and examined using scanning electron microscopy (SEM, Hitachi S 520).

Pollen stainability was tested by using malachite green-acid fuchsin-orange G stain (Alexander, 1969). Five hundred pollen grains per plant were examined.

Root tips for chromosome counts were cut from living plants. They were pretreated with 2 mM 8-hydroquinoline for 4 h at a temperature of 18-20℃, then fixed in a 3:1 mixture of absolute alcohol and glacial acetic acid overnight, macerated with 1% pectinase, stained with acetic orcein, squashed and observed using a Leica microscope.

RESULTS

External morphology

D. miaoliensis is characterized by its stem purple, glabrous or pubescence surface (Figs. 1A and D); leaf base truncate, leaves glabrous or pubescent (Figs. 2A and D); stipule hispid on margin only (Figs. 3A and D); petiole glabrous (Fig. 4A); style and gynophore glabrous (Fig. 5A); pods falcate, black and glabrous and seeds yellowish brown (Figs. 6A and E), oblong, with reticular and columns wax surface (Figs. 7A-C).

D. villosa subsp. *bicolor* is characterized by its stem green, soft hairy surface (Figs. 1C and F); leaf base acute, leaves soft hairy (Figs. 2C and F); stipule hispid on entire surface (Figs. 3C and F); petiole villous (Fig. 4C); style and gynophore villose (Fig. 5C); pods terete, yellowish brown, villose and seeds blue (Figs. 6C, D, G and H), elliptical, with reticular and layered like wax surface (Figs. 7G-I).

The putative hybrid is characterized by its stem half purple, half green, glabrous or pubescence surface (Figs. 1B & E); leaf base truncate, leaves glabrous or pubescent (Figs. 2B

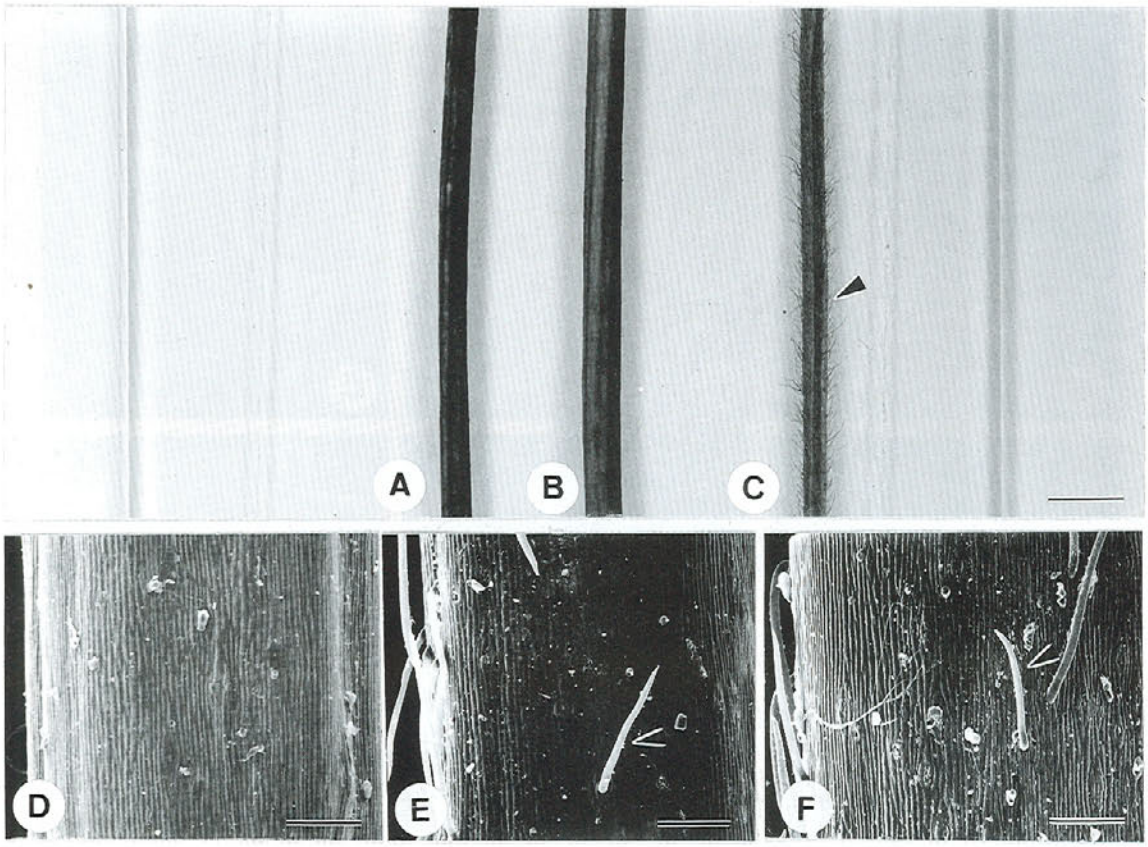

Fig. 1. Surface of stems of *Dumasia*. A and D: *Dumasia miaoliensis*; B and E: putative hybrid; C and F: *Duamsia villosa* subsp. *bicolor*. A: purple and glabrous; B: half purple, half green and glabrous; C: green and soft hairy (arrow head). D and E: glabrous or pubescent; F: soft hairy. A-C: LM micrographs; D-F: SEM micrographs. A-C: bar = 5 mm; D-F: bar = 0.5 mm.

and E); stipule hispid on margin only (Figs. 3B and E); petiole pubescent (Fig. 4B); style and gynophore villose (Fig 5B); pods terete, black and glabrous and seeds yellowish brown (Figs. 6B and F), oblong, with reticular and columns wax surface (Figs. 7D-F).

The putative hybrid is more similar to *D. miaoliensis*, but its terete pods and short gynophore similar to that in *D. villosa* subsp. *bicolor* (Figs. 1-7, Table 1).

Pollen morphology

Pollen grains in all three taxa are similar. They are 6-porate, prolate to oblate in equatorial view, subangular in polar view and have reticulate to rugulate surface ornamentation. The tectum is 1-1.5 μm thick. The foot layer is thin and interrupted. The endexine is about 1.5-2.0 μm thick. The intine is about 1-1.3 μm thick (Fig. 8, Table 2).

Pollen viability

Pollen stainability for *D. miaoliensis*, the putative hybrid and *D. villosa* subsp. *bicolor* is 92.5 %, 55.3 % and 87.4 % respectively (Fig. 9).

Reproductive evidence

Hybrid expriment between putative parents was taken without success.

Chromosome counts

The chromosome number is $2n = 20$ in all three taxa (Fig. 10).

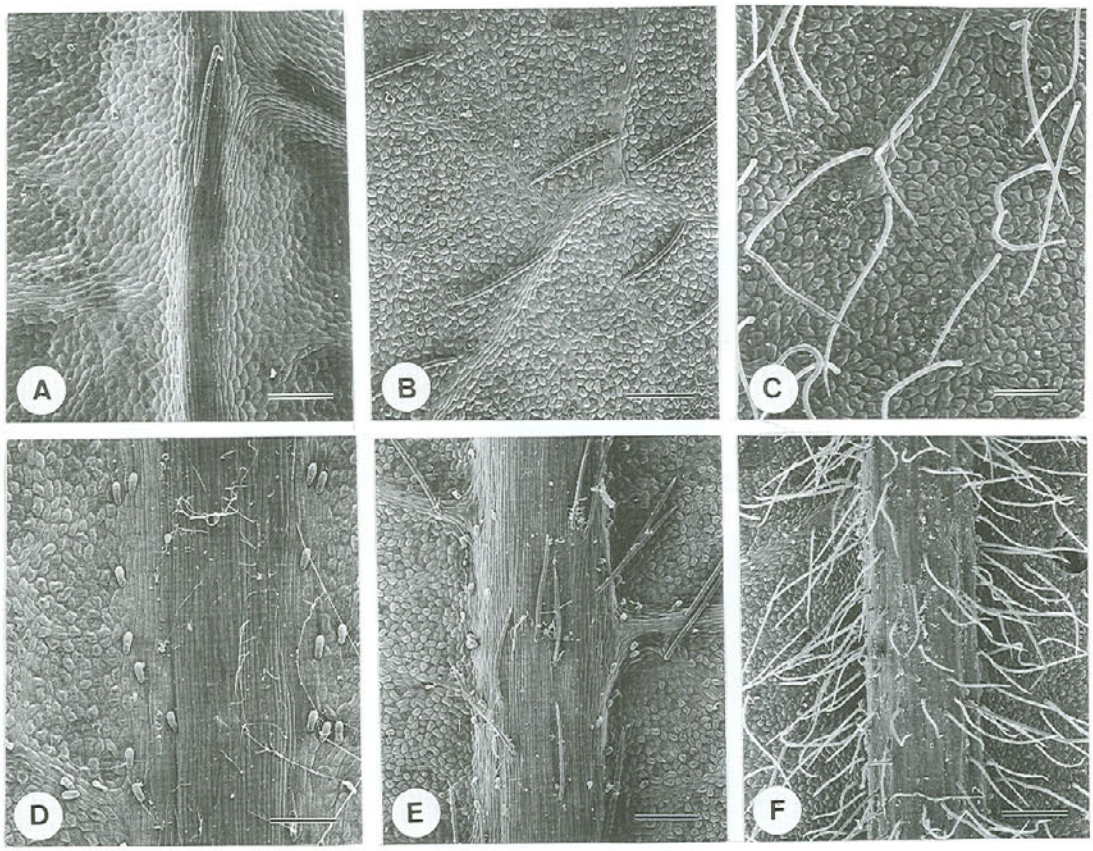

Fig. 2. Surface of leaves of *Dumasia*. A and D: *Dumasia miaoliensis*; B and E: putative hybrid; C and F: *Duamsia villosa* subsp. *bicolor*. A-C: Upper epidermis; D-F: Lower epidermis; A, B, D and E: Pubescence only on upper and lower leaf surfaces along midrib; C and F: Hairs on both upper and lower leaf surfaces. bar = 0.15 mm.

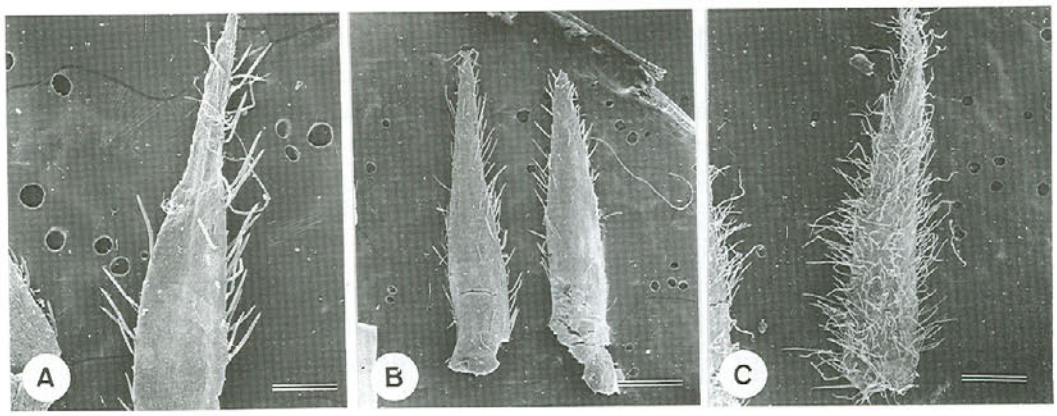

Fig. 3. Stipules of *Dumasia*. A: *D. miaoliensis*; B: putative hybrid; C: *D. villosa* subsp. *bicolor*. A and B: Hispid on margin only; C: Hispid on entire surface. A and B: bar = 0.15 mm; C: bar = 0.3 mm.

Geographical distribution

Dumasia villosa subsp. *bicolor* occurs throughout the mountains between 500 and 2500 m. *Dumasia miaoliensis* occurs only at Chingchieng and Erpenshong between 1100 and 1500 m. The putative hybrid grows intermixed with or near *D. villosa* subsp. *bicolor* and *D. miaoliensis* (Fig. 11).

Table1. Characteristic of *D. miaoliensis*, *D. villosa* subsp. *bicolor* and their putative hybrid.

Taxa / Characters	*D. miaoliensis*	Putative hybrid	*D. villosa* subsp. *bicolor*
Stem	glabrous or pubescent	glabrous or pubescent	soft hairy
Leaflets	glabrous or pubescent	glabrous or pubescent	soft hairy
Leaflets apex	truncate	truncate	acute
Stipules	2-2.5 mm long	2-2.5 mm long	5 mm long
Gynophore	2-3 mm	1 mm	1 mm
Pods	falcate, black, glabrous	terete, black, glabrous	terete, yellowish brown, villose
Seeds	yellowish brown	yellowish brown	blue
Chromosome numbers	20	20	20
Pollen stainability	92.5 %	55.3 %	87.4 %

Table 2. Pollen morphology of. *D. miaoliensis*, *D. villosa* subsp. *bicolor* and their putative hybrid.

Taxa / Characters	*D. miaoliensis*	Putative hybrid	*D. villosa* subsp. *bicolor*
Shape			
Equatorial view	prolate to oblate	prolate to oblate	prolate to oblate
Polar view	subangular	subangular	subangular
Pollen size(μm)			
Polar view	34.6-55.2 (45.1)	34.6-55.2 (45.1)	34.6-55.2 (45.1)
Equatorial view	24.1-54.0 (38.7)	24.1-54.0 (38.7)	24.1-54.0 (38.7)
Average P/E ratio	1.16	1.15	1.16

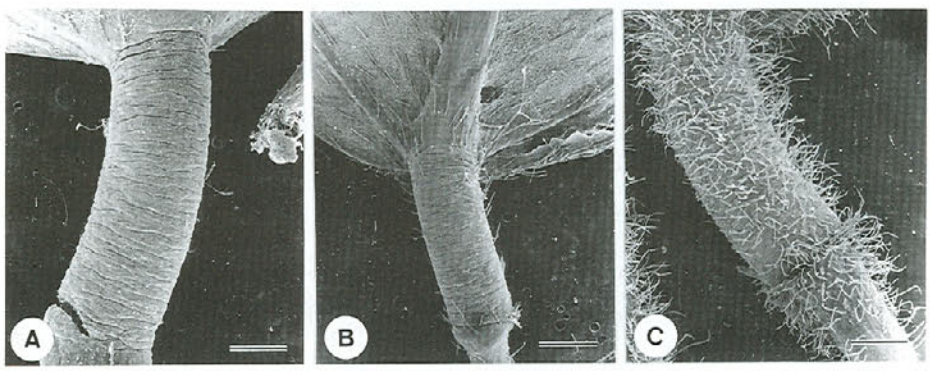

Fig. 4. Petioles of *Dumasia*. A: *D. miaoliensis*; B: putative hybrid; C: *D. villosa* subsp. *bicolor*. A: Petiole glabrous; B: Petiole pubescent C: Petiole villous. A-C: bar = 0.5 mm.

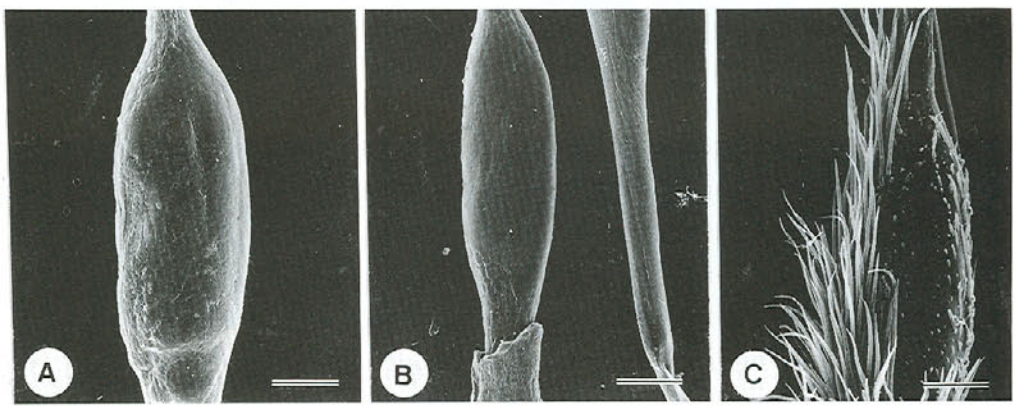

Fig. 5. Surface of ovary of *Dumasia*. A: *D. miaoliensis*; B: putative hybrid; C: *D. villosa* subsp. *bicolor*. A and B: Glabrous ovary surface; C: Villose ovary surface. A-C: bar = 60 μm.

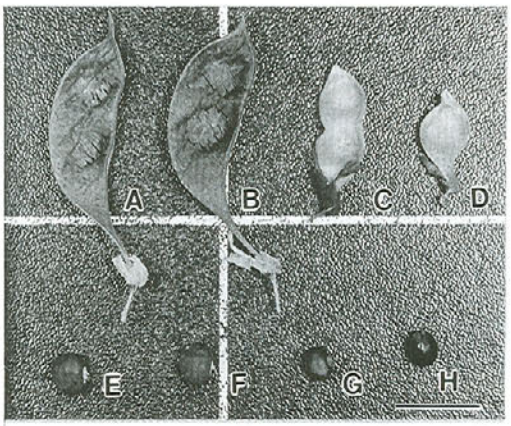

Fig. 6. Pods and seeds of *Dumasia*. A and E: *D. miaoliensis* ; B and F: putative hybrid; C, D, G and H: *D. villosa* subsp. *bicolor*.A and E: Showing black falcate glabrous pod with 1-2 darkish brown seeds; B and F: Showing black falcate glabrous pod with 1-2 darkish brown seeds; C, D, G and H: Showing brown cylindrical villose pod with 1-blueish green seed. bar = 1 cm.

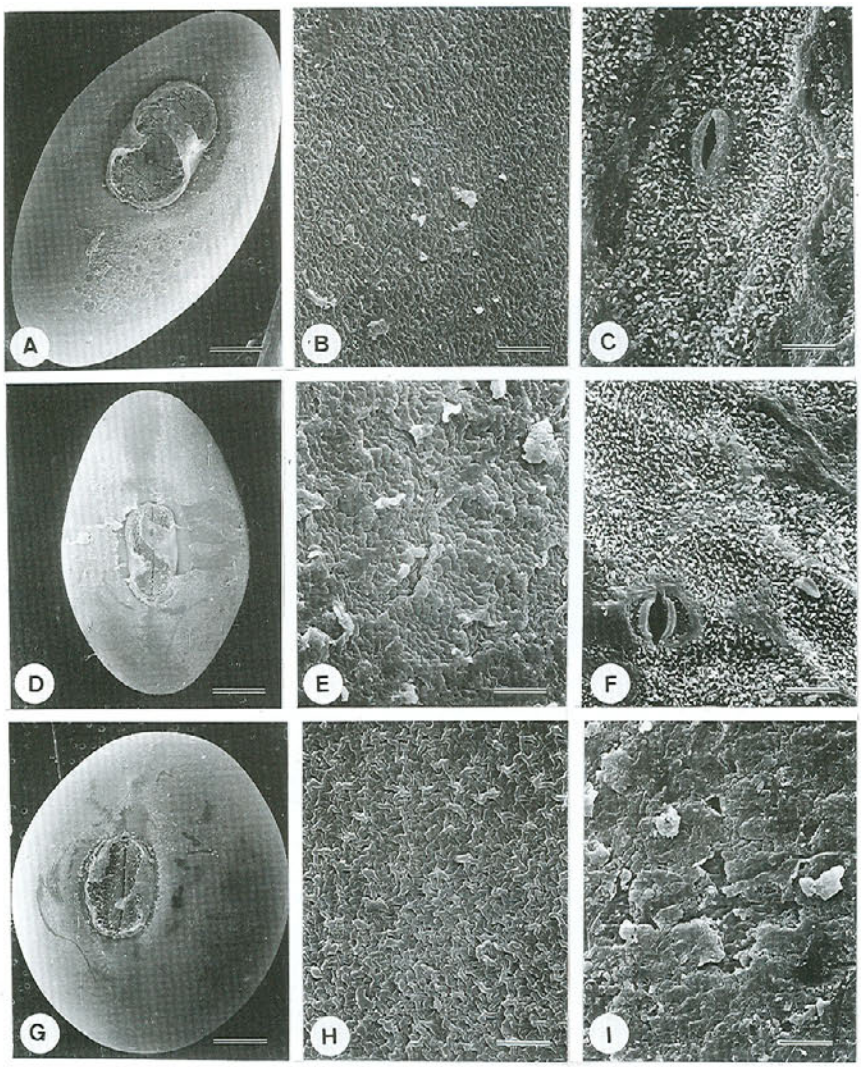

Fig. 7. Seeds of *Dumasia*. A-C: *D. miaoliensis*; D-F: putative hybrid; G-I: *D. villosa* subsp. *bicolor*. A, D and G: Ornamentation of exocarp; B, C, E, F, H and I: Seed surface. A, D and G: bar = 0.75 mm; B, E and H: bar = 23 μm; C, F and I: bar = 15 μm.

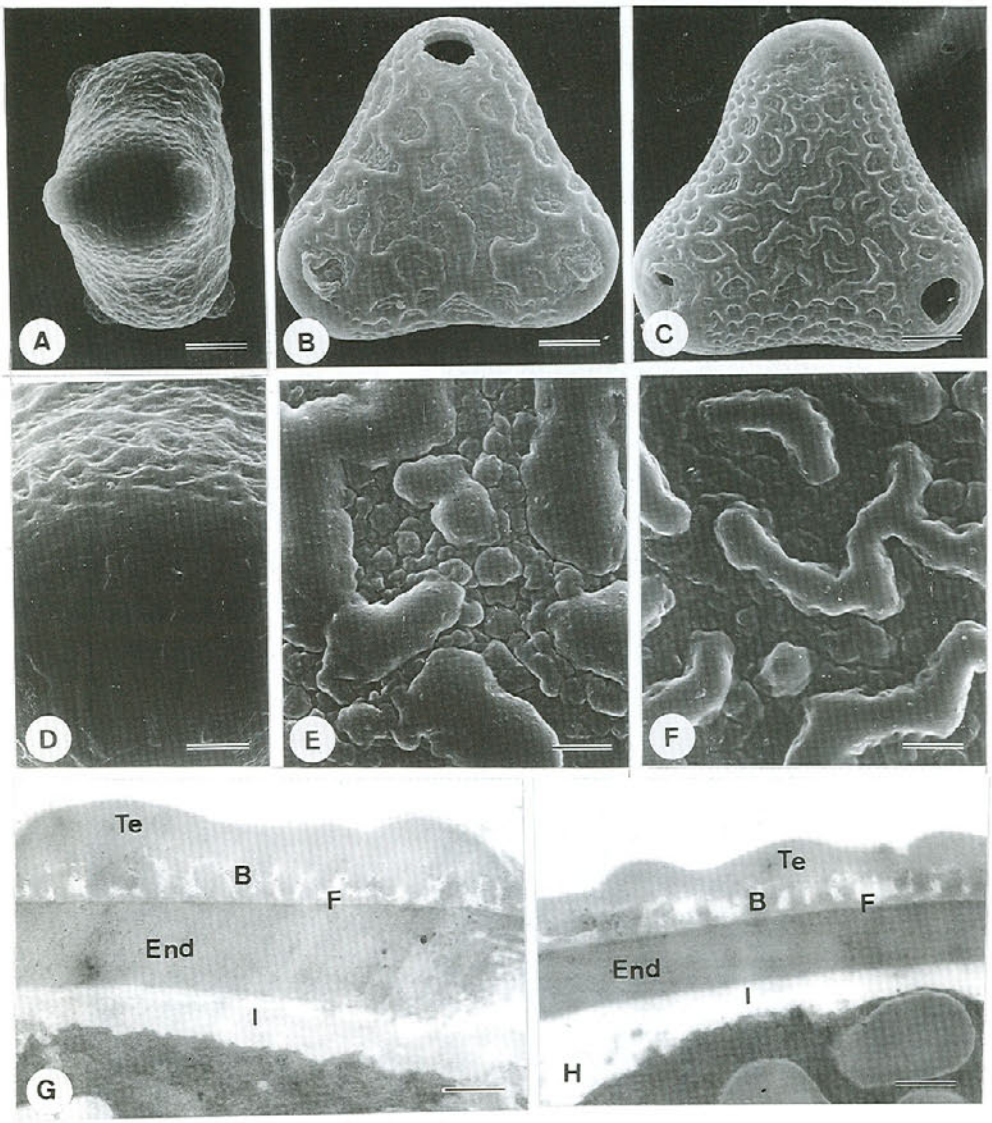

Fig. 8. Pollen grains of putative hybrid. A: Pollen grain prolate in equatorial view; B and C : Pollen grain subangular in polar view; D: High magnification of the pore area; E and F: High magnification of the ornamentation of apocolpium area; G: The stratification of the pollen wall; tectum (Te) thick and continuous, bacula (B) solid, foot layer (F) thin and discontinuous, endexine (End) and intine (I) thick. H: The stratification of the pollen wall; tectum (Te) thick and discontinuous, bacula (B) solid, foot layer (F) thin and discontinuous, endexine (End) and intine (I) thick.A-F: SEM; G-H: TEM- micrographs. A-C: bar = 6 μm; E-F: bar = 1.5 μm; G-H: bar = 0.67 μm.

DISCUSSION

Predeep and Nayar (1990, 1991), who studied *Dumasia* in the Indo-Burmese region, emphasized pubescence at flower length, leaves and petioles, color of pods and numbers of seeds as characters for identification. Huang and Ohashi (1993) emphasized the shape and pubescence of seeds, length of gynophore and shape of leaves as their criteria for distinguishing species in Taiwan. Wei (1995) emphasized the shape and pubescence of

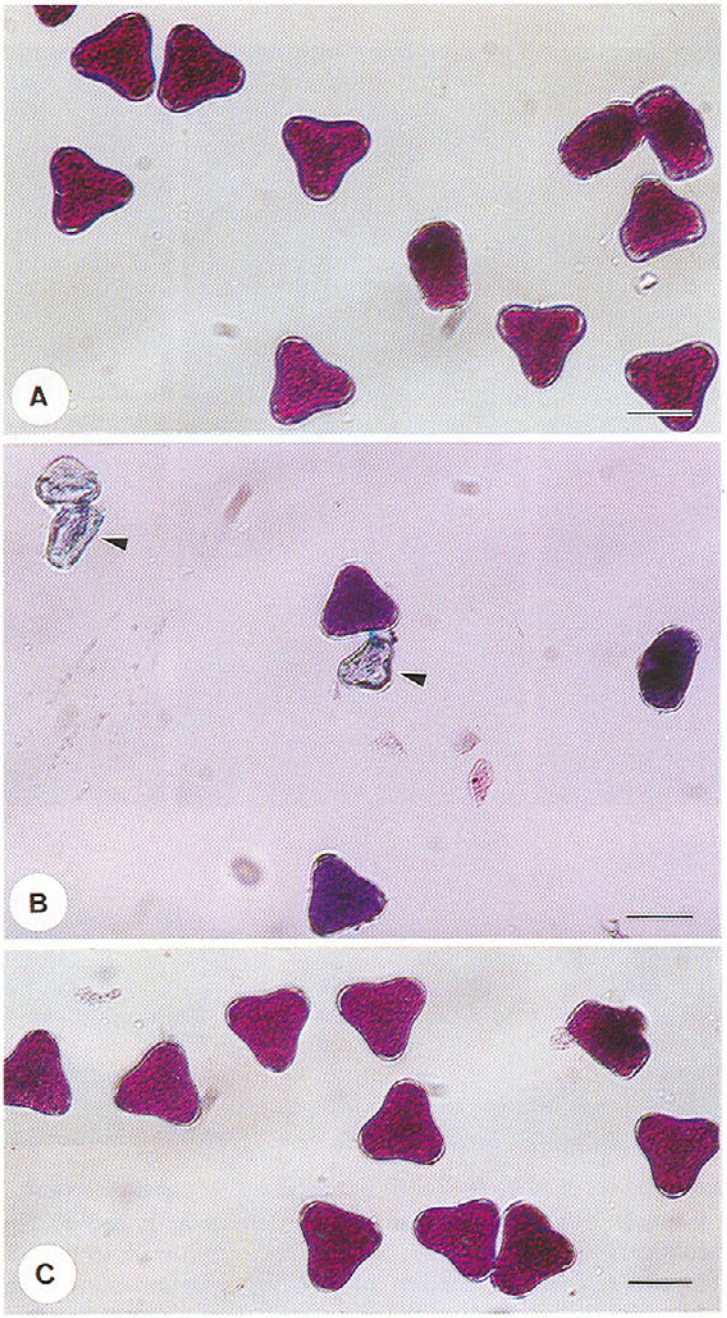

Fig. 9. Pollen stainability of *Dumasia*. A: *D. miaoliensis*. B: putative hybrid. C: *D. villosa* subsp. *bicolor*. Aberrant and aborted pollen grains indicated by arrow head. A-C: bar = 30 μm.

the leaves, stem and petioles, the length of the bracts and the number of seeds as his criteria for identifying species in mainland China. In our study, pubescence of the stem, leaf, stipule, ovary and pods, stipule and gynophore length, and number, color and ultrastructure of the seeds were useful characters for distinguishing the three taxa from each other in Taiwan.

Pollen grains of *D. truncata* and *D. villosa* were reported as 6-porate and subangular in polar view by Ikuse (1954) and Huang (1972). Variation in pollen in *Dumasia* in Taiwan is not useful for distinguishing species, but they are easily distinguished from other genera.

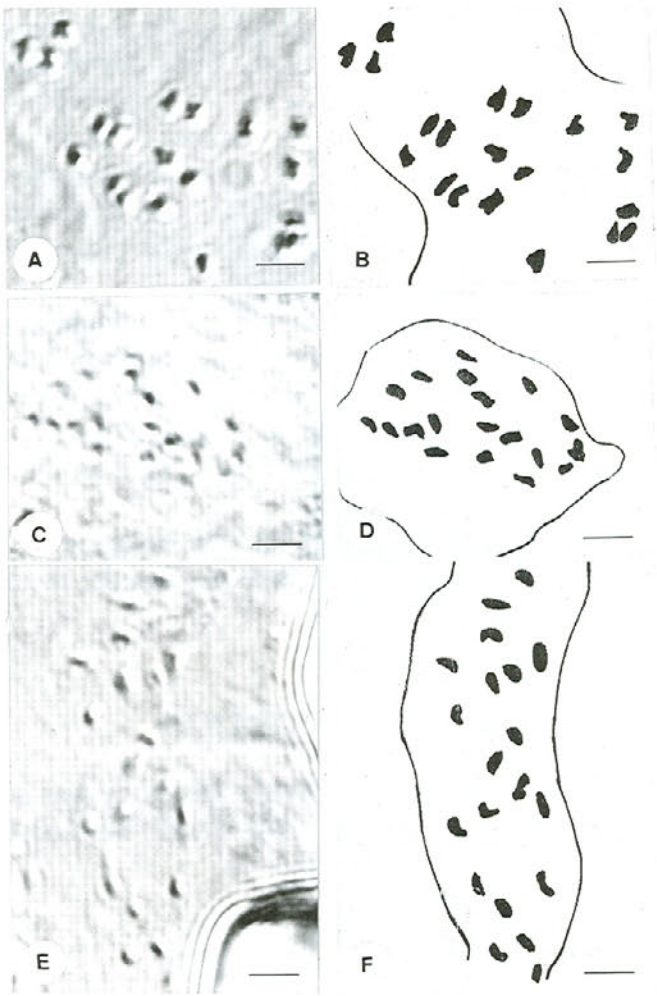

Fig. 10. Chromosomes of *Dumasia* ($2n=20$). A and B: *D. miaoliensis*; C and D: putative hybrid; E and F: *D. villosa* subsp. *bicolor*. A, C and E: LM micrographs; B, D and F: Camera lucid drawings. A-F: bar = 3 μm.

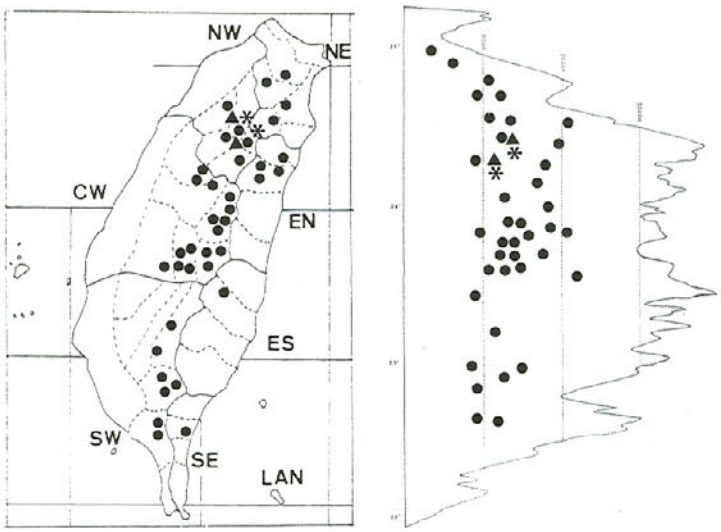

Fig. 11. Geographical and elevational distribution of *Dumasia*. *D. miaoliensis* (∗), putative hybrid (▲) *D. villosa* subsp. *bicolor* (●).

Wang and Huang (1992) and Peng and Sue (2000) reported that pollen grains of the putative hybrid of *Viola adenothrix* x *V. arcuata* and *Begonia* x*taipeiensis* were aberrant, and had low stainability indicating that they were aborted. Our study reconfirmed the low pollen stainability and found aborted, aberrant pollen grains in the suspected hybrid:

Bir and Kumari (1975) reported a chromosome number of $2n = 22$ in *D. cordifolia* and Yeh *et al.,* (1983) and Kumar and Hymowitz (1989) reported a chromosome number of $2n = 20$ for *D. truncata* and *D. villosa*. We counted $2n = 20$ in all three taxa in Taiwan, and the report of the chromosome number in the genus *Dumasia* as $2n = 20$ or 22 by Lackey (1981) is here accepted.

Information on hybrid origin can also be deduced from geographical distribution of *Nupha* x *rubrodisca* and *Begonia* x*taipeiensis* in comparison to distribution of the putative parents (Padgett *et al.*, 1998; Peng and Sue, 2000). *Dumasia villosa* subsp. *bicolor* occurs throughout the mountains between 500 and 2500 m, while *Duamsia miaoliensis* occurs only in Chingchieng and Erpenshong between 1100 and 1500 m. Plants of the putative hybrid occur only in the area of sympatric between them.

The putative hybrids are intermediate in several morphological characters and also the sympatric distribution between the parents, therefore, we consider them to represent another example of natural hybridization in Taiwan.

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臺灣產山黑扁豆屬雜交種之形態學證據

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摘　　要

本文利用形態學的證據來研究疑似苗栗野豇豆和臺灣山黑扁豆之間的雜交種。苗栗野豇豆葉基截形、葉光滑或具毛；花柱及子房柄長且光滑無毛；果莢鐮刀狀、果皮平滑無毛、成熟時黑色；種子呈黃褐色。臺灣山黑扁豆葉基銳尖、具柔毛；花柱及子房柄短且具毛；果莢圓柱狀、果皮披棕色柔毛、成熟時綠色；種子呈深藍色。但在二本松發現兩種間有一疑似雜交種，此疑似雜交種和苗栗野豇豆外部形態類似，但其莢果圓柱狀、子房柄短則和臺灣山黑扁豆相似。苗栗野豇豆的平均花粉可孕性染色比率為 92.5%，臺灣山黑扁豆的平均花粉可孕性染色比率為 87.4%，疑似雜交種的平均花粉可孕性染色比率只有 55.3%。染色體數均為 $2n=20$。苗栗野豇豆目前分佈只侷現於新竹清泉及苗栗二本松海拔介於 1100-1500 公尺處，臺灣山黑扁豆分佈於全省 500-2500 公尺之山地，疑似雜交種發現和苗栗野豇豆與臺灣山黑扁豆混生於一起。

關鍵詞：形態學，雜交，山黑扁豆屬，豆科，臺灣。

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