Different Ploids of *Pteris grevilleana* Wall. *ex* Agardh var. *ornata* Alderw. (Pteridaceae) in Taiwan and Vietnam

Yi-Shan Chao[(1)], Yi-Han Chang[(1)], Lu Thi Ngan[(2)], Do Van Truong[(2)], Ho-Yih Liu[(3*)] and Wen-Liang Chiou[(1*)]

1. Division of Botanical Garden, Taiwan Forestry Research Institute, Taipei 10066, Taiwan.
2. Department of Biology, Vietnam National Museum of Nature, Hanoi, Vietnam.
3. Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan.
** Corresponding authors. Email: hoyih@mail.nsysu.edu.tw; chiou@tfri.gov.tw*

(Manuscript received 24 April 2012; accepted 9 July 2012)

ABSTRACT: *Pteris grevilleana* Wall. *ex* Agardh var. *ornata* Alderw. has a white stripe at the center of the blade, which is the marked difference from *P. grevilleana* var. *grevilleana*. New records of this variety in Taiwan are reported. Using flow cytometry, tetraploids of *P. grevilleana* var. *ornata* were found in Vietnam, and diploids were found in Taiwan. Their spore characteristics are also given herein. The spore sizes of the two ploids significantly differ.

KEY WORDS: New record, ploidy levels, *Pteris*, *Pteris grevilleana*, spore, taxonomy, Taiwan, Vietnam.

INTRODUCTION

Pteris grevilleana Wall. *ex* Agardh is distributed in eastern and southern Asia, including Taiwan and Vietnam. An ornamental variety, *P. grevilleana* Wall. *ex* Agardh var. *ornata* Alderw., was named for its "whitish or pale-grey-green bands running down the centre of the pinnae" (Alderwerelt van Rosenburgh, 1909). This variety was recorded in similar geographic areas with *P. grevilleana* var. *grevilleana*, such as China, Vietnam, Myanmar, Thailand, Malaysia, India, the Philippines, and Indonesia (Wu *et al.*, 1932; Wu, 1990), but was never found in Taiwan (Shieh, 1994).

Recently, two populations of *P. grevilleana* var. *ornata* in northern Taiwan were discovered. All plants in these populations consistently have white-striped pinnae (Fig. 1) and grow with *P. grevilleana* var. *grevilleana* (without white-striped pinnae). In Vietnam, the sympatric distribution of these two varieties was also observed.

In this paper, we describe this newly recorded variety in Taiwan and compare the characteristics, including morphology, ploids, and spores, of *P. grevilleana* var. *ornata* plants between Taiwan and Vietnam. A lectotype is also designed herein (Fig. 2).

MATERIALS AND METHODS

The materials of *P. grevilleana* var. *ornata* include five plants from Hsintien and Wulai, northern Taiwan (*Y.-H. Chang 20100127-028*, *-029*, *-030*, *-031*; *P.-F. Lu 23294*), and eight plants from Mt. Tam Dao, Vietnam (*Y.-S. Chao 1665*, *1683*, *1684*, *1685*, *1701*, *1702*, *1703*, *1723*). All plants were used for ploidy determination, and their spore numbers/sporangium were counted.

Ploid levels were determined by FACScan (BD Technologies, Franklin Lakes, NJ, USA), using *Nicotiana tabacum* L. 'Xanthi' (genome size 4X=20.08 pg; Johnston *et al.*, 1999) as the calibration standard. The plant, *P. grevilleana* var. *grevilleana* with a known chromosome number, was used as the control (Chao *et al.*, 2010). Nuclei were extracted using a CyStain PI absolute P kit (Partec, Münster, Germany). About 100 mm^2 of fresh leaf tissue was chopped up with a razor blade in 0.5 mL of extraction buffer, incubated for 10-15 min, filtered through a 50-mm nylon mesh (Partec), and processed in staining solution (containing propidium iodide). Preparations were kept in the dark for 30 min.

In terms of the spore characteristics, the spore number/sporangium and sizes were determined. Five mature sporangia were randomly selected from each plant from Taiwan and Vietnam to count the number of spores per sporangium. To determine the spore size, equatorial diameters of 30 spores from each specimen were measured. T-test (IBM SPSS Statistics 19) was used to compare the means of spore sizes of the two taxa from Taiwan and Vietnam. To observe details of the spores, a scanning electron microscope (SEM; TM3000, Hitachi, Tokyo, Japan) was used.

RESULTS

In the protologue, Alderwerelt van Rosenburgh (1909) cited specimens from "Malacca, Borneo, the Philippines; Siam, North India". We have checked the possible herbaria where these specimens are deposited, B and BO (Stafleu and Cowan, 1976). Only one above specimen was found in BO. The label showed that the specimen was collected from Borneo and determined

臺灣大學學術期刊資料庫



Fig. 1. Photographs of *Pteris grevilleana*. var. *ornata* in Taiwan. A: Growing under an evergreen broadleaf forest. B: Pinna with broad, white, central stripe.

by Alderwerelt van Rosenburgh (signed as "v. A. v. R.") in 1907 (Fig. 2). We here designed it as the lectotype. After examining the frond morphologies of *P. grevilleana* var. *ornata* from different areas, no distinct differences were observed except that plants from Taiwan were smaller. Based on the genome size determined by flow cytometry, we found that plants of *P. grevilleana* var. *ornata* had two different values. Compared to *N. tabacum* and plants with known ploidy levels, they were 24 and 48 pg, i.e., diploids and tetraploids, respectively (Fig. 3). Diploids were found in Taiwan, and tetraploids were from Vietnam.

In all samples, each sporangium contained 32 spores, which indicated that they might reproduce apogamously, as is the case of *P. grevilleana* var. *grevilleana* (Chao *et al.*, 2010). All spores of both ploids were tan-colored and tetrahedral. The perispores had a depressed aperture and prominent tubercles, which were covered by digitation (Fig. 4). The spore characteristics observed were similar to descriptions of *P. grevilleana* (Huang, 1980; Tryon and Lugardon, 1990). The average equatorial diameter of diploid spores (42.4 ± 2.0 μm; Taiwan) was smaller than that of tetraploid spores (47.7 ± 2.6 μm; Vietnam). T-test showed that spore sizes of the diploids were significantly larger than those of tetraploids ($p = 0.037 < 0.05$). Therefore, in this case, it is clearer and easier to distinguish different ploids of *P. grevilleana* var. *ornata* by spore size than by frond morphology.

TAXONOMIC TREATMENTS

Pteris grevilleana Wall. ***ex*** J. Agardh var. ***ornata*** Alderw., Malayan Ferns 364. 1909.-TYPE: Borneo, 1907, *Alderwerelt van Rosenburgh s.n.* (lectotype, BO1526493!; here designated!)

白斑翅柄鳳尾蕨 Fig. 2

Rhizomes short, erect, apex clothed with scales; scales linear-lanceolate, 1-2 mm long, 0.2-0.5 mm wide, bicolorous, centers dark-brown, margins narrow, light-brown, apex long-acuminate, margin entire. Fronds 10-70 cm long, clumped, nearly dimorphic, blade larger and segment narrower on sterile one, stipe of fertile one about twice as long; stipes glabrous, dark-red or adaxially green and abaxially dark-red, 5-55 cm long, sparsely scaly at base, grooved on adaxial surface; blades pentagonal to lanceolate, 1/4-1/2 frond length, 3-20 cm long, 4-16 cm wide; terminal pinnae conforming to lateral ones, lateral pinnae 1-3 pairs, basal pinnae with basiscopic pinnules; rachises winged and pinnae decurrent along upper stipes; pinnae pinnatifid, lanceolate, green but with whitish or pale-grayish-green central bands on adaxial surface, 7-9cm long, 2-4 cm wide, pectinate, straight or incurved, sessile or short-petiolate, apex caudate or acute; segments of pinnae oblong, slightly oblique to falcate, 3-6 mm wide, margins almost entire to serrulate; veins forked, free, with linear false veins; distance between adjacent junctions of costa and costule 5-9 mm; antrorse awns 0.5-3(4) mm, persistent on adaxial costa and junctions of costa and costule; sori continuous along margins of segment except serrate portions at apex; spores tetrahedral, tan.

臺灣大學學術期刊資料庫



Fig. 2. Lectotype of *Pteris grevilleana* Wall. *ex* J. Agardh var. *ornata* Alderw. The right leaf shows adaxial face with white stripes on the center of pinna, but they are not very clear.

臺灣大學學術期刊資料庫

Very similar to the typical variety but pinnae with broad, white, central stripe.

Poloidy: Taiwanese plants diploid and Vietnamese plants tetraploid.

Distribution and habitat: Taiwan, China (Guangdong and Guangxi), Vietnam, Myanmar, Thailand, Malaysia, India, the Philippines, and Indonesia; in shaded places, under evergreen broadleaf forests, less than 1000 m in elevation. This variety can occur with *P. grevilleana* var. *grevilleana* and has similar habitats. There is no different habitat preference between these two varieties and the two ploids.

Specimens examined: TAIWAN. New Taipei City: Hsintien District, Mt. Chitang, *Y.-H. Chang 20100127-028*, *-029*, *-030*, *-031* (TAIF); Wulai District, Mt. Luofeng, *P.-F. Lu 23294* (TAIF).

VIETNAM. Thai Nguyen: *M. Colani 3442* (P).—Tonkin: *M. l'abbe Bon. 4334* (SING); Dam-bs, Lung Wan Village, Sai Wong Mo Shan, *W. T. Tsang 29986* (SING).—Vinh Phuc: Tam Dao, from Tam Dao Town to Cai Keng Road and Bac Thai Road, *Y. S. Chao 1665*, *1683*, *1684*, *1685*, *1701*, *1702*, *1703*, *1723* (TAIF, VNMN.B).

ACKNOWLEDGEMENTS

The authors thank Ms. Pi-Fong Lu for her assistance in collecting plant samples, Dr. Dedy Darnaedi and Mr. Arief Hidayat for assisting the examination of the specimens in BO, Cheng-Wei Chen for photographs of lectotype, Chien-Yu Lin for statistical assistance, and Wei-Hsiu Wu for photographs of plants in field. This research was supported by Taiwan Forestry Research Institute (96AS-11.1.2-FI-G1, 97AS-11.2.2-EI-W4, and 98AS-8.2.1-F1-G1).

LITERATURE CITED

Alderwerelt van Rosenburgh, C. R. W. K. V. 1909. Malayan Ferns: Handbook to the Determination of the Ferns of the Malayan Islands. Landsdrukkerij, Batavia.

Chao, Y.-S., H.-Y. Liu, Y.-M. Huang and W.-L. Chiou. 2010. Reproductive traits of *Pteris cadieri* and *P. grevilleana* in Taiwan: Implications for their hybrid origins. Bot. Stud. **51**: 209-216.

Huang, T.-C. 1980. Spore Flora of Taiwan. Dept. Bot., NTU, Taipei. 111 pp.

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**: 609-613.

Shieh, W.-C. 1994. Pteridaceae. In: Huang, T.-C., et al. (eds.), Flora of Taiwan, 2nd ed. **1**: 222-233. Editorial Committee, Dept. Bot., NTU, Taipei, Taiwan.

Stafleu, F. A. and R. S. Cowan. 1976. Taxonomic Literature: A selective guide to botanical publications and collections with dates, commentaries and types, 2nd ed. **1**: 28. Utrecht, UtrechtBohn, Scheltema & Holkema.

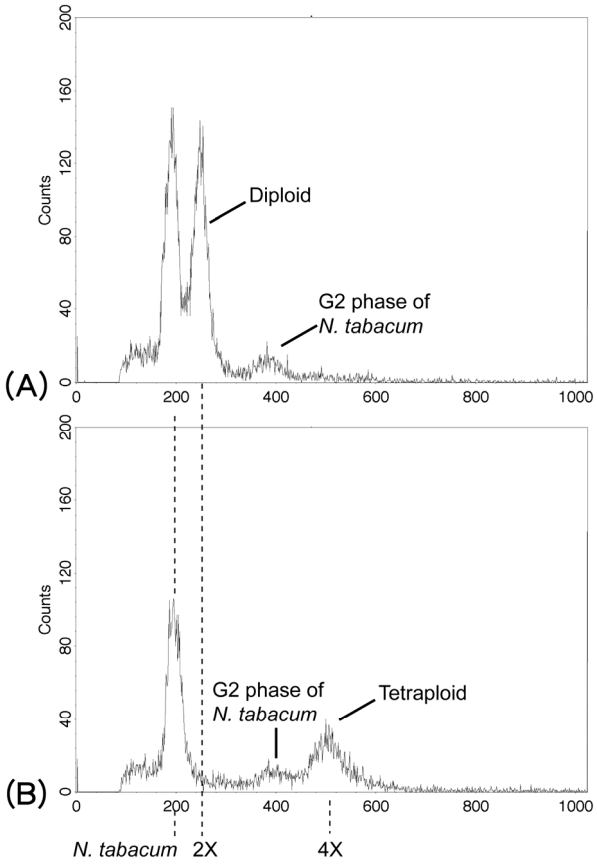


Fig. 3. Ploidy levels of *Pteris grevilleana* var. *ornata* (2x, 4x) determined by flow cytometry. A: Diploidy level. B: triploidy level. The horizontal axis indicates the fluorescence intensity. *Nicotiana tabacum* was used as a calibration standard. There was a 2:4 ratio between diploid and tetraploid values. The vertical axis indicates the number of cells.

Tryon, A. F. and B. Lugardon. 1990. Spores of the Pteridophyta: surface, wall structure, and diversity based on electron microscope studies. Springer-Verlag, New York. 648 pp.

Wu, Y.-C., K.-K. Wong and S.-M. Pong. 1932. Polypodiaceae Yaoshanensis, Kwangsi. Bull. Dept. Biol. Sun Yatsen Univ. **3**: 236.

Wu, S.-W. 1990. Pteridaceae. In: Ching, R.-C. and K.-H. Shing (eds.), Flor. Reipubl. Pop. Sinicae, **3**: 15-91. Science Press, Beijing.

臺灣大學學術期刊資料庫

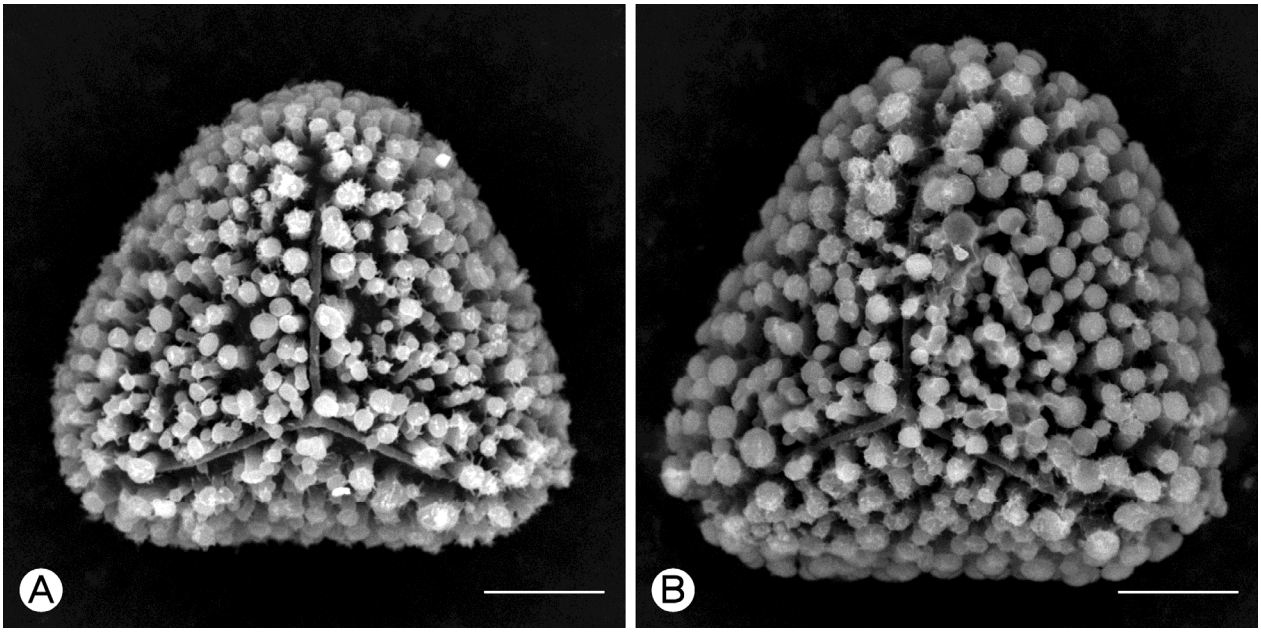


Fig. 4. Scanning electron micrographs of diploid (A, *P.-F. Lu 23294*, TAIF) and tetraploid (B, *Y.-S. Chao 1685*, TAIF) spores of the *Pteris grevilleana* var. *ornata*. Scale bars = 10 μm.

產於台灣與越南的白斑翅柄鳳尾蕨（鳳尾蕨科）具不同之倍體性

趙怡姍[(1)]、張藝翰[(1)]、Lu Thi Ngan[(2)]、Do Van Truong[(2)]、劉和義[(3*)]、邱文良[(1*)]

1. 林業試驗所植物園組，10066 台北市南海路 53 號，台灣。
2. 越南國家自然博物館生物系，河內，越南。
3. 國立中山大學生物科學系，80424 高雄市蓮海路 70 號，台灣。
* 通信作者。*Email: hoyih@mail.nsysu.edu.tw; chiou@tfri.gov.tw*

(收稿日期：2012年4月24日；接受日期：2012年7月9日)

摘要：白斑翅柄鳳尾蕨 (*Pteris grevilleana* Wall. *ex* Agardh var. *ornata* Alderw.) 與承名變種翅柄鳳尾蕨 (*P. grevilleana* Wall. *ex* Agardh var. *grevilleana*) 的差異在前者羽片中部具白色斑紋，於台灣為新紀錄。利用流式細胞儀，發現越南產的白斑翅柄鳳尾蕨為四倍體，台灣產為二倍體。兩者的孢子形態亦於本文中報導，二倍體與四倍體的孢子大小具明顯差異。

關鍵詞：新紀錄、倍體數、鳳尾蕨屬、翅柄鳳尾蕨、孢子、分類、台灣、越南。

臺灣大學學術期刊資料庫