

TAXONOMIC VALIDITY OF *DRYOPTERIS LAOSHANENSIS* J. X. LI ET S. T. MA AND *DRYOPTERIS CHAMPIONII* (BENTH.) C. CHR. FROM CHINA

XIAO-JUAN LI^{1,2}, JIAN-XIU LI³, KANG SUN² AND XIAO WANG^{1*}

*Qilu University of Technology [Shandong Academy of Sciences],
Shandong Analysis and Test Center, Jinan 250014*

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Abstract

A combination of plant taxonomy, micromorphological and palynological characters was used to systematically compare and study the status of the two closely related species, namely *Dryopteris laoshanensis* and *D. championii*. Results showed that the sporophylls of *D. laoshanensis* were triangular-ovate, with 1 alternate pair of pinnae at the base of the sporophylls, not shortened, and each alternate pair of pinnae gradually shortened upward; rhizome and petiole are densely covered with reddish brown ovate-lanceolate scales; the sori are distantly placed from the main vein; spore perispore with curved ridges protrusions, which is clearly different from the *D. championii*. The leaves of *D. championii* were ovate-oblong, 1 pair of pinnae at the base of the leaves slightly shortened, and the upper 2-4 pairs of pinnae were extended in sequence, which is the widest part of the leaves at the middle, and then the pinnae gradually shrinking upward; rhizome and petiole are densely covered with dark brown lanceolate scales; the sori close to main vein; spore perispore with tuberculiform and tuberculiform-rugulate protrusions. It provided micromorphological and palynological evidences for the clear identity of *D. laoshanensis* and *D. championii* as distinct species.

Introduction

Dryopteris Adans. is a large world-wide genus belonging to Dryopteraceae and known as wood fern. About 230 species were known to be widely distributed in both the hemispheres, mainly in Asia, especially from the Himalaya to China, Japan and Korea (China Flora Editorial Board 2000). It is one of the large genera of the Pteridophyte in China which is represented by 127 species and 12 species from Shandong. *Dryopteris laoshanensis* J. X. Li et S. T. Ma, a new species published in 1983 (Li and Ma 1983) was recorded in the Flora of Shandong (Chen 1990), Flora Reipublicae Popularis Sinicae (China Flora Editorial Board 2000) and Flora of China humped *D. laoshanensis* with *D. championii* and adopted the *D. championii* (Benth.) C. Chr. as a valid species. There were many complexes in the species of *Dryopteris*, and the morphological characteristics of sporophytes in some groups are very similar to those of related species and appeared to be difficult to distinguish based only on morphological characters of sporophytes. In recent years, many scholars have done a lot of useful work by combining the submicroscopic structure of spore morphology with morphological taxonomy, Zhang and Xi (1976), Zhang (1979), Li *et al.* (1996, 2013), Liu and Zhao (1999), Lu *et al.* (2007), Wang *et al.* (2010) and Li *et al.* (2019). They conducted palynological studies on more than 50 species of this genus. Zhou *et al.* (1985), Ding *et al.* (1990) and Guo *et al.* (1999) studied the morphology and anatomy of 11 species of the *Dryopteris*. These studies provide the basis of palynology and micromorphological submicroscopic structure for the classification and identification of *D. laoshanensis* and *D. championii*.

*Author for correspondence: <wxjn1998@126.com>. ¹Qilu University of Technology [Shandong Academy of Sciences], School of Pharmacy, Jinan 250014. ²Shandong Xiandai University, Jinan 250104. ³Shandong University of Traditional Chinese Medicine, Jinan 250014.

Materials and Methods

The mature sporophylls and well-developed spores of *D. laoshanensis* J. X. Li et S. T. Ma and *D. championii* (Benth.) C. Chr. were identified by Prof. Jianxiu Li of the Shandong University of Traditional Chinese Medicine, and all specimens were preserved in Shandong Xiandai University. The classification name of *Flora of China* was adopted, spore perispore ornamentations were described according to the concept of palynology (Wang *et al.* 2010). The species name, collection locality and voucher specimen information are presented in Table 1.

The sporophylls, rhizomes and scales on petioles of *D. laoshanensis* and the *D. championii* were observed under the dissecting microscope. The picture of position of the sori on the back of both sporophylls on both sides of the pinnae main vein was taken.

The dorsal and ventral characters of *D. laoshanensis* and *D. championii* were taken from the pinnae respectively. After SC7620 metal spraying for coating for 2 min, the materials were placed under SUPRATM55 thermal field emission scanning electron microscope (SEM) to observe the materials, the magnification was from high ($\times 1200$) to low ($\times 800$). When the voltage was stable, focal length was adjusted to take the pictures.

The mature and well-developed spores were taken from *D. laoshanensis* and *D. championii* respectively, and temporary water-packed tablets were made, the size of spores was observed and measured under an optical microscope, the 10 spores were selected for each species, and the length of polar axis and equatorial diameter were measured respectively, and the average value was taken. Then the spores dispersed evenly on the specimen holder double-sided adhesive paper, after SC7620 metal spraying for coating for 2 min, the materials were placed under SUPRATM55 thermal field emission SEM to observe the proximal side, distal side and equatorial side of the spores, the magnification was from high ($\times 5000$) to low ($\times 1500$). When the voltage was stable, focal length was adjusted to taken the pictures.

Results and Discussion

The sporophyll of *Dryopteris laoshanensis* are triangular-ovate, 60 cm long, 23-25 cm wide, with 1 pair of pinnae at the base of the leaves are 15 cm long, not shortened, the second alternate pair of pinnae were 14 cm long, slightly shortened, and tapered upwards (Fig. 1A); rhizome and petiole were densely covered with reddish brown ovate-lanceolate scales, thin scales with sharp teeth on the edges (Fig. 1B); the sori are located at midpoint between the edge of the pinnae and the main vein, and distantly placed from the main vein (Fig. 1C).

The periclinal wall of the upper and lower epidermis of pinnules protruded outwards, the cells long and straight, banded in shape; there are regular thick teeth on both sides of the anticlinal wall (Fig. 3A-D, Table 2).

The polar view of spores are oblong, the equatorial view is semicircular, and the perispore have curved ridge protrusions, and there are scales between the protrusions surface and the protrusions, forming a reticular sculpture (Fig. 4A-D; Table 2).

The sporophyll of *Dryopteris championii* (Benth.) C. Chr. are ovate-oblong, 80 cm long, 22-24 cm wide, 1 pair of pinnae at the base of the leaves are 14 cm long, slightly shortened, and the upper 2-4 pairs of pinnae were extended in sequence, which is the widest part of the leaves, and the pinnae gradually shrinking upward (Fig. 2 A); rhizome and petiole are densely covered with dark brown lanceolate scales; thick scales, long lanceolate, margin entire or small teeth (Fig. 2 B); the sori were located on both sides of the main vein of the pinna, but very close to the main vein (Fig. 2 C).



Fig. 1. *D. laoshanensis* J. X. Li et S. T. Ma A. plant; B. scales; C. pinnae.



Fig. 2. *D. championii* (Benth.) C. Chr. A. plant; B. scales; C. pinnae.

The periclinal wall of the upper and lower epidermis of pinnules are protruded outwards, the cells short and curved, polygonal in shape; irregularly polygonal on both sides of the anticlinal wall are observed (Fig. 3E-H; Table 2).

The polar view of spores are oblong, the equatorial view is semicircular, and the perispore have tuberculiform and tuberculiform-rugulate protrusions, and there are small scales on the surface of the protrusions and between the protrusions, forming fine reticular sculpture (Fig. 4E-H; Table 2), which is not found in *D. laoshanensis*.

Distinguishing features of the species:

Rhizome and petiole are densely covered with reddish brown ovate-lanceolate scales of *D. laoshanensis* (Fig. 1B), the sporophylls of *D. laoshanensis* are triangular-ovate, with 1 pair of pinnae at the base of the leaves, not shortened, and each pair of pinnae gradually shrinking upward (Fig. 1A); the sori are distantly placed from the main vein (Fig. 1C); which is clearly different from the *D. championii*, where rhizomes and petiole are densely covered with dark brown lanceolate scales (Fig. 2: B); the sporophylls of *D. championii* were ovate-oblong, 1 pair of pinnae at the base of the sporophyll slightly shortened, and the upper 2-4 pairs of pinnae were extended in sequence, which is the widest part of the leaves, and the pinnae gradually shrinking upward (Fig. 2 A); the sori close to main vein (Fig. 2C). This provides a strong basis for the establishment of *D. laoshanensis* as a distinct species.

The study of the spore morphology and perispore ornamentations of pteridophytes by Zhang and Xi (1976) and Zhang (1979) is of great significance to the taxonomy of pteridophytes, not only as an important basis for finding their position in plant taxa, but also as an important voucher of the genetic relationship and phylogenetic evolutionary sequence between taxa and their relatives. Warre and Wagner (1974) proposed that the perispore ornamentations of pteridophytes can be used as an important basis for judging the evolutionary relationship of pteridophytes at the species and genus levels. In the same species, the mature spore morphology and perispore ornamentations were consistent, its characteristics were stable, and there were differences between different species. Jermy (1980) believed that the spore morphology of pteridophytes contribute to the discovery of some new species. For example, *Dryopteris guanchica* of *Dryopteris* was a new species discovered through the ornamentation of the perispore wall. With the development of science and technology, SEM is widely used in palynology, it is rapid, simple and accurate, and gives people a clear effect (Li *et al.* 2019). The submicroscopic structure of spore morphology of different pteridophytes taxa under SEM is of great significance in taxonomy, which provides a palynological basis for the establishment of new species. The spore perispore of *D. laoshanensis* was curved ridges protrusions, while the spore perispore of *D. championii* were tuberculiform and tuberculiform-rugulate protrusions. These were two completely different perispore ornamentations, which provides a palynological basis for the establishment of *D. laoshanensis*. According to the remarkable characteristics of the submicroscopic structure of the perispore, combined with other characteristics of the sporophyll, one cannot accept the treatment opinion of Flora of China and suggest restoring the status of *D. laoshanensis* J. X. Li et S. T. Ma as a valid name in taxonomy.

Table 1. Source of materials.

Species	Locality	Collection time	Voucher or typus
<i>D. laoshanensis</i>	Laoshan, qingdao	1982.09.16	J. X. Li-02013 (typus)
<i>D. championii</i>	Mengshan, linyi	2020.08.15	X. J. Li 002-6 (voucher)

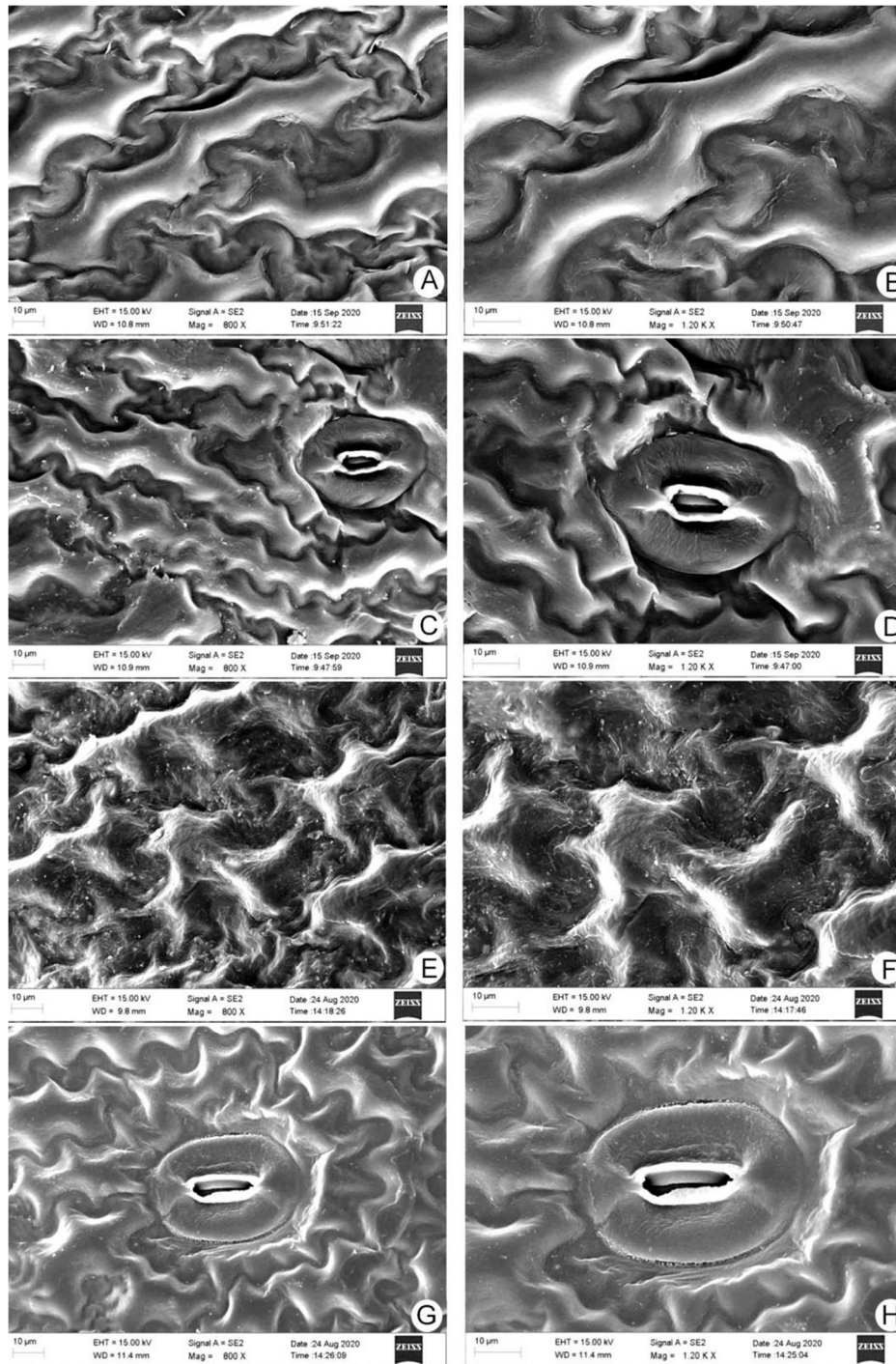


Fig. 3. Pinnule epidermis (SEM) A-D. *D. laoshanensis*; E-H. *D. championii*; A-B, E-F. Upper epidermis; C-D, G-H. Lower epidermis.

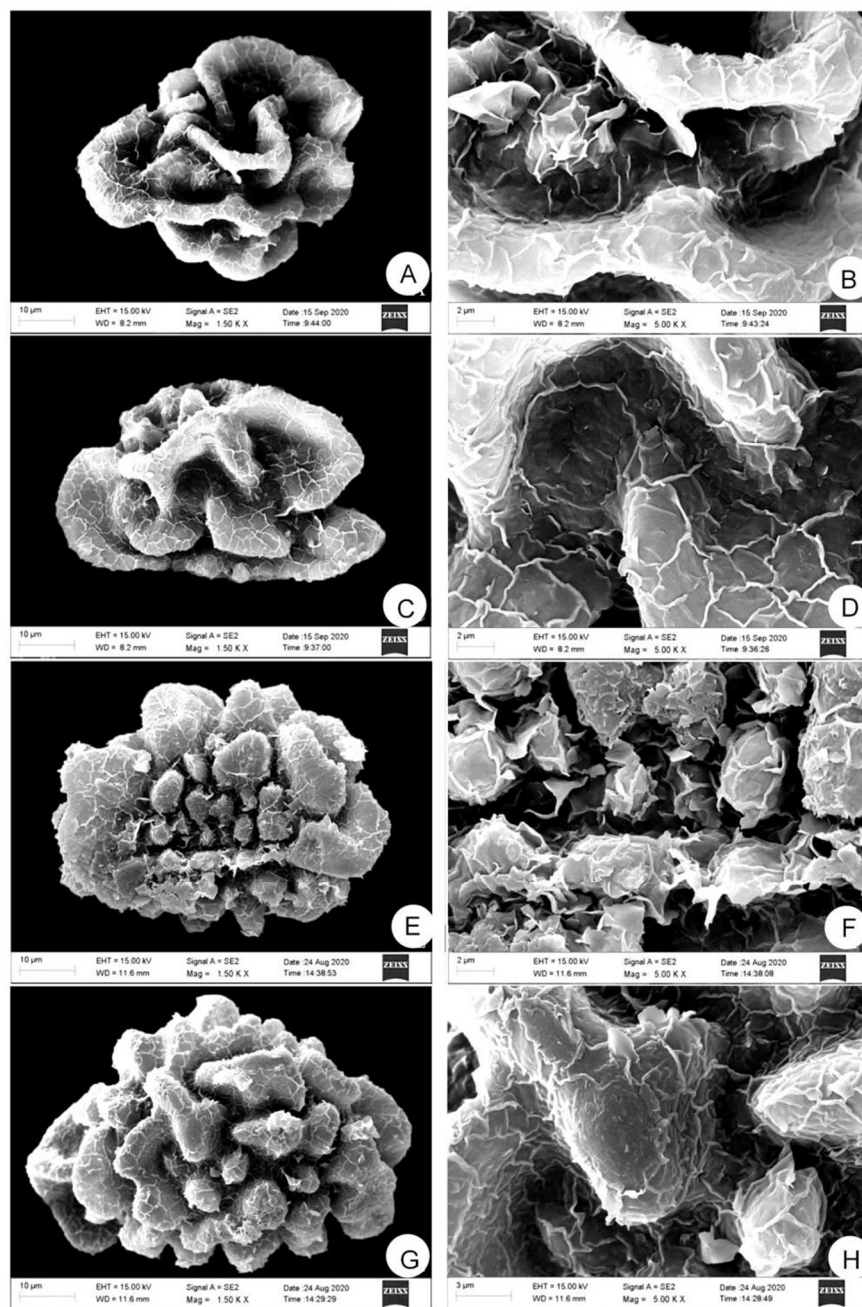


Fig. 4. Spore morphologies of two *Dryopteris* species (SEM). A. Spore in proximal view of *D. laoshanensis* (1500x); B. Detail of spore in proximal view of *D. laoshanensis* (5000x); C. Spore in distal view of *D. laoshanensis* (1500x); D. Detail of spore in distal view of *D. laoshanensis* (5000x); E. Spore in proximal view of *D. championii* (1500x); F. Detail of spore in proximal view of *D. championii* (5000x); G. Spore in distal view of *D. championii* (1500x) and H. Detail of spore in distal view of *D. championii* (5000x).

Table 2. Comparison of the main characteristics of *D. laoshanensis* and *D. championii*.

Species	Scales		Plant (Sporophylls)		Pinnae	
	Colour	Figure	Shape	Figure	Long and short	Figure
<i>D. laoshanensis</i> J. X. Li et S. T. Ma	Reddish brown	Fig. 1: B	Triangular- ovate	Fig. 1: A	1 pair of pinnae at the base of the sporophylls not shortened, and each pair of pinnae gradually shorten upward	Fig. 1: A
<i>D. championii</i> (Benth.) C. Chr..	Dark brown	Fig. 2: B	Ovate- oblong	Fig. 2: A	1 pair of pinnae at the base of the leaves slightly shortened, and the 2-4 pairs of pinnae were extended in sequence	Fig. 2: A

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Pinnule epidermis		Sori location			Spore	
Cell shape	Figure	Location	Figure	Ornamentation	Figure	
Cells long and straight, banded in shape; with regular thick teeth on both sides of the anticlinal wall	Fig.3: A-D	Located between the edge of the pinnae and the main vein , and away from the main vein	Fig. 1: C	Curved ridges protrusions	Fig.4:A-D	
Cells short and curved, polygonal in shape; with irregularly polygonal on both sides of the anticlinal wall	Fig.3: E-H	Closely located on both sides of the main vein of the pinna	Fig. 2: C	Tuberculiform and tuberculiform-rugulate protrusions	Fig.4: E-H	

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