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SEM STUDY OF LEAF EPIDERMIS AND SPORES OF NINE SPECIES OF *DRYOPTERIS* ADANS. FROM SHANDONG PROVINCE, CHINA

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Abstract

Scanning Electron Microscopy (SEM) structures of leaf epidermis and spores of 9 species of *Dryopteris* Adans. from Shandong Province of China were studied. These species are: *Dryopteris erythrosora* (Eaton) O. Ktze., *D. fuscipes* C. Chr., *D. tsoongii* Ching, *D. crassirhizoma* Nakai and *D. championii* (Benth.) C. Chr., *D. lijianxiuii* X. J. Li (new species), *D. shandongensis* J. X. Li & F. Li, *D. laoshanensis* J. X. Li & S. T. Ma and *D. parachinensis* Ching & F. Z. Li. The study showed that the perispore ornamentation and submicroscopic structural characteristics of peripheral wall of leaf epidermal cells were stable within each species and have significant differences between species. It does not only provide evidences of submicroscopic structural characteristics for its morphological classification, but also provides a palynological basis for the classification and identification of new pteridophyte resources of Shandong area. A new Identification Key to 9 species of *Dryopteris* Adans. is prepared using both morphological and SEM characters of spores and leaf epidermis. Therefore, the submicroscopic structural characteristics of palynology and the leaf epidermis of the genus were of great significance in taxonomy.

Introduction

Dryopteris Adans. is one of the largest genera of Dryopteriaceae, widely distributed by about 400 species. In both hemispheres, mainly in Asia, especially from the Himalaya to China, Japan, and Korea. The 167 species (60 endemic) in four subgenera in China (www.eFloras.org; FOC Vol. 2-3 Page 4, 5, 7, 541, 542, 571) were recorded.

The genus is known to be represented in Shandong by 12 species (Chen 1990). The relationship between the species of *Dryopteris* is complicated, and there are many complexes, the morphological characteristics of the sporophyte of some groups are very similar to those of related species, it is difficult to distinguish from the morphological characteristics of the sporophyte, and has been regarded as to classify difficult populations of Pteridophytes. In recent years, many scholars have carried out in-depth research on it from taxonomy, palynology, anatomy and molecular systematics, such as Li *et al.* (1983, 1985, 1988, 1996), Chen (1990) and Li (1985) conducted taxonomic research; Zhang *et al*. (1976), Zhang (1979), Liu *et al*. (1992), Li *et al.* (1997), Liu *et al*. (1997, 1999), Wang and Dai (2010), Lu *et al*. (2007) and Li *et al*. (2019) conducted palynological research of 50 species of the *Dryopteris*, accounting for only 1/3 of the genus; Zhou *et al*. (1985), Ding *et al.* (1990) and Guo *et al*. (1999) conducted anatomical studies on the rhizomes and petiole bases of 11 species of *Dryopteris* Adans. distributed in Shandong. Through field investigation and collection of specimens, the classical classification combined with SEM and submicroscopic structures of leaf epidermis and spores of the species of *Dryopteris* of Shandong, the studies provided scientific basis for the construction of the natural classification

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system of this genus, and were of great significance for species diversification and enrichment of new resources of Pteridophyte. Hence it was necessary to combine scanning electron microscopy with the submicroscopic structure of spores to solve some populations problems.

Materials and Methods

Samples of 9 species of *Dryopteris* were collected from different localities of Shandong area of China (Table 1, Figs 1 and 2), and the voucher specimens and type specimens of new species were examined and identified by consulting local experts (Li and Ma 1983, Li and Li 1988),. The specimens have been presered in the Herbarium of Shandong University of Traditional Chinese Medicine. The voucher specimens along with their collection localities are listed in Table 1. The species names were adopted from the *Flora of China*, and for new species from the *Bulletin of Botanical Research* (Li and Ma 1983) and *Acta Phytotaxonomica Sinica* (Li and Li 1988). The terms related to'perispore ornamentation' were adopted from Zhang and Xi (1976) and Wang and Dai (2010).

Scanning electron microscopy (SEM) was used to study the micromorphology of spores and leaf epidermis. Samples were dehydrated and were then placed on aluminium stubs using double-sided adhesive tape and sputter coated with gold in a Hitachi E-1010 Ion Sputter Coater, following Wen and Nowicke (1999). The materials were subsequently observed and photographed under a SUPRATM55 SEM. The magnification of the leaves were from high (×1200) to low (×800), the magnification of the spores were from high (×5000) to low (×1500).

Table 1. List of voucher (or typus) specimens.

Species	Locality	Voucher or typus specimen
Dryopteris erythrosora (Eaton) O. Ktze.	Mengshan	X. J. Li-0836
D. fuscipes C. Chr.	Mengshan	X. J. Li-0813
D. tsoongii Ching	Mengshan	X. J. Li-0865
D. shandongensis J. X. Li & F. Li	Mengshan	J. X. Li-108 (typus)
D. lijianxiuii X. J. Li	Weihai	J. X. Li-08123 (typus)
D. parachinensis Ching & F. Z. Li	Mengshan	J. X. Li-820561
D. championii (Benth.) C. Chr.	Mengshan	X. J. Li-0832
D. laoshanensis J. X. Li & S. T. Ma	Laoshan	J. X. Li-02013-1 (typus)
D. crassirhizoma Nakai	Weihai	J. X. Li-0106

Results and Discussion

The flat peripheral walls of the upper and lower epidermal cells of the leaves protruded outward, smooth and without texture, and the cells were inlaid with each other. The vertical walls were all wavy or deep wavy bends, forming typical lobed stagger, overlapping and inlaid with each other. The degree of cell wall curvature and cell size were obviously different among species of *Dryopteris*, the stomatal apparatus was only distributed on the lower epidermis, most of the stomatal apparatus was the actinocytic type, followed by the diacytic type. The upper and lower epidermal cells of the leaves were more consistent in shape and divided into three types: *Dryopteris erythrosora* and *D. laoshanensis* with long ribbon polygons; *D. tsoongii*, *D. parachinensis* and *D. lijianxiuii* with oblong polygons; *D. championii* and *D. shandongensis* with irregular polygons, only the upper and lower epidermal cells of *D. fuscipes* were inconsistent in shape, the upper epidermal cells were irregular polygons, and the lower epidermal cells were long ridged polygons (Table 2, Fig. 3).

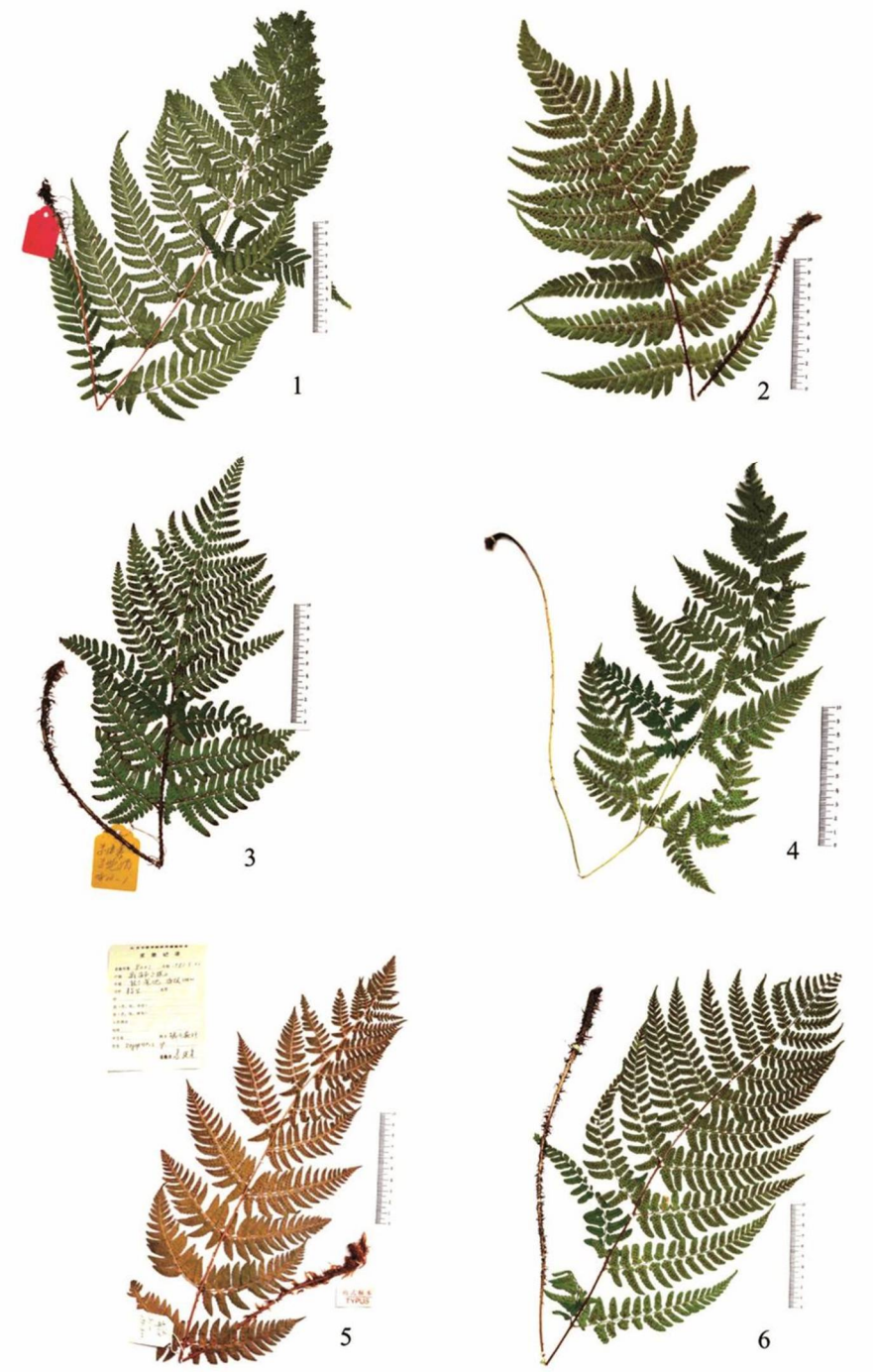

Fig. 1. New resources of *Dryopteris.* 1. *Dryopteris erythrosora*; 2. *D. fuscipes*; 3. *D. tsoongii*; 4. *D. parachinensis*; 5. *D. lijianxiuii*; 6. *D. championii.*

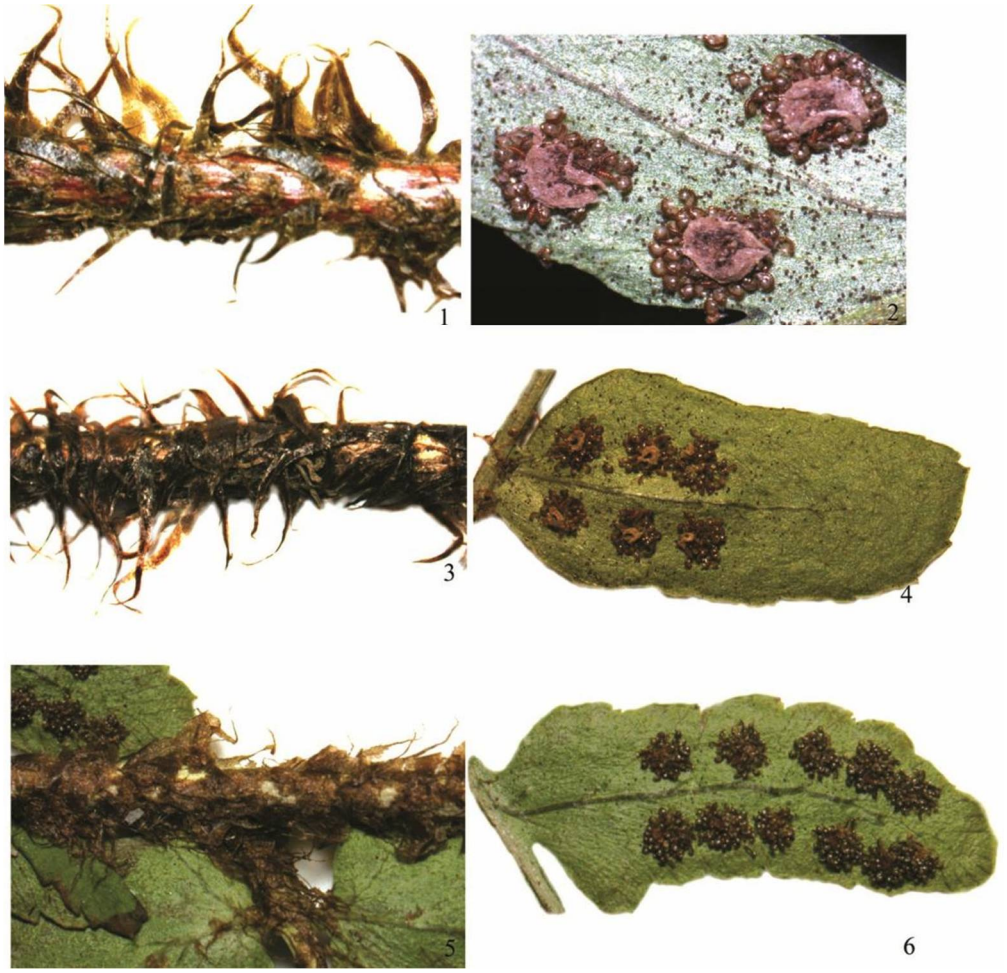

Fig. 2. Comparison petioles, rachis and pinnules of *Dryopteris***.** 1-2. *Dryopteris erythrosora*; 3-4. *D. fuscipes*; 5-6. *D. tsoongii*; 1,3. Petioles with lanceolate entire scales; 2, 4, 6. Pinnules; 5. Rachis.

The spores of *Dryopteris* were oblong in polar view, semicircular or super semicircular in equatorial view, single slit, symmetrical. *Dryopteris erythrosora*, *D. fuscipes*, *D. tsoongii*, *D. parachinensis*, *D. championii* and *D. laoshanensis* have larger spores, while the *D. shandongensis*, *D. lijianxiuii* and *D. crassirhizoma* have smaller spores. There were 5 types of perispore ornamentation: *Dryopteris erythrosora*, *D. parachinensis*, *D. tsoongii*, *D. fuscipes* and *D. championii* with tuberculate or tuberculate-massive protrusions, the perispore ornamentation of the first 3 species with tuberculate protrusions, small and dense, while the *D. championii* and *D. fuscipes* with tuberculate and tuberculate-massive protrusions, large and sparse, reflecting their close relationship; *D. laoshanensis* with curved long ridges protrusions; *D. shandongensis* and *D. crassirhizoma* have small spore, and with tuberculate and tuberculate-massive protrusions, with scale ornamentations of its surface of the above 8 species, and forming quasi-reticulate ornamentations; *D. lijianxiuii* with verrucate protrusions, and surfaces with melting ice and snow ornamentation. (Table 3, Fig. 4, Key to species).

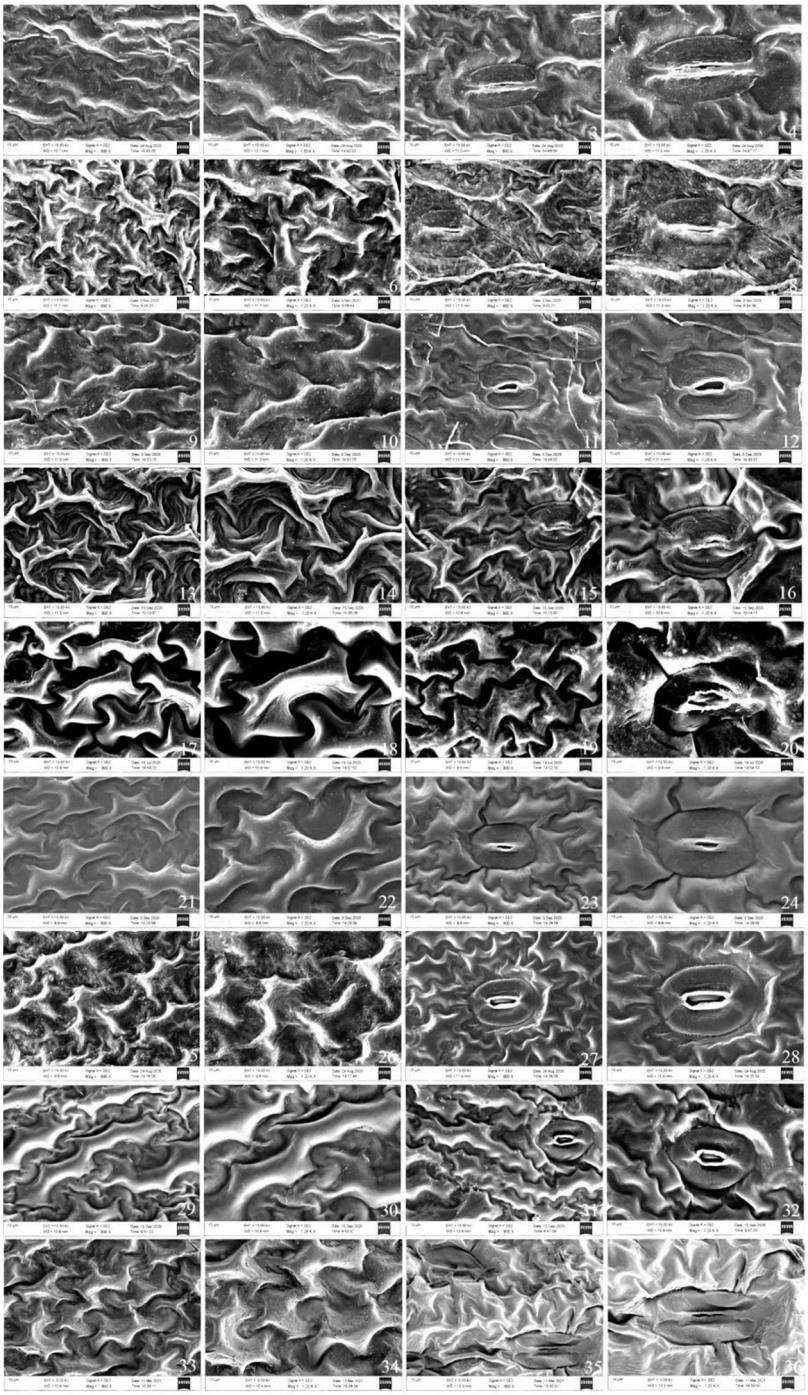

Fig. 3. Pinna epidermis**.** 1-4. *Dryopteris erythrosora*; 5-8. *D. fuscipes*; 9-12. *D. tsoongii*; 13-16. *D. shandongensis*; 17-20. *D. lijianxiui*; 21-24. *D. parachinensis*; 25-28. *D. championii*; 29-32. *D. laoshanensis*; 33-36. *D. crassirhizoma.*

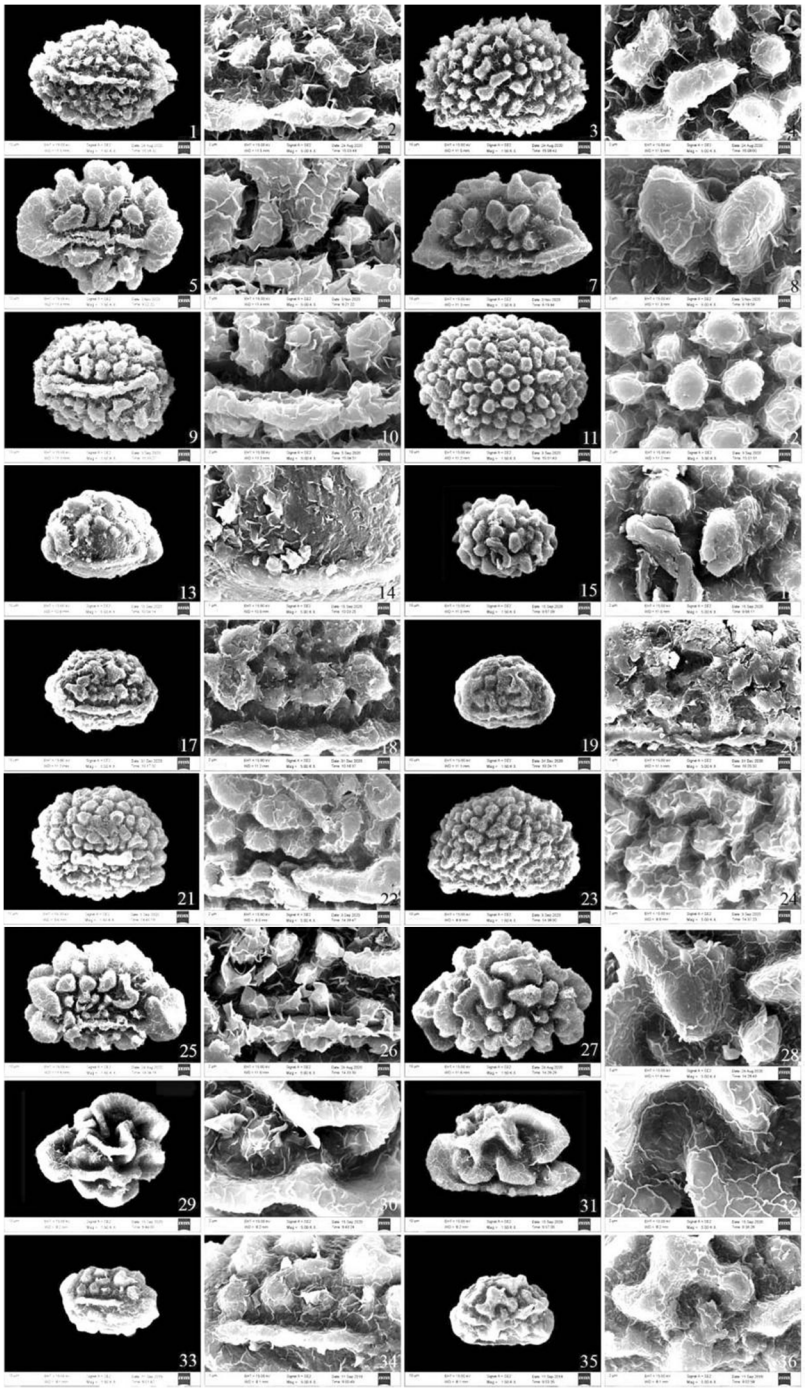

Fig. 4. Spore morphology of *Dryopteris***.** 1-4. *Dryopteris erythrosora*; 5-8. *D. fuscipes*; 9-12. *D. tsoongii*; 13-16. *D. shandongensis*; 17-20. *D. lijianxiuii*; 21-24. *D. parachinensis*; 25-28. *D. championii*; 29-32. *D. laoshanensis*; 33-36. *D. crassirhizoma.*

Table 2. Comparison of pinna in 9 species of *Dryopteris* Adans.

Species	Upper epidermal	Lower epidermal	Figure 3
Dryopteris erythrosora	Long ribbon polygon	Long ribbon polygon	1-4
D. fuscipes	Irregular polygon	Long ridge polygon	5-8
D. tsoongii	Oblong polygon	Oblong polygon	9-12
D. shandongensis	Irregular polygon	Irregular polygon	13-16
D. lijianxiuii	Oblong polygon	Oblong polygon	17-20
D. parachinensis	Oblong polygon	Oblong polygon	21-24
D. championii	Irregular polygon	Irregular polygon	25-28
D. laoshanensis	Long ribbon polygon	Long ribbon polygon	29-32
D. crassirhizoma	Oblong polygon	Broad ribbon polygon	33-36

Table 3. Spore morphology and perispore ornamentation.

Species	Ornamentation under SEM	Polar view	Equatorial view	Figure 4
Dryopteris erythrosora	Tuberculate	Oblong	Oblong	1-4
D. fuscipes	Tuberculate and tuberculate-massive	Circular	Super semicircular	5-8
D. tsoongii	Tuberculate	Circular	Circular	9-12
D. shandongensis	Tuberculate and tuberculate-massive	Circular	Circular	13-16
D. lijianxiuii	rucate, surface with melting ice and snow	Circular	Super semicircular	17-20
D. parachinensis	Tuberculate	Circular	Super semicircular	21-24
D. championii	Tuberculate and tuberculate-massive	Circular	Circular	25-28
D. laoshanensis	Curved long ridges	Circular	Super semicircular	29-32
D. crassirhizoma	Tuberculate and tuberculate-massive	Oblong	Super semicircular	33-36

Key to species of *Dryopteris* from Shandong

1a. Pinna rachis and rachiole with lanceolate small scales.

2a. Frond oblong, oblong-lanceolate, ovate-lanceolate or oblanceolate; bipinnate or tripinnate-pinnatifid.

3a. Sori were only born under the pinna above the middle part of the frond, or under the pinna and upper pinnule of the 1-3 pairs of pinna at the base of the frond.

4a. Sori were only born under the pinna above the middle part of the frond.

5a. Frond oblanceolate, and the number of pinnaes at the base gradually narrowed, about 1/2 of the length of the central pinna *D. crassirhizoma*

5b. Frond oblong or oblong lanceolate, base pinna not narrowed.

6a. Sori were born under the pinna above the middle part of the frond, about 1/2 of the frond, and pinnaes not narrowed *D. peninsulae*

6b. Sori were born under the pinna near the top of the frond, about 1/3 of the frond, and pinnaes sharply narrowed; perispore ornamentation with tuberculate protrusions, with scales between protrusions *D. lacera*

4b. Sori were only born in the 1-3 pairs of pinna at the base of the frond, and below the upper pinnule and the above pairs of pinna; perispore ornamentation with verrucate protrusions, and surfaces with melting ice and snow ornamentation *D. lijianxiuii*

3b. Sori were all over the under side of the frond.

7a. Terminal pinnule do not split; both surfaces of frond with glandular hair *D. woodsiisora*

7b. Terminal pinnule pinnatipartite; both surfaces of frond without glandular hair.

8a. Pinnule slightly narrowed on the under side of the base of a pair of pinna in the lower part of the frond; perispore ornamentation with curved short ridges and verrucate protrusions *D. goeringiana*

8b. Pinnule slightly stretched on the under side of the base of a pair of pinna in the lower part of the frond; perispore ornamentation with tuberculate and tuberculate-massive protrusions *D. shandongensis*

2b. Frond pentagonal or ovate pentagonal; tripinnate or quadripinnate-pinnatifid.

9a. Petiole thin, upward; rachis with brown lanceolate scales *D. chinensis*

9b. Petiole thick, upward; rachis nearly glabrous *D. gymnophylla*

1b. Vesicular small scales under the pinna rachis (that is, the base spherical and the upper long-diamond shaped).

10a. Rhizomes, petioles and rachides densely covered with reddish brown or brown scales.

11a. Frond triangular ovate, ovate-lanceolate or oblong-lanceolate; a pair of pinnaes on the lower part of the frond not narrowed or slightly narrowed.

12a. Rhizomes, petioles and rachides densely covered with reddish brown or brown scales; a pair of pinnaes on the lower part of the frond not narrowed.

13a. Rhizomes, petioles and rachides densely covered with reddish brown scales; frond triangular- ovate; sori were located away from the main vein; perispore ornamentation with curved long ridges protrusions *D. laoshanensis*

13b. Rhizomes, petioles and rachides densely covered with brown scales; frond ovate-oblong or oblong-lanceolate.

14a. Rhizomes, petioles and rachides densely covered with brown, narrowly lanceolate, entire scales; frond ovate-oblong or oblong-lanceolate; sori near the main vein; perispore ornamentation with tuberculate or tuberculate-massive protrusions.

15a. Sori red in central; perispore ornamentation with tuberculate protrusions *D. erythrosora*

15b. Sori brown; perispore ornamentation with tuberculate and tuberculate-massive protrusions *D. fuscipes*

14b. Petioles and rachides densely covered with toothed-ovate scales; frond ovate; sori near frond margin; perispore ornamentation with tuberculate protrusions *D. tsoongii*

12b. Rhizomes, petioles and rachides densely covered with brown scales; a pair of pinnaes on the lower part of the frond slightly narrowed; ovate-lanceolate; sori near the main vein; perispore ornamentation with tuberculate and tuberculate-massive protrusions *D. championii*

11b. Frond pentagonal or ovate-pentagonal; tripinnate-pinnatifid; the lower part

of the frond with the largest pair of pinnaes; rhizomes, petioles and rachides sparsely clothed with brown scales *D. parachinensis*

10b. Rhizomes, petioles and rachides densely covered with dark brown scales

16a. Frond tripinnate; the base of the petioles densely covered with bicolor scales (usually brown at the base and edges, black in the center and upper part of the scales).

17a. Rachis and rachiole sparsely clothed with vesicular small scales *D. sacrosancta*

17b. Rachis and rachiole densely covered with vesicular small scales *D. setosa*

16b. Frond bipinnate; terminal pinnule do not split (except the base pinna) *D. immixta*

The study of the spore morphology and perispore ornamentation of pteridophytes by Zhang (1979), Zhang and Xi (1976) 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. In the same species, the mature spore morphology and perispore ornamentation were consistent, its characteristics were stable, and there were differences between different species, which can be used as an important characteristics to distinguish different taxa (Li *et al*. 1997). Jermy believed that the spore morphology of pteridophytes contributes to the discovery of some new species. For example, *Dryopteris guanchica* of *Dryopteris* was a new species discovered through the perispore ornamentation (Jermy 1980). 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). Scanning electron microscopy observed the spore morphology and perispore ornamentation of the *Dryopteris*, its characteristics were stable within species and have significant differences between species. This provides basis for the morphological classification and related species of the *Dryopteris*, such as the *D. laoshanensis* with curved long ridges protrusions (Fig. 4: 29-32), while the *D. championii* with tuberculate or tuberculate-massive protrusions (Fig. 4: 25-28), this was two completely different spore perispore ornamentation characteristics (Li *et al*. 2019); the *D. lijianxiuii* with verrucate protrusions, and surfaces with melting ice and snow ornamentation (Fig. 4: 17-20), this obviously different from the *D. lacera, the D. lacera* has tuberculate protrusions, with scales between protrusions, scales forming quasi-reticulate ornamentations. Therefore, the spore morphology and its perispore ornamentation provide an important palynological basis for the classification and identification of species and related species (Li *et al*. 1997).

The upper and lower epidermal cells of the leaves of pteridophyte belong to the protective tissue of plants, it was formed in a long time to adapt and evolve around the surrounding natural conditions. The flat peripheral walls of the upper epidermal cells of the leaves protruded outward, and the cells were inlaid with each other, with different degrees of curvature of the vertical wall formed a long banded polygons, such as the *Dryopteris erythrosora* and *D. laoshanensis*; formed oblong polygons, such as the *D. tsoongii*, *D. parachinensis* and *D. lijianxiuii*; formed irregular polygons, such as the *D. championii* and *D. shandongensis*. Only the upper and lower epidermal cells of *D. fuscipes* were inconsistent in shape, the upper epidermal cells were irregular polygons, and the lower epidermal cells were long ridged polygons. These characteristics were stable within species, and there were significant differences between species, therefore, the differences morphology of the upper epidermal cells of the leaves under the scanning electron microscope can be used as the basis for identifying the submicroscopic characteristics of species and related species (Guo *et al*. 1999).

Acknowledgments

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