

PALYNOLOGY AND ANATOMY OF *DRYOPTERIS* ADANS. FROM SHANDONG, CHINA AND THEIR SIGNIFICANCE IN CLASSIFICATION

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Abstract

The palynology, rhizome and petiole-base anatomy of 12 species of *Dryopteris* Adans. from the Shandong Province of China were investigated using Scanning Electron Microscopy (SEM). The results showed that the spores of *Dryopteris* Adans. were reniform, bilaterally symmetrical, and possessed a perispore. The morphology was stable at the genus level. The perispore ornamentation was stable within species, with clear distinctions between species. Anatomical study of the rhizome and petiole base revealed that in the surrounding basic tissue of the reticular vascular bundle, there were many intercellular spaces, with each space containing intercellular space glandular hairs (ISGHs). The glandular hairs were found to be two types: Spheroidal-shaped and rod-shaped. Spheroidal-shaped glandular hairs of the head was present in six species of the subgenus *Dryopteris*, while the long rod-shaped glandular hairs of the head was present in five species of the subgenus *Erythrovariae*. This study provided new data as well as palynological basis for the classification and identification of related species. The regular distribution of ISGHs provided an anatomical basis for the classical taxonomy of the subgenera *Dryopteris* and *Erythrovariae* for the first time, hence this study is of great significance in taxonomy.

Introduction

Dryopteris Adans. is a large world-wide genus of the family Dryopteraceae and known as wood fern. About 230 species were known to be widely distributed in both hemispheres, mainly in Asia, especially from the Himalaya to China, Japan and Korea (China Flora Editorial Board 2000). It is one of the large genus of the Pteridophyte in China which is represented by 127 species and distributed in 3 sub-genera: *Pycnopteris*, *Dryopteris* and *Erythrovariae* (China Flora Editorial Board 2000). In the *Flora of China* 5(1) (China Flora Editorial Board 2000), no species of Subgenus *Pycnopteris* was recorded from Shandong while six species of subgenus *Dryopteris* and five species of subgenus *Erythrovariae* were recorded from Shandong. There were many complexes in the species of *Dryopteris*, and the morphological characteristics of sporophytes in some groups were very similar to those of related species and appeared to be difficult to distinguish based on morphological characters of sporophytes.

In recent years, many researches on taxonomy, palynology, anatomy, cytology and molecular systematics of Pteridophytes including *Dryopteris* Adans. of China have been made by a number of workers, such as, Li *et al.* (1983, 1985, 1988, 1990, 1996, 1997), Li (1985), Zhang *et al.* (1976), Zhang (1979), Liu *et al.* (1992, 1997), Liu and Zhao (1999), Wang and Dai (2010), Lu *et al.* (2007), Zhou *et al.* (1985), Ding *et al.* (1990) and Guo (1999). It had been reported that there were intercellular space glandular hairs (ISGHs) in these species, and the two types of ISGHs were regularly distributed in the subgenus *Dryopteris* and the subgenus *Erythrovariae*, and its taxonomic significance has not yet attracted the attention of scholars (Zhou *et al.* 1985, Ding *et al.* 1990).

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The submicroscopic structural characteristics of spore morphology provided a palynological basis for the classification and identification of the species from Shandong and their related species of the *Dryopteris* Adans. (Li and Ma 1983, Li 1985, Li and Li 1988). Two intercellular space glandular hairs (ISGHs) not only provided the anatomical basis for the classification of subgenus *Dryopteris* and the subgenus *Erythrovariae* for the first time, but also provided significant basis for construction of a natural classification with the species of this genus.

Materials and Methods

Samples of *Dryopteris* were collected from different localities of Shandong area of China (Li 1990). A total of 12 species of *Dryopteris* Adans. were collected and voucher specimens and type specimens of new species were identified by consulting expert, Prof. Jianxiu Li of the Shandong University of Traditional Chinese Medicine, and all specimens were preserved in the Medicinal Herbaria of Shandong University of Traditional Chinese Medicine and Shandong Forestry School. The voucher specimens along with their distribution are listed in Table 1. The species names were taken from the published new species model specimens of Bulletin of Botanical Research (Li and Ma 1983, Li 1985) and Acta Phytotaxonomica Sinica (Li and Li 1988). The terms related to 'perispore ornamentation' presented by Wang *et al.* (Wang and Dai 2010) were adopted and the terms 'ISGHs' presented by Zhou *et al.* (Zhou *et al.* 1985) and Ding *et al.* (Ding *et al.* 1990) were adopted.

Table 1.List of voucher specimens.

Taxon	Specimen	Locality
<i>Dryopterispeninsulae</i> Kitag.	J. X. Li-0836	Linyi (Mengshan)
<i>D. lacera</i> (Thunb.) O. Ktze.	J. X. Li-0813	Linyi (Mengshan)
<i>D. woodsii</i> sora Hayata	J. X. Li-0865	Taian (Taishan)
<i>D. goeringiana</i> (Kunze) Koidz.	J. X. Li-0832	Taian (Taishan)
<i>D. shandongensis</i> J. X. Li et F. Li	J. X. Li-108	Linyi (Mengshan)
<i>D. gymnophylla</i> (Bak.) C. Chr.	J. X. Li-08123	Qingdao (Laoshan)
<i>D. chinensis</i> (Bak.) Koidz.	J. X. Li-0846	Linyi (Mengshan)
<i>D. laoshanensis</i> J. X. Li et S. T. Ma	J. X. Li-02013-1	Qingdao (Laoshan)
<i>D. parachinensis</i> Ching et F. Z. Li	F. Z. Li-820561	Weihai (Shidao)
<i>D. sacrosancta</i> Koidz.	J. X. Li-08-11	Linyi (Tashan)
<i>D. bissetiana</i> (Baker) C. Chr.	J. X. Li-08-26	Yantai (Kunyushan)
<i>D. immixta</i> Ching	J. X. Li-08-19	Linyi (Mengshan)

The well-developed and fully-matured spores of *Dryopteris* were collected and placed at a dust proof site for air drying over 96 hrs. The dried spores were then examined under a dissection microscope. Meanwhile, the average size of the spores was measured from ten randomly chosen evenly spread on a wood-free printing paper on a specimen holder. After spraying gold particles for 30 s, the spores were placed under SUPRATM55 thermal field emission Scanning Electron Microscope (SEM) to observe the ornamentations. Typical and representative spores were selected and observed first at magnification of 5000× and then of 1500×. When the voltage was stable, focal length was adjusted to collect the pictures.

The fresh rhizomes and petiole bases of 11 species of *Dryopteris* were collected and were fixed by FAA fixative for permanent paraffin sections, voucher sections were preserved in the Medicinal Herbarium of Shandong University of Traditional Chinese Medicine. The sections were observed under Optical Microscope, first at magnification of 800 \times and then of 400 \times , and the intercellular space glandular hairs (ISGHs) were gradually drew, and all observed characters are presented in Fig. 1.

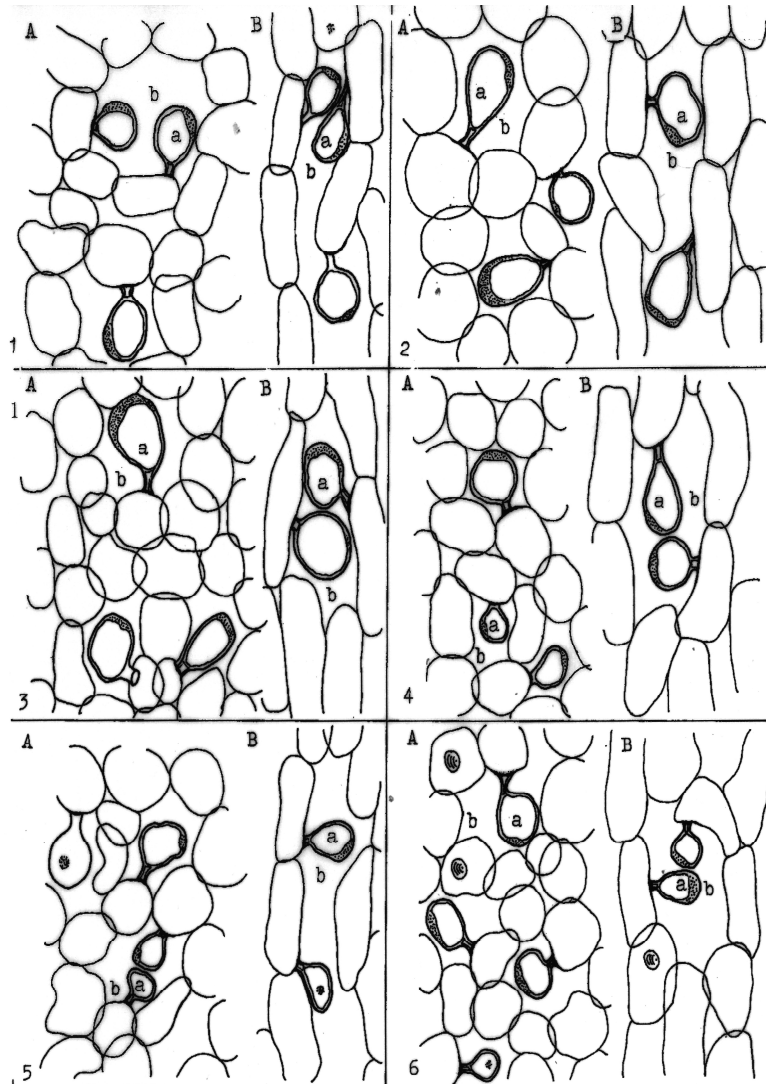


Fig. 1. The ISGHs of rhizome and petiole base of *Dryopteris*. A: Transverse section; B: Longitudinal section; a: Intercellular space glandular hairs (ISGHs); b: Intercellular space (IS); 1: *Dryopteris crassirhizoma*, 2: *D. woodsii-sora*, 3: *D. peninsulae*, 4: *D. shandongensis*, 5: *D. gymnophylla* and 6: *D. goeringiana*. (Contd.)

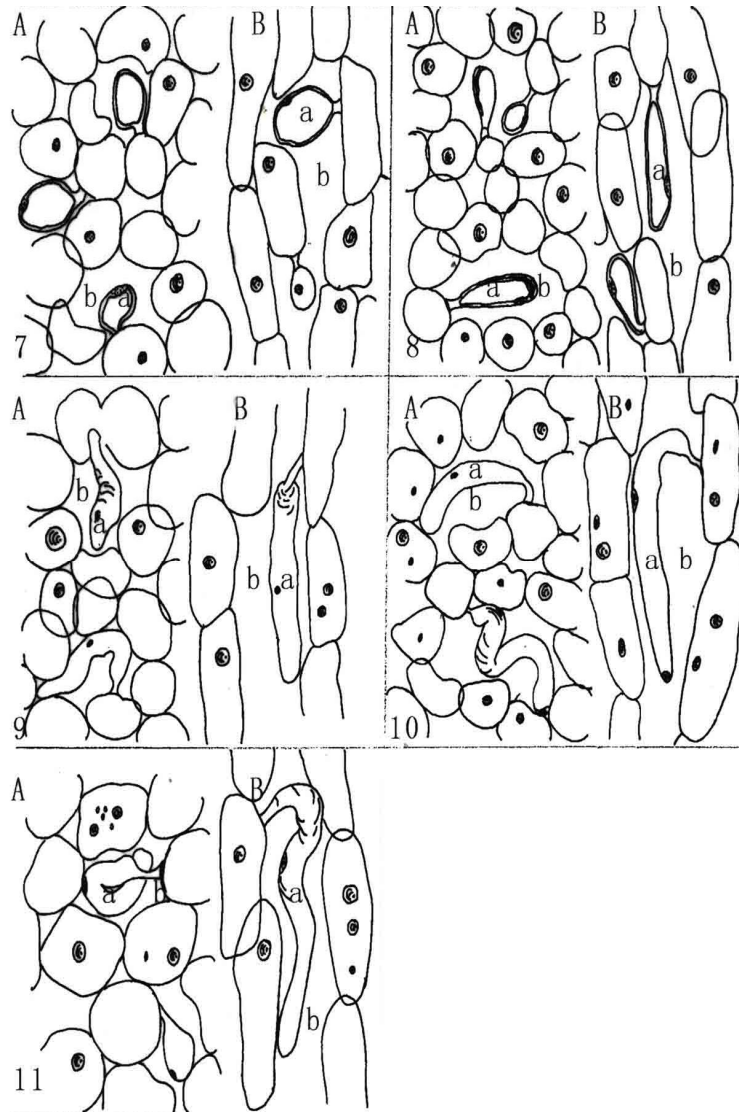


Fig. 1. The ISGHs of rhizome and petiole base of *Dryopteris* (Cont.). A: Transverse section; B: Longitudinal section; a: Intercellular space glandular hairs (ISGHs), b: Intercellular space (IS), 7: *D. lacera*, 8: *D. laoshanensis*, 9: *D. sacrosancta*, 10: *D. immixta* and 11: *D. bissetiana*.

Results and Discussion

The spores of *Dryopteris* were reniform, bilaterally symmetrical. The polar view was elliptical and the equatorial view was reniform. Single crack, the crack length was about $2/3$ of the spore pole axis and possessed a perispore. The types of perispore ornamentation were tuberculate, verrucate, verrucate-rugulate and costate. The spore morphology was stable at the genus level. Table 2 and Figs 2 - 3 revealed that the characteristics of perispore ornamentations of the *Dryopteris* distributed in Shandong Province was stable within species, with clear distinctions between species.

Table 2. Spore morphology of 12 species of *Dryopteris* of Shandong under SEM (5000× and 1500×).

Species	Size/μm	Ornamentation under SEM	Polar view	Equatorial view	Fig.2 (1-20) Fig.3 (1-22)
<i>Dryopteris peninsulae</i> Kitag.	27.0 × 41.6	Verrucate-rugulate	Round	Oval	1 - 4
<i>D. lacera</i> (Thunb.) O. Ktze.	27.1 × 41.6	Tuberculate-rugulate	Round	Semicircular	5 - 8
<i>D. woodsiiisora</i> Hayata	47.0 × 67.0	Spinate	Oval	Semicircular	9 - 12
<i>D. goeringiana</i> (Kunze) Koidz.	26.1 × 60.8	Spinate, tuberculate	Round	Round	13 - 14
<i>D. gymnophylla</i> (Bak.) C. Chr.	27.6 × 39.9	Verrucate, Verrucate-rugulate	Oval	Super semicircular	15 - 16
<i>D. shandongensis</i> J. X. Li et F. Li	27.0 × 45.0	Verrucate, scaly	Oval	Super semicircular	17 - 20
<i>D. chinensis</i> (Bak.) Koidz.	45.0 × 63.0	Verrucate	Round	Super semicircular	1 - 4
<i>D. laoshanensis</i> J. X. Li et S. T. Ma	32.6 × 51.8	Curved-costate	Oval	Oval	5 - 8
<i>D. parachinensis</i> Ching et F. Z. Li	40.5 × 61.4	Rugulate	Round	Super semicircular	9 - 12
<i>D. sacrosancta</i> Koidz.	40.5 × 60.8	Rugulate	Round	Round	13 - 16
<i>D. bissetiana</i> (Baker) C. Chr.	42.2 × 62.1	Curved-spinate, rugulate	Round	Round	17 - 20
<i>D. immixta</i> Ching	40.0 × 56.3	Verrucate-rugulate	Round	Super semicircular	21 - 22

Anatomic observation of the rhizome and petiole base (Fig. 1) revealed that in the surrounding basic tissue of the reticular vascular bundle, there were many intercellular spaces, with each space containing intercellular space glandular hairs (ISGHs). The ISGHs had single cell short handles and the gland heads were divided into two types: One a spheroidal shape and the other a long rod shape. However, there was only one type of gland head in the same plant, its characteristics were stable. Spheroidal shaped glandular hair of the head was present in 6 species of the subgenus *Dryopteris* (Wang and Dai 2010), including the *D. crassirhizoma* Nakai (was not distributed in Shandong, as a comparison), *D. woodsiiisora* Hayata, *D. peninsulae* Kitag., *D. shandongensis* J. X. Li et F. Li, *D. gymnophylla* (Bak.) C. Chr. and *D. goeringiana* (Kunze) Koidz., while the long rod-shaped glandular hair of the head was present in 5 species of the subgenus *Erythrovariae* (Wang and Dai 2010), including the *D. lacera* (Thunb.) O. Ktze., *D. laoshanensis* J. X. Li et S. T. Ma, *D. sacrosancta* Koidz., *D. immixta* Ching and *D. bissetiana* (Baker) C. Chr. The regular distribution of ISGHs provided an anatomical basis for the classical taxonomy of the subgenera *Dryopteris* and *Erythrovariae* for the first time (Fig. 1).

The spore morphology of Pteridophyte was of great significance in taxonomic and phylogenetic studies. The outer wall of pollen and types of perispore ornamentation have great differences among different groups and can be used as important features to identify different groups. Foreign fern botanists have applied scanning electron microscopy (SEM) to the study of pollen in Pteridophyte (Lu *et al.* 2007). The palynology of 12 species of *Dryopteris* from the Shandong Province were investigated using scanning electron microscopy (SEM), and referred to previous researches (Liu *et al.* 1992, 1997, Wang and Dai 2010, Lu *et al.* 2007), the results showed that the spores of *Dryopteris* were reniform, bilaterally symmetrical, single crack, the polar view was elliptical and the equatorial view was reniform. The types of perispore ornamentation were

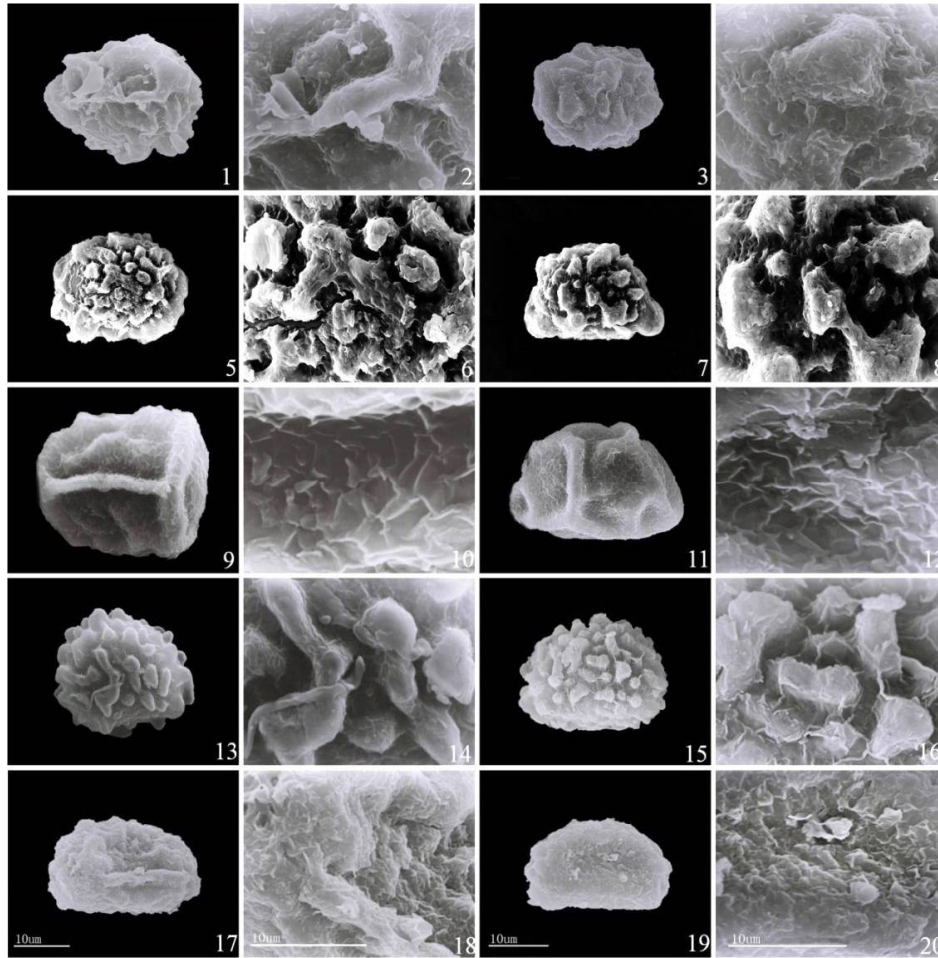


Fig. 2 (1-20). Spore morphology of 6 species of *Dryopteris* (SEM). (1-4:*D. peninsulae*; 5-8: *D. lacera*; 9-12: *D. woodsii-sora*; 13-14: *D. goeringiana*; 15-16: *D. gymnophylla*; 17-20: *D. shandongensis*; 1,5,9,13,17: polar view; 3,7,11,15,19: equatorial view; 2,4,6,8,10,12,14,16,18,20 : local view)

tuberculate, verrucate, verrucate-rugulate and costate. The perispore ornamentation was stable within species, with clear distinctions between species. Therefore, the submicroscopic structure characteristics of the spores of the *Dryopteris* were significant in taxonomy. Combined with the classical taxonomy, the submicroscopic structure characteristics of the perispore was an important basis for the identification of related species (Li and Ding 1988, Li *et al.* 1988, 1989, 2016). The perispore of *D. laoshanensis* has a prominent curved-costate ornamentation (Li and Ma 1983) (Figs 3, 5-8), while the perispore of *D. championii* has a verrucate-rugulate ornamentation (Wang and Dai 2010). These were two distinct submicroscopic structure features of the perispore, *D. laoshanensis* was incorporated in *D. championii* in the Flora of China5(1), using the name of *D. championii* (Benth.) C. Chr. (Wang and Dai 2010). 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 China5(1) and suggest restoring the status of *D. laoshanensis* J. X. Li et S. T. Ma in taxonomy.

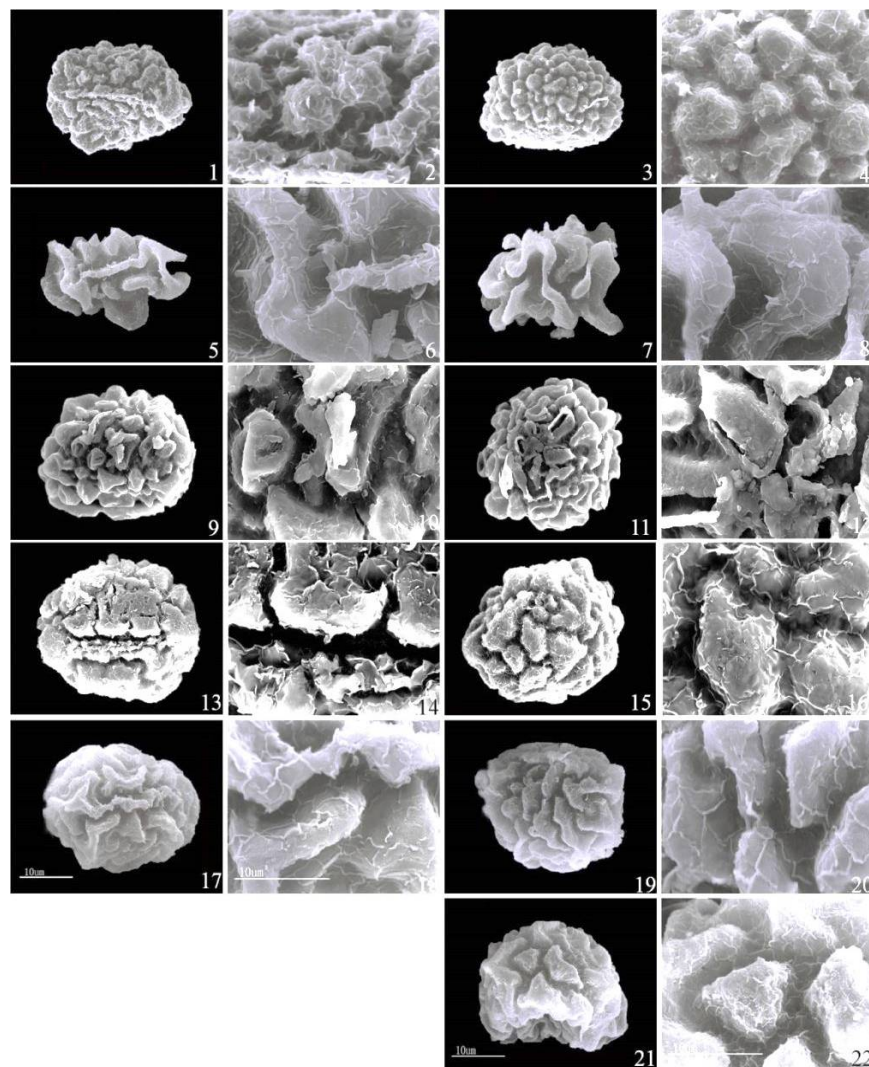


Fig. 3 (1-22). Spore morphology of 6 species of *Dryopteris*(SEM). (1-4: *D. chinensis*; 5-8: *D. laoshanensis*; 9-12: *D. parachinensis*; 13-16: *D. sacrosancta*; 17-20: *D. bissetiana*; 21-22: *D. immixta*; 1,5,9,13,17: polar view; 3,7,11,15,19,21: equatorial view; 2,4,6,8,10,12,14,16,18,20,22:local view).

The rhizome anatomy and petiole base of 11 species of *Dryopteris* from the Shandong Province were investigated, including the *D. crassirhizoma* Nakai (not distributed in Shandong, taken only for comparison). There were many intercellular spaces glandular hairs (ISGHs), there are two types of the ISGHs, it was first discovered that it was regularly and stably distributed in the subgenera *Dryopteris* and *Erythrovariae*, spheroidal shaped glandular hair of the head was present in 6 species of the subgenus *Dryopteris*, while the long rod-shaped glandular hair of the head was present in 5 species of the subgenus *Erythrovariae*. The ISGHs provided an anatomical basis for the morphological classification of the two subgenera for the first time (Zhou *et al.* 1985, Ding *et al.* 1990).

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