Study on the spore morphology of *Ceratopteris* Brongn.

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Abstract. Based on the significance of palynology in plant taxonomic identification, the purpose of this paper is to provide palynology basis for taxonomy, interspecific identification and resource protection of *Ceratopteris* Brongn. plant system. The spores of *C. thalictroides* and *C. pteridoides* of domestic *Ceratopteris* Brongn. were observed and compared by scanning electron microscope. The two types of spores of *Ceratopteris* Brongn. are radially symmetrical, tetrahedral, trilete suture, spherosome in polar view and equatorial view, and have common morphological characteristics such as perispore and ridge-like bulge of perispore, but there are obvious differences such as density of ridge-like bulge and ornamentation among bulges between two types of spores. The spore morphology is of great significance in the interspecific taxonomy of *Ceratopteris* Brongn.. It does not only provide the palynology scientific basis for the taxonomy and identification of *Ceratopteris* Brongn., but also provides a series of feasible measures for the exploitation and the resource conservation of *Ceratopteris* Brongn..

1. Introduction
Parkeriaceae *Ceratopteris* Brongn. is a year of aquatic summer green herbs, there are about 6-7 species in the world, distributed in the subtropical and tropical regions. There are two kinds in China including *C. thalictroides* and *C. pteridoides*, which are widely distributed in the waters of the provinces south of the Yangtze River, while in the north, only distributed in Weishan Lake in Shandong Province and Nanyang Lake, belonging to rare and endangered plants [1].

Some scholars have studied it from the aspects of resources and plant taxonomy [2-7], palynology [8-9], genetics [10] and endangered plants [11]. On the basis of previous studies, we observed the spores of two species of *Ceratopteris* Brongn. by scanning electron microscope, with purpose to providing the palynology basis for phylogeny, interspecific taxonomy and resource conservation of *Ceratopteris* Brongn..

2. Material and methods
2.1. Test Materials
The test materials are all derived from the voucher specimen. The voucher specimens of *C. thalictroides* and *C. pteridoides* are shown in Table 1. The voucher specimen was identified by Li Jianxiu of Shandong University of Traditional Chinese Medicine and collected in Traditional Chinese Medicine Laboratory of Shandong Xiandai University.
TABLE 1. Voucher specimen of Ceratopteris thalictroides and Ceratopteris pteridoides

| Species name               | Collector and specimen number | Collecting location | Collecting time |
|----------------------------|-------------------------------|---------------------|-----------------|
| Ceratopteris thalictroides | Hou Yuantong 201608           | Nanyang Lake        | August 2016     |
| Ceratopteris pteridoides   | Li Jianxiu 198508             | Weishan Lake        | August 1985     |

2.2. Test Methods
Well-developed and mature spores of voucher specimen of C. thalictroides and C. pteridoides were taken to be distributed evenly on double sided tape paper of specimen holder, after SC7620 metal spraying for coating for 30 s, placed under the scanning electron microscope of the hot field emission of ZEISS SUPRATM55, observe the spores in equatorial view and polar view, 10 typical and representative spores were selected for each, the magnification was from high to low, the voltage was stable at 15KV, the focal length was adjusted, take a picture and make a plate.

3. Results
3.1. Ceratopteris thalictroides (L.) Brongn.
Micromorphological characteristics of spores under scanning electron microscope are shown in Fig. 1.

![Figure 1](image_url)
3.2. *Ceratopteris pteridoides* (Hook.) Hieron.
The spores are radially symmetrical, tetrahedral, trilette suture, and the length of sutures is about 1/2 of the spore radius. Spherosome in proximal face view, oval in equatorial view. The perispore has a ridge-like bulge, and the gap between the two bulges is equivalent to 1/3 of the width of ridge. The ridge-like bulge in proximal face view is three parallel tangents along the spheroidal contour, and intersects into triangles in the middle; the ridge-like striations in equatorial view are nearly parallel to the diameter, slightly curved with a few branches. The gap between the bulges is small and the pattern structure is not obvious (Fig. 2).

![Figure 2](image_url)

**Figure 2.** Micromorphological characteristics of spores of *Ceratopteris pteridoides* under scanning electron microscope. 1). Polar View (×800); 2). Local magnification of polar view (×1500); 3). Equatorial view (×800); 4). Local magnification of equatorial view (×1500).

4. Discussion
The study of the spore morphology and sporoderm pattern of pteridophytes by Zhang Jintan [12] and Zhang Yulong [13] 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, Wagner [14] proposed that the outer sporoderm pattern of pteridophytes can be used as an important basis for judging the evolutionary relationship of pteridophytes at the species and genus levels. The trilette suture is evolutionary by comparing with single suture, the parallel ridge is evolutionary by comparing with non-ridge, the thick sporoderm is evolutionary by comparing with thin sporoderm pattern, and the sporoderm coarse pattern is evolutionary by comparing with fine pattern. Li et al. [15-18] studied the spore of pteridophytes of different taxa under scanning electron microscope, and the submicroscopic structure of spore morphology is of great significance in taxonomy.
Some scholars have different understandings on the sporoderm of Ceratopteris Brongn.. In the description of the spores of Ceratopteris thalictroides in Flora Reipublicae Popularis Sinicae [19], it was considered that the spores of Ceratopteris thalictroides had no perispores and the sporoderm were very thick, and Liu [20] et al. considered that all the spores of pteridophytes had sporoderm, while Dai [21] et al. observed by scanning electron microscope and transmission electron microscope, and the tapetum residues of the sporoderm of the spores were found in the process of spore development and maturation. The surface of the sporoderm is deposited into the perispores. By observing the spores of two species of Ceratopteris Brongn., it is further proved that the spores of Ceratopteris Brongn. have a perispore as proposed by Dai Xiling.

The main difference of perispores between C. thalictroides and C. pteridooides. Under scanning electron microscope, the spores of the two species were observed from different angles in several views. The morphology of the spores of the two species was similar, and they were radially symmetrical, tetrahedral, spherosome in polar view and equatorial view; trilete suture, with ridge-like bulge on the surface of perispores. The common characteristics of the above-mentioned spores show that they are close relatives to each other. The spore of C. thalictroides [9] is round and three-square in shape, the branches of ridge-like bulge are few and arranged sparsely, the distance between the ridges is equal to 2 to 3 ridges, and the perispores of the ridge-like bulge have small and short filaments (or branches), and the grains are intersected into a network; the spore of C. pteridooides is nearly spherical, the ridge bulge is not uniform, slightly curved and closely arranged, the gap between the ridges is small, equal to 1/3 of the ridge width, no fine patterns were seen. With the characteristics of trilete suture and parallel ridges, it indicates that the plant of Ceratopteris Brongn. is in a higher level of evolution; C. thalictroides has a more coarse small rod-shaped and granular bulge by comparing with C. pteridooides, so it is in a higher level of evolution. The difference of spores between C. thalictroides and C. pteridooides provides the palynology basis for the morphological taxonomy of them.

Ceratopteris was approved by the State Council in 1999 to be listed in A list of wild plants under special state protection as the first group of wildlife resources [1] under special state protection. They are not only two important plant resources in terms of plant system evolution and habitat benefit, but also important medicinal plant resources. In the north of China, C. thalictroides is only found in Weishan Lake, Nanyang Lake, Dushan Lake and other water areas or stream wetlands in Shandong Province with a small distribution [2-4]. Because it is an annual summer green plant, small aquatic or wetland fern, the distribution area is narrow, the habitat is fragile, so it is a rare and endangered group [11] in Shandong, facing extinction.

In order to effectively save this precious species from extinction, and strive to make it flourish in the shortest time, the following measures are suggested: a. to strengthen the awareness of resource conservation, take the endangered species in A list of wild plants under special state protection as an important task, and protect them in a timely and orderly manner. b. Recommend that the Shandong Institute of Botany, the Shandong Society of Traditional Chinese Medicine and the government’s plant protection departments work together and organize relevant experts to make plans with several institutions of higher learning in Shandong (the fourth national survey of traditional Chinese medicine resources currently under way) to find out the current distribution and endangered status of C. thalictroides and C. pteridooides resources and make plans, the species of endangered and important medicinal plants needed to be protected in the Shandong region shall be compiled into A list of wild plants under special protection in Shandong province, which shall be submitted to the provincial people’s congress for approval, in the name of the provincial people’s congress, together with A list of wild plants under special state protection, be issued to the governments at or above the county level to mobilize social forces and carry out effective protection under the leadership of the functional departments of the governments at all levels. c. Recommend that provincial and municipal governments set up functional departments and full-time technical managers for the special protection of wild plants, and include their tasks in the performance appraisal. d. Recommend that the Provincial Science and Technology Department set up the “Special Protection of Wild Rare and Endangered Plant Resources” Fund, as a special project, supported by the special fund, to carry out systematic
research so that the wild rare and endangered plant resources can be effectively protected. To establish a breeding base for *C. thalictroides* and carry out the transplanting in vivo. Based on the fact that the *C. thalictroides* propagates by mature spores, while the *C. thalictroides* spore is large, and its life history alternates, the whole process can be completed in the laboratory, and the difficulty is relatively small. From the beginning of June to the end of August of each year, it is an effective measure for the protection of *C. thalictroides* by carrying out live field water or wetland transfer in a planned way, and breeding in many waters of Shandong within 2-3 years.

5. Conclusion

*C. thalictroides* and *C. pteridoides* of *Ceratopteris* Bronn. are aquatic pteridophytes widely distributed in tropical and subtropical regions of the world. In China, they are distributed in Guangdong, Guangxi, Fujian, Taiwan, Yunnan, Sichuan and other tropical and sub-tropical areas in the south; in the north of the Yangtze River, they are only found in Weishan Lake and Nanyang Lake pond shallow water city in Shandong Jining, which is the northernmost distribution city in China, the distribution zone is narrow, the habitat is fragile, has been listed as China’s rare and endangered species, and is significant for the study of endangered plant habitat ecology, the protection of plant resources diversity, at the same time, *C. thalictroides* can be used medicinally and edible, it has important application value to its resource conservation and exploitation for use.

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