TAXONOMY AND PALYNOLOGY OF SOME CYRTOMIUM SPECIES IN SHANDONG, CHINA

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Abstract

Using SEM spore morphology combined with the palynological studies, four new species of *Cyrtomium* have been identified and added for the fern flora of China. The new species are *C. polypterum* (Diels) J. X. Li and X. J. Li stat. nov., *C. shandongensis* J. X. Li, *C. reflexosquamatum* J. X. Li *et* F. Q. Zhou and *C. confertiserratum* J. X. Li , H. S. Kung *et* X. J. Li. Among the four species, the later three have been considered as specific species for Shandong province in center esthern China. In addition, two more species, namely *C. yamamotoi* Tagawa and *C. yamamotoi* var. *intermedium* (Diels) Ching *et* Shing *were* recorded as new for the distribution of ferns in Shandong. While two other species of *Cyrtomium*, namely *Cyrtomium falcatum* (L. f.) Presl and *C. fortunei* J. Sm. have been described taxonomically further based on their spore SEM and palynological characteristics. This study not only gathers new information for the taxonomy and palynology of *Cyrtomium* spp. from this area, but also provides the basis for the development, utilization and protection of their medicinal values.

Introduction

In China, the fern genus *Cyrtomium* E. Presl is represented by about 35 species (Wu 2013). Detailed taxonomy of the genus was studied by Ching (1936). Later on, workers on its various aspects were also carried out (Shing 1965, Kong 2001, Christensen 1930, Lu *et al.* 2007, 2010, Dai *et al.* 2010). Some of the studies related to the taxonomy, morphology, anatomy and palynology of *Cyrtomium* from Shandong province have been done from 1984 until recently (Li 1984, 1985, 1994, Li *et al.* 1996, 2012, 2013, Chen *et al.* 1990, Zhou *et al.* 1999, Li 2004). All these reports not only provided the basis for the germplasm diversity, taxonomy, utilization and conservation of *Cyrtomium*, but also provided the related information for writing the monograph on medicinal lycophytes and ferns discovered in Shandong. The present research aimed at carrying out further investigation on *Cyrtomium* of Shandong areas by considering its tremendous medicinal value, conservation and studying spore morphology.

Materials and Methods

Samples of *Cyrtomium* were collected from different localities of Shandong area of China. A total of seven species and one variety of *Cyrtomium* were collected and the voucher specimens were preserved in the Traditional Chinese Medicine Resource Center Laboratory, Beijing Normal University. The name of the voucher specimen along with their distribution has been presented in Table 1.

The well-developed and fully-matured spores of *Cyrtomium* 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 were measured from ten randomly chosen

^{*}Author for correspondences <mfy@bnu.edu.cn; jianxiu_li@163.com>. ¹Beijing Normal University, Faculty of Geographical Science BNU, Beijing 100875, China. ²Shandong Hongjitang Museum, Jinan 250103, China. ³College of Chinese Medicine of Shandong University of Traditional Chinese Medicine, Jinan 250355, China. spores for each species with three replicates to obtain the data. Subsequently, the spores were

evenly spread on a wood-free printing paper on a specimen holder. After spraying gold particles for 30s, 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 $5000 \times$ and then of $1500 \times$. When the voltage was stable, focal length was adjusted to collect the pictures.

Table 1. List of the voucher specimens.

Taxon	Specimen	Locality
Cyrtomium falcatum (L. f.) Presl	J. X. Li 87826	Qingdao (Laoshan)
C. fortunei J. Sm.	A. X. Hou96001	Taian (Dawenkou)
C. polypterum (Diels) J. X. Li & X. J. Li stat. nov.	J. X. Li 850086	Jinan (Dafotou, Huangshiya)
C. shandongensis J. X. Li	J. X. Li 02023-1	Linyi (Tashan)
C.reflexosquamatum J. X. Li et F. Q. Zhou	J. X. Li 960521	Jinan (Xiying)
C. confertiserratum J. X. Li, H. S. Kung et X. J. Li	J. Q. Sun 88-131	Taian (Liangzhuang)
C. yamamotoi Tagawa	J. X. Li et X. J. Li 20121105	Taian (Dawenkou)
C. yamamotoi var. intermedium (Diels) Ching et Shing ex Shing	J. X. Li et X. J. Li 20121106	Taian (Dawenkou)

Results and Discussion

On the basis of morphology and other systematic characters, following keys for the identification of species have been developed as follows:

1a.	Leathery; lateral pinnae margins entire; coastal cliff or offshore area	
		1. C. falcatum
1b.	Herbaceous or papery; lateral pinnae margins tooth; land.	
2a.	Lateral pinnae more 20 pairs; smooth decoration on the surface of	
	bend, cristate protuberance	2. C. polypterum
2b.	Lateral pinnae less 20 pairs; short, cristate, verrucate, auricular fold.	
3a.	Lateral pinnae margins serrulate	3. C. confertiserratum
3b.	Lateral pinnae margins sparse serrate or corrugated coarse teeth.	
4a.	Densely clothed with anatropous, linear-lanceolate scales on upper	
	basal part of rachis.	4. C. reflexosquamatum
4b.	Without anatropous scales on upper basal part of rachis.	
5a.	1 piece large pinnae with 2 - 3 fork split on the top of the blade; with sori borne on the back of pinnae.	
ба.	Lateral pinnae sickle lanceolate.	
7a.	Lateral pinnae margins carse teeth wavy, ear flake protrusions	5. C. yamamotoi
	var. intermedium	
7b.	Lateral pinnae margins tooth, sparse, irregular, short, cristate and	
	tuberculate protuberance	6. C. fortunei
6b.	Lateral pinnae ovoid, round or oval block protuberance	7. C. yamamotoi
5b.	1 piece large pinnae on the top of the blade; more or less pinnae	

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division; sori borne on both sides of the main vein near the pinnae allied each arranged in 1-2 rows 8. *C. shandongens*is

1. Cyrtomium falcatum (L. f.) Presl

Plants 30 - 40 cm tall, rhizome erect, densely covered with lanceolate brown scales. Stipe stramineous, 15 - 27 cm, 3 - 4 mm in diam. at base, lower portion densely scaly; scales pale brown, sometimes blackish brown at middle, ovate, lower portion fimbriate. Lamina broadly lanceolate, 22 - 35×12 - 15 cm, base contracted, 1-imparipinnate, apex acute; rachis with lanceolate brown dentate scales or glabrous. Lateral pinnae 5-14 pairs, alternate, spreading or ascendant, shortly stalked, lanceolate or ovate-lanceolate, often curved acroscopically; middle pinnae 6 - $10 \times 2.5 - 3$ cm, base obliquely rounded-cuneate, margins entire or repand, sometimes dentate, apex long acuminate or caudate; upper pinna ovate-lanceolate, forked or trifurcate, $4.5 - 8 \times 2 - 4$ cm; leathery, glabrous on both surfaces; venation pinnate, slightly raised abaxially, indistinct adaxially, veinlets anastomosing to form 3 or 4 rows of areoles. Sori throughout abaxial surface of pinnae; circular indusia, peltate, indusia margins slightly incised. Spores oval, smooth decoration on the surface of regular, verrucate protuberance (Figs 1a-b).

Distribution: Qingdao (Laoshan, Jiaonan) (Jianxiu Li87826), Weihai (Liugongdao, Shidao), Yantai. Jiangsu, Zhejiang, Fujian, Taiwan, Guangdong [China] and Japan.

 Cyrtomium polypterum (Diels) J. X. Li & X. J. Li stat. nov. (Figs 1e-f, 3a-c) *Polystichum folcatum* Diels var. *polypterum* Diels; Cyrtomium fortunei J. Sm. f. *polypterum* (Diels) Ching.

Lateral pinnae 22-32 pairs, small sori, on both sides of the main vein in pinnae near the edge of each arranged in 2-3 lines, indusia margins with teeth, especially smooth decoration on the surface of bend, cristate protuberance (Figs 1e-f). Those features were different from *Cyrtomium* fortunei J. Sm.

Distribution: Jinan (Huangshiya) (Jianxiu Li850086), new record in Shandong province (or new state). Shanxi, Shaanxi, Gansu, Jiangxi, Henan, Hubei etc. [China].

3. C. confertiserratum J. X. Li, H. S. Kung et X. J. Li in Plant Diversity and Resources 34(1):17-21. 2012. (Figs 1m-p, 2b)

Plants 50 - 60 cm high, rhizome erect, densely scaly; scales brown, broadly ovate. Fronds caespitose, stipes 8 - 10 cm, base 2 mm wide, stramineous, ventral narrowly grooved, with broadly lanceolate scales, fimbriate-tooth at margin, upward scales sparse; lamina narrow-lanceolate, 40 - $45 \times 10 - 12$ cm, acuminate at the apex, slightly narrow at the base, 1-pinnate; lateral pinnae 15 - 17 pairs, alternate, obliquely ascendant, with short stalk, falcate-lanceolate, middle pinnae 6 - 8×2 cm, apices gradually acuminate, base oblique, sub-rounded, upward obtuse auriculate, downward cuneate, margin with finely sharp serrate, spreading forward; veins pinnate, veinlet joined into many rows of areoles, obscure adaxially, slightly convex abaxially; upper pinnae rhombic, 6 cm long, 4 cm wide, basal 1 - 2 deeply lobed, segment 3 - 4×1 cm. Lamina hard herbaceous, glabrous adaxially, clothed with hairlike scales abaxially; rachis narrowly grooved on ventral side, clothed with brown, linear-lanceolate scales. Sori dorsal; indusia rounded, peltate, with erose margin, caduceus. Spores reniform, thick stripped ridges and granule decoration among the irregular tuberculate protuberance (Figs 1m-p).

Distribution: Taian (Liangzhuang) (Jiquan Sun 88-131 TYPUS). Shandong specific species.

(Figs 1a-b, 2a).

Species	Size/µm	Ornamentation under SEM	Voucher specimen	Figure 1
Cyrtomium falcatum (L. f.) Presl	43.5×61.2	Smooth decoration on the surface of regular, verrucate protuberance	J. X. Li 87826, Qingdao (Laoshan)	a-b
C. fortunei J. Sm.	38.6×52.6	Thin flake decoration on the surface of sparse, irregular, short, cristate and tuberculate protuberance	A. X. Hou 96001, Taian (Dawenkou)	c-d
C. <i>polypterum</i> (Diels) J. X. Li & X. J. Li stat. nov.	38.1×50.8	Smooth decoration on the surface of bend, cristate protuberance	J. X. Li 850086, Jinan (Dafotou, Huangshiya)	e-f
C. shandongensis J. X. Li	42.1×60.4	Flake decoration on the surface of irregular, short, cristate and verrucate protuberance	J. X. Li 02023-1, Linyi (Tashan)	g-h
<i>C. reflexosquamatum</i> J. X. Li et F. Q. Zhou	41.4×56.1	Reticulate and granule decoration among the auricular protuberance	J. X. Li 960521, Jinan (Xiying)	Ŀ
C. confertiserratum J. X. Li, H. S. Kung et X. J. Li	36.2×51.6	Thick stripped ridges and granule decoration among the irregular tuberculate protuberance	J. Q. Sun 88-131, Taian (Liangzhuang)	d-m
C. <i>yamamotoi</i> Tagawa	40.5×52.4	Scale decoration on the surface of round or oval block protuberance	J. X. Li et X. J. Li 20121105, Taian (Dawenkou)	q-r
var. <i>intermedium</i> (Diels) Ching et Shing ex Shing	41.2×53.8	Irregular filamentous fold decoration on the surface of auricular flake fold	J. X. Li et X. J. Li 20121106, Taian (Dawenkou)	s-t

Table 2. Spore morphology of seven species and one varity of Cyrtomium from Shandong area.



Fig. 1. Spore morphology of seven species and one variety of *Cyrtomium*. a-b: *C. falcatum*; c-d: *C. fortunei*; e-f: C. *polypterum*; g-h: *C. shandongensis*; i-l: *C. reflexosquamatum*; m-p: *C. confertiserratum*; q-r: *C. yamamotoi*; s-t: var. *intermedium*; a, c, e, g, i, m, q, s: equatorial view; k-l;o-p: polar view; b, d, f, h, j, l, n, p, r, t: local view.

C. reflexosquamatum J. X. Li *et* F. Q. Zhou in Plant Diversity and Resources 34(1):17-21. 2012. (Figs 1i-l, 2 c)

Plants 40 - 60 cm high, rhizome erect, densely scaly; scales brown, oblong-ovate. Fronds caespitose, stipes 8 - 10 cm, base ca. 2 mm wide, stramineous, ventral narrowly grooved, with densely oblong lanceolate scales at the base, fimbriate-tooth at margin; lamina linear-lanceolate, 30 - 50×6 - 8 cm, acuminate at the apex, slightly narrower at the base, 1-pinnate; lateral pinnae 15 - 29 pairs, alternate, horizontally spreading, with very short stalk, falcate-lanceolate, middle pinnae $4.5 - 5.5 \times 1$ cm, apices gradually caudate acuminate, base oblique or round-cuneate, upside obtuse or triangular auriculate, downside cuneate, margin with irregular sparse serrate; veins pinnate, veinlet joined into 2 rows areoles, obscure adaxially, slightly convex abaxially; upper pinnae narrow-ovate, basal 1 - 2 deeply lobed, segment 3 - 4×2 - 2.5 cm. Lamina herbaceous, glabrous

adaxially, green, densely hairlike scales abaxially; greygreen; ventral side of rachis narrowly grooved, densely clothed with anatropous, brown, linear-lanceolate scales on upper basal part. Sori in 1 - 2 rows on each side of costa near to margin; indusia rounded, peltate, margin undulatly serrulate. Spores reniform, with ear flake and fine reticulate perine (Figs 1i-l).

Distribution: Jinan (xiying) (Jianxiu Li960521 TYPUS). Shandong specific species.



Fig. 2. Plant morphology of species of *Cyrtomium*. a. *Cyrtomium falcatum* (1. frond; 2. pinna; 3. indusium); b. *C. confertiserratum* (1. frond; 2. pinna; 3. scale at the base of the stipe; 4. indusium; 5. scale in the middle of the rachis; 6. multicellular cell on the underside of the lamina. (drawn by J. X. Li *et al.* from the holotype, J. Q. Sun 88-131(SDCM); c. *C. reflexosquamatum* (1. frond; 2. pinna; 3. scale at the base of the stipe; 4. indusium; 5. scale in the middle of the rachis); d. Frond of *C. fortunei*; e. *C. shandongensis* (1. plants; 2. pinna; 3. scale upper the stipe; 4. indusium)

5. C. fortunei J. Sm.

(Figs 1c-d, 2d).

Plants 25 - 50 cm tall, rhizome erect, densely covered with brown scales. Stipe stramineous, 12 - 26 cm, 2 - 3 mm in diam. at base, lower portion densely scaly; scales brown, sometimes with a dark brown central stripe, ovate or lanceolate, dentate. Lamina oblong-lanceolate, $20 - 42 \times 8 - 14$

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cm, base not contracted or slightly contracted, 1-imparipinnate, apex obtuse; rachis with sparse lanceolate or linear brown scales. Lateral pinnae 7 - 16 pairs, alternate, nearly spreading, shortly stalked, lanceolate, \pm falcate; middle pinnae 5 - 8 × 1.2 - 2 cm, base oblique, acroscopic margins subtruncate and sometimes with weak, blunt auricles, basiscopic margins cuneate, margins entire or sometimes serrulate, apex acuminate or rarely caudate; terminal pinna ovate-lanceolate, sometimes lower portion with 1 or 2 lobes, 3 - 6 × 1.5 - 3 cm; papery, glabrous on both surfaces. Sori throughout abaxial surface of pinnae; circular indusia, peltate, entire. Spores oval, thin flake decoration on the surface of sparse, irregular, short, cristate and tuberculate protuberance (Figs 1c-d).

Distribution: Taian (Dawenkou, Huangqian, Feicheng) (Aixia Hou96001); Jinan (Lingyansi). Hebei, Shanxi, Shaanxi, Gansu, Jiangsu, Anhui, Zhejiang, Jiangxi [China].



Fig. 3. Three species of *Cyrtomium. C. polypterum* (a. plant; b. pinna; c. indusium); d. Frond of *Cyrtomium yamamotoi*; C. var. *intermedium* (e. frond; f. pinna).

6. C. yamamotoi Tagawa

(Figs 1q-r, 3d).

Plants 40 - 60 cm tall, rhizome erect, densely covered with lanceolate blackish brown scales. Stipe stramineous, 22 - 30 cm, 2 - 3 mm in diam. at base, densely scaly; scales blackish brown, or blackish brown with a brown edge, ovate or lanceolate, denticulate. Lamina ovate or ovate-lanceolate, 24 - 44 \times 12 - 18 cm, base slightly contracted, 1-imparipinnate, apex obtuse; rachis with sparse, lanceolate, blackish brown or brown scales. Lateral pinnae 4 - 14 pairs, alternate,

slightly ascendant, shortly stalked, lanceolate or broadly lanceolate, \pm falcate; middle pinnae 8 - 12 × 3 - 3.5 cm, base rounded-cuneate or inequilateral and broadly cuneate, acroscopic margins with semicircular or acute auricles, margins entire or distally denticulate, apex acuminate or caudate; terminal pinna ovate or rhombic-ovate, forked or trifurcate, 8 - 12 × 6 - 8 cm; papery, glabrous on both surfaces; venation pinnate, slightly raised abaxially, indistinct adaxially, veinlets anastomosing to form 3 or 4 rows of areoles. Sori throughout abaxial surface of pinnae; indusia dark brown in center, margins with incised teeth. Scale decoration on the surface of round or oval block protuberance (Figs 1q-r).

Distribution: Taian (Dawenkou) (Jianxiu Li, Xiaojuan Li20121105). New record of Shandong, Anhui, Shaanxi, Gansu, Zhejiang, Jiangxi, Hubei, Henan, Guangxi [China] and Japan.

7. C. yamamotoi var. intermedium (Diels) Ching *et* Shing *ex* Shing (Figs 1s-t, 3e-f)

This species was different from *Cyrtomium yamamotoi* in pinnae edge with wave or blunt tooth. irregular filamentous fold decoration on the surface of auricular flake fold (Figs 1s-t).

Distribution: Taian (Dawenkou) (Jianxiu Li, Xiaojuan Li20121106). New record of Shandong distribution. Anhui, Shaanxi, Zhejiang, Jiangxi, Hubei, Hunan, Guangxi [China] and Japan.

8. **C. shandongensis** J. X. Li in Bull. Bot. Res. 4(2): 142. pl.1.1984; Jianxiu Li, Flora of Shandong (volume 1): 129. Picture 68. 1990.; Xianxu Kong, Flora of China, 5(2): 207.2001.

(Figs 1g-h, 2e)

Plants 20 - 35 cm tall, rhizome erect, densely covered with ovate-lanceolate brown scales. Fronds caespitose, stipes 8 - 10 cm, base 2 mm wide, stramineous, ventral narrowly grooved, with densely oval lanceolate scales at the base, fimbriate-tooth at margin; upward smooth; lamina oblong-lanceolate, 18 - 30×6 - 8 cm, acuminate at the apex, slightly narrower at the base, 1-pinnate; lateral pinnae 8 - 15 pairs, alternate, slightly oblique upward, with very short stalk, falcate-lanceolate, middle pinnae 3 - 4×1 - 1.5 cm, apices gradually acuminate, base oblique, upside obtuse, downside cuneate, margin with small forward tooth; veins pinnate, veinlet joined into 2 rows areoles, obscure two sides, upper pinnae narrow-ovate, more or less pinnae split, segment $3-5 \times 2-3$ cm; papery, glabrous on both surfaces; ventral side of rachis narrowly grooved, sparsely lanceolate or linear brown scales. Sori in 1 - 2 rows on each side near to margin; indusia rounded, peltate, fimbriate-tooth at margin. Spores oval, flake decoration on the surface of irregular, short, cristate and verrucate protuberance (Figs 1g-h).

Distribution: Shandong (Tashan) (Jianxiu Li02023-1 TYPUS). Shandong specific species.

Polystichum folcatum Diels var. polypterum Diels was regarded as *C. fortunei* J. Sm. f. polypterum (Diels) Ching by Ching in 1935. According to the Flora of China (Kong 2001), the main difference between *C. fortunei* J. Sm. f. polypterum and *C. fortunei* is that pinnae smaller and more, $2 - 3 \times 0.6 - 1$ cm, 19 - 32 pairs. We systematically searched on them by plant taxonomy combined with palynology, we concluded that lateral pinnae 22 - 32 pairs, small sori, on both sides of the main vein in pinnae near the edge of each arranged in 2 - 3 lines, indusia margins with teeth, especially smooth decoration on the surface of bend, cristate protuberance (Figs 1e-f). Those features were different from *Cyrtomium fortunei* J. Sm. and showed that *Cyrtomium polypterum* (Diels) J. X. Li and X. J. Li stat. nov., was a distinct species, which should be restored to a species level position rather than *C. fortunei* J. Sm. f. polypterum (Diels) Ching.

Cyrtomium was recorded in the document (Xing 1965). It rangs from the north by the Qin mountains in a south slope to Shanxi, the south of Henan and southwest of Hebei from the geographical distribution. *C. falcatum* (L. f.) Presl was recorded in the 1980 years in Shandong.

Then we investigated the resources of medicinal plants of *Cyrtomium* from Shandong. We collected specimen, performed the classification and the identification, and the plant taxonomy combined with palynology. Overall the spore ornamentations had been examined by means of scanning electron microscope, there are 8 species at present, including *C. shandongensis* (Li 1984), *C. reflexosquamatum, C. confertiserratum* (Li 2012) and *C. polypterum* (Diels) J. X. Li & X. J. Li stat. nov. of four new species; *C. yamamotoi* Tagawa and *C. yamamotoi* var. *intermedium* (Diels) Ching *et* Shing *ex* Shing were recorded as new for the distribution of ferns in Shandong. These documents have shown that the medicical plant resources of *Cyrtomium* from Shandong province are rich. Most species live in damp well except *Cyrtomium falcatum*, which lives in the coast. This might be due to long time drought for years because the plants might have died in the land, and spores spreaded in the damp well in order to save or reproduce. This study not only has provided new information for the taxonomy and palynology of *Cyrtomium* spp. from the area, but also considered as the basis for the development, utilization and protection of their medicinal values.

C. shandongensis J. X. Li has been considered as a new species, which was published in Bull. Bot. Res. **4**(2): 142. pl. 1. Then, it was recorded in Flora of China (Kong 2001) and Flora of Shandong (Volume1) (Chen 1990), yet the contributors merged *C. shandongensis* J. X. Li into *C. fortunei* J. Sm.. Therefore the former was different from the latter, which showed the lateral pinnae falcate-lanceolate, upper pinnae narrow-ovate, more or less pinnae split, fimbriate-tooth at margin; Sori in 1 - 2 rows on each side near to margin; indusial with fimbriate-tooth at margin. Spores with flake decoration on the surface of irregular, short, cristate and verrucate protuberance. Based on the above characteristics, the spore ornamentation characteristics showed that *C. shandongensis* J. X. Li was a distinct species, which should be restored to a species level position rather than incorporated with *C. fortunei* J. Sm..

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