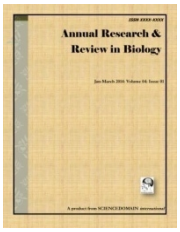

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Spore Morphology of 34 Species of Monilophyta from Northern Parts of Iran

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Authors' contributions

Authors may use the following wordings for this section: This work was carried out in collaboration between two authors. Author AM designed the study, collected the materials identified them and performed the research. Author FS managed the literature searches and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study is to analyze the 34spores species by using SEM that grown in Northern parts of Iran as a contribution to the knowledge about the general morphology and assess if these characteristics could be useful for systematic purpose.
Methodology: For Scanning Electron Microscope, the material treated with hot 3% sodium carbonate, for 2 min, washed, dehydrated, suspended in 96% ethanol and then transferred to acetate plates and finally coated with gold. The shape, ornamentation, the equatorial and polar diameter, and the number of cells in annulus in 38 spores were studied.
Results: ornamentations of spores consist of rugulate, ornate, echinate or microechinate, verrucate, perforate and spinule or spinuluse. Based on our results the identification key was devised using spore characteristics.
Conclusion: Based on our results, the spore sculpture could be useful for systematic purpose, specially in Polypodiaceae, Thelypteridaceae and Hydrofilicinae ferns.

Keywords: Micro morphology; Fern; SEM; Iran; Spores.

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1. INTRODUCTION

The ferns are a group of lower tracheophyta that range of distribution extends throughout the temperate regions. According to Mazooji et al., 45 species belonging to 21 genera are represented in Iran specially in the northern parts [1]. Contributions on Sinopteridaceae spore morphology were made by Khosravi, who studied the relationship between species by using Scanning Electron Microscope [2]. Cubasa and Pardo using SEM, described the spore in *Polystichum* as trilete with a verrucate exospores [3]. Using both LM and SEM, Erdtman and Sorsa described 45 species of spores and commented that, in some species, the spores were devoid of perispore, and others that those which had periospore, were ornamented by pleated with echinates or granules [4]. Tryon and Lugardon in their work on spores of the Pteridophyta described and illustrated with SEM and TEM the spore that characterized the genera of the Cyatheaceae, Grammitidaceae, Aspleniaceae, and Polypodiaceae [5]. Denk recorded the *Cystopterisregia* from Iran based on spore sculpture [6]. Spore morphology of six genera of Polypodiaceae from the Northwestern Argentina were analyzed by Giudice et al. According to this study, the spores were monolete with elliptic to oblong in polar view [7]. Morphology and ultrastructure of the spores of Grammitidaceae from Argentina were analyzed by Ramos Giacosa et al. [8,9]. The ornamentation of the five species of *Grammitis* were tuberculate, papillate or verrucate. Multivariate analyses based on morphological and anatomical characters performed by Wannachai et al. to investigate the phenetic relationship in *Lepisorus* genus and its related genera [10]. Also, spore morphology of twenty species of the genus *Lindsaea* from Japan with SEM using were illustrated by Lin et al. [11]. They described that spores were trilete and had with verrucose sculpture. The variation in the spore size is in relation to the ploidy of cytotypes. Oliver studied fifty spore's fern in India by using SEM [12]. He diagnostic that most species may can be distinguished by differences in shape, and in the structure and sculpture of the perispore and exospore layer. As a result, of the diagnostic key was elaborated devised based on using spore morphology. The aim of this study in to analyze the 34 spores species by using SEM that grown in Northern parts of Iran as a contribution to the knowledge about the general morphology and assess if these characteristics could be useful for systematic purpose.

2. MATERIALS AND METHODS

The studies were conducted on herbarium material provided by the VezarateJahad Keshavarzi Herbarium (IRAN). Origin of species and their voucher numbers examined are given in Table 1. For SEM study, the material treated with hot 3% sodium carbonate, for 2 min, washed, dehydrated, suspended in 96% ethanol and then transferred to acetate plates and finally coated with gold. The observation were made a JEOL ISMT–100 Scanning Electron Microscope. Shape, ornamentation, the equatorial and polar diameter, and the number of cells in annulus were studied. The terminology used for spore sculpturing is based on the work by Moore et al. (Table 2) [13].

Table 1. Materials and collecting data for micro morphological studies on spores in 34 species of Pteridophyta

Species	Locality	Voucher number
Adiantum capilus-verneris L.	Mazandaran : Royan road to Noshahr, 350 m	2009
Asplenium adiantumnigrum L.	Gilan : Asalem to Khalkhal, 2300m	2023
Asplenium onopteris L.	Mazandaran : Lahijan to siyahkoh, 250m	2042
Asplenium ruta – muraria L.	Gilan : Asalem to Khalkhal, 2200m	2045
Asplenium septentrionale (L.) Hoffm.	Gilan : Asalem to Khalkhal, 2200m	2037
Asplenium officinarum D.C.	Gilan : Masoleh, 950m	2051
*Athyrium distentifolium*Tausch ex. Opiz.	Mazandaran : Abasabad to klardasht, 900m	2001
Athyrium filix – femina (L.) Roth.	Gilan : Asalem to Khalkhal, 1850m	2008
Azolla filiculoides Lam.	Gilan : Bandar Anzali, -10m	2048
Blechnum spicant (L.) roth.	Gilan : Shaft road to Lask, 900m	2049
Cystopteris fragilis (L.) Bernh.	Tehran : Darband, 2000m	2058
Cystopteris regia (L.) Desvaux.	Tehran : Dizin, 2500m	2052
Dryopteris affinis (Lowe). Frazer – Jankins	Mazandaran : Haraz road, 200m	2140
D. carthusiana (Vill.) H.P. Fuchs.	Gilan : Asalem to Khalkhal, 550m	2080
D. caucasica (A. Braun.) Frazer – Jankins&Jermy.	Gilan : Asalem to Khalkhal, 900m	2069
D. dillatata (Hoffm.) A. Gray	Gilan : Asalem to Khalkhal, 450m	2075
D. expansa (C. Presl.) Frazer – Jankins&Jermy.	Mazandaran : Ramsar to Javaherdeh, 700m	2076
D. filix – mas (L.) schott.	Gilan : Asalem to Khalkhal, 700m	2066
D. pallida (Bory.) Fomin.	Ardebil : Fandoglo forest, 1900m	2062
D. remota (A. Braun ex. Doll) Drice.	Mazandaran : Alamdeh to Marzanabad, 300m	2071
*Matteuccia struthiopteris*Tod.	Mazandaran : Abasabad to Klardasht, 900m	2083
Ophioglossum vulgatum L.	Mazandaran : Sangdeh, 2500m	16821
Oreopteris limbosperma (Bellard. ex all.) Y. Holub	Gilan : shaft, Lask road, 950m	2084
*Polypodium interjectum*Shivas.	Gilan : Asalem to Khalkhal, 100m	2095
P. vulgare L.	Gilan : Asalem to Khalkhal, 1800m	2092
Polystichum aculeatum (L.) Roth.	Gilan : Asalem to Khalkhal, 450m	2103
P. braunii (spenner) Fee.	Mazandaran : Abasabad to Klardasht, 900m	2104
P. woronowii Fomin in Mon.	Gilan : Gizvin to Rasht, 350m	2117
Pteridium aquilinum (L.) Kuhn.	Gilan : roadbar, 350m	2122
Salvinia natans (L.) All.	Gilan : Lahijan, -20m	2130
Thelypteris palustris	Gilan : Lahijan to Langroad, -27m	2132
Woodsia alpina (Bolton.) S.F.Gray.	Gilan : Asalem to Khalkhal, 2400m	2133

3. RESULTS AND DISCUSSION

The species studied had general characteristic given below. In equatotial view, the spore sizes varied between 27.44–70.50 µm. The spores are monolete or trilete and had bean, circular or elliptic shape (Fig. 1).The largest spores were showed in *Matteucia struthiopteris* Tod. and the smallest lowest ones were in *Dryopteris pallida* (Bory.) Fomin.

The ornamentations of spores were rugulate, ornate, echinate or microechinate, verrucate , perforate and spinule or spinuluse (Fig. 1). Based on our results the identification key was devised using spore characteristics as followed:

1.+ spore bean shape.....2
- spore orbicular or elliptic.....14
2.+ sporangium without annulus..... *Salvinia* groups (*Salvinia,Azolla*)
- sporangium with annulus.....3
3.+ the equatorial diameter > 60 µm.....4
- the equatorial diameter < 60 µm 6
4.+ spore ornate..... *Matteucia*
- sporeverrucate.....5
5.+ cell numder of annulus is 10 – 11..... *Polypodium interjectum*
- cell number of annulus is 14 – 45..... *Polypodium vulgar*
6.+ spore rugulate..... *Blechnum spicant*
- different from above 7
7.+cell number of annulus <19 8
- cell number of annulus >19 9
8.+ spore spinule .....*Thelypteris palustris*
- sporespinuluse ..... *Athyrium distentifolium*
9.+ the polar diameter > 30 µm, spore echinate or microechinate.....
Asplenium groups (*A. onopteris*, *A. trichomanes*, *A. ruta-muraria*, *A.septenterional*, *A. adiantum-nigrum*)
- the polar diameter < 30 µm 10
10.+ the equatorial diameter > 40 µm.....11
- the equatorial diameter < 40 µm 12
11.+ spore rugular reticulate..... *Oreopteris limbosperma*
- sporespinule ..... *Cystopteris* groups (*C. fragilis, C. regia*)
12.+ spore echinate- reticulate 13
- sporespinuluse .....*Polysticum aculeutum*
13.+ polar diameter< 25 µm, spines shorter ..... *Asplenium sclopendrium*
- polar diameter> 25 µm, spines longer ..... *Polystichum woronowii*
14.+ spore orbicular or subrobicular.....15
- spore elliptic.....18
15.+ the equatorial diameter < 40 µm, spore perforate.....
..... *Ophioglossum vulgatum*
- the equatorial diameter > 40 µm 16
16.+ cell number of annulus > 20 .....*Adiantum capilus – veneris*
- cell number of annulus < 20 17
17 .+ spore ornate ..... *Asplenium officinarum*
- sporeechinate..... *Woodsia alpina*
18.+ spore ornate ..... *Dryopteris* group (*D. dillatata, D. carthusiana*, *D. affinis, D. caucasica, D. expansa, D. filix-mas, D. pallida*)
- spore different from above.....19
19.+ the equatorial diameter > 40 µm..... *Dryopteris remota*
- the equatorial diameter < 40 µm 20
20.+ spore echinate .....*Polystichumbraunii*
- sporedifferent from above 21
21.+ spore rugulate, the polar diameter 28.28±0.01 µm ..... *Pteridium aquilinum*
- sporespinuluse, the polar diameter 26.1±0.01 µm..... *Athyriumfilix – femina*

Table 2. Spore characteristics by SEMin Pteridophytaspecies

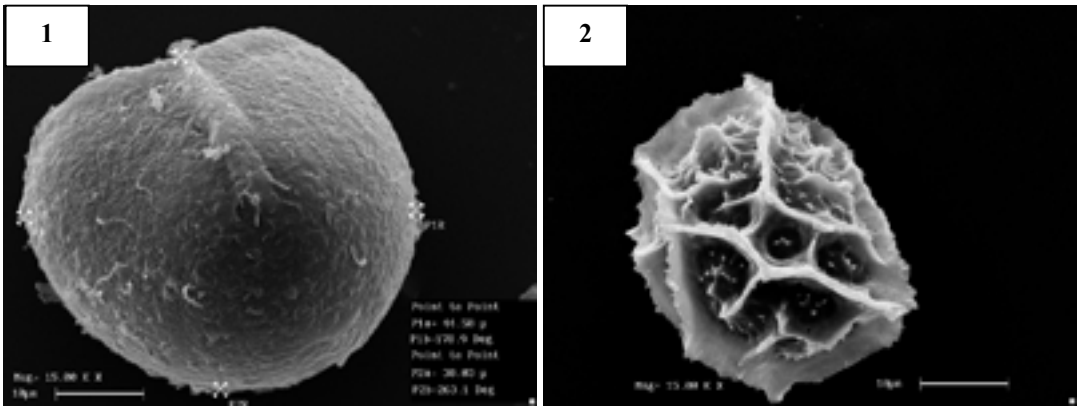
Species	Shape	Aperture	Ornamentation	Cell Number of Annulus	Polar Diameter (µm)	Equatorial Diameter (µm)
Adiantum capilus-veneris	Circular	trilete	Rugulate	19-23	38.03	44.5
Asplenium adiantum-nigrum	Bean	monolete	Echinate	19-20	33.3	43.2
A. onopteris	Bean	monolete	Echinate	19-20	31.85	42.14
A. rutamuraria	Bean		Echinate	18-20	-	-
A. septentrinal	Bean	monolete	Echinate	18-19	34.62	55.44
A. trichomanes	Bean	monolete	Echinate	19-20	32.9	40.90
Athyrium distentifolium	Bean	monolete	Spinuluse	16-18	29.3	34.5
A. filix-femina	Elliptic	monolete	Rugulate	13-14	26.1	31.1
Azolla filiculoides	Bean	monolete	Ornate	-	19.45	45.82
Blechnum spicant	Bean	monolete	Rugulate	20-22	31.98	39.44
Asplenium officinarum	Circular	monolete	Ornate	17-19	36.54	44.04
Cystopteris fragilis	Bean	monolete	Spinule	16-18	31.12	41.05
C. regia	Bean		Spinule	21-22	16.19	39.51
Dryopterisaffinis	Elliptic	monolete	Ornate	15-16	28.13	35.87
D. carthusiana	Elliptic	monolete	Ornate	14-15	28.63	33.26
D. caucasica	Elliptic	monolete	Ornate	15-16	38.82	49.5
D. dillatata	Elliptic	monolete	Ornate	13-15	31.4	45.5
D. expansa	Elliptic	monolete	Ornate	22-24	30.1	37.4
D. filix – mas	Elliptic	monolete	Ornate	15-16	25	35
D. pallida	Elliptic	monolete	Ornate	15-17	20.40	27.44
D. remota	Elliptic	monolete	Verrucate	16-17	33.55	43.15
Matteucia struthiopteris	Bean	monolete	Ornate	-	55.21	70.50
Ophioglossum vulgatum	Circular	trilete	Perforate	14-16	31.42	31.47
Oreopteris limbosperma	Bean	monolete	Echinate	20-21	26.81	38.83
Asplenium scolopendrium	Bean	monolete	Spinuluse	21-23	24.28	34.03
Polypodium interjectum	Bean	monolete	Verrucate	10-11	48.82	65.09
P. vulgar	Bean	monolete	Verrucate	20-22	30.12	63.69
Polystiehum aculeatum	Bean	monolete	Spinuluse	23-24	23.41	35.22
P. braunii	Elliptic	monolete	Echinate	-	23.26	41.18
P. woronowii	Bean	monolete	Echinate- reticulate	19-20	29.99	33.99
Pteridium aquilinum	Elliptic	trilete	Rugulate	14-16	28.28	29.88
Salvinia natans	Bean	monolete	Ornate	-	35.15	44.48
Thelypteris palustris	Bean	monolete	Spinule	16-18	23.80	38.42
Woodsia aplina	Circular	monolete	Echinate	16-17	41.41	46.16

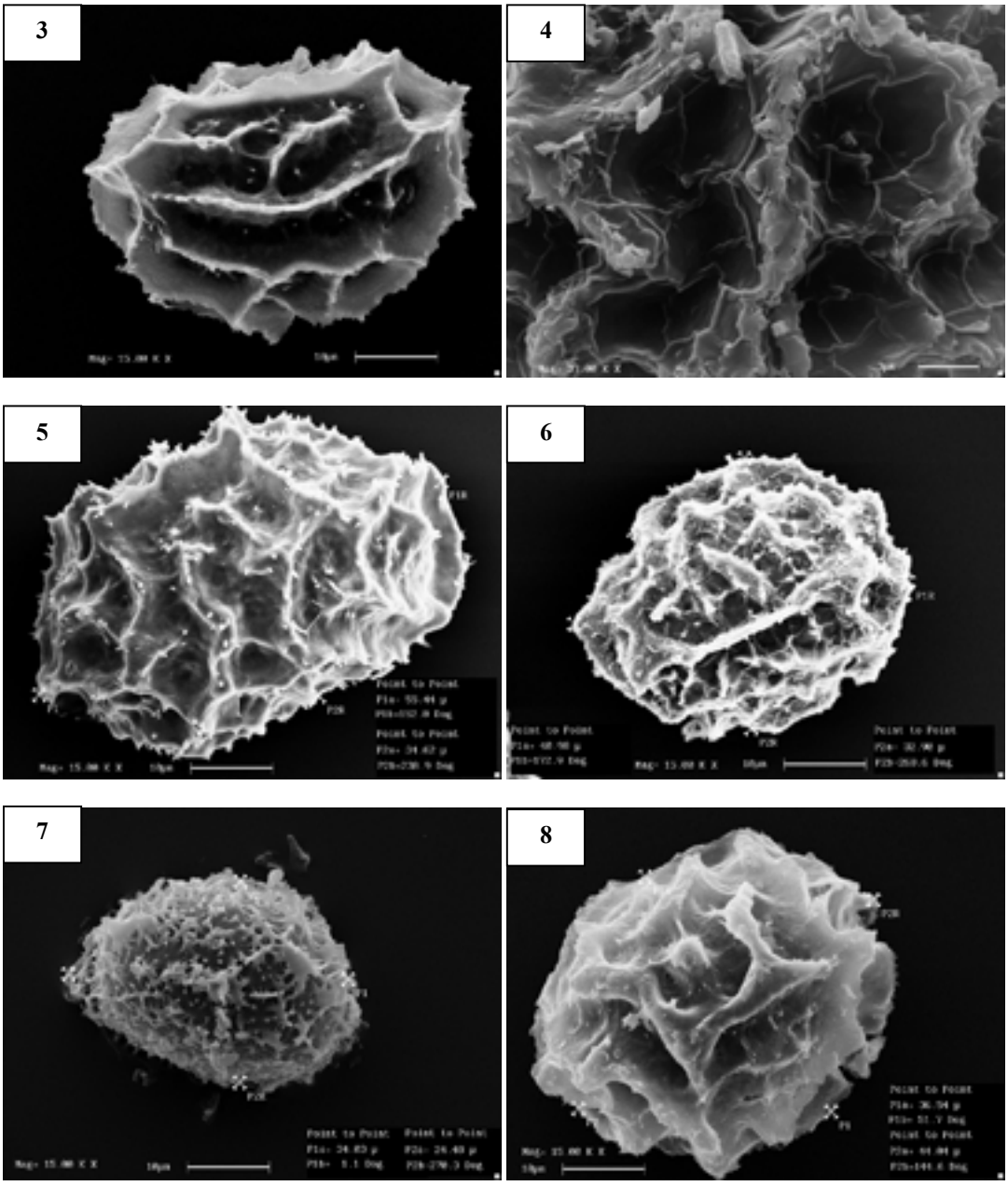
According to our observations, the spore sculpture could be useful for systematic purpose. *Polypodium vulgar* and *P. interjectum* have similarity specially in rhizome anatomy, orbicular sori and morphology of spore but annulus cells can separate them. Also, the spore ornamentation of could be useful for distinguishing of *Thelypteris palustris* from *Orepteris limbosperma*. This species was named *Thelypteris limbosperma* in the past but the shape and sculpture confirmed Mazooji porposed. He separated these two species using cross section of rhizomes anatomy and morphology.

However, the spore characters couldn't separate *Dryopteris* species very well. It seems that morphology and the kind of steles are better for distinguished them, because most of species are apogame and have similarity especially in frond. Ivanova and Mirkowa (2003) noted that *D. palliada* and *D. carthusiana* have similarity in chromosome numbers [14]. In our study, only *D. remota* is differed from the other species by used spore ornamentation.Aspleniaceae is one of the few families that are nearly homogenous by cytologyically and morphology. Most of them have linear sorus, real indusium and pinnate or pinnatifid frond. Based on this analysis, it is verified that these are an ultrastractural homogeneity of the *Asplenium* species spores. Also, spore characteristics could be useful for separate the *Polystichum* species especially similar species means of *P. aculeatum* and *P. worronowii.* Two species of *Cystopteris* are very similar by with the different mean of size.

4. CONCLUSION

Based on our results, the spore sculpture could be useful for systematic purpose, specially in Polypodiaceae, Thelypteridaceae and Hydrofilicinae ferns. However, the results derived from our studies point out the need of further researches on spore especially by using TEM in order to obtain more information about spore ultra structure.

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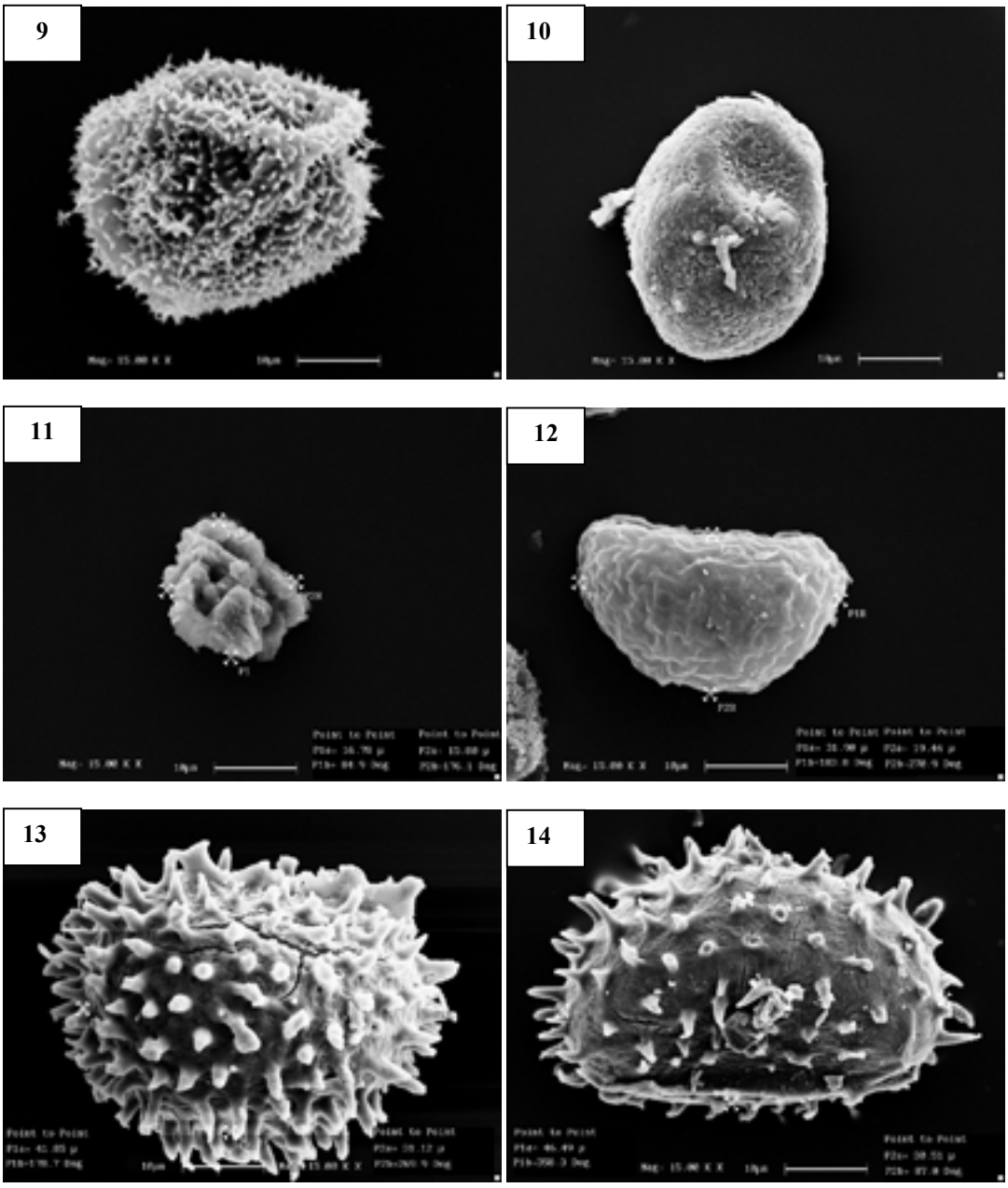
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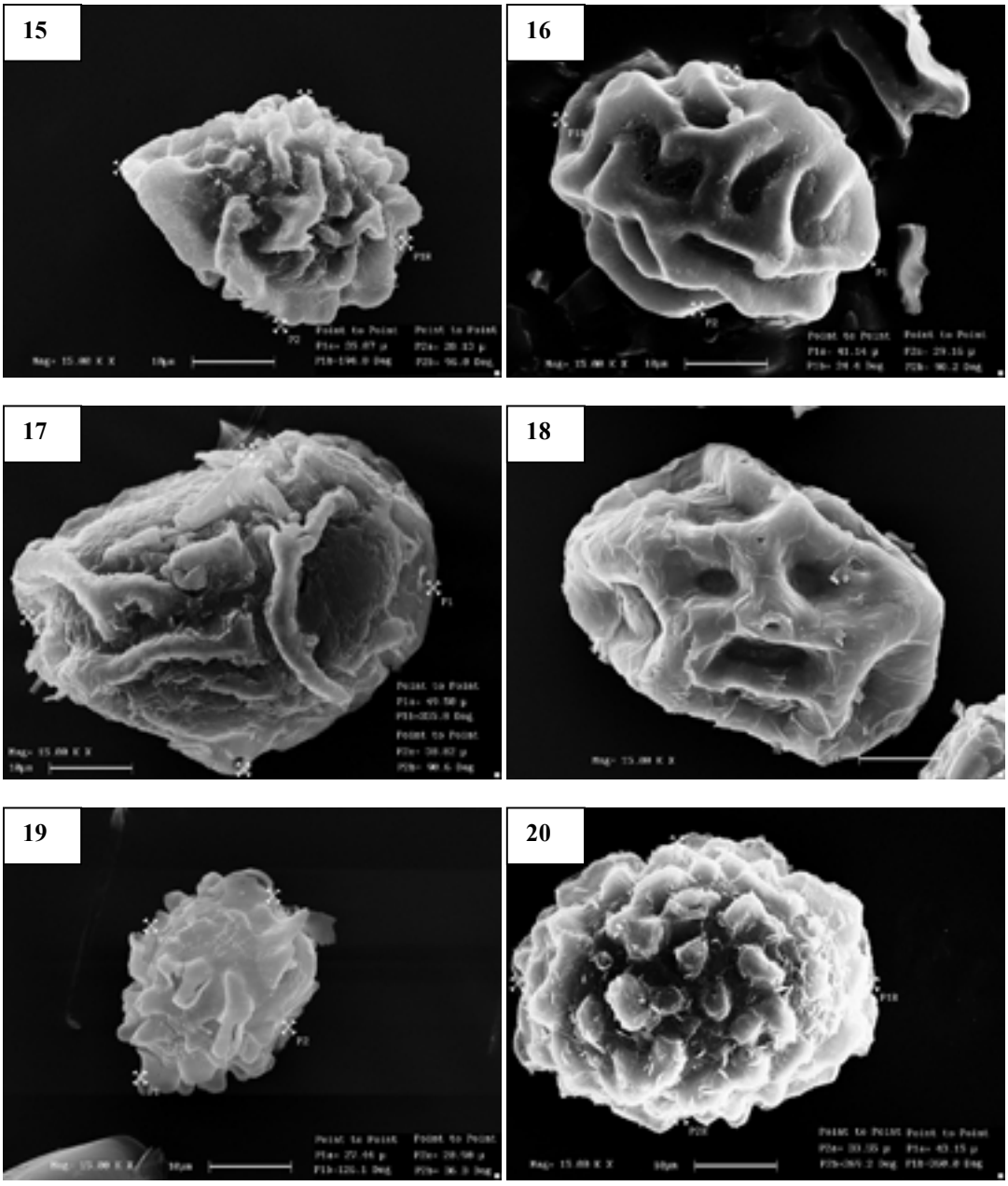
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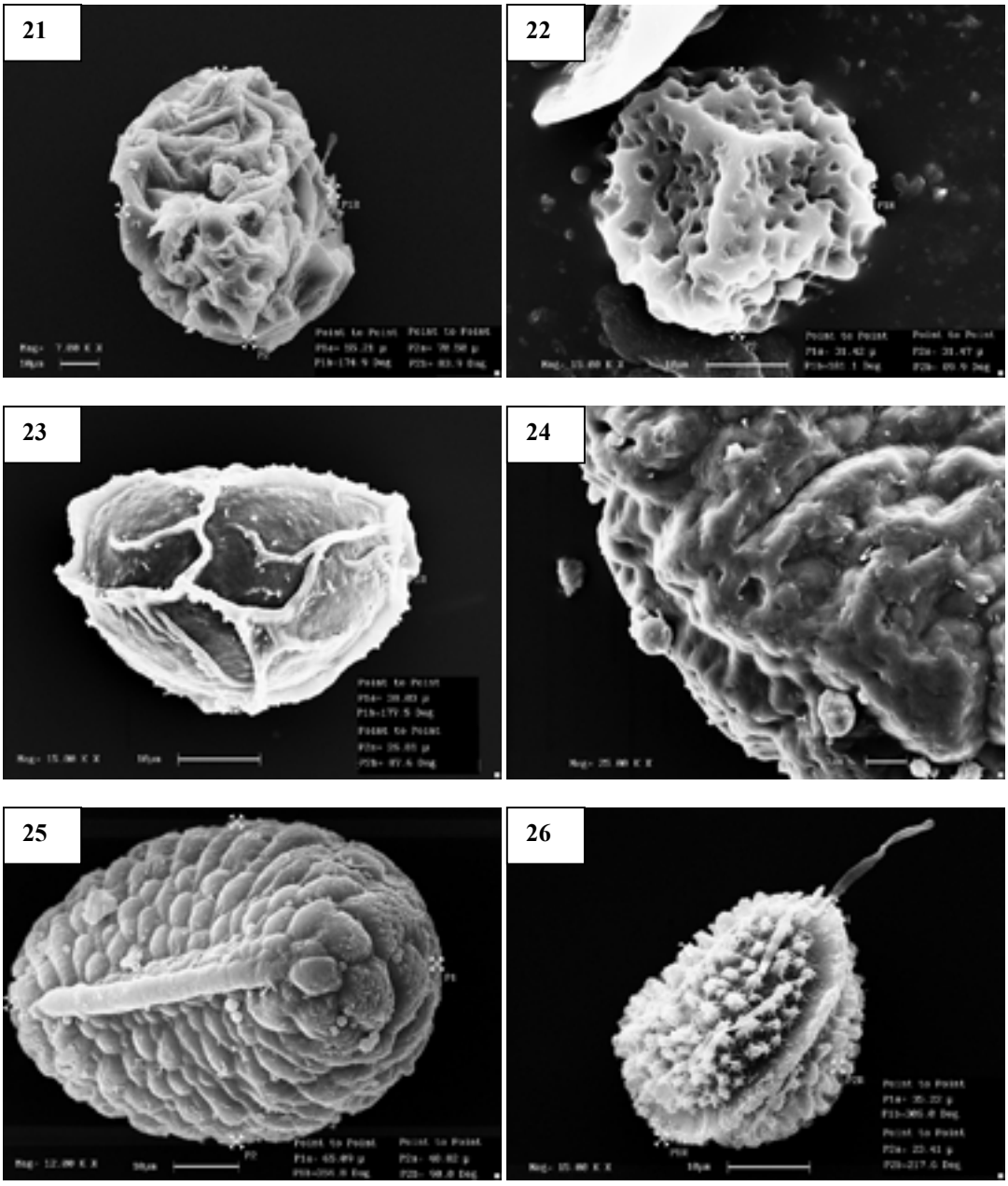
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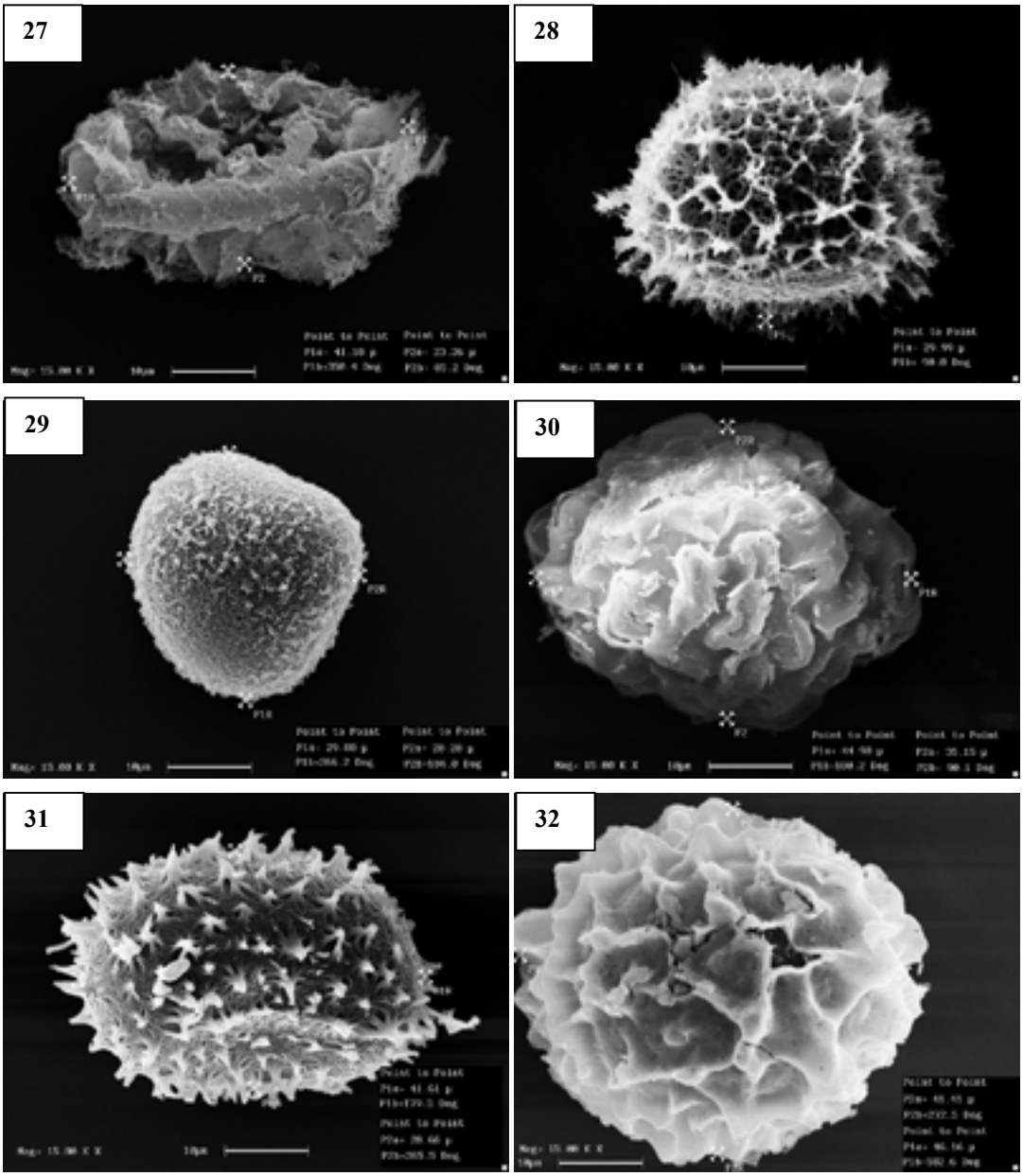

Fig. 1. Scaning electron microscopic photographs of spores (15000x, SEM).
1- *Adianthumcapilus-veneris*; 2- *Aspleniumadiantum-nigrum*; 3- *Aspleniumonopteris*; 4- *Aspleniumrutamuraria*; 5- *Aspleniumseptenterional*; 6- *Aspleniumtrichomanes*; 7- *Aspleniumscolopendrium*; 8- *Aspleniumceterach*; 9- *Athyriumdistentifolium*; 10- *Athyriumfilix-femina*; 11- *Azollafilicoloides*; 12- *Blechnumspicant*; 13- *Cystopterisfragilis;* 14- *Cystopterisregia*; 15- *Dryopterisaffinis*; 16- *Dryopteriscarthusiana*; 17- *Dryopteriscaucasica*; 18- *Dryopterisdillatata*; 19- *Dryopterispallida*; 20- *Dryopterisremota*; 21- *Matteuciastruthiopteris*; 22- *Ophioglossumvulgatum*; 23- *Oreopterislimbosperma*; 24- *Polypodiuminterjectum*; 25- *Polypodiumvulgare*; 26- *Polysticumaculeatum*; 27- *Polysticumbraunii*; 28- *Polysticumworronowii*; 29- *Pteridiumaquilinum*; 30- *Salvia natans*(microspore); 31- *Thelypterispalustris*; 32- *Woodsiaalpina*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mazooji A., Fallahian F, Nejadsatari T. Systematic study of fern in Northern parts of Iran. PhD Thesis. 2004.
2. Khosravi AR. Spore variation in Sinopteridaceae From Iran, Iran Jour. Bot.1998;7(2):207–213.
3. Cubasa P, Pardo C. Perispore structure in *P. setiferum*, *P. aculeatum* and their hybrid *P. xbicknellii*. American fern Journal. 1993;82(6):128–152.
4. Erdtman G, Sorsa P. Pollen and spore morphology/Plant taxonomy. Pteridophyta (test and additional illustrations). Almqvist and wiksell, Stockholm; 1971.
5. Tryon R, Lugardon B. Spores of Pteridophyta. Springer– Verlag, New york. 1991.
6. Denk TA. Record of *Cystopteris regia* from Iran, Iran Jour. Bot. 1998;7(2):254–267.
7. Giudice GE, Morbelli MA, Piñeiro MR, Copello M, Erra G. Spore morphology of the Polypodiaceae from North western Argentina, American fern Journal. 2004;94(1):9–27.
8. Ramos Giacosa JP, Morbelli MA, Giudice GE. Morphology and ultrastructure of the spores of the Grammitidaceae from Argentina, Review of palaeobotany and palynology. 2007;143:155–166.
9. Jermy C, Josephine C. The illustrated field guide to ferns and allied plants of the British isles. London, Published by HSMO.1993;
10. Wannachai C, Thaweesakdi B, Bernard RB. Phenetic relationship between *Lepisorus* (J.sm.) Ching (Pteridophyta, Polypodiaceae) and its related genera. Bangladesh Journal of Plant Taxonomy. 2009;16(2):99-113.
11. Lin SJ, Kato M. Iwatsuki K. Spore morphology of the fern Genus *Lindsaea.* Journal of Japanese Botany. 1999;74(6):353–366.
12. Oliver JC. A study of spore characteristics of the ferns of Indiana, American fern Journal. 1968;58(1):5–12.
13. Moore PD, Webb JE, Elinson ME. Pollen analysis, 2nd edition. Blackwell Scientific; 1991.
14. Ivanova D, Mirkowa HP. Chromosomes numbers of Polish Ferns. Acta Biologia Cracoviensia series Botanica. 2003;45(2):93-99.

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