

# Palynological Studies of Some Species of Aspleniaceae-Pteridophyta

Gamal M. A. Lashin

Department of Botany, Faculty of Sciences, Zagazig University, Zagazig, Egypt.  
Email: gamalsabaa@yahoo.com

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## ABSTRACT

Palynology of six fern species belonging to the family Aspleniaceae from Saudi Arabia are investigated by light microscope (LM). These are: *Asplenium aethiopicum*, *Asplenium trichomanes*, *Asplenium adiantum-nigrum*, *Asplenium* sp.1, *Asplenium* sp.2 and *Ceterach officinarum*. All studied spores are oblate-spheroidal in shape class, with 11 - 50  $\mu\text{m}$  in equatorial diameter and with 12 - 49  $\mu\text{m}$  in polar diameter. Two types of spores are recognized: monolete spores in *Asplenium aethiopicum*, *Asplenium trichomanes*, *Asplenium adiantum-nigrum*, *Asplenium* sp.2 and trilete spores in *Asplenium* sp.1 and *Ceterach officinarum*. The exospore (perine) is homogeneous and apparently double-layered in all the studied spores. The perispore is single or double-layered with a microlacunose structure. The perispore layers can be distinguished by their different contrast, structure and thickness. The exospore sculpture varies from cristates-ridged (*Asplenium aethiopicum*), cristates granulate (*Asplenium adiantum-nigrum*), cristates scabrate (*Asplenium trichomanes*), regulate-scabrates (*Asplenium* sp.1 and *Ceterach officinarum*) to papillate or tuberculate sculpture (*Asplenium* sp.2). Comparison between the earlier studies of some species from Saudi Arabia is given. General characteristics like spore type, ornamentation and laesurae features as well as the number of exospore layers could be useful for taxonomy of Aspleniaceae.

**Keywords:** Aspleniaceae; Ferns; Palynology; Pteridophyta; Saudi Arabia

## 1. Introduction

The Aspleniaceae (spleenworts) is a family of ferns, included in the order Polypodiales or in some classifications as the only family in the order Aspleniales. All Members of this family have intramarginal, linear sori with a flap-like indusium arising along one edge [1]. Most pteridologists today consider this family as consisting of just one to three genera. Others still maintain segregate genera such as *Phyllitis* and *Ceterach*; however, the species segregated into these genera all hybridize readily with undisputed *Asplenium* species. A phylogenetic study of Aspleniaceae, shows that species segregated as *Camptosorus* and *Neottopteris* are nested within *Asplenium* [2]. It also recommends that they be included in that genus, but suggests that *Hymenasplenium* (including *Boniniella*) and *Phyllitis* are distantly related to other *Asplenium* species and should be recognized at the generic level. The type species for the genus is *Asplenium marinum*. Some botanists treat Aspleniaceae as comprising a single genus, *Asplenium*, but up to nine small segregate genera are recognized by other botanists [2,3]. Many groups of species have been separated from *Asplenium* as segregate genera. These include *Ceterach*,

*Phyllitis*, and *Tarachia*, but these species can form hybrids with other *Asplenium* species and because of this are usually included in a more broadly defined *Asplenium* [4,5]. The newer classifications place it in the subordinal group called eupolypods within the order Polypodiales. *Asplenium* is one of the largest fern genera, of about 700 species, distributed worldwide from Greenland and Europe to South America and New Zealand [6]. It occurs in a wide variety of exposed or partly shaded habitats, e.g., on rocks, in low-altitude semi-deciduous woodland, wet evergreen forest and (sub) Montana rain forest. Various segregates have been proposed (e.g. *Camptosorus*, *Phyllitis*, *Ceterach*, *Pleurosorus*), but numerous “intergeneric” hybrids occur. The most unusual allopolyploid phytogeographically is *Asplenium adiantum-nigrum*, the parents of which are known only in the Old World [7]. Polyploidy is widespread in *Asplenium* and the chromosome numbers vary from  $2\times$  to  $6\times$ . Two species, *Asplenium trichomanes* and *A. heterochroum*, occur in different levels of polyploidy— $2\times$  and  $4\times$ , and  $4\times$  and  $6\times$ , respectively. The highest chromosome number known for *Asplenium* in North America is  $2n = 216$  (in *A. trichomanes-dentatum* and the hexaploid form of *A.*

*heterochroum*) [2,3]. Mickel [8] mentioned that the spore characteristics were useful to differentiate some genera and he compared to other spore-bearing vascular plants. Spores of *Asplenium* are monolete; perispore typically winged, spiny, reticulate, or perforate.

This contribution is part of the study of Pteridophyta spores that grow in South West Saudi Arabia. The work aims to provide palynological data, as a complement to the general knowledge of the species [9,10]. The aim of this study is to investigate the spore morphology and wall ultrastructure of six species of family Aspleniaceae growing in the South West Saudi Arabia by using LM. The results will be used for the general morphology, and the ultrastructure studies to see if the spore characteristics could be used successfully for systematic purposes.

## 2. Materials and Methods

Spores were obtained from herbarium specimens of biological department, Faculty of Sciences, King Khaled University, **Table 1**. The plant specimens were identified according to [11-13]. Spores of the different specimens were studied by using light microscope (LM). For LM the spores were treated with 3% hot sodium carbonate for 2 min and acetolyzed according to [14,15]. All the observations were performed with Olympus BH2 and BHB light microscopes. Slides housed at the biological department, Faculty of Sciences, King Khaled University.

## 3. Results

The Fern spores examined are monolete type (four species) and trilete types (two species). Spore colour is brown, yellow, or black-brown. Various types of spore surface are found including cristate, folded, granulate, papillates and scabrates or some combination of these features. The spores produced by the selected species are spheroidal-shape, 11 - 50  $\mu\text{m}$  in equatorial diameter and 12 - 49  $\mu\text{m}$  in polar diameter, the exospore is 0.5 - 3  $\mu\text{m}$  thick, smooth and the perispore is 0.3 - 1  $\mu\text{m}$  thick and ornamented (**Tables 2 and 3, Figures 1 and 2**).

## 4. Spore Descriptions

1) *Asplenium aethiopicum* (Burm. P.) Bech.

*Acrostichum filar* Forssk.

*Asplenium filar* Forssk.

*Asplenium lanceolatum* Forssk.

Spores are monolete, pteroidal, sometimes large and compressed, with an irregularly cristate perine (exospore) and winged folds sometime with few ridges. Spores are light-brown or yellowish in colour. The spores averaged and varied in size from 32.5 - 35  $\mu\text{m}$  in polar diameter and 35 - 37.5 - 40  $\mu\text{m}$  in equatorial diameter (**Figure 1(a)**,  $\times 1000$  and see **Table 2**). The sori and the underside of the frond are covered with reddish scales on the young fronds and 1 - 3 cm length.

**Table 1. The sites of collection of the studied species.**

Family	No.	Species	Localities	Herbarim No.
Aspleniaceae	1	<i>Asplenium aethiopicum</i> (Burm. P.) Bech.	Aqbet al-samma	138-81
	2	<i>Asplenium adiantum-nigrum</i> L.	Al-Suoda-road	138-83
	3	<i>Asplenium trichomanes</i> L.	Al-Suoda-road	138-82
	4	<i>Asplenium</i> sp.1	NW. Abha	138-84
	5	<i>Asplenium</i> sp.2	Al-Mukadda-sauda	138-85
	6	<i>Ceterach officinarum</i> Willd.	Al-Suoda-road	138-75

**Table 2. Palynological features of the studied species.**

No.	Species	P. $\mu\text{m}$	E. $\mu\text{m}$	P/E ratio	Exospore (perine) thickening $\mu\text{m}$	Sculptures of exospore	Shape types
1	<i>Asplenium aethiopicum</i>	32.5 - 35	35 - 37.5 - 40	0.9	4.0 - 5.0	cristates	monolete
2	<i>Asplenium trichomanes</i>	20 - 22.5	20 - 22.5	1.0	2.5 - 2.3	cristates scabrates	monolete
3	<i>Asplenium adiantum-nigrum</i>	20 - 25 - 30	25 - 27.5 - 32.5	0.86	2.3 - 2.5	Cristates granulate	monolete
4	<i>Asplenium</i> sp.1	20 - 22.5 - 32.5	20 - 30 - 37.5	0.85	2.5 - 3.8	regulate scabrates	Trilete
5	<i>Asplenium</i> sp.2	37.5 - 42.5 - 49	35 - 42.5 - 50	1.01	2.4 - 2.5	papillate to tuberculate	monolete
6	<i>Ceterach officinarum</i>	12 - 14	11 - 12 - 13 - 14	1.04	1 - 1.5	Cristates granulate	Trilete

**Table 3. Comparison between the studied specimens (2) and the specimens studied by AL Shehri, 2002 (1). (+) Species studied before but no data, (-) species not studied before.**

Species	<i>Asplenium aethiopicum</i>		<i>Asplenium trichomanes</i>		<i>Asplenium adiantum-nigrum</i>		<i>Ceterach officinarum</i>		<i>Asplenium</i> sp.1	<i>Asplenium</i> sp.2
	1	2	1	2	1	2	1	2		
Diameter size $\mu\text{m}$	70 × 50	33 × 38	50 × 53	21 × 21	+	25 × 29	80 × 90	11 × 14	-	-
Shape class	prolate	subspheroidal	kidney shape	spheroidal	+	subspheroidal	spherical	spheroidal	-	-
Shape type	monoporates or monosulcates	monolates	monosulcate	monolates	+	monolates	Monoporates or monosulcates	triletes	-	-
Exospores sculptures	nearly regulates	crustates	irregular reticulates	crustates scabrates	+	Crustates granulate	Regulates reticulates	Crustates granulate	-	-

### 2) *Asplenium adiantum-nigrum* L.

*Asplenium adiantum-nigrum* L. (= *A. lucidum* Burm.f.)

*Asplenium adiantum-nigrum* ssp. *adiantum-nigrum* (Tausch) Koch.

*Asplenium adiantum-nigrum* ssp. *serpentina* (Tausch) Koch.

Black spleenwort (*A. adiantum-nigrum*), Spores are monolet, oblate-spheroidal, sometimes large and compressed, with an irregularly granules to cristate perine and winged folds with a few ridges. Spores are brown and varied in size from 20 - 30  $\mu\text{m}$  in polar diameter and 25 - 32.5  $\mu\text{m}$  in equatorial diameter (**Figures 1(b)**,  $\times 1000$  and see **Table 2**). The sori and the underside of the frond are covered with brownish scales on the young fronds and 1 - 2 cm in length.

### 3) *Asplenium trichomanes* L.

*Asplenium trichomanes* ssp. *quadrivalens* D.E. Meyer.

*Asplenium trichomanes* ssp. *trichomanes* L.

*Asplenium trichomanes* subsp. *coriaceifolium* D.E. Meyer.

Spores are monolet, spheroidal, sometimes large and compressed, with an scabrates to cristate perine and wavy folds with a few ridges. Spores are brown or yellowish colour. The spores averaged and varied in size from 20 - 22.5  $\mu\text{m}$  in polar diameter and 20 - 22.5  $\mu\text{m}$  in equatorial diameter (**Figure 1(c)** and see **Table 2**). The sori are covered with reddish scales on the young fronds and 1 - 4 cm in length.

### 4) *Asplenium* sp.1

Spores are trilete, oblate-spheroidal, sometimes large and compressed, with a regularly scabrates exospores and winged folds are absent. Non-perinate, smooth or slightly granulates. Spores are light-brown or yellowish and varied in size from 20 - 32.5  $\mu\text{m}$ . in polar diameter and 20 - 37.5  $\mu\text{m}$ , in equatorial diameter (**Figure 2(a)**,  $\times 1000$  and see **Table 2**). The sori and the underside of the frond are covered with yellowish brown scales on the young fronds and 1 - 3 cm in length.

### 5) *Asplenium* sp.2

Spores are monolet, sub-spheroidal, sometimes large

and compressed, with an irregularly papillate to tuberculate perine and wavy folds. The spores averaged and varied in size from 37.5 - 49  $\mu\text{m}$  in polar diameter and 35 - 50  $\mu\text{m}$  in equatorial diameter (**Figure 2(b)**,  $\times 1000$  and see **Table 2**). The sori and the underside of the frond are covered with brownish scales on the young fronds and 1 - 3 cm length.

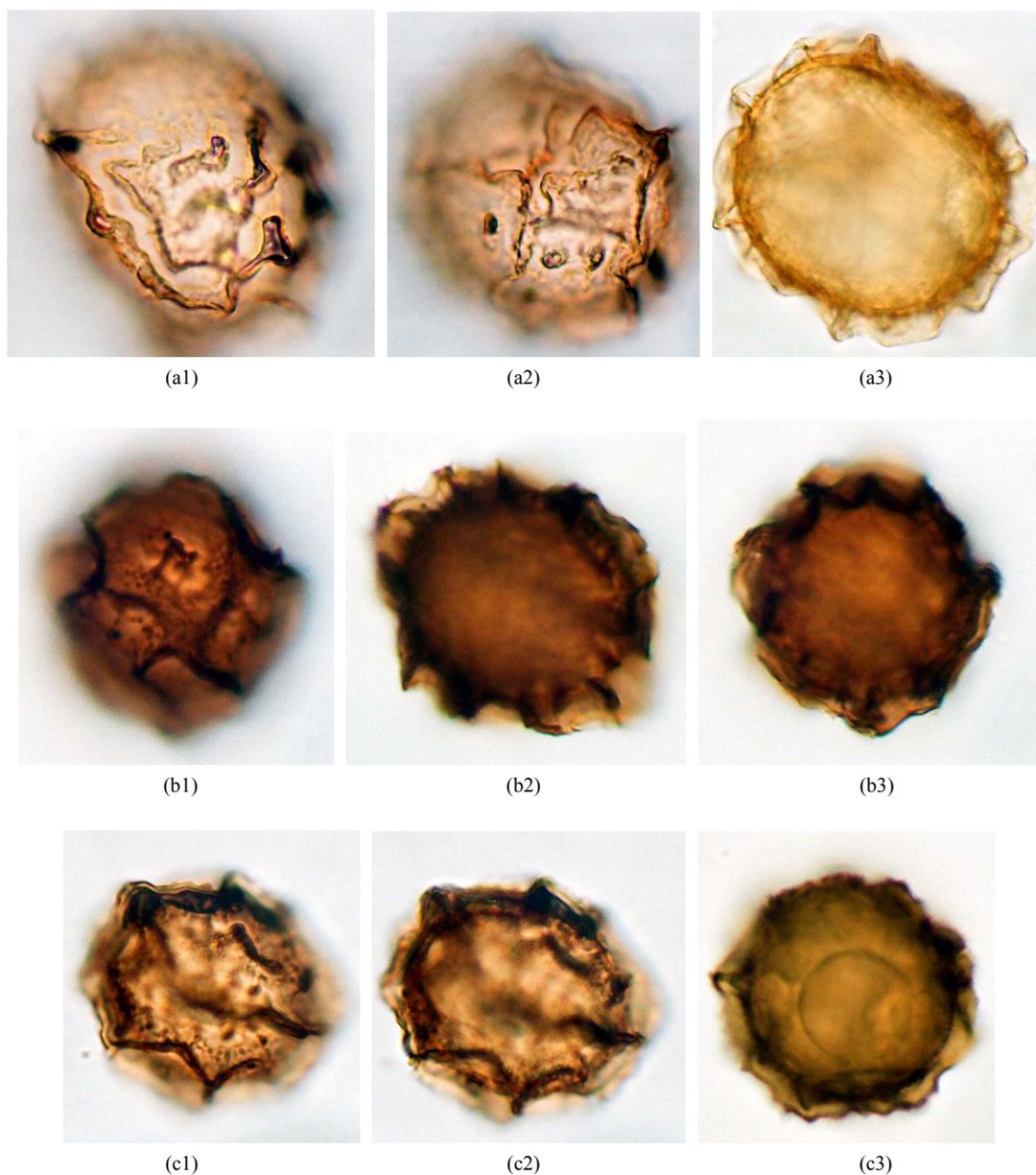
### 6) *Ceterach officinarum* Willd.

*Asplenium ceterach* Willd.

Spores are trilete, sub-spheroidal, sometimes large and compressed, with an irregularly papillate to tuberculate perine and winged folds. The spores averaged and varied in size from 12 - 14  $\mu\text{m}$  in polar diameter and 11 - 14  $\mu\text{m}$  in equatorial diameter (**Figure 2(c)**,  $\times 1000$  and see **Table 2**). The sori and the underside of the frond are covered with silvery scales on the young fronds which become rusted coloured with age and 1 - 3 cm in length.

## 5. Discussion

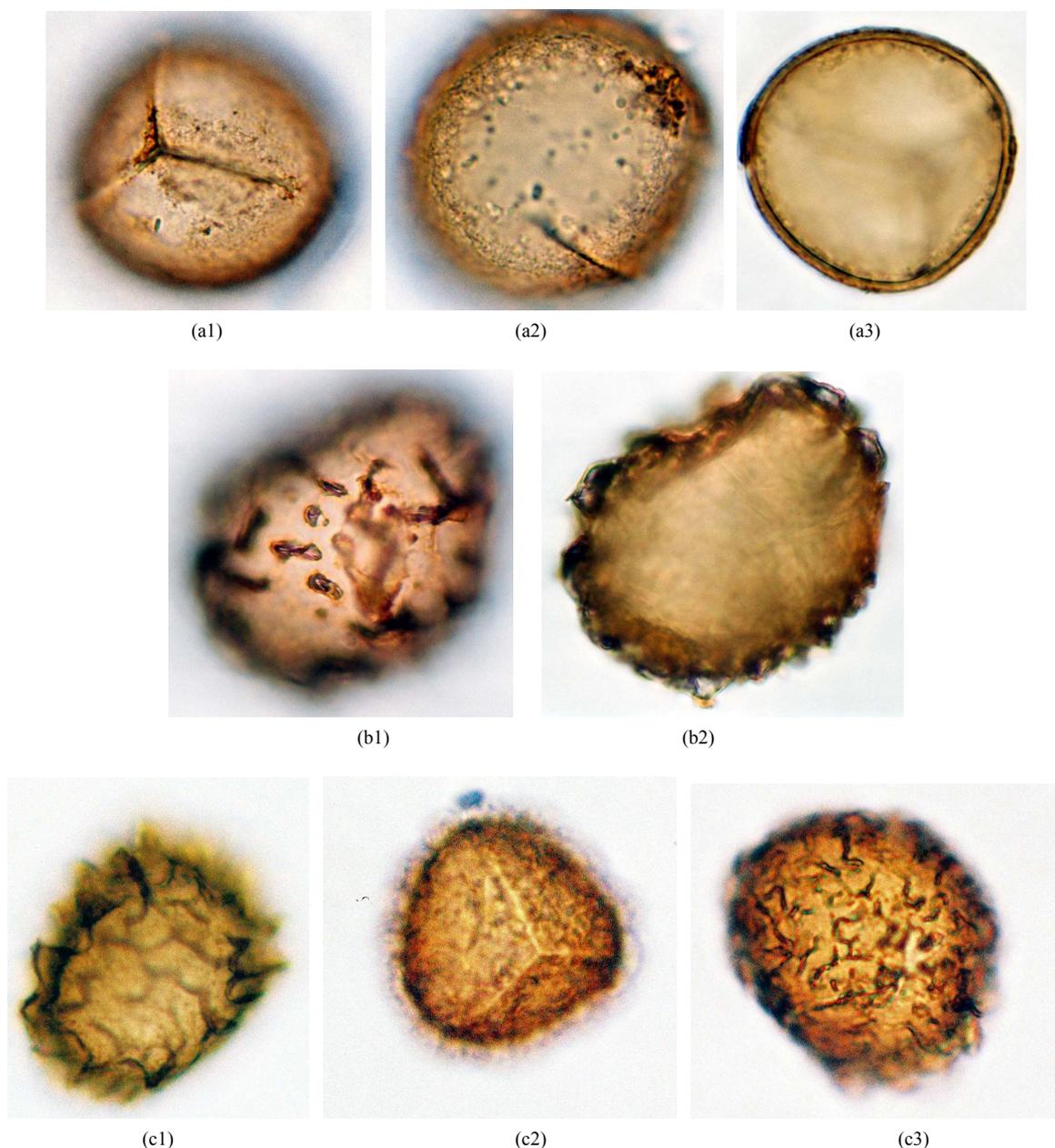
*Asplenium* species present in southern West Saudi Arabia produce ridged or cristate ridged spores. These observations are agree with those of several studies for the species spores of the New World [16-19] and for species of the Old World [3,6,20-22]. According to the present observations, the spores sculpture in the six taxa analysed is formed by the outer perispore layer and characteristics of the ridges such as their form, margin and fusion could be useful for systematic purposes. *Asplenium aethiopicum* spores are monolet in shape type, cristate perine sculptures, winged ridges and characteristic by very thick exospores wall 4.0 - 5.0  $\mu\text{m}$ . According to pervious works there many differences as in **Table 3**, spores are monoculcates and regulate sculptures [23]. *Asplenium adiantum-nigrum* spores are monolet in shape type but its characters has no previous records [23]. But according to [24] he studied the Chinese variety (*Asplenium adiantum-nigrum* var. *yuanum* (ching) Ching), he found that the sculptures was alar-lophate and echinate used SEM observations. The size diameters was 30.1 - 46.4  $\mu\text{m}$  but in our specimens the sculptures are cristate-granulates



**Figure 1.** (a) *Asplenium aethiopicum* (Burm. P.) Bech. ((a1)-(a3)),  $\times 1000$ , (a1) Equatorial view showing cristate sculptures; (a2) Polar view showing monolete mark; (a3) Equatorial view showing folded perine; (b) *Asplenium adiantum-nigrum* L. ((b1)-(b3)),  $\times 1000$ , (b1) Equatorial view showing cristate sculptures, and monolete mark; (b2) and (b3) Equatorial view showing folded perine; (c) *Asplenium trichomanes* L. ((c1)-(c3)),  $\times 1000$ , (c1) Equatorial view showing cristate and verrucate sculptures; (c2) Polar view showing monolete mark; (c3) Equatorial view showing folded perine.

and the size diameter is smaller than the Chinese variety (20 - 32  $\mu\text{m}$ ). There are few references about the structure of the exospore in *Asplenium*. [25] described the exospore in *Asplenium adiantum-nigrum* L. as homogeneous, granular or completely undifferentiated. *Asplenium trichomanes* spores are monolete cristate-scabrate and very small in size. By [23], the spores were monoculcate and reticulate sculptures, size  $21 \times 21 \mu\text{m}$  in diameter. The Chinese species according to [24], the sculptures alar-lophate and size, 24.3 - 34.3  $\mu\text{m}$  were medium. *As-*

*plenium* sp.2 not described by [23], but in general characters the species belong to genus *Asplenium*. *Ceterach officinarum* spores are trilete in shape type and it is the main difference between the genus *Asplenium*. Because all the species of *Asplenium* are monolete type [6,22]. I agree with the separation of *Ceterach officinarum* species from the genus *Asplenium* and I don't agree with opinion which fixes it under *Asplenium officinarum*. In [23], the size of *Asplenium officinarum* is very large (80 - 90  $\mu\text{m}$ ) but in present studies it is very small 11 - 14  $\mu\text{m}$  in di-



**Figure 2.** (a) *Asplenium* sp.1 ((a1)-(a3)),  $\times 1000$ , (a) Polar view showing trilete mark; (b) Equatorial view showing verrucate sculptures; (c) Polar view showing exospore thickness; (b) *Asplenium* sp.2 ((b1) and (b2)),  $\times 1000$ , (b1) Polar view showing papillate to tuberculate sculptures and monlete mark; (b2) Equatorial view showing perispore thickness; (c) *Ceterach officinarum* Willd. ((c1) and (c2)),  $\times 1000$ , (c1) and (c2) Equatorial view showing cristate sculpture; (c3) Polar view showing trilete mark and exospore thickness.

ameters. By [24], the sculptures fibrate-alate and the size was medium between Chinese and Al-shehri species. *Asplenium* sp.1 spores are trilete in shape it could better put under the genus *Ceterach*. [26], described the exospore in Aspleniaceae as blechnoid type. The perispore ultrastructure in the species examined here are compared with those other genera of Filicophyta in former studies [1,23], considered the perispore structure in *Asplenium serra* as pillared and pointed out that this particular

structure resembled the columellate characters, typical of the Angiosperm pollen exine. The ultrastructure of *Asplenium serra* was found similar to that of *Oleandra articulata* (Sw.) Pr. (Oleandraceae) according to the records which given by [6]. With regard to the perispore layer observed here in the present specimens [26,27] was described in some species of Aspleniaceae that layer with similar characters. Studies of spore development are needed in order to understand the sequence of events that

determine the formation of the perispore layers and strata as well as to understand their possible functions (formation of the gametophyte generation).

## 6. Conclusions

1) *Cetraech officinarum* is trilete spores for this reasons it's disputed to put under the genus *Asplenium* (*Asplenium officinarum*), because *Asplenium* species are monolete spores as recorded before. 2) *Asplenium* sp.1 also is trilete in spores type it could be under the genus *Cetraech* for the same reasons. 3) All features and main ferns spores characters which recorded before by [23] need more revision according to the main and the principles terminology of the Palynologists.

The characteristics shared by all the species are: 1) the general structure and stratification of the exospore; 2) the presence of cristate sculptures on the exospore at the inner part of the perispore.

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