SPORE MORPHOLOGY OF CERTAIN MOSSES OF NORTHERN TEHRAN-IRAN: TAXONOMICAL AND ECOLOGICAL IMPLICATIONS

R. Khoshravesh & Sh. Kazempour Osaloo

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During the bryophyte floristic study of northern Tehran, spore morphology of 15 moss species belonging to families *Amblystegiaceae, Brachytheciaceae, Encalyptaceae, Funariaceae, Grimmiaceae, Mniaceae, Orthotrichaceae,* and *Pottiaceae* were examined and photographed by scanning electron microscopy. Spore shape of all the studied species was spheroid. The spore size was ranging from 7.50 (±0.06) × 8.07 (±0.06) µm in *Schistidium flaccidum* to 35.84 (±3.67) × 37.51 (±4.55) µm in *Encalypta intermedia*. No sulcus was detected on the exine surface of the examined species. Based on surface ornamentation, the spores were divided into six following types: verrucate, scabrate-gemmate, gemmate, pilate, retipilate and rugulate. The examined mosses species are belonging to two habitat types: saxicolous type and terrestrial type. The taxonomical and ecological implications of the spore morphology of the species were discussed.

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ریخت‌شناسی هاگ در برخی گونه‌های خزه‌ای شمال تهران، ایران

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طی بررسی فلوریستیک خزگیان ارتفاعات شمال تهران، ریخت‌شناسی هاگ در ۱۵ گونه از خزه‌های منطقه متعلق به خانواده‌های *Amblystegiaceae*، *Brachytheciaceae*، *Encalyptaceae*، *Funariaceae*، *Grimmiaceae*، *Mniaceae*، *Orthotrichaceae* و *Pottiaceae* با میکروسکوپ نوری و الکترونی نگاره مورد مطالعه قرار گرفتند. شکل هاگ در کلیه گونه‌های مورد مطالعه گرد بود و اندازه آنها از (±۰/۰٦) ۸/۰۷ × (±۰/۰٦) ۷/۵۰ میکرومتر در *Schistidium flaccidum* تا (±٤/۵۵) ۳۷/۵۱ × (±۳/٦۷) ۳۵/۸٤ در *Encalypta intermedia* متغیر است. در سطح اگزین هیچ یک از گونه‌ها شیار و یا منفذ مشاهده نشد. بر اساس آراستار سطح اگزین، هاگ‌ها به شش نوع تقسیم می‌شوند: انواع زگیلی (verrucate)، جوانه‌ای-سمباده‌ای (scabrate-gemmate)، جوانه‌ای (gemmate)، چوگانی (pilate)، چوگانی شبکه‌ای (retipilate) و چین خورده‌ای (rugulate). گونه‌های مطالعه شده به دو نوع رویشگاه صخره زی و خاکزی تعلق دارند. اهمیت تاکسونومیکی و بوم‌شناختی ریخت‌شناسی هاگ گونه‌ها مورد بحث قرار گرفت.

Introduction

Spore exine ornamentation in bryophytes is similar to that of pollen grains in higher plants. The spore morphology is of taxonomical, ecological and paleobotanical values. Because of the same rule in preventing desiccation, chemical composition of spore exine, it is comparable with that of pollen exine (Faegri et al. 1989). As in the seed plants, where the exine plays an important role to protect the male gametophyte during its journey between anther and stigma, in bryophytes (and pteridophytes) it also protects gametophyte tissue while its dispersal from the sporophyte to a moist location suitable for germination.

Despite these functional similarities, there are some differences in their structures. Pollen grains of gymnosperms and angiosperms appear to have similar wall stratification (layers have been tentatively assigned to sexine and nexin), but spores of pteridophytes and bryophytes do not resemble structurally. The exine of their spores often appears to be laminated throughout their thickness and there is no layer containing columella. As there is no sexine and nexine division, any aperture that they possess cannot be assigned strictly to colpi and pori, as these are sexine features (Moore et al. 1991). However, Boros et al. (1993) reported monolete spores and described sexine and nexine features in some European bryophytes.

Moss spores are typically single-celled but in some cases, the spores can be multicellular (Buck and Goffinet 2002). The tetrad form is somewhat more characteristic for liverworts but most of moss spores emerged from the spore mother cells as monads. In contrast to tetrad form, that they are generally tetragonal or rhomboidal, non-united spores are most frequently radiosymetric and are spheroid in shape (Boros et al. 1993). For the most part, moss spores are papillate, but ranging from essentially smooth to highly rigid, spiny or reticulate. Strongly ornamented spores are often large and associated with cleistocarpous capsules. Spores are ranging in size from 5μm to over 100μm (Buck and Goffinet 2002). Spore output per sporophyte is in the range of 500000-600000 in many moss species with spores of 15-25 μm in diameter. In mosses with somewhat larger spores, numbers are in the range of 5000-10000 per sporophyte (During and Van Tooren 1987). Among the bryophytes, few members like the moss belonging to the family *Splachnaceae*, produce sticky spores that are dependent on flies for their dispersal. In contrast, many bryophytes disperse over great distances by wind (Tan and Pocs 2002). Few large sized spores (>25μm) are of short-range dispersal than many small sized spores (<25μm) providing many light spores to increase chance of successful dispersal (During 1992). The smaller spores are more easily transported over longer distances by wind, while larger spores will establish more easily once they have arrived at a locality (Söderström and During 2005).

Identification of bryophyte spores is rather difficult, because few morphological works with adequate illustrations are available. Only few works of these kinds have appeared since 1990. Illustration, identification and description of some European bryophyte spores are recently published (Boros et al. 1993). Main studies are about spores of a taxonomic group (like *Dicranaceae* species of Brazil, Pereira Luizi-Ponzo and Monika Barth 1999), or works on life strategy and dispersal patterns of bryophytes (During and Van Tooren 1987; During 1992; Taylor 1995; Gao et al. 2000; Tan & Pocs 2002). The spore size and dispersal chance [described by During's (1992)] are considered by many sociological and conservational researches. Three ecological traits, light, temperature and soil acidity, in addition to four life-history traits including minimum spore size, life expectancy, types of gametophyte and papillose leaf cell walls are well used for predicting distribution range of mosses that have an important function to estimate future ecological disturbance and bryophytes conservation. (Kürschner 1999, 2004; Vanderpoorten and Engels 2002; Söderström and During 2005).

The Iranian bryophyte spores are completely unknown yet. In spite of having ca. 440 bryophyte species in 149 genera and 57 families (Akhani and Kürschner 2004) and suitable habitat for growing at least on floor and tree trunk of the Hyrcanian forests in north of Iran, bryology in Iran is in the infancy stage. This study is therefore, an attempt to examine spore morphology of certain mosses inhabited in southern slops of northern Tehran (Iran) in relation to their taxonomy and ecology.

Materials and Methods

Spore of 15 species were collected from natural habitat and the voucher specimens of the examined species were deposited in Tarbiat Modares University Plant Collection. List of spore examined by SEM are given in the Table 1. Mature capsules used as source of spores. Since the acetolysis method has been reported as causing swelling, all spores were studied without the acetolysis. Howevere reserving the specimens in ethanol 96% for the dehydration was necessary for all of them (Moore et al. 1991). Capsules transferred into ethanol 96% three weeks before the examination. Then capsule walls were physically crashed and spore solutions were divided into two parts. One portion transferred to glycerin jelly as reference slide and deposited at plant biology lab in Tarbiat Modares University. The remaining part transferred on stubs and dried in vacuum, then coated by gold with a sputter coater (model SCDOOS). All spores examined using scanning electron microscopy (Philips XL30) and photographed in two scales, general view and close-up view. For each species, at least 20 spores were measured under light microscopy. In addition, standard error was calculated for spore measurements. Because polar axis and equatorial diameter were not detectable for studied spores, the long diameter/short diameter ratio were measured instead of P/E for each spore and spore shape identified based on the Erdtman’s system

Table 1: Species examined in this study

Species	Voucher specimens & Herbarium[a]
Amblystegium serpens (Hedw.) Bruch, Schimp. & Gümbel	Tehran, Darband valley, in beginning of Shirpala way, around the coffees, Apr.26. 2005, Khoshravesh (TMUPC 169).
Brachythecium velutinum Bruch, & W. Gümbel.	Tehran, Darband valley, Near the small waterfall before Shirpala station, on dry rocks N: 35˚ 50′ 50″ E: 51˚ 25′ 33″ Alt. 2622, Aug. 20. 2004, Khoshravesh (TMUPC50). - Darband valley, around the spring before Shirpala station on way toward Shirpala, on dry rocks N: 35˚ 51′ 11″ E: 51˚ 25′ 40″ Alt: 2620m, Apr.15. 20, Khoshravesh (TMUPC 108).
Brachythecium collinum (Schleich. ex C. Müll.) Bruch, Schimp. & Gümbel.	Tehran, Darband valley, before the spring before Shirpala station on way toward Shirpala, on moist rocks N: 35˚ 51′ 08″ E: 51˚ 25′ 67″, Alt. 2484m, Apr.15. 20, Khoshravesh (TMUPC 113).
Encalypta intermedia Jur.	Tehran, Darake valley, in way toward Palang-chal, on moist rocks, N: 35˚ 49′ 76″, E: 51˚ 22′ 87″, Alt. 2065m, Nov. 22. 2005, Khoshravesh (TMUPC 257).
Entosthodon attenuatus (Diks.) Bryhn	Tehran, Darband valley, near the spring before Shirpala station on way toward Shirpala, on moist soil N: 35˚ 51′ 11″ E: 51˚ 25′ 40″ Alt. 2622m, Apr. 22. 2005, Khoshravesh (TMUPC 143).
Funaria hygrometrica Hedw.	Tehran, Drake valley toward Palang-chal station, near Alborz coffee on moist soil, Jun. 4. 2005, Khoshravesh (TMUPC 241).
Grimmia ovalis (Hedw.) Lindb.	Darake valley, before the bridge near the river on dry rocks, Apr. 30. 2004, Khoshravesh (TMUPC 29).
Coscinodon cribrosus (Hedw.) Spruce	Tehran, Darake valley, on front of ghandil coffee on moist rocks, Apr. 30. 2004, Khoshravesh (TMUPC 39).
Schistidium flaccidum (De Not.) Ochyra	Tehran, Touchal way, Around 2nd station, on dry rocks, March. 27. 2005, Khoshravesh (TMUPC 70).
Melichhopheria melichhoferiana (Frunck ex Hornsch.) Loesk.	Tehran, Darake valley, on the moist split of rocks, in shadow, Apr. 30. 2004, Khoshravesh (TMUPC 43).
Orthotrichum cupulatum Brid.	Tehran, Darake valley, on way toward Palang-chal, on moist rocks, N: 35˚ 49′ 76″, E: 51˚ 22′ 87″, Alt. 2065m, Nov. 22. 2005 Khoshravesh (TMUPC 261).
Hymenostylium recurvirustre (Hedw.) Dixon	Tehran, Darake valley, on way toward Palang-chal, Nov. 22. 2005, Khoshravesh (TMUPC 243).
Phascum cuspidatum Schreb. Ex. Hedw.	Tehran, Darband valley, before the spring near Shirpala station, on moist rocks, N: 35˚ 51′ 08″ E: 51˚ 25′ 67″, Alt. 2484m, Apr.15. 2005, Khoshravesh (TMUPC 126)
Tortula revolvens (Shimp.) G. Roth.	Tehran, Darband valley, in beginning of Shirpala way, around the coffees, Apr.26. 2005, Khoshravesh (TMUPC 162).
Tortula subulata Hedw.	Tehran, Touchal way, Around 2nd station, on dry rocks, March. 27. 2005, Khoshravesh (TMUPC 69); Tehran, Darband valley, before the spring near Shirpala station, on moist rocks, N: 35˚ 51′ 08″ E: 51˚ 25′ 67″, Alt. 2484m, Apr.15. 2005, Khoshravesh (TMUPC 122)

[a]TMUPC is an acronym of Tarbiat Modares University Plant Collection.

(Punt et al. 1994). The spore terminology followed Punt et al. (1994), Boros et al. (1993), Moore et al. (1991) and Faegri et al. (1989). Habitat types of examined species were determined according to Smith (1982).

Results

Spore morphological characteristics of 15 studied species summarized in Table 2. Spore shape of all the studied species were spheroid. Spore size is ranging from 7.50 (±0.06) × 8.07 (±0.06) µm in *Schistidium flaccidum* (De Not.) Ochyra to 35.84 (±3.67) × 37.51 (±4.55) µm in *Encalypta intermedia* Jur. All species, except for *Encalypta intermedia* Jur., have small-sized spores, < 20 µm. No sulcus is detected on the exine surface of the species examined (Figs. 1-4). Based on the exine surface ornamentation, six spore types can be recognized here. The most common spore type is that with verrucate exine surface, which is found in six

Table 2. Spore characteristics of the examined species

A: *Amblystegiaceae*, B: *Brachytheciaceae*, E: *Encalyptaceae*, F: *Funariaceae*, G: *Grimmiaceae*, M: *Mniaceae*, O: *Orthotrichaceae*, and P: *Pottiaceae*.

Taxon	Family	Spore size	Spore Type	Habitat type
Amblystegium serpens	A	14.30 (±.14) × 14.94 (±0.10) µm	I	Saxicolous
Brachythecium velutinum	B	12.17 (±.28) × 13.38 (±0.005) µm	I	Saxicolous
Brachythecium collinum	B	14.14(±0.21) × 14.89 (±0.23) µm	I	Saxicolous
Coscinodon cribrosus	G	8.74 (±0.17)×9.91 (±0.17) µm	I	Saxicolous
Encalypta intermedia	E	35.84 (±3.67) × 37.51 (±4.55) µm	II	Saxicolous
Entosthodon attenuatus	F	18.60 (±0.49) ×20.12 (±0.54) µm	VI	terrestrial
Funaria hygrometrica	F	16.90 (±0.52) × 17.36 (±0.53) µm	II	terrestrial
Grimmia ovalis	G	8.35 (± 0.12) × 9.13 (± 0.12) µm	IV	Saxicolous
*Hymenostylium recurvirustre**	P	12.5 × 10.1 µm	I	Saxicolous
Melichhopheria melichhoferiana	M	12.95 (±0.22) ×13.85 (±0.25) µm	II	Saxicolous
Orthotrichium cupulatum	O	15.53 (±0.36) × 14.63 (±0.21) µm	II	Saxicolous
Phascum cuspidatum	P	16.77 (±0.48) × 18.53 (±0.54) µm	I	Saxicolous
Schistidium flaccidum	G	7.50 (±0.06) × 8.07 (±0.06) µm	III	Saxicolous
Tortula revolvens	P	8.385 (±0.08) × 9.38 (±0.17) µm	V	Saxicolous
Tortula subulata	P	11 (±1.3) × 11.1 (±0.0001) µm	III	Saxicolous

*Only one spore was measured due to the lacking sufficient spores during the specimen preparation.

species, and the type with gemmate exine surface is the next one detected in five species. The spore type having scabrate-gemmate surface is seen in three species; while the types with retipilate, rugulate and pilate surfaces each are restricted to one species. Below we describe characteristics of these six spore types in further details.

I. Verrucate exine surface

This is the largest group and contains six species *Amblystegium serpens* (Hedw.) Bruch, Schimp. & Gümbel, *Brachythecium velutinum* Bruch & W. Gümbel., *B. collinum* (Schleich. ex C. Müll.) Bruch, Schimp. & Gümbel, *Coscinodon cribrosus* (Hedw.) Spruce, *Hymenostylium recurvirustre* (Hedw.) Dixon, and *Phascum cuspidatum* Schreb. ex. Hedw. The species *Amblystegium serpens*, *Brachythecium velutinum* and *B. collinum* are the members with a clear common spore features. In these three species, exine surface is irregularly covered by rather scattered verrucae that subpatterned by small granules on the surface. In *Coscinodon cribrosus*, the processes are dense and wiedly different in size and intermixed with microperforations. *Hymenostylium recurvirustrum*, densely covered by mixed large and small processes . Spore surface in *Phascum cuspidatum* covered by sparsely and rare dispersed verrucae (Fig. 1. a-h and Fig. 2. a-d).

II. Gemmate exine surface

Encalypta intermedia Jur., *Orthotrichium cupulatum* Brid., *Melichhopheria melichhoferiana* (Frunck ex Hornsch.) Loesk. and *Funaria hygrometrica* Hedw**.** are belonging to this type. *Encalypta intermedia* has the largest spore (see Table 2), covered by regularly arranged macrogemmae with poorly constricted base similar to shortened pillae. Size of each gemma is about 4-4.4 µm. The exin surface of *Orthotrichium cupulatum* is like to that of the former species with the exception that gemmae are smaller, almost 1 µm in diameter. *Funaria hygrometrica* has microgemmate exine surface that are densely arranged on the exine (Fig. 2. e-h and Fig. 3. a-b). During preserving the specimens in ethanol 96% for the dehydration, all samples of *Melichhopheria melichhoferiana* were destroyed and it was not possible to study it by SEM. By light microscopy studies, rod-like structure was found on the exine surface of this species.

III. Scabrate-gemmate exine surface

Schistidium flaccidum (De Not.) Ochyra and *Tortula subulata* Hedw. are belonging to this type. The spore surface, in this group, is covered by regular or irregular somewhat dense gemma-like processes of < 1 µm and perforations among them (Fig. 3: c-f).

IV. Retipilate exine surface

This type contains *Grimmia ovalis* (Hedw.) Lindb. The exine surface in this type is composed of small pilae

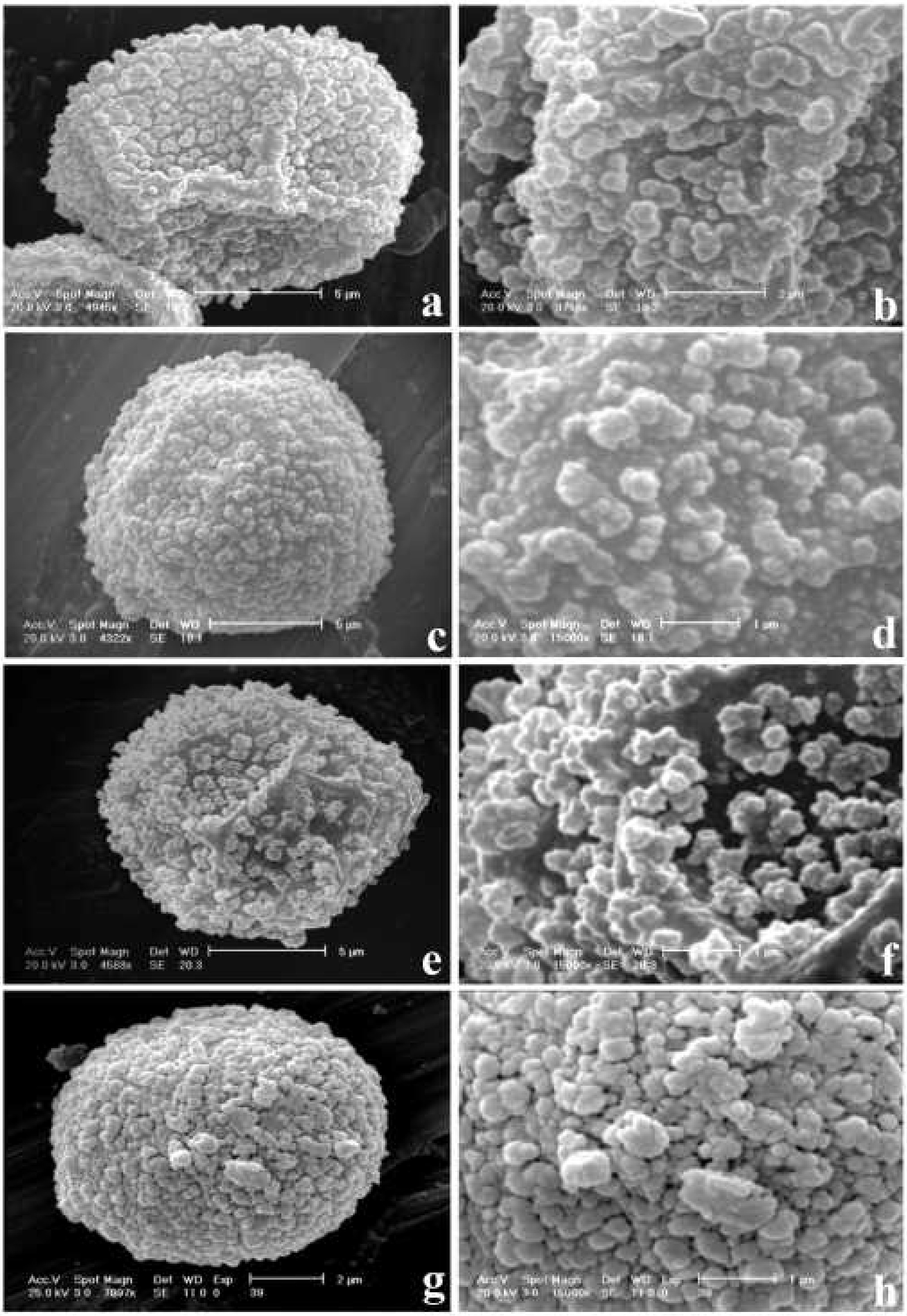

Fig. 1. a and b *Amblystegium serpens*, (a): spore view, (b): irregular-sized and shaped verruca with poorly constricted base. c and d *Brachythecium collinum*, (c): spore view, (d): scabrate surface of spore and verruca. e and f *B. velutinum*, (e): spore view, (f): clava-like verruca with a small stalk. g and h *Coscinodon cribrosus*, (g): spore view, (h): spore surface with irregular-sized veruca-like processes.

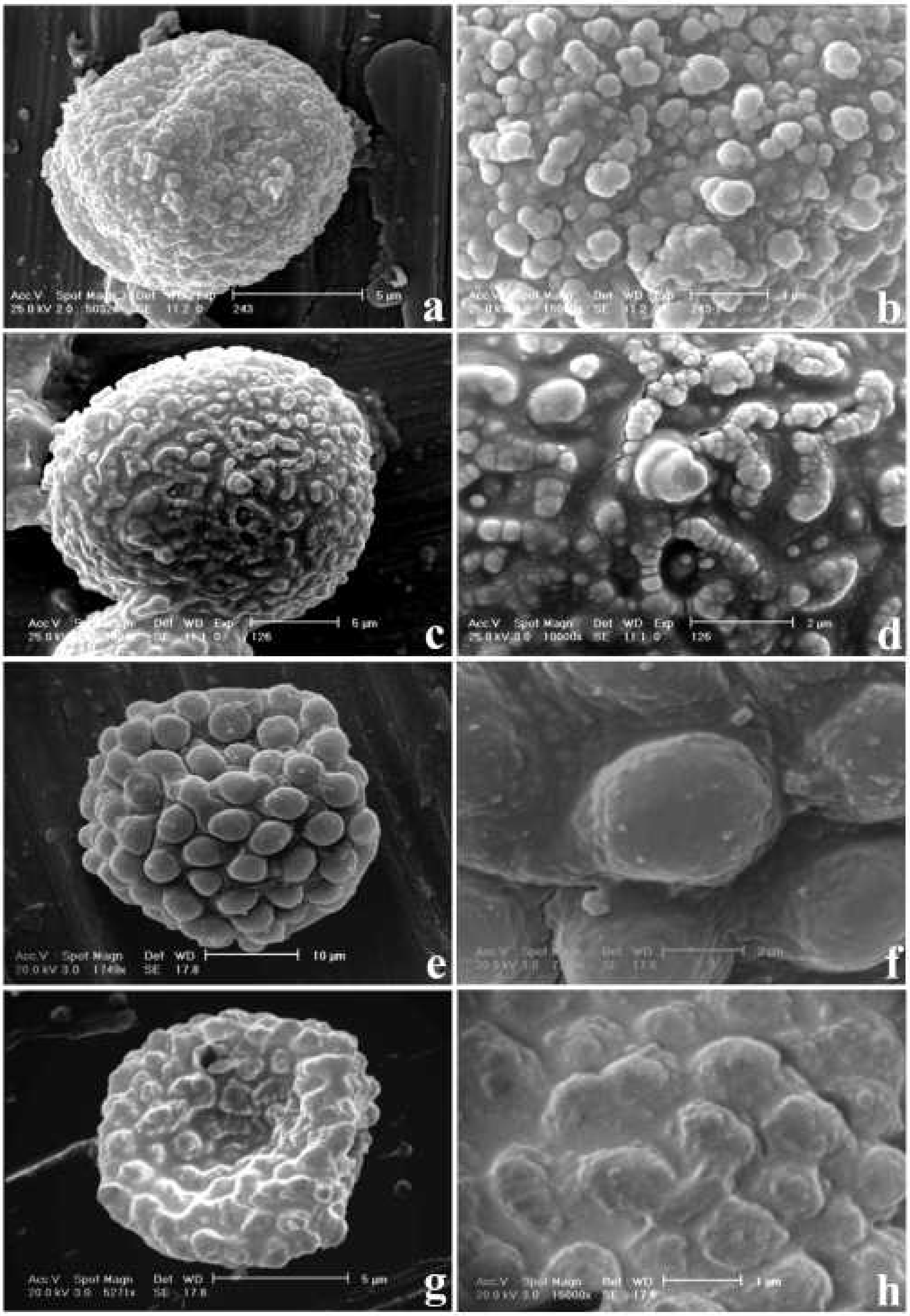

Fig. 2. a and b *Hymenostylium recurvirustre*, (a): spore view, (b): exine surface shows non similsr pillum like processes with somewhat conjunct heads. c and d *Phascum cuspidatum*, (c): spore view, (d): spore surface with rare and irregular-sized verruca. e and f *Encalypta intermedia*, (e): spore view, (f): a single gemmae that is more than 2 µm with a very short stalk (arrow). g and h *Orthotrichum cupulatum*, (g): spore view, (h): irregular gemma.

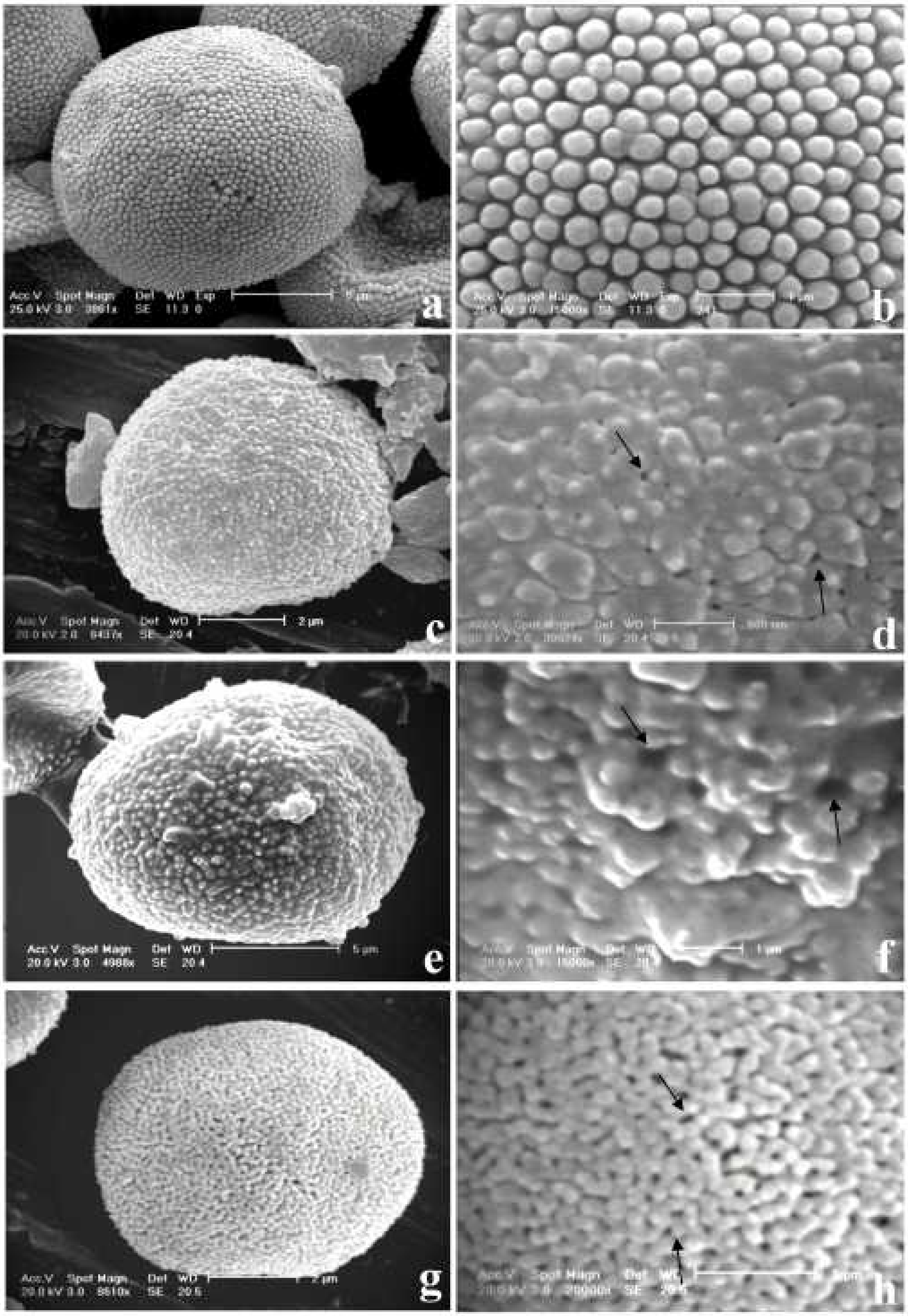

Fig. 3. a and b *Funaria hygrometrica*, (a): spore view, (b): pilla on exine surface. c and d *Schistidium flaccidum*, (c): spore view, (d): scabrate surface with very small processes, arrows show perforations. e and f *Tortula subulata*, (e): spore view, (f): irregular verruca like processes on spore surface and perforations. g and h *Grimmia ovalis*, (g) spore view, (h): muri like structure of exine surface, arrow shows jointed heads of pilla.

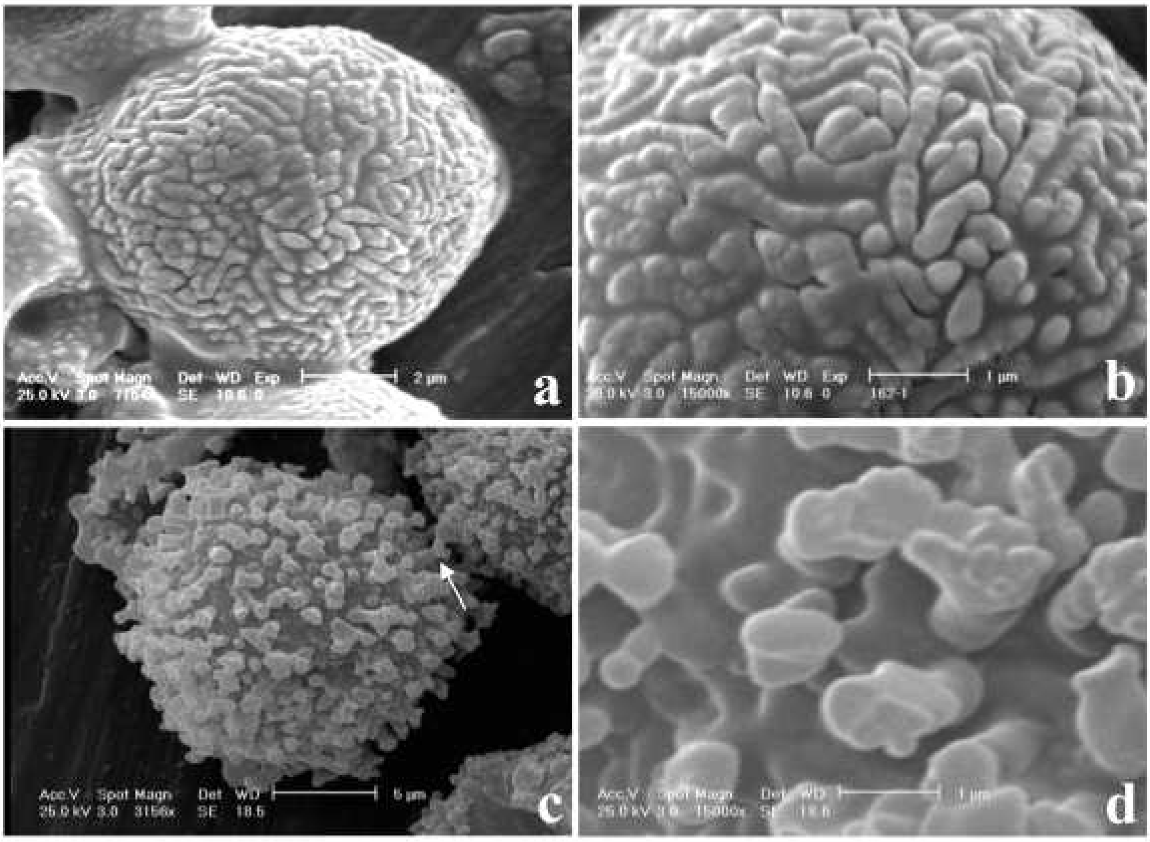

Fig 4. a and b *Tortula revolvens,* (a): spore view, (b): muri like structures of exine surface. c and d *Entosthodon attenuatus*,, (c): triangular spore-shape (arrow shows single clava), (d): sub–patterned heads of clava.

that their heads are tightly packed and yield pattern of tectal circles (Fig. 3: g and h).

V. Rugulate exine surface

This type contains *Tortula revolvens* (Schwägr.) Venturi ex Broth. The exine surface is ornamented by elongated elements arranged in an irregular elongated pattern (Fig. 4: a and b).

VI. Pilate exine surface

This type contains *Entosthodon attenuatus* (Diks.) Bryhn. The spore shape is slightly triangular with rounded angles. The exine surface is covered by loosely arranged rod-lik elements with swollen apical part, known as pila, and gemmae among them (Fig. 4: c and d).

Discussion

As described in the results, six spore types were determined for 17 species studied. All spores are spheroidal, and in all taxa, but *Encalypta intermedia,* spores are small-sized (see Table 2). Spore morphology of six of our 17 species analyzed here was reported previousely (Boros et al. 1993). General spore morphology of most of those six species, *Funaria hygrometric, Grimmia ovalis* and *Tortula subulata* is the same that Boros et al. (1993) illustrated using light microscopy. In *Amblystegium serpens* and *Brachythecium velutinum* spore surfaces are ornamented by irregular verrucate like elements as depicted here; whereas in their work, the former species has pilum like processes and the second one showed reticulum-like pattern. The SEM based analysis of spore morphology for *Brachythecium collinum, Coscinodon cribrosus, Encalypta intermedia, Entosthodon attenuatus*, *Hymenostylium recurvirustrum, Orthotrichium cupulatum, Shistidum flaccidum, Tortula revolvens*, is first reported herein.

The spore surface ornamentations are of diagnostic value for identification the examined taxa at least in the generic and somewhat in the specific level within the family. For instance, our present finding illustrates that four species of three different genera including

Hymenostylium recurvirostre and *Phascum cuspidatum, Tortula subulata* and *T. revolvens,* (all of *Pottiaceae*) are belonging to four spore types I (verrucate), III (scabrate-gemmate) and V (rugulate), respectively. Similarly, three different species *Coscinodon cribrosus*, *Schistidium flaccidum* and *Grimmia ovalis* (all of *Grimmiaceae*) are members of spore types I, III and IV (retipilate), respectively. Spore ornamentation is distinguishable between the two funariaceous taxa, *Funaria hygrometrica,* and *Entosthodon attenuatus*, having microgemate and pilate surface ornamentations, respectively. However, in some cases, spore morphology could not differentiate the taxa either congeneric species like *Brchythecium collinum* and *B. velutinum*, or heterogeneric ones such *H. recurvirostre* and *Phascum cuspidatum*. Furthermore, it should be noted that most of all six recognized spore ornamentation types can not distinguish the taxa at the familial level, indicating that the types are evolved in parallel among the families *Amblytegiaceae*, *Brachytheciaceae, Encalyptaceae, Funariaceae, Grimmiacaea, Mniaceae, Orthotrichaceae* and *Pottiaceae*.

Our examined mosses species are belonging to two habitats, saxicolous, the species inhabited on the rock surface, and moist soil (terrestrial), (Table 2; Khoshravesh 2006). There are some correlation between exine surface ornamentations and the vegetation substratum. All the species having verrucate, scabrate-gemmate, rugulate and retipilate exine ornamentations are saxicolous. With the exception of *Amblystegium serpens*, *Brachythecium collinum* and *B. velutinum* that growing under the running water on rock surface, the remaining saxicolous species are sun exposed and adapted to the dry condition (see Table 2). The species with gemmate spore ornamentation are belonging to either saxicolous or terrestrial habitats. The saxicolous members including *Encalypta intermedia* and *Orthotrichum cupulatum* produce their sporophytes under condition of high humidity and short-period sun light mainly during winter season. *Melichhopheria melichhoferiana* is adapted to grow in cliffs of highly humid rocks under the running water. Whereas, *Funaria hygrometrica* grows on the moist soil and sometimes on animal remains. *Entosthodon attenuatus*, a species having spores of pilate exine ornamentation, is exclusively inhabited to the moist soils. It is noteworthy that the sun exposed saxicolous species possess spores that densely ornamented by exine elements, while the moisture dependent species have spores that loosely covered by subpatterned exine surfaces (see Figs. 1-4). Other morphological adaptations including spore size, life forms and life strategies, related to habitat conditions have been already illustrated in the Near and Middle East Bryophytes (Kürschner 2004).

Furthermore, there is little correlation between spores size and shape of the examined species and their habitat. Based on During's (1992) ecological classification of bryophytes, estimating the spore dispersal strategies can be possible. All the species, except *Encalypta intermedia* that has the largest spores, possess small-sized spores and common sporophytes that increase chance of successful dispersal and occupying new localities. These characters related to a common strategy of drought-resistant. The strategy that characterized by a longer life span, monoecy, regular sporophyte production, and the production of large quantities of small spores. This functional type is typical for saxicolous bryophytes, which compensate the high mortality rates of the gametophytes, often caused by the summer-drought or erosion effects, by a regular formation of sporophytes (Kürschner 2004).

A critical survey about bryophytes flora and populations in Iran would be a major step forward for understanding morphological diversities and ecoadaptations and dispersal patterns as well as estimating correlation between spore morphology with the relevant taxonomical groups and ecological conditions. These kinds of investigations help us to predict bryophytes rarity, future ecological disturbance and conservation.

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