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SPORE MORPHOLOGY OF SOME GRIMMIACEAE ARN. SPECIES BELONGING TO MOSS FLORA OF TURKEY

FILIZ SAVAROGLU[1], ISMUHAN POTOGLU ERKARA[1*] AND FATIH OZCELIK[2]

[1]*Eskisehir Osmangazi University, Faculty of Science and Art, Department of Biology, 26480, Meselik, Eskisehir, Turkey*
[2]*Eskişehir Osmangazi University, Graduate School of Applied and Natural Science, 26480, Meselik, Eskisehir, Turkey*
[*]*Corresponding author's e-mail: ismuhan@ogu.edu.tr*

Abstract

This study includes spore morphology of seven Grimmiaceae taxa from Turkey. The spores of *Schistidium trichodon* (Brid.) Poelt, *S. confertum* (Funck) Bruch & Schimp., *Grimmia ovalis* (Hedw.) Lindb., *G. pulvinata* (Hedw.) Sm., *G. trichophylla* Grev., *G. dissimulata* E.Maier and *G. decipiens* (Schultz) Lindb. were examined by light and scanning electron microscopy. The apertural region forms from a leptoma in all spores. The spore morphology of the examined taxa of the family is verrucate type. The spore shape of all studied species is prolate-spheroid. Spore size ranges from 6 µm to 17 µm in the family of Grimmiaceae. The spore wall of the family Grimmiaceae includes sclerine and intine. The examined species of mosses are belonged to saxicolous habitat type. The taxonomical and ecological implications of the family Grimmiaceae are discussed on the basis of their spore morphology.

Key words: Bryophyta, Grimmiaceae, spore morphology, light microscope (LM), scanning electron microscope (SEM), Turkey.

Introduction

Geographically, Anatolia is located at the intersection of Europe and Asia, and climatically, it is situated in an area where the climates of the Mediterranean, Iran – Turan and Europe – Siberia overlap. This variety is increased due to the presence of structures such as mountains, plains, streams and valleys at short distances from each other, and as a result of all of these characteristics, the phytogeographical elements of Anatolia displays multiple varieties. This varied elements consist of nonvascular plants as well as vascular ones. The bryophytes, possess an important role in the flora of Anatolia.

Bryophytes, which are the most basic members of the plant realm, constitute the second largest group after seed plants, with close to 23.000 species, and are spread over a wider area across the world than seed plants (Yıldız & Aktoklu, 2012; Goffinet & Shaw, 2009). Till to-date, 163 species and sub-species in the Hepaticae class, 3 species in the Anthocerotae classis and 721 species and taxon sub-species in the Musci classis have been recorded (Kürschner & Erdağ, 2005).

Bryophytes can survive in various different climates and habitats. They survive not only in terrestrial environments, but have also developed the required adaptation to live in sand dunes, on rocks, in marshes, on the surface of water, on roof tiles in areas of man-made structures, on gravestones, on pavement stones, and in extremely dry and extremely humid atmospheres. The plant stems of bryophytes are generally small. They grow by clinging on to the soil, trees and rocks, with thread-like single cell or multi-cell structures known as rhizoids. While acrocarpous bryophytes form lumps or clusters and pleurocarpous ones spread out like a carpet. Grimmiaceae Arn. family are acrocarpous mosses. As bryophytes are mostly small in size they are not noticed when they are on their own in an area, and their generation of clusters or lumps in this way makes them noticeable (Alataş, 2006).

In our study, we have examined the spore morphology of 7 taxa belonging to the Grimmiaceae family of the Bryophyta division. The polar and equatorial measurements of the bryophytes are useful for the purposes of the determination of species as a result of this study, and the ornamentation shapes on the surface of the spores make identification easier.

The Grimmiaceae family and its genera have been separated according to their gametophytic and sporophytic characteristics. Spore characteristics are widely used in the taxonomic analyses and descriptions of bryophytes. These characteristics within the systematic of bryophytes can be a model for spore morphology applications. Spore morphology is helpful in solving the taxonomic problems. At the same time, this is also a potential source of information for the evolutionary processes which result in the determination of the biological and taxonomic boundaries (Carrion *et al.*, 1995). Some sources (Gambardella *et al.*, 1994; Carrion *et al.*, 1995; Estebanez *et al.*, 1997; Luizi-Ponzo & Barth, 1998, 1999; Khoshravesh & Kazempour Osaloo, 2007; Potoglu Erkara & Savaroglu, 2007; Savaroglu *et al.*, 2007; Savaroglu & Potoglu Erkara, 2008; Medina *et al.*, 2009; Aşçı *et al.*, 2010; Caldeira *et al.*, 2013) in recent years have proved that the intine structure and external spore morphology are useful in the characterization of bryophytes, in respect of generic and species delimitation. However, there is still a need for further studies in this field.

The bryophyte spores in Turkey are not fully known. The detailed spore morphologic characteristics of certain Grimmiaceae species have been examined in this study, with a light microscope (LM) and a scanning electron microscope (SEM). The aim of this study is to characterize the spore morphology of the seven species in the Grimmiaceae family, and thereby shed light on studies in the areas of taxonomy, ecology and paleobotany.

Materials and Methods

Plant materials: All of the specimens were collected from their natural habitats, as detailed below:

Grimmiaceae Arn.

Schistidium trichodon (Brid.) Poelt A1 Osmaneli (Bilecik): Düzmeşe-Orhaniye, Göksu river (Avdan), waterfalls, reef, 96 m, N 40°21'07.2", E 029°54'08.4", 17.04.2006, on rocks, Savaroğlu 911.

S. confertum (Funck) Bruch & Schimp. A1 Osmaneli (Bilecik): Belenalan village, rocky area, 713 m, N 40°19'02.7", E 029°53'57.5", 28.02.2007, on rocks, Savaroğlu 1183.

Grimmia ovalis (Hedw.) Lindb. B7 Sündiken Mountains (Eskişehir): Arıkaya, *Pinus nigra* subsp. *pallasiana-Quercus cerris* var. *cerris* forest, 1200 m, 23.07.2000, on rocks, Savaroğlu 446.

G. pulvinata (Hedw.) Sm. A2 Osmaneli (Bilecik): Ciciler village, by the side of the road, 92 m, N 40°26'31.5", E 030°03'30.3", 10.04.2006, on rocks, Savaroğlu 858.

G. trichophylla Grev. A2 Osmaneli (Bilecik): Medetli-Kazancı village road, by the sides of graves, 101 m, N 40°15'33.8", E 030°05'06.2", 10.04.2006, on rocks, Savaroğlu 893.

G. dissimulata E.Maier A1 Osmaneli (Bilecik): Balçıkhisar village – rural areas, 584 m, N 40°20'43.4", E 029°58'09.0", 01.05.2008, on rocks, Savaroğlu 1346.

G. decipiens (Schultz) Lindb. Sündiken Mountains (Eskişehir): Tandır, *Pinus nigra* subsp. *pallasiana-Quercus cerris* var. *cerris* forest, 1400 m, 11.06.2000, on rocks, Savaroğlu 278.

Morphological studies: The spore materials are employed in this study was obtained from the Faculty of Science and Arts of Osmangazi University Herbarium. The external surface was observed using LM and SEM. The spores were prepared untreated with glycerin jelly on microscope slides (Wodehouse, 1935), using the acetolysis method (Erdtman, 1957) for LM. Measurements of the shortest and the largest diameters (in polar view), as well as the polar axis and the equatorial diameter (in equatorial view), were taken in 25 randomly selected spores. The mean, standard deviation, standard error and range were then established. The sclerine thickness and the largest length of the aperture region were based on 25 measurements, with only the mean presented. For SEM investigations, the unacetolyzed spores were directly placed onto stubs. The stubs were then coated with carbon and gold in a vacuum evaporator to a total thickness of 7.5–15.0 nm and examined with a JEOL 5600LV SEM at an accelerating voltage of 20 kV. The first exsiccate listed under the ‘‘Specimens examined’’ is the reference specimen, while the others are the comparisons. The terminology for spore morphology was proposed by Erdtman (1957); Boros & Járai-Komlódi (1975); Blackmore & Barnes (1991); Punt *et al.* (1994) and Kapp *et al.* (2000).

Results

General remarks: The sporoderm of the Grimmiaceae family contains perine, exine and intine. It may be difficult to identify the difference between exine and perine. Therefore, sclerine is a more appropriate term to use. Ornamentation is different in each genus and it may be possible to identify species according to these characteristics. The aperture region may be comprised of an open space. One or more of the ornamentation elements may, or may not generate hoops.

The spores of the taxa examined in the family are uniform. For this reason the morphological characteristics of the spores display features which are more or less similar. All of the spores possess radial symmetry, are isopolar, and the spores are of a prolate-spheroid shape (Figs. 1-4). The ornamentation of all 7 taxa of the spores is of verrucous type.

The measurements of the reference specimens is consistent with the measurements of the compared specimens. However, the mean is a little different. This reflects the existence of a variation within the species. The polar axis measurements (P) in the equatorial aspect has been assessed in accordance with preparates prepared according to the Erdtman method (Table 1). The morphometric data of the spores is given in Tables 1-3.

Descriptions of the spores

Schistidium trichodon (Brid.) Poelt; Verrucous, irregular spores, sub-spheric, from time to time plano-convex, dimensions 8, 0-11, 0 μm (mean value 9,8 μm) (Fig. 1a-c).

S. confertum (Funck) Bruch & Schimp.; Verrucous, spores are sub-spheric – plano-convex or concave convex, diameter 9, 0-11, 0 μm (mean value 10, 2 μm) (Fig. 1d-f).

Grimmia ovalis (Hedw.) Lindb.; Verrucous, ellipsoid spores, from time to time clearly plano-convex, varied dimensions 8, 0-12, 0 μm (mean value 9,8 μm) (Fig. 2a-c).

G. pulvinata (Hedw.) Sm.; Verrucous, spores are sub-spheric – plano-convex or concave convex, diameter 7, 0-11, 0 μm (mean value 9, 4 μm) (Fig. 2d-f).

G. trichophylla Grev.; Verrucous, ellipsoid spores, from time to time clearly plano-convex, varied dimensions 8, 0-12, 0 μm (mean value 9, 7 μm) (Fig. 3a-c).

G. dissimulata E.Maier; Verrucous, spores are sub-spheric – plano-convex or concave-convex, diameter 7, 0-11, 0 μm (mean value 9,1 μm) (Fig. 3d-f).

G. decipiens (Schultz) Lindb.; Verrucous, irregular spores, sub-spheric, from time to time plano-convex, dimensions 8, 0-11, 0 μm (mean value 10, 0 μm) (Fig. 4a-c).

The taxon spores which were examined are varied on the distal surface due to being verrucous (verruca, papilla). The sides of the proximal surfaces do not appear to be developed. Spores of smaller dimensions (6-17 μm) are bilateral, sometimes ranging from radial symmetric to asymmetric, heteropolar, circular to semi-circular amb, and convex to concave in shape. The exine surface is decorated with verruca like elements (Figs. 1-4). The aperture area is comprised of a less resolute area and this is rendered to be leptoma. The verruca like elements of these taxa are larger and more rarely dispersed, and this area is assessed as being an aperture. The SEM is helpful for spore type characterizations. This does not permit a clear differentiation among the examined taxa. In addition to the creation of an aperture or leptoma, the most important features of these spores which make it easier for them to be distinguished is their diameter measurements (Table 2). Some of the morphological differences observed in the verruca elements can also appear in some other taxa. However, we have observed, large differences within the species themselves in terms of these characteristics, when differentiating between species these features are not a reliable characters.

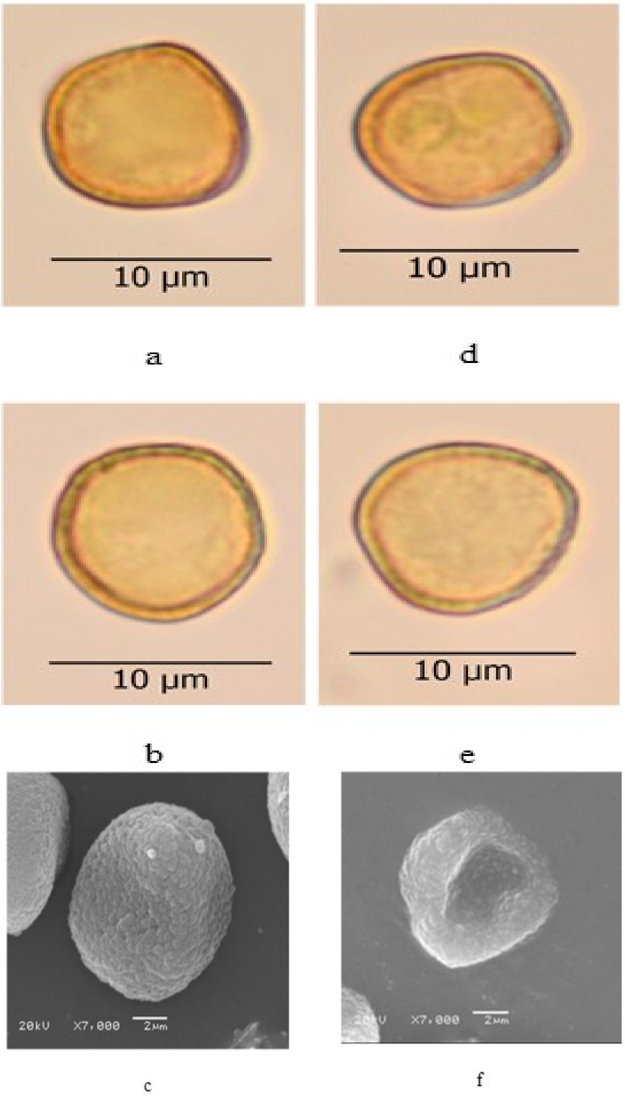

Fig. 1. a-c: *Schistidium trichodon*. a. proximal view (IM, N), b. proximal view (IM, A), c. distal surface (SEM). d-f: *S. confertum*. d. proximal view (IM, N), e. proximal view (IM, A), f. proximal view (SEM).

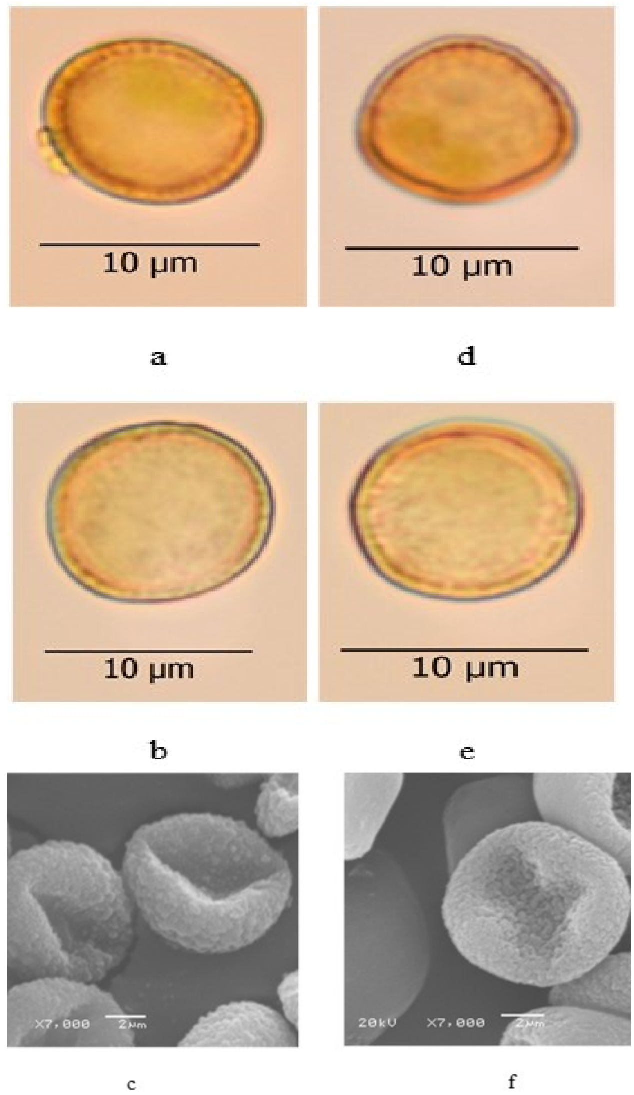

Fig. 2. a-c: *Grimmia ovalis*. a. proximal view (IM, N), b. proximal view (IM, A), c. proximal view (SEM). d-f: *G. pulvinata*. d: proximal view (IM, N), e. proximal view (IM, A), f. proximal view (SEM).

Table 1. Morphometric data of the Grimmiaceae spores (equatorial view).

Taxa	Measurements							
	P (µm)				E (µm)			
	R	$X \pm S_x$	S	V (%)	R	$X \pm S_x$	S	V (%)
Schistidium trichodon (N)	8,0-11,0	10,1-0,1	0,7	0,5	8,0-11,0	9,6-0,09	0,6	0,4
S. trichodon (A)	8,0-11,0	9,8-0,09	0,7	0,4	8,0-11,0	9,6-0,1	0,8	0,6
S. confertum (N)	8,0-11,0	9,8-0,09	0,6	0,4	8,0-11,0	9,7-0,09	0,6	0,4
S. confertum (A)	9,0-11,0	10,2-0,09	0,6	0,4	8,0-11,0	9,9-0,1	0,7	0,5
Grimmia ovalis (N)	9,0-12,0	10,1-0,1	0,7	0,5	6,0-12,0	9,5-0,1	1,1	1,2
G. ovalis (A)	8,0-12,0	9,8-0,1	0,8	0,8	8,0-11,0	9,5-0,1	0,8	0,7
G. pulvinata (N)	8,0-11,0	9,9-0,1	0,8	0,8	7,0-11,0	9,1-0,1	0,8	0,7
G. pulvinata (A)	7,0-11,0	9,4-0,1	0,9	0,9	7,0-11,0	9,1-0,1	0,9	0,8
G. trichophylla (N)	8,0-11,0	9,6-0,1	0,7	0,5	7,0-11,0	9,5-0,1	0,8	0,6
G. trichophylla (A)	8,0-12,0	9,7-0,1	0,9	0,8	8,0-11,0	9,5-0,1	0,7	05
G. dissimulata (N)	8,0-17,0	10,4-0,2	1,5	2,5	8,0-16,0	10,0-0,2	1,4	2,2
G. dissimulata (A)	7,0-11,0	9,1-0,1	0,8	0,6	7,0-11,0	9,1-0,1	0,8	0,6
G. decipiens (N)	10,0-13,0	10,9-0,1	0,8	0,7	9,0-13,0	10,8-0,1	0,8	0,6
G. decipiens (A)	8,0-11,0	10,0-0,09	0,6	0,4	8,0-12,0	9,8-0,1	0,7	0,5

Abbreviations: P: polar axis, E: equatorial diameter, R: range, X: mean, S_x: standard error, S: standard deviation, V: variation, N: non-acetolyzed spores, A: acetolyzed spores

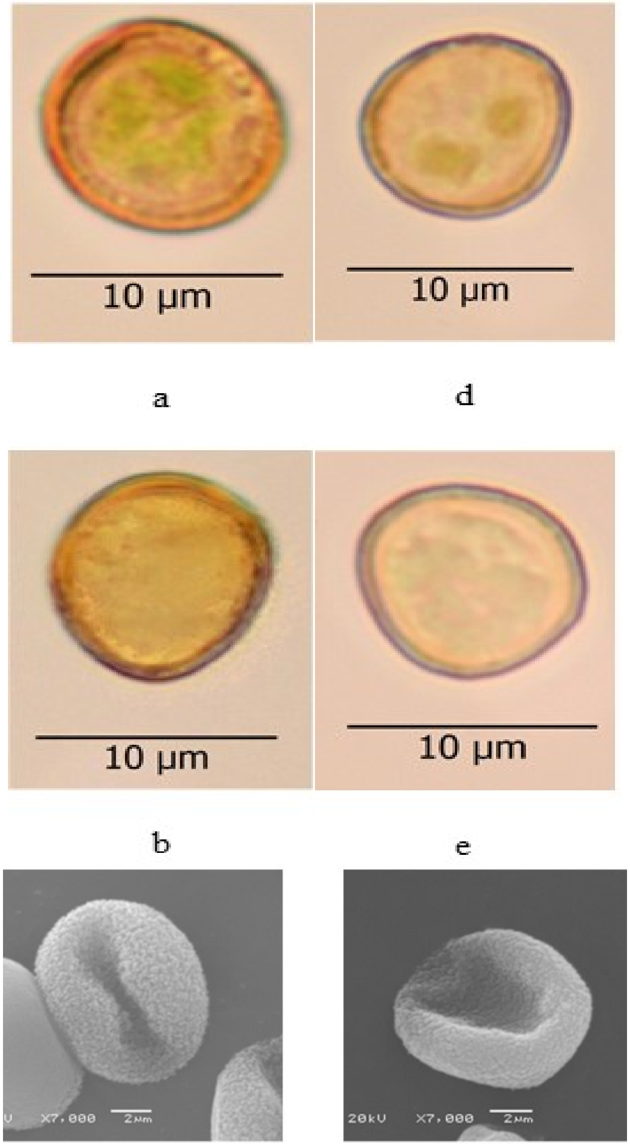

Fig. 3. a-c: *Grimmia trichophylla*. a. proximal view (IM, N), b. proximal view (IM, A), c. proximal view (SEM). d-f: *G. dissimulata*. d: proximal view (IM, N), e. proximal view (IM, A), f. proximal view (SEM).

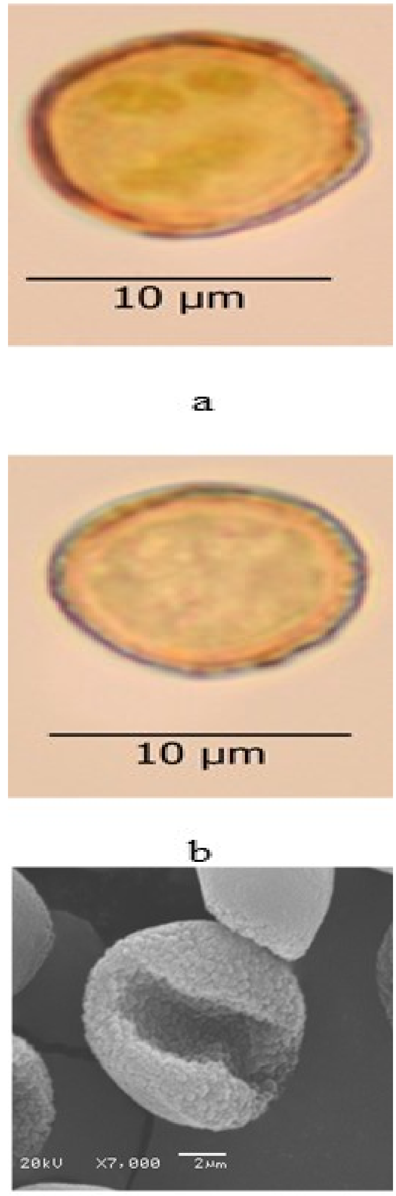

Fig. 4. a-c: *Grimmia decipiens*. a. proximal view (IM, N), b. proximal view (IM, A), c. proximal view (SEM).

Table 2. Morphometric data of the Grimmiaceae spores (polar view).

Taxa	Measurements							
	D_M (µm)				D_m (µm)			
	R	X ± S_x	S	V (%)	R	X ± S_x	S	V (%)
Schistidium trichodon (N)	8,0-12,0	9,8-0,1	1,0	1,0	7,0-12,0	9,2-0,1	1,1	1,3
S. trichodon (A)	8,0-12,0	10,1-0,1	0,8	0,7	6,0-12,0	9,2-0,1	1,2	1,5
S. confertum (N)	8,0-12,0	10,0-0,1	0,8	0,7	7,0-11,0	9,2-0,1	0,8	0,7
S. confertum (A)	8,0-11,0	9,9-0,1	0,7	0,5	7,0-11,0	9,4-0,1	1,0	1,0
Grimmia ovalis (N)	8,0-12,0	10,1-0,1	0,8	0,7	7,0-12,0	9,8-0,1	0,9	0,9
G. ovalis (A)	7,0-12,0	9,6-0,1	0,9	0,9	6,0-11,0	8,8-0,1	1,1	1,2
G. pulvinata (N)	7,0-11,0	9,2-0,1	0,9	0,9	6,0-10,0	9,0-0,1	0,9	0,9
G. pulvinata (A)	7,0-11,0	9,3-0,1	1,1	1,2	7,0-11,0	8,9-0,1	1,1	1,4
G. trichophylla (N)	8,0-11,0	9,4-0,1	0,7	0,5	7,0-10,0	9,0-0,1	0,8	0,6
G. trichophylla (A)	8,0-12,0	10,1-0,1	0,8	0,7	6,0-12,0	9,2-0,1	0,8	0,7
G. dissimulata (N)	8,0-11,0	9,9-0,1	0,7	0,5	6,0-11,0	9,2-0,1	1,0	1,2
G. dissimulata (A)	7,0-11,0	8,8-0,1	1,0	1,0	6,0-10,0	8,8-0,1	1,0	1,0
G. decipiens (N)	10,0-14,0	11,4-0,1	0,9	0,9	9,0-13,0	10,9-0,1	0,9	0,8
G. decipiens (A)	8,0-12,0	9,8-0,1	1,0	1,0	8,0-12,0	10,2-0,1	1,2	1,5

Abbreviations: D_M: largest diameter, D_m: smallest diameter, R: range, X: mean, S_x: standard error, S: standard deviation, V: variation, N: non-acetolyzed spores, A: acetolyzed spores

Table 3. Morphometric data of the sclerine and apertural region of the Grimmiaceae spores.

Taxa	Measurements	
	st (µm)	a (µm)
Schistidium trichodon (N)	1,0	1,0
S. trichodon (A)	1,0	1,0
S. confertum (N)	1,0	1,0
S. confertum (A)	1,0	1,0
Grimmia ovalis (N)	1,0	1,2
G. ovalis (A)	1,0	1,2
G. pulvinata (N)	1,0	1,1
G. pulvinata (A)	1,0	1,2
G. trichophylla (N)	1,0	1,0
G. trihophylla (A)	1,0	1,0
G. dissimulata (N)	1,0	1,1
G. dissimulata (A)	1,0	1,0
G. decipiens (N)	1,0	1,2
G. decipiens (A)	1,0	1,2

Abbreviations: st: sclerine thickness, a: largest length of the apertural region, N: non-acetolyzed spores, A: acetolyzed spores

Discussion

The spore morphology for the species is based on peristome morphology. The taxa which were examined possessed uniform spores. It has been determined that the spores of the 7 examined species displayed verrucous type ornamentation. The spores of the examined species are prolate-spheroid. It has also been previously reported by Boros *et al.* (1993) and Kapp *et al.* (2000) that the spore types are verrucous. The general spore morphology of these taxa are consistent with the studies carried out by Boros *et al.* (1993), using a LM. On the other hand, the spore morphologies of the widespread species of the *Schistidium* and *Grimmia* genera in our country are being reported in this study, which has used LM and SEM.

Spore surface ornamentations possess distinguishing significance in the identification of the taxa which have been examined within the family, at least at the level of species or genus. For instance, our findings show that the seven species possess a single (verrucous) spore type. The species of bryophyte which were examined have a single type, in respect of their habitat. These species can be widely found on calcerous rocks, in acidic areas, on old roofs and tiles, in man-made areas, on walls and on gravestones. The species on rocks, which have been examined, are subjected to intense sunlight. These species have adapted to drought conditions. These are also a correlation between the exine surface ornamentation and the habitats of species of bryophytes. In general species possessing verrucous spore ornamentation live on rocks. These produce sporophytes, primarily in the winter, during periods of high humidity and shorter sunlight. The spores belonging to rock based species which are subjected to continuous sunlight have a dense ornamentation on the exine surface, while the spores of species which live in humid areas display a looser ornamentation. As also stated in Near and Middle East Bryophytes, other morphological adaptations such as spore dimensions, life forms and life strategies, are related to habitat conditions (Kürschner, 2004; Khoshravesh & Kazempour Osaloo, 2007).

Further, there is very little relationship between the shape and size of the spores and their habitats, in the species which were examined. All of the species have a successful distribution of spores, and possess small spores and widespread sporophytes in order to increase their chances of invading new areas. These characteristic features are related to the general drought resistance strategy. This strategy is characterized with a longer lifespan, being monoicous, regular sporophyte production and the large amounts of production of small spores. This functional type is typical of rock based bryophytes and is used to balance the high mortality rate among gametophytes, due to the frequent effects of drought in the summer or erosion (Kürschner, 2004). There is an estimated relationship between the taxonomic groups and ecological conditions of the bryophytes in the area, and the spore morphology of the species. These types of studies will hold a light to researchers into the rare and endangered species of bryophytes, in respect of any future ecological disruption and the protection of the species.

The spores of the bryophytes of the Grimmiaceae family may not reveal many of the morphological features used to distinguish among the taxa. The manner of ornamentation of the spores has taxonomic significance. This is proof of the distribution of different types of spores among species (Estebanez *et al.*, 1997; Luizi-Ponzo & Barth, 1998, 1999; Khoshravesh & Kazempour Osaloo, 2007; Potoglu Erkara & Savaroglu, 2007; Savaroglu & Potoglu Erkara, 2008). Verrucous type spores have been found in all the 7 species. The spores of the *Grimmia* and *Schistidium* genera of the Grimmiaceae family were studied by Erdtman (1957), Boros & Járai-Komlódi (1975), Punt *et al.* (1994) and Kapp *et al.* (2000). The results reached in this study are consistent with these authors. However, the features in the spore surface ornamentation of the species *S. trichodon*, *S. confertum* and *G. dissimulata* were not included in previous literature. They are only contained in our present study. There are, from time to time, variations in the means in the analysis of different samples for each taxon. However, the range in the measurements of the compared samples does show consistency with that of the reference specimens. These results are also similar to those of Olesen & Mogensen (1978). This proves to us that there is a need to use more than one specimen for comparison purposes in order to identify the spore dimensions of a taxon. This study has revealed that the spores of the 7 Grimmiaceae species are of a prolate-spheroid shape. Further, it has been determined that their exine structures are of verrucous type. These characteristics and the fact that the exine structure is among the essential criteria in order to determine the phylogenetic relationships between Grimmiaceae species. It was determined with the differences in the measurements that the analyses of all of the species possessed genetic differences. This appears to complete the claim that spore character within taxonomy have a valid morphological structure (Estebanez *et al.*, 1997).

The spore morphologies between the Grimmiaceae family and its relatives exhibit significant differentiating characteristics for taxonomic studies (Sorsa & Koponen, 1973; Vitt & Hamilton, 1974; Boros & Járai-Komlódi, 1975; Olesen & Mogensen, 1978; Brown & Lemmon, 1988; Blackmore & Barnes, 1991; Estebanez *et al.*, 1997; Luizi-Ponzo & Barth, 1998, 1999; Khoshravesh & Kazempour Osaloo, 2007; Potoglu Erkara & Savaroglu, 2007; Savaroglu *et al.*, 2007; Savaroglu & Potoglu Erkara, 2008; Medina *et al.*, 2009; Aşçı *et al.*, 2010; Caldeira *et al.*, 2013).

This study will at the same time provide a basis to the phylogenetic relationship among taxa. The conclusion is that the morphological structures of spores possess distinguishing characteristics for the identification of taxa. Significant findings have been obtained from the spore morphology studies which have been performed, and we carry the belief that, with comparisons between the species to be collected from the region and other taxa within the family, and interpretations from these comparisons, they will make significant contributions to taxonomy.

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