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OBSERVATIONS OF SPORE MORPHOLOGY OF SOME SPECIES OF HYPNACEAE SCHIMP. (BRYOPHYTA) IN TURKEY

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Abstract

The spores of *Hypnum cupressiforme* Hedw. var. *cupressiforme*, *H. cupressiforme* Hedw. var. *lacunosum* Brid., *H. cupressiforme* Hedw. var. *resupinatum* (Taylor) Schimp. and *H. jutlandicum* Holmen and E. Warncke were studied. The apertural region consists of a leptoma in all of the spores. Taxa of the family are uniform, characterized by their surface ornamentation, reflecting the species' taxonomic relationships. The spore shape of all of the species is spheroid. The spore size ranged from 7 to 19 μm in the genus *Hypnum*. The surface ornamentation is verrucate in all species of *Hypnum*. The spore walls of Hypnaceae include sclerine (the distinction between exine and perine difficult to define) and intine. The Moss species belong to corticolous habitat type. The taxonomic and ecological implications of the genus *Hypnum* were discussed on the basis of its spore morphology.

Introduction

The Hypnaceae Schimp. family is small to robust pleurocarpous mosses in tight mats to lax coarse wefts. *Hypnum* is a cosmopolitan genus, in Hypnaceae in the major group of Bryophytes. Both the division of Hypnaceae and the separation of the genera were made according to their gametophytic and sporophytic characteristics. Some of these characters included leaf and its margins shape, alar cells shape, capsules shape, stem epidermal cells, stems and plants color (Smith 2004).

The plants of this genus are suitable for stuffing pillows and thus inducing sleep; moreover, the creeping habit of the moss suggests a sleeping posture. The name was also applied in ancient times to some epiphytic mosses or lichens used as a sleep-inducing medication (Ando 2012).

The capsules of *Hypnum* are erect to horizontal, obloid to cylindrical, straight or curved. Capsules ripen in autumn to winter. The species investigated in this study grows on tree trunks and lower branches of trees, logs, rocks, walls, tombstones, very rarely on soil, in dry exposed or sheltered situations. The species are epiphytic moss. *H. cupressiforme* prefers acidic environments and is fairly tolerant of pollution. It is calcifuge and is a widespread and common moss species belonging to its genus. *H. jutlandicum* is medium-sized plants forming pale green lax patches or wefts. Stems complanately pinnately branched, green colour (Smith 2004).

The spore characteristics are widely used in taxonomic analyses and descriptions of bryophytes and in bryophyte systematics. Spore morphology has been of limited value in taxonomy; however, it has been useful in resolving taxonomic problems. It is also a potential source of information regarding the evolutionary processes, which may lead to the definition of biological or taxonomic boundaries (Carrion *et al*. 1995). In some recent reports the intine structure and the spore external morphology have proved to be useful to characterize moss taxa at

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the generic and specific levels. Therefore studies of bryophyte spores have increased during the last few years (Sorsa and Koponen 1973, Vitt and Hamilton 1974, Boros and Járai-Komlódi 1975, Olesen and Mogensen 1978, Brown and Lemmon 1988, Blackmore and Barnes 1991, Gambardella *et al.* 1994, Carrion *et al.* 1995, Estebanez *et al.* 1997, Luizi-Ponzo and Barth 1998, 1999, Khoshravesh and Kazempour Osaloo 2007, Potoglu Erkara and Savaroglu 2007, Savaroglu *et al.* 2007, Savaroglu and Potoglu Erkara 2008, Medina *et al.* 2009, Caldeira *et al.* 2013). There is, however, still a lot of research necessary in this area of study.

The Turkish bryophyte spores were studied by Potoglu and Savaroglu (2007), Savaroglu *et al.* (2007), Savaroglu and Potoglu (2008). In this study, the detailed spore morphological characteristics of some Hypnaceae species have been studied for the first time with light microscopy (LM) and scanning electron microscopy (SEM). The aim of this study was to characterize the spore morphology of four species of the Hypnaceae.

Materials and Methods

The spore materials of this study were 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 apertural 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 5600 LV scanning electron microscope 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 and Járai-Komlódi (1975), Blackmore and Barnes (1991), Punt *et al.* (1994) and Kapp *et al.* (2000).

Results and Discussion

The sporoderm of the Hypnaceae consists of perine, exine and intine. The separation between the exine and perine may be difficult to define, so sclerine is a more appropriate term. The ornamentation is different in each genus, and it is sometimes possible to distinguish species based on these features. The apertural region consists of an aperture surrounded or not by one or more rings of ornamentation elements. The spores can be placed into one group on the basis of their morphology: the verrucate type. The range of measurements which is found in the reference specimens is in accordance with that of the comparison specimens, but the mean may be somewhat different. This difference reflects intraspecific variation. All of the spore morphometric data are presented in Tables 1 - 3.

The spore shape of all the studied species varied from almost spherical to angular. The spore size ranged from 9.7 (± 0.2) × 13.0 (± 0.3) µm in *H. cupressiforme* var. *resupinatum* to 13.0 (± 0.2) × 15.3 (± 0.2) µm in *H. cupressiforme* var. *cupressiforme* (Table 1). Based on the exine surface ornamentation, one spore type can be recognized here. The spore type having a verrucate exine surface was observed in four species. Below, the characteristics of this spore type are described in further detail.

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

Taxa	Measurements							
	P (µm)				E (µm)			
	R	$X \pm S_x$	S	V (%)	R	$X \pm S_x$	S	V (%)
Hypnum cupressiforme var. *cupressiforme* (N)	9.0 - 14.0	12.2 ± 0.3	1.3	1.8	13.0 -17.0	14.3 ± 0.3	1.3	1.7
Hypnum cupressiforme var. *cupressiforme* (A)	11.0 - 14.0	13.0 ± 0.2	0.8	0.7	14.0 - 17.0	15.3 ± 0.2	0.9	0.9
H. cupressiforme var. *lacunosum* (N)	8.0 - 17.0	12.3 ± 0.4	2.2	4.9	12.0 - 19.0	16.8 ± 0.3	1.7	3.1
H. cupressiforme var. *lacunosum* (A)	8.0 - 14.0	10.4 ± 0.3	1.6	2.6	12.0 - 18.0	14.7 ± 0.3	1.7	2.9
H. cupressiforme var. *resupinatum* (N)	7.0 - 12.0	9.7 ± 0.2	1.2	1.4	10.0 - 15.0	13.0 ± 0.3	1.3	1.8
H. cupressiforme var. *resupinatum* (A)	8.0 - 12.0	9.8 ± 0.2	1.0	1.0	10.0 - 15.0	13.0 ± 0.2	1.1	1.2
H. jutlandicum (N)	7.0 - 15.0	10.6 ± 0.4	2.1	4.3	10.0 - 18.0	13.2 ± 0.5	2.2	5.1
H. jutlandicum (A)	10.0 - 14.0	11.4 ± 0.2	1.0	1.2	13.0 - 17.0	14.2 ± 0.2	1.0	1.0

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.

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

Taxa	Measurements							
	D_M (µm)				D_m (µm)			
	R	$X \pm S_x$	S	V (%)	R	$X \pm S_x$	S	V (%)
Hypnum cupressiforme var. *cupressiforme* (N)	15.0-23.0	18.3±0.3	1.5	2.4	14.0-22.0	17.6±0.3	1.6	2.6
Hypnum cupressiforme var. *cupressiforme* (A)	15.0-19.0	16.8±0.2	1.1	1.2	14.0-17.0	15.6±0.2	0.9	0.9
H. cupressiforme var. *lacunosum* (N)	13.0-20.0	16.3±0.4	1.8	3.3	12.0-19.0	15.3±0.4	1.8	3.2
H. cupressiforme var. *lacunosum* (A)	15.0-19.0	17.0±0.2	0.9	0.9	14.0-18.0	15.9±0.2	1.2	1.4
H. cupressiforme var. *resupinatum* (N)	10.0-16.0	12.5±0.4	1.9	3.8	9.0-15.0	11.7±0.4	1.9	3.7
H. cupressiforme var. *resupinatum* (A)	12.0-18.0	13.9±0.3	1.6	2.4	11.0-17.0	12.9±0.3	1.6	2.4
H. jutlandicum (N)	10.0-16.0	12.4±0.3	1.4	2.0	9.0-15.0	11.3±0.3	1.4	1.9
H. jutlandicum (A)	10.0-15.0	12.3±0.3	1.3	1.6	9.0-14.0	11.2±0.3	1.4	1.8

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.

In examined species, the exine surface is irregularly covered by rather scattered verrucae that are subpatterned by small granules on the surface. The spores are small ranging from 9.7 to 13.0 µm (Table 1), bilaterally and sometimes radially symmetric to asymmetric, heteropolar and plane-convex to concave-convex in shape with a rounded to sub-rounded amb. The exine surface is ornamented by verruca-like elements (Figs 2A-D, 3A-D). The apertural region consists of a less resistant area in the majority of the taxa, and it was interpreted to be a leptoma. The verruca-like elements are larger and rarely dispersed; in these taxa, this field is evaluated as an aperture. The SEM studies are useful for spore type characterization but do not permit a clear differentiation of

the investigated taxa. In addition to the occurrence of an aperture or a leptoma, the most important properties that facilitate the discrimination of these spores are the measurements of their largest diameter (Table 2). Some morphological variations that were observed in the verrucate elements may occur in one taxa, but as far as can see, these elements are not a reliable method to distinguish between species because there is a great intraspecific variation in these characteristics.

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

Taxa	Measurements	
	st (µm)	a (µm)
Hypnum cupressiforme var. *cupressiforme* (N)	0.9	6.9
Hypnum cupressiforme var. *cupressiforme* (A)	0.8	7.1
H. cupressiforme var. *lacunosum* (N)	0.9	7.9
H. cupressiforme var. *lacunosum* (A)	0.9	7.5
H. cupressiforme var. *resupinatum* (N)	0.9	6.2
H. cupressiforme var. *resupinatum* (A)	0.9	5.9
H. jutlandicum (N)	0.8	6.8
H. jutlandicum (A)	0.8	5.8

st: Sclerine thickness. a: Largest length of the apertural region. N: Non-acetolyzed spores.
A: Acetolyzed spores.

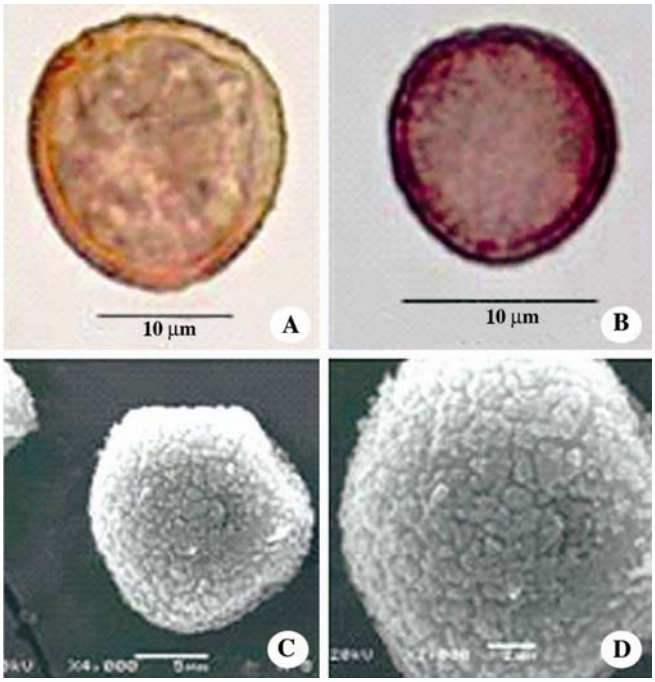

Fig. 1. LM and SEM microphotographs of the spores (A-D). Spore microphotography of *Hypnum cupressiforme* var. *cupressiforme*, (A) proximal view of a non-acetolysed spore under LM, (B) proximal view of an acetolysed spore under LM, (C) distal surface of a non-acetolysed spore in SEM, (D) close up of a non-acetolysed spore in SEM.

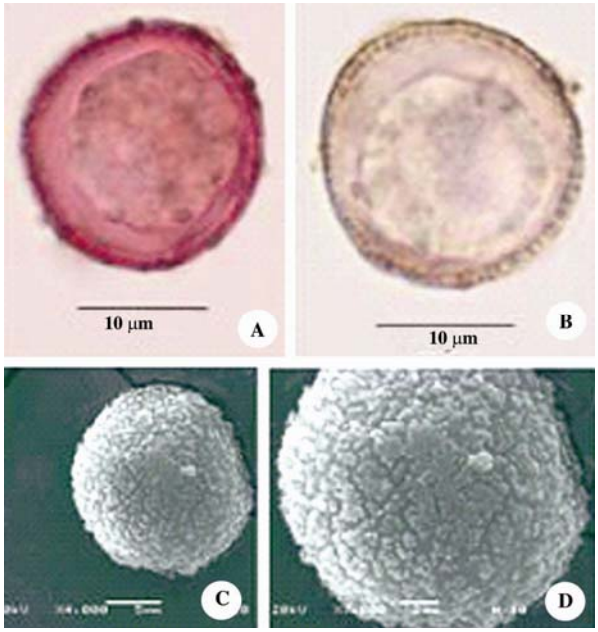

Fig. 2. LM and SEM microphotographs of the spores (A-D). Spore microphotography of *H. cupressiforme* var. *lacunosum*, (A) proximal view of a non acetolysed spore under LM, (B) proximal view of an acetolysed spore under LM, (C) distal surface of a non-acetolysed spore in SEM, (D) close up of a non-acetolysed spore in SEM.

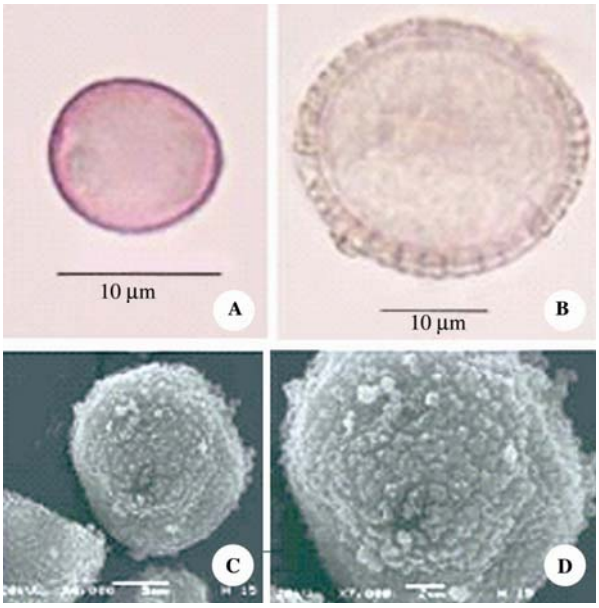

Fig. 3. LM and SEM microphotographs of the spores (A-D). Spore microphotography of *H. cupressiforme* var. *resupinatum*. (A) proximal view of a non-acetolysed spore under LM, (B) proximal view of an acetolysed spore under LM, (C) distal surface of a non acetolysed spore in SEM, (D) close up of a non-acetolysed spore in SEM.

The spore morphology of the species was based on the peristome morphology. The investigated taxa have short spores with verrucate types of spore. The examined spores were spheroidal. The spore morphology of the species that were analyzed here has previously been reported (Boros *et al.* 1993, Kapp *et al.* 2000). The general spore morphology of most of these four species is the same as that which Boros *et al.* (1993) illustrated using light microscopy. The SEM-based analysis of the spore morphology of four *Hypnum* species in Turkey is reported here for the first time. The spore surface ornamentation is of diagnostic value in the identification of the four examined taxa, at least at the genus level and somewhat at the species level within the family. For instance, the present findings illustrate that these four species belong to only one spore type (verrucate).

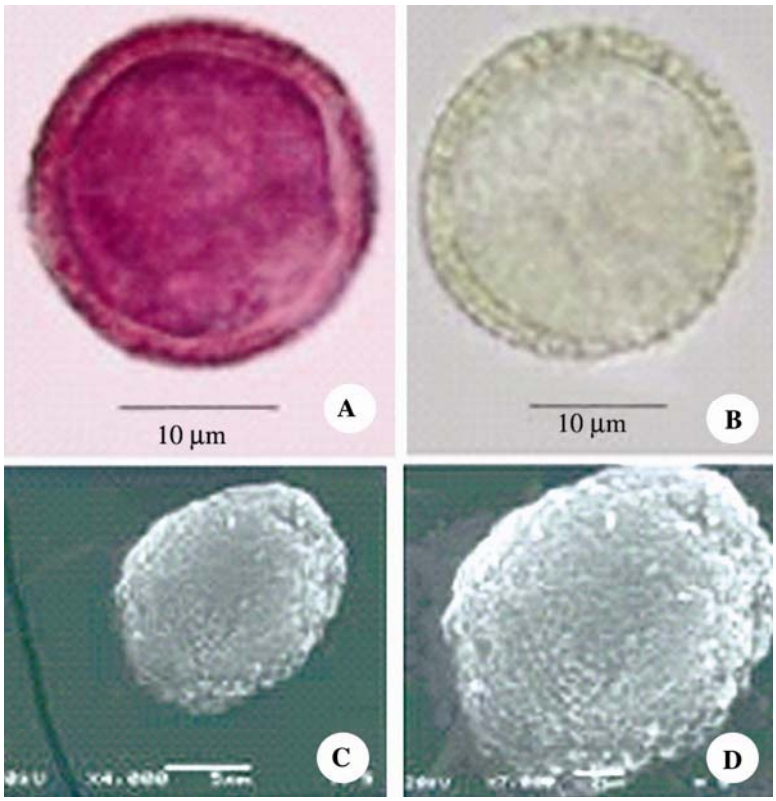

Fig. 4. LM and SEM microphotographs of the spores (A-D). Spore microphotography of *H. jutlandicum*. (A) proximal view of a non acetolysed spore under LM. (B) proximal view of an acetolysed spore under LM. (C) distal surface of a non acetolysed spore in SEM. (D) close up of a non-acetolysed spore in SEM.

The moss species that were examined are of one type with respect to their habitat: corticolous species that inhabit epiphytic surfaces. There is some correlation between the exine surface ornamentation and the vegetation substratum. The verrucate species exine surfaces prefer corticolous habitats. Other morphological adaptations, including spore size, life forms and life strategies, that are related to the habitat conditions have already been illustrated in the Near and middle east bryophytes (Kürschner 2004, Khoshravesh and Kazempour 2007).

Furthermore, there is little correlation between the size and shape of the spores of the examined species and their habitats. All of the species possess small spores and common sporophytes that increase their chance of successful dispersal and of occupying new localities. There is a predicted correlation between the spore morphology of the region with the relevant taxonomic groups and the ecological conditions. These types of investigations help us to predict the rarity, future ecological disturbance, and conservation of bryophytes.

The relatively simple spores of Hypnaceae do not offer many morphological characteristics that are useful for distinguishing between taxa. The ornamentation pattern of the spores is of taxonomic importance, as is evident from the distribution of the different spore types among the species (Luizi-Ponzo and Barth 1998, 1999, Khoshravesh and Kazempour 2007, Potoglu and Savaroglu 2007, Savaroglu and Potoglu 2008). Spores of the verrucate types are found in four species. The spores of some species of the Hypnaceae genera were described by Erdtman (1957), Boros and Járai-Komlódi (1975), Punt *et al.* (1994) and Kapp *et al.* (2000). Spore ornamentation and size are quite variable.

The results that are presented here are in conformity with those found by the above mentioned authors. However, diversities that have not previously been mentioned in the literature but were based on the present study include the surface ornamentation in investigated species. There was sometimes some variability in the mean in the different specimens that were analyzed for each taxon, but the range of the measurements for the comparison specimens were always in accordance with that of the specimen reference. These results confirm those of Olesen and Mogensen (1978) and demonstrate the need to examine more than one specimen to characterize the spore size of a taxon.

Present study agrees with Sorsa and Koponen (1973), Vitt and Hamilton (1974), Boros and Járai-Komlódi (1975), Olesen and Mogensen (1978), Brown and Lemmon (1988), Blackmore and Barnes (1991), Estebanez *et al.* (1997), Luizi-Ponzo and Barth (1998, 1999), Khoshravesh and Kazempour (2007), Potoglu and Savaroglu (2007), Savaroglu *et al.* (2007), Savaroglu and Potoglu (2008), Medina *et al.* (2009), and Caldeira *et al.* (2013) that spore morphology in Hypnaceae and its relatives show distinctive properties that are important for taxonomic studies.

Specimens investigated

All the specimens were collected from Turkey.

Hypnaceae Schimp.

Hypnum cupressiforme Hedw. var. *cupressiforme* A1 Osmaneli (Bilecik): Duzmese out village, 156 m, N 40°22'37.8", E 029°54'54.4", 24.04.2006, on *Platanus* sp., Savaroglu 974.

H. cupressiforme Hedw. var. *lacunosum* Brid. A2 Osmaneli (Bilecik): Osmaneli-Kazanci road, in forest, 156 m, N 40°20'42.4", E 030°03'58.0", 10.04.2006, on soil, Savaroglu 884.

H. cupressiforme Hedw. var. *resupinatum* (Taylor) Schimp. A2 Osmaneli (Bilecik): Mekece-Osmaneli road, in forest, on *Pinus nigra*, 160 m, N 40°25'13.5", E 030°01'25.2", 01.05.2006, on tree, Savaroglu 1003.

H. jutlandicum Holmen and E. Warncke A1 Osmaneli (Bilecik): Beyce entry, stream edge, on *Populus* sp., 324 m, N 40°13'43.7", E 029°56'59.1", 16.10.2008, on tree, Savaroglu 1396.

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Environs with Studies on Pollen Morphology of Some Flowering Plants" (Project Number: 2006/19005).

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