Bangladesh J. Bot. **46**(1): 139-145, 2017 (March)

SPORE MORPHOLOGY, TAXONOMICAL AND ECOLOGICAL IMPORTANCE OF SOME ENCALYPTACEAE SCHIMP. SPECIES (BRYOPHYTA) FROM TURKEY

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Key words: Bryophyta, Encalyptaceae, Spore morphology, Scanning electron microscope

Abstract

Detailed taxonomical, morphological, ecological and paleobotanical characterizations of genus *Encalypta* in Turkey have been provided. The spores of *Encalypta streptocarpa* Hedw., *E. alpina* Sm., *E. rhaptocarpa* Schwagr. and *E. vulgaris* Hedw. were examined by light and scanning electron microscopy. The apertural region forms a leptoma in all spores. The spore morphology of the taxa belonged to four gemmate types (smooth, finely papillose, warty, coarsely papillose). The spore shape of all species was spheroid. Spore size ranged from 11 to 23 µm in the genus *Encalypta*. The spore wall of the family Encalyptaceae included sclerine and intine. The examined species of mosses belonged to two habitat types: the saxicolous and terrestrial. Authors also discussed the taxonomical and ecological implications of the genus *Encalypta* with respect to its spore morphology.

Introduction

The Encalyptaceae Schimp. is primarily dispersed in the northern Hemisphere and is a genus that is common to montane and arctic habitats. Species of Encalyptaceae can be observed spreading on dry or seasonally moist soil on ledges and in crevices of base-rich montane rocks, walls, and cliffs, and on sand-dunes (Horton 1983a, 1983b, 1988, Smith 2004). Both the division of Encalyptaceae and the partition of the genera have been made according to gametophytic and sporophytic characters. Some of these characters include leaf shape, laminal cells, capsule shape and spore ornamentation (Smith 2004). The genus *Encalypta* is exceptional spore characteristics have proven the taxonomic value at the genus as well as the species level (Carrion *et al.* 1995). The spore morphology of bryophytes is an important character for use in taxonomy. It has been useful in resolving taxonomic problems. It is also a potential source of information about evolutionary processes, information which may prove to be useful for the definition of biological or taxonomic boundaries (Carrion *et al.* 1995). Therefore, studies of bryophyte spores have increased in recent years (Vitt and Hamilton 1974, Horton 1983a, 1983b, 1988, Blackmore and Barnes 1991, Gambardella *et al.* 1994, Carrion *et al.*1995, Luizi-Ponzoand Barth 1998, 1999, Magill 2007, Potoglu and Savaroglu 2007, Savaroglu *et al.* 2007, Savaroglu and Potoglu 2008, Calderia *et al.* 2013). Nevertheless, more study is needed in this field.

The elaborated spore morphological structures of some Encalyptaceae were studied for the first time by light microscopy (LM) and scanning electron microscopy (SEM). The purpose of this study was to define the spore morphology of four species of the Encalyptaceae to aid studies in taxonomy, ecology and paleobotany.

Materials and Methods

The spore material was supplied by the Faculty of Science and Arts of the Osmangazi University Herbarium (OUFE). The outward surface was investigated with LM and SEM. The spores were prepared untreated with glycerin-jelly on microscope slides (Wodehouse 1935), using

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the acetolysis method (Erdtman 1957) for LM. Measurements of the shortest and the largest diameters (in the polar view), as well as the polar axis and the equatorial diameter (in the equatorial view), were taken of 25 randomly selected spores. The mean, the standard deviation, the standard error, and the range were then established. The sclerine thickness, as well as the largest length of the apertural region, was 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 then examined with a Jeol 5600 LV scanning electron microscope at an accelerating voltage of 20 kV. The specimen listed under 'Specimens examined' is the reference specimen, whereas the others are the collations. Terminologies for spore morphology suggested by Erdtman (1957), Blackmore and Barnes (1991), Punt *et al.* (1994) and Kapp *et al.* (2000) were used throughout.

Results and Discussion

The sporoderm of Encalyptaceae contains the perine, the exine and the intine. The distinction between the exine and the perine may be difficult to describe, and thus, sclerine is a more suitable term for use. The ornamentation is different in each genus, and it is sometimes possible to recognize species based on these properties. The apertural region is composed of an aperture, which may or may not be enclosed by one or more rings of ornamentation elements. The examined taxa of the family are uniform in their spore morphology. The spores of the four taxa are of the gemmate type. The ranges of measurements established in the reference specimens are in line with those of the comparison specimens, but the mean may be somewhat different. This reflects the occurrence of intraspecific variation. The morphometric data of the spores are found in Tables 1 - 3. The spore morphology of the examined taxa of Encalyptaceae belonged to four gemmate types (smooth, finely papillose, warty and coarsely papillose).

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

Taxa	Measurements							
	P				E			
	R (µm)	$X \pm S_x$ (µm)	s (µm)	V (%)	R (µm)	$X \pm S_x$ (µm)	s (µm)	V (%)
Encalyptastreptocarpa (N)	13-17	14.9-0.2	0.9	0.9	15-18	16.5-0.1	0.8	0.7
E. streptocarpa (A)	11-16	14-0.3	1.3	1.8	14-19	16.9-0.3	1.3	1.7
E. alpina (N)	14-19	16.2-0.3	1.3	1.6	16-20	17.7-0.2	1	1
E. alpina (A)	14-19	16.8-0.3	1.3	1.8	16-22	19-0.3	1.3	1.8
E. rhaptocarpa (N)	15-20	18.2-0.2	1	1	17-23	20.7-0.3	1.3	1.7
E. rhaptocarpa (A)	15-19	17.2-0.2	1.1	1.2	18-22	19.5-0.2	1.2	1.3
E. vulgaris (N)	13-17	15-0.2	1.1	1.2	13-18	15.9-0.3	1.3	1.8
E. vulgaris (A)	14-19	16.8-0.3	1.3	1.8	16-21	18.9-0.3	1.3	1.7

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.

E. streptocarpa (Type I). Spores small, smooth. 11 - 19 µm, ornamentation ± similar at proximal and distal ends; calyptraeerose at base, papillose towards apex (Fig. 1).

E. alpina (Type II). Spores 14-22 µm in diameter, circular to elliptic, the proximal and distal faces distinguished by ornamentation forms. No ridges or trilete markings are present. The ornamentation of the distal surface consists of small, irregularly, fine papillose, verrucate papillae 0.5 - 1.5 µm in diameter, which often fuse to form a more or less reticulate pattern when viewed at high magnification. The proximal surface often seems caved-in when using SEM, possibly indicating a thinner wall than that of the distal face. It is coated with widely spaced, irregularly armed papillae. The line of demarcation between the proximal and distal surfaces is usually quite sharp. Spore papillose, variable in size, 28 - 40 µm, ornamentation similar at proximal and distal ends (Fig. 2).

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

Taxa	Measurements							
	D_M				D_m			
	R (µm)	$X \pm S_x$ (µm)	s (µm)	V (%)	R (µm)	$X \pm S_x$ (µm)	s (µm)	V (%)
Encalypta streptocarpa (N)	16-20	18.2-0.2	1.1	1.1	15-19	17.3-0.2	1	1
E. streptocarpa (A)	16-20	17.4-0.2	1.2	1.3	15-19	16.6-0.2	1.1	1.2
E. alpina (N)	16-21	18.4-0.3	1.3	1.7	15-20	17.4-0.3	1.3	1.7
E. alpina (A)	15-22	18.8-0.3	1.6	2.4	14-21	17.8-0.3	1.5	2.2
E. rhaptocarpa (N)	21-27	23.8-0.3	1.5	2.1	20-26	22.8-0.3	1.5	2.2
E. rhaptocarpa (A)	17-27	21.8-0.5	2.4	5.5	16-26	20.7-0.5	2.3	5.5
E. vulgaris (N)	17-21	18.5-0.2	0.9	0.9	16-20	17.6-0.2	0.9	0.8
E. vulgaris (A)	17-21	18.8-0.2	0.9	0.9	16-20	17.9-0.2	0.9	0.9

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 Encalyptaceae spores.

Taxa	Measurements	
	st (µm)	a (µm)
Encalyptastreptocarpa (N)	1	6.6
E. streptocarpa (A)	0.9	6.9
E. alpina (N)	1	7
E. alpina (A)	0.9	6.8
E. rhaptocarpa (N)	0.9	7.4
E. rhaptocarpa (A)	0.9	7
E. vulgaris (N)	1	7.1
E. vulgaris (A)	1	7.2

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

E. rhaptocarpa (Type III). Spores 15 - 23 µm in diameter, triangular, with the proximal and distal surfaces greatly differentiated. The proximal face is irregularly, coarsely papillose, with the papillae arising from numerous (18 - 22) ridges, which radiate outward from the center. The distal surface is ornamented by very large (5 - 6 µm in diameter), warty, clavate, closely spaced papillae. The ridges of the proximal side often terminate in one of the marginal clavate papillae. No ridges are present on the distal face. The large, clavate papillae of the distal surface, which are warty, and the ridged proximal surface are characteristic of this species and to a lesser extent, of *E. vulgaris* (Fig. 3).

E. vulgaris (Type IV). Spores 13 - 21 µm in diameter, elliptic to triangular-elliptic, often somewhat straightened. The proximal and distal faces are recognizable by both ridges and ornamentation. The distal face is coated by large (4 - 5 µm in diameter), widely spaced gemmate to verrucate papillae, roughened by small projections and varying from tall, squarrose structures to smaller, rounded projections. The proximal face is usually ridged and contains smaller gemmate ledges, which vary in size and number (Fig. 4).

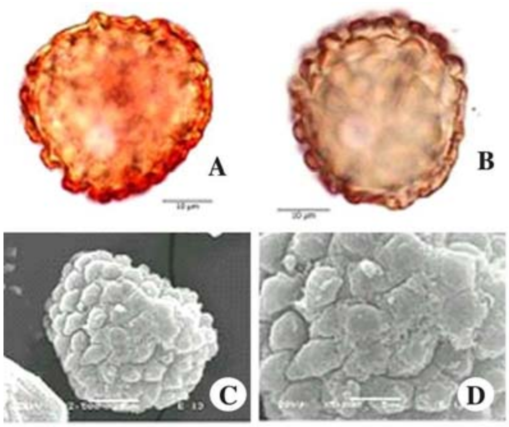

Fig. 1. *E. streptocarpa*

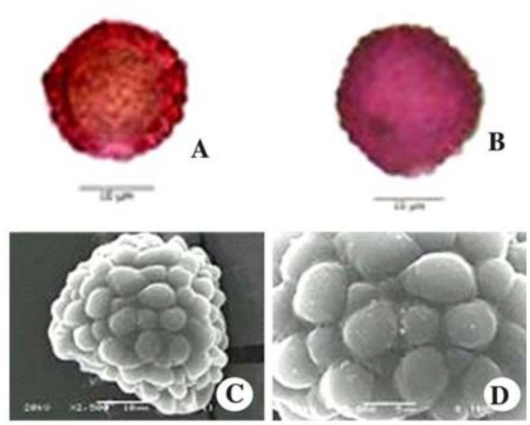

Fig. 2. *E. alpina*

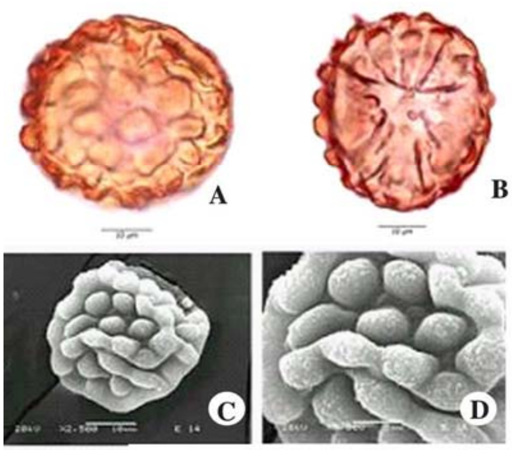

Fig. 3. *E. rhaptocarpa*

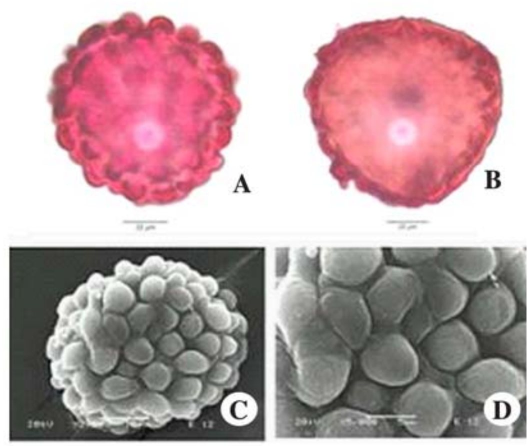

Fig. 4. *E. vulgaris*

Figs 1 - 4. 1. *Encalypta streptocarpa* spores; 2. *E. alpina* spores; 3. *E. rhaptocarpa* spores; 4. *E. vulgaris* spores. A- proximal view (NA; LM), B- proximal view (A; LM), C- distal surface (SEM), D- close-up of spore (SEM).

The spores of *E. vulgaris* diverge from those of *E. rhaptocarpa* in having verrucate or gemmate, widely spaced papillae on the distal face. The ridges of the proximal face do not appear as well developed as they are in *E. rhaptocarpa*. Like the latter species, *E. vulgaris* has warty papillae and markedly polar spores. It is the most unstable of the species studied in its spore structure and is clearly close in its morphological features to *E. rhaptocarpa*.

The spores of *E. streptocarpa* diverge from those of *E. alpina* in having verrucate or gemmate, widely spaced papillae on the distal face. The ridges of the proximal face do not appear as well developed as they are observed in *E. streptocarpa*. Like the latter species, *E. alpina* has fine papillose and markedly polar spores. It is the most unstable of the species studied in its spore structure and is clearly close in its morphological features to *E. streptocarpa*.

Small (ranging from 11 to 23 µm) sized spores (Tables 1 - 3), bilaterally and sometimes radially symmetric to asymmetric, heteropolar, rounded to subroundedamb, plane-convex to concave-convex in shape. The exine surface is ornamented by gemmae-like elements (Figs 1-4). The apertural region consists of a less resistant area in the majority of the taxa and it was interpreted as a leptoma (Figs 1-4). 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 discriminating of these spores are the measurements of their largest diameter (Table 2). Some morphological variations observed in the gemmae elements may occur in some taxa, but as far as we can see, these are not a reliable source to use when establishing a distinction between species because there is a great intraspecific variation in these characteristics.

The spore morphology of the species was based on the peristome morphology. Investigated taxa have short spores with four gemmate types (smooth, finely papillose, warty, coarsely papillose) of spore morphology. As described in the results, gemmate spore types were determined for four species. The examined spores were spheroidal. The spore morphology of the gemmate of the four species analyzed here has previously been reported (Boros *et al.*1993). 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 *Encalypta* species is reported here. The spore surface ornamentations are of diagnostic value in the identifications of the four examined taxa, at least to the genus level and somewhat to the species level within the family. For instance, present findings illustrate that the four species included belong to just one spore type (gemmate).

The species of mosses examined are of two types with respect to their habitat: saxicolous species that inhabit rock surfaces, and terrestrial species that inhabit moist soil. There is some correlation between the exine surface ornamentations and the vegetation substratum. The species with gemmate spore ornamentations belong to either saxicolous or terrestrial habitats. The saxicolous members produce their sporophytes under conditions of high humidity and short-period sunlight, primarily during the winter season. It is noteworthy that the saxicolous species possess spores that are densely ornamented by exine elements, whereas the moisture-dependent species have spores that are loosely covered by subpatterned exine surfaces. Other morphological adaptations, including spore size, life forms and life strategies, that are related to habitat conditions have already been illustrated in the Near and Middle East Bryophytes. Furthermore, there is little correlation between the size and shape of the spores of the examined species and the species' habitat. All the species possess small spores and common sporophytes that increase their chance of successful dispersal and occupying of new localities. These characteristics are related to a common strategy of drought resistance. This strategy is 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 and is used to compensate for the high mortality rate of the gametophytes, which is often caused by summer drought or erosion effects (Kürschner 2004). There is a predicted correlation between the spore morphology of the region with the relevant taxonomical groups and the ecological conditions. These types of investigations help us to predict the rarity, future ecological disturbance, and conservation of bryophytes.

The ornamentation pattern of the spores is of taxonomic importance, as is evident from the distribution of the different spore types among the species (Horton 1983a, 1983b, 1988, Luizi-Ponzoand Barth 1998, 1999). Spores of the gemmate types (smooth, finely papillose, warty, coarsely papillose) are found in all four species. The spores of some species of the Encalyptaceae genera were described by Erdtman (1957), Punt *et al.* (1994), Kapp *et al.*(2000) and Magill (2007).

The results presented here are in conformity with other authors. However, diversities that have not previously been mentioned in the literature but were based on the present study include the surface ornamentation in *Encalypta streptocarpa, E. alpina, E. rhaptocarpa* and *E. vulgaris*. There is sometimes some variability in the mean found in the different specimens analyzed for each taxon, but the range of the measurements for the comparison specimens were always in accordance with the specimen reference. The authors agrees with Vitt and Hamilton (1974), Horton (1983a, 1983b, 1988), Blackmore and Barnes (1991), Luizi-Ponzoand Barth (1998, 1999), Magill (2007), Potoglu and Savaroglu (2007), Savaroglu *et al.* (2007), Savaroglu and Potoglu (2008) and Caldeira *et al.* (2013) that spore morphology in Encalyptaceae and its relatives show distinctive properties that are important for taxonomic studies.

Specimens investigated

All specimens are from Turkey. Encalyptaceae Schimp.

Encalypta streptocarpa Hedw. A2 Osmaneli (Bilecik): Kazanci village, in the valley, canyon, 218 m, N 40°20'08.6", E 030°12'46.1", 26.05.2006, on rock, Savaroglu 1090.

Encalypta alpina Sm. A1 Osmaneli (Bilecik): From Egerce-Orhaniye, Ataturk’s path, 422 m, N 40°20'31.5", E 029°51'39.1", 18.05.2006, on rock, Savaroglu 1044.

E. rhaptocarpa Schwagr. A2 Osmaneli (Bilecik): Balcikhisar road, 299 m, N 40°20'58.0", E 030°00'03.1", 04.04.2007, on rock, Savaroglu 1246.

E. vulgaris Hedw. A2 Osmaneli (Bilecik): Kozan village, highland, *Pinusnigra, Quercus* sp., *Juniperus oxycedrus*, 189 m, N 40°26'45.4", E 030°07'48.4", 10.04.2006, on soil, Savaroglu 872.

Acknowledgement

This study was supported by Eskişehir Osmangazi University Scientific Research Projects Commission in the name of “Flowering Plants and Moss Flora of Osmaneli (Bilecik-Turkey) and Environs with Studies on Pollen Morphology of Some Flowering Plants” (Project Number: 2006/19005).

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(Manuscript received on 30 December, 2015; revised on 12 March, 2016)