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POLLEN MORPHOLOGY AND ITS RELATIONSHIP TO THE TAXONOMY OF THE SOME TAXA OF *HELICHRYSUM* GAERTNER (ASTERACEAE) IN TURKEY

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Abstract

Palynological studies of *Helichrysum chionophilum*, *Helichrysum noeanum*, *Helichrysum arenarium* subsp. *aucheri*, *Helichrysum graveolens, H. plicatum* subsp. *plicatum* and *H. plicatum* subsp. *polyphyllum* (Asteraceae) from Turkey have been carried out. *H. chionophilum, H. noeanum* and *H. arenarium* subsp. *aucheri* are endemics to Turkey. Pollen features of both taxa were observed with the Light and Scanning Electron Microscopy (SEM). The pollen type of both taxa were determined as tricolporate, pollen shapes were spheroidal and oblate-spheroidal, reticular pollen ornamentation was observed. The studies show that the pollen of the six taxa are very similar regarding pollen shapes, apertures, and ornamentation, but may be distinguished by colpus length (Clg), pore width (Plt) and pore length (Plg). The findings obtained from the study are discussed with each other and the genus patterns.

Key words: *Helichrysum*, Asteraceae, Pollen morphology, LM, SEM.

Introduction

The tribe Gnaphalieae of Asteraceae comprises 185 genera and about 1240 species; it is cosmopolitan, but most diverse in the southern hemisphere. *Helichrysum* is the largest genus of this tribe, with about 600 species, occurring in Europe, Asia, Africa and Madagascar (Bayer *et al.*, 2007; Şenol *et al.*, 2011). This genus is represented by 24 species, 30 taxa of which, 17 are endemic in Turkey (Guner, 2012). A high level morphological polymorphism including anatomical characters among *Helichrysum* species was noted by Jahn *et al*., (Öztürk *et al*., 2014).

Helichrysum species have been used as folk medicine for at least 2000 years against gall bladder disorders in the form of medicinal teas, because of their bile regulatory and diuretic effects (Lindemann, 1973). In Turkey several *Helichrysum* species are used in folk medicine for removing the kidney stones and as diuretics. The diuretic and bile regulatory effects of the *Helichrysum* species are due to presence of the flavonoids that they contain (Cubukcu, 1992).

The Mediterranean, European, western Asian, and central Asian *Helichrysum* species are morphologically characterized by the presence of homogamous or heterogamous capitula, with hermaphroditic flowers outnumbering the pistillate ones; phyllaries with a fenestrated stereome; smooth or alveolate receptacle; cypselae glabrous or with duplex hairs; monomorphic uniseriate pappus, consisting of several free scabrid bristles, with patent cilia at the base (Galbany-Casals *et al*., 2011).

Helichrysum species are generally known under the names ‘‘ölmez çiçek or altınotu” and are widely used as herbal teas in Turkey (Albayrak *et al*., 2010).

Helichrysum is a large and taxonomically difficult genus, and its circumscription has undergone notable changes, because the traditional concept of the genus included an assemblage of numerous heterogeneous taxa. It has been repeatedly stated that Australian and New Zealand species traditionally known as *Helichrysum* certainly belong to other taxonomic groups, mainly to genera from either the subtribe Angianthinae or the subtribe Cassiniinae (Anderberg, 1991; Bayer *et al.,* 2002, Galbany-Casals *et al.,* 2009). A considerable number of changes, based on morphology, have been made concerning the generic delimitation of African *Helichrysum* species and several related genera. Many species have been placed in other genera (e.g., *Syncarpha* DC. [Anderberg, 1991; Nordenstam, 2003], *Edmondia* Cass., *Dolichothrix* Hilliard & B. L. Burtt, *Atrichantha* Hilliard & B. L. Burtt, and *Plecostachys* Hilliard & B. L. Burtt [Hilliard and Burtt, 1981]), while others have been transferred into *Helichrysum* from related genera (e.g., Leontonyx Cass. (Hilliard & Burtt 1981). Finally, some genera closely related to *Helichrysum*, such as *Achyrocline* (Less.) DC. and *Pseudognaphalium* Kirp., cannot be segregated from *Helichrysum* by any qualitative morphological character, and some authors suggest that their circumscription should be revised (Hilliard & Burtt, 1981; Anderberg, 1991).

The number of taxa and the taxonomic rank of *Helichrysum* populations is still widely debated, due to the high morphological variability and to the fragmentary distribution, so that many different interpretations and nomenclatural proposals are available in the literature including Flora of Turkey and the East Aegean Islands. To disentangle such a critical taxonomic issue, important additional information can be obtained through the application of techniques and methods other than the mere, albeit accurate, comparison of micro- and macromorphological structures (Maggio *et al*., 2016). For this purpose, it was decided to collect a representative number of specimens from the *Helichrysum* populations found in the loci classici of the nomenclatural types of Turkey taxa, to check the pollen morphology which is a well known useful complementary tool in a taxonomic treatment and also to characterize individual species or populations.

The ability to identify plants from their pollen has enabled botanists and ecologists to reconstruct past assemblages of plants and identify periods of environmental changes. Morphological characteristics of pollen grains also can be useful characters in studies of plant taxonomy because many pollen traits are influenced by the strong selective forces involved in various reproductive processes, including pollination, dispersal, and germination. At the same time, characters subject to strong selection can be misleading if they reflect convergent evolution – similar evolutionary responses by unrelated taxa to similar environmental conditions. Thus, the use of pollen morphology as a taxonomic character is challenging and pollen characteristics must be considered in concert with other characteristics in evolutionary reconstructions (Oswald *et al*., 2011).

The main objectives of the present study are to provide a detailed account of the pollen morphology of the some species of *Helichrysum*, using light (LM) and scanning electron microscopy (SEM) and to assess the utility of the palynological data for classification at infraspecific and sectional level within the genus.

Materials and Methods

Plant material: The aerial and underground parts of *Helichrysum* taxa used in this study were collected in the flowering season from the different regions of Turkey in 2010 (Table 1 and Fig. 1). The plants were identified with the help of Flora of Turkey (Davis & Kupicha 1975) and also confirmed by the herbarium specimens of the examined taxa in ANK, HUB and GAZİ.

Palynological studies: Pollen grains of 6 taxa of the genus *Helichrysum* were studied by LM and SEM. Pollen material was obtained from plant specimens collected from Turkey 2010. Faegri & Iversen's (1975) terminology was used for naming the exine layers. For light microscopic (LM) investigations, the method of preparation described by Wodehouse (1935) was used. Pollen counts and identifications were done using a Prior binocular microscope with a 10 x ocular lens and 10, 40 and 100 x plain oil immersion objectives. The exine and intine thicknesses of pollen were measured using 50 replicates. From the measurements, a natural mathematical mean was calculated.

The pollens were placed under a stereomicroscope on a metal holder with double-sided tape attached with the aid of a pair of clamps and clean needles. Later, the pollen covered with gold with Denton brand coating device in Fırat University Electron Microscope Laboratory was scanned at 10.00 kV with JEOL JSM 7001F brand scanning electron microscope. Examples of polar appearance, equatorial appearance, ornamentation, and spins of poles are preferred, and 5-8 pictures are drawn for each taxon.

Statistical method: Latent class cluster analysis was used to identify segments of plants who were similar in their pollen measure got from 8 different criteria (Table 2). We used Bayesian Information Criteria (BIC) to select optimum number of class as a maximum likelihood estimator (Tables 3, 4).

Table 2. Cluster analysis results.

	Bayesian information criteria (BIC)
1 cluster	206.86
2 cluster	204.83
3 cluster	205.53

Table 3. Loadings for each taxa to each class for 2-class solution.

No.	Taxa	Loadings	
		1st cluster	2nd cluster
1.	*H. chionophilum*	**0.9933**	0.0067
2.	*H. noeanum*	0.0017	**0.9983**
3.	*H. arenarium* subsp. *aucheri*	**0.9978**	0.0022
4.	*H. plicatum* subsp. *polyphyllum*	**1.0000**	0.0000
5.	*H. plicatum* subsp. *plicatum*	0.0000	**1.0000**
6.	6-*H. graveolens*	**1.0000**	0.0000

Table 1. Locations, voucher and specimen code of *Helichrysum* taxa.

	Taxon	Locality, Voucher and specimen code
1.	*H. chionophilum* *	B6 Sivas: 2000 m., 26.07.2010, *Elkiran* 1046
2.	. *H. noeanum* *	B4 Ankara: 1200m., 14.06.2010, *Elkiran* 1001
3.	*H. arenarium* subsp. *aucheri* *	B5 Kırşehir: 1100m., 17.07.2010, *Elkiran* 1045
4.	*H. graveolens*	A2 Bursa: 1850 m., 24.06.2010, *Elkiran & Evren & Bagci* 1041
5.	*H. plicatum* subsp. *plicatum*	B6 Malatya: 1400 m., 21.06.2010, *Elkiran & Evren & Bagci* 1005
6.	*H. plicatum* subsp. *polyphyllum*	C3 Isparta: 2000 m., 22.06.2010, *Elkiran & Evren & Bagci* 1018

* =Taxa endemic to Turkey

Table 4. Coefficient of variation (CV) values of each criterion for each taxa cluster.

	Criteria-1 (L)	Criteria-2 (Equatorial axes)	Criteria-3 (Polar axes)	Criteria-4 (Clg)	Criteria-5 (Clt)	Criteria-6 (Plt)	Criteria-7 (Plg)	Criteria-8 (Exine)
1st cluster	0.09	0.09	0.08	**0.03**	0.27	0.07	0.08	0.08
2nd cluster	12.19	9.34	7.72	21.15	10.84	**4.38**	**4.48**	9.66

Fig. 1. Geographical location of study area.

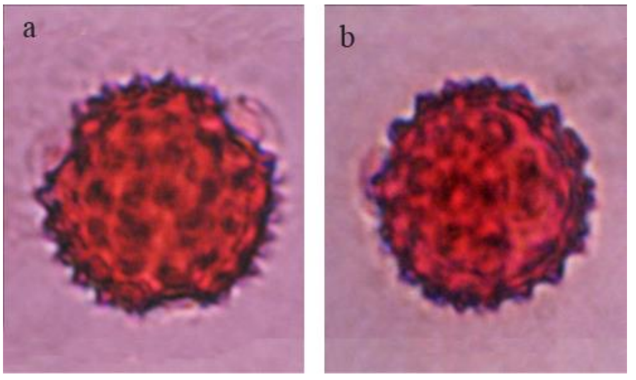

Fig. 2. Light Microscopy micrographs of pollen grains of *H. chionophilum*. a. Polar view, b. Equatorial view.

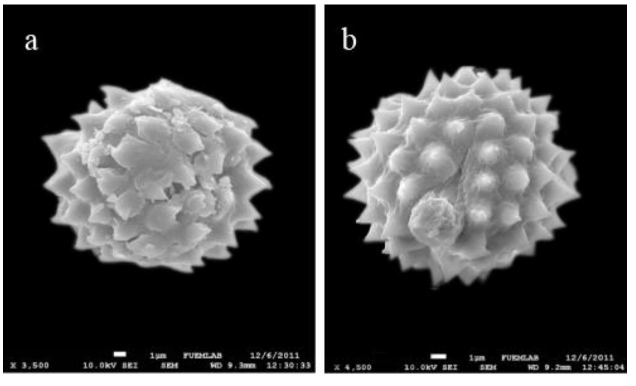

Fig. 3. Scanning electron microscopy micrographs of pollen grains of *H. chionophilum*. a. Polar view, b. Equatorial view.

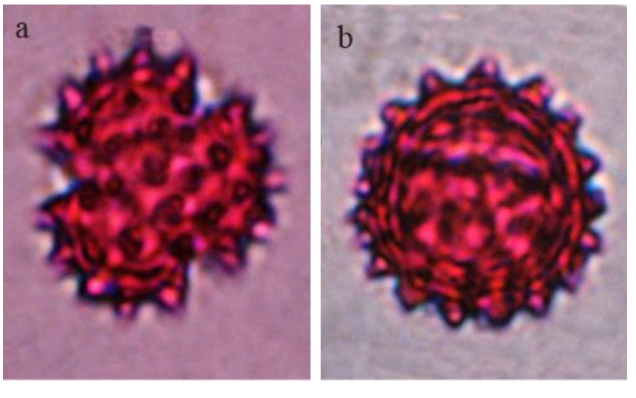

Fig. 4. Light Microscopy micrographs of pollen grains of *H. noeanum*. a. Polar view, b. Equatorial view.

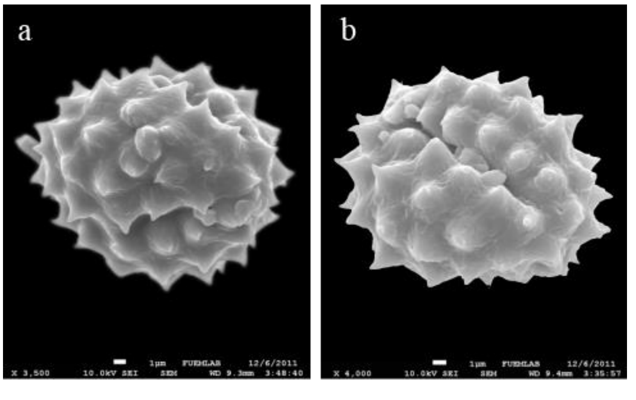

Fig. 5. Scanning electron microscopy micrographs of pollen grains of *H. noeanum*. a. Polar view, b. Equatorial view.

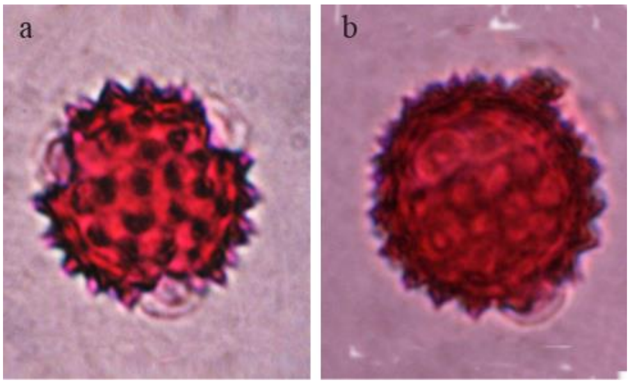

Fig. 6. Light Microscopy micrographs of pollen grains of *H. arenarium* subsp. *aucheri*. a. Polar view, b. Equatorial view.

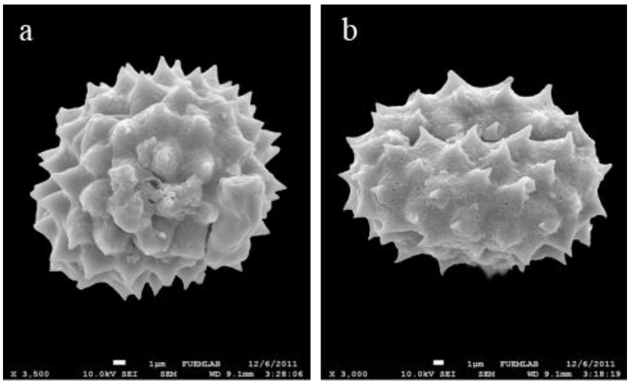

Fig. 7. Scanning electron microscopy micrographs of pollen grains of *H. arenarium* subsp. *aucheri*. a. Polar view, b. Equatorial view.

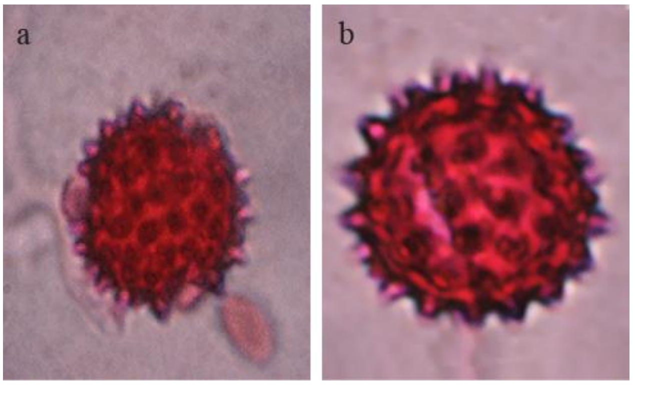

Fig. 8. Light Microscopy micrographs of pollen grains of *H. plicatum* subsp. *plicatum*. a. Polar view, b. Equatorial view.

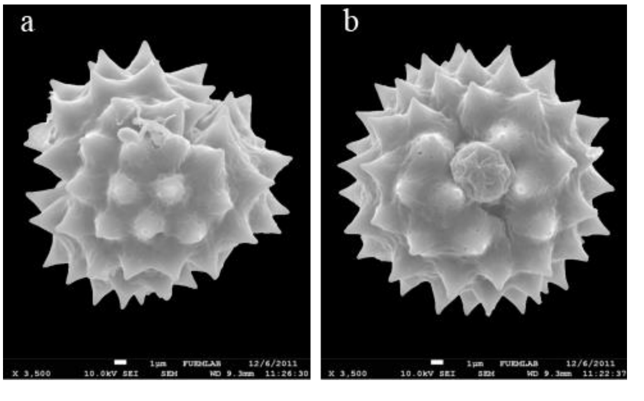

Fig. 9. Scanning electron microscopy micrographs of pollen grains of *H. plicatum* subsp. *plicatum* a. Polar view, b. Equatorial view.

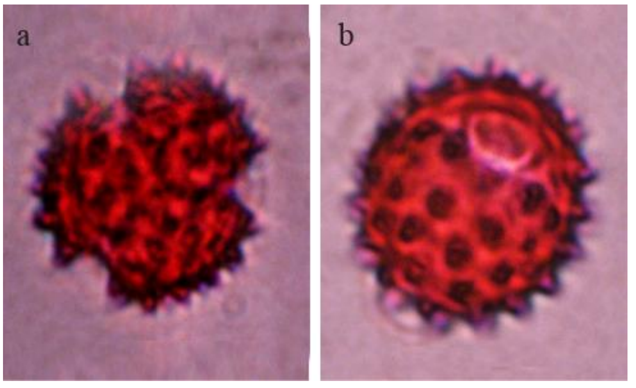

Fig.10. Light Microscopy micrographs of pollen grains of *H. plicatum* subsp. *polyphyllum*. a. Polar view, b. Equatorial view.

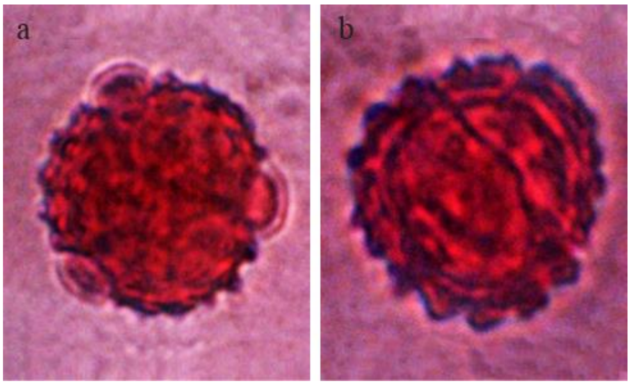

Fig. 12. Light Microscopy micrographs of pollen grains of *H. graveolens*. a. Polar view, b. Equatorial view.

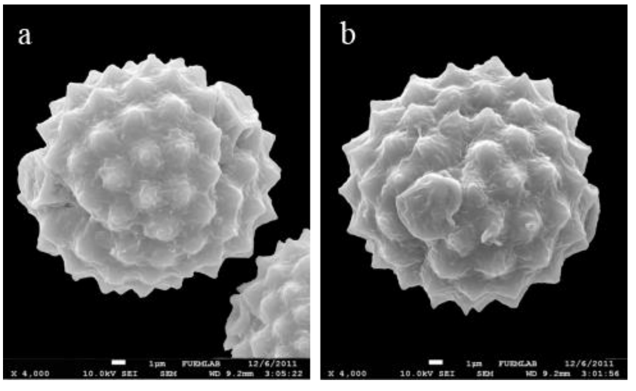

Fig. 11. Scanning electron microscopy micrographs of pollen grains of *H. plicatum* subsp. *polyphyllum* a. Polar view, b. Equatorial view.

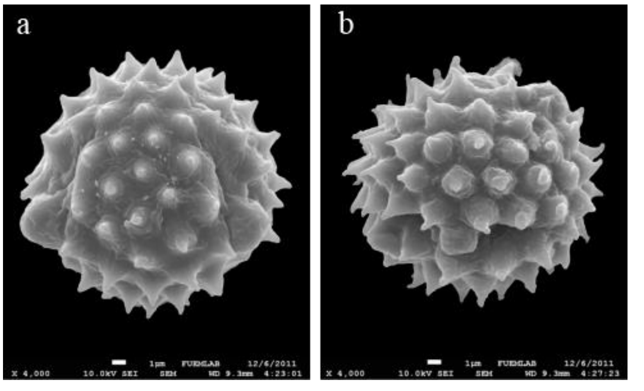

Fig. 13. Scanning electron microscopy micrographs of pollen grains of *H. graveolens* a. Polar view, b. Equatorial view.

Discussion

Pollen morphology remains an ideal tool for systematics at the family level or below. The more prominent features such as aperture morphoform, shape, relative size, exine wall pattern and ornamentation are characteristic to various taxa and are used extensively in taxonomy, volution and palaeontology (Akhila *et al.*, 2015).

In the present study, the detailed pollen morphological structure of the some species of *Helichrysum* taxa are given. The pollen type of studied taxa were determined as trycolporate, pollen shape was oblate- spheroidal and spheroidal, reticulate pollen ornamentation. So, we can say that, the some taxa of the genus *Helichrysum* showed similarities in palynological characters, but showed differences in the size of pollen. The maximum P and E values were observed in *Helichrysum arenarium* subsp. *aucheri* and minimum values in *Helichrysum noeanum*. Walker (1976) regarded pollen size as a tertiary character in phylogenetic studies and was of not much phylogenetic significance. The pollen size is the result of a multitude of physiological, morphological, ecological and historical constraints on a plant species (Torres, 2000). According to Blackmore *et al*. (2007), exine is the most complex structure and its vast morphological diversity forms the basis of the discipline of palynology. In the present investigation, it was found that exine thickness was higher in *Helichrysum chionophilum* as compared to the other species.

However, Ok (2009) reported that, pollen shapes were oblate- spheroidal of *H. chionophilum* and *H. arenarium* subsp. *aucheri*, but pollen shape was prolate-spheroidal of *H. noeanum*. And other hand, Pınar *et al*. (2000) reported that, *H. noeanum* pollen shape was spheroidal and *H. chionophilum* pollen shape was prolate-spheroidal. In another study, İnceoğlu *et al*. (1977) reported that, *H. plicatum* subsp. *plicatum* pollen shape was oblate-spheroidal.

In summary, the systematical study into *H. chionophilum*, *H. noeanum* and *H. arenarium* subsp. *aucheri*, *H. plicatum* subsp. *plicatum*, *H. plicatum* subsp. *polyphyllum*, *H. graveolens* might shed light on further studies with regard to palynological characteristics of these taxa.

Results

H. chionophilum: Pollen grains are tricolporate, oblate-spheroidal. Polar view triangular, amb 15±1.32 µm. Polar axis 24.69±2.15 µm, equatorial diameter 24.77±1.98 µm, colpus length 14.90±1.91 µm, colpus width 4.40±1.77 µm, Porus length 6.00±1.00 µm, porus width 5.00±1.00 µm. Exine 2.4±0.26 µm. Spines are perforated (Figs. 2, 3).

H. noeanum: Pollen grains are tricolporate, oblate-spheroidal. Polar view triangular, amb 16.25±1.50 µm. Polar axis 20.53±1.14 µm, equatorial diameter 21.20±1.34 µm, colpus length 14.73±3.67 µm, colpus width 3.54±1.03 µm, porus length 4.25±0.95 µm, porus width 3.25±1.25 µm. Exine 1.81±0.53 µm. Spines are perforated (Figs. 4, 5).

H. arenarium **subsp.** ***aucheri:*** Pollen grains are tricolporate, oblate-spheroidal.Polar view triangular, amb 17.71±1.49 µm. Polar axis 25.81±1.76 µm, equatorial diameter 26.65±1.79 µm, colpus length 15.06±1.83 µm, colpus width 3.40±1.12 µm, Porus length 6.14±1.00 µm, Porus width 4.89±0.87 µm. Exine 2.11±0.19 µm. Spines are perforated (Figs. 6, 7).

H. plicatum **subsp.** ***plicatum:*** Pollen grains are tricolporate, spheroidal. Polar view triangular, amb 14, 83±1, 16 µm. Polar axis 24, 67±1, 18 µm, equatorial diameter 24, 67±1, 29 µm, colpus length 13, 77±2, 10 µm, colpus width 3, 11±1, 02 µm, porus length 5, 50±0, 92 µm, porus width 4, 50±0, 92µm. Exine 2, 2 µm. Spines are perforated (Figs. 8, 9).

H. plicatum **subsp.** ***polyphyllum:*** Pollen grains are tricolporate, oblate-spheroidal. Polar view triangular, amb 14, 66±2, 41 µm. Polar axis 21, 90±1, 56 µm, equatorial diameter 23, 03±1, 64 µm, colpus length 14, 17±2, 16 µm, colpus width 2, 28±0, 78 µm, porus length 5, 14±0, 94 µm, porus width 4, 21±0, 89 µm. Exine 2 µm. Spines are perforated (Figs. 10, 11).

H. graveolens: Pollen grains are tricolporate, oblate-spheroidal. Polar view triangular, amb 13, 66±1, 07 µm. Polar axis 21, 96±1, 67 µm, equatorial diameter 22, 18±1, 98 µm, colpus length 14, 66±2, 52 µm, colpus width 4, 33±1, 37 µm, porus length 5, 54±1, 03 µm, porus width 4, 63±0, 92 µm. Exine 2, 3 µm. Spines are perforated (Figs. 12, 13).

We used Bayesian Information Criteria (BIC) to select optimum number of class as a maximum likelihood estimator. According to BIC it was decided that optimum class size was 2. So we concluded that 1; 3; 4; 6 and 2; 5 taxa were in same class (Table 3). To evaluate the variation of criterions according to clusters, the coefficient of variation (CV) was calculated and interpreted as extent of variability (Table 4). While looking CV of criterions for each cluster we concluded that criteria-4(Clg) measures pollen with least variability for 1st cluster and criteria-6 (Plt) and criteria-7 (Plg)measure pollen with least variability for 2nd cluster.

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