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MICROMORPHOLOGICAL CHARACTERISTICS OF POLLEN GRAINS OF *RUELLIA* L. (ACANTHACEAE) IN SAUDI ARABIA

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Abstract

This paper aims to provide an accurate morphological description of pollen grains of *Ruellia* L. (Acanthaceae), which is widely spread in Saudi Arabia, as a first study in the region. Eight species of *Ruellia*, *Ruellia* sp. Abha (*Ruellia brittoniana*), *Ruellia* sp. Aseer (*Ruellia*.sp.sh2010), *Ruellia* sp. Jazan (*Ruellia* carolinensis), *Ruellia malacosperma*, *Ruellia patula*, *Ruellia* sp. Taif rose (*Ruellia tweediana* Rose), *Ruellia* sp. Taif violet (*Ruellia tweediana* Violet) and *Ruellia* sp.Taif white (*Ruellia tweediana*.white) were collected from the western and southwestern regions of Saudi Arabia for microscopic characterization using a light microscope and scanning electron microscopy. The size and shape of the grains were recorded, as well as the number of apertures and pollen wall sculpture, in addition to a genus key based on pollen characteristics. The results showed that in all studied species pollen were large, and the sculpturing was reticulate in each, but there were some differences in other parameters which divided the species into two groups according to the number of apertures, the grain genral shape, granules in the tectum layer, and lumina cavity size and shape in each species.

Key word: *Ruellia*, Acanthaceae, Pollen grain, SEM, Saudi Arabia.

Introduction

Ruellia L. is the second-largest genus, after *Justicia* L., in the Acanthaceae family (Daniel, 1995; Graham, 1988). There are 897 species names in the genus *Ruellia* are listed in the plant list (2022) but only 269 species names are accepted. Many species in the genus are significant medically due to the presence of bioactive phytomolecules such as glycosides, alkaloids, and triterpenoids. Because of this various species of the genus have historically been used to treat a number of diseases, including diabetes, eczema, high blood pressure, bronchitis, and bronchial asthma (Afzal *et al*., 2015). Around the world, tropical to temperate climates are home to the majority of *Ruellia* species (Tripp *et al*., 2013). A variety of microscopic methods can be used to identify pollen. The most frequently employed features are morphological traits, particularly shape characteristics (length, breadth, circularity, and form factor or length/width ratio) (del Pozo-Baos *et al*., 2015; Pospiech *et al*., 2019). The pollen's form and surface features (exina) are also used in light microscopy for identification. Li *et al*., 2004Scanning electron microscopy is another microscopic technique that enables a more precise classification of the type of pollen particle based on variations in surface characteristics (Li & Flenley, 1999; Jones & Bryant, 2014). Pollen grains are an effective tool that researchers can use to classify and identify plant species at various taxonomic levels due to the variability of their phenotypic forms. (Radford *et al*., 1974) The size, form, and pollen wall sculpting type of the grain, as well as the existence and number of pores and grooves in a single grain, all play a role in the taxonomic significance of pollen (Erdtman, 1971). There have been few investigations into the morphological properties of pollen grains from *Ruellia* species. Early research by Furness & Grant (1996) characterized the pollen morphology of 31 species of *Ruellia* from Africa and Madagascar. The pollen morphology of eight *Ruellia* species from the Malay Peninsula having been examined (Nurulaini *et al*., 2017). Pollen morphology of five species of *Ruellia* L. (*R. prostrata, R. patula, R. grandiflora, R. insignis, and R. dioscoridis*) were studied Yemen (Al-Hakimi & Latiff, 2015; Al-Hakimi *et al*., 2015). According to Chaudhary (2000), there are 4 species of *Ruellia* in the Saudi Arabian flora: *R. grandiflora, R. malacosperma, R. prostrata, and R. patula*. Where as Collenette (1999) found 7 species, 4 of which are yet to be unidentified. This study used microscopic techniques to describe the shape of the pollen grains of eight Saudi Arabian *Ruellia* species. The presented study will provide an accurate morphological description of pollen grains of *Ruellia* L. (Acanthaceae), in Saudi Arabia, as a first study in the region.

Material and Methods

In this investigation, samples were gathered from various places (Table 1) According to Clark & Jones (1977), the pollen was prepared for both the light microscope and the scanning microscope as follows: Use an autopsy needle to pry open the mature stalks for light microscopy studies. Next, a drop of the safron dye was added to the pollen before it was sprinkled directly onto glass slides. Then, a drop of glycerol was placed on it and covered with the slide cover and examined under force (40x).

While examining the pollen with a scanning electron microscope, the pollen was directly on the adhesive carbon tape of the two sides of numbered metal cylinders, encased in a thin layer of coating gold by a device—Auto Fine Coater JFC-1600—and then examined the pollen with different zoom forces to study the nature of the pollen surface, using a JEOL-JSM-6060LV device. The images were taken using a Samsung Gal A52S mobile camera.

Table 1. List of the *Ruellia* species studied and their localities in Saudi Arabia.

Taxa	Accession number	Collection place	date of collection	GPS coordinates
Ruellia brittoniana	OP893975	Abha	December, 2021	18°16'37.6"N 42°43'23.5"E
Ruellia sp. sh2010	OP867066	Aseer	December, 2021	19°07'44.5"N 41°55'42.2"E
Ruellia carolinensis	OP867067	Jazan	September, 2021	16°59'30.7"N 42°42'59.0"E
Ruellia malacosperma	OP745457	Riyadh, Jazan, Madinah	September, 2021	24°43'07.9"N 46°37'24.4"E
Ruellia patula	OQ106916	Jazan	September, 2021	16°54'50.1"N 42°33'20.4"E
Ruellia tweediana Rose	OP745451	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E
Ruellia tweediana Violet	OP712208	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E
Ruellia tweediana White	OP712206	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E

Table 2. General pollen characteristics of *Ruellia* species in Saudi Arabia.

Taxa	Dimensions (µm)			Shape class	Pollen size (P+E/2) µm		Tectum		Lumina (µm)
	P	E	P/E		Number	Shape	Apertures	Granules	
Ruellia brittoniana	71.42	41.78	1.709	Prolate	56.6	Large	4	More	18.8
Ruellia sp. sh2010	109.3	63.3	1.726	Prolate	86.3	Large	3	More	61.7
Ruellia carolinensis	135.4	61.8	2.191	Perprolate	98.6	Large	3	Less	258
Ruellia malacosperma	123.6	57.3	2.158	Perprolate	90.4	Large	3	None	191
Ruellia patula	106.2	49.37	2.152	Perprolate	77.8	Large	3	More	46.25
Ruellia tweediana Rose	120	56	2.142	Perprolate	88	Large	3	Less	114.4
Ruellia tweediana Violet	63.6	33.5	1.894	Prolate	48.6	Large	3	Very less	43.4
Ruellia tweediana White	126.7	55.3	2.889	Perprolate	91	Large	3	More	182.35

Measurements and analysis: The polar length (p) and equatorial length (l) of 5 to 10 pollen grains from each species were measured (E). The equation 2/E+P, multiplied by 100, was used to calculate the pollen grain size as the ratio of the polar length (P) to the equatorial diameter (E) As like (Fig. 5). The exine surface and the sculpturing pattern were examined, according to Erdtman (1966). The procedure outlined by Punt *et al*., (2007) served as the foundation for the definitions. The acquired data was numerically analyzed using the XLSTAT Statistical Software program (Fig. 6).

Results

The pollen grains of *Ruellia* species are monads, with isopolar and triporate of simple circular aperture, with the exception of *R. brittoniana*, which are tetraporate (Figs. 1, 2, and 3) with coarsely reticulate tectum. Granules are present, and the number of them varies between species. The lack of granules on the surface distinguishes *R. malacosperma*. Both the tectum layer and the coleumella layer can be thought of as hollow networks with a basal surface that may or may not be granular. Each reticulum cavity, also known as a lumina, is enclosed between five to six "Muri" columns (walls). Many of the pollen characteristics in *Ruellia* have uncertain functional importance. It appears that exine-bound materials like lipids and recognition proteins are stored in the lumina and the gaps between the columellae (Heslop-Harrison, 1976).

Different species of *Ruellia* have different lumina cavity sizes (Table 2) and (Fig. 4). *R. carolinensis* was found to have the biggest area, measuring 258 µm, while *R. brittoniana* had the smallest area, measuring 18.8 µm.

The huge size of every *Ruellia* species under study served as a defining characteristic. Three species were found to have "unusual oval shapes," ranging from oval to extended oval (*R. brittoniana*, *R.*sp.sh2010, and *R. tweediana* Rose).

The length of the polar diameter was largest for *R. carolinensis*, measuring 135.4 µm, and lowest for *R. tweediana*-violet, measuring 63.6 µm. The rest of the species had an elongated oval shape. *Ruellia* sp. sh2010 had the maximum equatorial diameter measurement at 63.3 µm, while *R. tweediana*-violet had the lowest measurement at 33.5 µm.

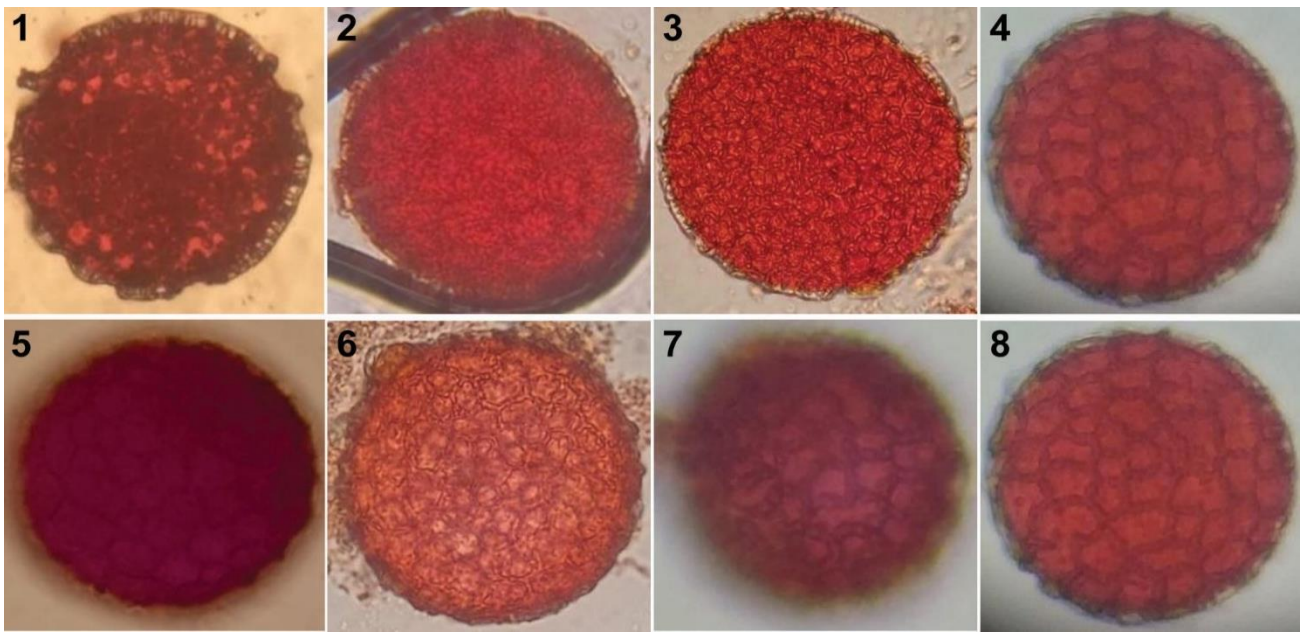

Fig. 1. Light micrographs under force 40x: *Ruellia* species in Saudi Arabia. **1)** *Ruellia brittoniana*, **2)** *Ruellia* sp. sh2010, **3)** *Ruellia carolinensis*, **4)** *Ruellia malacosperma*, **5)** *Ruellia patula*, **6)** *Ruellia tweediana* rose, **7)** *Ruellia tweediana* violat, **8)** *Ruellia tweediana* white.

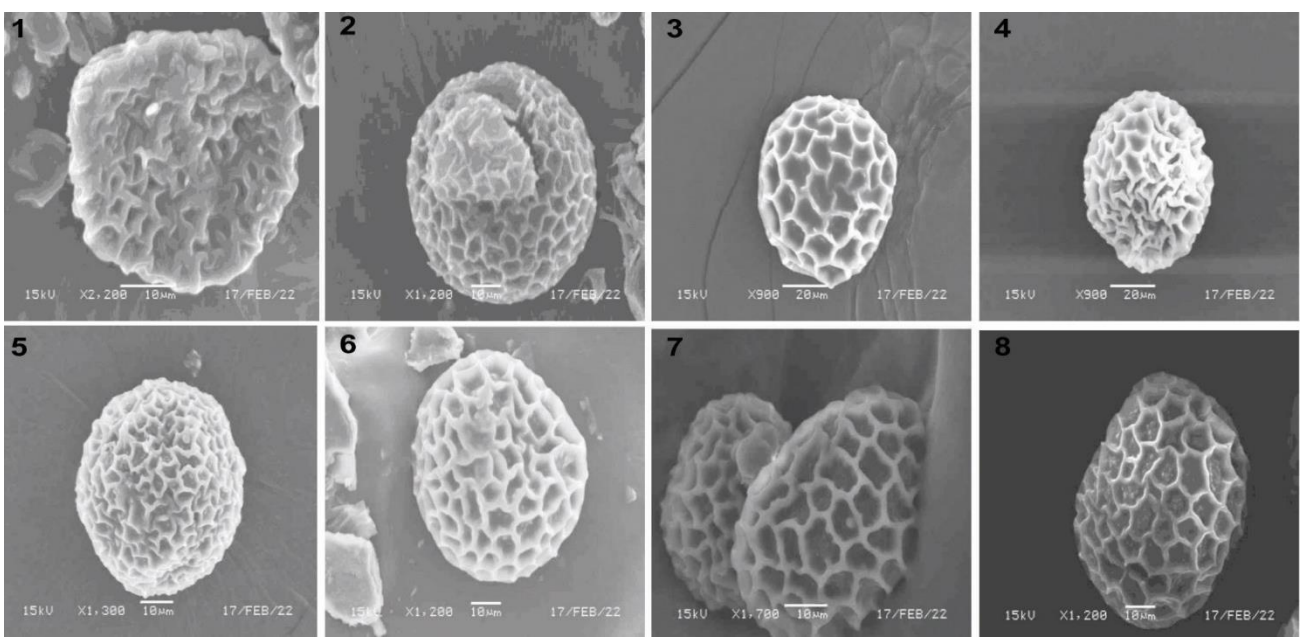

Fig. 2. Scanning micrographs *Ruellia* species equatorial view. **1)** *Ruellia brittoniana*, **2)** *Ruellia* sp. sh2010, **3)** *Ruellia carolinensis*, **4)** *Ruellia malacosperma*, **5)** *Ruellia patula*, **6)** *Ruellia tweediana* Rose, **7)** *Ruellia tweediana* Violat, **8)** *Ruellia tweediana* White. Scale bar = 1.2.5.6.7.8=10 *µm*, 3.4=20 *µm*.

Discussion

The results of this study revealed similarities among the pollen grains of the 8 *Ruellia* species. However, microscopic analysis revealed a number of differences of taxonomic significance. As each *Ruellia* species is symmetrically isopolar with coarsely reticulate sculpturing. These traits confirmed earlier findings (Al-Hakimi & Latiff, 2015; Nurulaini *et al*., 2017; Raza *et al*., 2020) and demonstrated stability at the species level. Regarding the distinctions between the eight *Ruellia* species, only *R. brittoniana* has four pollen apertures, compared to three in all the other species. Our findings support those of Raza *et al*., (2020), Al-Hakimi & Latiff (2015). However, *Ruellia patula* was shown to have five germination openings in the form of straightforward spherical pores by Shamso (2013) and Furness & Grant (1996). Different *Ruellia* species have different tectum. These variations allow us to separate the kinds into two groups: those with smooth dermis, found exclusively in *Ruellia malacosperma*, and those with rough dermis, found in the remaining seven *Ruellia* species. Al-Hakimi & Latiff (2015) previously reported on the significance of the lumina cavity's area as a taxonomic measure. In our study, we found clear distinctions between the studied species.

The 8 *Ruellia* species had pollen prolate – perpolate, similar to species found in the Malay Peninsular (Nurulaini *et al*., 2017). However, a spherical shape was reported by Al-Hakimi & Latiff (2015) and Perveen & Qaiser (2010). The huge size of *Ruellia species* pollen grains, which is a defining characteristic at the species level in our study, supports Shamso's (2013) findings. Although Furness & Grant (1996) and Al-Hakimi & Latiff (2015) reported pollen grains of an average size. The increased level of chromosomal set doubling in polyploid plants may be the cause of these variations in pollen grain size (Meo *et al*., 1988). Additionally, the high relative humidity levels in various locations have an impact on the size and form of pollen grains. The pollen grains are larger and more spherical in a humid environment, but in a dry environment, they may be smaller and cracked with fissures (Haler & Hess, 2004). Previous studies have emphasized how significant pollen shape and its features are to the classification of the Acanthaceae family (Al-Hakimi & Latiff, 2015; Scotland, 1992; Scotland & Vollesen, 2000; Vollesen, 2008). The results of our study give information about Saudi Arabian *Ruellia* species that could help future phylogenetic research and our understanding of pollen morphology in different settings.

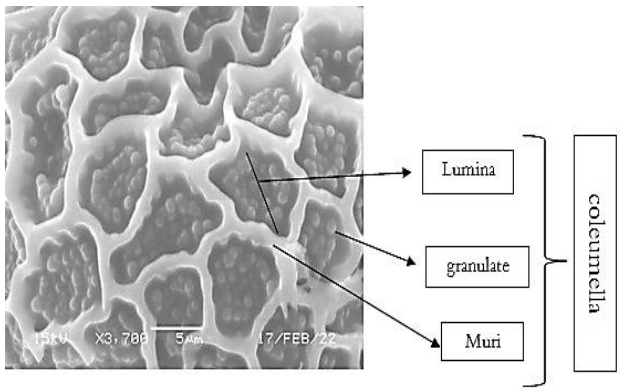

Fig. 3. The exine of pollen grain in *Ruellia* species elucidates columella components.

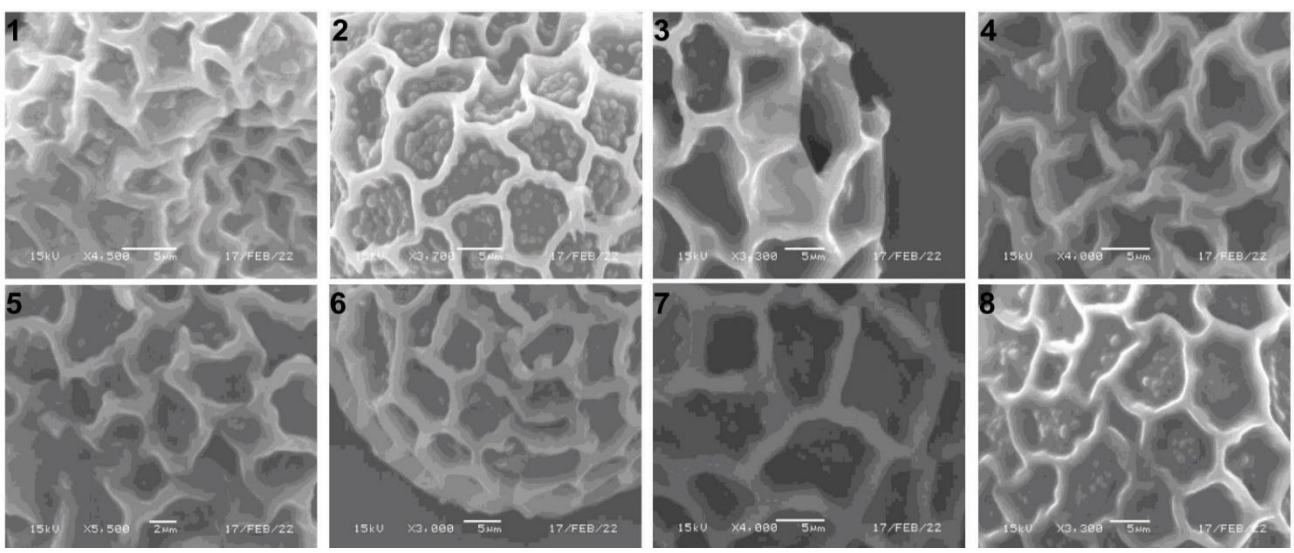

Fig. 4. Scanning micrographs: *Ruellia* species differences for shape and size of lumina: **1)** *Ruellia brittoniana*, **2)** *Ruellia* sp. sh2010, **3)** *Ruellia carolinensis*, **4)** *Ruellia malacosperma*, **5)** *Ruellia patula*, **6)** *Ruellia tweediana.* rose, **7)** *Ruellia tweediana* Violat, **8)** *Ruellia tweediana* White.

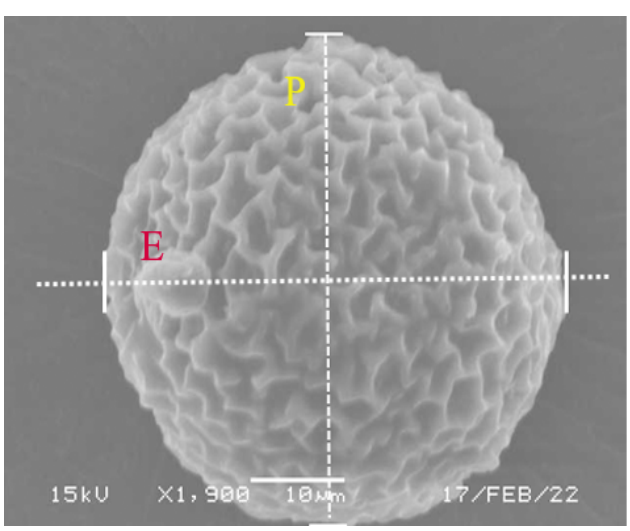

Fig. 5. Clarification of the polar length (P) and equatorial diameter (E) of a pollen grain to determine the measurement of size and infer the shape of the pollen

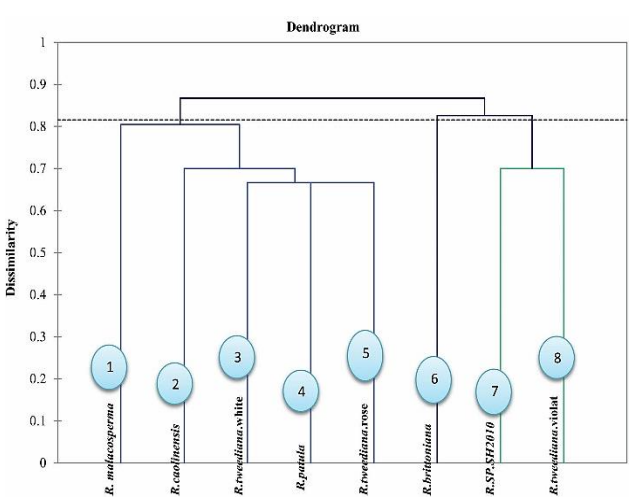

Fig. 6. dendrogram tree relation, Values on the horizontal axis are species: **1**-*R. malaccosperma*, **2**-*R. carolinensis*, **3**-*R. tweediana*. white, **4**-*R. patula*, **5**-*R. tweediana* Rose, **6**-*R. brittoniana*, **7**-*R.* sp. sh2010, **8**-*R. tweediana*.violat

Key to the species of *Ruellia* through the results

1- The number of germination apertures
1-a. (4) Germination apertures ..... *Ruellia brittoniana*
1-b. (3) Germination apertures …..... (2)
2- Presence of granules in exine
2-a. No granules …….... *R. malacosperma*
2-b. Presence of granules …..... (3)
3- Lumina area
3-a. An area of 258 µm ..... *R. carolinensis*
3-b. An area of 182.35 µm ..... *R. tweediana* White
3-c. An area of 114.4 µm ..... *R. tweediana* Rose
3-d. An area of 61.7 µm ..... *Ruellia* sp. sh2010
3-e. An area of 46.25 µm ……..... *R. patula*
3-f. An area of 43.4 µm ..... *R. tweediana* Violat

Conclusion

Species that were recorded in Flora Saudi Arabia for the first time: *R. brittoniana, R. sp. sh 2010, R. carolinensis*, and *R. tweediana 2* were found in the study. The following species, which were discovered for the first time in the Flora of Saudi Arabia, were named by the study. The results of the morphological study of pollen under light and electron microscopy demonstrated that the distinction of species depends on the number of pores, the size of the lumina, and the type of surface on the tectum.

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