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PALYNOLOGICAL DIVERSITY OF POLLEN MORPHOLOGY IN ENDEMIC NORTHERN IRAQI *HYPERICUM* SPECIES (HYPERICACEAE)

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SUMMARY

The presented research investigated the pollen morphology of endemic Iraqi *Hypericum* species. The study revealed phenotypic features of pollen grains in the polar and equatorial views and their quantitative and qualitative characteristics. The results showed that the pollen grains of the genus *Hypericum* were radially symmetrical and isopolar, and their apertures were simple and tricolporate, except the species *H. davisii,* distinguished as tetracolprate. Dividing the studied species based on pollen grain sizes comprised two groups. Small pollen grains with an average length of the equatorial view ranged between 10–16 µm in *H. lysimachioides* and *H. vermiculare*. Medium-sized pollen grains with an average extent between 17–26 µm emerged in the species, i.e., *H. retusum, H. triquetrifolium, H. perforatum, H. lydium, H. tetrapterum, H. asperulium, H. scabrum,* and *H. davisii***.** Pollen grains, also divided according to the P/E ratio, had two main groups. The pollen grains were subprolate and spherical, with the exine sculpturing examined by a scanning electron microscope (SEM), and isolated into four groups: 1) microreticulate reticulum, including *H. asperulium, H. lydium,* and *H. vermiculare*, 2) perforate, i.e., *H. retusum, H. scabrum, H. tetrapterum,* and *H. triquetrifolium*, 3) psilate with *H. perforatum* and *H. davisii*, 4) and reticulate exine sculpturing in *H. lysimachioides,* all isolated from the rest of the species. SEM images showed the thickness of exine ranged from 0.5 to 2 µm. The topical study also added *H. davisii* as a new species with no previous records in Iraq. This pollen study added valuable data for taxonomy and suggested that it should proceed with its first-time registration in Iraq. The pertinent research could provide a taxonomical key for *Hypericum* species in Iraq.

Keywords: *Hypericum* L., Hypericaceae, endemic Iraqi species, palynological diversity, pollen grains, SEM

Key findings: A comparative palynological study for the endemic Iraqi *Hypericum* species by light microscopy (LM) and scanning electron microscopy (SEM) determined the shape and size of the pollen grains, which were subprolate and sph prolate with small and medium size. Additionally, the ornamentation on the exine were microreticulate, perforate, psilate, and reticulate. The thickness of

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the exine was 0.5–2 µm. The number and shapes of apertures provide useful taxonomic information to distinguish the species. *H. davisii* was a new addition, having no previous records in Iraq.

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INTRODUCTION

Hypericum L. (Hypericaceae) has a wide distribution and is one of the most remarkable plants due to its several pharmaceutical properties. The genus *Hypericum* has served as a medicinal herb for more than 2,000 years. The most well-known species of *Hypericum* is St. John's wort (*Hypericum perforatum*). This plant became an anti-diarrheal substance, a treatment for skin diseases, a fever reducer, a cut-off for bleeding, and a cure for snake bites in the past. It also helped enhance blood circulation (Irak *et al*., 2019).

Ancient peoples used the *Hypericum* species to treat worms and parasites. *Hypericum perforatum* was a focus in several past studies, and the plant became one of the most widespread medicinal plants in healing depression and mental disorders as a common herbal remedy, widely accepted in traditional medicines (Sirvent and Gibson, 2008). In various countries, drenching it in oil is a way of treating eczema, biliary disorders, burns, bruises, inflammation of the bronchi or trachea, the genitourinary system, and common headaches, as well as migraines and diabetes (Bombardelli and Moraazzoni, 1995).

The genus *Hypericum* contains several active compounds with biological efficacy that are vital in diagnosing many important biofunctions, such as, anti-inflammatory, antioxidant, and carcinogenic anti-mutagenic activities (Taker, 2009). These phytochemicals have enormous structural diversity, providing complex defense strategies against insects, fungi, animals, abiotic stressors, and pollination or dispersal attractions. Considered the most widely consumed medicinal plant, *H. perforatum*, in recent years, has dramatically increased the consumption of products derived from it.

Commercially available products derived from *H. perforatum* include phytopharmaceuticals, nutrients, teas, tinctures, syrups, oily infusions, cosmetics, ointments, and an added ingredient to alcoholic beverages. In the past 30 years, *H. perforatum* has undergone extensive clinical and laboratory testing, making it one of the most valuable medicinal herbs used in Europe and the USA to treat several chronic diseases (Monteiro *et al.*, 2022).

Globally, the genus *Hypericum* L. includes 400 species (Townsend and Guest, 1980), 360 species (Judd *et al*., 1999), and according to Meseguer and Sanmartín (2012), the *Hypericum* comprises about 484 species. Chakravarty (1976), in his book Plant Wealth of Iraq, reported the existence of about 400 species present in temperate regions worldwide, of which 11 species occur in Iraq. Several researchers have been studying the diverse Hypericaceae (Guttiferae) taxa according to their morphological, anatomical, palynological, and pharmacological characteristics till today. Additionally, no previous studies on some Hypericaceae taxa exist, and investigations on *Hypericum* species are relatively limited (Asan, 2019). Ocak *et al*. (2013) examined the detailed pollen morphological structures of some endemic Turkish *Hypericum* taxa by LM and SEM.

The data on pollen morphology and taxonomy can serve as an essential tool in various scientific researches of systematic botany, such as aeropalynology, allergy, copropalynology, criminology, honey improvement in the field, or mellito palynology, paleobotany, paleoecology, pollen analysis, and stratigraphic (Meo, 2005; Kumar *et al*., 2015; Azka *et al*., 2021). Pollen analysis by SEM offers to record a high-resolution image of the pollen exine, proving the significance of

taxonomic classification of the pollen taxa (Al-Anbari, 2016; Gins *et al*., 2022). The micromorphological characteristics or phenotypic characteristics of pollen grain in plant taxonomy are the best evidence in flowering plants to solve various problems (Al-Anbari, 2019), and therefore, it has a vast taxonomic value (Erdtman, 1943). In light of the above discussion, the presented research investigated pollen morphology of the endemic Iraqi *Hypericum* species through LM and SEM.

MATERIALS AND METHODS

For the latest study, collecting endemic Iraqi *Hypericum* fresh species grown in a natural environment ensued in the summer of 2021 in the mountainous regions of Iraq (Erbil, Dohuk, and Sulaymaniyah Governorates). Newly opened flowers and mature flower buds attained fixing with Carnoy's solution for 18-24 h, with the samples washed later with 70% ethyl alcohol and kept till use for study. The *Hypericum* species samples' diagnosis transpired in the National Herbarium (BAG) and the University of Salah al-Din Herbarium (ESUH), Iraq.

Following Sass's (1958) method, about 25–50 pollen grains underwent study for each species in the plant laboratory of the Biology Department, Education College for Pure Sciences, Diyala University, Iraq. Documenting the polar axis and the equatorial axis of the pollen grain progressed. The pollen grain wall's thickness measurement used the precise scale of the ocular lens Ocullar micrometer, with the pollen grains depicted through a camera type NSZ-606 of the optical microscope installed under the lens (40×). For scanning electron microscopy (SEM), the BPC analysis center sent the pollen grains to Iran, prepared as follows: dry pollen grains transferred onto stubs, coated with gold, and then investigated and photographed with an (FE-SEM) Model: S-4800, FB-2100 (Hitachi). For pollen terminology, the study followed the methodology of Walker and Doyle (1975). The recorded data on measuring the P/E ratio, polar and equatorial views, and decorations on the surfaces appear in Table 1 and in Figures 1a, 1b, 2a, and 2b.

Table 1. Pollen morphology of *Hypericum* species in Iraq.

No.	Species	Polar axis (P) (µm)	Equatorial axis (E) (µm)	Rate P/E	Size	Shape	Aperture	Exine Sculpture
1.	*H. asperulium*	15-25(23)	8-20(18)	1.38	M*	Subprolate	TRC	Mic
2.	*H. davisii*	18-30(22)	17-30(22)	1	M	Sphprolate	TTC	PST
3.	*H. lydium*	20-31(25)	10-23(20)	1.33	M	Subprolate	TRC	Mic
4.	*H. lysimachioides*	13-16(15)	10-14(12)	1.25	S	Subprolate	TRC	RET
5.	*H. perforatum*	14-28(26)	13-27(25)	1.07	M	Sphprolate	TRC	PST
6.	*H. retusum*	15-29(26)	14-24(23)	1.25	M	Subprolate	TRC	PER
7.	*H. scabrum*	15-30(28)	12-27(21)	1.33	M	Subprolate	TRC	PER
8.	*H. tetrapterum*	12-27(25)	10-25(20)	1.25	M	Subprolate	TRC	PER
9.	*H. triquetrifolium*	13-26(25)	10-23(19)	1.31	M	Subprolate	TRC	PER
10.	*H. vermiculare*	10-16(13)	5-10(10)	1.3	S	Subprolate	TRC	Mic

* Size of grains: M = medium-sized grains, S = small-sized grains; Aperture types: TRC = tricolporate, TTC = tetracolpopate; Types of exine sculpturing; Mic = Microreticulate, PER = perforate, PST= Psilate, and RET= Reticulate.

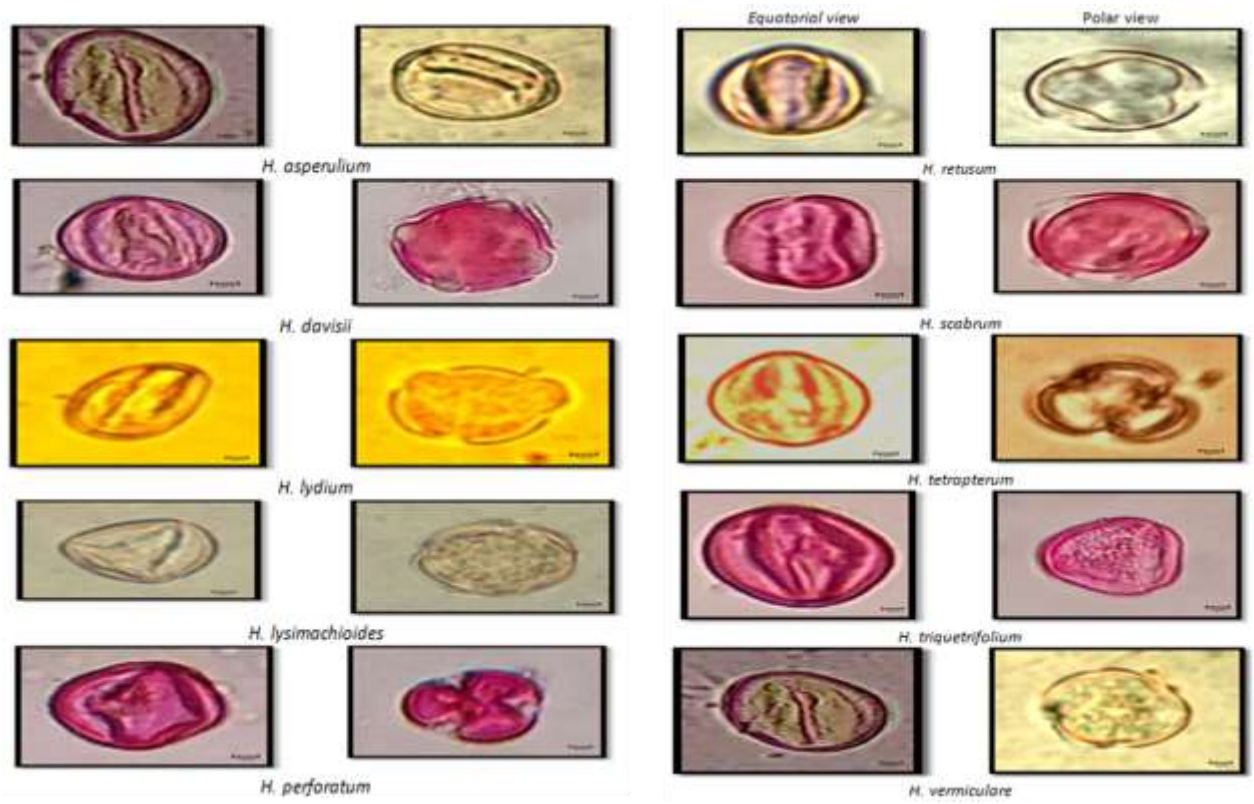

Figure 1. (a). The Equatorial view (E) and the Polar view (P) in the pollen grain of *Hypericum* species *H. asperulium*, *H. davisii*, *H. lydium, H. lysimachioides,* and *H. perforatum in Iraq by LM;* **(b)** The Equatorial view (E) and the Polar view (P) in the pollen grain of *Hypericum* species*, H. retusum, H. scabrum, H. triquetrifolium, H. tetrapterum,* and *H. vermiculare* in Iraq by LM.

RESULTS AND DISCUSSION

In the genus *Hypericum* species, the pollen grains were all radially symmetrical and isopolar, and their apertures were simple. However, the species *H. davisii* differed from other species under study, having tetracolpopate apertures, while others were tricolporate. With trizonocolporate, the grooves of the pollen grain do not extend from one pole to the second pole, and the ends of the furrows do not connect (Table 1). The presented results corresponded and were in analogy with the findings of Özbek *et al.* (2019), who studied the *Hypericum* L. species, except for the species *H. davisii*.

The pollen grains' sizes ranged from small to medium, and according to Erdtman (1971), it was possible to divide the Hypericum species based on their sizes into two groups. The *H. lysimachioides* and *H. vermiculare* were small in pollen grain size, ranging between 10–16 µm on the E axis; however, the rest of the taxa were medium, reaching about 17–26 µm, and the size of pollen grains agree with the findings of Otaghvari *et al.* (2015). In this study, the species' pollen differed in P/E, which could diverge into two groups. The first group was subprolate, which includes the species *H. asperulium, H. lydium, H. lysimachioides, H. retusum, H. scabrum, H. tetrapterum, H. triquetrifolium, and H. vermiculare.* The second group was sph prolate, composed of two species, i.e., *H. davisii and H. perforatum***.**

Mazari *et al.* (2017) also obtained similar results and mentioned that in the polar view, the pollen grains' shape in all the species was circular to semi-angular; however, in the

equatorial view, the pollen grains were oblate-spheroidal. According to the surface decoration of pollen grains examined by SEM, the isolated species under study were divided into four groups***:***

- Microreticulate, including the species *H. asperulium, H. lydium,* and *H. vermiculare;*
- Perforate includes the species H*. retusum, H. scabrum, H. tetrapterum,* and *H. triquetrifolium;*
- Psilate includes the species *H. perforatum* and *H. davisii;*
- Reticulate includes the species *H. lysimachioides* isolated from the rest of the Hypericum species

The significant results contradicted the past findings of Harami *et al*. (2006), who reported the exine sculpturing of pollen grains was symmetrical and smooth. However, Özbek *et al.* (2019) confirmed that *Hypericum* was microreticulate ornamentation surfers. Mazari *et al.* (2017) divided the exine sculpturing in *Hypericum* species and were foveolate, sculpturing, reticulate, and scabrate in *H. perforatum.* The thickness of the exine ranged from 0.5 µm (*H. retusum* and *H. lysimachioides*) to 2 µm (*H. davisii*), and the rest of the seven species in the pertinent study ranged between these two limits.

Confirmations centered on the aspects of comparison of pollen grains, especially on the diameter of the hole between the colpe grooves of the pollen grain in the polar axis, as it reached 1.5 µm in *H. tetrapterum* and 7 µm for *H. vermiculare*, with rest of the eight species fluctuating between these two values. Overall, the importance of using pollen grains as micromorphological features proved to distinguish the species from each other in the same genus under study in general and among the species in particular, using exine thickness, distance between furrows, and surface textures of pollen grains as a good taxonomy tool.

CONCLUSIONS

Based on the scanning microscope, the pollen grains of the different species of *Hypericum* under study showed a significant variation in size, shape, and surface decoration. The present classification of endemic Iraqi *Hypericum* species can benefit as an influential tool to isolate the species.

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