



Original Article

Pollen Morphology of the Genus *Trigonella* L. (Fabaceae) in Kurdistan/Iraq

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ABSTRACT

Pollen morphology provides essential diagnostic traits for species delimitation in the genus *Trigonella* (Fabaceae) in the Kurdistan Region of Iraq. Pollen grains of eight species were examined using light microscopy (LM) and scanning electron microscopy (SEM), and quantitative traits were analyzed with ANOVA and Tukey's HSD tests. Equatorial diameter showed the highest interspecific variation ($P < 0.0001$), confirming its strong diagnostic value, with colpus length and mesocolpium width also showing significant interspecific differentiation. Grains were predominantly tricolporate with triangular polar outlines. Significant variations were observed in colpus length (16.99 μm in *T. monspeliaca* to 22.34 μm in *T. filipes*) and mesocolpium width (12.97 μm in *T. monspeliaca* to 18.32 μm in *T. uncinata*). Exine ornamentation varied from finely reticulate to coarse. These pollen characters provide reliable taxonomic markers for distinguishing *Trigonella* species in the region and contribute valuable baseline data for future systematic and phylogenetic studies.

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Keywords: Fabaceae, Genus, *Trigonella* L., Pollens, Micromorphology.

1 Introduction

The Fabaceae (Leguminosae) family is one of the largest and most economically significant plant families comprising over 700 genera and nearly 20,000 species ^[1]. Members of this family including *Trigonella* are widely distributed across diverse habitats and are recognized for their roles in nitrogen fixation, soil improvement, and agricultural production ^[2]. The genus *Trigonella* commonly known as fenugreek within the Fabaceae family encompasses over 70 species widely distributed across temperate, arid, and semi-arid regions of the Mediterranean, Central Asia, and the Indian subcontinent ^[3].

These herbs: annual and perennial are renowned for their nutritional, medicinal, and ecological significance ^[4]. The *Trigonella* species display considerable variation in growth habits, leaf structures, and reproductive traits, including pollen grain features ^[5]. Pollen grains within this genus vary in size, shape, aperture structure, and exine ornamentation, making them key tools for species identification and classification. Beyond their economic and pharmacological importance ^[6], *Trigonella*

species exhibit notable morphological diversity in their vegetative and reproductive structures. Pollen morphology has proven to be valuable for taxonomic classification and evolutionary studies ^[7,8]. The pollen grains of the *Trigonella* typically exhibit variations in size, shape, aperture types, and surface ornamentation patterns, which reflect adaptations to different pollination strategies and environmental conditions ^[9]. Their shapes are usually tricolporate or rarely tricolpate, pantocolporate, tetracolporate and with various ornamentation types ^[10]. The shape and aperture types have been observed as important morphological characters to delimit the taxa. The palynological characteristics of selected *Trigonella* species using Light Electron Microscopy (LEM) and Scanning Electron Microscopy (SEM) were explored ^[11]. These techniques enable a detailed investigation of both macroscopic and microscopic pollen traits ^[12], contributing to a refined understanding of morphological diversity and taxonomic relationships within the genus ^[13]. The objective of this study is to investigate pollen grain micromorphology to figure out the species identification and evolutionary relationships of the *Trigonella* species.

2 Materials and Methods

Pollen grains were hydrated in 70% ethanol (10 min) and stained with methyl green solution (1 g in 100 mL 95% ethanol) for 2–3

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min, then briefly rinsed in 95% ethanol. Grains were mounted in glycerol on clean slides and coverslips sealed with DPX. Observations were made with an OLYMPUS CX21 microscope and images captured using a Sony 8.2-MP digital camera. For each species, 20 randomly selected grains were measured and all quantitative pollen traits analyzed using one-way analysis of variance (ANOVA) to test for significant differences among the eight *Trigonella* species. When the ANOVA indicated significant differences ($P < 0.05$), pairwise comparisons were performed using Tukey's Honest Significant Difference (HSD) post-hoc test to identify which species differed significantly^[14]. for polar length (P), equatorial diameter (E), P/E ratio, mesocolpium width, apocolpium width, colpus length, exine thickness, and aperture characters. Whereas Scanning electron microscopy

(SEM). Air-dried pollen was sprinkled onto aluminum stubs with carbon adhesive tabs, then sputter-coated with Au-Pd (~15–20 nm). Grains were imaged using FEI Quanta 450 SEM at the University of Soran Research Center at 10–15 kV and working distance. Digital micrographs at multiple magnifications (~1–5 k $\times$) The morphological observations including polar length (P) and equatorial diameter (E), P/E ratio, number of apertures, mesocolpium (in the equatorial view), apocolpium, colpus length, exine thickness, apertural shape were examined. The mean, average, and maximum and minimum for each character were calculated based on 20 observations. Size classes were assessed, and the shape classes were followed according to^[15], and the pollen terminology in general follows^[16]. A stage micrometer was used for calibration before each session.

Table 1. Details of specimens used for this study.

Taxa	Locality	altitude	Latitude	Longitude
<i>T. foenum-graecum</i> L.	Suliamaniya/ Qaradakh mountain	900m	4212843.3N	5063604.7E
<i>T. aurantiaca</i> Bioss.	Erbil/safeen mountain	700m	4212801.7N	5063605.8E
<i>T. filipes</i> Bioss.	Suliamaniya/azmar mountain	1100m	4246770.2N	5062055.4E
<i>T. monantha</i> C.A.Mey.	Duhok/zawita mountain	1300m	4246774.4N	5062050.1E
<i>T. strangulate</i> Bioss.	Duhok /baadre	1500m	4254782.5N	5083607.7E
<i>T. spruneriana</i> Bioss.	Zakho/sharans mountain	800m	42467880.2N	5063635.8E
<i>T. monspeliaca</i> L.	Suliamaniya/qaradakh mountain	1400m	42267860.2N	5063457.7E
<i>T. uncinata</i> Banks & Sol.	Amedi/amedi mountain	800m	4242770.1N	5062053.4E

2.1 Statistical Analysis

All analyses were conducted in IBM SPSS Statistics v26. For each trait, data were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test). When assumptions were met, a one-way ANOVA tested differences among species including polar length, equatorial diameter, exine thickness, polar/equatorial length ratio, apocolpium width, mesocolpium width, and colpus length. This test determined whether variations in these morphological traits were statistically significant across different species.

3 Results and Discussion

The pollen grain characteristics of *Trigonella* taxa observed under LM and SEM are presented in Table 2 and Figures 1–9. Morphological data of the eight species revealed significant variation across several parameters. *T. foenum-graecum* (Figure 2) displayed spheroidal to circular polar views with uniform exine outlines, three distinct colpi, and elliptic equatorial shapes, with SEM revealing tricolpate apertures and a reticulate-to-granulate exine surface. *T. aurantiaca* (Figure 3) exhibited sub-triangular

and slightly compressed polar LM views with partially open apertures, while SEM showed sub-oblate polar shapes, prolate equatorial contours, and microreticulate ornamentation. *T. filipes* (Figure 4) had circular to slightly elliptic polar LM views and sub-oblate equatorial forms, with SEM highlighting tricolpate grains and microechinate exine sculpturing. *T. monantha* (Figure 5) showed LM polar views with tricolpate apertures and elliptical equatorial shapes, and SEM confirmed elongate equatorial grains with striate to finely reticulate exine surfaces. *T. strangulata* (Figure 6) exhibited subprolate to spheroidal equatorial LM views, with SEM showing isopolar tricolporate grains, faintly reticulate ornamentation, and smooth psilate surfaces. *T. spruneriana* (Figure 7) displayed spheroidal polar LM views and sub-oblate equatorial shapes, with SEM revealing tricolpate apertures and fine reticulate exine patterns. *T. monspeliaca* (Figure 8) had sub-oblate to prolate-spheroidal LM forms, with SEM showing distinct opercula, elongated equatorial shapes, and localized exine thickening. *T. uncinata* (Figure 9) exhibited oblate-spheroidal LM views with slightly tapering poles, and SEM revealed rugulate surfaces, visible colpi, and faintly perforate psilate ornamentation. Overall, variations in polar and equatorial shapes, aperture configurations, and exine

Table 1: Statistics for pollen morphological characters(um).

Characteristic	Measurement	<i>T. foenum graecum</i>	<i>T. aurantiaca</i>	<i>T. filipes</i>	<i>T. monantha</i>	<i>T. strangulata</i>	<i>T. spruneriana</i>	<i>T. monspeliaca</i>	<i>T. uncinata</i>	F Value	P Value
Colpus Length (µm)	Minimum	17.3	16.9	17.8	15.9	13.6	15.6	13.5	13.5		
	Mean ± SEM	22.13 ^a ±0.86	20.52 ^b ±0.75	22.34 ^a ±0.93	20.73 ^{ab} ±0.90	21.88 ^a ±1.37	21.37 ^a ±0.94	16.99 ^c ±0.45	19.68 ^b ±0.95	3.546	0.0017
	Maximum	27.2	27.1	29.4	27.2	29.6	28.4	19.6	28.1		
Mesocolpium (µm)	Minimum	9.21	11.22	9.87	9.86	9.78	10.21	10.87	15.78		
	Mean ± SEM	13.65 ^a ±0.70	13.86 ^a ±0.40	13.95 ^a ±0.46	13.86 ^a ±0.55	13.44 ^{ab} ±0.60	13.85 ^a ±0.66	12.97 ^b ±0.46	18.32 ^c ±0.30	9.657	<0.0001
	Maximum	19.16	17.21	17.98	17.54	17.89	17.78	17.12	20.21		
Apocolpium (µm)	Minimum	11.68	12.25	8.96	12.18	11.21	10.27	11.37	11.27		
	Mean ± SEM	14.67 ^a ±0.51	17.18 ^b ±0.42	11.27 ^c ±0.42	15.67 ^d ±0.65	15.69 ^d ±0.62	16 ^{bd} ±0.64	15.32 ^d ±0.64	15.82 ^{bd} ±0.62	9.089	<0.0001
	Maximum	18.26	19.42	15.27	19.27	19.32	19.21	19.32	19.25		
Polar Length (µm)	Minimum	36.03	37.2	31.78	29.38	32.28	31.29	28.39	26.28		
	Mean ± SEM	40.37 ^a ±0.57	40.77 ^a ±0.50	38.27 ^b ±0.90	36.8 ^c ±0.93	39.1 ^b ±0.91	39 ^{bc} ±1.25	41.3 ^a ±1.5	32.9 ^d ±1.12	7.043	<0.0001
	Maximum	44.29	44.28	42.67	42.38	44.27	48.32	52.11	41.29		
Equatorial Diameter (µm)	Minimum	29.39	31.34	30.76	26.87	27.62	28.87	31.98	21.61		
	Mean ± SEM	34.09 ^a ±0.79	39.13 ^b ±1.12	37.76 ^b ±1.06	31.08 ^d ±0.75	32.79 ^{ad} ±0.81	33.62 ^a ±0.84	40.93 ^b ±1.79	29.06 ^c ±0.98	14.75	<0.0001
	Maximum	39.28	43.89	43.64	36.87	39.63	39.65	65.21	37.94		
Exine Thickness (µm)	Minimum	1.21	1.23	1.21	1.12	1.23	1.23	1.22	1.11		
	Mean ± SEM	1.56±0.05	1.5±0.049	1.55±0.062	1.52±0.059	1.56±0.05	1.63±0.062	1.48±0.057	1.39±0.057	1.506	0.1714
	Maximum	1.91	1.87	1.98	1.91	1.98	1.97	1.92	1.87		
P/E Ratio	Minimum	1.21	1.02	1.19	1.11	1.22	1.13	1.11	1.23		
	Mean ± SEM	1.48±0.06	1.28±0.07	1.4±0.07	1.45±0.057	1.22±0.065	1.42±0.06	1.17±0.064	1.51±0.07	0.5774	0.7723
	Maximum	1.89	1.91	1.98	1.89	1.98	1.87	1.38	1.92		
Shape	-	Prolate	Subprolate	Prolate	Prolate	Subprolate	Prolate	Spheroidal	Prolate		
Size	-	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium		

Different superscript letters (a–d) in Table 2 indicate groups that differ significantly at $P < 0.05$ according to HSD test.

ornamentation clearly distinguish *Trigonella* species and suggest functional adaptations for pollination in semi-arid and disturbed habitats.

The quantitative pollen traits are summarized in Table 2 and Figure 1 (A–H). ANOVA showed significant differences observed in most traits. Figure 1A (Colpus length) showed differences among taxa ($F = 3.546$, $P = 0.0017$), with the longest colpi in *T. foenum-graecum* (22.1 μm) and *T. filipes* (22.3 μm), and the shortest in *T. monspeliaca* (17.0 μm). Figure 1B (Mesocolpium width) varied significantly ($F = 9.657$, $P < 0.001$), widest in *T. uncinata* (18.3 μm) and narrowest in *T. monspeliaca* (13.0 μm). Figure 1C (Apocolpium width) also differed ($F = 9.089$, $P < 0.001$), being widest in *T. aurantiaca* (17.2 μm) and narrowest in *T. filipes* (11.3 μm). Figure 1D (Polar length) showed significant variation ($F = 7.043$, $P < 0.001$), longest in *T. monspeliaca* (41.3 μm) and shortest in *T. uncinata* (32.9 μm). Figure 1E (Equatorial diameter) exhibited the highest differentiation ($F = 14.75$, $P < 0.001$), largest in *T. monspeliaca* (40.9 μm) and smallest in *T. uncinata* (29.1 μm). In contrast, Figure 1F (Exine thickness) did not differ significantly ($F = 1.506$, $P = 0.171$), ranging from 1.39 μm in *T. uncinata* to 1.63 μm in *T. spruneriana*. Figures 1G–H show qualitative differences in exine ornamentation, with finely reticulate patterns in *T. foenum-graecum*, coarse reticulation in *T. aurantiaca*, and sharply triangular polar outlines in *T. filipes*. Overall, colpus length, mesocolpium width, and equatorial diameter were the most diagnostic quantitative traits for distinguishing *Trigonella* species, while exine thickness remained relatively conserved, a pattern consistent with many angiosperms where exine architecture shows less variability compared to other pollen traits^[17]. These observations have both taxonomic and ecological significance. Colpus length, mesocolpium width, and exine ornamentation are widely recognized as reliable diagnostic characters in Fabaceae^[18] in this study, these characters contributed to confirming species delimitation and supported morphological distinctions. When compared with regional studies, the pollen dimensions and ornamentation patterns of *Trigonella* species in the Kurdistan Region were broadly consistent with reports from Turkey and Iran^[19], although some variations were observed, likely reflecting microhabitat differences^[20]. For example, species occurring at higher elevations exhibited slightly smaller pollen grains, which may represent adaptive responses to reduced pollinator availability or climatic constraints. Ecologically, the pollen traits observed suggest adaptations for effective pollination. The elongated colpi and finely reticulate exine surfaces likely facilitate adhesion to insect vectors, enhancing cross-pollination efficiency. These functional adaptations align with the widespread occurrence of

Trigonella in disturbed and semi-arid habitats, where efficient reproduction is critical for survival^[21]. Overall, integrating pollen morphology with macromorphological and ecological observations strengthens species delimitation within the genus, reduces misidentification risk, and provides baseline data for future taxonomic and phylogenetic studies in the region. This study contributes to the broader understanding of *Trigonella* diversity and its ecological adaptations in the Middle east^[22].

Conclusion

The current study revealed considerable morphological diversity among the pollen grains of eight *Trigonella* species from the Kurdistan Region of Iraq. The observed variations in pollen traits, such as polar and equatorial dimensions, colpus length, mesocolpium, apocolpium width, and surface ornamentation, highlight the diagnostic importance of palynological characters in species identification and classification within the genus. These differences reflect ecological adaptation and evolutionary divergence among species, supporting the use of pollen morphology as a reliable tool in taxonomic and systematic studies. The combined morphological and micromorphological data provide a clearer understanding of species relationships and offer a foundation for further phylogenetic and ecological research within the genus.

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Authors contribution

All parts of this article were conducted by the author.

Conflict of interests

None.

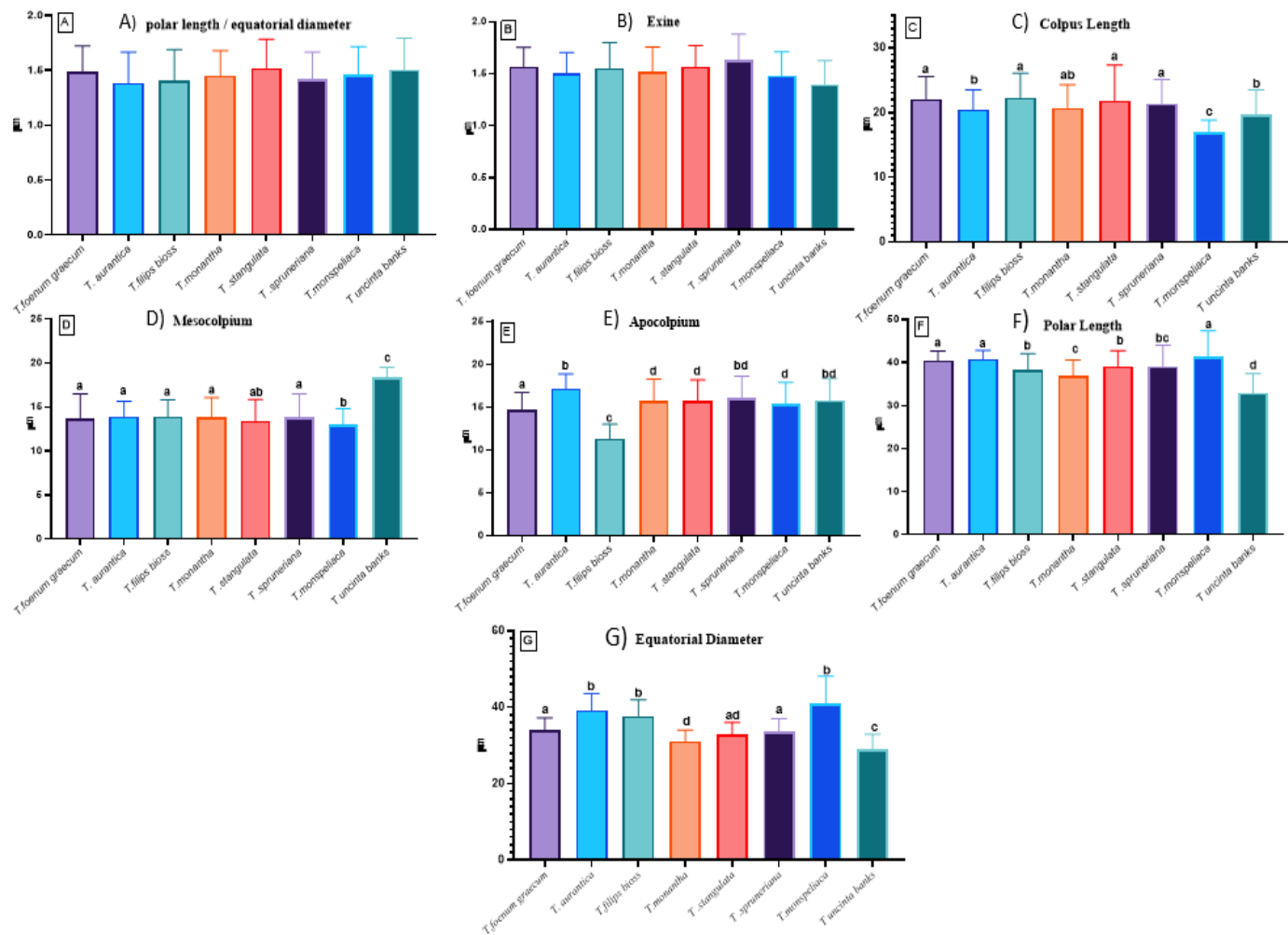


Figure 1: Bar charts depict(A-G) seven pollen traits (P/E ratio), exine thickness, colpus length, mesocolpium width, apocolpium width, polar length, equatorial diameter) across eight *Trigonella* species. Superscript letters indicate statistically significant differences based on Tukey’s HSD test (P < 0.05): shared letters denote no significant difference, whereas differing letters indicate significant variation.

Table 3: Percentage of repeat morphological shape of pollen grains. *1-20 %, ** 20-30%, *** 30- 40 % and **** more 40%, - not present.

Taxon	Triangular P	Ovate P	Circular P	Elliptic P	Tetrangular P	Triangular E	Ovate E	Circular E	Elliptic E
T. foenum-graecum	***	*	**	***	-	**	*	-	***
T. aurantiaca	**	**	*	-	-	**	***	*	-
T. filipes	****	*	***	**	-	**	*	**	*
T. monantha	*	**	*	-	-	***	*	**	-
T. stragulata	**	***	**	*	-	*	**	*	**

<i>T. spruneriana</i>	**	*	**	-	-	***	**	***	*
<i>T. monspeliaca</i>	*	***	*	**	-	*	**	*	-
<i>T. uncinata</i>	**	*	***	*	-	****	**	*	*

The table presents the morphological variability of pollen grains across eight *Trigonella* species, categorizing shapes into Pollen (P) and Equatorial (E) views, including Triangular, Ovate, Circular, Elliptic, and Tetrangular forms. Using symbols (*: 1–20%, **: 20–30%, ***: 30–40%, ****: >40%, "-": absent), the data reveal distinct patterns, such as *T. filipes* * exhibiting high Triangular P abundance (****) and *T. uncinata* * showing dominance in Triangular E (****), while Tetrangular P is absent in all species.

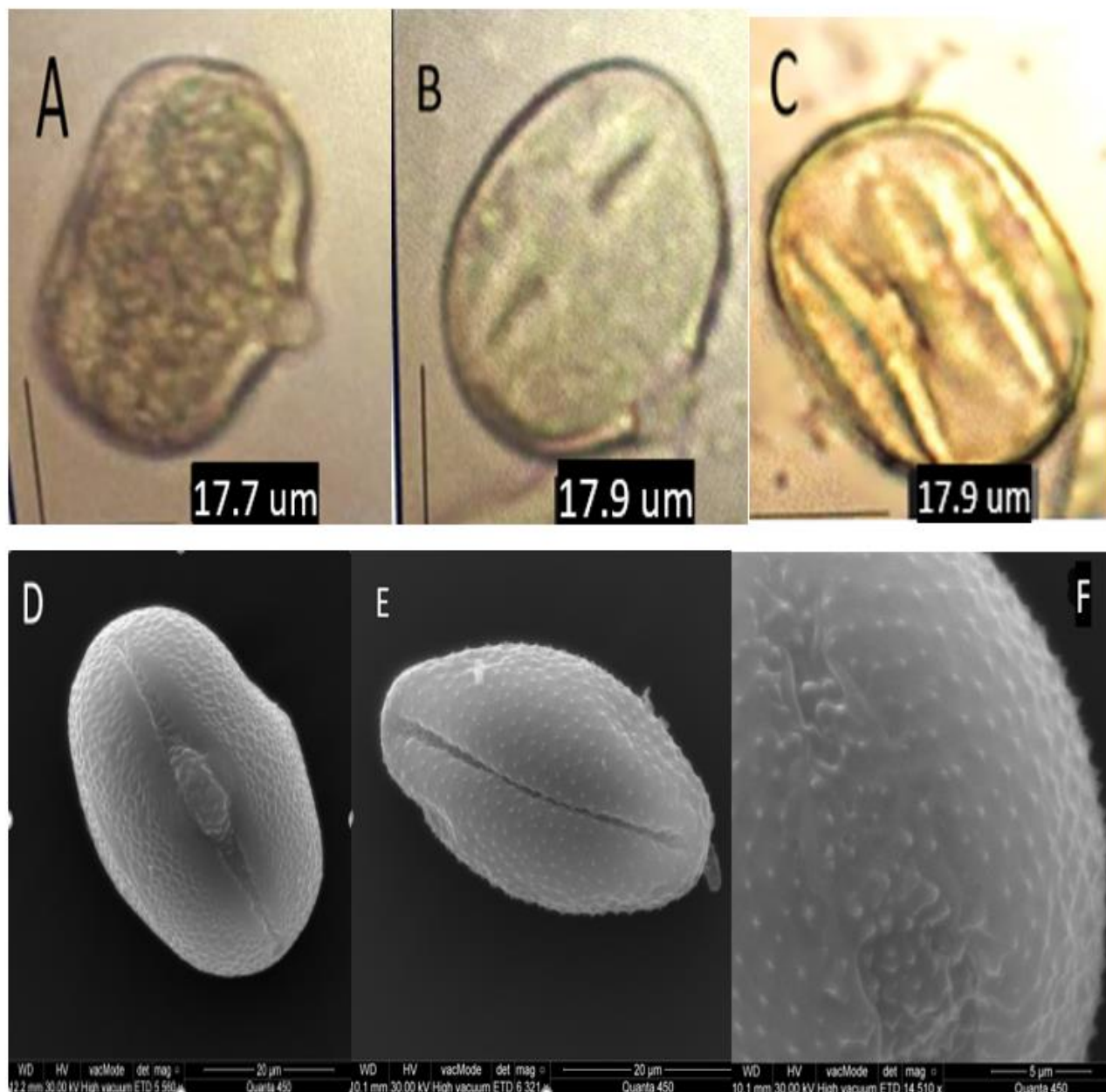


Figure 2: LM and SEM micrographs of pollen grains of *T. fenugraecum* A. Spheroidal polar view, B. Sub-oblate polar view colpi, C. Elliptic. D. Circular E. Elongated equatorial view displaying colpus margins, G. Exine surface.

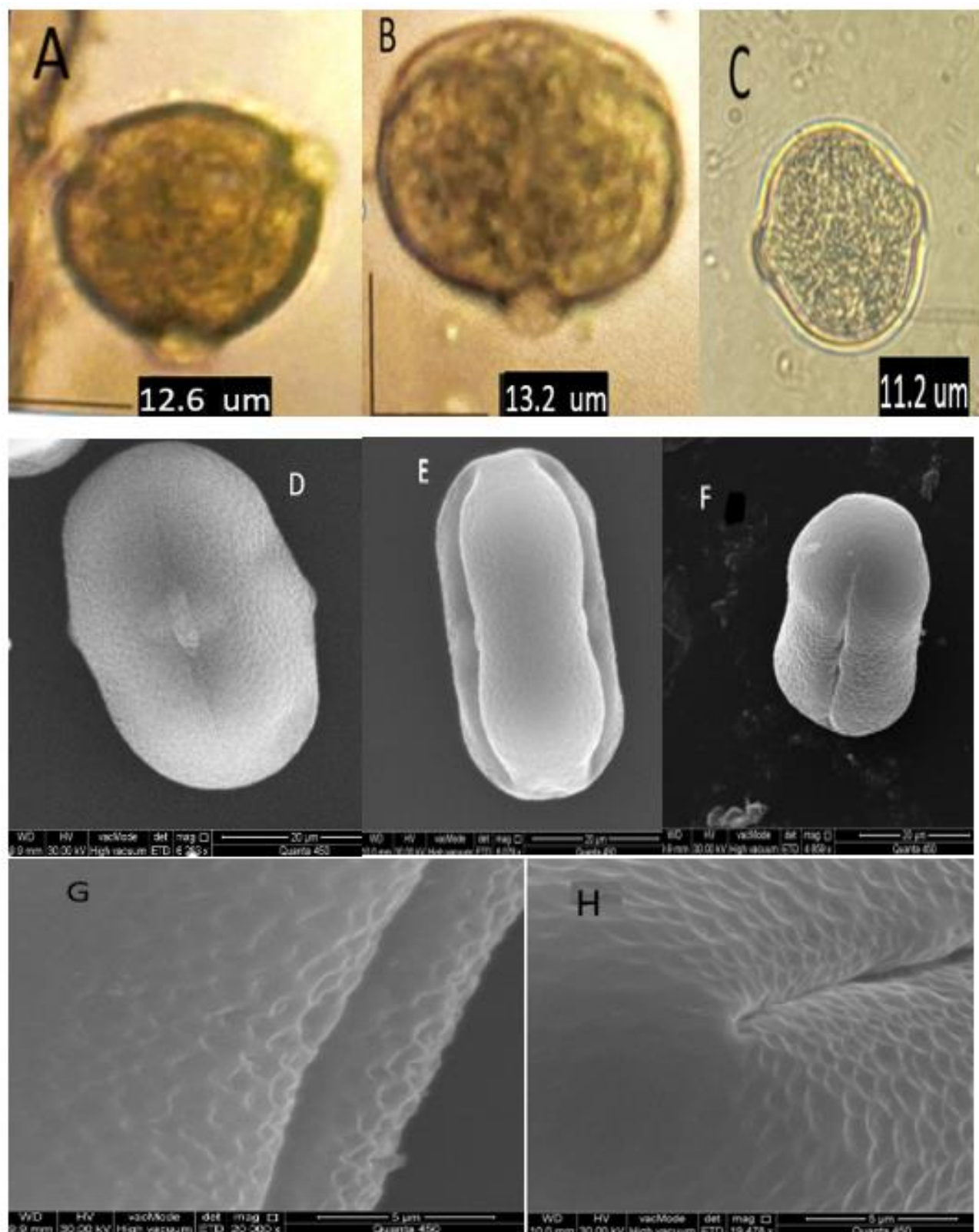


Figure 3: LM and SEM micrographs of pollen grains of *T. aurantiaca*: A. Sub-triangular polar view, B. polar view with, C. circular outline.: D. Polar view of a tricolpate pollen grain, E. Equatorial view F. Equatorial view elongated shape and H visible colpi, G. Exine sculpture showing microreticulate ornamentation.

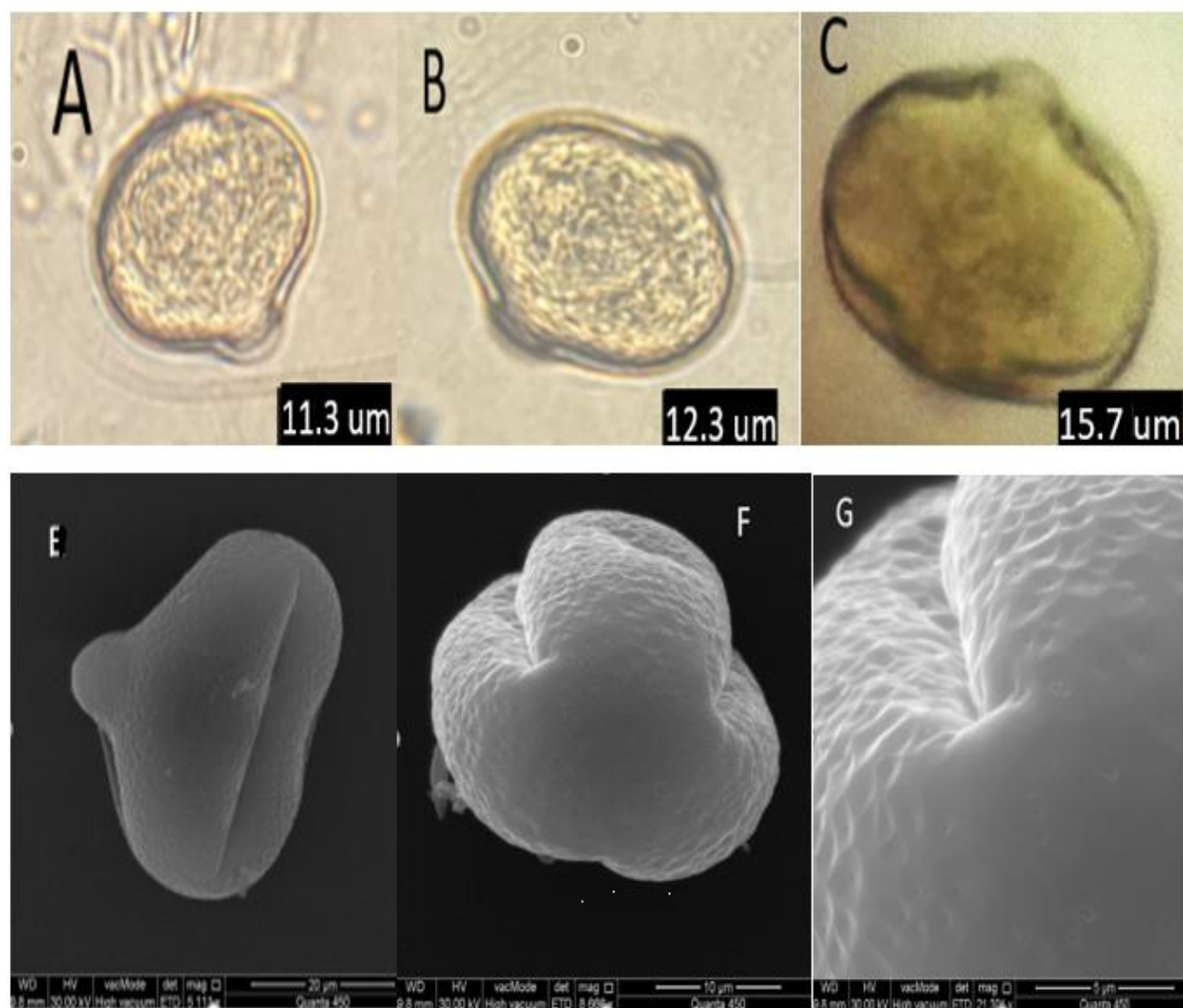


Figure 4: LM and SEM micrographs of pollen grains of *T. filipes*: A and B. Circular to slightly elliptic polar views; C. equatorial view; F. Tricolpate pollen grain in polar view, H. Semi-elliptic to elongate equatorial view, G. Exine sculpture.



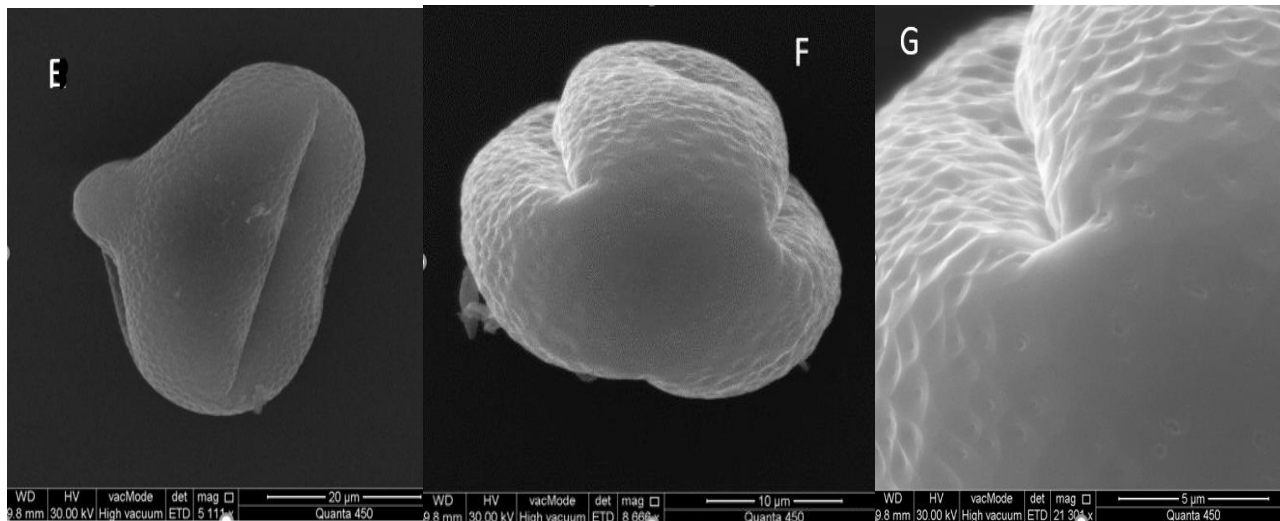


Figure 4: LM and SEM micrographs of pollen grains of *T. filipes*: A and B. Circular to slightly elliptic polar views; C. equatorial view; F. Tricolpate pollen grain in polar view, H. Semi-elliptic to elongate equatorial view, G. Exine sculpture.

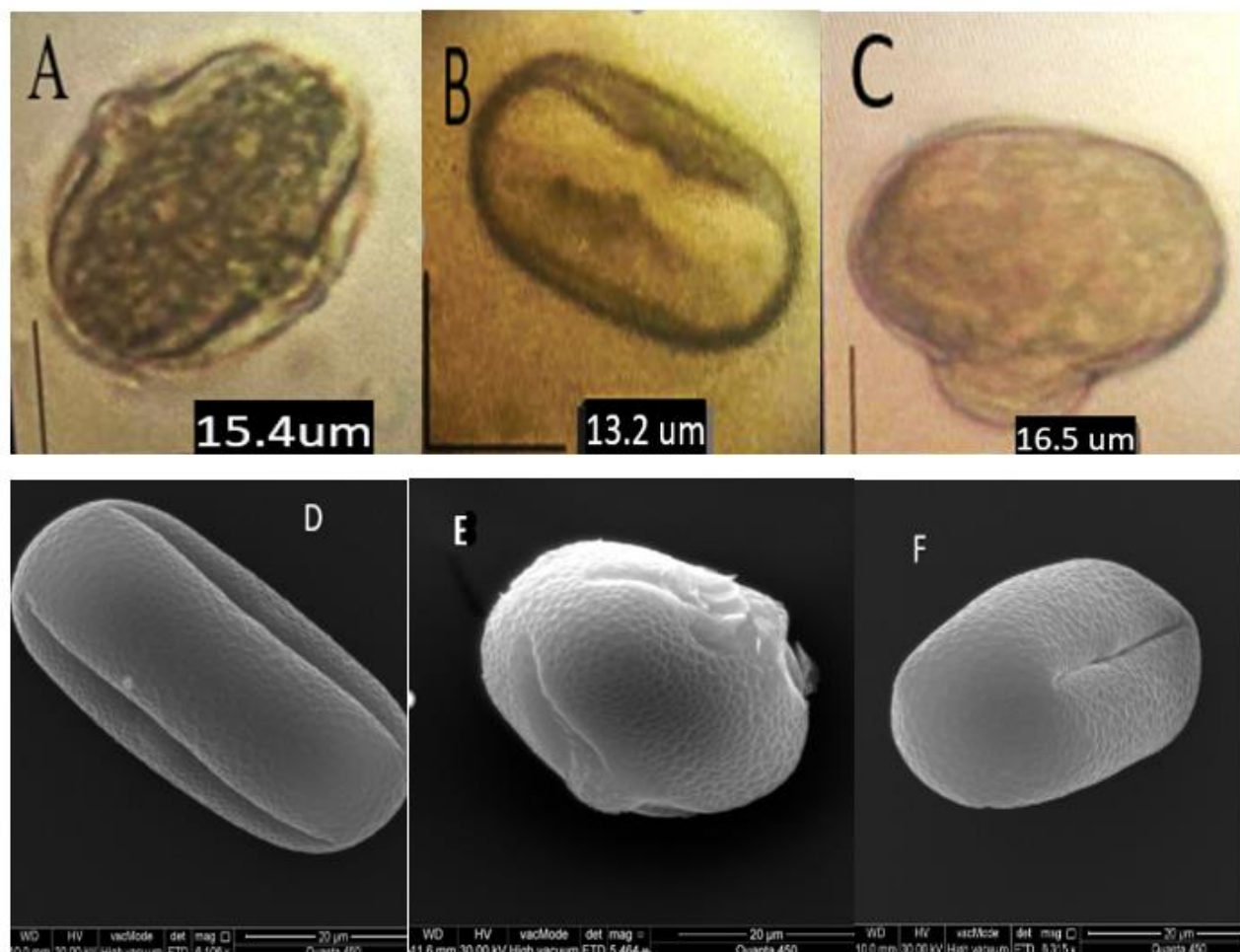


Figure 5: LM and SEM micrographs of pollen grains of *T. monantha*. **(A)** a tricolpate aperture type **(B)** Equatorial view of a pollen grain. **(C)** Oblique or possibly. **(D)** Equatorial view displaying the elongate form **(E)** Polar view with visible colpus; the exine surface shows a scabrate to finely reticulate texture. **(H)** Oblique view showing the overall spheroidal **(F)** exine surface .

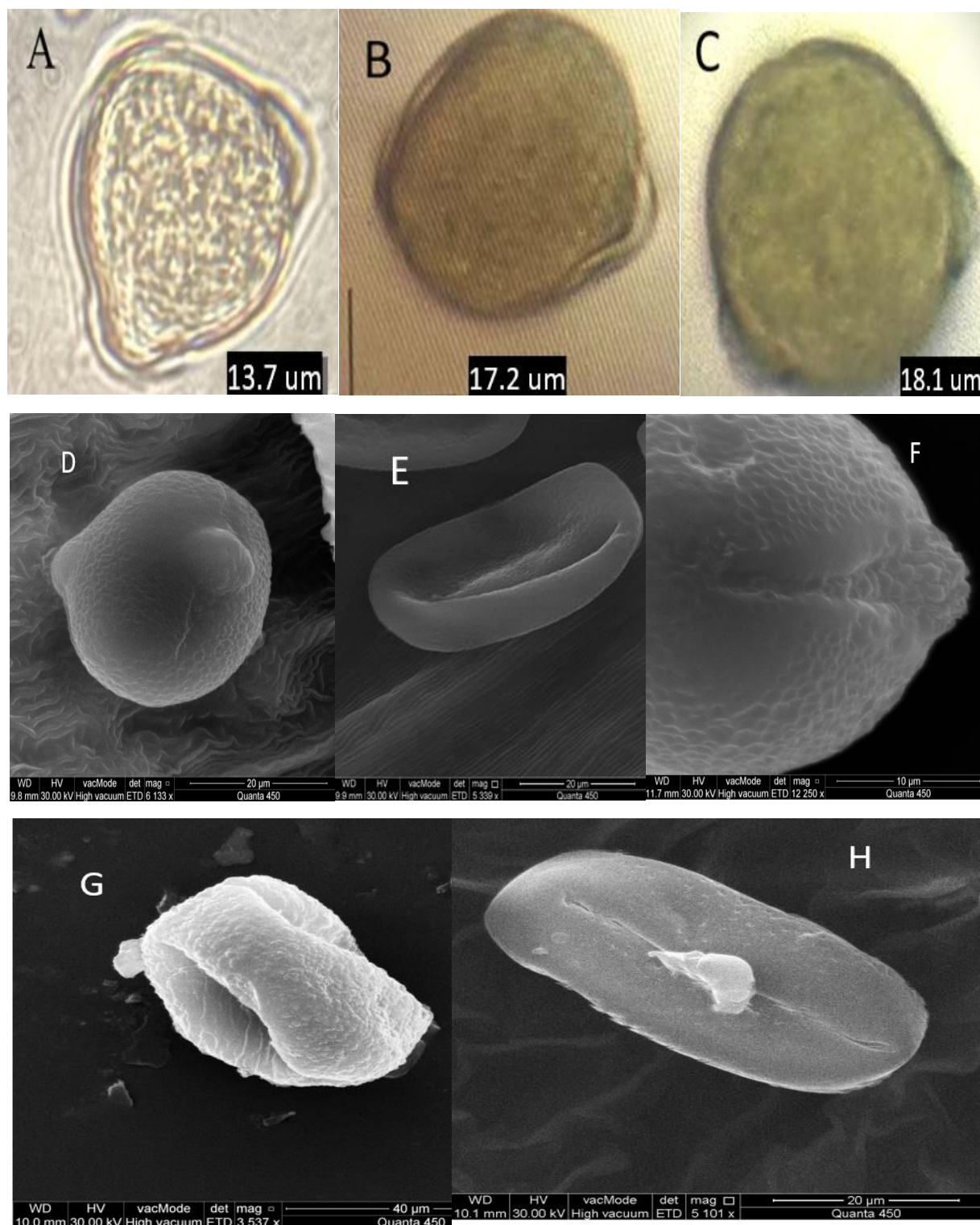


Figure 6: LM and SEM micrographs of pollen grains of *T. strangulata* (A) Polar view of a single pollen grain (B) Equatorial view showing subprolate to spheroidal shape (C) Another equatorial view: (D) Polar view showing isopolar, tricolporate grain (E) Equatorial view showing elliptic contour and colpus outline, (F) Close-up of exine ornamentation displaying microreticulate sculpturing (G) Oblique view illustrating grain folding and overall tricolporate aperture (H) is elongated with a distinct sulcus extending along its length. The exine appears smooth (psilate).

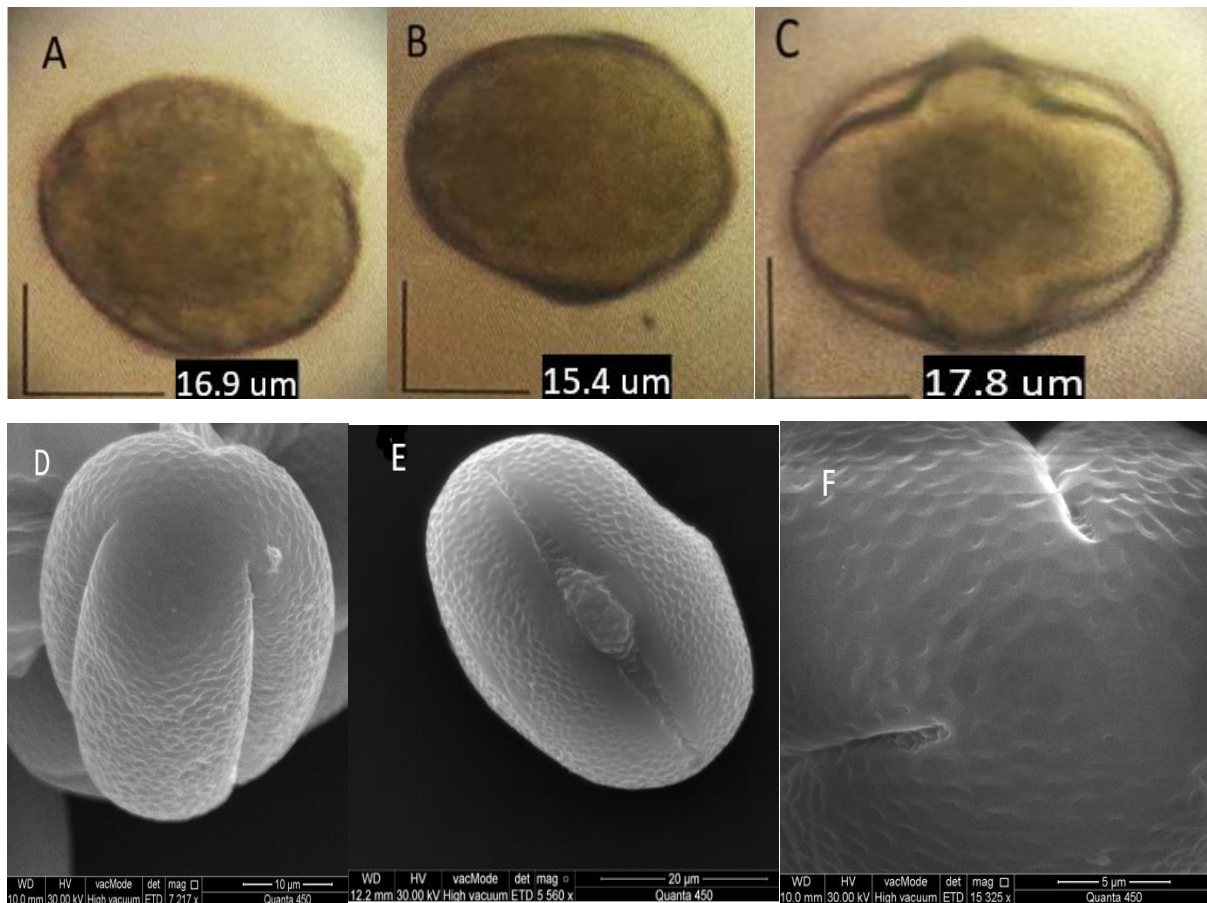
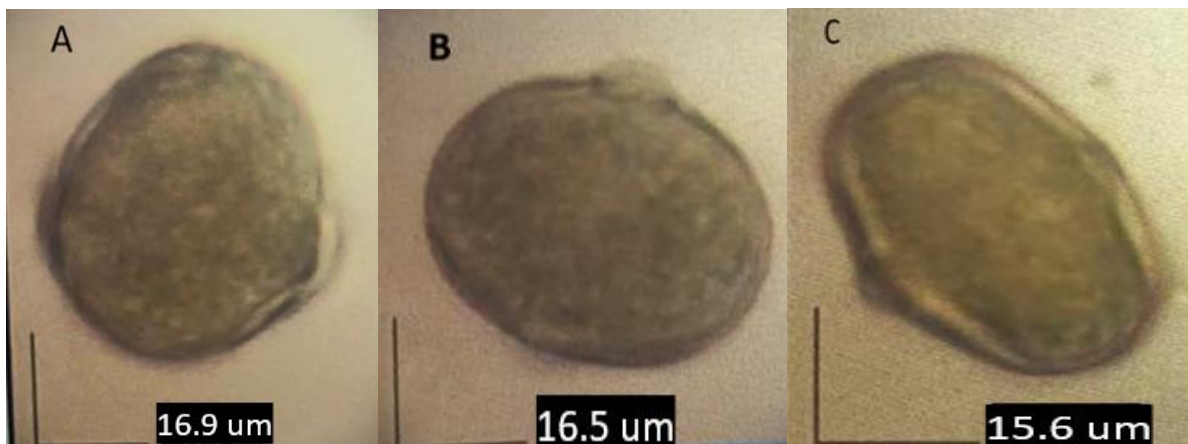


Figure 7: LM and SEM micrographs of pollen grains of *T. spruneriana* (A) Polar view showing the spheroidal shape, (B) Equatorial view displaying suboblate outline (C) Optical focus showing the presence of colpi and internal structure D. Polar view exhibiting three prominent colpi, (E) Equatorial view displaying elongated shape with a distinct aperture, (f) Close-up of the exine surface.



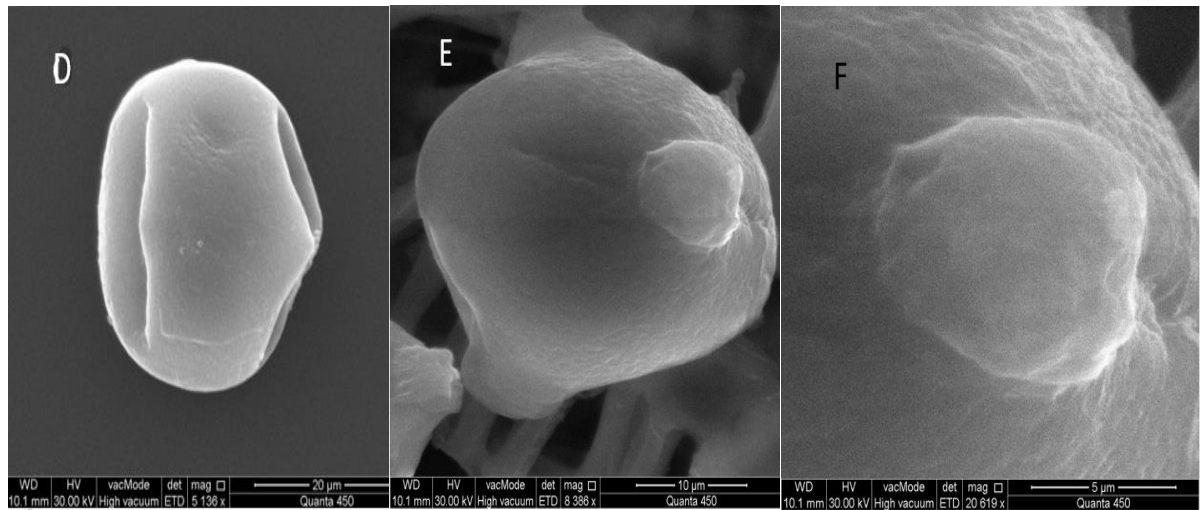
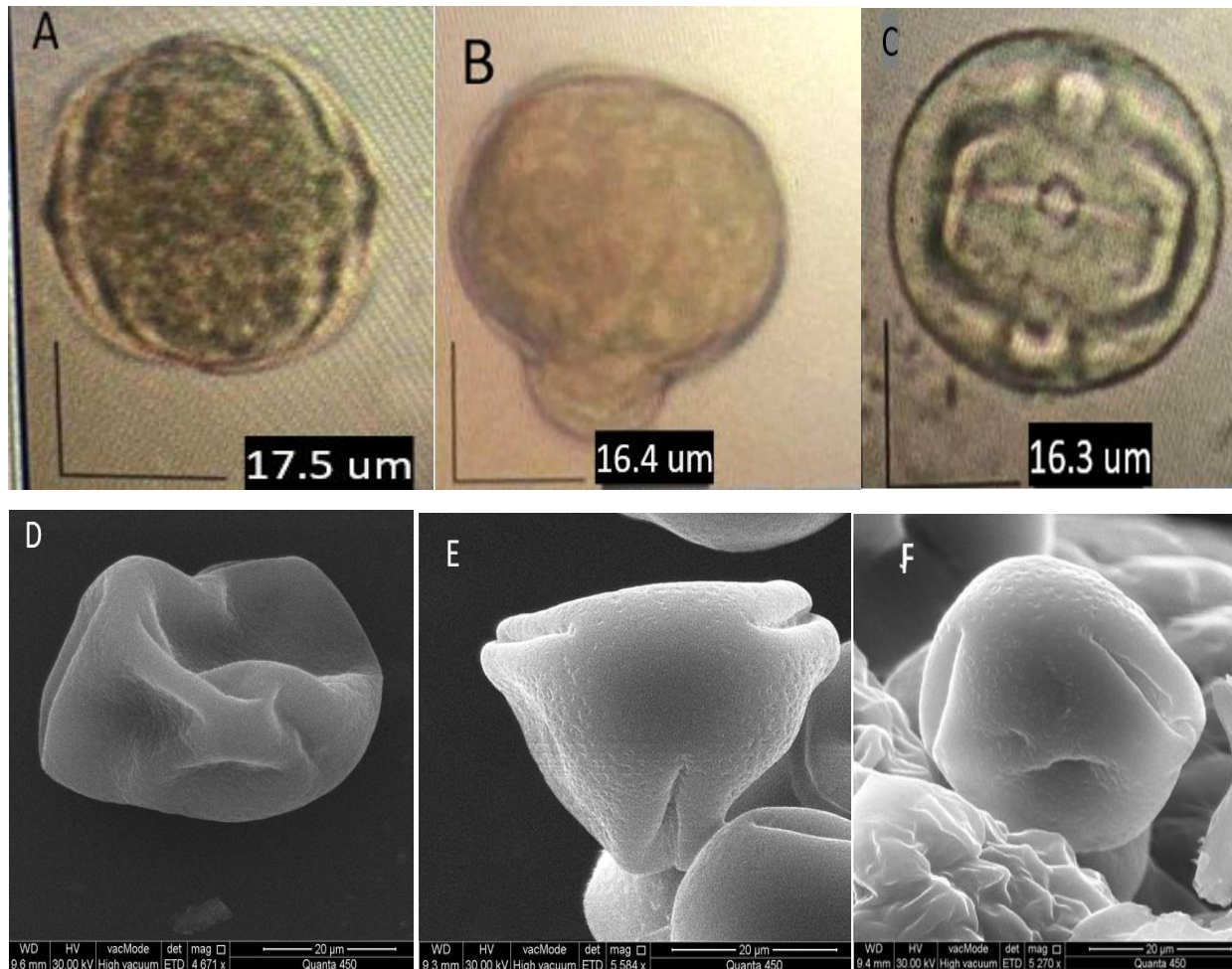


Figure 8: LM and SEM micrographs of pollen grains of 7. *T. monspeliaca* (A) Sub-ovoid, (B) Oblate-spheroidal, (C) Prolate-spheroidal forms., (D) Polar view with distinct operculum, (E) Close-up of aperture region showing exine thickening, (F) Equatorial view showing elongated form.



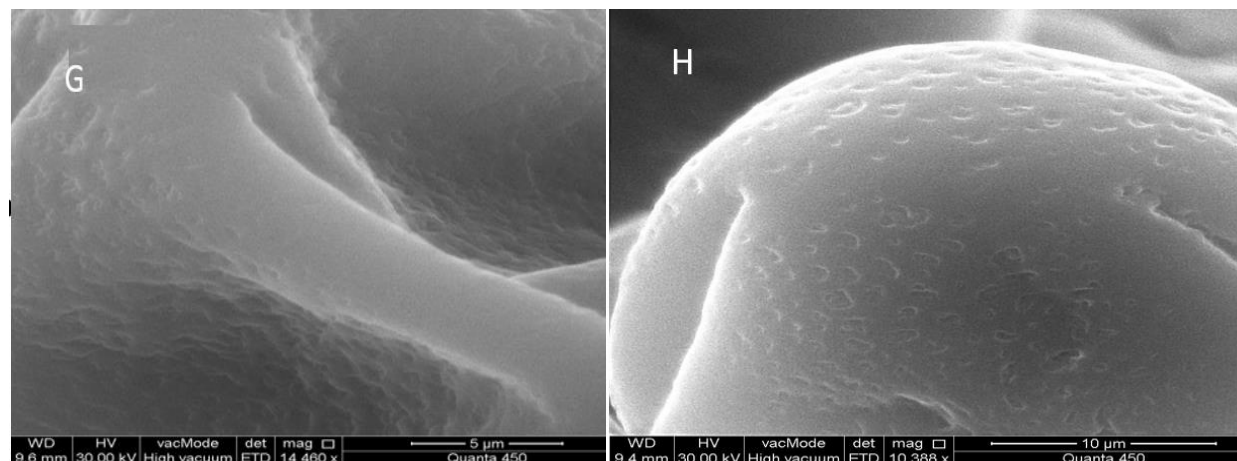


Figure 9: LM and SEM micrographs of pollen grains of 8.*T uncinata* (A) Equatorial view of a pollen grain showing an oblate-spheroidal (B) showing a subcircular. (D), clearly showing the tricolpate apertures and the circular outline with a slightly thickened exine. (E) a single pollen grain. (F) visible colpi and s. (G) (H) showing fine surface ornamentation and a slightly elongated body.

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