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Research Article

Pollen Morphology of Some *Galium* L. (Rubiaceae) Taxa

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ABSTRACT

This study investigated the palynological characteristics of eleven *Galium* L. (Rubiaceae) taxa (*G. setaceum, G. spurium subsp. spurium, G. aparine, G. rotundifolium, G. odoratum, G. rivale, G. incanum* subsp. *elatius, G. humifusum, G. verum subsp. verum, G. album* subsp. *pycnotrichum*, and *G. paschale*) collected from different localities in Eskişehir Province, Türkiye. Pollen grains were examined using scanning electron microscopy (SEM). For each taxon, the polar axis (P), equatorial axis (E), P/E ratio, pollen shape, aperture number, colpus length (Cl), colpus width (Cw), and exine ornamentation were measured. Across the examined taxa, the smallest pollen grains were observed in *G. paschale* (P = 12.79 µm), whereas the largest were recorded in *G. aparine* (P = 28.20 µm). The equatorial axis (E) ranged from 8.70 to 19.90 µm, and the P/E ratio ranged from 1.25 to 2.20. Pollen grains were predominantly prolate in *G. setaceum, G. spurium* subsp. *spurium, G. aparine, G. rotundifolium, G. odoratum, G. rivale, G. verum* subsp. *verum*, and *G. album* subsp. *pycnotrichum,* whereas *G. incanum* subsp. *elatius, G. humifusum,* and *G. paschale* exhibited subprolate pollen. Aperture numbers ranged from penta- to nonacolpate, and exine ornamentation was consistently microechinate or microechinate–perforate. Although slight quantitative differences were observed among the examined taxa, the extensive overlap in pollen morphological characters indicates that palynological features alone provide limited taxonomic resolution for species-level discrimination within *Galium*.

Keywords: Aperture morphology, Exine ornamentation, Palynotaxonomy, SEM, Pollen micromorphology

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1 Introduction

Rubiaceae Juss. is one of the largest angiosperm families, ranking fifth globally with approximately 615 accepted genera and 14,266 species distributed worldwide. Members of the family are characterized by opposite leaves, interpetiolar stipules, and diverse growth forms ranging from herbs to shrubs and trees [13].

Within the tribe Rubieae (subfamily Rubioideae, Rubiaceae), the genus *Galium* L. is the largest. Commonly known as bedstraw or cleavers, the genus comprises approximately 650 accepted species and exhibits a broad geographic distribution across North and South America, extratropical Eurasia, Australia, Arctic highlands, and subtropical to tropical regions [5,6,10]. The Turkish flora comprises 125 *Galium* taxa (species, subspecies, and varieties) assigned to 10 sections, of which 64 are endemic to Türkiye. The recent description of a new *Galium* species further demonstrates that the taxonomy of the genus remains an active field of research [3].

The taxonomic interpretation of *Galium* is often challenging, as species belonging to different sections frequently share similar growth habits. Moreover, the extensive distribution and pronounced polymorphism of many species result in species groups that are often poorly differentiated both morphologically and geographically [9].

The genus *Galium* mainly consists of herbaceous plants or low subshrubs, and includes both annual and perennial species. Whorled leaves and small flowers characterize these plants, and the genus has been subdivided into ten sections based on morphological characteristics [4]. Despite these well-defined general morphological characteristics, delimitation among closely related species remains problematic because of extensive morphological overlap and phenotypic variability.

Due to the taxonomic complexity of the genus *Galium*, additional characters beyond traditional morphological traits are often required for reliable species delimitation. Palynological features have been widely employed in systematic studies and may provide valuable supplementary characters for taxonomic assessment. Consequently, pollen morphology can contribute significantly to clarifying relationships among taxa within the genus. Because species belonging to different sections of *Galium* often exhibit overlapping vegetative and reproductive morphological characters, comparative studies including representatives from multiple sections are particularly valuable. Evaluating pollen morphology across taxa assigned to different sections may provide additional diagnostic evidence and improve the understanding of interspecific relationships within the genus.

The pollen morphology of *Galium* has been investigated in several palynological studies from different regions of the world, emphasizing the potential taxonomic value of these characters [1,2,7,8,12]. However, palynological data remain limited for many *Galium* taxa, particularly for species distributed in Türkiye. Therefore, the present study investigates the pollen morphology of eleven *Galium* taxa representing different sections of the genus using scanning electron microscopy (SEM). By comparing taxa from different infrageneric groups, this study evaluates whether pollen morphological characters possess sufficient diagnostic value for species discrimination and taxonomic interpretation. In addition, graphical visualization of pollen measurements was incorporated to facilitate direct comparisons among taxa. The findings provide detailed SEM-based palynological data and contribute to a better understanding of pollen diversity and its taxonomic significance within *Galium*.

2 Method

2.1 Sampling and Palynological Methods

Plant specimens were collected by M. Kalas from natural populations in Eskişehir, Türkiye, during the vegetation periods between 2021 and 2024. Locality information and voucher data of the examined taxa are presented in Table 1. Taxonomic identification of the specimens was performed by M. Kalas and A. Kaya using Flora of Turkey and the East Aegean Islands (Vol. 7) [4]. Voucher specimens were deposited in the ESSE (Herbarium of the Faculty of Pharmacy, Anadolu University) and CUEF (Herbarium of the Faculty of Pharmacy, Çukurova University) herbaria.

For palynological analyses, unopened flower buds were carefully selected to prevent contamination by foreign pollen. Anthers were dissected manually, and pollen grains were transferred onto aluminum SEM stubs covered with double-sided conductive carbon tape using a clean, fine-tipped needle. For each taxon, measurements were performed on at least 20 pollen grains to ensure reliable and representative results. Pollen dimensions were measured directly from SEM micrographs using ZEISS ZEN imaging software. Before examination, the samples were sputter-coated with a thin layer of platinum. Scanning electron microscope (SEM) micrographs were obtained using a Zeiss

Ultra Plus microscope at the SEM Laboratory of the Faculty of Science, Eskişehir Technical University. Pollen morphological terminology and descriptive criteria followed Punt et al. [11].

Table 1. Section and taxa names, locality, date and herbarium numbers of the collected *Galium* specimens.

Section	Taxa	Locality	Date	ESSE NO	CUEF NO
Jubogalium	*G. setaceum*	B3 Eskişehir: Mihalıççık, between Bahtiyar and Sekiören villages, rocky slope on the left side of the bridge, 692 m., 39°56'51''K, 31°41'20'' D	23.04.2024	16335	1999
Kolgyda	*G. aparine*	B3 Eskişehir: Odunpazarı, Sümer neighborhood, 800 m., 39°45'30''K, 30°29'32''D	19.06.2021	16336	2001
	G. spurium subsp. *spurium*	B3 Eskişehir: Mihalıççık, between Bahtiyar and Sekiören villages, rocky slope on the left side of the bridge, 691 m., 39°56'52'' K, 31°41'20'' D	23.04.2024	16337	2000
Platygalium	*G. rotundifolium*	B3 Eskişehir: Türkmen Mountain, forest interior, flat areas, 1670 m., 39°27'42''K, 30°21'32''D	11.07.2023	16321	1985
Hylaea	*G. odoratum*	B3 Eskişehir: Türkmen Mountain, forest floor, shade areas, 1470 m., 39°30'5''K, 30°22'28''D	08.06.2023	16323	1986
Trachygalium	*G. rivale*	B3 Eskişehir: Türkmen Mountain, forest inner, 39°29'47''K, 1145 m., 30°23'52''D	20.07.2023	16325	1988
Orientigalium	*G. incanum* subsp. *elatius*	B3 Eskişehir: 20. km of Eskişehir-Kütahya road, left side of the road, marsh hills, 880 m., 39°43'29''K, 30°20'26''D	25.05.2021	16333	1997
Galium	*G. humifusum*	B3 Eskişehir: Sivrihisar, inner road of Ahiler village, edges of stones, 803 m., 39°11'41''K, 31°37'4''D	18.06.2024	16327	1990
	G. verum subsp. *verum*	B3 Eskişehir: Odunpazarı, Gültepe neighborhood, near the forest of Kent entrance, road sides, 850 m., 39°44'42''K, 30°30'23''D	28.06.2021	16328	1992
Leiogalium	*G. album* subsp. *pycnotrichum*	B3 Eskişehir: Türkmen Mountain, forest inferior, shade areas, 1150 m., 39°29'47''K, 30°23'50''D	27.07.2022	16330	1994
	G. paschale	B3 Eskişehir: Türkmen Mountain, forest inner, 1150 m., 39°29'46''K, 30°23'53''D	29.06.2021	16331	1995

3 Results and Discussion

The palynological characteristics of the studied *Galium* taxa are illustrated by SEM microphotographs showing the polar and equatorial views of the pollen grains, together with detailed images of their exine ornamentation (Figure 1). Quantitative pollen morphological data, including the minimum, mean, and maximum values of the polar axis (P) and equatorial axis (E), P/E ratio, pollen shape, aperture number, colpus length (Cl), colpus width (Cw), and exine ornamentation types, are summarized in Table 2. To facilitate direct comparisons among the studied taxa, the minimum, mean, and maximum values of the polar axis, equatorial axis, and colpus length are further illustrated using range plots (Figures 2–4).

3.1 Symmetry, Polarity, Shape, and Size

All examined pollen grains were single, radially symmetrical, and isopolar. The pollen shape varies from prolate to subprolate–prolate. The polar axis (P) ranges from 12.79 to 28.20 µm, while the equatorial axis (E) ranges from 8.70 to 19.90 µm. The smallest pollen grains were observed in *G. paschale* (P = 12.79–20.49 µm; E = 10.2–14.6 µm), whereas the largest pollen grains were recorded in *G. aparine* (P = 22.63–28.20 µm; E = 14.73–19.90 µm).

3.2 Aperture Characteristics

The pollen grains were predominantly hexacolpate; however, penta-, hepta-, octa-, and nonacolpate types were also observed. Colpus length (Cl) ranges from 7.09 to 23.37 µm, while colpus width (Cw) generally exhibits low values,

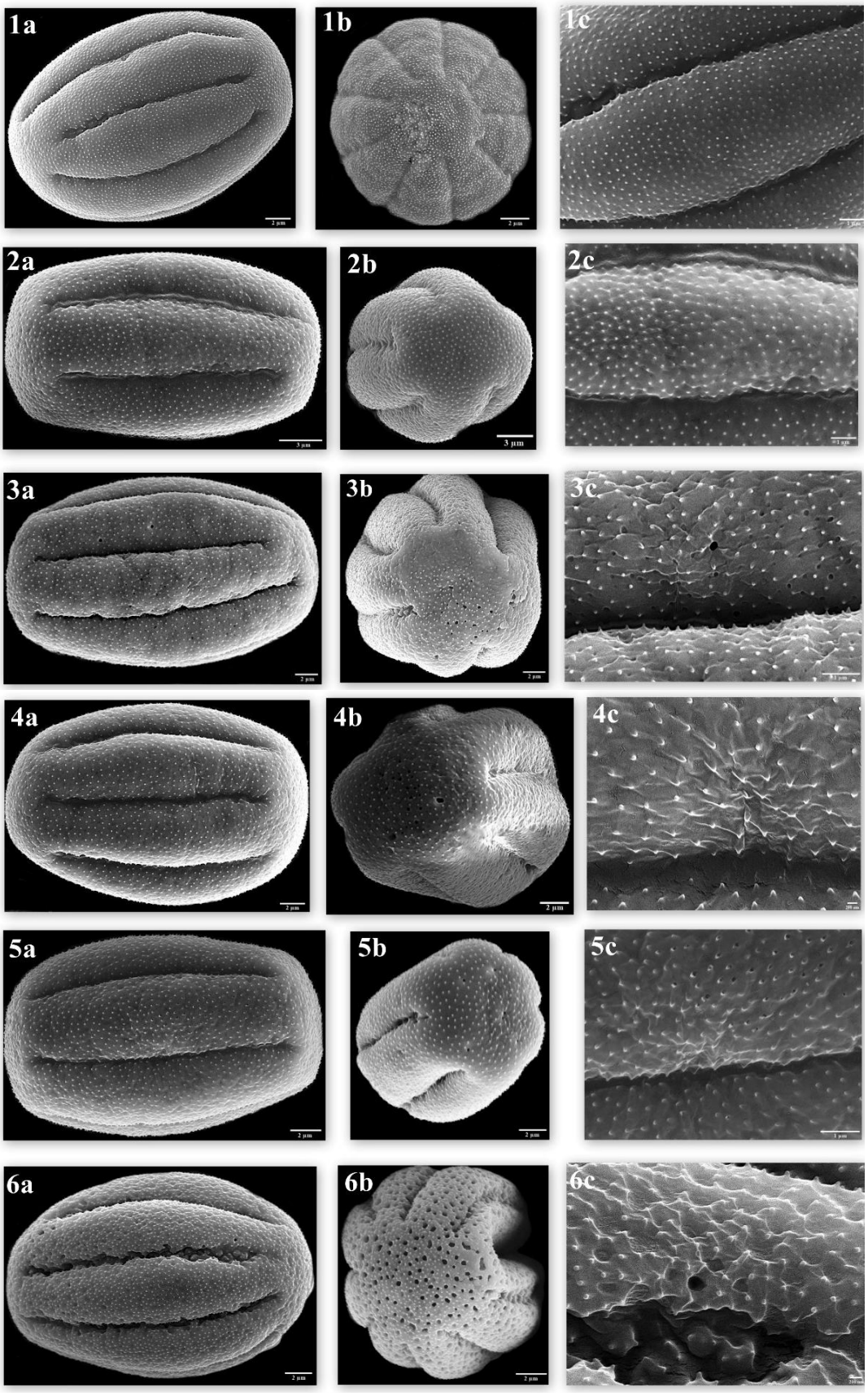
with a minimum of 2.25 µm. The shortest colpus was observed in *G. paschale* (7.09 µm), whereas the longest colpus was recorded in *G. aparine* (23.37 µm).

3.3 Exine Ornamentation

The exine ornamentation of the pollen grains in the studied *Galium* taxa was microechinate or microechinate–perforate. The perforations vary in both size and density, ranging from small and sparse to large and densely distributed.

Table 2. Pollen morphological characteristics of the studied *Galium* taxa, including size, shape, aperture number, colpus dimensions, and exine ornamentation.

Taxa name	Polar axis (µm)			Equatorial axis (µm)			P/E (µm) ratio	Pollen shape	Aperture number	Colpus (µm)			Exine ornamentation
										Length		Width	
	Min	Max	Avg	Min	Max	Avg				Min	Max		
G. setaceum	20.28	23.67	21.98	12.85	18.0	15.43	1.31±1.58	prolate	hepta–nonacolpate	11.33	20	<1	microechinate
G. spurium subsp. *spurium*	19.61	23.53	21.57	12.3	15.53	13.92	1.51±1.59	prolate	penta–hexacolpate	13.88	19.41	<1	microechinate
G. aparine	22.63	28.20	25.33	14.73	19.90	17.32	1.41±1.54	prolate	hexa–heptacolpate	15.45	23.37	<1	microechinate–perforate (perforates sparse and small)
G. rotundifolium	21.73	23.41	22.57	12.17	16.36	14.27	1.43±1.78	prolate	penta–hexacolpate	12.5	19.62	<1	microechinate–perforate (perforates sparse and small)
G. odoratum	15.28	20.30	17.79	10.87	15.09	12.98	1.34±1.4	prolate	penta–hexa–heptacolpate	11.58	16.66	<1	microechinate–perforate (perforates sparse and small)
G. rivale	16.2	21.11	18.66	8.76	15.36	12.06	1.37±1.84	prolate	hexa–octa–nonacolpate	8.82	17.85	<1	microechinate–perforate (perforates dense and large)
G. incanum subsp. *elatius*	17.31	22.82	20.07	13.85	16.43	15.14	1.25±1.39	subprolate	hexa–octacolpate	11.76	20	<1.76	microechinate
G. humifusum	13.91	21.87	17.89	8.70	17.39	13.05	1.26±1.6	subprolate	penta–hexacolpate	8.69	19.13	<2.25	microechinate–perforate (perforate sparse and variable sizes)
G. verum subsp. *verum*	14.9	17.9	16.40	10.0	12.4	11.20	1.44±1.49	prolate	penta–hexacolpate	7.69	13.46	<1	microechinate
G. album subsp. *pycnotrichum*	21.0	24.5	22.75	11.6	17.0	14.30	(1.44–)1.51± 1.89(–2.2)	prolate	hexa–heptacolpate	13.7	20.62	<1	microechinate
G. paschale	12.79	20.49	16.64	10.2	14.6	12.40	1.25±1.40	subprolate	heptacolpate	7.09	16.92	<1	microechinate–perforate (perforates sparse and large)


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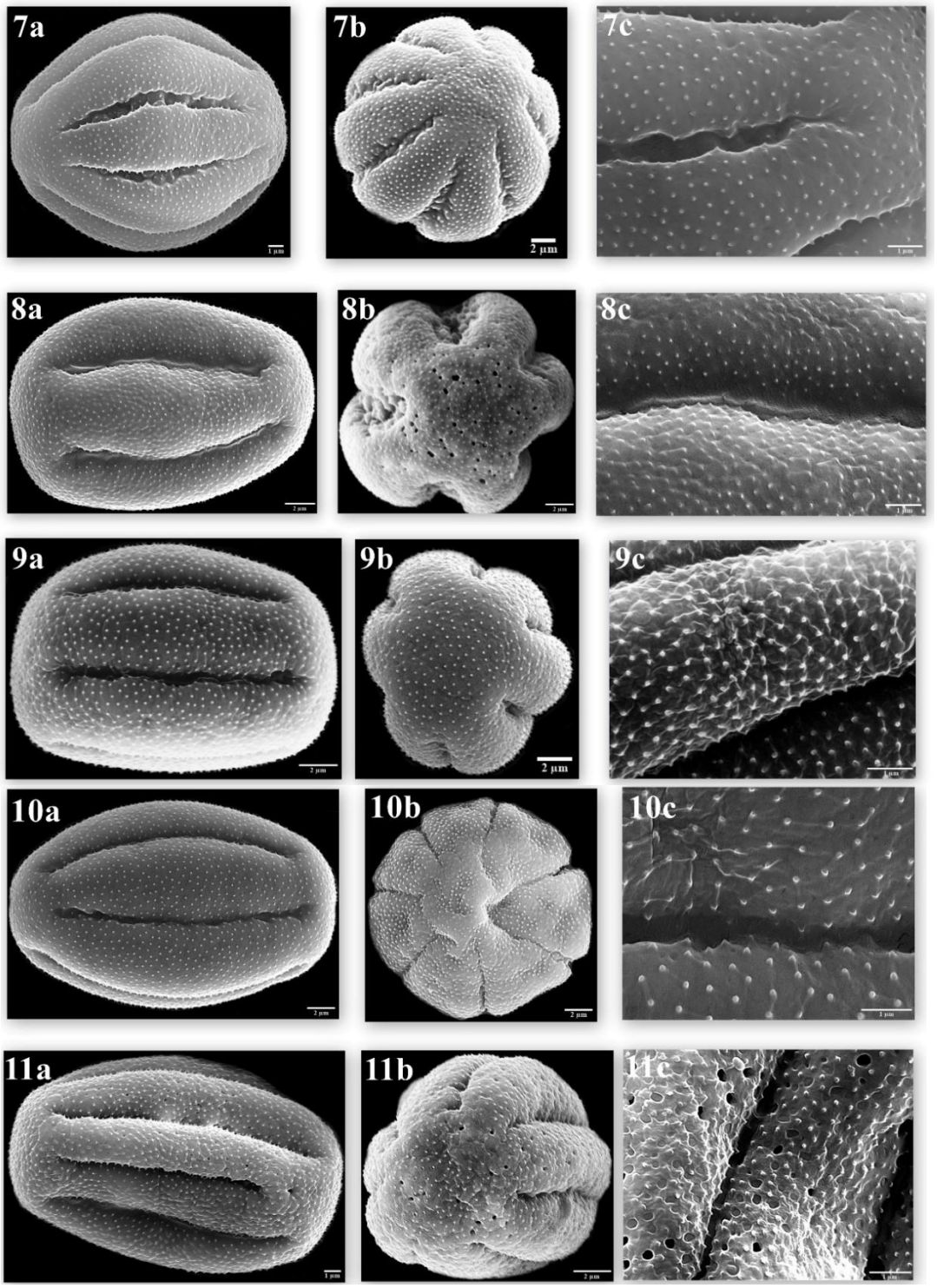


Figure 1. SEM microphotographs of the studied *Galium* taxa 1a, 1b, 1c: *G. setaceum*; 2a, 2b, 2c: *G. spurium* subsp. *spurium*; 3a, 3b, 3c: *G. aparine*; 4a, 4b, 4c: *G. rotundifolium*; 5a, 5b, 5c*: G. odoratum*; 6a, 6b, 6c: *G. rivale*; 7a, 7b, 7c: *G. incanum* subsp. *elatius*; 8a, 8b, 8c: *G. humifusum*; 9a, 9b, 9c: *G. verum* subsp. *verum*; 10a, 10b, 10c: *G. album* subsp. *pycnotrichum*; 11a, 11b, 11c: *G. paschale*

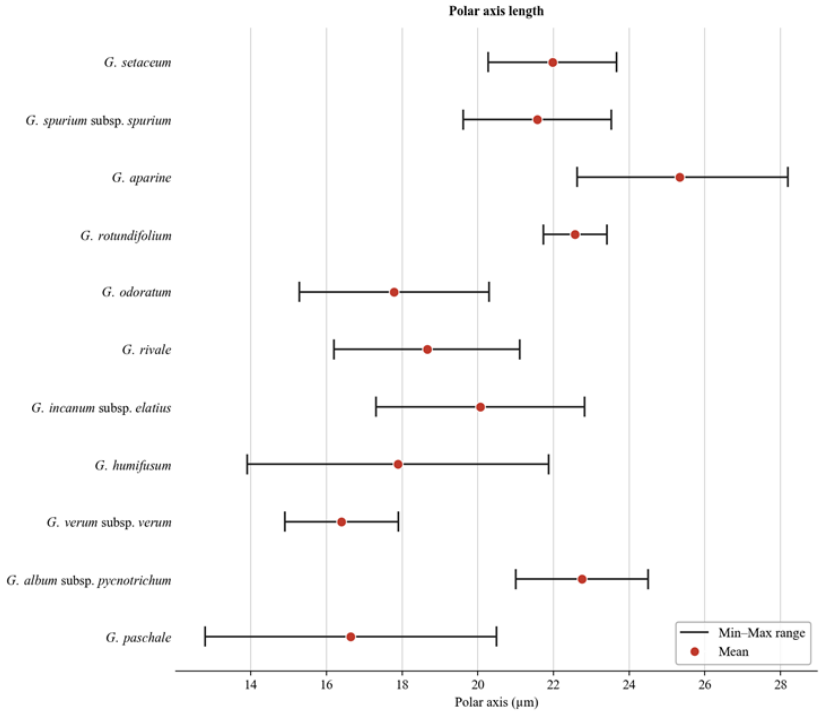


Figure 2. Range plot showing the minimum, maximum, and mean values of the polar axis (P) length in the studied *Galium* taxa

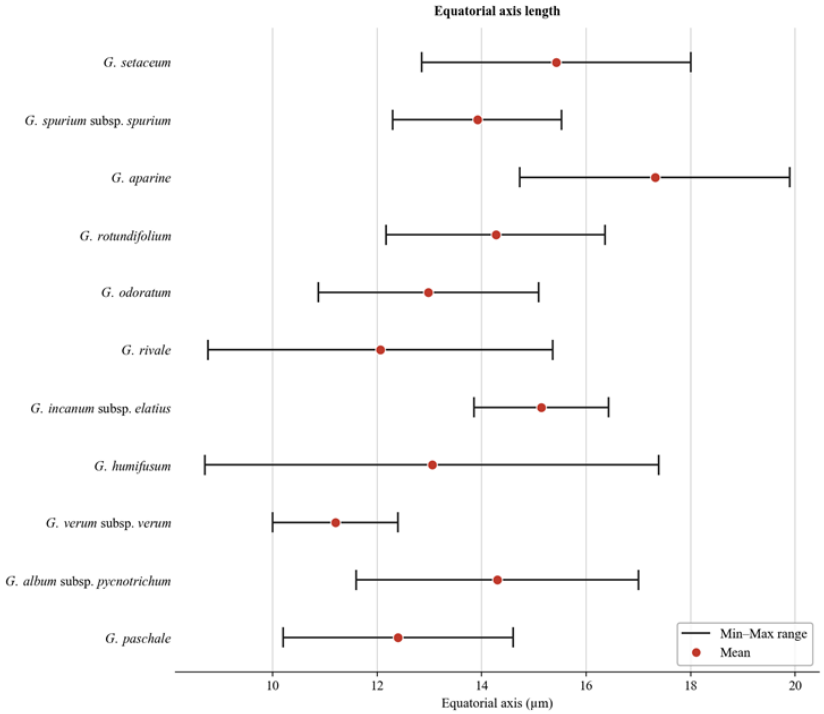


Figure 3. Range plot showing the minimum, maximum, and mean values of the equatorial axis (E) length in the studied *Galium* taxa

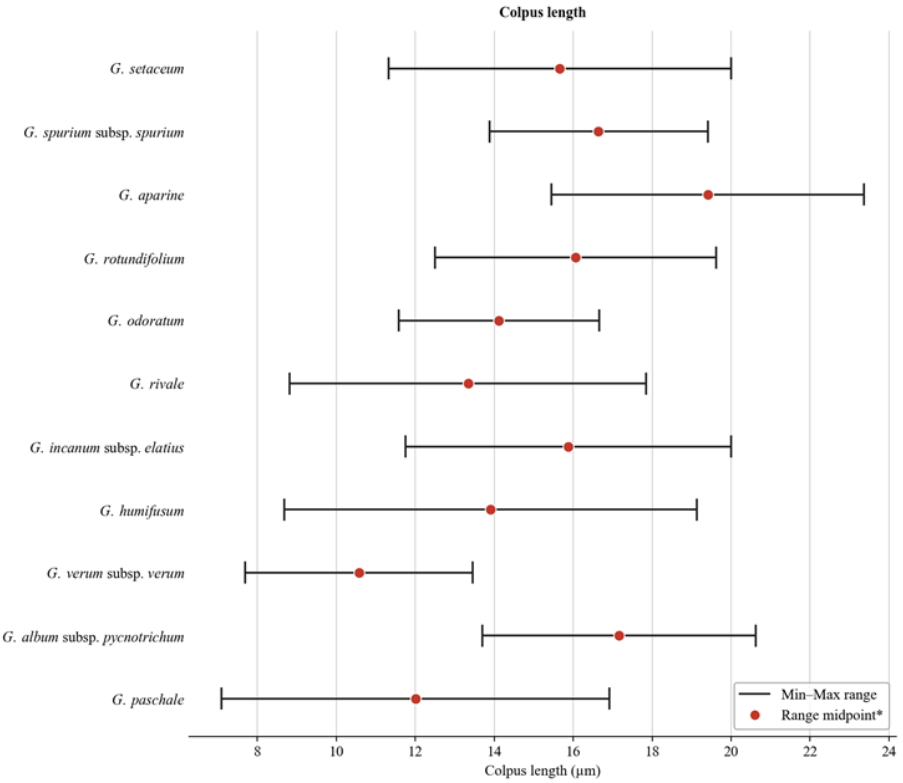


Figure 4. Range plot showing the minimum, maximum, and mean values of the colpus length in the studied *Galium* taxa

The SEM observations (Figure 1), quantitative pollen morphological data (Table 2), and the range plots illustrating the minimum, mean, and maximum values of the polar axis, equatorial axis, and colpus length (Figures 2–4) consistently demonstrate a high degree of similarity among the examined *Galium* taxa. Although slight quantitative differences were observed in pollen size, aperture number, and colpus dimensions, the extensive overlap of measurement ranges among taxa indicates that these characters do not provide clear diagnostic boundaries for reliable species discrimination. Likewise, the predominance of similar pollen shapes, aperture types, and exine ornamentation suggests that pollen morphology is relatively conserved within the genus. Therefore, the present findings indicate that palynological characters alone have limited taxonomic resolution at the species level and should be considered complementary to other taxonomic evidence.

Previous palynological studies on *Galium* have likewise reported considerable similarity in pollen morphological characters despite quantitative variation among taxa. Huysmans et al. [7], based on LM and SEM observations of several Rubiaceae genera in northwestern Europe, described pollen grains of *G. aparine* (P: 17–19 µm; E: 16–19 µm), G. *odoratum* (P: 18–21 µm; E: 17–20 µm), *G. spurium* (P: 18–24 µm; E: 18–22 µm), and *G. verum* (P: 18–21 µm; E: 17–20 µm) as predominantly spheroidal to suboblate, with generally similar aperture numbers (5–7) and perforate–microspinate exine ornamentation. Similarly, Abdel-Khalik et al. [1] reported prolate-spheroidal to spheroidal pollen grains, comparable aperture numbers (6–9), and perforate–microspinate exine ornamentation for *G. aparine* (P: 15–24 µm; E: 17–25 µm) and *G. spurium* (P: 15–19 µm; E: 14–19 µm). In contrast, the present study revealed predominantly prolate pollen grains with relatively higher P/E ratios and microechinate or microechinate–perforate exine ornamentation in the examined *Galium* taxa. Nevertheless, the quantitative differences observed among the present and previous studies show substantial overlap and do not alter the overall conclusion that pollen morphology provides only limited discriminatory power for distinguishing species within the genus.

According to Perveen and Qaiser [8], a comprehensive palynological study conducted in Pakistan investigated the pollen morphology of 17 *Galium* taxa using light microscopy (LM) and scanning electron microscopy (SEM). The authors recognized two pollen types, namely the *G. elegans* type and the *G. tenuissimum* type, and described the pollen grains as radially symmetrical and isopolar, with shapes ranging from prolate-spheroidal to subprolate. Exine

ornamentation was reported as scabrate–punctate or spinulose–punctate, and the number of colpi varied between five and ten. Three taxa examined in their study (*G. setaceum, G. verum*, and *G. aparine*) were also included in the present investigation. While Perveen and Qaiser [8] described the pollen grains of these taxa as predominantly subprolate with six or seven apertures, the present study identified mainly prolate pollen grains with comparable aperture numbers and predominantly microechinate or microechinate–perforate exine ornamentation. Although differences were observed in pollen shape and exine ornamentation, the quantitative pollen characters showed considerable overlap among the studied taxa. This pattern further supports the conclusion that pollen morphology alone provides limited diagnostic value for reliable species discrimination within *Galium*.

In Türkiye, Başer et al. [2] investigated the palynological characteristics of several *Galium* taxa using LM and SEM. Three taxa included in their study (*G. humifusum*, *G. spurium* subsp. *spurium*, and *G. incanum* subsp. *elatius*) were also examined in the present study. Başer et al. [2] described these taxa as having predominantly oblate-spheroidal to prolate-spheroidal pollen grains with 6–8-zonocolpate apertures and scabrate–perforate exine ornamentation. In contrast, the present study identified predominantly subprolate to prolate pollen grains and microechinate or microechinate–perforate exine ornamentation. Differences were also observed in the reported aperture numbers and colpus dimensions. Nevertheless, the overall pollen morphology remained broadly comparable between the two studies. As demonstrated by the range plots (Figures 2–4), the quantitative variation observed among the examined taxa largely overlaps, supporting the conclusion that pollen morphology alone provides limited taxonomic resolution at the species level.

More recently, Ullah et al. [12] investigated the pollen morphology of twenty *Galium* taxa using LM and SEM. They described the pollen grains of *G. aparine, G. setaceum*, *G. spurium*, and *G. verum* as predominantly oblate-spheroidal to prolate-spheroidal, with relatively low P/E ratios, aperture numbers ranging from five to nine, and comparatively short, broad colpi. Exine ornamentation varied among taxa and included microechinate–punctate, microechinate–verrucate, and microspinulate–perforate patterns. In comparison, the present study revealed predominantly prolate pollen grains with relatively higher P/E ratios, longer and narrower colpi, and mainly microechinate or microechinate–perforate exine ornamentation. Despite these differences, the pollen grains shared a common structural organization, particularly with respect to aperture type and general pollen architecture. The broad overlap of quantitative pollen characters illustrated in the range plots (Figures 2–4) further indicates that these characters are of limited value for distinguishing closely related *Galium* taxa. Overall, the present findings are consistent with previous studies in demonstrating that pollen morphology is relatively conserved within the genus and should therefore be interpreted as complementary, rather than independent, evidence in taxonomic studies.

4 Conclusions

The present study provides a comprehensive SEM-based assessment of the pollen morphology of eleven *Galium* taxa representing different sections of the genus. Although slight quantitative and qualitative differences were observed among the examined taxa, the extensive overlap in pollen morphological characters indicates that these variations do not provide reliable diagnostic features for species-level discrimination. Comparative evaluation with previous palynological studies further confirms that pollen morphology is relatively conserved within *Galium*. Consequently, palynological characters should be regarded as complementary rather than primary taxonomic evidence and interpreted together with morphological, anatomical, molecular, and other systematic data for reliable species delimitation within the genus.

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Author Contribution Statements

M.K. conceived and designed the study, performed the experimental analyses, collected and interpreted the data, conducted the literature review, and prepared the original manuscript draft. A.K. supervised the research process, contributed to the interpretation of the results, critically reviewed and edited the manuscript, and provided academic guidance throughout the study. Both authors read and approved the final version of the manuscript.

Declaration of Competing Interests

The authors declare that there are no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethical Statement

This study does not involve experiments on humans or animals. Plant materials were collected and analyzed in accordance with relevant scientific and ethical standards. The authors confirm that all procedures were conducted responsibly and ethically.

Declaration of Generative Artificial Intelligence Use

During the preparation of this manuscript, generative artificial intelligence tools were used solely to improve the language, grammar, and readability of the text. All AI-generated suggestions were carefully reviewed and edited by the authors, who take full responsibility for the final content of the manuscript.

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