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Palynomorphic Characteristics of the Heteroblastic Inflorescence, *Lamium amplexicaule* L.: A Case Study

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IN EGYPT, the genus *Lamium* L. (Family Lamiaceae) is represented by *L. amplexicaule* L., which possesses a heteroblastic inflorescence in which two breeding systems coexist. These two breeding systems are the cleistogamous system that processes self-pollinated, closed flower (CL) and the chasmogamous system with cross-pollinated, opened flower (CH). Although the pollen grains of the family Lamiaceae and *Lamium* spp. have been extensively investigated, the palynomorphological data distinguishing the pollen features for the CL and CH flowers are still lacking. This study aimed to (1) add more information about the palynomorphology of CL and CH flowers; (2) investigate the similarities and differences in palynomorphology between the pollen of these two flower types; in the same and different *L. amplexicaule* morphs. Our results showed that the heteroblastic inflorescence in *L. amplexicaule* L. in Egypt was represented by five pollen grain types. The scanning electron microscopy (SEM) results showed that all the pollen grain types were monads, tri-zonocolpate, isopolar, radially-symmetrical and reticulate, or microreticulate sculpture. The colpus length/colpus width ratios, P/E, apocolpium, and mesocolpium widths in the studied heteroblastic inflorescence were significantly different in the CL and CH flowers in the same and different morphs. The present study developed a key differentiating the five pollen grain types. In conclusion, this study confirms that the pollen characteristics were significantly different between and among the CL and CH flowers that coexist in the heteroblastic inflorescences of *L. amplexicaule;* this feature requires future clarification in different taxa.

Keywords: Cleistogamous, Chasmogamous, Heteroblastic-inflorescence, *Lamium amplexicaule*, Pollen, SEM.

Introduction

Lamiaceae (mint family) is the seventh-largest flowering plant family worldwide (Firdous et al., 2015). The Mediterranean basin has one of the highest species diversity of the family Lamiaceae. Members of this family are used as kitchen herbs, for ornamental purposes (Denisow & Bozek, 2008) and for honey production (Harley et al., 2004). Moreover, *Lamium* spp. are used to treat several ailments (Gul et al., 2021). Lamioideae is the second-largest subfamily, comprising 63 genera and approximately 1,260 species worldwide (Gul et al., 2021).

Genus *Lamium* L. (sec. *amplexicaule* Mennema; Azimishad et al., 2018) includes approximately 38 annual and perennial species. Its diversity is centered in the IranoTuranian and Mediterranean regions (Atalay et al., 2016a). *L. amplexicaule* L., was reported as invasive species whenever it was introduced and threatened the croplands and natural ecosystems (Azimishad et al., 2018). In Egyptian flora, it is a common weed in winter crops and canal bank species of the Nile River region (Boulos, 2009) and has not yet changed to an invasive form. *Lamium amplexicaule* is an annual herb; up to 30cm in height with numerous ascending stems. Leaves 1-2.5 x 1.2cm, orbicular-ovate, crenate, rounded. Petiole 1-3cm, bracts 1-2

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x 1.2-3.0cm sessile classing the stem. Inflorescence verticillate, flowers bisexual, zygomorphic. Calyx 5-7mm, tubular shorter than the tubular purple corolla (1.2-1.8cm). Stamens 4-epipetalous. Nutlet 2-2.5mm dark brown (Boulos, 2002).

Lamium amplexicaule L. possesses a heteroblastic inflorescence in which two breeding systems coexist. These two breeding systems are the cleistogamous system that contains closed, autogamous or self-pollinated flowers (CL) and the chasmogamous system with opened, allogamous or xenogamous pollinated or cross-pollinated flowers (CH), as reported earlier (Lord, 1979, 1982; Sato et al., 2013; Azimishad et al., 2018, 2019; Stojanova et al., 2020). Both the CL and CH flowers have been observed in the same individual in earlier studies (Lord, 1979; Sato et al., 2013; Woolam, 2016).

Palynological investigations are widely used in plant systematics (Atalay et al., 2016b). Palynotaxonomy is used for taxonomic and evolutionary studies of plants (Firdous et al., 2015). Analysis of pollen micromorphology helps resolve the taxonomic questions regarding Lamiaceae from the family to the species level (Gul et al., 2019b). Scanning electron microscopy (SEM) is extensively used in systematics and evolution to understand the taxonomy at the family level (Carlo & Paula, 2004; Ashfaq et al., 2018; Sufyan et al., 2018; Naz et al., 2019; Bahadur et al., 2019, 2020; Amina et al., 2020; Paul & Chowdhury, 2020).

The pollen grains of the Lamiaceae family have been thoroughly researched since the studies by Erdtman (1945, 1963). Gul et al. (2019b) and Özaltan & Koçyiğit (2022), reported that this family was divided into two subfamilies: Lamioideae (including the species with tricolpate) and Nepetoideae (with six colpi pollen). *Lamium* pollen is tri-zonocolpate, oblate-spheroidal to subprolate shape, and tiny-medium size (P= 21.65–39.96μm). Three primary exine sculpturing patterns, including reticulate, granulate, and microreticulate, have been reported (Atalay et al., 2016b), while, *L. amplexicaule* has tri-zonocolpate pollen, consistent with that reported by Abu-Asab (1991), and oblate-spheroidal pollen (Gul et al., 2019b).

Nevertheless, CL flowers have been reported in 56 Angiospermae families from 56 genera. Of these, three genera belong Lamiaceae family, namely, *Ajuga, Lamium,* and *Salvia.* A sufficient morphological or developmental description of the CL flowers is still lacking (Culley & Klooster, 2007). However, several comprehensive palynomorphological studies have been conducted for Lamiaceae and Lamioideae (Azizian & Moore, 1982; Bassett & Munro, 1986; Cantino & Sanders, 1986; Abu-Asab & Cantino, 1992; Demissew & Harley, 1992; Trudel & Morton, 1992; Abu-Asab & Cantino, 1994; Carlo & Paula, 2004; Ashfaq et al., 2018; Doaigey et al., 2018; Sufyan et al., 2018; Bahadur et al., 2019, 2020; Gul et al., 2019a, b; Naz et al., 2019; Amina et al., 2020; Paul & Chowdhury, 2020; Gul et al., 2021). The pollen grains of *Lamium* spp. have also been extensively investigated (Denisow & Bozek, 2008; Baran & Özdemdr, 2013; Kallajxhiu et al., 2014; Atasagun et al., 2015; Firdous et al., 2015; Atalay et al., 2016b). Despite these studies, comprehensive information about the pollen grains from heteroblastic inflorescences, specifically regarding the palynomorphology of the CL and CH flowers, is still lacking.

This study is the first to investigate CL and CH flowers of *L. amplexicaule* using SEM. It intended to achieve the following aims: (1) to understand further the palynomorphology of CL and CH flowers; (2) to investigate the differences and similarities in the palynomorphology of the pollen grains from the CL and CH flowers in the same and different *L. amplexicaule* morphs.

Materials and Methods

Plant sampling

Based on the flower type, the heteroblastic inflorescence (two types of flowers in the same inflorescence) of *L. amplexicaule* was grouped under three flower morphs as follows: (1) Morph 1: dimorphic cleistogamy (CL + CH), where the individual plant carried both CL and CH flowers simultaneously. (2) Morph 2: dimorphic cleistogamy with spots (CL + CHs) in which the individual plant carried CL and CH flowers simultaneously and also displayed purple spots on the petals of the CH flowers. and (3) Morph 3: cleistogamous (true cleistogamy; CL), in which the individual plant carried only CL flowers throughout its lifespan.

These three morphs included five pollen grain types. The pollen from the two flower types of Morph 1 was named Morph 1-CL and Morph 1-CH. Similarly, the pollen type from Morph

2 was Morph 2-CL and Morph 2-CH. Finally, Morph 3-CL was from Morph 3 with only CL flowers (Fig. 1).

Fresh plant materials were collected from the five flower types corresponding to the five pollen types (Table 1), during spring 2021, 5 individuals/ morphs were collected from five locations (one plant/morph/locality) to investigate and verify the pollen grains. For further investigations, parts of the fresh samples were preserved in formol-acetic-alcohol (FAA: ethyl alcohol 50mL, formaldehyde 10mL, glacial acetic acid 5mL, and distilled water 35mL; Amer et al., 2019). Meanwhile, voucher herbarium specimens were placed at Cairo University (CAI) and Beni-Suef University Herbaria.

Samples investigation

Flowers (n= 25, five plants/locality) were selected from the localities outlined in (Table 1). Before performing SEM, the specimens were examined under an OLYMPUS BX-51 light microscope (LM) to visualize the pollens (mounted using glycerol jelly) according to Pavlova et al. (2016). For each of the five flower types, 30 randomly selected pollen grains were examined using JEOL-JSM-5510 SEM microscope, and the apocolpium (A), mesocolpium (M), polar diameter (P), equatorial diameter (E), the P/E ratio, colpus width (ColpS), and colpus length (ColpL) were measured according to Atalay et al. (2016b). The pollen grains were suspended in a drop of 95% ethanol and then gold-coated. Specific SEM images were taken with a ZEISS Axiocam erc5 camera and were used to investigate the exine ornamentation, lumina features, and muri thickness (Pavlova et al., 2016). The retrieved data for the different morphological traits are outlined in Tables (2–4). The pollen terminology is based on previous studies (Walker & Doyle, 1975; Faegri & Iversen, 1975; Abu-Asab & Cantino, 1994; Punt et al., 2007).

Statistical analysis

GraphPad Prism software (version 8.0.1) was used to analyze and plot the pollen lumen and muri data, which was represented as means ± standard error. All the statistical comparisons were made using a one-way analysis of variance (ANOVA) and Tukey's post hoc test analysis, values with $P \leq 0.0001$ to 0.05 were treated as significant.

Results

This study investigated the differences between CL and CH flowers of *L. amplexicaule* by visualizing the SEM micrographs of the pollen grains. The three *L. amplexicaule* morphs studied were Morph 1 (CL+ CH), Morph 2 (CL+ CHs), and Morph 3 (CL). These morphs co-existed in time and space, as observed in the field (Fig. 1). Their flowers correspond to the five pollen grain types (Morph 1-CL and Morph 1-CH; Morph 2-CL and Morph 2-CH, and Morph 3-CL). The SEM micrographs of all five pollen grain types shared the following features: monads, tri-zonocolpate, isopolar, radially-symmetrical, and with either an oblate-spheroidal or a prolate-spheroidal shape (Fig. 2 and Table 2).

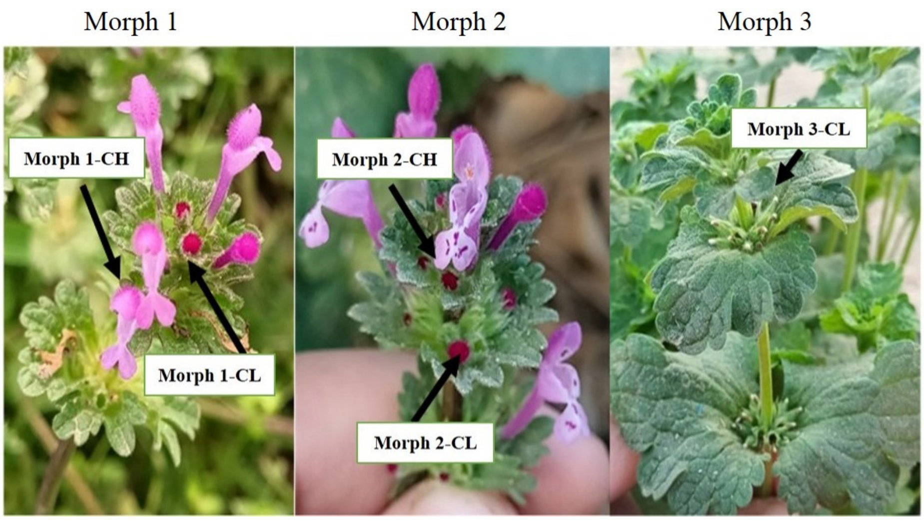

Fig. 1. The studied five flower types in the three morphs of *L. amplexicaule* (CL: cleistogamous flower and CH: chasmogamous flower)

TABLE 1. Localities for the studied *L. amplexicaule* morphs

Locality	GPS coordinates		Date of collection
	N°	E°	
Metobus-Kafr El Shiekh Governorate	31°29'75"	30°52'33"	9.3.2022
Abu Sleem, El-Menoufia Governorate	30°06'45"	31°12'54"	27.1.2022
El Saf, Giza Governorate	29°34'57"	31°15'17"	10.12.2021
El-Siliene spring, Faiyum Governorate	29°24'48"	30°51'27"	18.12.2021
Belyfa, Beniseuf Governorate	29°07'27"	31°02'56"	18.2.2022

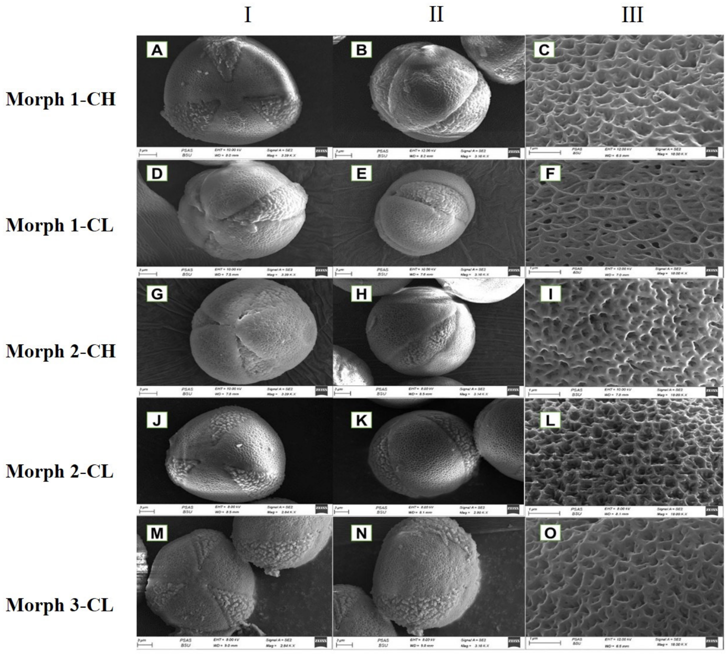

Fig. 2. Scanning electron microscope micrographs of the *L. amplexicaule* pollen grains of the five flower types (I: polar view, II: equatorial view, and III: magnified exine)

TABLE 2. Pollen classes and pollen shapes in the five flower types of *L. amplexicaule* L.

Name of specimen	Pollen class	Polar axis (µm)		Equatorial axis (µm)		P/E ratio		Pollen shape
		Mean	Range	Mean	Range	Mean	Range	
Morph 1-CH	Monad Tricolpate	24.40±0.27	(23.20 - 25.27)	24.69±0.71	(21.81- 26.60)	0.99±0.02	(0.96- 1.09)	Oblate-spheroidal
Morph 1-CL	Monad Tricolpate	20.58±0.59	(18.17 - 21.78)	19.10±0.90	(17.67 - 21.46)	1.08±0.03	(1.01- 1.22)	Prolate-spheroidal
Morph 2-CH	Monad Tricolpate	22.13±1.26	(17.95 - 25.70)	22.63±1.45	(16.85 - 26.43)	0.98±0.07	(0.93- 1.12)	Oblate-spheroidal
Morph 2-CL	Monad Tricolpate	25.65±1.04	(21.24 - 28.40)	25.14±0.98	(20.91 - 26.95)	1.03±0.03	(1.02- 1.22)	Prolate-spheroidal
Morph 3-CL	Monad Tricolpate	19.04±1.15	(18.82 - 21.20)	19.86±1.25	(17.28 - 22.37)	0.96±0.03	(0.88- 1.09)	Oblate-spheroidal

Pollen size and shape

The sizes of the five pollen grain types were grouped under "small-size pollen"; the mean value of its polar axis (P) varied from 19.04 ± 1.15µm in Morph 3-CL to 25.65 ± 1.04µm in Morph 2-CL (Table 2). Additionally, Morph 1-CL showed the smallest equatorial axis (E) length (19.1 ± 0.9µm), while the largest was for Morph 2-CL (25.14 ± 0.98

µm). Therefore, the Morph 2-CL pollen has longer P and E axes.

The five studied pollen types were oblate-spheroidal to subprolate, and their P/E ranged from 0.96 ± 0.03 in morph 3-CL to1.08 ± 0.03 in morph 1-CL (Table 2 and Fig. 3A). Three of the pollen types, i.e., Morphs 1-CH, 2-CH, and 3-CL, had oblate-spheroidal pollen shapes with the mean P/E values of 0.99 ± 0.02, 0.98 ± 0.07, and 0.96 ± 0.03, respectively. While Morphs 1-CL and 2-CL displayed prolate-spheroidal shapes with mean P/E values of 1.08 ± 0.03 and 1.03 ± 0.03, respectively (Table 2). The P/E of the three pollen types from the CL flowers of Morphs 1, 2, and 3 differed significantly while non-significant differences were observed in the pollen types of the CH flowers from Morphs 1 and 2. On the other hand, significant differences were reported in P/E between two pollen types from the same morph using the ANOVA test where the F-value was 68.4 (Fig. 3A).

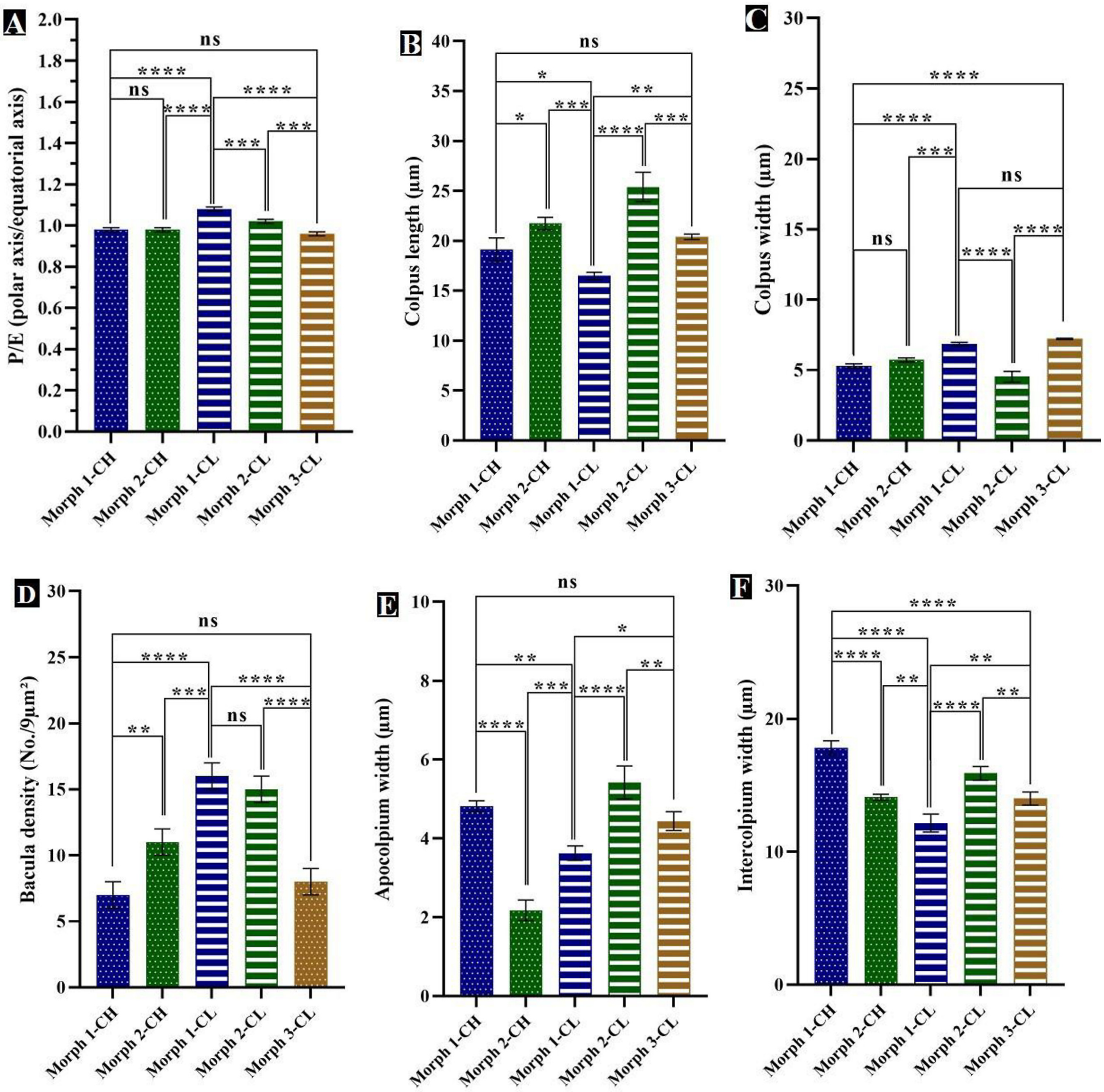

Fig. 3. Histograms of different pollen morphological characters in the five flower types of *L. amplexicaule* with significations and Ƥ value; * = (Ƥ ≤ 0.05), ** = (Ƥ ≤ 0.01), *= (Ƥ≤ 0.001) and ****= (Ƥ ≤ 0.0001) [A: Polar/equatorial ratio (P/E); B: Colpus length; C: Colpus width; D: Bacula density in colpus membrane; E: Apocolpium width and F: Intercolpium width]**

Apertures

The five studied pollen types were trizonocolpate, isopolar, with free colpi. The colpus was elongated and tapered toward the distal and proximal poles, forming acute to blunt ends. Morph 1-CL pollen had the shortest colpus (16.79 ± 0.12µm), while pollen of Morph 2-CL had the longest (25.05 ± 0.75µm). The ColpS ranged from 4.52 ± 0.22 to 7.22 ± 0.03µm in Morph 2-CL and Morph 3-CL pollen, respectively (Fig. 2 and Table 3). The ratio of the ColpL to ColpS in the pollen grains from Morphs 1-CH and 2-CH was comparable, at 3.62 ± 0.11 and 3.63 ± 0.14, respectively (Table 3). However, the pollen types of the CL flowers (Morphs 1-CL, 3-CL, and 2-CL) showed variable ratios of 2.45 ± 0.09, 2.76 ± 0.18, and 5.45 ± 0.23, respectively. The three pollen types of the CL flowers in Morphs 1, 2, and 3 showed significantly different colpus lengths and widths. Whereas the ColpS of the pollen types from the CH flowers of Morphs 1 and 2 showed a nonsignificant difference. Nevertheless, the ColpL and ColpS significantly differed between the two pollen types in the same morph (Fig. 3B and C).

The sculpture of the colpus membrane (intine sculpture) showed the presence of bacula with irregular shapes and different densities (9µm^2) in the five pollen grain types (Fig. 3D and Table 3). Four ornamentation types were observed for the colpi membranes in these pollen types; granulate with sparse bacula (8/9µm²) in Morph 3-CL, papillate with sparse bacula (7/9µm²) in Morph 1-CH, rugate granulate with dense bacula (16/9µm²) in Morph 1-CL and with moderate density (11/9µm²) in Morph 2-CH and baculate with dense bacula (15/9µm²) in Morph 2-CL. The bacula density on the colpus membrane was significantly different within the three morphs' CL and CH pollen types (Fig. 3D and Table 3). The colpus end varied from acute (Morphs 1-CL and 2-CL) to sharp acute (Morphs 2-CH and 3-CL) and acute-blunt (less sharp) in Morph 1-CH (Fig. 2).

The apocolpium (A) and mesocolpium (M), widths of the studied pollen grain types showed notable variability. The narrowest A (2.17 ± 0.15µm) and the widest A (5.41 ± 0.24µm) were measured in the pollen Morphs 2-CH and 2-CL, respectively. The sculpture of A was microreticulate, where the muri width reduced toward poles in all the pollen types (Fig. 3E and Table 3) and the widest M area (19.98 ± 0.16µm) and the narrowest (12.15 ± 0.39µm) were measured in the Morphs 3-CL and 1-CL, respectively (Fig. 3F and Table 3).

The apocolpi and mesocolpi (A and M) were significantly different in the pollen types of the CL flowers in the three Morphs 1, 2, and 3. Also, A and M of the CH flowers in Morphs 1 and 2 showed significant differences within the CL and CH pollen in the same morph (Fig. 3E and F).

TABLE 3. Features of the colpi, apocolpium and mesocolpium of the pollen grains in the five flower types of *L.amplexicaule* L., with measurements (µm) with (mean ± standard error)

Name of specimen	Colpi								Apocolpium		Intercolpium (mesocolpium) width (µm)
	State	Length (µm)	Width (µm)	L/W	End	Membrane	Bacula density	Bacula shape	Width (µm)	Texture	
Morph 1-CH	Free	19.16±0.78	5.29±0.09	3.62±0.11	Acute-blunt	Papillate	Sparse (7/9µm²)	Irregular	4.81±0.08	Micro reticulate	17.82±0.30
Morph 1-CL	Free	16.79±0.12	6.85±0.07	2.45±0.09	Acute	Rugate granulate	Dense (16/9µm²)	Irregular	3.62±0.10	Micro reticulate	12.15±0.39
Morph 2-CH	Free	20.82±0.56	5.73±0.08	3.63±0.14	Sharp acute	Rugate granulate	Moderate (11/9µm²)	Irregular	2.17±0.15	Micro reticulate	14.09±0.13
Morph 2-CL	Free	25.05±0.75	4.52±0.22	5.45±0.23	Acute	Baculate	Dense (15/9µm²)	Irregular	5.41±0.24	Micro reticulate	15.89±0.29
Morph 3-CL	Free	19.98±0.16	7.22±0.03	2.76±0.18	Sharp acute	Granulate	Sparse (8/9µm²)	Irregular	4.43±0.13	Micro reticulate	19.98±0.16

Bacula density: sparse (1-10); moderate (11-14) and dense (15-20).

Exine sculpture pattern

The SEM micrographs showed a reticulate pattern for all the studied pollen types, except Morph 2-CL, which showed a reticulate granulate pattern. The reticulate sculpturing pattern varied among the four pollen types with reticulate sculpture (Table 4). The Morph 1-CH pollen had triangular/hexagonal-shaped lumina with a foveolate membrane and muri thickness of 0.38± 0.05µm. Whereas the lumina of the Morph 1-CL pollen shape was pentagonal/hexagonal with a porate membrane and a muri thickness of 0.42 ± 0.07µm. Morph 2-CH had a triangular/tetragonal lumina with a pitted membrane and muri thickness of 0.32 ± 0.04 µm. Morph 3-CL displayed a pentagonal lumina with ridged membrane and muri thickness of 0.31 ± 0.04µm. Only, the Morph 2-CL pollen grains possessed a reticulate granulate sculpturing pattern with hexagonal lumina, and striate membrane, and a muri thickness of 0.32 ± 0.03µm (Table 4).

The mesocolpium (M) area in Morph 1-CL showed the largest lumina (1.02 ± 0.06µm), while the smallest lumina (0.38 ± 0.05µm) was found in Morph 2-CH. The number of lumina/µm² across the M area varied between 5 and 8 in Morph 1-CL and both Morph 2-CL and Morph 3-CL, respectively. Whereas the narrowest muri width was measured as 0.31 ± 0.04µm in Morph 3-CL, while the widest was 0.42 ± 0.07µm in Morph 1-CL (Fig. 4 and Table 4).

TABLE 4. Exine sculpture, muri and lumina features of the pollen grains in the five flower types of *L. amplexicaule* L.

Name of specimen	Lumina						Muri width (µm)
	Exine sculpture	Length (µm)	Width (µm)	No of lumina/ µm²	Shape	Intine ornamentation	
Morph 1-CH	Reticulate	0.55±0.03	0.48±0.03	7/1µm²	Triangular/hexagonal	Foveolate	0.38±0.05
Morph 1-CL	Reticulate	1.02±0.06	0.67±0.02	5/1µm²	Pentagonal/hexagonal	Porate	0.42±0.07
Morph 2-CH	Reticulate	0.38±0.05	0.23±0.02	6/1µm²	Triangular/tetragonal	Pitted	0.32±0.04
Morph 2-CL	Reticulate granulate	0.48±0.07	0.37±0.05	8/1µm²	Hexagonal	Striate	0.32±0.03
Morph 3-CL	Reticulate	0.43±0.05	0.30±0.04	8/1µm²	Pentagonal	Ridged	0.31±0.04

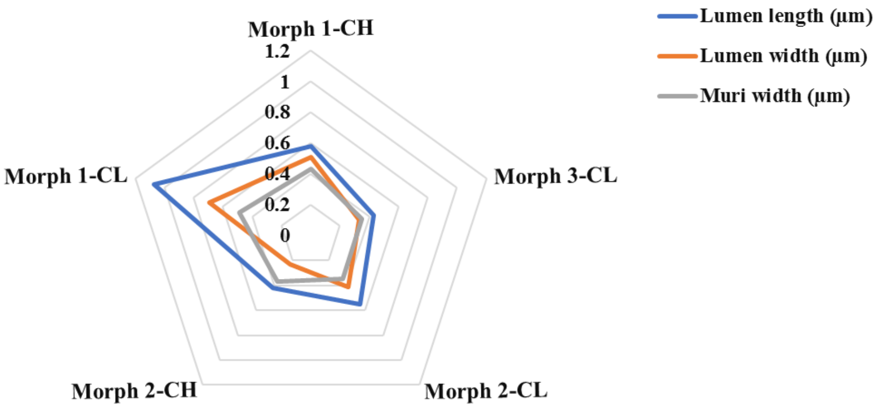

Fig. 4. Radar plot for the lumina and muri in different types of pollen grains of *L. amplexicaule* in the studied five flower types

The differential key of the five pollen grain types based on the SEM data

1a. The sculpture of exine is reticulate granulate……………………….**Morph 2-CL**

b. The sculpture of the exine is reticulate…………………………….................. **2**

2a. The lumina shape is pentagonal with ridged membrane …………...................... **Morph 3-CL**

b. The lumina shape is not so………………....**3**

3a. The lumina shape is triangular/tetragonal with a pitted membrane ……............ **Morph 2-CH**

b. The lumina shape is not so………...………**4**

4a. The lumina shape is pentagonal/hexagonal and porate membrane ….............. **Morph 1-CL**

b. The lumina shape is triangular/hexagonal and foveolate membrane……...............**Morph 1-CH**

Discussion

Lamium amplexicaule is one of the three genera in the family Lamiaceae with heteroblastic inflorescence (Culley & Klooster, 2007). To date, there is a lack of palynomorphic data about heteroblastic inflorescence. This species possesses both CL and CH flower types, as shown in previous studies investigating the pollen of these flowers (Denisow & Bozek, 2008; Baran & Özdemdr, 2013; Kallajxhiu et al., 2014; Atasagun et al., 2015; Firdous et al., 2015; Atalay et al., 2016b; Gul et al., 2019b, 2021). However, these studies did not mention the flower type. The analyzed palynomorphic traits from the five pollen grain types (Morphs 1-CL and 1-CH, Morphs 2-CL and 2-CH, and Morph 3-CL), including the exine sculptures, apocolpus (A), mesocolpus (M), polar axis (P), equatorial axis (E), P/E, ColpS, ColpL, lumina width, muri thickness showed substantial diversity among and between the morphs. Hence, our examination of the pollen grains from the CL and CH flowers from the heteroblastic inflorescence of three *L. amplexicaule* morphs represents a pioneer model.

The general features of the five pollen types of *L. amplexicaule* morphs included tri-zonocolpate, isopolar, radially-symmetrical, and reticulate sculpture, which is consistent with previous studies for this species (Abu-Asab, 1991; Firdous et al., 2015; Atalay et al., 2016b; Gul et al., 2021) and Lamiaceae (Ashfaq et al., 2018; Doaigey et al., 2018; Sufyan et al., 2018; Bahadur et al., 2019, 2020; Naz et al., 2019; Amina et al., 2020; Paul & Chowdhury, 2020). The detected pollen shape was either oblate-prolate-spheroidal, as reported by Gul et al. (2021), or subprolate congruent, as shown by Firdous et al. (2015).

The size of the five pollen types varied considerably which is consistent with Firdous et al. (2015). However, the prior studies did not recognize the CL and CH flower types. The exine sculpture in all pollen types (Fig. 2) showed a reticulate pattern except that of the Morph 2-CL, which showed a reticulate granulate pattern. The reticulate sculpture was reported only by Gul et al. (2021), while Firdous et al. (2015) mentioned the microreticulate exine sculpture. Atalay et al. (2016b) reported the presence of three different pollen sculpture patterns in the subgenus *Lamium.* Gul et al. (2021) reported that the A region was microreticulate, consistent with our observations of the five pollen types. These pollen types showed variability in lumina shape, membrane sculpture, and muri thickness, which has been reported earlier (Atalay et al., 2016b). Although the study distinguished the species' exine pattern into subtypes, it did not mention flower type (CL and/or CH). The detected infraspecific difference in pollen sculpture confirms the taxonomic significance of pollen sculpture, which is supported by Doaigey et al. (2018).

The colpus length/width ratio of the five pollen types varied from 2.45 ± 0.09 and 5.45 ± 0.23 indicating that the length is approximately two times the width. This is consistent with the study by Firdous et al. (2015) concerning acute or blunt colpus ends. Significant differences were detected for P/E and the colpus length/width ratio between the CL flowers in Morphs 1, 2, and 3 and between the pollen types of the same morph. The CH flowers of the Morphs 1 and 2 showed non-significant differences.

The colpi membrane in the five pollen types showed four types of ornamentation (papillate, rugate-granulate, baculite, and granulate). This disagreed with Gul et al. (2021), who reported that the colpus membrane was densely verrucate to gemmate. The detected variability in pollen micromorphology of *L. amplexicaule* was predicted by Gul et al. (2019b), who reported the presence of pollen variability in Lamioideae taxa at various levels, including species and up to the generic level.

Conclusion

This study pioneers in recommending the importance of the flower type identity (CL & CH flowers) during the pollen investigation, especially in species that possess heteroblastic inflorescences. The investigation elucidated the heteroblastic inflorescences in *L. amplexicaule* and revealed significant differences in most pollen traits between and among the CL and CH flowers that coexist in the same individual in space and time. Moreover, some pollen traits varied in the CL and CH flowers from the same morph/ individual. This work also highlights the need for further studies clarifying the palynomorphological features of the CL and CH flowers in different taxa.

Conflict of interest: The authors declare no conflict of interest.

Authors contributions': Maha H. Khalaf collected the plant specimens; carried out the practical work, prepared the draft manuscript and wrote the manuscript. Wafaa Amer proposed the paper idea, supervised the practical work, and refined the manuscript. And Mahmoud O. Hassan supervised the field work and draft preparation.

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صفات حبوب اللقاح في النورات غير المتجانسة: حالة دراسية على نبات فم السمكة

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جنس اللاميم (الفصيلة الشفوية) يُمثل في مصر بنوع واحد هو اللاميم الأبيض أو فم السمكة، وهذا النبات يتميز بوجود نورات عديدة الأشكال وهي التي تحمل في نفس الوقت نوعين من الأزهار بنظامين في التلقيح على النبات. هذان النظامان للتلقيح هما: نظام الأزهار المغلقة ذاتية التلقيح (CL)، والزهرة المفتوحة ذات التلقيح الخلطي (CH). وعلى الرغم من أن حبوب اللقاح من الفصيلة الشفوية وتحت الفصيلة القراصية قد تم دراستها على نطاق واسع، إلا إن حبوب اللقاح الموجوده في كل نوع من الأزهار المغلقة والمفتوحة (CL & CH) مازالت غائبة. وتهدف هذه الدراسة إلى (1) إضافة المزيد من المعلومات حول أشكال حبوب اللقاح في الأزهار المغلقة والمفتوحة. (2) التحقيق في أوجه التشابه والاختلاف في أشكال حبوب اللقاح في الأزهار المغلقة والمفتوحة؛ في نفس الصنف من اللاميم وفي الأصناف المختلفة. وقد أظهرت النتائج أن الإزهار المفتوحة والمغلقة بها عدم تجانس شديد بين أنواع الحبوب اللقاح في الأزهار المفتوحة والمغلقة في نفس الصنف وبين الأصناف المدروسة وبعضها. وفي هذه الدراسة تم تقسيم اللاميم الي خمسة أنواع من حبوب اللقاح. وقد أظهرت نتائج الفحص بالمجهر الإلكتروني الماسح (SEM) أن جميع أنواع حبوب اللقاح كانت أحادية، ثلاثية الشقوق، متساوية القطب، متناظرة شعاعيًا، شبكية الإكذين. كما أظهرت النتائج أن نسب طول/ عرض الحبوب (P / E)، المنطقة القطبية بين الشقوق، وعرض وكذلك منطقة الإستواء بين الشقوق في حبوب اللقاح للأزهار الخمسة غير المتجانسة ومختلفة بشكل كبير بين أزهار CL وCH في الصنف الواحد والأصناف المختلفة. كما تم عمل مفتاحًا يميز بين أنواع حبوب اللقاح الخمسة. في الختام، تؤكد هذه الدراسة أن خصائص حبوب اللقاح كانت مختلفة بشكل كبير بين أزهار CL وCH التي توجد في نورات اللاميم وتتطلب هذه الخاصية توضيحًا مستقبليًا في انواع اخرى من النباتات.