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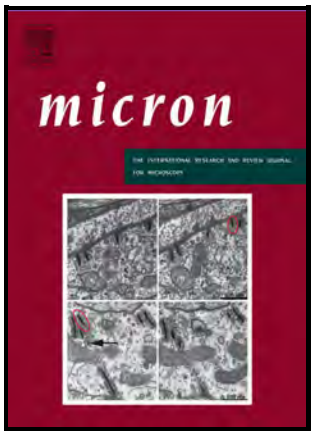


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Micrometer Insights into *Nepeta* Genus: Pollen Micromorphology Unveiled

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Micrometer Insights into *Nepeta* Genus: Pollen Micromorphology Unveiled

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Abstract

This study provides a comprehensive pollen micromorphology within the *Nepeta* genus, revealing intricate details about the pollen grains' structure and characteristics. The findings shed light on the evolutionary and taxonomical aspects of this plant genus, offering valuable insights for botanists and researchers studying *Nepeta* species. The pollen grains of 18 Nepeta species were studied using scanning electron microscopy (SEM) and light microscopy (LM) in

Northern Pakistan. At the microscale, pollen quantitative measurements, qualitative traits, and diverse sculpturing patterns were reported and compared. Significant differences in pollen size, shape, ornamentation, and sculpturing patterns were discovered among the Nepeta species. Our data show that exine sculpturing is quite diverse, with most species exhibiting a reticulate perforate pollen pattern. *Nepeta connata, Nepeta discolor, Nepeta elliptica,* revealed a distinct bireticulate perforate exine stratification. Hexazonocolpate pollen is the most common. Furthermore, the surface membrane attributes of the colpus varied greatly, ranging from rough, scabrate, psilate, to sinuate patterns. Principal Component Analysis (PCA) was used to discover the key factors influencing pollen diversity. PCA results showed that polar and equatorial diameters, colpi size, and exine thickness were the most influential pollen features between *Nepeta* species. This study adds to our understanding of pollen morphology in the *Nepeta* genus, offering information on the vast range of characteristics found in this economically important group. The extensive characterization of pollen features provides useful insights for the categorization and differentiation of *Nepeta* species, adding to the Lamiaceae micromorphology.

Keywords:

Hexacolpate, Lamiaceae, Micromorphology, Palynology, SEM, Taxonomy

1. Introduction

The pollen of the Lamiaceae family has been thoroughly investigated, leading to suggest splitting it into two subfamilies based on matured pollen grain features such as the number of nuclei and apertures (Erdtman, 1945). The pollen of the first subfamily, Lamioideae, has three apertures and two nuclei, while the pollen of the second subfamily, Nepetoideae, has six apertures and three nuclei. The subfamily Nepetoideae is the largest, with over 3400 species divided into three tribes: Ocimeae, Mentheae, and Elsholtzieae. The Mentheae tribe is the most important and economically important among the Nepetoideae. It is divided into three subtribes: the Nepetinae, the Salviinae, and the Menthinae. Mentheae consist of approximately 65 genera and 2300 species (Harley et al., 2004). Nepetinae is a subtribe of Mentheae that includes 12 genera and around 350 species found in Eurasia and North America. The differences in the length of the posterior pair of stamens and the number of calyx nerves

distinguish Nepetinae (Wagstaff and Van Doorslaer, 1992). Despite the fact that Nepetinae is a member of the Mentheae family, nothing is known about its pollen features. To fill this knowledge gap, we undertook an in-depth investigation of pollen from the *Nepeta* genus within the Mentheae tribe.

Nepeta is a member of the tribe Mentheae and the subfamily Nepetoideae (Cantino, 1992). *Nepeta* L. is a prominent genus within the Nepetoideae family, having roughly 300 species of high commercial worth. The *Nepeta* species are found primarily in North Africa, Eurasia, the Canary Islands, and North and Central America. Southwest Asia and the western Himalayas, particularly around the Hindu Kush, have the greatest diversity and number of *Nepeta* species (Hedge, 1986; Jamzad et al., 2000; Pojarkova, 1954). *Nepeta* species have gained importance in traditional medicine due to their various beneficial properties such as antitussive, antispasmodic, diuretic, expectorant, antiseptic, and anti-asthmatic effects. They have been extensively used for medicinal purposes. One of the well-known species, catnip (*Nepeta cataria*), has a long history of being used as tea in Europe, preceding the arrival of tea from the Asia. Furthermore, compounds obtained from the plant's blossoming tips have been used for their soothing effects (Baser and Kirimer, 2006; Newall et al., 1996). *Nepeta* species also contribute to bee population support since numerous species provide nectar and pollen to bees. The *Nepeta* genus can be complex due to a mix of influencing factors such as frequent hybridization and introgression, as well as variations in age and habitat variation (Sammataro and Avitabile, 1998).

According to (Boissier, 1875), (Shishkin and Borisova, 1954), (Rechinger, 1982), (Hedge et al., 1982) and (Dirmenci, 2003) *Nepeta* have different infrageneric classification. These studies largely focus on plant identification based on growth patterns, leaf shapes, flower arrangements, and the features of their little seed-like aspects (Bentham, 1848; Briquet, 1897; Budantsev, 1993; Budantsev and Lobova, 1997; Pojarkova, 1954). In the work of (Jamzad et al., 2003) an analysis of molecular data indicated that *Nepeta* forms a monophyletic group. (Jamzad et al., 2003) proposed reviewing *Nepeta* present classification below the genus level. (Ubera, 1982) examined the pollen microstructural traits of 20 *Nepeta* species in the Iberian Peninsula. In Iran (Jamzad et al., 2000; Jamzad et al., 2003) studied the pollen structure of selected *Nepeta* species. (Perveen and Qaiser, 2004) described the morpho-palynology of *Nepeta* species from Pakistan. (Moon et al., 2008) analyzed the palyno-morphological features of 21 *Nepeta* species.

Studying the micromorphology of pollen within the *Nepeta* genus is essential for several reasons. First, it plays a crucial role in plant reproduction and is intimately connected to pollination mechanisms, which influence the genetic diversity and adaptation of these plants. Understanding the intricate plants microscale details can provide insights into their adaptability and ecological interactions (Majeed et al., 2022b). Additionally, pollen micromorphology serves as a valuable tool for taxonomic classification and species differentiation, aiding in the accurate identification and categorization of *Nepeta* species. Furthermore, palynological insights can have broader implications for the conservation of these plants and their role in various ecosystems, making it an important study for both botanical research and environmental preservation (Majeed et al., 2022a)

The primary aims of this study are to investigate and document the micromorphological characteristics of pollen grains across multiple *Nepeta* species. Through this analysis, we aim to elucidate the palynological relationships within the *Nepeta* genus and enhance the taxonomic classification at micro level using scanning electron microscopy. Additionally, the study seeks to contribute valuable data for better understanding pollination mechanisms and the ecological roles of *Nepeta* species in their respective habitats.

2. Materials and Methods

2.1. Collection, Identification and Preservation

A total of 18 *Nepeta* species were collected during various field surveys throughout Northern Pakistan during July 2022 to February 2023, including Lower Dir, Swat, Quetta, Kashmir, Murree, Hazara, Ayyubia National Park, Chitral, and Gilgit (Figure 1 & Table.1). About five to ten specimens of each species were collected, and the identification of species was assisted by examining ISL Herbarium specimens and published literature from the Flora of Pakistan (https://www.tropicos.org/project/Pakistan). The botanical names were further confirmed and validated through World Flora Online catalogue (https://www.worldfloraonline.org/). The *Nepeta* specimens were pressed, dried, and maintained according to herbarium procedures. They were then mounted on herbarium sheets with voucher accession numbers and submitted to the ISL Herbarium.

2.2 Light Microscopic Palynological Study

The floral part containing a single anther, a few anthers, or an entire flower was taken apart and placed in tiny sample bags for palynological examination. For light microscopic slide preparations, a single to a few anthers from each sample were placed in drops of acetic acid on microscopic glass slide. Following the procedure of (Erdtman, 1986) acetolyzed materials on slides were crushed for a short time to soften the anthers, release the pollen grains and remove any remaining organic material from the pollen grain surfaces. The pollen grains were then stained with glycerin jelly in order to improve their visibility. The pollen grains were measured using an eyepiece (ocular; 10X). The measurements taken were then multiplied by 2.5 to convert them into micrometers (µm). To find the mean value and standard error, 20–25 grains were measured from each prepared slide.

2.3 Scanning microscopic (SEM) analysis of *Nepeta* pollen

Nepeta species pollen grains were prepared according to protocol for scanning electron microscopy (Butt et al., 2018). The procedure required suspending the pollen in a 90% ethanol acetolyzed solution, and then affixing it to metal stubs. The pollen was subsequently coated with a thin layer of gold palladium particles to improve its appearance under SEM (Zafar et al., 2022). The examination of pollen through SEM used descriptive pollen terminology (Halbritter et al., 2018). Regarding the categorization of pollen shapes (P/E), the definitions of (Nilsson and Praglowski, 1992), were adopted. The pollen grains size was measured according to (Erdtman, 1971), classifying sizes as follows: "very small" (< 10 µm), "small" (10–25 µm), "medium" (25–50 µm), "large" (50–100 µm), "very large" (100–200 µm), and "huge" (> 200 µm).

2.4 Statistical analysis

Statistical software (SPSS 16.00) was used to examine the data and determine the maximum, minimum, mean, and standard error. For the statistical analysis of mean (minimum-maximum) SE given in (Table 2), approximately 15 to 20 measurements of each parameter were taken. The principal component analysis is a statistical method for determining similarities and differences between a group of species (Rozina et al., 2022). The variance between seven variables for the PCA, equatorial diameter, polar diameter, P/E ratio, exine thickness, colpi length and breadth, and mesocolpium of the analyzed species, was evaluated using the statistical program XLSTAT version 2018, and cluster analysis was performed using Past software version 4.13.

Fertility and sterility were analyzed employing the subsequent formula.

2.4.1 $\boldsymbol{Fertility = F/F + S \times 100}$

F indicates the number of fertile pollen on the ocular, and S indicates the number of sterile pollen on the same ocular.

2.4.2 $\boldsymbol{Sterility = S/S + F \times 100}$

S indicates the number of sterile pollen on the ocular, and F indicates the number of fertile pollen on the same ocular.

2.4.3 ***P/E ratio***

The P/E ratio for each species was calculated by considering the polar-to-equatorial diameter of the corresponding pollen, using the formula (Butt et al., 2018):

$$\boldsymbol{P/E = PD / ED \times 100}$$

In this equation, PD stands for the pollen's polar diameter and ED stands for equatorial diameter.

3. Results and discussion

The scanning electron microscope (SEM) and light microscope (LM) were used to study a total of 18 *Nepeta* species. Plates 1-9 show light and scanning electron micrographs. Micromorphological variations were observed within pollens of the genus *Nepeta*, such as pollen size and shape, pollen outline and colpi apex. Symmetry, polarity, aperture orientation, lumina shape, number of secondary lumina per primary lumen, aperture sculpturing, and exine sculpturing are all important considerations. Tables 1 and 2 show quantitative and qualitative pollen measurements and characteristics.

[Plate 1-9]

[Table 1 & 2]

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Pollen grains are mostly 6-colpate or 6-zonocolpate, and pollen grains are frequently isopolar. The largest polar diameter was found in *Nepeta distans* (83.7-88.0=86.5±.75 µm), and smallest in *N. praetervisa* (19.5-21.2=20.3±.34 µm). The largest equatorial diameter was found in *N. distans* (55.7-76.2=66.0±3.6 µm), and smallest in *N. griffithii* (13.0-18.7=15.3±1.2 µm) **(**Figure 2). Six types of pollen shapes were observed in the present research i.e., oblate-spheroidal, sub-oblate, prolate, spheroidal, per-prolate and sub-prolate. The highest P/E ratio was found in *N. linearis (*2.05%), and lowest in *N. elliptica* (0.72%) **(**Figure 3). Pollen shapes in polar and equatorial view are spherical and sub-spheroidal except in *N. govaniana* in which equatorial view is irregular *(*Plate 4A, B), in *N. laevigata* the polar view is broad shape and equatorial view is rhomboid (Plate 7D, E), and in *Nepeta linearis* the polar view is hexagonal and equatorial view is ellipsoid (Plate 8A, B).

[Figure 2]

[Figure 3]

The aperture sculpturing in the current study was mostly granulated in 13 species, followed by verrucate in 5 species. Length of colpi ranges from (32.5-36.2=34.1±.700 µm), highest in *N. distans* to smallest (8.7-9.7=9.2±.17 µm), in *N. nervosa* and *Nepeta praetervisa*. Width of colpi ranges from (8.0-13.7=10.7±1.1 µm), highest in *N. distans* to smallest (4.5-5.5=5.0±.176 µm), in *N. graciliflora* (Figure 4). Mesocolpium distance ranges (31.2-34.2=32.8±.53 µm), maximum in *N. distans* and minimum (8.0-9.0=8.5±.17 µm), in *N. praetervisa.* SEM observation revealed three types of exine stratification i.e., reticulate perforate, bi-reticulate perforate and only reticulate in three species. Exine thickness varies from (6.2-8.0=7.1±.32 µm), maximum in *N. distans* to (2.2-3.0=2.7±.13 µm), minimum in *Nepeta raphanorhiza* (Table 2).

3.1 *Nepeta cataria*

Pollen is found as monad and are mostly hexacolpate, isopolar. Pollen size is medium, pollen shape oblate spheroidal. The shape of pollen in polar and equatorial view is sub-spherical and spherical respectively. The pollen outline is rough, pollen apex acute. Aperture ornamentation and aperture sculpturing is concave and verrucate respectively. Lumina shape is elongated, number of secondary lumina per primary lumen is not clear and exine sculpturing is reticulate perforate not coincide with the results of (Celenk et al., 2008). Lumina shape and

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exine sculpturing of the present study shows inconsistent with (Talebi et al., 2020). According to (Perveen and Qaiser, 2004) the exine sculpturing is coarsely reticulate and the pollen shape of *N. cataria is* prolate-spheroidal which is different from our findings (Plate. 4C). According to (Moon et al., 2008) the pollen shape and exine sculpturing of *Nepeta fissa* show similarity with our results. According to (KILIC, 2013) the pollen shape of *N. cateria* is sub prolate with micro reticulate exine sculpturing not consistent with our study.

3.2 *Nepeta connata*

Type of pollen hexazonocolpate and are isopolar. Pollen size is medium, pollen shape sub oblate. The shape of pollen in polar and equatorial view is spherical. Pollen outline is scabrate, pollen apex acute (Plate. 4D-F). Aperture ornamentation and aperture sculpturing is concave and granulate respectively. Lumina shape and number of secondary lumina per primary lumen is not clear and exine sculpturing is bireticulate perforate not agreed with (Perveen and Qaiser, 2004).

3.3 *Nepeta discolor*

Pollen size is small- medium, pollen shape prolate. The shape of pollen in polar and equatorial view is spherical. The pollen outline is slightly rough, pollen apex round (Plate. 4G-I). Pollen is radially symmetrical and isopolar. Type of aperture and ornamentation is hexazonocolpate and slightly convex. Lumina shape is elongated (Azizian et al., 2001). The number of secondary lumina per primary lumen is 10-20. Aperture sculpturing is granulated and exine sculpturing is bireticulate perforate. Previous studies by (Moon et al., 2008) the shape of pollen, exine sculpturing, and lumine number of *N. eremophila* and *N. pungens* coincides with our results but disagreed with the shape of lumine.

3.4 *Nepeta distans*

Pollen size is large, pollen shape prolate. According to (Moon et al., 2008) the size of *N. longibracteata* and *N. sibirica* is large in pollen shape similar to our research but different in size. The shape of pollen in polar and equatorial view is sub-spherical and spherical respectively. The pollen outline is rough, pollen apex acute. Pollen is radially symmetrical and isopolar. The type of aperture and ornamentation is hexacolpate and concave. Lumina shape is circular-oval and number of secondary lumina per primary lumen is 10-13 (Plate. 5A-C).

Aperture sculpturing is gemmate and exine sculptural elements are reticulate perforate not coincides with the results of (Perveen and Qaiser, 2004) in pollen shape and exine sculpturing. Yet they neglected the remaining characteristics for intense, lumen shape, aperture sculpturing etc.

3.5 *Nepeta elliptica*

Pollen is found as monad. Pollen size is medium, pollen shape sub oblate. The shape of pollen in polar and equatorial view is spherical. The pollen outline is rough, pollen apex acute. Pollen is radially symmetrical and isopolar. The type of aperture and ornamentation is hexacolpate and convex. Lumina shape is Irregular and number of secondary lumina per primary lumen is 10-14 (Plate. 5E-F). Aperture sculpturing is gemmate and exine sculpturing is bi-reticulate perforate. The present study showed similarity with Celenk et al., (2008) in pollen and lumina shape of *N. stenantha.* According to (KILIÇ, 2014) the shape of *N. fissa* is sub oblate similar to our studies.

3.6 *Nepeta erecta*

Pollen is found as monad. Pollen size is medium, pollen shape spherical. The shape of pollen in polar and equatorial view is spherical. The pollen outline is rough, pollen apex acute. Pollen is radially symmetrical and isopolar. The type of aperture and ornamentation is hexacolpate and slightly convex. Lumina shape is circular and lumina number per reticula is not clear (Plate. 5H-I). Aperture sculpturing is gemmate and exine peculiarities are reticulate perforate. According to (Perveen and Qaiser, 2004) pollen shape and exine ornamentation of *N. erect* is sub spheroidal and coarsely reticulate not agreed with our results.

3.7 *Nepeta govaniana*

Monad, pollen size is medium, pollen shape oblate-spheroidal similar to (ÖZALTAN and KOÇYİĞİT, 2022). The shape of pollen in polar and equatorial view is sub-spherical and irregular respectively. The pollen outline is sinuate, pollen apex acute. Pollen is radially symmetrical and isopolar. The type of aperture and ornamentation is hexacolpate and Sunken. Lumina shape is Oval and lumina number per reticula is 12-17 (Plate. 6A-C). Aperture sculpturing is verrucate and exine sculpturing is reticulate perforate-verrucate. According to

(Perveen and Qaiser, 2004) the pollen shape and exine ornamentation of *N. govaniana* is prolate spheroidal and rough regulate not coincides with our results.

3.8 *Nepeta graciliflora*

Pollen size is medium, pollen shape sub-oblate. The shape of pollen in polar and equatorial view is spherical. The pollen outline is rough, pollen apex acute. Pollen is radially symmetrical and isopolar. The type of aperture and ornamentation is hexacolpate and convex. Lumina shape is irregular similar with (Jamzad et al., 2003) in *N. gloeocephala* and *N. Ispahani*. Lumina number per reticula is 6-9 (Plate. 6D-F). Aperture sculpturing is granulated and exine sculpturing is reticulate perforate.

3.9 *Nepeta griffithii*

Pollen is found as monade. Pollen size are *small to medium*, pollen shape prolate. The shape of pollen in polar and equatorial view is spherical. The pollen outline is rough, pollen apex acute. Pollen is radially symmetrical and isopolar. The type of aperture and ornamentation is hexacolpate and slightly convex. Lumina shape is circular-oval and number of secondary lumina per primary lumen is 5-9 (Plate. 6H-I). Aperture sculpturing is granulated and exine ornamentation is reticulate perforate-sparsely gemmate. According to (Perveen and Qaiser, 2004) the pollen shape and exine ornamentation of *N. griffithii* is prolate spheroidal and coarsely reticulate not agreed with our results.

3.10 *Nepeta hindostana*

Pollen is found as monad. Pollen size is small, pollen shape prolate. The shape of pollen in polar and equatorial view is oblong and widely oval respectively. The pollen outline is psilate, pollen apex acute. Pollen symmetry was found to be bilateral and heteropolar. The polar region was observed as small and smooth. The type of aperture and ornamentation is hexacolpate and sunken. Lumina shape is irregular number of secondry lumina per primay lumen is 6-10 (Plate. 7A-C). Aperture sculpturing is Verrucate and exine sculpturing is bi-reticulate perforate. According to (Perveen and Qaiser, 2004) the pollen shape and exine ornamentation of *N. hindostana* is prolate spheroidal and coarsely reticulate not agreed with our results. Earlier studies of (Dong et al., 2015) investigate the pollen shape and exine surface

of *Nepeta wuana* i.e., prolate-spheroidal and bi-reticulate not consistent to our observed specie *Nepeta hindostana.*

3.11 *Nepeta laevigata*

Pollen is found as monad. Pollen size is small- medium, pollen shape prolate. The shape of pollen in polar and equatorial view is broad and Rhomboid respectively. The pollen outline is psilate, pollen apex acute. Pollen symmetry was found to be bilateral and heteropolar. The polar region was observed as medium and smooth. The type of aperture and ornamentation is hexazonocolpate and bulged. Lumina shape and number of secondary lumina per primary lumen is absent (Plate. 7D-F). Aperture sculpturing is granulated and exine sculpturing is reticulate. Previous studies by (Perveen and Qaiser, 2004) observed pollen shape and exine ornamentation of *N. laevigata* was prolate-spheroidal and coarsely reticulate not agreed with our results. Earlier studies of (Kılıç, 2013) the pollen shape *of N. baytopii* is spheroidal with reticulate exine sculpturing similar to our studied specie *Nepeta laevigata* in exine sculpturing but dissimilar in pollen shape. According to (Sultan et al., 2021) the shape of *N. sulaimanica* is sub prolate not consistent with the shape of *N. laevigata*.

3.12 *Nepeta leucolaena*

Monad, pollen size medium, shape spherical. The shape of pollen in polar and equatorial view was spherical and sub-spherical. The pollen outline is scabrate, pollen apex acute. Pollen symmetry was found to be radial and isopolar. The type of aperture and ornamentation is hexazonocolpate and slightly raised. Lumina shape circular and number of secondary lumina per primary lumen is 10-15 (Plate. 7G-I). Aperture sculpturing is granulated and exine sculpturing is reticulate perforate. Previous studies of (Perveen and Qaiser, 2004) the observed pollen shape and exine ornamentation of *N. leucolaena* was prolate-spheroidal and coarsely reticulate not agreed with our results. Our research explores extra characteristics that may serve to differentiate between these closely linked taxa.

3.13 *Nepeta linearis*

Monad, pollen size small-medium, shape per-prolate. The shape of pollen in polar and equatorial view is Hexagonal and Ellipsoid (Plate. 8A-C). The pollen outline is scabrate, pollen apex acute. Pollen symmetry was found to be radial and isopolar. The type of aperture and

ornamentation is hexazonocolpate and Sunken respectively. Lumina shape Irregular and number of secondary lumina per primary lumen was not clear. Aperture sculpturing is granulated and exine stratification is fine reticulate perforate. Previous studies by (Perveen and Qaiser, 2004) the observed pollen shape was prolate-spheroidal and ornamentation of *N. linearis* was coarsely reticulate not agreed with our results.

3.14 *Nepeta nervosa*

Monad, pollen size small-medium, shape sub-prolate. The shape of pollen in polar and equatorial view is spherical. The pollen outline is rough, pollen apex acute. Pollen symmetry was found to be radial and isopolar. The type of aperture and ornamentation is hexazonocolpate and sunken respectively. Lumina shape elongated and number of secondary lumina per primary lumen is 14-19 (Plate. 8D-F). Aperture sculpturing is granulated and exine sculpturing is reticulate perforate. Earlier studies of (Perveen and Qaiser, 2004) observed pollen shape and exine ornamentation of *N. nervosa* i.e., prolate spheroidal and coarsely reticulate not agreed with our results.

3.15 *Nepeta podostachys*

Monad, pollen size medium, shape sub prolate. The shape of pollen in polar and equatorial view is spherical. The pollen outline is rough, pollen apex acute. Pollen symmetry was found to be radial and isopolar. The type of aperture and ornamentation is hexazonocolpate and bluged respectively (Plate. 8G-H). Lumina shape irregular and number of secondry lumina per primay lumen is 15-18Aperture sculpturing is verrucate and exine sculpturing is reticulate perforate. Earlier studies of (Perveen and Qaiser, 2004) investigate the pollen shape and exine ornamentation of *N. podostachys* oblate-spheroidal and coarsely reticulate not agreed with our results. According to (Jamzad et al., 2003) the exine surface of *N. gloeocephala* and *N. ispahanica* are reticulate perforate with irregular lumina shape similar to our work.

3.16 *Nepeta praetervisa*

Monad, pollen size small, shape sub-prolate. The shape of pollen in polar and equatorial view is spherical. The pollen outline is rough, pollen apex acute. Pollen symmetry was found to be radial and isopolar. The type of aperture and ornamentation is hexazonocolpate and sunken respectively. Lumina shape circular-elongated and number of secondary lumina per

primary lumen is 4-9 (Plate. 9 A-C). Aperture sculpturing is granulated and exine sculpturing is reticulate-perforate. According to (Sultan et al., 2021) studied the pollen shape *of N. sulaimanica* is subprolate similar to the shape of our observed *N. praetervisa.*

3.17 *Nepeta raphanorhiza*

Monad, pollen size medium, shape prolate. The shape of pollen in polar and equatorial view is hexagonal-spherical and spherical respectively (Plate. 9D-F). The pollen outline is scabrate, pollen apex acute. Pollen symmetry was found to be radial and isopolar. The type of aperture and ornamentation is hexazonocolpate and raised respectively. Lumina shape circular-oval and number of secondary lumina per primary lumen was not clear. Aperture sculpturing is granulated and exine sculpturing is reticulate- perforate/foveolate. Earlier studies of (Perveen and Qaiser, 2004) researched the pollen shape and exine ornamentation of *N. raphanorhiza* was prolate-spheroidal and coarsely reticulate not agreed with our results. According to (Myoung and Yuon, 2012) observed the pollen size and shape of *N. nuda* is medium and prolate with bi-reticulate exine surface similar to *Nepeta raphanorhiza* in size and shape but dissimilar in exine surface.

3.18 *Nepeta schmidii*

Monad, pollen size small-medium, shape prolate. The shape of pollen in polar and equatorial view is sub-spheroidal and spherical respectively. The pollen outline is rough, pollen apex acute. Pollen symmetry was found to be radial and isopolar. The type of aperture and ornamentation is hexazonocolpate and sunken respectively. Lumina shape and number of secondary lumina per primary lumen not clear (Plate. 9G-I). Aperture sculpturing is verrucate and exine sculpturing is reticulate. Previous studies of (Al-Watban et al., 2015) observed prolate pollen shape and reticulate perforate exine sculpturing of *N. deflersiana* coincides with our results in pollen shape but not in exine sculpturing.

3.19 Pollen Fertility and Sterility

Pollen fertility plays an important role in determining the diversity of plants and their distribution in various ecosystems. Their stability is confirmed by the highest and lowest fertility proportions. The highest pollen fertility was found in *N. laevigata* (90.9%) and lowest

in *N. hindostana* (64.2%) while, the highest sterility was observed in *N. hindostana* (35.8%) and lowest in *N. laevigata* (9.7%).

3.20 Principal Component Analysis (PCA) and Dendrogram Clustering

The similarity index among the studied *Nepeta* species were analyzed using UPGMA cluster analysis. The pollen quantitative data divides the dendrogram into three major clusters (Figure 5). The first cluster consists of *N. distans* which is entirely varied from the other studied species of the genus in terms of quantitative data. The second cluster is divided into four sub-clusters i.e., sub-cluster1, sub-cluster 2, sub-cluster 3 and sub-cluster 4. The sub-cluster 4 is again divided into two cluster, in which *N. laevigata* and *N. schmidii* were the most similar as compared to the other studied species. The third cluster is divided into one sub-cluster which is again divided into two clusters. The third subdivided cluster showed similarity between *N. connata* and *N. graciliflora* but less similar than *N. laevigata* and *N. schmidii*.

One of the most significant statistical tests for analyzing a set of pollen traits are principal component analysis (PCA), which aims to represent the variation that exists among *Nepeta* species. The observed data is represented in two or dimensional projection with axis components (Ashfaq et al., 2019). Active variables of pollen of the principal component analysis biplot was presented (Figure 6). In the present study, the pollen size, (polar diameter, equatorial diameter), colpi size, (colpi length, colpi width), exine thickness and mesocolpium distance among 18 *Nepeta* species were used to explore pollen variability. PCA variable loadings for first seven components were illustrated in Table 4. Total accumulative variance of (85.05 %) was presented (Figure 7). PC1 and PC2 are regarded as the most significant because the eigen value is higher than one. Additionally, PC1 showed a variability of (66.51 %) and PC2 showed a variability of (18.54 %), with a large positive loading element of the pollen quantitative traits. Polar diameter followed by colpi length, mesocolpium and colpi width are the most valuable variables in PC1. On the first axis positive side, *N. distans* were found with the largest polar diameter followed by colpi length, colpi width and mesocolpium. *N. linearis* is also found on the positive side of first axes with high value of P/E ratio. Whereas *N. cataria*, *N. connata, N. erecta*, *N govaniana* and *N podostachys* were observed on negative side of first axes. Equatorial diameter followed by exine thickness are the significant components in PC2. On the second axis positive side, *N. raphanorhiza*, *N. hindostana*, *N. griffithii*, *N. nervosa*, *N. laevigata*, *N. discolor* and *N. schmidii* were examined while, *N. praetervisa*, *N. leucolaena*, *N.*

elliptica and *N. graciliflora* were found on the negative side of second axis. Additionally, the semantic differential chart of the PCA biplot were displayed in Figure 8 to better visualize and assess the relationships between variables.

3.21 Dichotomous Taxonomic Keys of *Nepeta* Species

1	a	Pollen shape per-prolate	*Nepeta linearis*
	b	Pollen shape other than per-prolate	2
2	a	Pollen oblate-spheroidal	3
	b	Pollen oblate other than oblate-spheroidal	4
3	a	Equatorial view spheroidal	*Nepeta cataria*
	b	Equatorial view irregular	*Nepeta govaniana*
4	a	Pollen shape spheroidal	5
	b	Pollen shape other than spheroidal	6
5	a	Lumina shape circular	*Nepeta leucolaena*
	b	Lumina shape not clear	*Nepeta erecta*
6	a	Pollen shape sub-oblate	7
	b	Pollen shape other than sub-oblate	9
7	a	Exine sculpturing reticulate perforate	*Nepeta graciliflora*
	b	Exine sculpturing bireticulate perforate	8
8	a	Aperture orientation concave	*Nepeta connata*
	b	Aperture orientation convex	*Nepeta elliptica*
9	a	Pollen sub-prolate	10
	b	Pollen prolate	12
10	a	Aperture sculpturing verrucate	*Nepeta podostachys*
	b	Aperture sculpturing granulate	11
11	a	Number of secondary lumina per primary lumen 14-19	*Nepeta nervosa*
	b	Number of secondary lumina per primary lumen 4-9	*Nepeta praetervisa*
12	a	Pollen in equatorial view rhomboid	*Nepeta laevigata*
	b	Pollen in equatorial view other than rhomboid	13
13	a	Pollen apex round	*Nepeta discolor*
	b	Pollen apex other than round	14

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14	a	Lumina absent	*Nepeta schmidii*
	b	Lumina present	15
15	a	Exine sculpturing reticulate-perforate/ foveolate	*Nepeta raphanorhiza*
	b	Exine sculpturing other than reticulate-perforate/foveolate	16
16	a	Aperture orientation concave	*Nepeta distans*
	b	Aperture orientation slightly convex	*Nepeta griffithii*
17	a	Aperture orientation sunken	*Nepeta hindostana*

4. Conclusion:

This study elaborates previously unexplored pollen characteristics of *Nepeta* species within the tribe Mentheae (Lamiaceae). The detailed analysis using scanning microscopic imaging tools unveiled the intricate variations in pollen morphotypes at micrometer dimensions helps accurate in species identification. The PCA and cluster analysis provided insights into the relationships between different species based on their pollen traits. This research contributes to a broader understanding of the *Nepeta* genus and its significance within the larger context of the Lamiaceae family. Pollen were hexazonocolpate, with a spherical mostly in circular view. SEM sculptural elements revealed exine was bi-reticulate. The size of pollen ranged from 13 to 88 µm. This pollen study of the *Nepeta* genus adds to our understanding of the morphological diversity of the Lamiaceae and emphasizes the need for further pollen analysis of the *Nepeta* genus using transmission electron microscopy at the micromorphological level. The difficulty in distinguishing some Nepeta species using a traditional approach was easily solved using micrometer scale pollen traits as the sculptured pattern of pollen was unique and different species were statistically comparable, thus representing a tool for identifying *Nepeta* species.

Conflict of Interest:

We all authors have no conflict of interest to declare.

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Authors Contributions:

Shaista Jabeen; Writing Original Draft and Methods, Muhammad Zafar; Supervision, Review Editing, Mushtaq Ahmad; Supervision and Validation of Results, M. Ajmal Ali and Mohamed S Elshikh; Funding Acquisition and Sofware, Trobjon Makhkamov; Visualization, Oybek Mamarakhimov; Method analysis, Akramjon Yuldashev; Conceptualization, Khislat Khaydarov; Statistical software, Yusufjon Gafforov; Data curation, Babir Baysunov; Formal analysis, Afat O. Mammadova; English language editing, Laziza Botirova; Software, Shazia Sultana; Methodology, Salman Majeed; Review Editing and Language editing, Rozina; Interpret the results, Shabir Ahmad; Visualization, Aqsa Abid; Microphotography analysis, Abdurashid Rahmatov; Identification and Discussion

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Figure and Plates Captions:-

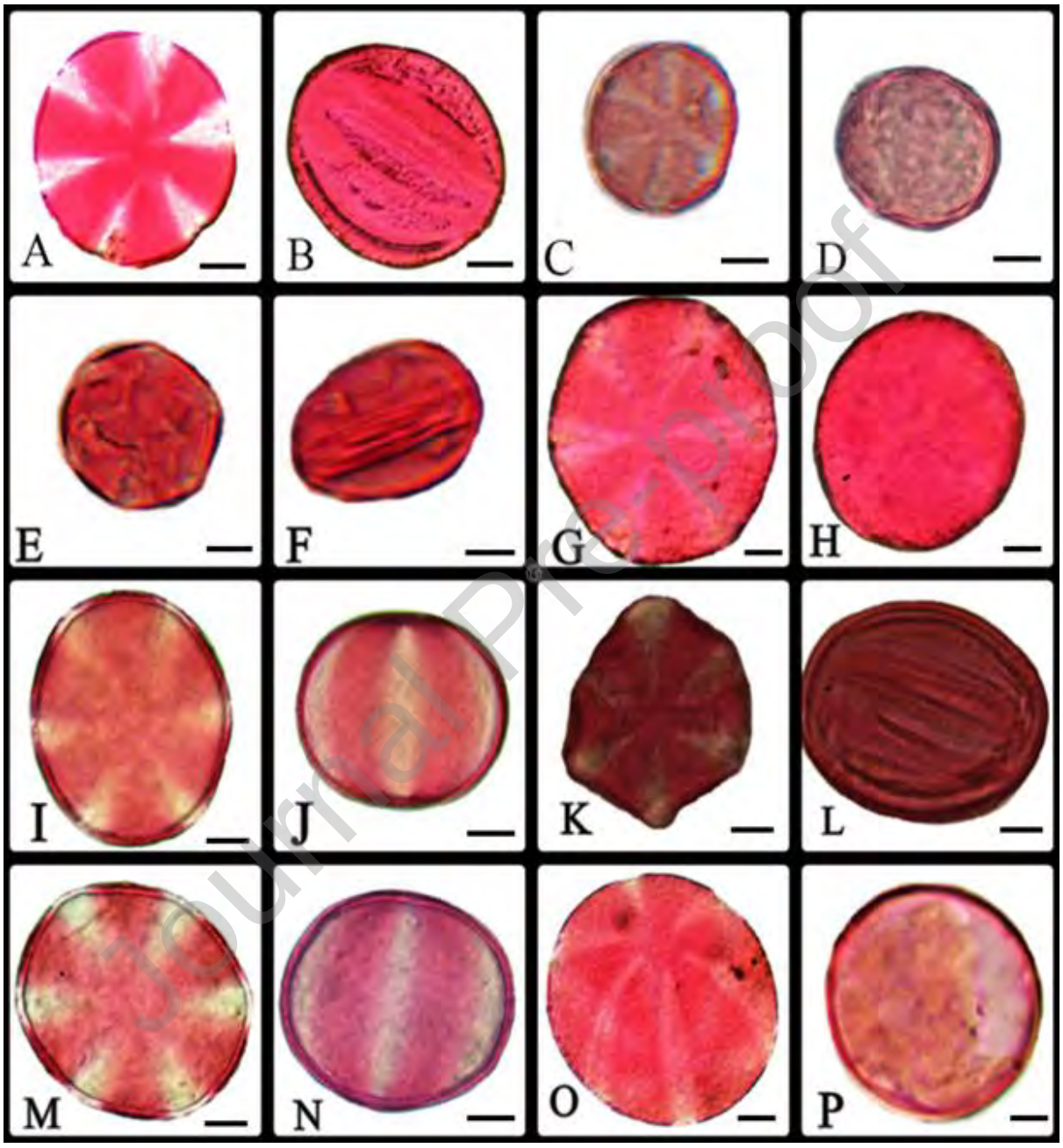


Plate. 1: Pollen light micrographs illustrated polar view, equatorial view of genus *Nepeta* species (scale bar 10 µm). *Nepeta cataria* (A, polar view; B, equatorial view). *N. connata* (C, polar view; D, equatorial view). *N. discolor* (E, polar view; F, equatorial view). *N. distans* (G, polar view; H, equatorial view). *N. elliptica* (I, polar view; J, equatorial view). *N. erecta* (K,

polar view; L, equatorial view). *N. govaniana* (M, polar view; N, equatorial view). *N. graciliflora* (O, polar view; P, equatorial view).

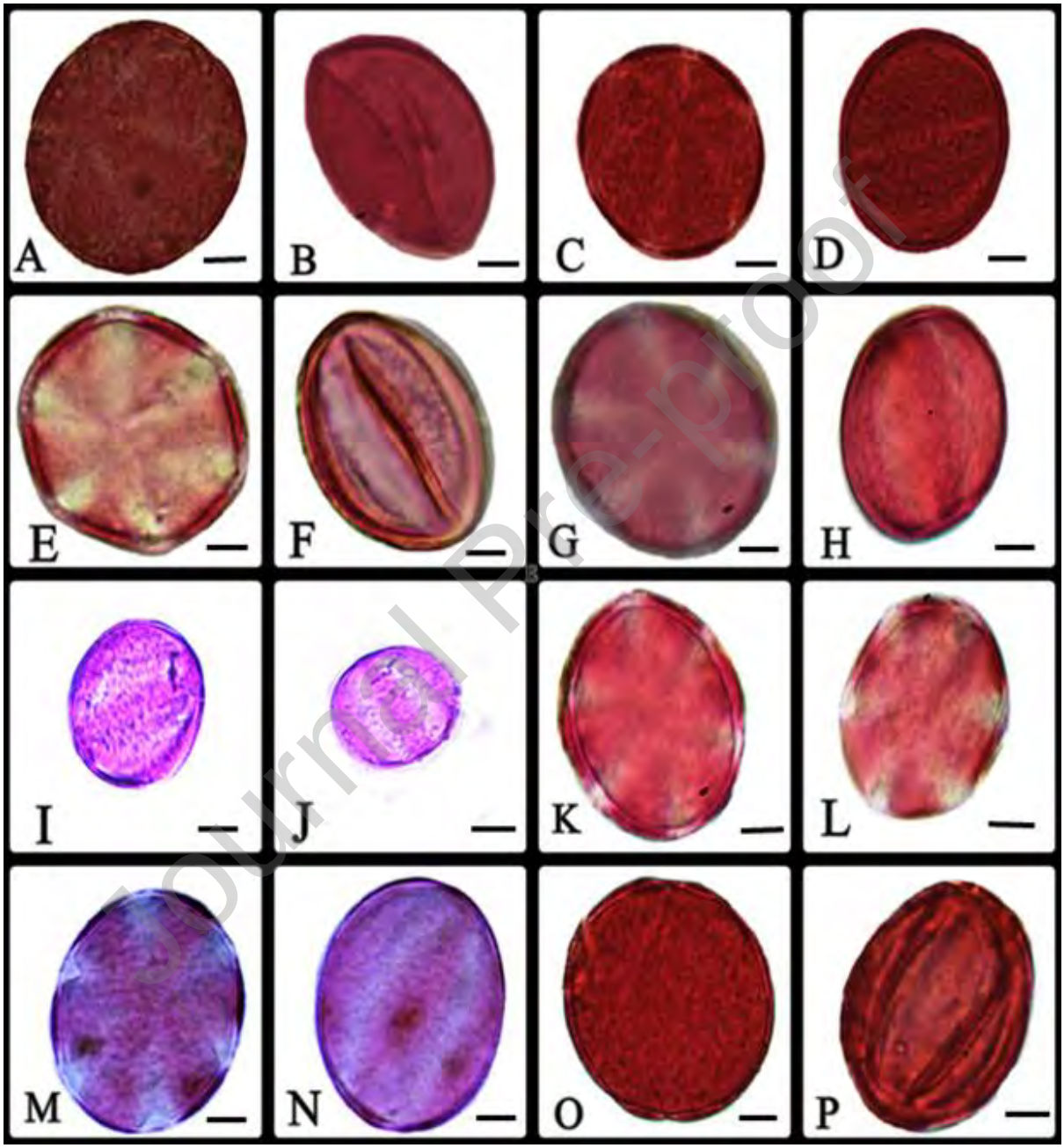


Plate. 2: Pollen light micrographs illustrated polar view, equatorial view of genus *Nepeta* species (scale bar 10 µm). *Nepeta griffithii (*A, polar view; B, equatorial view). *N. hindostana* (C, polar view; D, equatorial view). *N. laevigata* (E, polar view; F, equatorial view). *N. leucolaena* (G, polar view; H, equatorial view). *N. linearis* (I, polar view; J, equatorial view).

*N. nervosa (*K, polar view; L, equatorial view). *N. podostachys* (M, polar view; N, equatorial view). *N. praetervisa* (O, polar view; P, equatorial view).

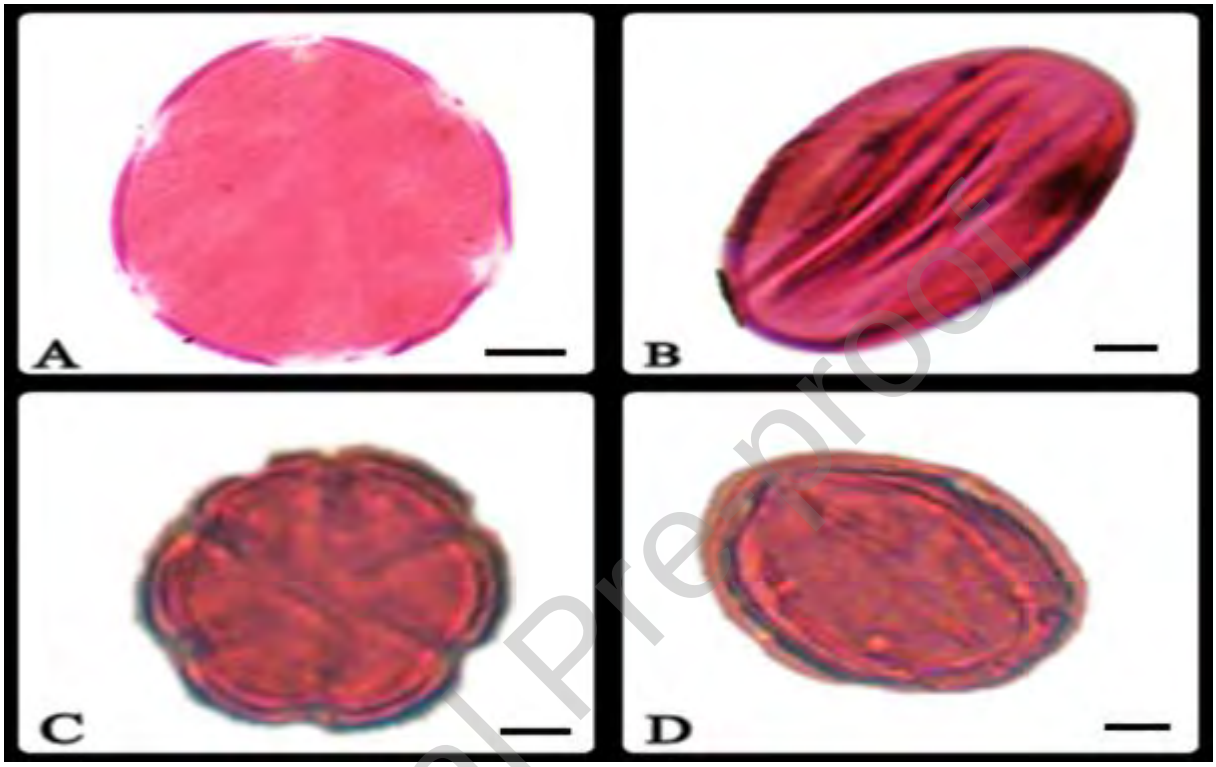


Plate. 3: Pollen light micrographs illustrated polar view, equatorial view of genus *Nepeta* species (scale bar 10 µm). *Nepeta raphanorhiza* (A, polar view; B, equatorial view). *N. schmidii* (C, polar view; D, equatorial view).

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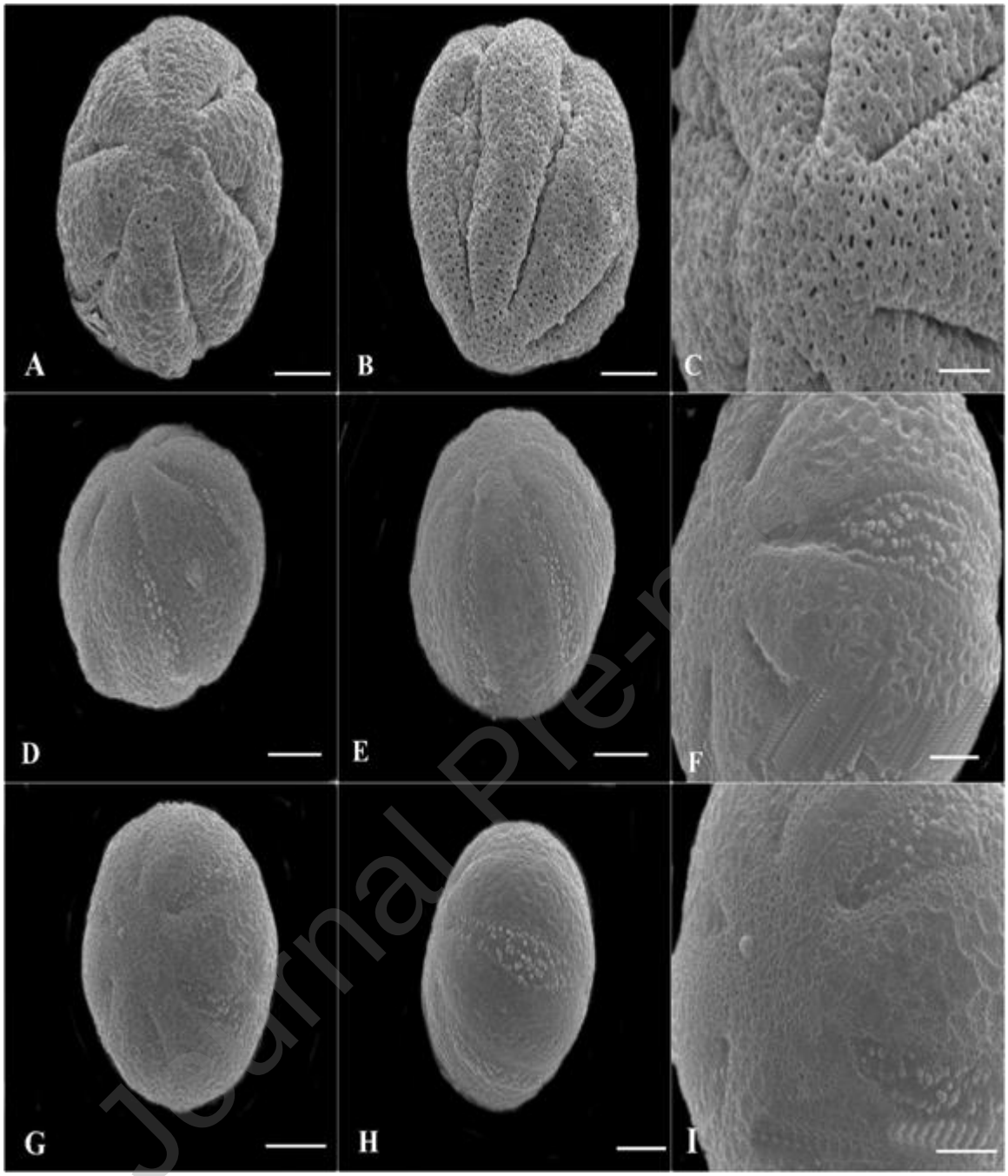


Plate. 4: Scanning electron micrographs of pollen illustrated polar view, equatorial view (scale bar = 5 μm) and exine sculpturing (scale bar = 1 μm) of genus *Nepeta* species. (A-C) *Nepeta cataria* (A) Polar view (B) equatorial view (C) exine sculpturing. (D-F) *N. connata* (D) Polar view (E) equatorial view (F) exine sculpturing. (G-I) *N. discolor* (G) Polar view (H) equatorial view (I) exine sculpturing.

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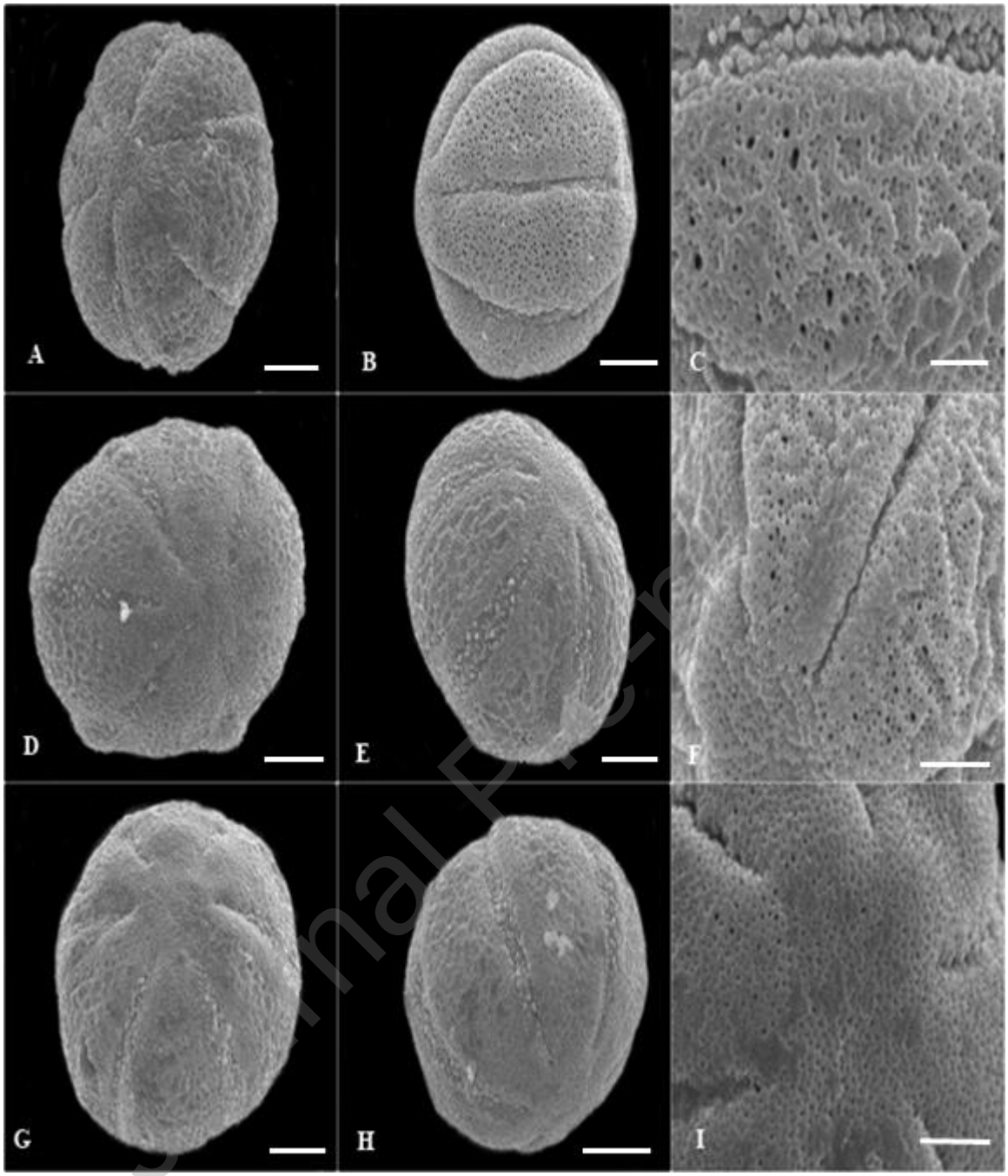


Plate. 5: Scanning electron micrographs of pollen illustrated polar view, equatorial view (scale bar = 5 µm) and exine sculpturing (scale bar = 1 µm) of genus *Nepeta* species. (A-C) *N. distans* (A) Polar view (B) equatorial view (C) exine sculpturing. (D-F) *N. elliptica* (D) Polar view (E) equatorial view (F) exine sculpturing. (G-I) *N. erecta* (G) Polar view (H) equatorial view (I) exine sculpturing.

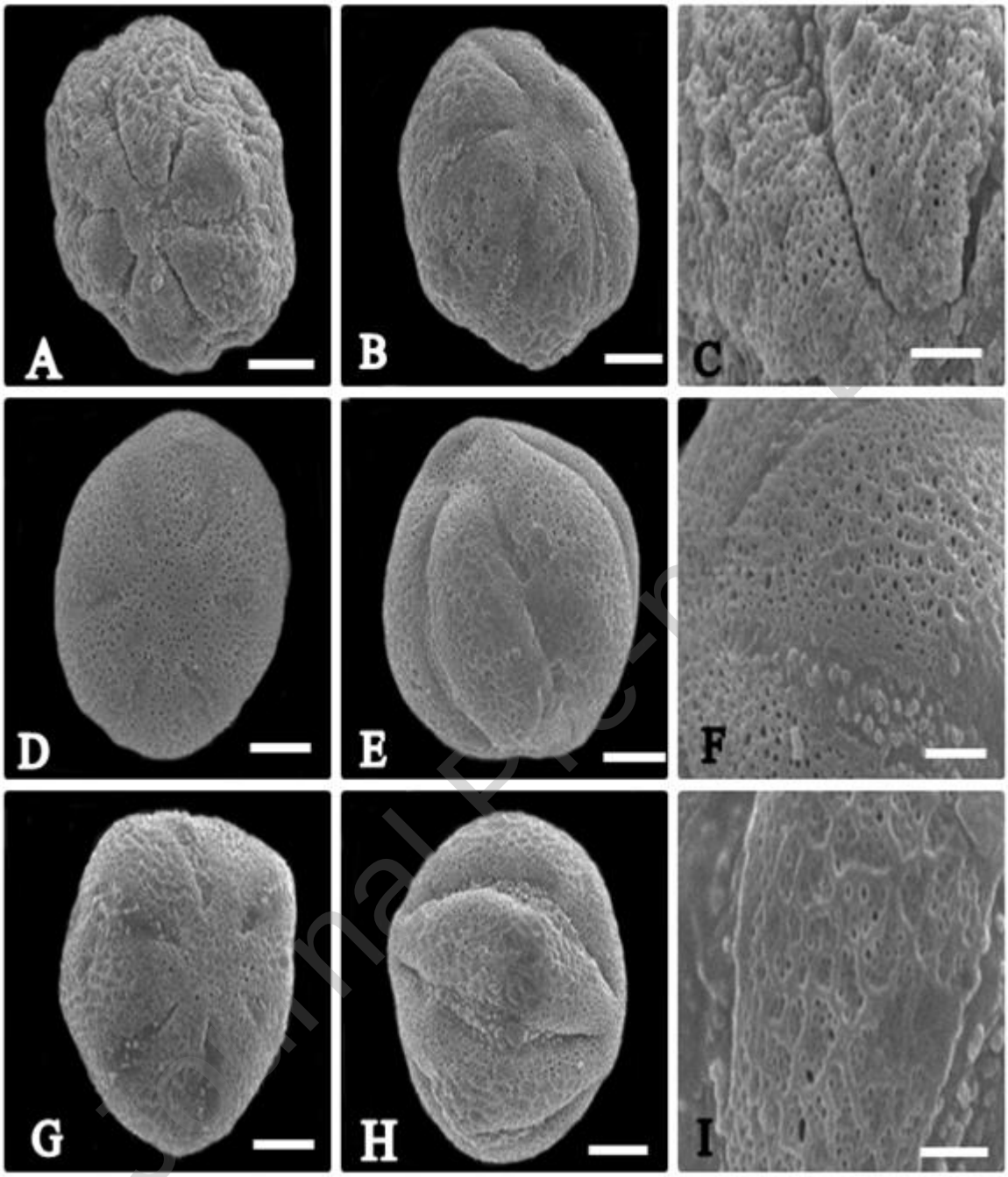


Plate. 6: Scanning electron micrographs of pollen illustrated polar view, equatorial view (scale bar = 5 µm) and exine sculpturing (scale bar = 1 µm) of genus *Nepeta* species. (A-C) *N. govaniana* (A) Polar view (B) equatorial view (C) exine sculpturing. (D-F) *N. graciliflora* (D) Polar view (E) equatorial view (F) exine sculpturing. (G-I) *N. griffithii* (G) Polar view (H) equatorial view (I) exine sculpturing.

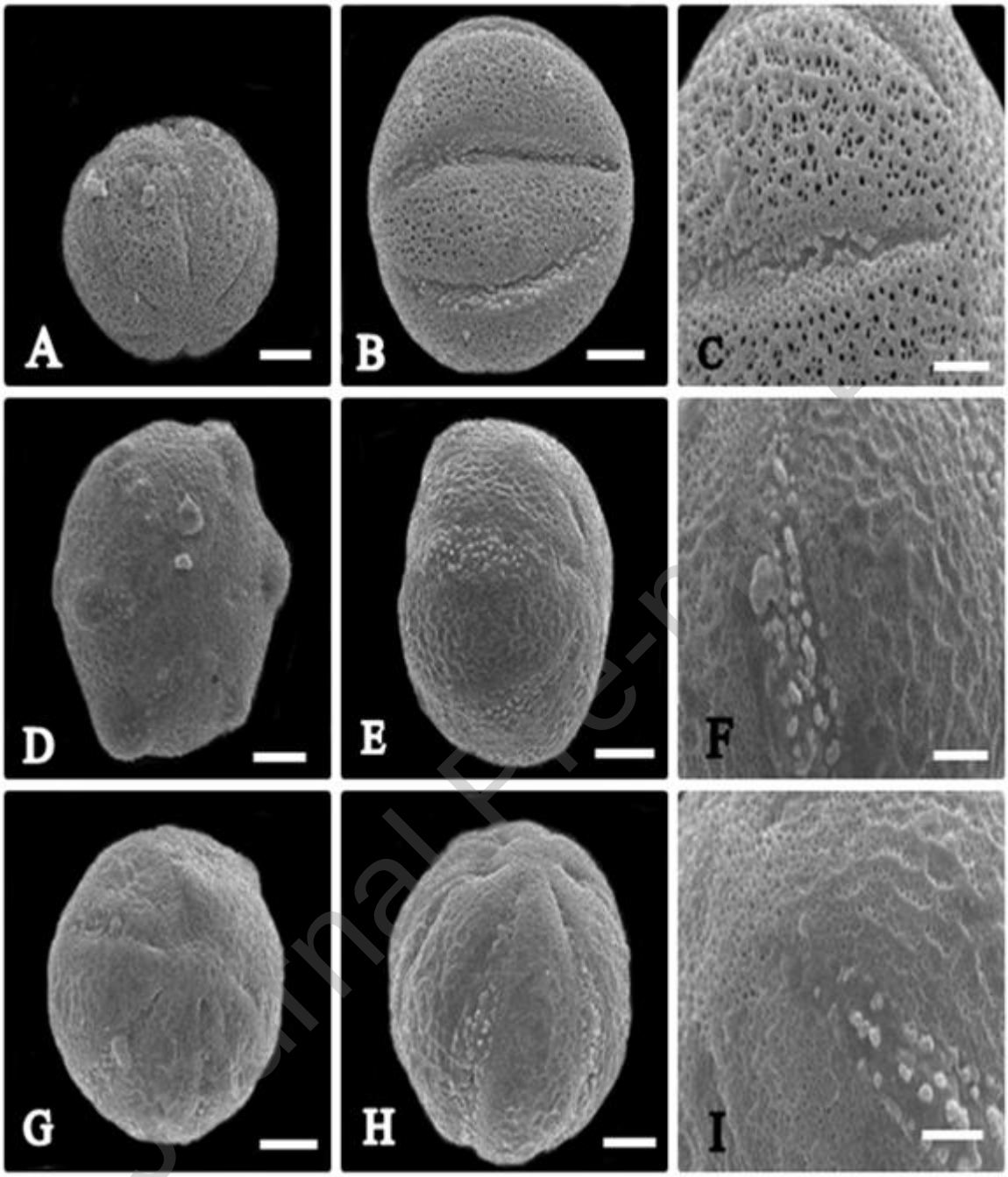


Plate. 7: Scanning electron micrographs of pollen illustrated polar view, equatorial view (scale bar = 5 µm) and exine sculpturing (scale bar = 1 µm) of genus *Nepeta* species. (A-C) *N. hindostana* (A) Polar view (B) equatorial view (C) exine sculpturing. (D-F) *N. laevigata (*D) Polar view (E) equatorial view (F) exine sculpturing. (G-I) *N. leucolaena* (G) Polar view (H) equatorial view (I) exine sculpturing. Scale bar 5µm for polar and equatorial micrographs, scale bar 2µm for exine sculpturing.

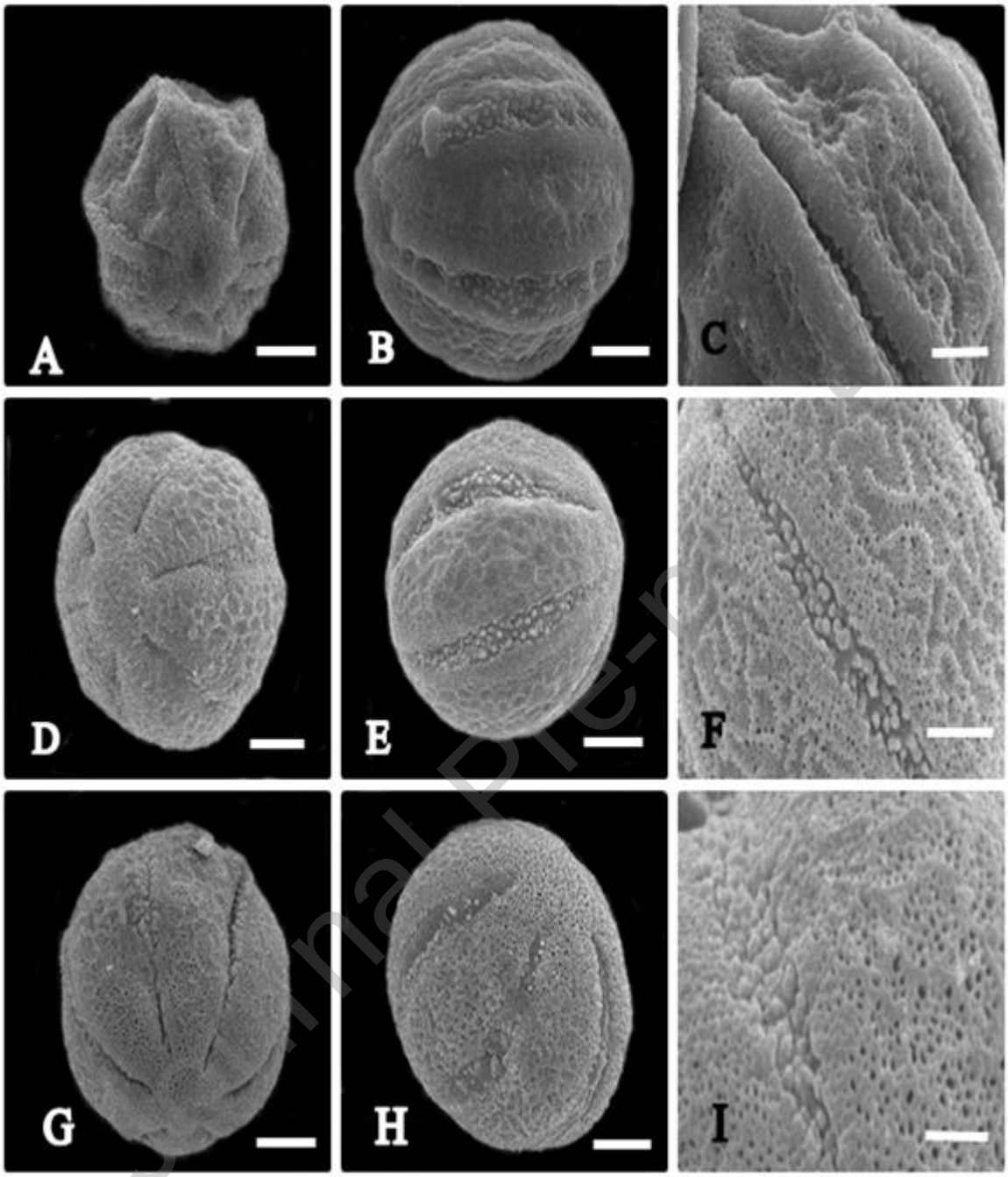


Plate. 8: Scanning electron micrographs of pollen illustrated polar view, equatorial view (scale bar = 5 µm) and exine sculpturing (scale bar = 1 µm) of genus *Nepeta* species. (A-C) *N. linearis* (A) Polar view (B) equatorial view (C) exine sculpturing. (D-F) *N. nervosa* (D) Polar view (E) equatorial view (F) exine sculpturing. (G-I) *N. podostachy (*G) Polar view (H) equatorial view (I) exine sculpturing. Scale bar 5µm for polar and equatorial micrographs, for *(*A, G) 10 µm, scale bar 2µm for exine sculpturing, for (I) 1µm.

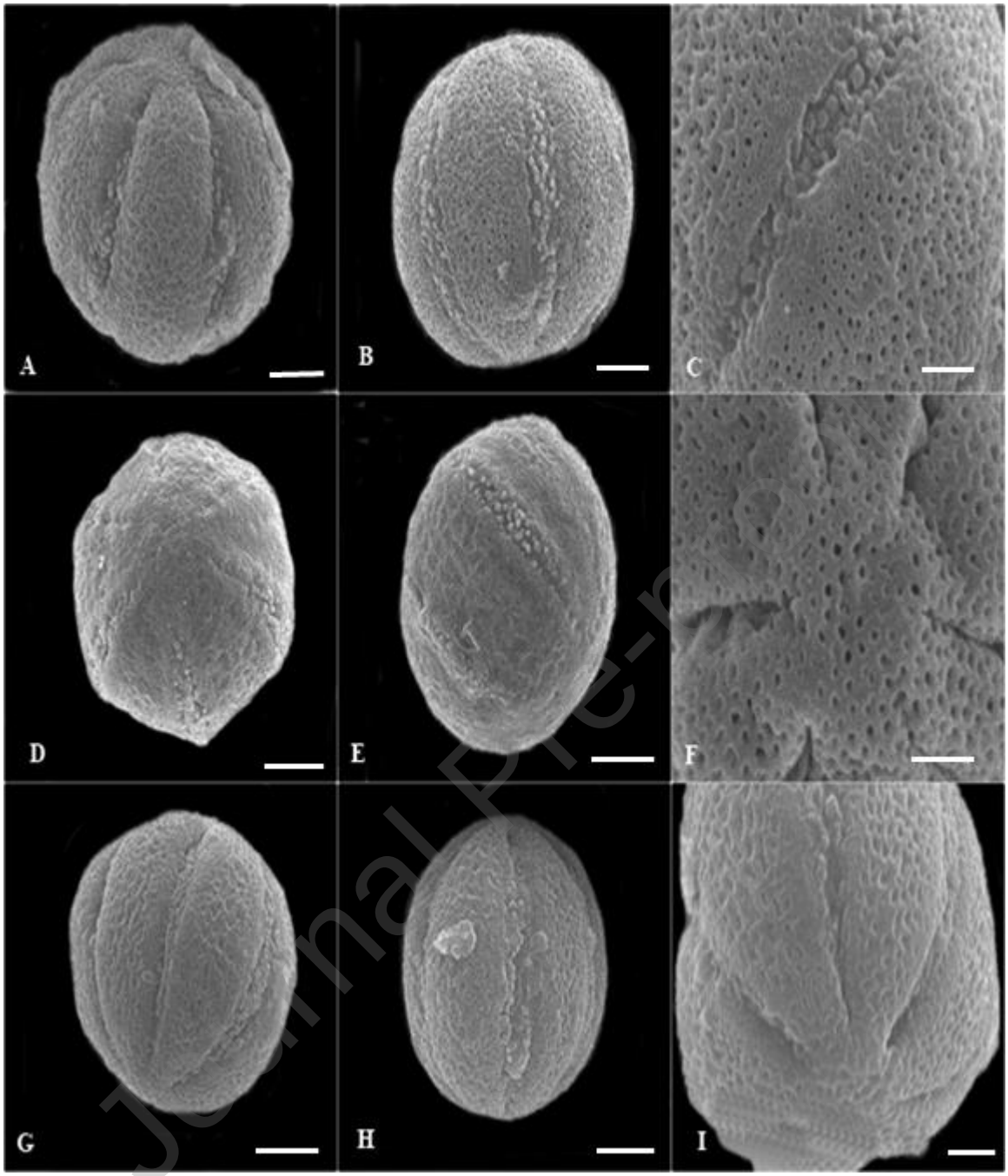


Plate. 9: Scanning electron micrographs of pollen illustrated polar view, equatorial view (scale bar = 5 µm) and exine sculpturing (scale bar = 1 µm) of genus *Nepeta* species. (A-C) *N. praetervisa* (A) Polar view (B) equatorial view (C) exine sculpturing. (D-F) *N. raphanorhiza* (D) Polar view (E) equatorial view (F) exine sculpturing. (G-I) *N. schmidii* (G) Polar view (H) equatorial view (I) exine sculpturing. Scale bar 5µm for polar and equatorial micrographs, scale bar 2µm for exine sculpturing.

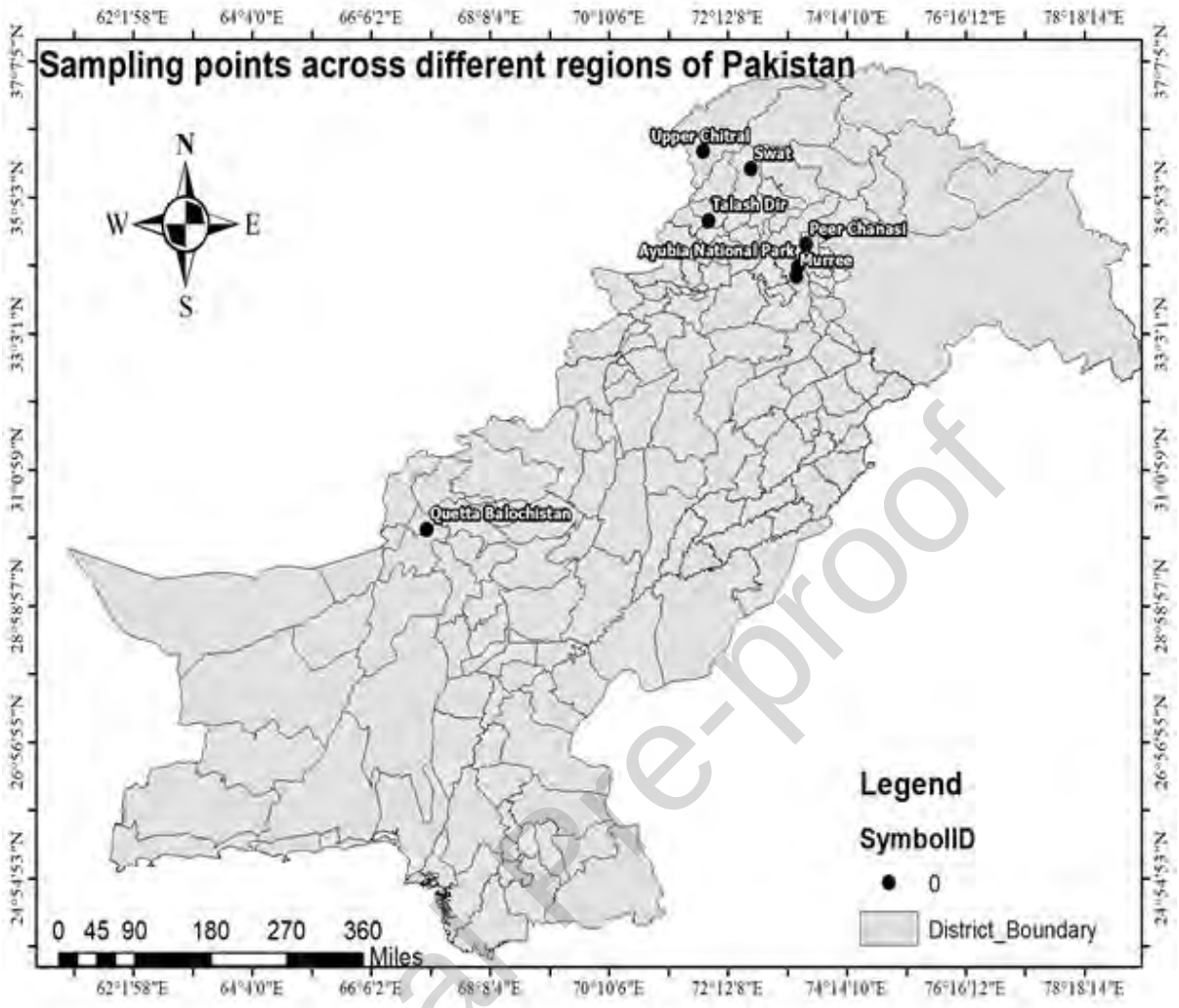


Figure 1. Map of the study area: Northern-Pakistan

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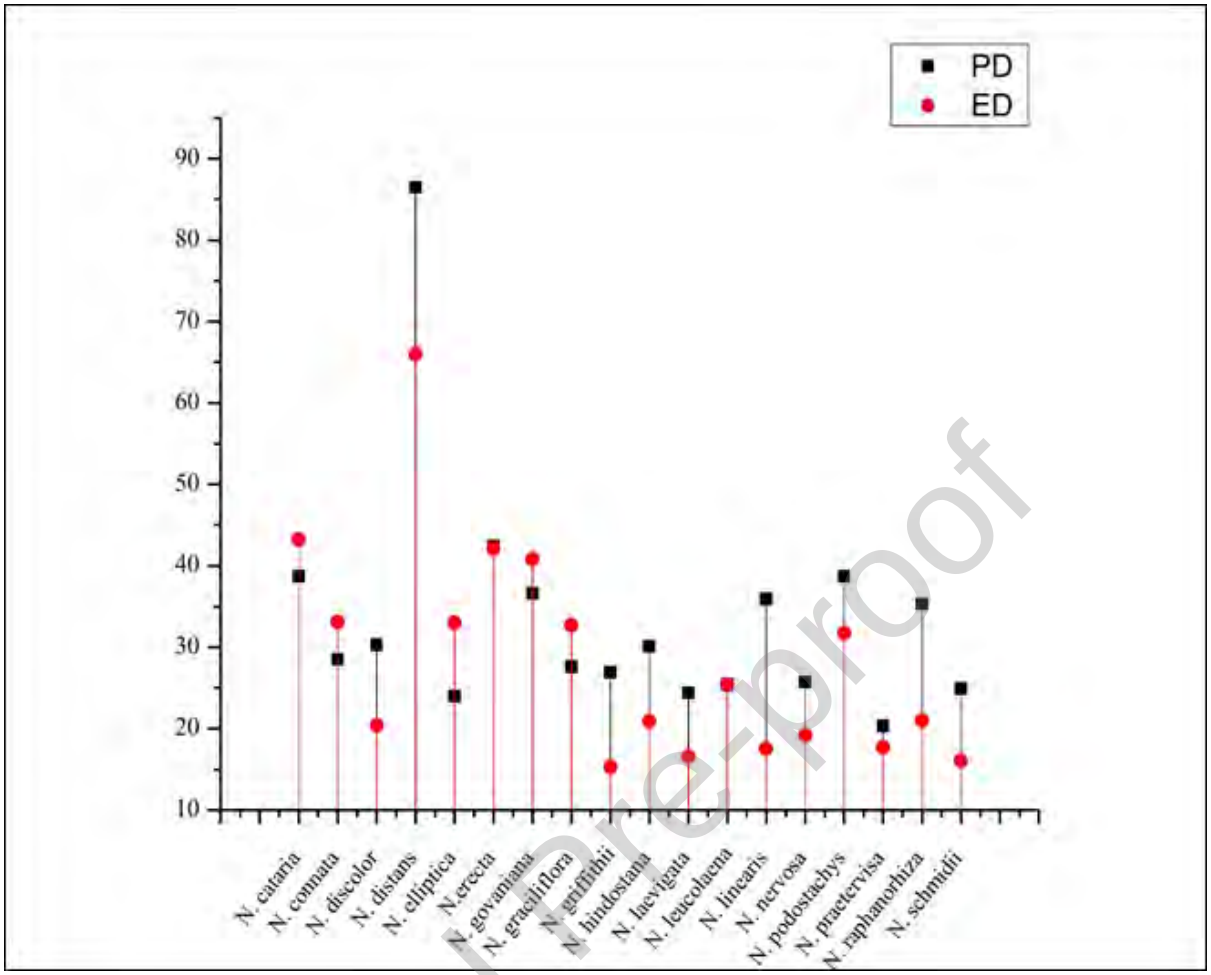


Figure 2. Comparative mean variations in polar and equatorial diameter among *Nepeta* species

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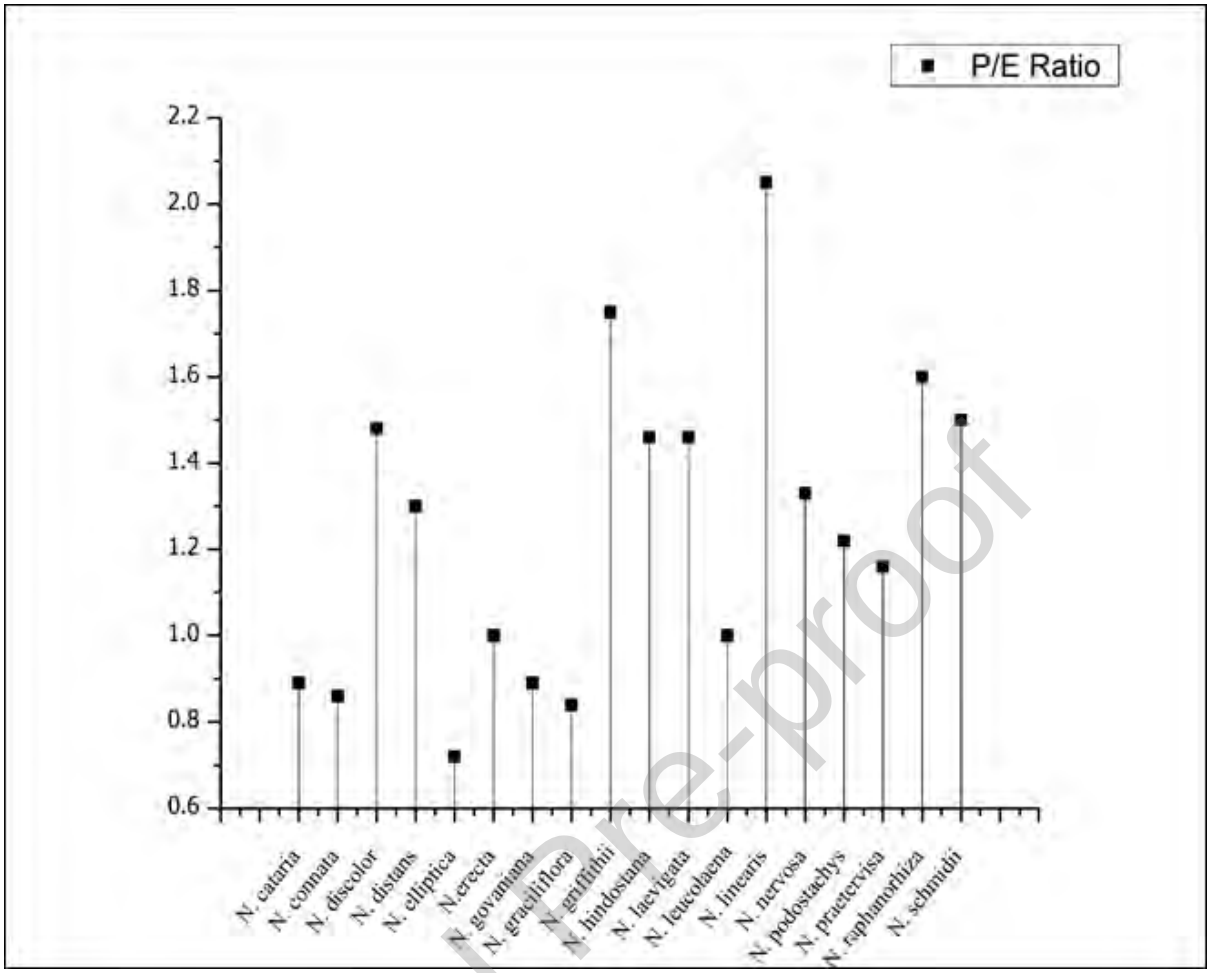


Figure 3. P/E ratio index variability among *Nepeta* species

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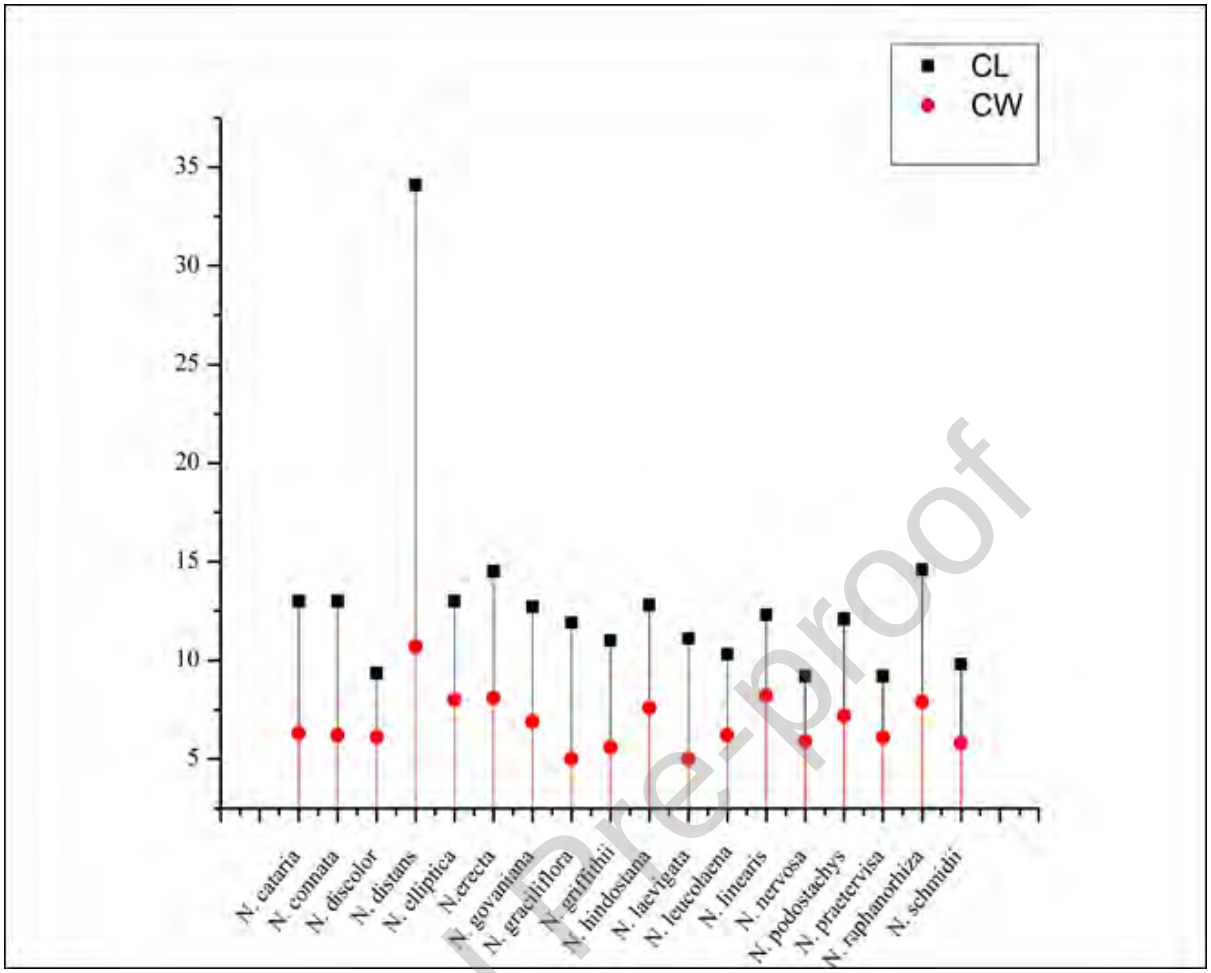


Figure 4. Average colpi length and width variations among *Nepeta* species

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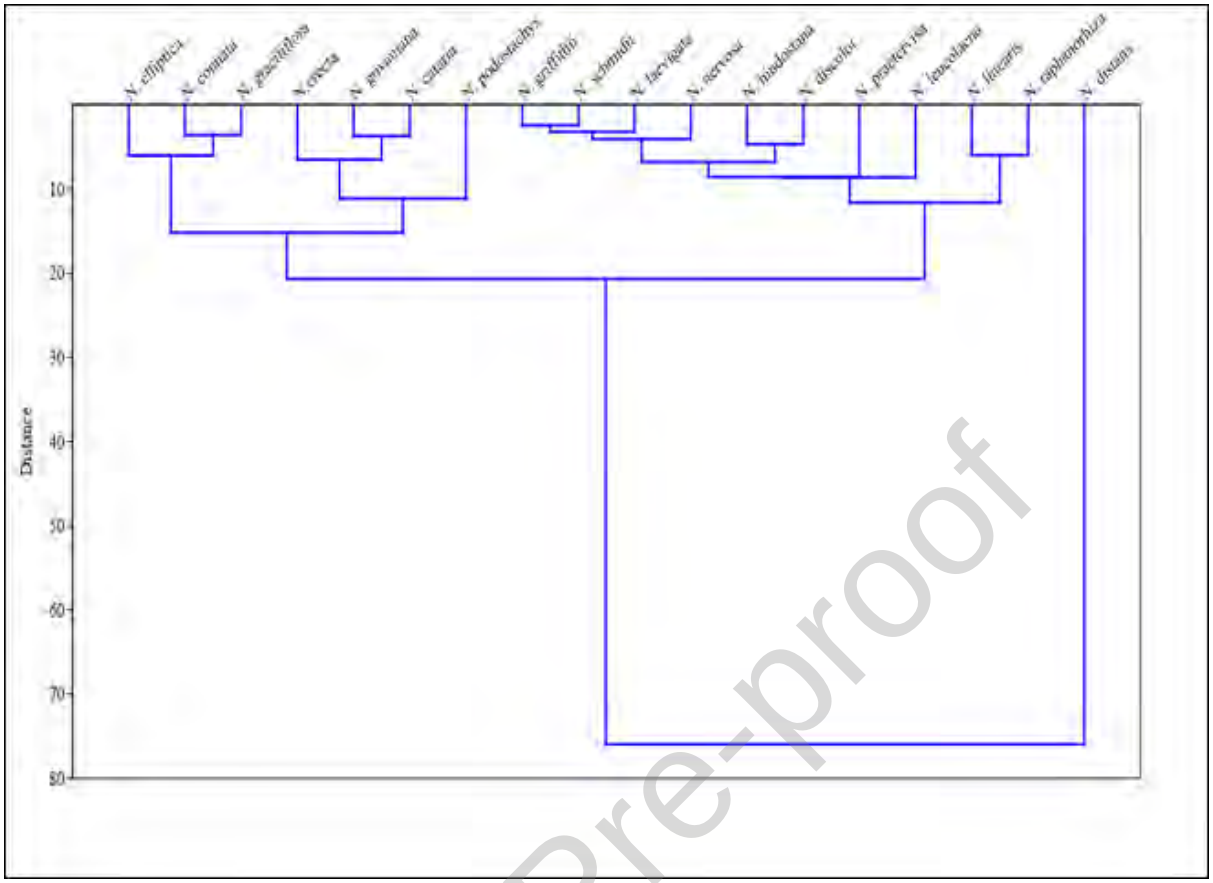


Figure 5. Dendrogram clustering analysis among *Nepeta* species based pollen quantitative data

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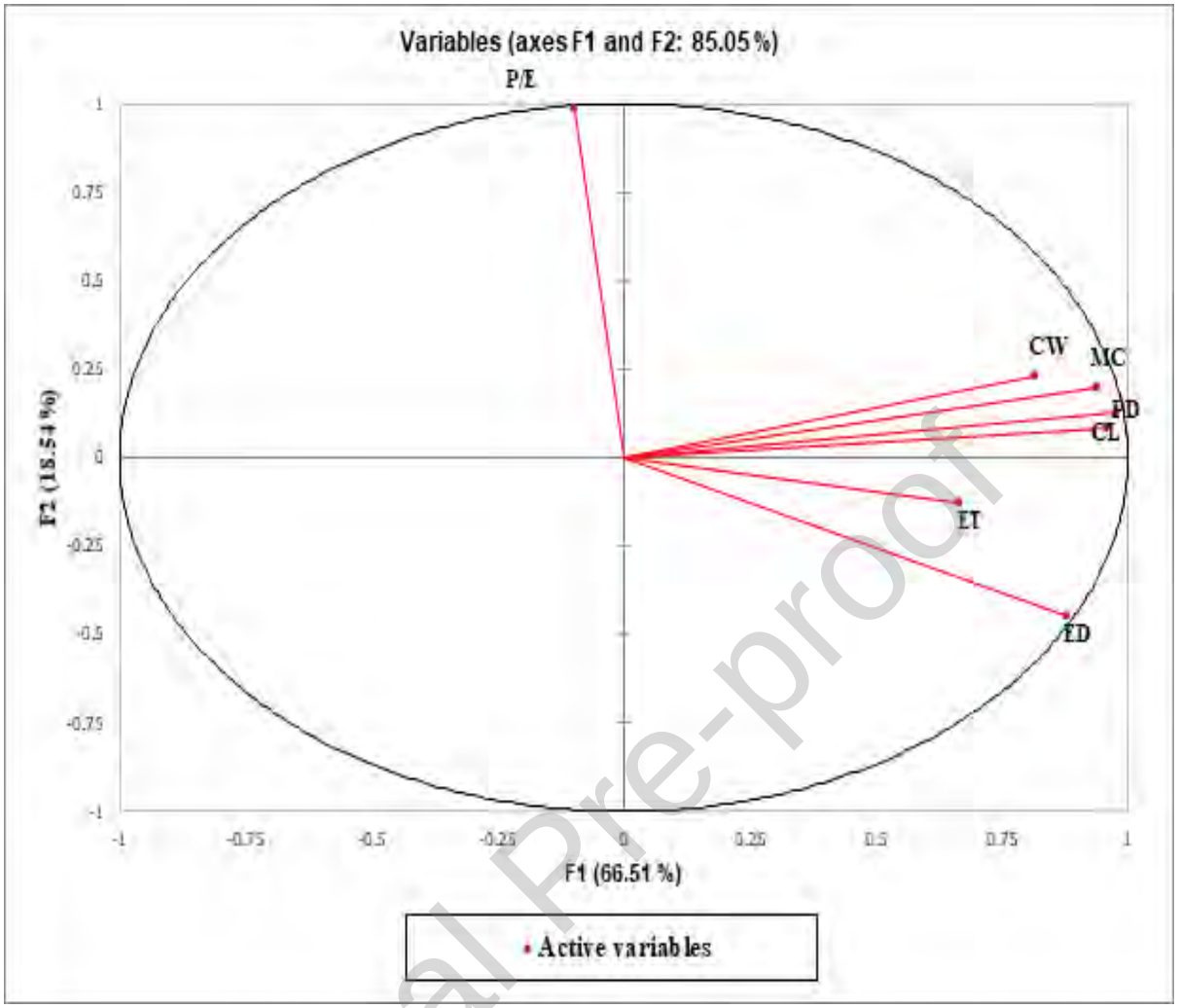


Figure 6. PCA variables of *Nepeta* pollen of the principal component biplots

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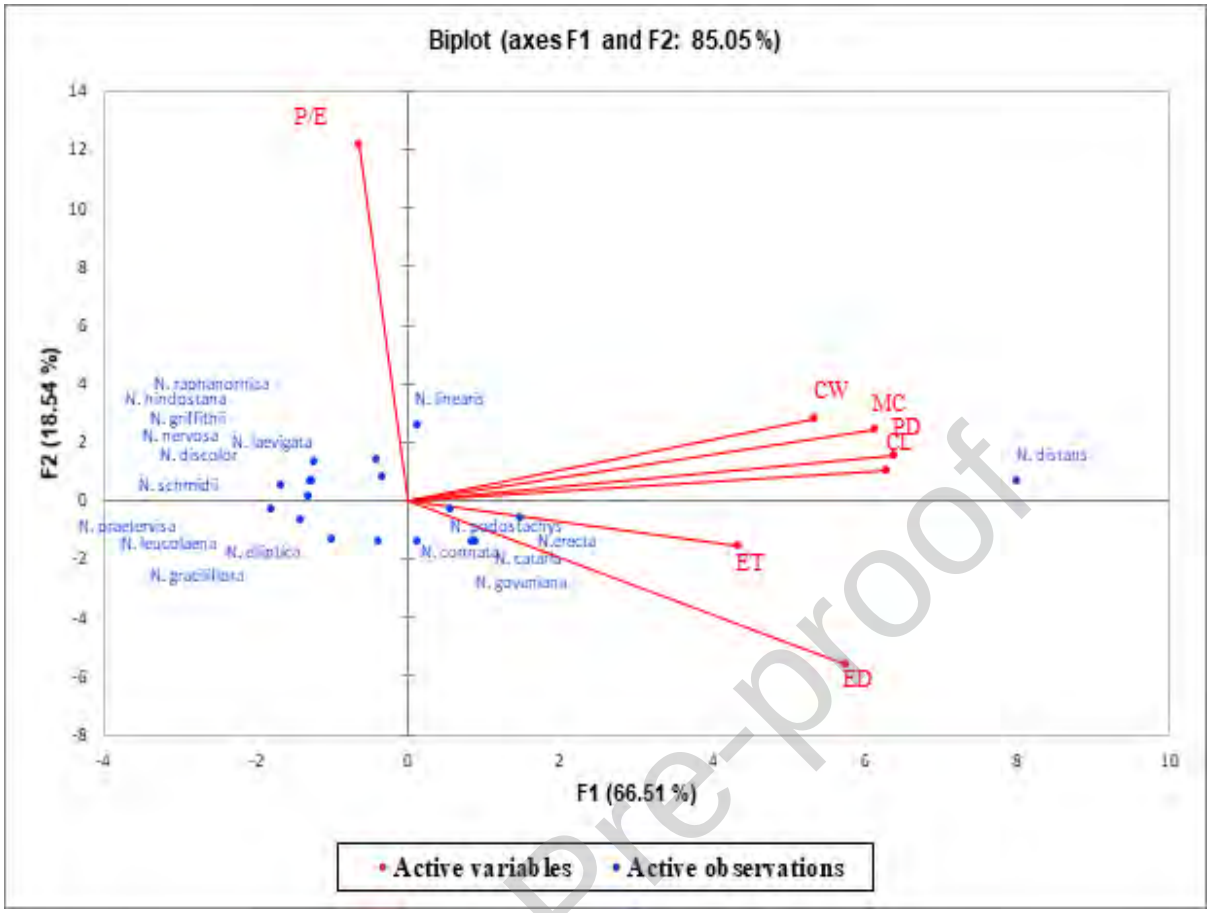


Figure 7. PCA based *Nepeta* pollen traits variations in terms of Eigen values.

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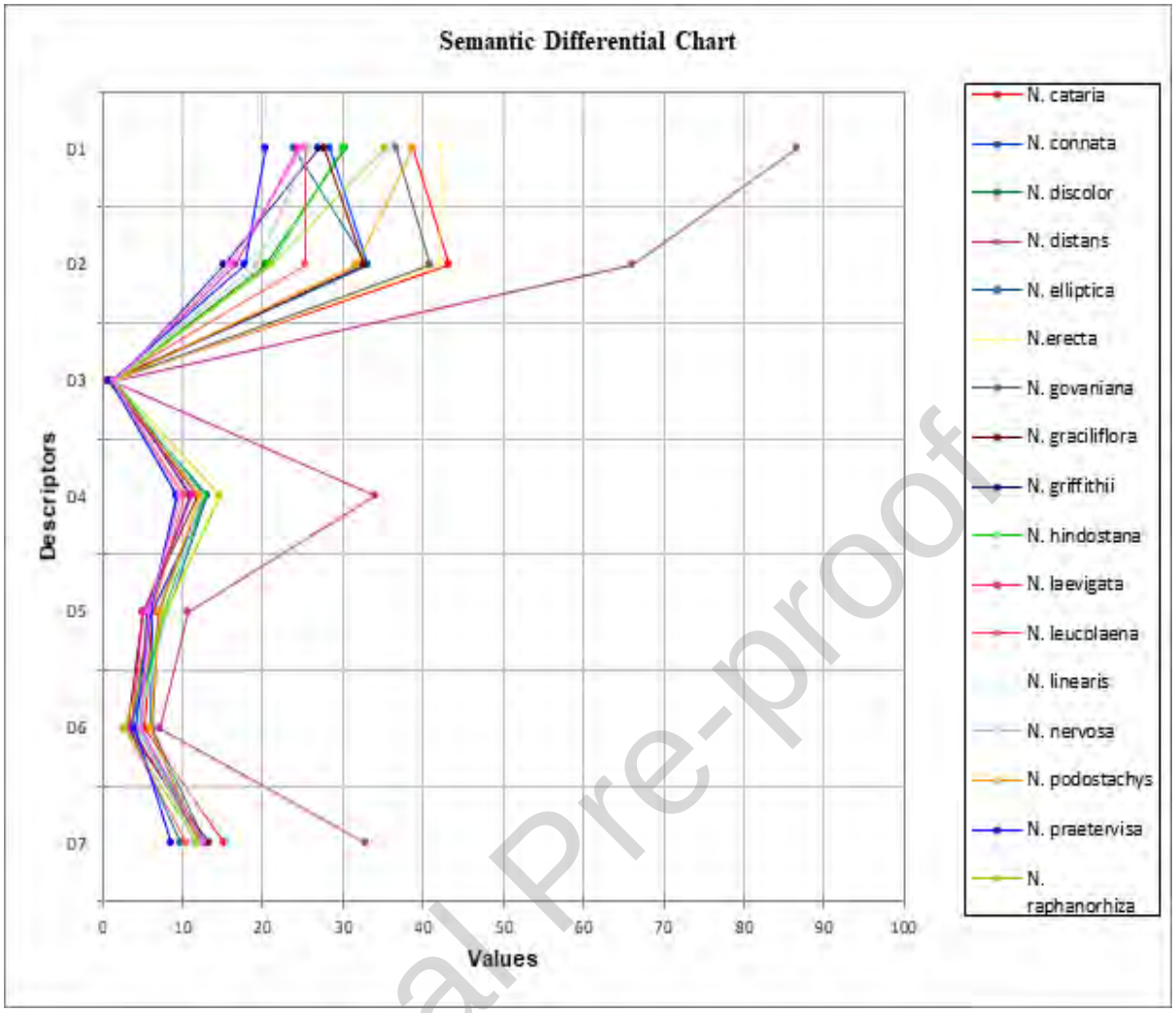


Figure 8. Semantic differential chart of seven pollen variables among *Nepeta* species

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Table. 1: Checklist, plant sampling, locality GPS coordinates and Herbarium accession details of *Nepeta* species.

S.no	*Nepeta* species	Habit	Status	Collector	Locality	Date	Altitude (ft)	Coordinates	Voucher specimen no/ Accession no
1.	*Nepeta cataria* L.	Herb	Wild	Shaista Jabeen	Galoch (Swat)	15-4-2021	4900	35°07'05"N 76°19'18"E	SJ 28/ 131890
2.	*Nepeta connata* Royle ex Benth.	Herb	Wild	Shaista Jabeen	Panjkot vally Kashmir	10-7-2022	6875	35°30'40.05"N 72°36'15.80"E	SJ 39/ 131480
3.	*Nepeta discolor* Royle ex Benth.	Herb	Wild	Shaista Jabeen	Khaplu Gilgit Baltistan	25-8-2021	4117	34°09'26.56"N 73°12'45.20"E	SJ 1/ 131482
4.	*Nepeta distans Royle*	Herb	Wild	Shaista Jabeen	Sarlara (Lower Dir)	5-5-2021	2501	34°41'01.87"N 72°07'38.74"E	SJ 87/ 133481
5.	*Nepeta elliptica* Royle ex Benth.	Herb	Wild	Shaista Jabeen	Peerchanasi (Kashmiir)	3-9-2021	8857	34°22′54.6"N 73°33'08.41"E	SJ 2/ 133480
6.	*Nepeta erecta* (Royle ex Benth.) Benth.	Herb	Wild	Salman Majeed	Ayubia national park (KPK)	8-9-2021	2011	33°44'44.65"N 73°07'40.07"E	SJ 20/ 131898
7.	*Nepeta govaniana* (Wall. ex Benth.) Benth.	Herb	Wild	Shaista Jabeen	Peerchanasi (Kashmiir)	9-9-2021	9403	342316.63N 733308.41E	SJ 3/ 133482
8.	*Nepeta graciliflora* Benth.	Herb	Wild	Shaista Jabeen	Kashmiir	7-4-2021	4744	35°16'43.18"N 72°00'08.34"E	SJ 33/ 131483

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9.	*Nepeta griffithii* Hedge	Herb	Wild	Shaista Jabeen	Swat (KPK)	25-4-2021	1655	33°40'13.46 N 73°07'33.12 E	SJ 27/ 1318907
10.	*Nepeta hindostana* (B. Heyne ex Roth) Haines	Herb	Wild	Shaista Jabeen	Hazara (KPK)	18-4-2021	1613	33°53'44.95 "N 72°51'23.38 "E	SJ 11/ 131872
11.	*Nepeta laevigata* (D. Don) Hand. -Mazz.	Herb	Wild	Salman Majeed	Murree (Punjab)	10-9-2021	1488	34°30'04.30 "N 71°54'10.37 "E	SJ 50/ 1318903
12.	*Nepeta leucolaena* Benth. ex Hook.f.	Herb	Wild	Shaista Jabeen	Peerchanasi (Kashmir)	11-9-2021	8995	34°22'57.35 "N 73°32'45.88 "E	SJ 4/ 133483
13.	*Nepeta linearis* Royle ex Benth.	Herb	Wild	Shaista Jabeen	Kashmir (Neelum valley)	6-10-2022	4713	34°35'52.80 "N 73°55'27.17 "E	SJ 5/ 133484
14.	*Nepeta nervosa* Royle ex Benth.	Herb	Wild	Shaista Jabeen	Peerchanasi (Kashmir)	6-10-2022	7085	34°22'44.57 "N 73°31'30.66 "E	SJ 6/ 133485
15.	*Nepeta podostachys* Benth.	Herb	Wild	Shaista Jabeen	Chitral (KPK)	5-18-2022	4644	35°46'07.47 "N 71°46'45.53 "E	SJ 7/ 133486
16.	*Nepeta praetervisa* Rech.f.	Herb	Wild	Shaista Jabeen	Quetta Balochistan	5-4-2021	1622	33°53′47.44 "N 72°51′27.47 "E	SJ 25/ 131891
17.	*Nepeta raphanorhiza* Benth.	Herb	Wild	Tariq ullah	Talash (Lower Dir)	4-9-201	2746	34°44'27.66 "N 71°52'17.29 "E	SJ 8/ 133487
18.	*Nepeta schmidii* Rech .f.	Herb	Wild	Shaista Jabeen	Chitral and Kashmir	5-5-	5989	36°00'48.26 "N	SJ 9/ 133488

	2022	71°°45'47.23"E

Keywords: KPK = Khyber Pakhtunkhwa, ft =feet

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Table. 2: Quantitative pollen attributes micrometer measurements of *Nepeta* species.

S. no	*Nepeta* species	Polar diameter Min-Max = Mean±SE (µm)	Equatorial diameter Min-Max = Mean±SE (µm)	P/E ratio	Length of colpi Min-Max = Mean±SE (µm)	Width of colpi Min-Max = Mean±SE (µm)	Exine thickness Min-Max = Mean± SE (µm)	Mesocolpium distance Min-Max = Mean±SE (µm)	Fertility (%)	Sterility (%)
1.	*Nepeta cataria* L.	36.2-41.2=38.7±.85	41.2-46.2=43.2±.85	0.89	12.5-13.7=13.0±.21	5.7-7.0=6.3±.23	4.7-5.7=5.2±.17	13.7-16.2=15.2±.45	78.3	21.7
2.	*Nepeta connata* Royle ex Benth.	26.2-30.7=28.5±.880	31.0-35.5=33.1±.860	0.86	12.2-13.7=13.0±.289	5.7-6.7=6.2±.176	5.5-6.5=6.0±.176	11.7-12.7=12.2±.176	69.5	30.5
3.	*Nepeta discolor* Royle ex Benth.	29.5-31.2=30.3±.320	19.2-21.2=20.4±.384	1.48	8.7-10.0=9.35±.231	5.7-6.5=6.1±.127	3.2-4.0=3.6±.127	11.2-13.2=12.4±.331	75.4	24.6
4.	*Nepeta distans Royle*	83.7-88.0=86.5±.75	55.7-76.2=66.0±3.6	1.3	32.5-36.2=34.1±.700	8.0-13.7=10.7±1.1	6.2-8.0=7.1±.32	31.2-34.2=32.8±.53	80.3	19.7
5.	*Nepeta elliptica* Royle ex Benth.	19.7-28.7=24.0±1.5	32.0-34.5=33.0±.47	0.72	12.0-13.7=13.0±.29	7.2-8.7=8.0±.28	3.0-4.0=3.5±.17	9.2-1s0.5=9.8±.23	85.5	14.5
6.	*Nepeta erecta* (Royle ex Benth.) Benth.	41.2-43.7=42.4±.42	40.5-43.7=42.1±.56	1.0	13.7-15.2=14.5±.25	7.2-9.0=8.1±.32	3.7-4.7=4.2±.17	16.2-18.5=17.6±.40	89.9	10.1
7.	*Nepeta govaniana* (Wall. ex Benth.) Benth.	34.2-38.2=36.6±.69	37.7-42.7=40.8±.82	0.89	12.0-13.5=12.7±.26	6.2-7.2=6.9±.18	5.5-6.7=6.2±.21	12.0-13.5=12.9±.25	75.2	24.8
8.	*Nepeta graciliflora* Benth.	26.2-28.7=27.6±.45	31.2-34.7=32.7±.59	0.84	10.7-13.7=11.9±.54	4.5-5.5=5.0±.176	2.2-3.7=3.1±.26	12.2-14.0=13.3±.30	65.4	34.6
9.	*Nepeta griffithii* Hedge	25.5-28.2=26.9±.49	13.0-18.7=15.3±1.2	1.75	9.7-12.7=11.0±.52	5.0-6.2=5.6±.23	4.2-5.5=4.8±.23	12.0-13.2=12.6±.23	68.9	31.1
10.	*Nepeta hindostana* (B. Heyne ex Roth) Haines	29.2-30.7=30.1±.26	19.5-23.7=20.9±.77	1.46	12.0-13.7=12.8±.32	7.0-8.2=7.6±.23	3.7-5.2=4.3±.25	12.0-13.2=12.6±.23	64.2	35.8
11.	*Nepeta laevigata* (D. Don) Hand. - Mazz.	23.7-25.2=24.4±.26	15.0-18.2=16.6±.52	1.46	10.2-11.7=11.1±.26	4.5-5.5=5.0±.17	3.2-4.2=3.7±.17	12.0-13.2=12.6±.23	90.3	9.7

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12.	*Nepeta leucolaena* Benth. ex Hook.f.	24.7-26.2=25.4±.24	23.7-28.2=25.4±.76	1	9.5-11.2=10.3±.32	5.7-6.7=6.2±.17	2.2-3.5=3.0±.20	9.7-11.2=10.4±.26	72.4	27.6
13.	*Nepeta linearis* Royle ex Benth.	34.0-38.0=35.9±.70	15.7-19.2=17.5±.591	2.05	11.2-13.2=12.3±.358	7.7-8.7=8.2±.176	3.7-5.0=4.4±.231	14.7-17.0=16.0±.382	80.5	19.5
14.	*Nepeta nervosa* Royle ex Benth.	23.7-27.2=25.7±.67	16.2-21.2=19.2±.84	1.33	8.7-9.7=9.2±.17	5.5-6.2=5.9±.12	3.7-5.2=4.5±.25	10.7-12.7=11.7±.33	86.1	13.9
15.	*Nepeta podostachys* Benth.	34.0-41.5=38.7±1.3	22.2-26.2=31.7±2.5	1.22	10.7-13.5=12.1±.49	6.5-8.0=7.2±.25	5.2-6.7=6.0±.25	9.5-11.2=10.2±.31	89.9	10.1
16.	*Nepeta praetervisa* Rech.f.	19.5-21.2=20.3±.34	17.0-18.5=17.7±.26	1.16	8.7-9.7=9.2±.17	5.7-6.5=6.1±.12	3.7-4.5=4.2±.14	8.0-9.0=8.5±.17	70.3	29.7
17.	*Nepeta raphanorhiza* Benth.	31.2-45.2=35.3±2.5	17.7-25.2=21.0±1.38	1.6	13.7-15.7=14.6±.33	7.5-8.7=7.9±.21	2.2-3.0=2.7±.13	10.0-13.7=12.0±.64	78.4	21.6
18.	*Nepeta schmidii* Rech.f.	24.2-25.7=24.9±.26	15.0-16.7=16.0±.31	1.5	8.7-10.7=9.8±.35	5.5-6.2=5.8±.12	4.2-5.5=4.8±.21	12.0-13.0=12.5±.17	82.4	17.6

Keywords: Min= Minimum, Max= Maximum, SE= Standard Error, P= Polar Diameter, E= Equatorial Diameter, % = percentage, µm= Micrometer

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Table. 3: Qualitative pollen micromorphological characters of *Nepeta* species.

S. no	*Nepeta* species	Pollen size	Pollen shape	Polar view (Amb)	Shape	Colpi apex	Symmetry	Aperture type	Aperture orientation	Lumina shape	No of secondary lumina per primary lumen	Aperture surface	Exine sculpturing
1.	*Nepeta cataria* L.	Medium	Oblate-spheriodal	Sub spherical	Spherical	Acute	Radial	Hexacolpate	Concave	Slightly elongated	Not clear	Verrucate	Reticulate perforate
2.	*Nepeta connata* Royle ex Benth.	Medium	Sub oblate	Spherical	Spherical	Acute	Radial	Hexazonocolpate	Concave	Elongated	Not clear	Granulate	Bireticulate perforate
3.	*Nepeta discolor* Royle ex Benth.	Small-Medium	Prolate	Spherical	Spherical	Round	Radial	Hexazonocolpate	Slightly convex	Elongated	10-20	Granulate	Bireticulate perforate
4.	*Nepeta distans Royle*	Large	Prolate	Sub spherical	Spherical	Acute	Radial	Hexacolpate	Concave	Circular-Oval	10-13	Gammate	Reticulate perforate
5.	*Nepeta elliptica* Royle ex Benth.	Medium	Sub oblate	Spherical	Spherical	Acute	Radial	Hexacolpate	Convex	Irregular	10-14	Gammate	Bireticulate perforate
6.	*Nepeta erecta* (Royle ex Benth.) Benth.	Medium	Spherical	Spherical	Spherical	Acute	Radial	Hexacolpate	Slightly convex	Circular	Not clear	Gemmate	Reticulate perforate
7.	*Nepeta govaniana* (Wall. ex Benth.) Benth.	Medium	Oblate-spheroidal	Sub Spherical	Irregular	Acute	Radial	Hexacolpate	Sunken	Oval	12-17	Verrucate	Reticulate perforate-verrucate
8.	*Nepeta gracili flora* Benth.	Medium	Sub oblate	Spherical	Spherical	Acute	Radial	Hexacolpate	Convex	Irregular	6-9	Granulate	Reticulate perforate
9.	*Nepeta griffithii* Hedge	Small-Me	Prolate	Spherical	Spherical	Acute	Radial	Hexacolpate	Slightly	Circular-Oval	5-8	Granulate	Reticulate

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		dium							convex				perforate
10.	*Nepeta hindostana* (B. Heyne ex Roth) Haines	Small-Medium	Prolate	Spherical	Spherical	Acute	Bilateral	Hexacolpate	Sunken	Irregular	6-10	Verrucate	Bireticulate perforate
11.	*Nepeta laevigata* (D. Don) Hand. -Mazz.	Small-Medium	Prolate	Broad shape	Rhomboid	Round	Bilateral	Hexazonocolpate	Bulged	_	_	Granulate	Reticulate
12.	*Nepeta leucolaena* Benth. ex Hook.f.	Medium	Spherical	Spherical	Sub Spherical	Acute	Radial	Hexazonocolpate	Slightly raised	Circular	10-15	Granulate	Reticulate perforate
13.	Nepeta linearis Royle ex Benth.	Small-Medium	Perprolate	Hexagonal	Ellipsoid	Acute	Radial	Hexazonocolpate	Sunken	Irregular	Not clear	Granulate	Fine reticulate perforate
14.	*Nepeta nervosa* Royle ex Benth.	Small-Medium	Subprolate	Spherical	Spherical	Acute	Radial	Hexazonocolpate	Sunken	Elongated	14-19	Granulate	Reticulate perforate
15.	*Nepeta podostachys* Benth.	Medium	Subprolate	Spherical	Spherical	Acute	Radial	Hexazonocolpate	Bulged	Irregular	15-18	Verrucate	Reticulate perforate
16.	*Nepeta praetervisa* Rech.f.	Small	Subprolate	Spherical	Spherical	Acute	Radial	Hexazonocolpate	Sunken	Circular-elongated	4-9	Granulate	Reticulate perforate
17.	*Nepeta raphanorhiza* Benth.	Medium	Prolate	Hexagonal-spherical	Spherical	Acute	Radial	Hexazonocolpate	Raised	Circular-Oval	Not clear	Granulate	Reticulate-perforate/ foveolate
18.	*Nepeta schmidii* Rech.f.	Small-Medium	Prolate	Subspherical	Spherical	Acute	Radial	Hexazonocolpate	Sunken	_	_	Verrucate	Reticulate

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Table. 4: Principal component analysis variable loadings for first seven pollen components.

Variables/ Factors	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Polar diameter	0.979	0.128	-0.024	-0.091	-0.072	-0.097	-0.047
Equatorial diameter	0.880	-0.451	-0.073	-0.047	-0.108	-0.025	0.057
Polar/equatorial ratio	-0.097	0.982	0.145	-0.032	-0.043	-0.026	0.034
Colpi length	0.960	0.086	-0.130	-0.098	0.207	-0.027	0.017
Colpi width	0.817	0.228	-0.295	0.438	-0.017	0.033	-0.005
Exine thickness	0.666	-0.127	0.718	0.157	0.025	0.010	-0.002
Mesocolpium	0.939	0.195	-0.012	-0.257	-0.042	0.115	-0.014
Eigenvalue	4.655	1.298	0.646	0.303	0.064	0.026	0.007
Variability (%)	66.506	18.541	9.233	4.335	0.917	0.367	0.101
Cumulative %	66.506	85.047	94.280	98.615	99.531	99.899	100.000

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Highlights

- *Nepeta* genus pollen micromorphology assists in species differentiation and classification, offering distinctive features for taxonomic purposes.
- Pollen analysis provides insights into the evolutionary history and relationships among *Nepeta* species, aiding in phylogenetic studies.
- The micromorphological data reveal information about pollinators, reproductive strategies, and adaptation to changing environments within the Nepeta genus.

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