

POLLEN MORPHOLOGY IN THE TRIBE *NIGELLEAE* (RANUNCULACEAE): A WORLDWIDE PALYNOLOGICAL INVESTIGATION INTO THE SPECIES

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The pollen morphology of many collections of taxa of the tribe *Nigelleae* from the family Ranunculaceae which occur worldwide is presented in this study. A total of 88 specimens from 21 taxa, some of which were recently proposed, belonging to the genera *Komaroffia*, *Garidella*, and *Nigella* of *Nigelleae* were examined using light microscopy (LM) and scanning electron microscopy (SEM). In the tribe, the pollen type is mostly trizonocolpate, but in many taxa and specimens, both trizonocolpate and non-trizonocolpate types occur together. The pollen grains are small to medium (25–53.75 µm × 20–55 µm) in size and oblate to prolate in shape. The exine pattern at the mesocolpium in all the taxa investigated is similar: micro-echinate in LM and micro-echinate-punctate in SEM. The colpus membrane in *Komaroffia* and *Nigella* is micro-echinate in both LM and SEM. In *Garidella*, it is micro-echinate in LM but echinate (spinulose) in SEM.

In this study, multivariate analyses, principal component analysis (PCA), and unweighted pair group method with arithmetic mean (UPGMA), were used to evaluate relationships between the genera and species within the tribe with respect to pollen morphology. PCA results show three main groups in the tribe: *Garidella*, *Komaroffia*, and *Nigella*. Moreover, the UPGMA tree also chiefly supports generic segregation into the smaller genera. An overall synthesis of the pollen characteristics of the three genera is provided and discussed.

Keywords: *Garidella*, *Komaroffia*, multivariate analysis, *Nigella*, Ranunculaceae, pollen morphology

INTRODUCTION

Komaroffia, *Garidella*, and *Nigella* are morphologically similar taxa that were recently accepted as three separate genera of the tribe *Nigelleae*, belonging to the family Ranunculaceae (Tamura, 1993; Strid, 1997). However, the previous taxonomic treatments of the taxa by several authors varied, and they were based only on morphological grounds (Terraciano, 1897–1898; Krasheninnikov, 1937; Davis, 1965; Meikle, 1977; Zohary, 1983; Riedl, 1992; Tutin and Akeroyd, 1993). In the present study, the recent and most comprehensive revision of the tribe by Dönmez et al. (under revision) is followed, in which *Komaroffia*, *Garidella*, and *Nigella* are evaluated separately in the tribe *Nigelleae*. The revision is based on the

studies of several aspects of the species worldwide, including taxonomy, pollen morphology, cytology, seed morphology, numerical analyses, and biochemistry.

The tribe *Nigelleae* comprises 18 species from the three genera. *Komaroffia* and *Garidella* are small genera; the former having been recently proposed to be represented by two species (Dönmez et al., under revision), while the latter is accepted to be represented by two species. The genus *Nigella* is represented by 14 species, including one newly accepted species, *N. koyuncui* (Dönmez et al., 2015) and three taxa proposed as varieties.

The members of the tribe mainly occur throughout the Mediterranean region and western Asia. *Nigella* populations also extend westward to central Europe and eastward to central Asia

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(Zohary, 1983; Tamura, 1993). Studies of chloroplast DNA variation in the populations of the so-called *Nigella arvensis* complex revealed the presence of three major lineages, with largely non-overlapping distributions in the western, central, and eastern Aegean region (Bittkau and Comes, 2005; Comes et al., 2008). The flowers of the genera are mainly insect-pollinated (Weber, 1993; Zaitoun et al., 2008; Munawar et al., 2009). Some species in the *N. arvensis* complex are known to be autogamous (Ellmer and Andersson, 2004).

Nigella has several species of ethnopharmacological interest (*N. arvensis*, *N. damascena*, and *N. sativa*), as their seeds have substances with pharmacological activities (Baytop, 1999; Kökdil et al., 2006; Moghaddasi, 2011; Heiss et al., 2013). *Nigella sativa* is locally cultivated in southwestern Asia, northern Africa, and in Europe. Its aromatic seeds are also used to flavor bread and cheese.

The pollen morphology of Ranunculaceae has received a great deal of attention by research workers (Erdtman et al., 1961; Santisuk, 1979; Fernandez, 1986; Clarke et al., 1991; Lee and Blackmore, 1992; Khan, 1994; Tatlıdil et al., 2005; Perveen and Qaiser, 2006; Penny and Steven, 2009; Xie and Li, 2012; Zhang et al., 2015; Humphrey, 2016). However, previous studies on pollen morphology of the closely related genera from the family, i.e., *Komaroffia*, *Garidella*, and *Nigella*, included only some species and a few specimens (Wodehouse, 1936; Aytuğ, 1971; Skvarla and Nowicke, 1979; Al-Eisawi, 1986; Clarke et al., 1991; Liang-qian, 1995; Qureshi et al., 2002; Oybak Dönmez and Işık, 2008; Takhtajan, 2009; Oberschneider et al., 2016).

The aim of this study was to investigate pollen morphology of the *Nigelleae* species on a global scale. The study was based on the examination of many specimens by light and electron microscopy together with size measurements and multivariate analyses.

MATERIALS AND METHODS

PLANT MATERIAL

The pollen material used in the present study was either collected by Ali A. Dönmez (AAD) in the field in several countries in Eurasia, including Cyprus, Greece, Syria, and Turkey or taken from the herbaria of Ankara University-Pharmacy Faculty (AEF) and Hacettepe University (HUB) in Turkey, Coimbra (COI) in Portugal, and Austria National Herbarium (W) in Vienna. The voucher specimens are listed in Supplementary material S1. The plant specimens collected from Turkey are all deposited at HUB. In many cases more than one specimen

per taxon was investigated to try to determine any possible intraspecific variation in pollen morphology, but for few taxa adequate material was not available to enable such investigation.

The species and the taxonomic order given in this study followed the recent revision of the tribe *Nigelleae* by Dönmez et al. (under revision). In this revision, there are new proposals for some taxa that are treated differently from those given in the previous studies: *Nigella bucharica* N. Schipcz. is named as *Komaroffia bucharica*, while *N. arvensis* L. subsp. *aristata* Nyman is treated as a variety, *N. arvensis* L. var. *aristata*. In addition, *Nigella latisecta* P.H. Davis and *N. lancifolia* Hub.-Mor. are proposed as varieties of *Nigella oxypetala*, *N. oxypetala* var. *latisecta*, and *N. oxypetala* var. *lancifolia*, respectively. The genus *Nigella* is also treated according to infrageneric classification of three sections, *Erobathos*, *Nigellaria*, and *Nigellastrum*, following De Candolle (1817, 1824) and Willkomm and Lange (1880).

A total of 88 specimens from the accepted and proposed 21 taxa were investigated: *Komaroffia* (2 species, 4 specimens), *Garidella* (2 species, 13 specimens), and *Nigella* (17 species, 71 specimens) (Table 1 in Supplementary material).

LIGHT MICROSCOPIC (LM) STUDIES

For LM observations, the pollen was first treated with 70% ethyl alcohol to remove oily substances, and then embedded in glycerin-jelly and stained with safranin to prepare pollen slides (Wodehouse, 1935). Photomicrographs were produced using a Leica DFC 320 digital camera connected to a Leica DM 4000 B microscope. To determine pollen types, two pollen slides for each sample were examined from traverses covering the whole slide. Size measurements were made on the most frequent trizonocolpate type pollen grains. The following parameters were measured: pollen size, given by the polar axis (P) and equatorial axis (E); exine (sexine and nexine) and intine thickness in the polar area; mesocolpium and margo; and colpus width (Clw) in the equatorial view. The colpus length (Clg) could not be measured in the equatorial view because the colpi extend to almost the full length of the grain. The apocolpium index (polar area index) was calculated as the ratio of the mean distance between the apices of two colpi to its equatorial diameter. The pollen size (P and E) was measured for 50 pollen grains per specimen. Pollen grains from < 20–40 µm were defined as 'small' and 41–55 µm as 'medium'. The other measurements were made on ten grains per specimen. The means (M), standard deviations (SD), and ranges (V) for P and E measurements as well as the means and ranges for the other

measurements are given in Table 1 (Supplementary material). All statistical analyses were run by PASW (SPSS) Statistics 17.0 and IBM Minitab 18.1.

SCANNING ELECTRON MICROSCOPIC (SEM) STUDIES

For SEM study, the pollen was first treated with 70% ethyl alcohol, then air-dried before being mounted on SEM specimen stubs and subsequently coated with gold. The photomicrographs were taken using a Zeiss EVO 50 EP electron microscope.

The clearest LM and SEM photographs representing the main pollen features were selected for this paper. The palynological terminology mainly follows Punt et al. (2007) and Hesse et al. (2009).

MULTIVARIATE DATA ANALYSES

Individual value plot analysis was utilized for the mean polar (P) and equatorial (E) axes, while principal component analysis (PCA) was utilized for the following pollen characteristics: the mean polar and equatorial axes, apocolpium index, and the mean length of colpus. Exine and intine size values were excluded from the analyses. Cluster analysis was also performed using the unweighted pair group method with arithmetic mean (UPGMA) method using Past 3.2 version (Hammer et al., 2001). The taxa and their acronyms are as follows: *Garidella nigellastrum* GN, *G. unguicularis* GU, *Komaroffia integrifolia* KI, *K. bucharica* KB, *Nigella arvensis* var. *arvensis* NAVA, *N. arvensis* var. *aristata* NAVAR, *N. koyuncui* NK, *N. stellaris* NS, *N. fumariifolia* NF, *N. sativa* NSA, *N. turcica* NT, *N. gallica* NG, *N. papillosa* subsp. *atlantica* NPVA, *N. segetalis* NSE, *N. damascena* ND, *N. elata* NE, *N. orientalis* NO, *N. oxypetala* var. *oxypetala* NOVO, *N. oxypetala* var. *lancifolia* NOL, *N. oxypetala* var. *latisecta* NOLA, and *N. ciliaris* NC.

RESULTS

GENERAL POLLEN CHARACTERISTICS OF THE TRIBE NIGELLEAE

Pollen grains occur as monads. The most frequent pollen type is trizonocolpate (with three equatorially situated colpi) (Table 1 in Supplementary material, Figs. 1a–n, 2a,c). In some taxa and specimens both trizonocolpate and non-trizonocolpate pollen grains occur together. Non-trizonocolpate pollen grains are represented by some pollen types, including zona-aperturate (with a ring-like aperture), dicolpate, and synaperturate with various forms of colpi/apertures (Figs. 1o–t, 2g,h).

Trizonocolpate pollen grains are radially symmetrical, isopolar, and small to medium in

size; the polar axis (P) measures 25–53.75 μm , and the equatorial axis (E) measures 20–55 μm . The pollen is usually prolate-spheroidal, occasionally subprolate in *Komaroffia*; usually subprolate and occasionally prolate-spheroidal in *Garidella*; and usually oblate-spheroidal, occasionally prolate-spheroidal, or subprolate in *Nigella*. The shape in polar view is angular in *Komaroffia* and *Garidella* (Figs. 1a,f), while it is circular (Fig. 1k) or angular in *Nigella*. The apocolpium index ranges from 0.19 to 0.41. In trizonocolpate pollen grains the colpi are usually long and 1–16.25 μm wide, the colpi ends are usually obtuse, occasionally acute; the margins are distinct and irregular. In LM, the colpus membrane is micro-echinate in all *Nigelleae* species (Figs. 1e,j,n); in SEM, it is echinate (spinulose) in *Garidella* (Fig. 2d), while it is micro-echinate in *Komaroffia* (Fig. 2b) and *Nigella* (Fig. 2e). The exine is thick; sexine thickness is 1–5 μm in the mesocolpium, 1.5–4 μm in the polar area, and 1–3 μm in the margo. The sexine is usually thinner than the adjacent mesocolpium and it is thicker than the nexine; the nexine thickness is 0.5–1 μm in the polar area, 0.5–1.5 μm in the mesocolpium, and 0.5–2 μm in the margo. In all taxa, the exine is tectate-columellate; in LM, the exine pattern is micro-echinate (Figs. 1b,d,g,i,n), and in SEM it is micro-echinate-punctate (perforate) (Fig. 2f). The intine is quite thin, < 1 or 1 μm thick, having almost the same thickness throughout the pollen.

POLLEN MORPHOLOGY IN KOMAROFFIA

Pollen grains are usually trizonocolpate (Table 1 in Supplementary material, Figs. 1a–e, 2a,b). In *K. integrifolia*, both trizonocolpate and non-trizonocolpate pollen grains occur. The pollen is small to medium in size; P 25–48 μm , E 23–49 μm . The pollen shape is prolate-spheroidal, with the exception of one specimen of *K. integrifolia* (from the province of Dschebel-Seradsch in Afghanistan by A. Gilli 827) which has subprolate pollen grains. The shape in polar view is angular; the apocolpium index is 0.23–0.26. The colpi are 5–14 μm wide; the colpi ends are obtuse; the margins are distinct, irregular; the colpus membrane is micro-echinate in LM and SEM. The exine is thick; the sexine thickness is 1.5–2.75 μm in the polar area, 1–2 μm in the mesocolpium, 0.9–1.75 μm in the margo; the sexine is thicker than the nexine, and the nexine thickness is 0.50–1 μm in the polar area, mesocolpium and margo. The exine is tectate-columellate; the exine pattern is micro-echinate in LM and micro-echinate-punctate in SEM. The intine is thin; it is < 1 μm or 1 μm thick, having almost the same thickness throughout the pollen.

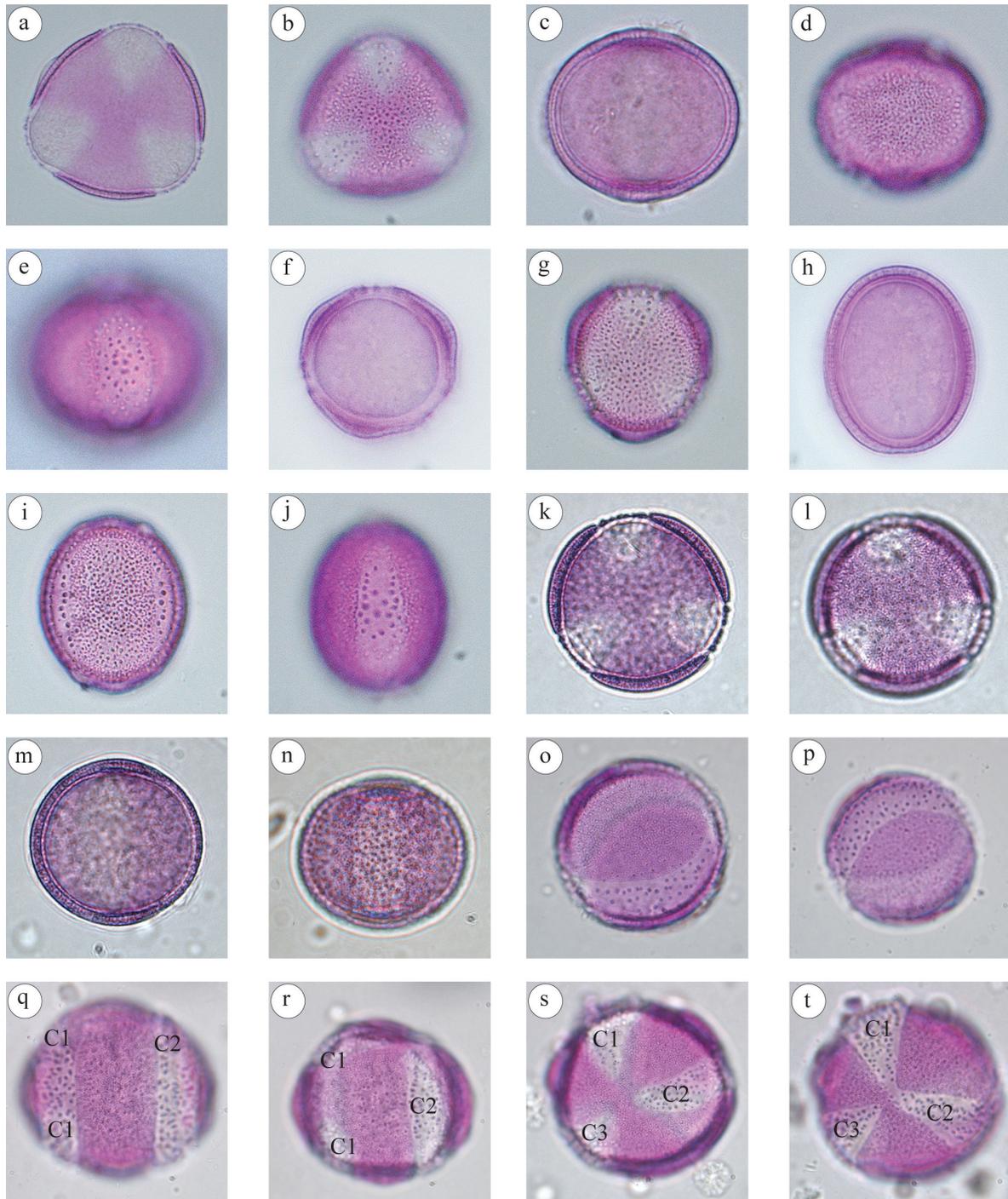


Fig. 1. Selected LM micrographs of pollen grains in the tribe *Nigelleae* (1000 \times). (**a–e**) trizonocolpate pollen in *Komaroffia integrifolia* (**a**) polar view, (**b**) micro-echinate exine pattern in polar area, (**c**) equatorial view in optical cross section, (**d**) micro-echinate exine pattern in mesocolpium, (**e**) colpus with micro-echinate membrane. (**f–j**) trizonocolpate pollen in *Garidella unguicularis*, (**f**) polar view, (**g**) micro-echinate exine pattern in polar area, (**h**) equatorial view in optical cross section, (**i**) micro-echinate exine pattern in mesocolpium, (**j**) colpus with micro-echinate membrane. (**k–n**) trizonocolpate pollen in *Nigella* (**k–l**; *N. arvensis* var. *aristata*) (**k**) polar view, (**l**) micro-echinate exine pattern in polar area, (**m**) equatorial view in optical cross section in *N. segetalis*, (**n**) micro-echinate exine pattern in mesocolpium and colpus membrane in *N. sativa*, (**o–t**) non-trizonocolpate in *N. orientalis*, (**o–p**) zona-aperturate pollen with a ring-like aperture at different foci, (**q–r**) dicolpate pollen at different foci (C – colpus, C1 and C2 represent two separate semi-circled colpi), (**s–t**) synaperturate pollen with two anastomosing colpi (C1, C2) and one free colpus (C3) at different foci.

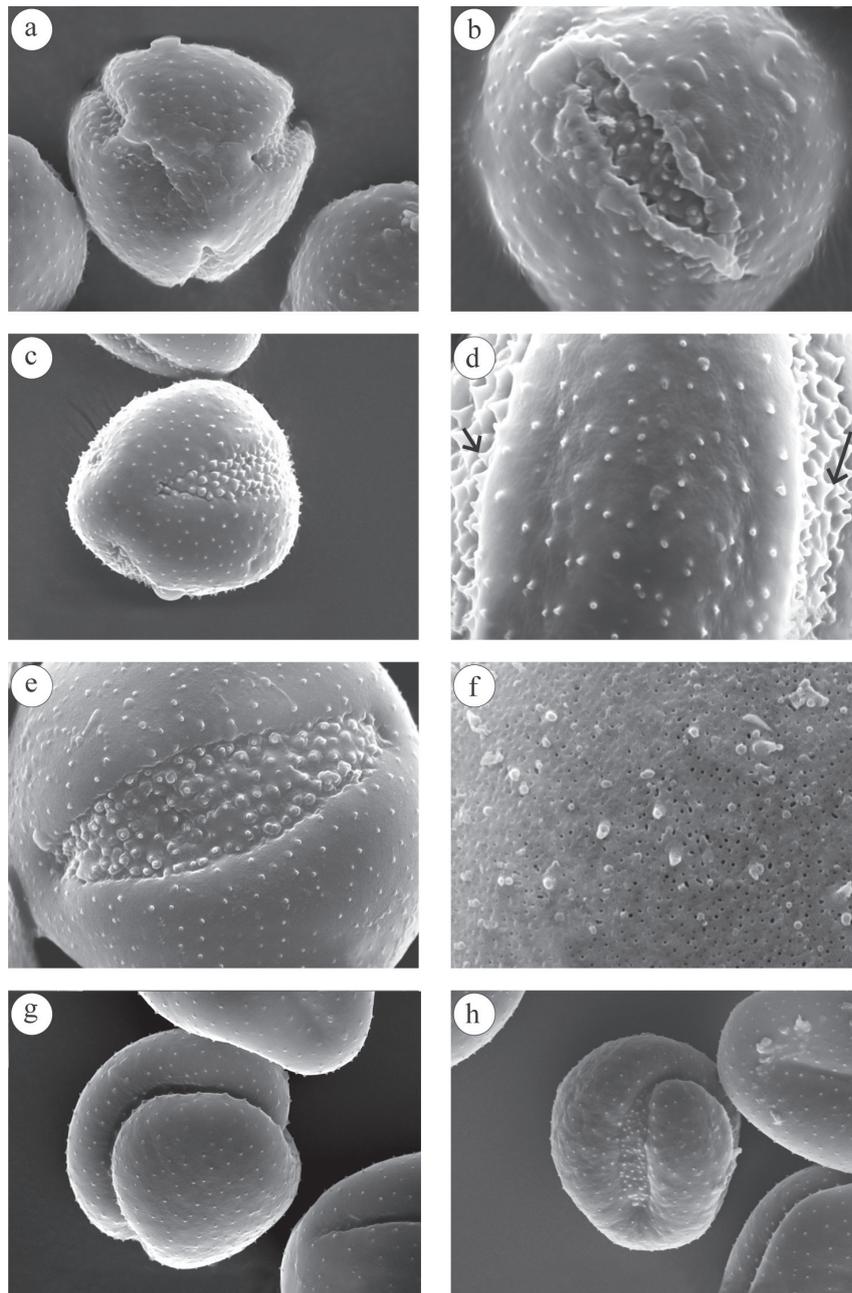


Fig. 2. Selected SEM micrographs of pollen grains in *Nigelleae*. (**a–f**) trizonocolpate pollen grains: (**a–b**) *Komaroffia integrifolia* (**a**) pollen in polar view (7330 \times), (**b**) micro-echinate colpus membrane (12080 \times), (**c–d**) *Garidella unguicularis* (**c**) pollen with echinate (spinulose) colpus membrane in polar view (7160 \times), (**d**) a closer view of echinate (spinulose) colpus membrane (arrowed) (17880 \times), (**e**) micro-echinate colpus membrane in *Nigella papillosa* subsp. *atlantica* (10000 \times), (**f**) micro-echinate-punctate exine pattern in mesocolpium in *N. arvensis* var. *aristata* (35000 \times), (**g–h**) non-trizonocolpate pollen grains in *N. elata* (**g**, 6100 \times and **h**, 4560 \times).

POLLEN MORPHOLOGY IN *GARIDELLA*

Pollen grains are usually trizonocolpate (Table 1 in Supplementary material, Figs. 1f–j, 2c–d). In one specimen of *G. nigellastrum* (from the province of Gazimagusa in Cyprus by AAD 15928) and two

specimens of *G. unguicularis* (from the province of Kahramanmaraş in Turkey by Z. Aytaç 4867-H. Duman and from the province of Damascus in Syria by AAD 15440), both trizonocolpate and non-trizonocolpate pollen grains occur. The pollen

is small in size; P 25–36.25 μm , E 20–32.5 μm . The pollen shape is subprolate, with the exception of one specimen of *G. nigellastrum* (from the province of Mersin in Turkey by AAD 15965) and of *G. unguicularis* (from the province of Damascus in Syria by AAD 15440), both of which have prolate-spheroidal pollen grains. The shape in polar view is angular; the apocolpium index is 0.24–0.36. The colpi are 6–10 μm wide; the colpi ends are obtuse; the margins are distinct, irregular; the colpus membrane is micro-echinate in LM and echinate (spinulose) in SEM. The exine is thick; the sexine thickness is 1.5–2.5 μm in the polar area and mesocolpium, 0.7–1.5 μm in the margo; the sexine is thicker than the nexine, and the nexine has almost the same thickness in the polar area (0.9–1 μm), mesocolpium and margo (0.5–1 μm). The exine is tectate-columellate; the exine pattern is micro-echinate in LM, micro-echinate-punctate in SEM. The intine is thin; it is < 1 μm or 1 μm thick, having almost the same thickness throughout the pollen.

POLLEN MORPHOLOGY IN NIGELLA

Pollen grains are usually trizonocolpate (Table 1 in Supplementary material, Figs. 1k–n, 2e–f). Both trizonocolpate and non-trizonocolpate (Figs. 1o–t, 2g–h) pollen grains occur in some specimens of many taxa (*N. damascena*, *N. elata*, *N. gallica*, *N. arvensis* var. *arvensis*, *N. arvensis* var. *aristata*, *N. segetalis*, *N. sativa*, *N. turcica*, *N. orientalis*, *N. oxypetala* var. *oxypetala*, *N. oxypetala* var. *latisecta*, *N. oxypetala* var. *lancifolia* and *N. ciliaris*) in the genus. The pollen is small to medium in size; P 25–53.75 μm , E 25–55 μm . The pollen shape is usually oblate-spheroidal. *N. damascena*, *N. gallica*, *N. papillosa* subsp. *atlantica* and *N. arvensis* var. *arvensis* have both

oblate-spheroidal and prolate-spheroidal pollen grains, while *N. fumariifolia* has both prolate-spheroidal and subprolate pollen grains. The shape is circular or angular in polar view; the apocolpium index is 0.19–0.41. The colpi are 1–16.25 μm wide; the colpi ends are usually obtuse, occasionally acute; the margins are distinct, irregular; the colpus membrane is micro-echinate in LM and SEM. The exine is thick; the sexine thickness is 2–4 μm in the polar area, 1–5 μm in the mesocolpium and 1–3 μm in the margo; the sexine is thicker than the nexine, and the nexine thickness is 0.5–1.5 μm in the polar area and mesocolpium, 0.5–2 μm in the margo. The exine is tectate-columellate; the exine pattern is micro-echinate in LM, micro-echinate-punctate in SEM. The intine is thin; it is < 1 μm or 1 μm thick, having almost the same thickness throughout the pollen.

EVALUATION OF MULTIVARIATE ANALYSES OF SOME POLLEN CHARACTERISTICS IN THE TRIBE NIGELLEAE

Individual value plot analysis shows that pollen grains of *Komaroffia* and *Garidella* are smaller than those of *Nigella* (Figs. 3a,b). The PCA result revealed affinities among the taxa; three main groups are clustered in the plot (Fig. 4). All *Nigella* taxa are loaded separately from the remaining taxa, while *Garidella* and *Komaroffia* are located closer to each other. Moreover, cluster analysis produced the phenogram with three groups with an insufficient bootstrap value (Supplementary material S2). However, *Garidella* and *Komaroffia* taxa are nested together with *N. arvensis* var. *aristata* and *N. sativa*. The other two groups included the remaining *Nigella* taxa with one individual of *Garidella nigellastrum*.

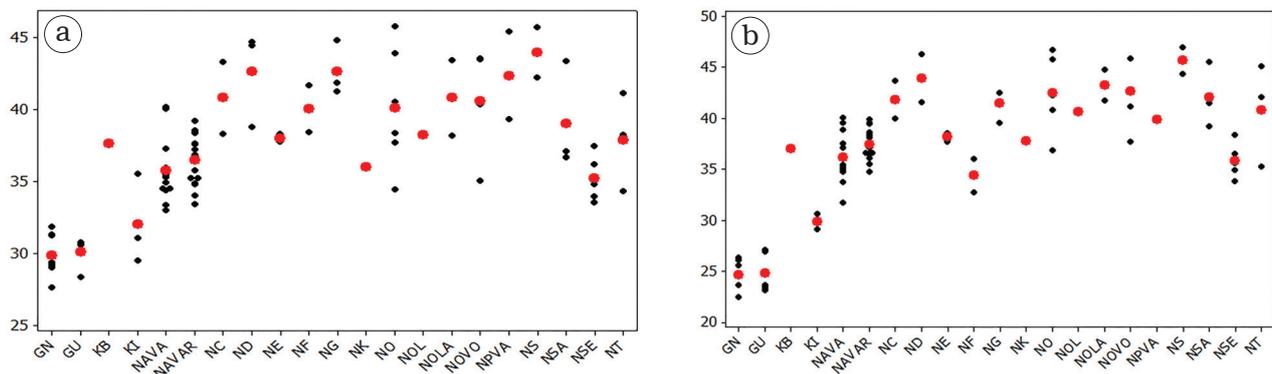


Fig. 3. Individual Value Plot of pollen polar axis (P) and equatorial axis (E) in *Nigelleae*. (a) individual value plot of polar axis (black dot = individual value of each value, red dot = mean value of polar axis), (b) individual value plot of equatorial axis (black dot = individual value of each value, red dot = mean value of equatorial axis).

characterized by phenotypic divergence, appear to be similar in their pollen characteristics.

In this study, it is revealed that many taxa and specimens from the species of the genera belonging to the tribe *Nigelleae* comprise other pollen types, along with trizonocolpate pollen within the same pollen sac. However, in several previous palynological studies, only the trizonocolpate pollen was recorded in the genera of the tribe (Wodehouse, 1936; Aytuğ, 1971; Al-Eisawi, 1986; Clarke et al., 1991). The co-occurrence of trizonocolpate and non-trizonocolpate pollen was noticed in several other genera of the family Ranunculaceae, such as *Actaea* L., *Adonis* L., *Anemone* L., *Caltha* L., *Clematis* L., *Ranunculus* L., and *Thalictrum* L. (Clarke et al., 1991; Tamura, 1993; Liang-qian, 1995; Humphrey, 2016). Pollen morphological variability, a fairly common phenomenon in modern angiosperms, may occur within the same individual and it may be related to several factors, such as functional specialization and ploidy within individuals (Borsch and Wilde, 2000). The aperture number, position, and form in pollen grains are the result of a series of developmental processes that comprise the type of microsporogenesis (related to the resulting tetrad shape), pollen polarity, and pollen wall formation (Furness and Rudall, 2004; Banks et al., 2007).

Based on the SEM studies, the pollen of *Komaroffia* and *Nigella* with a micro-echinate colpus membrane was distinguished from that of *Garidella* with a distinctly echinate (spinulose) colpus membrane. The exine is thick in all the genera. The presence of exinous structures on the colpus membrane in combination with the thick exine in the members of the genera would probably be an adaptive response to terrestrial conditions to protect the pollen protoplasm from dehydration since the species thrive mainly in dry habitats. They also probably function against the entry of pathogens into the apertures. They may also have a harmomegathic function, allowing expansion and contraction of the pollen grain in response to external conditions (Hesse et al., 2009).

With scanning electron microscopy, the exine pattern in the mesocolpium was shown to be micro-echinate-punctate in all the taxa within the tribe. It is widely accepted that elaborate exine surface patterns or pollen wall structures are associated with animal pollination (Chaloner, 1986; Hesse, 2000; Punt, 1986).

CONCLUSION

This study provided pollen data for a total of 88 specimens from 21 taxa belonging to the tribe *Nigelleae*, describing the characteristics of almost

all the taxa worldwide. We also described some intergeneric and intrageneric pollen variations in the tribe. In some species of the tribe, variability in the aperture form was recorded in single pollen sacs.

AUTHORS' CONTRIBUTIONS

The authors contributed in the following manner to the investigation of the subject. SI, EOD: pollen morphological studies, photographing, text writing and editing; ZUA: material collections in Turkey, taxonomic studies, evaluation of multivariate data analyses; AAD: field work and herbarium visits in Eurasia for extensive material collections, taxonomic studies, revision, and evaluation of multivariate data analyses. The authors declare that there are no conflicts of interest.

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REFERENCES

- AL-EISAWI DM. 1986. Pollen morphology of Ranunculaceae in Jordan. *Pollen et Spores* 28(3-4): 311-328.
- AYTUĞ B. 1971. *İstanbul Çevresi Bitkilerinin Polen Atlası*. İstanbul Üniversitesi Orman Fakültesi Yayınları, İstanbul.
- BANKS H, STAFFORD P AND CRANE PR. 2007. Aperture variation in the pollen of *Nelumbo* (Nelumbonaceae). *Grana* 46 (3): 157-162.
- BAYTOP T. 1999. *Türkiye'de Bitkiler ile Tedavi*. Nobel Tıp Kitapevleri, İstanbul.
- BITTKAU C, AND COMES HP. 2005. Evolutionary processes in a continental island system: molecular phylogeography of the Aegean *Nigella arvensis* alliance (Ranunculaceae) inferred from chloroplast DNA. *Molecular Ecology* 14: 4064-4083.
- BORSCH T, AND WILDE V. 2000. Pollen variability within species, populations, and individuals, with particular reference to *Nelumbo*. In: Harley MM, Morton CM, Blackmore S [ed.], *Pollen and spores: morphology and biology*, 285-299. Royal Botanical Gardens, Kew.
- CHALONER W. 1986. Electrostatic forces in insect pollination and their significance in exine ornament. In: Blackmore S,

- Ferguson IK [ed.], *Pollen and spores: form and function*, 103–108. Academic Press, London.
- CLARKE GCS, PUNT W, AND HOEN PP 1991. Ranunculaceae. In: Punt W, Blackmore S [ed.], *The Northwest European pollen flora VI*, 117–271. Elsevier Science Publishers, Netherlands.
- COMES HP, TRIBSCH A, AND BITTKAU C. 2008. Plant speciation in continental island floras as exemplified by *Nigella* in the Aegean Archipelago. *Philosophical Transactions of the Royal Society* 363: 3083–3096.
- CORNEANU GC, POPESCU M, SITORIS A, SIMDANU VD, AND POPRUCU C. 1987. Researches concerning the biology of the *Nigella* L. species (Ranunculaceae) from Romania. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 16: 151–163.
- DAVIS PH. 1965. *Nigella* L. (Ranunculaceae). In: Davis PH [ed.], *Flora of Turkey and the East Aegean islands*, vol 1, 98–105. Edinburgh University Press, Edinburgh.
- DE CANDOLLE AP. 1817. Règne végétabilis systema naturale. *Treuttel et Würtz, Paris* 1–3: 25–333.
- DE CANDOLLE AP. 1824. Prodromus systematics naturalis regni vegetabilis. *Treuttel et Würtz, Paris* 1: 48–50.
- DÖNMEZ AA, DEMİRCİ B, OYBAK DÖNMEZ E, AND UĞURLU Z. 2011. *Nigella* L. (Ranunculaceae) türleri üzerinde taksonomik ve kimyasal araştırmalar. Proje No 107 T 6862, TÜBİTAK [Taxonomical and chemical studies in *Nigella* L. (Ranunculaceae) species. Project No 107 T 6862, The Scientific and Technical Research Council of Turkey], Ankara.
- DÖNMEZ AA, UĞURLU Z, AND IŞIK S. 2015. A new species of *Nigella* (Ranunculaceae) from Northeastern Turkey. *Novon: A Journal for Botanical Nomenclature* 23(4): 411–415.
- ERDTMAN G, BERGLUND B, AND PRAGLOWSKI J. 1961. An introduction to a Scandinavian pollen flora. *Grana Palynologica* 2(2): 3–92.
- ELLMER M, AND ANDERSSON S. 2004. Inbreeding depression in *Nigella degenii* (Ranunculaceae): Fitness components compared with morphological and phenological characters. *International Journal of Plant Sciences* 165(6): 1055–1061.
- FERNANDEZ I. 1986. Contribución al conocimiento palinológico de la familia Ranunculaceae en Andalucía. II. Subfam. Ranunculoideae. *Candollea* 41(1): 75–85.
- FURNESS CA, AND RUDALL PJ. 2004. Pollen aperture evolution – a crucial factor for eudicot success? *Trends in Plant Science* 9(3): 154–159.
- HAMMER Ø, HARPER DAT, AND RYAN PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9.
- HEISS AG, STIKA H-P, DE ZORINI N, AND JURSA M. 2013. *Nigella* in the mirror of time: A brief attempt to draw a genus' ethnohistorical portrait. In: von Carnap-Bornheim C, Dörfler W, Kirleis W, Müller J, Müller U [ed.], *von Sylt bis Kastanas. Festschrift für Helmut Johannes Kroll*, 147–169. Wachholtz Verlag, Neumünster.
- HESSE M. 2000. Pollen wall stratification and pollination. In: Dafni A, Hesse M, Pacini E [ed.], *Pollen and pollination*, 1–17. Springer, Wien.
- HESSE M, HALBRITTER H, ZETTER R, WEBER M, BRUCHNER R, FROSCH-RADIVO A, AND ULRICH S. 2009. *Pollen Terminology: An Illustrated Handbook*. Springer, Wien.
- HUMPHREY RP. 2016. Pollen heteromorphism is pervasive in *Thalictrum* (Ranunculaceae). *Plant Systematics and Evolution* 302(8): 1171–1177. DOI: 10. 1007/s00606-016-1312-8
- IPCN (The Index to Plant Chromosome Numbers) 2011. www.tropicos.org/Project/IPCN.
- KHAN HA. 1994. Studies in the pollen morphology of Indian Ranunculaceae. *Journal of Palynology* 30: 51–72.
- KÖKDİL G, İLÇİM A, ÖZBİLGİN B, AND UYGUN C. 2006. Morphology and stem anatomy of some species of genus *Nigella* L. in Turkey. *Journal of Faculty of Pharmacy of Ankara* 35(1): 19–41.
- KRASHENINNIKOV IM. 1937. *Nigella* L. (Ranunculaceae). In: Komarov VL, Shishkin BK [ed.], *Flora URSS*, vol 7, 50–57. Botanical Institute of the Academy of Science of the USSR, Israel Program for Scientific Translations, Keter Press Binding, Jerusalem.
- LEE S, AND BLACKMORE S. 1992. A palynotaxonomic study of the genus *Trollius* (Ranunculaceae). *Grana* 31: 81–100.
- LIANG-QIAN LI. 1995. The geographical distribution of subfamily Helleboroideae (Ranunculaceae). *Acta Phytotaxonomica Sinica* 33(6): 537–555.
- MEIKLE RD. 1977. *Nigella* L. (Ranunculaceae). In: Meikle RD [ed.], *Flora Cyprus*, vol 1, 60–64. The Bentham-Moxon Trust, Royal Botanic Gardens, Kew.
- MOGHADDASI MS. 2011. *Nigella sativa* traditional usages (Black Seed). *Advances in Environmental Biology* 5(1): 5–16.
- MUNAWAR MS, SARWAR G, RAJA S, AND WAGHCHOURE ES. 2009. Pollination by honeybee (*Apis mellifera*) increases seed setting and yield in black seed (*Nigella sativa*). *International Journal of Agriculture and Biology* 11: 611–615.
- OBERSCHNEIDER W, WEBER M, AND HALBRITTER H. 2016. *Nigella arvensis*. In: PalDat – A Palynological Database. http://www.paldat.org/Nigella_arvensis/301726.
- OYBAK DÖNMEZ E, AND IŞIK S. 2008. Pollen morphology in Turkish *Nigella* L. (Ranunculaceae). *Abstracts of the Twelfth International Palynological Congress (IPC-XII) and Eighth International Organisation of Palaeobotany Conference (IOPC-VIII)*, 30 August–5 September 2008, 214. Bonn, Germany.
- PENNY RH, AND STEVEN JC. 2009. Sexual dimorphism in pollen grain size in cryptically dioecious *Thalictrum macrostylum*. *Plant Systematics and Evolution* 279: 11–19.
- PERVEEN A, AND QAISER M. 2006. Pollen Flora of Pakistan – L. Ranunculaceae. *Pakistan Journal of Botany* 38(3): 499–509.
- PUNT W. 1986. Functional factors influencing pollen form. In: Blackmore S, Ferguson IK [ed.], *Pollen and spores: form and function*, 97–101. Academic Press, London.
- PUNT W, HOEN PP, BLACKMORE S, NILSSON S, AND LE THOMAS A. 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143: 1–81.
- RIEDL H. 1992. *Nigella* L. (Ranunculaceae). In: Iranshahr M, Rechinger KH, and Riedl H [eds.], *Flora Iranica* 171, 24–34. Akademische Druck- und Verlagsanstalt, Graz, Austria.
- SANTISUK T. 1979. A palynological study of the tribe Ranunculaceae. *Opera Botanica* 48: 1–74.
- SKVARLA JJ, AND NOWICKE JW. 1979. The morphology of the exine in *Nigella* (Ranunculaceae). *American Journal of Botany* 66 (2): 162–165.

- STRID A. 1997. *Nigella* L. In: Strid A, and Tan K [ed.], *Flora Hellenica*, vol 2, 3–13. Koeltz Scientific Books, Germany.
- TAKHTAJAN A. 2009. *Flowering plants*. Second Edition, Springer Verlag+Business Media, www.springer.com.
- TATLIDİL S, BIÇAKÇI A, MALYER H, AND BAŞER KHC. 2005. Pollen morphology of *Thalictrum* L. species (Ranunculaceae) in Turkey. *Pakistan Journal of Botany* 37(2): 203–212.
- TAMURA M. 1993. Ranunculaceae. In: Kubitzki K, Rohwer JG, Bitrich V [ed.], *The families and genera of vascular plants*, vol 2, 563–583. Springer, Heidelberg.
- TERRACCIANO A. 1897–1898. Revisione monographica della species del genere *Nigella*. *Bollettino del R. Orto Botanico Di Palermo* 1: 122–153 (1897); 2: 19–43 (1898).
- TUTIN TG, AND AKEROYD JR. 1993. *Nigella* L. (Ranunculaceae) In: Tutin TG, Burges NA, Chater AO, Edmondson JR, Heywood VH, Moore DM, Valentine DH, Walters SMD, Webb DA [ed.], *Flora Europaea*, vol 1, 251–253. University Press, Cambridge.
- QURESHI SJ, KHAN MA, MOHAMMAD T, BANO S, ARSHAD M, AND RASHID A. 2002. Palynological studies of some problematical medicinal plants of Pakistan. *Hamdar Medicus* 45(3): 75–92.
- WEBER A. 1993. Struktur, Antheseverlauf und Bestäubung der Blüte von *Nigella arvensis* (Ranunculaceae). *Verhandlungen der Zoologisch-Botanischen Gesellschaft in Österreich* 130: 99–125.
- WILLKOMM HM, AND LANGE J. 1880. Prodrömus Florae Hispanicae seu Synopsis Methodica omnium Plantarum in Hispania Sponte Nascentium vel Frequentius Cultarum quae Innotuerunt Auctoribus. *Stuttgartiae* 3: 963–965.
- WODEHOUSE RP. 1935. *Pollen grains*. Mc Grew Hill, New York.
- WODEHOUSE RP. 1936. Pollen grains in the identification and classification of plants VII. The Ranunculaceae. *Bulletin of the Torrey Botanical Club* 63(9): 495–514.
- XIE L, AND LI L-Q. 2012. Variation of pollen morphology, and its implications in the phylogeny of Clematis (Ranunculaceae). *Plant Systematics and Evolution* 298(8): 1437–1453.
- ZAITOUN S, AL-GHZAWI AA, SAMARAH N, AND ALQUDAH A. 2008. Comparative study yield and flowers attractivity to bee visitors between *Nigella sativa* L. and *Nigella damascena* L. (Ranunculaceae) grown in semiarid conditions. *Italian Journal of Agronomy* 2: 125–130.
- ZAKHARYEVA OI. 1985. Chromosome numbers of some flowering plants from the Caucasus and Middle Asia. *Botanicheskii Zhurnal SSSR* 70: 1699–1701. (In Russian).
- ZHANG Y, HONG Y, REN C, TANG M, HOOT SB, AND YANG Q-E. 2015. Palynology, cytology, and molecular systematics of *Anemone* section *Begoniifolia* (Ranunculaceae). *Plant Systematics and Evolution* 301: 411–424.
- ZOHARY M. 1983. The genus *Nigella* (Ranunculaceae), taxonomic revision. *Plant Systematics and Evolution* 142: 71–107.