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Research Article

Comparative anatomy and pollen morphology of two endemic *Noccaea* species (Brassicaceae) and their taxonomic significance

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Abstract

This study was conducted for extensive and systemic investigation of anatomical, palynological and seed morphological properties of two endemic *Noccaea* species, naturally growing in Turkey. Independent sample T-test and box plot were carried out using quantitative characters of the studied species. The anatomical results showed that the species had similar characteristics, though there were significant differences in root cortex cells and trachea; stem epidermis, cortex cell and endodermis; leaf upper and lower epidermis, lower cuticle, mesophyll and palisade parenchyma. Pollen grains of two endemic species were observed as radially symmetric, isopolar, with tricolpate aperture, prolate pollen shape and had small size. Pollen surface ornamentation was micro-reticulate in both species. Considering palynological characters, equatorial axis, AMB exine and intine have taxonomic importance. Seeds of *N. birolmutlui* were ovate to orbicular in shape and orange-brown in color, with colliculate ornamentation; however, the seeds of *N. camlikensis* were ovate to oblong in shape and brown and shiny in color, with colliculate-reticulate ornamentation.

Keywords: box plot; endemic; *Noccaea*; pollen; Trkiye

Introduction

Brassicaceae is a large angiosperm family of plant kingdom, represented by a total of 348 genera and 4065 species distributed worldwide in all continents except for Antarctica (Appel and Al-Shehbaz, 2003; Al-Shehbaz *et al*., 2006; Özüdoğru *et al*., 2019). It is primarily concentrated in temperate regions, particularly the Mediterranean basin and southwest and central Asia (Kandemir *et al.,* 2017). The family Brassicaceae includes several agronomic and economic species, widely cultivated species, forage crops and medicinal plants (Koch and Mummenhoff, 2006).

Genus *Noccaea* Moench is a systematically problematic genera within the Brassicaceae family, and discussions about the generic circumscription of this genus still continue (Özüdoğru, 2017). Just because of the problems related to the systematics of the genus, several taxonomic studies have been carried out on the classification of the *Noccaea* (Al-Shehbaz, 2012; Al-Shehbaz, 2014; Al-Shehbaz *et al.,* 2006; Özgişi *et al.,* 2018a). In these studies, researchers mainly used morphological characters such as flower characteristics (leaves and fruit shapes) to constrain the generic circumscription of *Noccaea* species (Özgişi *et al.,* 2020). Özüdoğru *et al.,* (2019) investigated the phylogenetic analysis of the tribe Coluteocarpeae using ITS DNA sequences and

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plastidic trnL-F regions. The Brassicaceae family has 93 genera and 583 taxa in flora of Turkey (Al-Shehbaz *et al.*, 2007). In Turkey, the genus *Noccaea* is represented by 51 taxa according to Al-Shehbaz (2014), while Mutlu (2012) reported that it was represented by 16 species based on the delimitation by Warwick *et al*. (2006).

The Brassicaceae are quite complex with minor characteristic differences. When there are problems in terms of distinctive macromorphological characters, palynological, anatomical and karyological characters are occasionally used for systematic purposes to correct general and specific constraints (Özgişi *et al*., 2018b).

There are several studies conducted on pollen morphology of Brassicaceae family. Orcan and Binzet (2003) studied palynological and anatomical features of *Alyssum obtusifolium* DC. Pinar *et al*. (2009) analyzed pollen and seed morphology of *Hesperis* L. (Brassicaceae) in Turkey. Mutlu and Erik (2012) examined the pollen morphology of the genus *Arabis* L. (Brassicaceae) in Turkey. Karaismailoğlu and Erol (2019) investigated pollen morphology of some taxa of *Thlaspi* L. in Turkey. Erden and Menemen (2021) investigated pollen morphology of some taxa of Brassicaceae family in Turkey. However, only two palynological studies were found on *Noccaea* taxa examining pollen morphology of *Noccaea elegans* Al-Shehbaz and *N. cilicica* Al-Shehbaz. Bülbül *et al.,* (2022) analyzed palynological features of *Noccaea aghrica* Fırat and Özüdoğru.

Similarly, there are many studies on the anatomical features of the taxa of the Brassicaceae family. Çilden and Zare (2019) studied the anatomy of *Iberis* taxa grown in Turkey in detail and thus tried to to solve the complexity of taxonomical uncertainties of the genus. Çitak and Dural (2020) investigated anatomy of the vegetative and reproductive parts of Turkish *Iberis* L. species. Karaismailoğlu (2020) investigated in detail the petiole anatomy of 21 representatives of the *Alysseae* tribe. Karaismailoğlu and Erol (2020) studied anatomical characteristics of stems and leaves of 19 taxa of *Thlaspi* sensu lato. Mohamed *et al*. (2021) studied the flower anatomy of some Brassicaceae species. However, any anatomical studies about *Noccaea* taxa has not been encountered.

Many systematist agree that the macro and micro properties of seeds are crucial for the classification of the taxa in the Brassicaceae. In addition, many research have shown that the macro and micro characters of seeds have systematic importance (Bona, 2013; Moazzeni *et al.*, 2007; Özüdoğru *et al.*, 2016; Farouji *et al.*, 2018; Yılmaz-Çıtak and Dural, 2020).

Both *Noccaea birolmutlui* Özgişi & Özüdoğru and *Noccaea camlikensis* Aytaç, Nordt & Parolly are endemic Turkish species encountered in southwest of Turkey. *N. birolmutlui* species is distributed clearings in *Cedrus libani* Rich forest of Isparta Kızıldağ National Park. *N. birolmutlui* is distinguished from the closest species by various morphological characters such as basal and cauline leaves, fruit shape and number of ovules. *N. camlikensis* grows on dry, sun-exposed serpentine, partly clearings of *Pinus nigra* Businský forest. *N. camlikensis* differs from the closely related species in terms of leaves and carpological characters.

The purpose of the study was: i) to determine anatomical, palynological and micromorphological characteristics of endemic *Noccaea birolmutlui* and *Noccaea camlikensis* and ii) to shed light on the taxonomic significance of these characters in the *Noccaea*.

Materials and Methods

In this study, the plant specimens were collected from their natural distribution areas: *Noccaea birolmutlui* -Turkey. B3 Isparta: Şarkikaraağaç; Kızıldağ National Park, 1400-1500 m, clearings in *Cedrus libanii* forest, 18.05.2019, Aksoy 2298, B. Atasagun (Akdeniz University Herbarium). *Noccaea camlikensis* - Turkey. C3 Konya: Derebucak; Çamlık village, Kızıldağ, 1400-1500 m, clearings in *Pinus nigra* forest, 18.05.2019, Aksoy 3001, B. Atasagun (Akdeniz University Herbarium).

For anatomical studies, plant specimens were preserved in bottles containing 70% alcohol. Root, stem and leaves were cut into small pieces and paraffin method was applied (Johansen, 1940). Cross-sections of 10-

15 µm thick were taken from paraffin blocks by means of a microtome. Sectioned samples were stained with safranin-fast green method and turned into a fixed preparation using entellan. The best sections were selected and photographed using the Leica DM750 light microscope.

Table 1. Comparison of the anatomical features of the studied species

			Width (µm)			Length (µm)		
			Min.	Max.	Mean±SD	Min.	Max.	Mean±SD
N. birolmutlui	Root	Periderm	20.66	88.88	49.54±15.43			
		Cortex	12.17	64.6	25.29±11.4	5.61	29.96	16.58±6.61
		Trachea	2	19.5	8.72±5.23			
		Pith rays	2	5	3.53±0.97			
	Stem	Epidermis	12.38	36.19	24.69±5.11	11.42	24.76	17.77±3.5
		Cortex	7.14	38.57	23.61±7.72	6.42	35	17.28±5.51
		Trachea	2.14	30.71	11.88±6.28			
		Endodermis	22.85	65.21	38.9±11.61	9.52	27.61	17.04±4.94
		Pith	8.163	52.24	34.09±9.65			
	Leaf	Upper Epidermis	14.08	71.52	40.63±12.62	9.72	54.86	28.73±8.84
		Lower Epidermis	14.56	59.02	28.35±10.89	13.19	31.94	22.92±4.27
		Mesophyll	47.88	259.1	190.41±45.96			
		Palisade P.	7.511	25.69	14.41±3.62	31.25	69.48	50.19±8.55
		Upper Cuticle	2.11	141.1	9.58±24.89			
		Lower Cuticle	1.041	5.63	3.28±1.31			
N. camlikensis	Root	Periderm	29.16	96	49.57±15.89			
		Cortex	13.88	82.6	37.02±20.05	12.03	50.72	22.83±10.32
		Trachea	7.246	28.98	17.43±6.41			
		Pith rays	2	8	3.43±1.41			
	Stem	Epidermis	5.71	26.66	17.04±5.68	7.61	22.85	14.69±4.11
		Cortex	16.19	51.42	27.88±7.34	6.66	19.04	13.43±2.72
		Trachea	4.76	22.85	12.19±4.19			
		Endodermis	18.09	47.61	28.26±7.29	8.57	25.71	16.41±4.3
		Pith	11.42	54.28	35.47±10.32			
	Leaf	Upper Epidermis	14.28	64.76	32.08±13.49	9.52	50	24.78±9.62
		Lower Epidermis	13.33	45.71	24.32±8.3	7.61	34.28	19.42±8.21
		Mesophyll	79.04	210	162.5±35.05			
		Palisade P.	7.142	17.85	13.83±3.54	23.57	42.14	36.04±4.41
		Upper Cuticle	1.22	6.17	3.9±1.52			
		Lower Cuticle	1.22	12.38	4.63±3.06			

Pollen grains were obtained from dried herbarium specimens. Pollen slides were prepared according to the methods described by Wodehouse (1935) and then examined under the Leica DM750 light microscope. Approximately, 30 pollen grains were measured for each character. For the SEM study, dry pollen grains were transferred to stubs and coated with gold-palladium. LEO 440 digital scanning electron microscope was used for the examination. The pollen terminology of Punt *et al*. (2007) and Hesse *et al*. (2009) was followed.

For seed micromorphological investigations, mature seeds collected from natural populations of plants were used. Micromorphological examinations were performed with the use of Leica EZ4HD microscope and scanning electron microscope (LEO 440). For micromorphological characters, the terminology of Stearn (1985) was followed.

At least 30 measurements were taken from all quantitative characters to compare root, stem, leaf anatomy and pollen characters. Maximum, minimum, mean and standard deviations were determined (Tables 1-2). All statistical analyses were performed with the use of R 4.0.2 software. Box plots were drawn to present

quantitative measurements of root, stem and leaf characteristics of each species. Independent samples t-Test was performed to evaluate the significance of anatomical and palynological characters.

Table 2. Comparison of the palynological features of the studied species (P: polar axis, E: Equatorial axis, clt: colpus width, clg: colpus length, t: apocolpium, L=AMB: diameter of pollen at the polar view)

Species	Characteristics	Min.	Max.	Mean	SD
N. birolmutlui	**P (µm)**	13.36	16.00	14.76	0.65
	E (µm)	9.13	12.52	11.13	0.88
	P/E	1.18	1.53	1.33	0.10
	clg (µm)	10.05	13.86	11.74	1.26
	clt (µm)	1.89	4.86	3.24	0.81
	t (µm)	2.79	7.66	4.91	1.04
	L=AMB (µm)	10.79	16.13	13.63	1.53
	Exine (µm)	0.81	1.64	1.13	0.25
	Intine (µm)	0.48	1.01	0.69	0.12
N. camlikensis	**P (µm)**	13.8	18.02	15.66	0.96
	E (µm)	9.94	13.65	11.81	0.88
	P/E	1.16	1.51	1.33	0.09
	clg (µm)	9.27	14.97	11.09	1.39
	clt (µm)	2	4.59	3.01	0.71
	t (µm)	3.04	6.26	4.60	0.90
	L=AMB (µm)	10.37	18.06	14.98	2.14
	Exine (µm)	0.83	2.31	1.19	0.28
	Intine (µm)	0.63	0.978	0.82	0.10

Results

N. birolmutlui

Root

There is a periderm approximately 50 µm thick as the outermost protective tissue. Phellem cells consist of 4-5 rows of rectangular shaped cells. However, in the root sections, phellogen and phelloderm cells could not be clearly distinguished. The cortex parenchyma has rectangular-oval-shaped cells of 10-15 rows. The size of parenchymatic cells is 12.17-64.6 × 5.61-29.96 µm. After the cortex, there is a central cylinder consisting of phloem and xylem. Under the cortex, there is a vascular cylinder consisting of phloem and xylem. Cambium is clearly indistinguishable. The size of trachea cells of xylem is 2-19.5 µm. Pith rays consist of a series of 2-5 rows of cells. The xylem tissue has expanded to fill the pith region as well (Figure 1A-B).

Stem

Epidermis cells have assumed the role of protective tissue. The epidermis consists of a single-row of rectangular or oval-shaped cells measuring 12.38-36.19 × 11.42-24.76 µm. Under the epidermis, there are oval-shaped cortex parenchyma cells with abundant chloroplasts consisting of 4-6 layers. The size of parenchymatic cells is 7.14-38.57 × 6.42-35 µm. A single-row endodermis cells can easily be distinguished. In the vascular tissue, phloem and xylem elements can be distinguished, and xylem elements are quite well-developed. The diameter of trachea cells is 2.14-30.71 µm. The pith has a wide cavity and the pith cells are composed of parenchymatic cells (Figure 1C).

Leaf

There is an epidermis layer consisting of single-row, regularly arranged and polygonal cells on both surfaces. The surface of both epidermal cells is surrounded by the cuticle layer. The upper epidermis cells are larger than the lower epidermis cells. The sizes of the upper epidermis cells are 14.08-71.52 × 9.72-54.86 µm,

and the sizes of the lower epidermis cells are 14.56-59.02 × 13.19-31.94 µm. The mesophyll tissue is 47.8-259.10 µm thick. Mesophyll tissue consists of two types of cells: palisade and spongy parenchyma (bifacial). The size of the palisade parenchyma cells is 7.51-25.69 × 31.25-69.48 µm. The portion of the mesophyll occupied by the palisade parenchyma is 47-69%. Beneath palisade parenchyma, there are 2 rows of sponge parenchyma with excess intercellular spaces. The vascular bundles are collateral and the middle ones are larger than the others (Figure 1D).

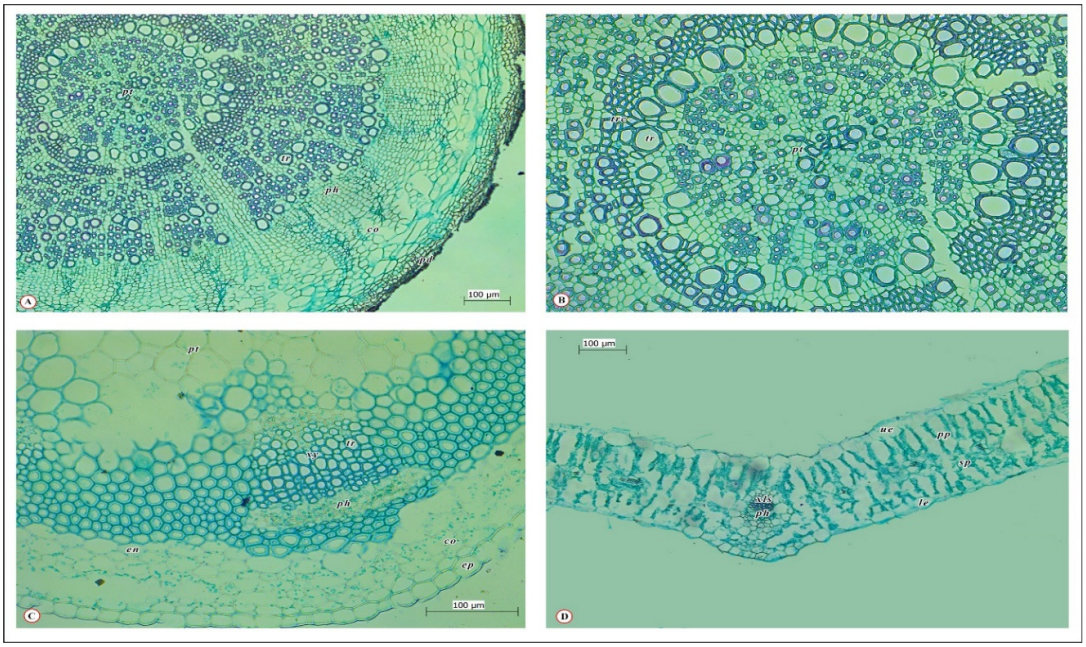

Figure 1. *N. birolmutlui,* Cross-section of the **A.-B.** Root **C.** Stem **D.** Leaf (pd: periderm, co: cortex, en: endodermis, ph: phloem, xls: xylem, tr: trachea, trc: tracheid, pt: pith, ep: epidermis, ue: upper epidermis, le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma)

N. camlikensis

Root

The outermost surface of the root consists of a multilayered periderm as a protective tissue. The thickness of the periderm layer is approximately 50 µm. In the periderm layer, phellogen and phelloderm cells could not clearly be distinguished. Under the periderm, there is a parenchymatic cortex with 10-20 rows of rectangular-oval cells. The size of the cortex parenchyma cells is 13.88-82.6 × 12.03-50.72 µm. Just under the cortex, there is a well-developed vascular cylinder consisting of phloem and xylem. Between the phloem and xylem, there is a vascular cambium with several layers of thin-walled and oblong shaped cells. Tracheal cells are 7.24-28.98 µm in size. The pith rays consist of 2-8 rows of parenchymatic cells. The pith is filled with xylem elements (Figure 2A-B).

Stem

The stem consists of the epidermis, cortex and vascular cylinder and a pith region in the center. The epidermis is single-layered, with oval-oblong cells and is covered with a thin layer of cuticula. The size of the epidermis cells is 5.71-26.66 × 7.61-22.85 µm. Beneath the epidermis layer, there is a cortex composed of 5-8 rows of parenchymatic cells. The cortex cells are 16.19-51.42 × 6.66-19.04 µm in size. The vascular bundles consisting of phloem and xylem are surrounded by 3-4 layers of sclerenchyma. Tracheal cells are well-developed and 4.76-22.85 µm in diameter. In the innermost part, there is a pith region composed of oval-shaped parenchymatic cells (Figure 2C).

Leaf

The abaxial and adaxial surfaces of the leaves are composed of flat or polygonal shaped single-row epidermis cells. The lower epidermis cells are 13.33-45.71 × 7.61-34.28 µm and the upper epidermis cells are 14.28-64.76 × 9.52-50 µm in size. The leaf is equifacial with a well-developed palisade parenchyma under both the lower and upper epidermis. Sponge parenchyma is quite scarce. The palisade parenchyma cells are 7.14-17.85 × 23.57-42.14 µm in size. Palisade parenchyma covers 51-77% of the mesophyll. The vascular bundle (collateral type) in the midvein is larger than the others (Figure 2D).

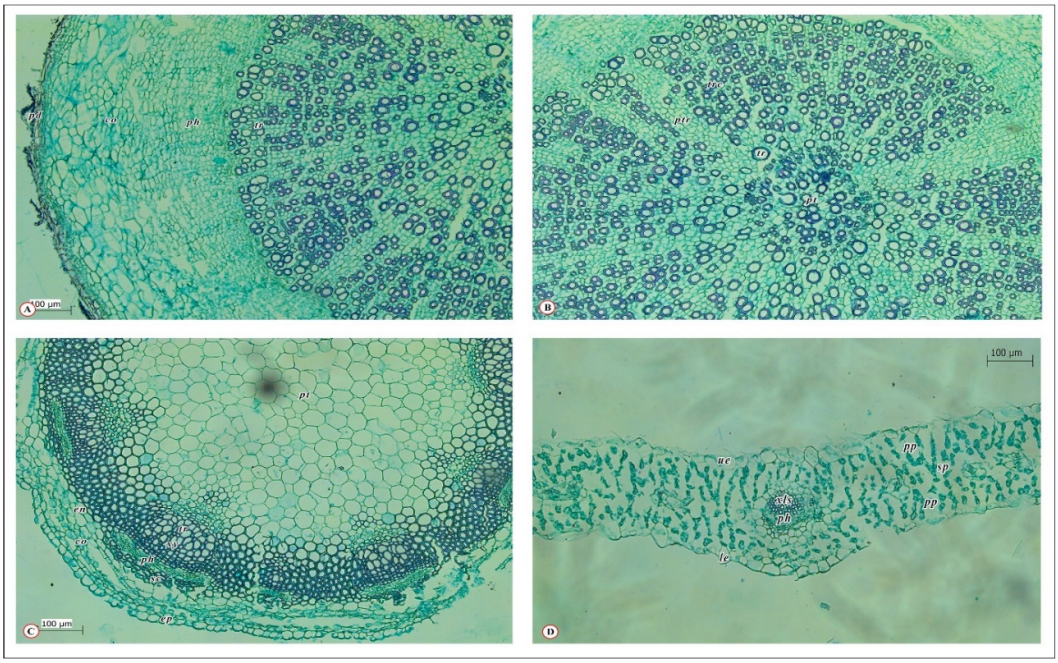

Figure 2. *N. camlikensis,* Cross-section of the **A.**-**B.** Root **C.** Stem **D.** Leaf (pd: periderm, co: cortex, en: endodermis, ph: phloem, xls: xylem, tr: trachea, trc: tracheid, pt: pith, ep: epidermis, ptr: pith rays, sc: sclerenchyma, ue: upper epidermis, le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma)

Pollen morphology

The pollen grains of *N. birolmutlui* are tricolpate, isopolar and radially symmetrical. Pollen shape is prolate, small sized, polar axis is 14.76±0.65 µm, and the equatorial axis is 11.13±0.88 µm, P/E 1.33. AMB is circular and 13.63 ± 1.53 µm. Colpus length is 11.74-±1.26 µm and colpus width is 3.24-±0.8 µm. The exine and intine thickness are measured as 1.13 µm and 0.69 µm, respectively. The ornamentation of exine is identified as micro-reticulate (Figure 3, Table 2).

The pollens of *N. camlikensis* are radial symmetrical, tricolpate and isopolar. Polar axis (P) is 15.66 ± 0.96 µm, equatorial axis (E) is 11.81±0.88 µm, P/E 1.33. The pollen shape is confirmed as prolate and small sized. The pollens are circular in polar view, and the AMB is 14.98±2.14 µm. Colpus length (Clg) is 11.09±1.39 µm and colpus width (Clt) is 3.01±0.71 µm. Exine is 1.19±0.28 µm and intine is 0.82±0.10 µm long. Pollens have micro-reticulate type surface ornamentation (Figure 4, Table 2).

Seed morphology

Seeds of *N. birolmutlui* are ovate to orbicular, 1.29- 1.57×0.86-1.22 mm in size and orange-brown in color. Epidermal cells are irregularly pentagonal. Surface ornamentation is colliculate (Figure 5).

Seeds of *N. camlikensis* are ovate to oblong, 1.70-2.38×1.11-1.81 mm in size and brown and shiny in color. Epidermal cells are irregularly pentagonal to elliptic. Cell surface ornamentation is colliculate-reticulate and beaked (Figure 6).

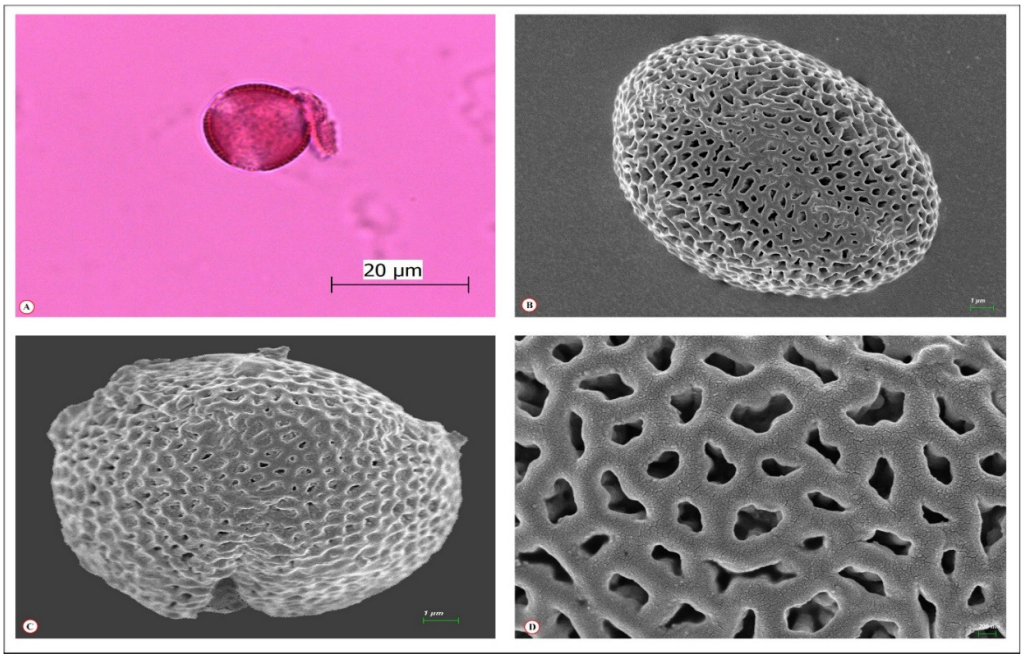

Figure 3. *N. birolmutlui* **A.** General view of pollen grain using LM. **B.** Equatorial view of pollen in SEM. **C.** Polar view of pollen in SEM. **D.** Structure of the pollen surface in SEM

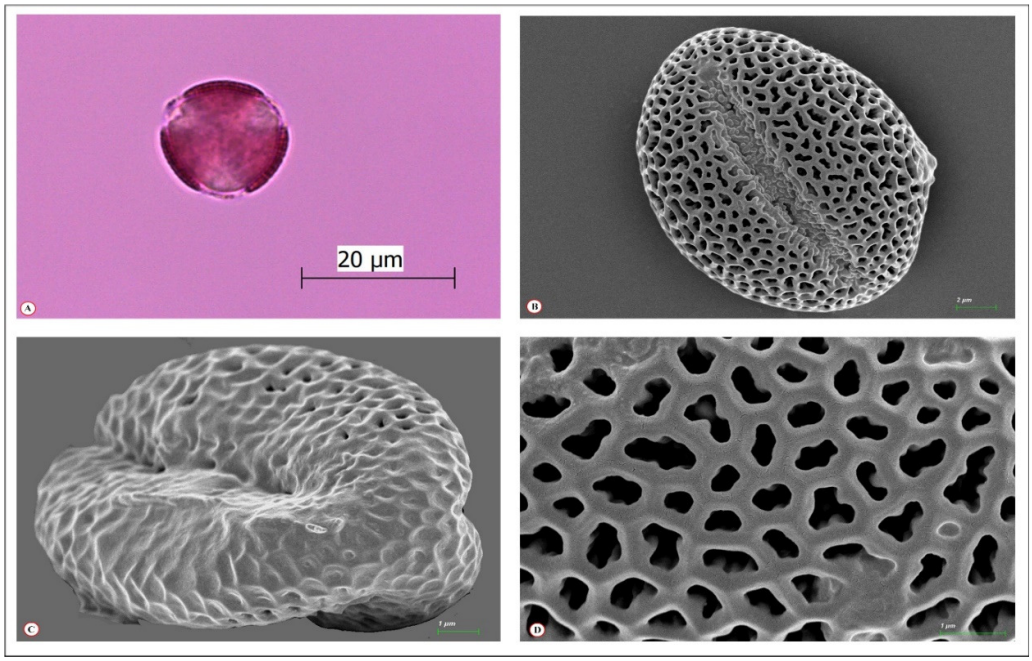

Figure 4. *N. camlikensis* **A.** General view of pollen grain using LM. **B.** Equatorial view of pollen in SEM. **C.** Polar view of pollen in SEM. **D.** Structure of the pollen surface in SEM

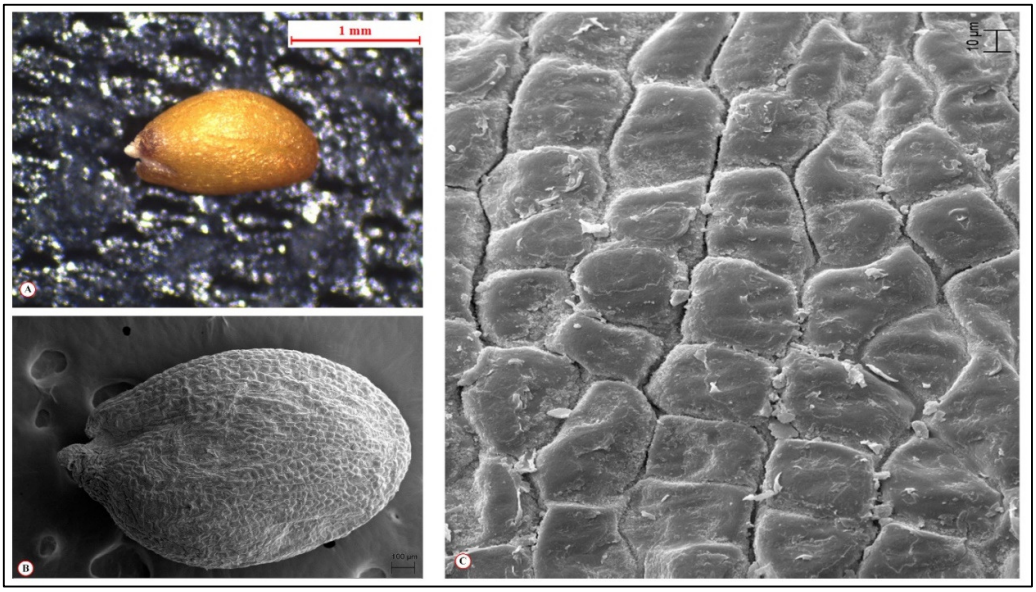

Figure 5. *N. birolmutlui* **A.** Seed general view in LM. **B.** General view in SEM. **C.** Seed surface in SEM

Figure 6. *N. camlikensis* **A.** Seed general view in LM. **B.** General view in SEM. **C.** Seed surface in SEM

Statistical analysis

Present independent sample T-test revealed that *N. birolmutlui- N. camlikensis* were significantly different from each other in terms of the cortex parenchyma row, cortex cell length and width and diameter of trachea in the cross-section of root. As for stem characters, *N. birolmutlui- N. camlikensis* were significantly different from each other in terms of epidermis width and length, cortex width and length, endodermis width. *N. birolmutlui- N. camlikensis* were significantly different from each other in terms of upper epidermis width, lower epidermis length, lower cuticle thickness, mesophyll cells width and palisade parenchyma cells length in the cross-section of leaf (Figures 7-9, Table 3).

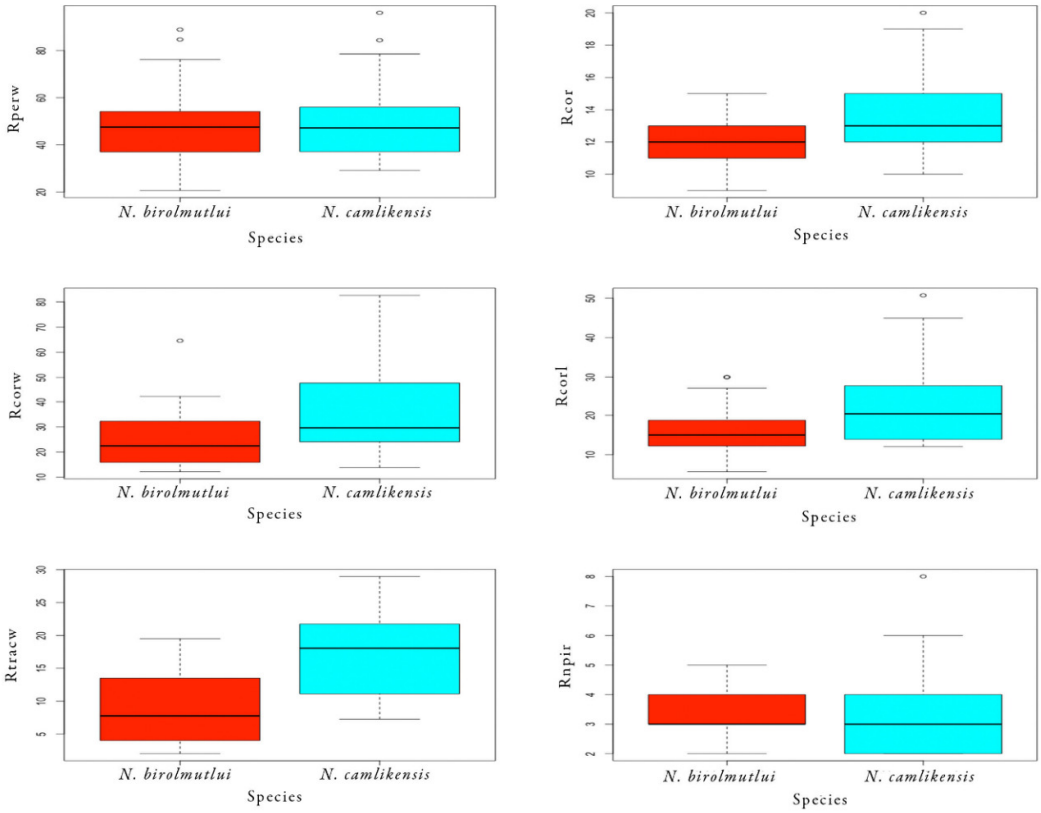

Figure 7. Box Plots of examined root characters (Rperw: peridermis cell width, Rcor: cortex parenchyma row, Rcorw: cortex cell width, Rcorl: cortex cell length, Rtracw: trachea width, Rnpir: number of pith rays)

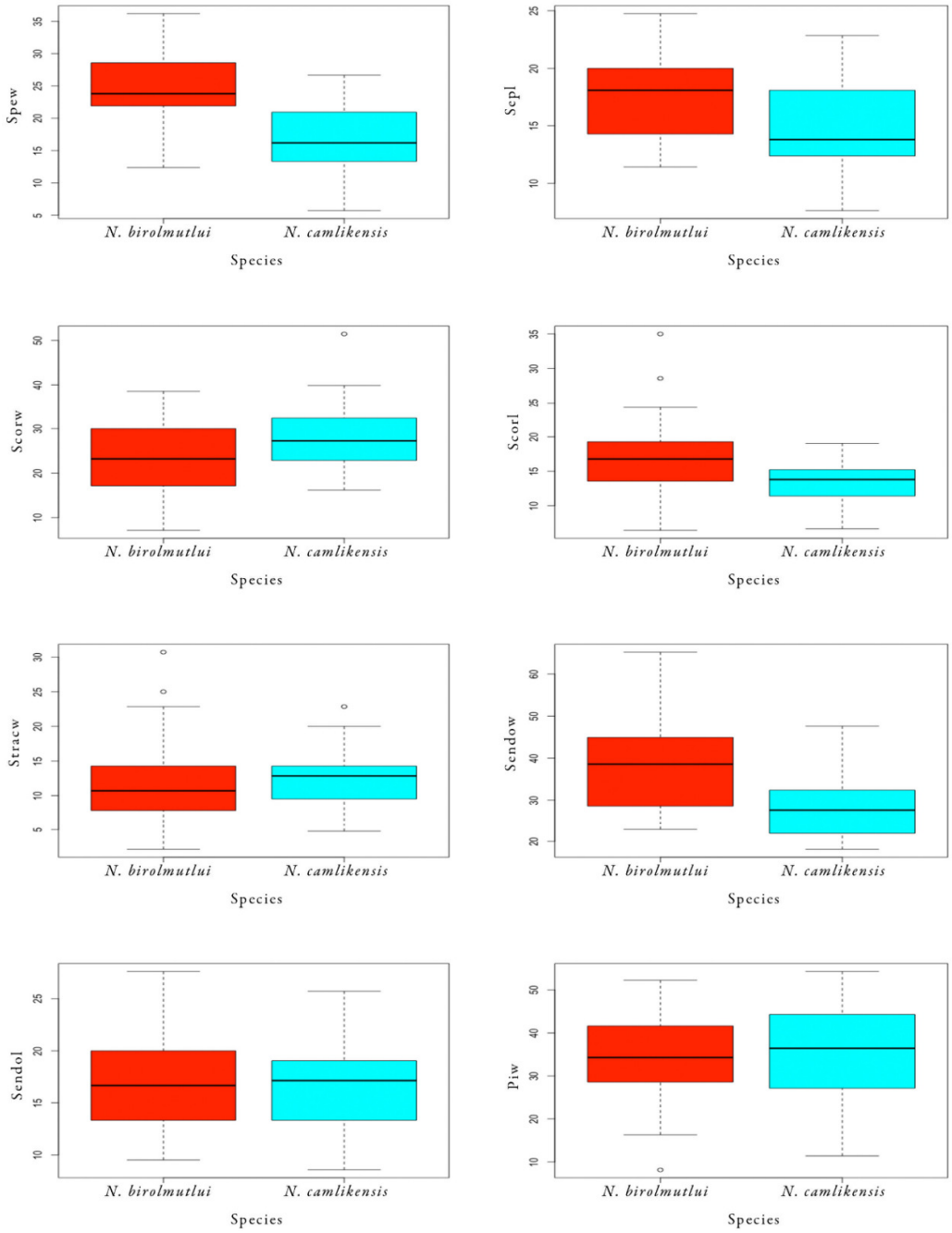

Figure 8. Box Plots of examined stem characters (Sepw: epidermis cell width, Sepl: epidermis cell length, Scorw: cortex cell width, Scorl: cortex cell length, Stracw: trachea width, Sendow: endodermis width, Sendo: endodermis length, Piw: pith cell width)

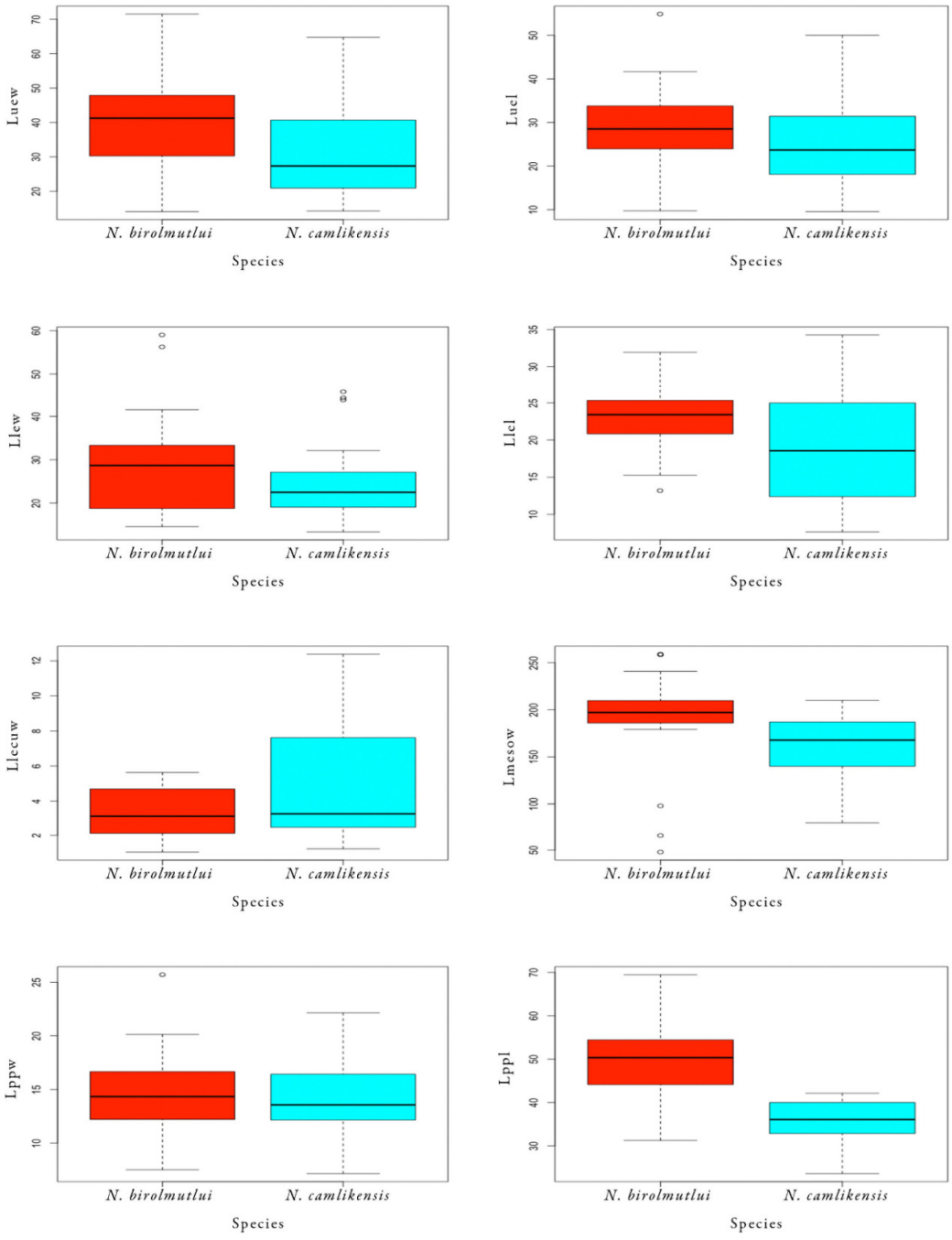

Figure 9. Box Plots of examined leaves characters (Luew: upper epidermis width, Luel: upper epidermis length, Llew: lower epidermis width, Llel: lower epidermis length, Llecuw: lower cuticula thickness, Lmesow: mesophyll cells width, Lppw: palisade parenchyma cells width, Lppl: palisade parenchyma cells length)

Table 3. Independent Samples t-Test according to anatomical features in the studied species

		N. birolmutlui-N. camlikensis
Root	Rperw	P>0.05 NS
	Rcor	P< 0.05 *
	Rcorw	P< 0.05 *
	Rcorl	P< 0.05 *
	Rtracw	P< 0.05 *
	Rnpir	P>0.05 NS
Stem	Sepw	P< 0.05 *
	Sepl	P< 0.05 *
	Scorw	P< 0.05 *
	Scorl	P< 0.05 *
	Stracw	P>0.05 NS
	Sendow	P< 0.05 *
	Sendol	P>0.05 NS
	Piw	P>0.05 NS
Leaf	Luew	P< 0.05 *
	Luel	P>0.05 NS
	Llew	P>0.05 NS
	Llel	P< 0.05 *
	Luecuw	P>0.05 NS
	Llcuw	P< 0.05 *
	Lmesow	P< 0.05 *
	Lppw	P>0.05 NS
	Lppl	P< 0.05 *

(Rperw: periderm cell width, Rcor: cortex parenchyma row, Rcorw: cortex cell width, Rcorl: cortex cell length, Rtracw: trachea width, Rnpir: number of pith rays); NS = non-significant. * Significant at the level of 0.05. Sepw: epidermis cell width, Sepl: epidermis cell length, Scorw: cortex cell width, Scorl: cortex cell length, Stracw: trachea width, Sendow: endodermis width, Sendo: endodermis length, Piw: pith cell width, Luew: upper epidermis width, Luel: upper epidermis length, Llew: lower epidermis width, Llel: lower epidermis length, Llecuw: lower cuticula thickness, Lmesow: mesophyll cells width, Lppw: palisade parenchyma cells width, Lppl: palisade parenchyma cells length). NS = non-significant. * Significant at the level of 0.05.

When evaluated in terms of palynological characters, *N. birolmutlui- N. camlikensis* were significantly different from each other in terms of equatorial axis, L=AMB (equatorial diameter), exine and intine thickness (Figure 10, Table 4).

Table 4. Independent Samples t-Test according to palynological features in the studied species

	N. birolmutlui-N. camlikensis
P	P>0.05 NS
E	P< 0.05 *
P/E	P>0.05 NS
clg	P>0.05 NS
clt	P>0.05 NS
t	P>0.05 NS
AMB	P< 0.05 *
Exine	P< 0.05 *
Intine	P< 0.05 *

(P: polar axis, E: Equatorial axis, clt: colpus width, clg: colpus length, t: Apocolpium, L=AMB: diameter of pollen at the polar view)

Box plot results complied with the t-test results. Mean trends for anatomical characters (cortex and trachea of the root; epidermis, cortex and endodermis of stem; epidermis, lower cuticle, mesophyll and palisade parenchyma of leaves) and palynological characters (equatorial axis, AMB, exine and intine) of taxonomic pairs were significantly different (Figures 7-10, Table 3-4).

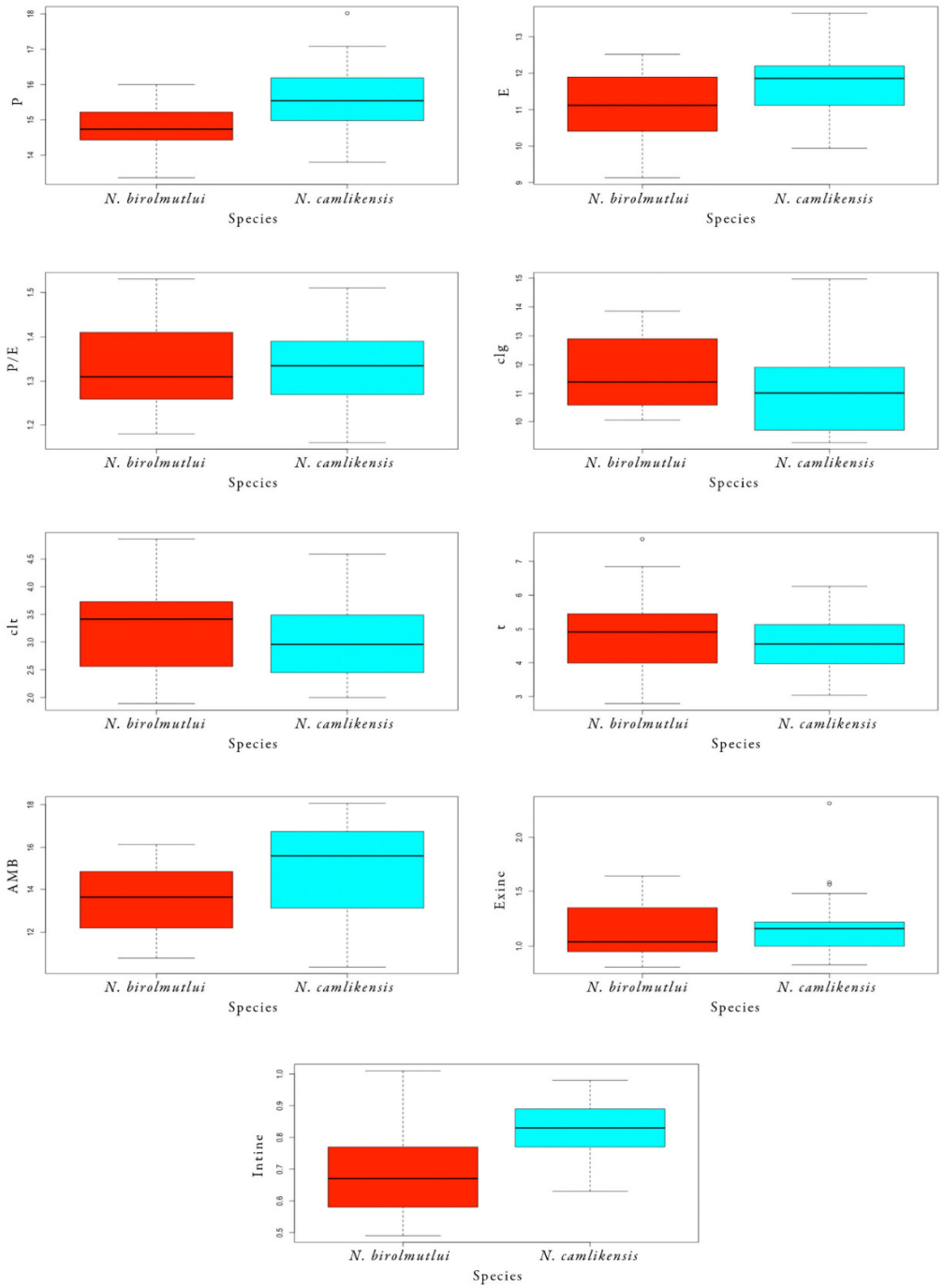

Figure 10. Box Plots of examined palynological characters (P: polar axis, E: equatorial axis, clt: the width of colpus, clg: the length of colpus, t: apocolpium, AMB: the measurements of polar view of pollen grains)

Discussion

Noccaea is systematically complicated and these problems have not been fully resolved yet. Especially in recent years, in addition to taxonomic features, anatomy, pollen morphology and seed micromorphology have been used extensively for generic delimitation of the Brassicaceae family (Farouji *et al*., 2018; Antkowiak *et al*., 2018; Çilden and Zare, 2019).

In present study, anatomical, palynological and seed micromorphological features of *N. birolmutlui* and *N. camlikensis* species were investigated. The taxonomic use of anatomical characters is useful for distinguishing closely related taxa within the Brassicaceae (Metcalfe and Chalk, 1957). However, the taxonomic use of these characters is quite limited (Yılmaz-Çıtak and Dural, 2020; Gönen *et al*., 2019; Karaismailoglu, 2019). There is no available data on the leaf, root and stem anatomy of the genus *Noccaea* and this study is the first report on the anatomy of *N. birolmutlui* and *N. camlikensis*. The root cross-section of *N. birolmutlui* and *N. camlikensis* have a similar secondary structure in terms of multi-layered periderm, cortex, phloem, xylem and pith region. However, there were some minor differences among the studied species. The average size of cortex parenchyma, tracheal cells of *N. camlikensis* were larger than *N. birolmutlui*.

In the stem sections, rounded, semirounded, rectangular, circular or irregular stem shapes were observed in the Brassicaceae family (Atçeken *et al*., 2016; Orcan and Binzet, 2003; Qader, 2018). The stem shape of the investigated *Noccaea* species was circular. *N. birolmutlui* and *N. camlikensis* share the similar stem anatomical features, characterized by a single-layered epidermis as the outermost protective tissue, the cortex parenchyma containing abundant chloroplasts, a well-developed vascular cylinder and the innermost pith. Similar results were also found in studies with the other genera of the Brassicaceae family (Metcalfe and Chalk, 1957; Karaismailoglu and Sirin, 2020; Karaismailoğlu and Osman, 2020; Yıldırım *et al*., 2021).

The upper cuticle layer of *N. birolmutlui* leaves is thicker than *N. camlikensis*. The epidermis cells on both sides of *N. birolmutlui* are larger than *N. camlikensis*. The mesophyll tissue is thicker in *N. birolmutlui* than in *N. camlikensis*. While bifacial leaves were observed in *N. birolmutlui*, the equifacial leaves were observed in *N. camlikensis*. The equifacial leaves are often a characteristic feature of xerophytic plants. In addition, palisade parenchyma in mesophyll covers more area in *N. camlikensis* (61.4%) than *N. birolmutlui* (58.7%). Other anatomical measurements given in Table 1 were similar in the species studied. Metcalfe and Chalk (1957) previously reported that the bifacial mesophyll was the most common in the Brassicaceae family, but in later studies, it was commonly observed in unifacial and equifacial types (Akbar and Begum, 2020; Yılmaz-Çıtak and Dural, 2020; Karaismailoğlu and Erol, 2020).

The box plots and T-test analysis revealed that there were significant differences in root, number of parenchymal cell layers in the cortex, size of cortex cells, and trachea diameter of two species. These root anatomical characters can be used to identify species with morphological characters. According to the stem anatomy results, the size of the epidermis cells, the size of parenchymal cells in the cortex and width of endodermis cells are taxonomically significant characters. As for the leaf characters, the width of the upper epidermis cells, the length of the lower epidermis cells, thickness of the lower epidermis cuticle, thickness of the mesophyll and the length of the palisade parenchyma cell have taxonomic importance. Other quantitative characters were not sufficient to discriminate the species of *N. birolmutlui- N. camlikensis*.

The pollen characteristics can provide useful information for the classification and identification of some closely-related taxa of the Brassicaceae family (Farouji *et al*., 2018; Karaismailoğlu and Erol, 2019; Özgişi *et al*., 2018b). Erdtman (1952) stated that Brassicaceae was a unipalynous family with pollen grains tricolpate, usually prolate to subprolate, oblate to oblate-spheroidal, or prolate-spheroidal and granulate or reticulate exine ornamentation. Erdtman *et al*. (1961) reported 2 pollen types as reticulate and granulate according to the exine structure of Brassicaceae. Khalik *et al*. (2002) divided the Brassicaceae family into 3 pollen types according to the size of lumina. Similarly, Khalik *et al*. (2002) point out that the family Brassicaceae had tricolpate grains

and reticulate exine. When *N. birolmutlui and N. camlikensis* pollens are evaluated in this context, they were classified as reticulate according to Erdtman *et al*. (1961) and type I (exine micro-reticulate) according to Khalik *et al*. (2002). Özgişi *et al*. (2018b) stated that the pollen grains of two endemic *Noccaea* were the tricolpate type, spheroid/suboblate shape and exine semitectate- reticulate ornamentation. Bülbül *et al*. (2016) reported that *Noccaea aghrica* pollens were isopolar, prolate-spheroidal, trizonocolpate and reticulate. In present study, *Noccaea* pollen grains were isopolar, radially symmetrical and tricolpate. The pollen shape was prolate and exine sculpture was micro-reticulate in both species. The palynological features of *N. birolmutlui* and *N. camlikensis* coincide the general characteristics of the Brassicaceae pollens.

T-test analysis and box plots for palynological characters revealed equatorial axis, AMB, and thickness of exine and intine as taxonomically significant characters. These palynological characters can be used as an additional tool for identification accurate species

The seed micromorphology is widely used for taxonomic problems and to observe species relationships in Brassicaceae (Koul *et al*., 2000). Present investigations of seed morphology showed that the seeds of *N. camlikensis* were larger than the seeds of *N. birolmutlui*, and the seed of *N. camlikensis* was ovate to oblong in shape, while that of *N. birolmutlui* was ovate to orbicular. In addition, the seed surface sculpture of *N. birolmutlui* was colliculate whereas that of *N. camlikensis* is colliculate-reticulate. In previous studies, it was stated that the dominant type of seed surface sculpture in Brassicaceae was reticulate (Moazzeni *et al.,* 2007; Tantawy *et al.,* 2004). Antkowiak *et al*. (2018) identified five types of seed surface sculpture as colliculate, reticulate, mixed (colliculate-reticulate), verrucate and foveolate in *Noccaea* species and stated that the collicate pattern was the dominant surface sculptural pattern of *Noccaea*. Özgişi *et al*. (2018c) reported that surface ornamentation of *N. birolmutlui* as colliculate. Özüdoğru (2018) stated that seed surface sculpture was regulate in *Noccaea rosularis* Al-Shehbaz. The general seed coat type of *N. granatanse* Boissier & Reuter and *N. perfoliata* Al-Shehbaz was reticulate (Antkowiak *et al.,* 2018). The outcomes of this study are in agreement with the general seed surface characteristics of *Noccaea.*

Conclusions

As a result, no detailed study was conducted on *N. birolmutlui* and *N. camlikensis* before this research. Accordingly, the present study is the first comprehensive study of the anatomy and pollen characteristics of both species. In this study, a total of 23 anatomical characters (six root, eight stem and nine leaf) and 9 palynological characters of two *Noccaea* species and its taxonomic implications were analyzed. The results of the study showed that two species were generally similar to each other in terms of pollen morphology. However, some differences were observed between species in terms of anatomical characters and seed micromorphology features. This study confirmed that comparative root, stem and leaf anatomical features can be used as an additional tool for accurate species identification. In addition, size of the equatorial axis, AMB, and thickness of exine and intine were identified as taxonomically significant characters.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The author declares that there are no conflicts of interest related to this article.

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