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# TAXONOMIC IMPORTANCE OF NUTLETS AND FLOWERS IN TRIBE CYNOGLOSSEAE (BORAGINACEAE)

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The macro and micro-morphology of flowers and nutlets of 35 species of Boraginaceae belonging to the Tribe Cynoglosseae have been studied by light and scanning electron microscopes. These include *Paracaryum* (8 species), *Mattiastrum* (11 species), *Microparacaryum* (4 species), *Rindera* (4 species), *Cynoglossum* (3 species), *Solenanthus* (2 species), *Trachelanthus* (1 species), and *Lindelofia* (1 species). Nutlets morphology of the examined species showed significant variation in shape, size, and surface ornamentation. Two main types of nutlets were defined: Type I, without a wing, nutlets densely glochidiate, was observed in *Cynoglossum*, *Solenanthus*, *Trachelanthus*, and *Lindelofia*; Type II, characterized by winged nutlets, was found in all examined species of *Paracaryum*, *Mattiastrum*, *Microparacaryum*, and *Rindera*. Our results highlight the importance of the floral and nutlet characters for the identification of the most studied species, based on cluster analysis. The main aim of the present study is to investigate the macro and micro-morphological characteristics of the studied species for the taxonomic identification and species delimitation at the tribal level. For quick and easy identification of the species, identification keys based on floral characters have been developed.

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**Keywords:** Floral structure; fruit morphology; Micro-morphology; PCA analysis; Boraginaceae; Cynoglosseae; Iran

**اهمیت آرایه‌شناسی گل و فندقچه در قبیله سینوگلوسه (خانواده گاوزبان)**

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**منصور میرتاج الدینی**: دانشیار دانشکده علوم، دانشگاه شهید با هنر کرمان

صفات ریخت‌شناسی و ریز ریخت‌شناسی در گل و فندقچه ۳۵ گونه از قبیله سینوگلوسه از خانواده گاوزبان با میکروسپ‌های نوری و الکترونی اسکنینگ مطالعه شده است. این جنس‌ها شامل Paracaryum (۸ گونه)، Mattiastrum (۱۱ گونه)، Microparacaryum (۴ گونه)، Rindera (۴ گونه)، Cynoglossum (۳ گونه)، Solenanthus (۲ گونه)، Trachelanthus (یک گونه) و Lindelofia (یک گونه) می‌باشند. ریخت‌شناسی فندقچه‌ها در گونه‌های مطالعه شده نشان داد که تنوع زیادی در شکل، اندازه و تزئینات سطح فندقچه‌ها وجود دارد. دو نوع تیپ اصلی در فندقچه‌ها

مشخص است: تیپ ۱: فندقچه‌های بدون بال و بطور متراکم پوشیده از کرکهای لنگری که در جنس‌های Cynoglossum، Solenanthus، Trachelanthus و Lindelofia؛ تیپ ۲: فندقچه‌های دارای بال که در جنس‌های Paracaryum، Microparacaryum، Mattiastrum و Rindera مشاهده می‌شود. نتایج ما اهمیت صفات گل و فندقچه را در تعیین و تحدید حدود گونه‌های مطالعه شده در آنالیز خوشه‌ای نشان می‌دهد. هدف اصلی مطالعه حاضر نشان دادن صفات ریخت‌شناسی و ریزشناسی گونه‌های مورد مطالعه برای تحدید حدود گونه‌ها در سطح طایفه است. برای شناسایی سریع و آسان آرایه‌ها کلید شناسایی براساس خصوصیات گل داده شده است.

## INTRODUCTION

The family Boraginaceae consists of approximately 131 genera and 2500 species, mainly distributed in dry, cliffy, and sunny habitats of Eurasia, the Mediterranean region, and western North America (Binzet and Akçin 2009). They are mainly annual, biennial, perennial herbs, shrubs, some trees, and a few lianes, distributed throughout the temperate and subtropical regions of the world (Retief and Vanwyk 1997), with a high distribution in Iran (Willis 1973).

Subfamily Cynoglossoideae Weigend is the largest subfamily, having about 900 species and 50 genera. Recent molecular studies have shown that a wide range of previously recognized tribes place into this subfamily (Chacón & al. 2016; Pourghorban & al. 2020; Sherafati & al. 2021). Tribe Cynoglosseae W.D.J.Koch, Subtribe Cynoglossinae Dumort. is entirely restricted to the Old World, with a center of diversity in western Asia and the Mediterranean region (Chacón & al. 2016; Attar & al. 2018). Morphologically this subtribe is characterized by having an ovate-acuminate ovary, four dorso-ventrally compressed nutlets; usually with a concave to slightly convex, rarely flat central disc, and distinct margin. Central disc glochidiate, papillose and rarely smooth; margin winged or un-winged; marginal wing flat (such as in *Mattiastrum* (Boiss.) Brand) or incurved (such as in *Paracaryum* Boiss.); margins un-winged such as in C*ynoglossum* L. and *Lindelofia* Lehm.

Different researchers worked on the taxonomic relationship among the taxa of the Boraginaceae for a synthetic approach of this family, that considers both phylogenetic and evolutionary aspects (Cronquist 1981; Takhtajan 1997; Selvi & al. 2006), and in most researches fruit morphology has been used as the most important character. Fruits possess several characteristics that offer valuable taxonomic characters for the tribe identification in the Boraginaceae, like the straight or incurved nutlet, a specialized form of emergence, the position of attachment scar, the distinctive form of prickles or glochids (Al-Shehbaz 1991; Baillon 1888; Gurke 1893; Hilger 2014; Riedl 1997). These characters are also valuable for the definition of genera, species, and subspecies (Danin 1995, 2000; Gray 1884; Johnston 1924, 1927, 1937; Langstrom and Chase 2002; Selvi and Bigazzi 2003; Selvi & al. 2006).

In some genera of Boraginaceae, e.g. *Cynoglossum* (Akçin 2008), *Lithospermum* L. (Weigend & al. 2009), *Onosma* L. (Akçin 2007; Binzet and Akçin 2009), nutlet surface ornamentation (using SEM) shows infra-generic variability and can be used to assess the relationships among the species. The morphology of nutlets has major taxonomic importance in *Cynoglossum* (Riedl 1978). Nutlets of *Cynoglossum creticum* Miller, *C. officinale* L., *C. montanum* L., and *C. glochidiatum* Wall. distributed in Turkey, were studied by Akçin (2008). Despite differences in fruit surface features, there are still some problems in the systematics of tribe Cynoglosseae.

A comprehensive study on morphological and micro-morphological characters in tribe Cynoglosseae is almost lacking, moreover, the potential application of these characters in the taxonomy of the tribe has not been illustrated yet. The objectives of the present study are:1) to provide detailed morphological and micro-morphological information on nutlets and flowers of the genera in tribe Cynoglosseae, and 2) to evaluate the application of these characters in the identification of the species as well as delimiting the genera within the tribe Cynoglosseae.

## MATERIALS AND METHODS

### Plant material

The nutlet and floral morphology and micro-morphology of 35 species including eight species of *Paracaryum*, eleven of *Mattiastrum*, three of *Microparacaryum* (Popov ex Riedl) Hilger & Podlech, 4 of *Rindera* Pallas, 3 of *Cynoglossum*, 2 of *Solenanthus* Ledeb., one of *Trachelanthus* Kunze and *Lindelofia* were investigated of the tribe Cynoglosseae using SEM (Scanning Electron Microscopy) and Dino-Lite digital microscope AM413T. The studied materials were obtained from TUH (Central Herbarium of Tehran University), FUMH (Ferdowsi University of Mashhad), and TARI (Research Institute of Forests and Rangelands) herbaria, and among these species, we also added some species from neighboring countries to

have more information about their nutlets and flowers variation (table 1). Identification was confirmed by matching with the available literature and type specimens of these species. One to three populations were studied in each species to recognize possible differences.

Table 1. List of the studied taxa, including collecting data of voucher specimens.

| | Taxa | Locality | Collector & Voucher |
|---|---|---|---|
| 1 | *Cynoglossum creticum* Mill. | Iran. Gilan, Bandar-e Anzali<br>Iran. East-Azarbaijan, Kaleybar | Saeedi 18714 TUH<br>Attar 17169 TUH |
| 2 | *Cynoglossum officinale* L. | Iran. Hariz, near Chorteh | Ghahreman 43255 TUH |
| 3 | *Cynoglossum teheranicum* Riedl | Iran. Gilan, Langerud, Chaff | Naghinezhad 27894 TUH |
| 4 | *Lindelofia kandavanensis* Bornm. & Gauba | Iran. Gilan, Bandar-e Anzali<br>Iran. Mazandaran, Nowshahr<br>Iran. Mazandaran, 40 km Tonekabon to janat abad | Mozaffarian, 6796 TARI,<br>Moradi 40051 TUH,<br>8464 TUH |
| 5 | *Mattiastrum crista-galli* Rech.f. & Riedl | Iran. Mashhad, Chenaran, Quchan, Hezareh<br>Iran. Mashhad, 59 km from Kashmar to Neyshabour | Hojjat and Zengooie 29291 FUMH; Ayatolahi & Joharchi, 12667 FUMH |
| 6 | *Mattiastrum cristatum* Brand | Iran. Hamedan, 20km s of Nahavand | Assadi & Mozaffarian 36999 TARI |
| 7 | *Mattiastrum densum* (Rech. f. & H. Riedl) Heller | Iraq. near Koma sang (Harran), Police post. NE of Mandali | Al-Shehbaz and A.R.Mayah, April 20, 1976 |
| 8 | *Mattiastrum dielsii* Bornm. | Afghanistan. Kabul, Bandi Kharghak, 2050 m | Rechinger 31241 |
| 9 | *Mattiastrum heratense* Rech.f. & Riedl | Afghanistan. Bamian and Bandi-Amir, 2800-3000m | Rechinger 18174 |
| 10 | *Mattiastrum honigbergeri* Rech. f. | Afghanistan. Province Khost: in SE slopes, Sata Kandao between Gardez and Khost, 2800-2200 m | Rechinger 32142 |
| 11 | *Mattiastrum leptophyllum* (DC.) Boiss. | Iran. West-Azarbaijan, Urumieh, Silvana | Siami 327 TARI |
| 12 | *Mattiastrum luristanicum* (Náb.) H. Riedl | Iran. Khorassan, Tang-e malavi | Foroughi 3289 TARI |
| 13 | *Mattiastrum modestum* (Boiss. & Hausskn.) Brand | Iran. Khuzestan, 9 km to Behbahan, Road of Dehdasht, Rocky region, 200 m | Assadi and Abohamzeh 17908, 38731 TARI |
| 14 | *Mattiastrum polyanthum* Rech. & Riedl | Iran. Semnan, 20 km NW of Shahrud | Assa di&Mozaffarian 40929 TARI |
| 15 | *Mattiastrum pygmaeum* Rech.f. | Iran. Semnan, 30km north-west of Shahrud, Shahvar mont. Above Tash, 3500 m | Assadi and Maassoumi 21080 TARI |
| 16 | *Mattiastrum turcomanicum* Brand | Iran. Gorgan, 32 km to Marave Tappeh on the road of Incheborum, 180 m | Assadi & Maassoumi 55450 TARI |
| 17 | *Microparacaryum bungei* (Boiss.) Khatamsaz | Iran. Hormozgan, Bandar-Abbas, 5 km to Hajiabad, to Kahkom, 850 m;<br>Hormozgan, Bandar-Abbas;<br>Esfahan, Ghamishleh, protected area, Kooh Dojdoon | Mozaffarian 52175TARI,<br>3968TARI,<br>1330TARI |
| 18 | *Microparacaryum intermedium* (Fresen.) Hilger & Podl. | Iran. Khorassan, Kashmar-darvaneh | Dini & Bazargan 33035TARI |
| 19 | *Paracaryum stellatum* H. Riedl | Iran. Khorassan: south Birjand, 1800 m | Aliabadi 22214TUH |
| 20 | *Microparacaryum salsum* (Boiss.) H.H. Hilger & D. Podlech | Iran. Semnan, Kavir protected area, Talheh Mont., 1110-1250m;<br>Khorasan, Shahrud, Turan protected area, 18 km to E of ES Delbar, near Ahmadabad | Runemark et al. 19513,<br><br>Rechinger 50616TARI |
| 21 | *Paracaryum cyclhymenium* (Boiss.) H. Riedl | Iran. Kerman, 50 km W of Ravar, Khajeh mt.<br>Iran. Tehran, road of Firozkuh. | Assadi and Bazgosha 56237TARI<br>Dini & Arazm 1570TARI |
| 22 | *Paracaryum persicum* subsp. *macrocarpum* H. Riedl | Iran. Tehran, Rudehen | Dini & Arazm 1631 TARI |

Table 1. Continued.

| | Taxa | Locality | Collector & Voucher |
|---|---|---|---|
| 23 | *Paracaryum persicum* subsp. *persicum* (Boiss.) Boiss. | Esfahan: ca. 20 km sw Ardestan on the road Taleghan, 1700 m | Wendelbo & Foroughi, 11504 TARI |
| 24 | *Paracaryum platycalyx* Riedl | Iran. 80 km from Zahedan on the road to Khash | Assadi 22793 TARI |
| 25 | *Paracaryum rugulosum* (DC.) Boiss. | Iran. Esfahan, 50 km Delijan to Meymeh, 2100 m | Ghahreman and Attar 19082 TUH |
| 26 | *Paracaryum sintenisii* Hausskn. ex Bornm. | Iran. Fars, 7 km from Evaz to Lar | Assadi & Sardabi 41683 TARI |
| 27 | *Paracaryum strictum* (C. Koch) Boiss. | Azerbaijan, Nakhitchevan, near Bichanak village. 2434-5000 m, 28.05.1971. | Gogin 1647 MHA |
| 28 | *Paracaryum undulatum* Boiss. | Iran. Tehran, Darakeh | Attar 29069 TUH |
| 29 | *Rindera albida* (Wettst.) Kusn. | Iran. Kordestan, Sanandaj | Attar 2465 TUH |
| 30 | *Rindera bungei* (Boiss.) Gürke | Iran. Razavi Khorasan, Kashmar, Kuhsorkh District | Joharchi 45219 FUMH |
| 31 | *Rindera cyclodonta* Bunge | Iran. Bojnord, Ghorkhod protected area | Joharchi 43655 FUMH<br>Joharchi 44782 FUMH |
| 32 | *Rindera lanata* (Lam.) Bunge | Iran. Kordestan, Sanandaj | Attar 14292 TUH |
| 33 | *Solenanthus circinatus* Ledeb. | Iran.Tehran, Damavand | Talebi 43264 TUH |
| 34 | *Solenanthus stamineus* (Desf.) Wettst. | Iran.Tehran, Damavand<br>Iran. Mazandaran: road of Karaj to Chalus, Pol-e Zanguleh, 2550 m<br>Iran. Mazandaran, Kandovan-Siahbisheh | Talebi 43263 TUH,<br>Nazarian 33418-TUH,<br>Ghahreman 5290 TUH |
| 35 | *Trachelanthus cerinthoides* (Boiss.) Kunze | Iran. Tehran, Dizin | Attar 45739 TUH |

For scanning electron microscopy, dry nutlets were directly mounted on metallic stubs using double adhesive tape and coated with gold for 6 minutes in the sputtering chamber (BAL-TEC, SCDOOS) and observed with SEM. Coating with gold by the physical vapor deposition method (PVD) was restricted to 100 Å. The SEM examination was carried out with a TESCAN microscope.

In total, 4 quantitative and 10 qualitative traits of the flowers and nutlets were studied (table 2). These were: calyx length, calyx width, corolla length, corolla shape, corolla color, faucal appendages, nutlet shape, nutlet length, nutlet surface ornamentation, stamens position, style position, nutlet margin, disc, and sepal indumentum. For grouping of the studied species based on petal and nutlet morphology and micro-morphology, mentioned data were standardized (mean = 0, variance = 1). For morphological analysis, quantitative characters were coded as multistate characters and used for further analysis (Podani 2000).

All measurements and observations taken from the corolla and nutlet (table 2) were done with a Dino-Lite digital microscope. The general terminology follows Riedl (1967) and Davis (1988). Observations were done on the surface patterns of nutlets (Stearn 1973).

**Multivariate analysis.** multi-state qualitative characters were converted into presence-absence descriptions, whereas continuous quantitative measures were treated as such they were averaged on 15 to 20 nutlets for each taxon. Agglomerative cluster analysis was performed on the Average Taxonomic Distance dissimilarity matrix, after linear standardization by a range of each variable of the original data set. The unweighted pair-group average algorithm (UPGMA) yielded the highest coefficient of cophenetic correlation, which was performed using the Mantel T-Test (Rohlf 1993). Ordination by Principal Component Analysis (PCA) was then performed to summarize the correlational structure among the variables and to display it in a nonhierarchical way (Vezey & al. 1988). Eigenvectors and eigenvalues were extracted from the correlation matrix. These analyses were performed using PAST version 2.17 (Hammer & al. 2012).

Table 2. Morphological characters and coding of diagnostic nutlet and flower characteristics in studied species.

| **Taxa** | Calyx length (mm) | Calyx width (mm) | Corolla length (mm) | Corolla shape: 1. funnel-shaped, 2. cylindrical-campanulate, 3. campanulate to infundibular, 4. cylindrical to infundibular, 5. cylindrical | Corolla color: 1. dark purple, 2. pink or whitish, 3. dark maroon to deep pink,4. pale yellow, 5. bluish-purple, 6. red or reddish-purple, 7. violet-purple | Faucal appendages (scales): 1. in throat, 2. in tube | Nutlet shape: 1. sub-orbicular – rarely ovate, 2. ovate, 3. ovate-orbicular, 4. orbicular, 5. sub-orbicular | Nutlet length (mm) | Nutlet surface ornamentation: 1. tuberculate, 2. granulate, 3. tabular papillae | Stamens position: 1. exserted, 2. included | Style position: 1. exserted, 2. included | Nutlet margin: 1. type I unwinged, 2. type II winged | Disc of nutlet: 1. smooth, 2. smooth or sparsely echinulate 3. densely or sparsely glochidiate-aculeate | Sepal indumentum: 1. sparsely hairy, 2. softly tomentose or pilose, 3. glabrous |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Paracaryum persicum* subsp *persicum* | 3.5–5 | 1.2–1.5 | 5–8.5 | 2 | 7 | 1 | 1 | 5–6 | 2 | 1 | 2 | 2 | 2 | 1 |
| *P. persicum* subsp *macrocarpum* | 4.4–4.5 | 1.5–1.9 | 6.1–6.3 | 2 | 7 | 1 | 1 | 6.7–7.5 | 2 | 1 | 2 | 2 | 2 | 1 |
| *P. cyclhymenium* | 3.8–7.2 | 1–1.4 | 5.8–8 | 2 | 7 | 1 | 1 | 4.1–6.8 | 2 | 1 | 2 | 2 | 2 | 1 |
| *P. platycalyx* | 4.2–7 | 1.5–2.9 | 6–8 | 2 | 7 | 1 | 1 | 7.1–7.9 | 2 | 2 | 2 | 2 | 2 | 1 |
| *P. sintenisii* | 3–4.7 | 1–1.5 | 3.5–5 | 2 | 7 | 1 | 1 | 5–8 | 2 | 2 | 2 | 2 | 2 | 1 |
| *P. rugulosum* | 3.5–4.4 | 1.2–1.5 | 4–5 | 2 | 7 | 1 | 1 | 4–6 | 2 | 2 | 2 | 2 | 2 | 1 |
| *P. undulatum* | 3–4 | 1–1.4 | 3.7–5 | 2 | 7 | 1 | 1 | 4.7–6.8 | 2 | 2 | 2 | 2 | 2 | 1 |
| *P. strictum* | 2.3–3.6 | 0.5–0.8 | 3–3.1 | 2 | 7 | 1 | 1 | 3.6–3.9 | 2 | 2 | 2 | 2 | 2 | 1 |
| *P. intermedium* | 2.1–2.2 | 0.5–0.6 | 2–2.1 | 2 | 7 | 1 | 2 | 3.1–3.4 | 2 | 2 | 2 | 2 | 2 | 1 |

Table 2. Continued.

| **Taxa** | Calyx length (mm) | Calyx width (mm) | Corolla length (mm) | Corolla shape: 1. funnel-shaped, 2. cylindrical-campanulate, 3. campanulate to infundibular, 4. cylindrical to infundibular, 5. cylindrical | Corolla color: 1. dark purple, 2. pink or whitish, 3. dark maroon to deep pink,4. pale yellow, 5. bluish-purple, 6. red or reddish-purple, 7. violet-purple | Faucal appendages (scales): 1. in throat, 2. in tube | Nutlet shape: 1. sub-orbicular – rarely ovate, 2. ovate, 3. ovate-orbicular, 4. orbicular, 5. sub-orbicular | Nutlet length (mm) | Nutlet surface ornamentation: 1. tuberculate, 2. granulate, 3. tabular papillae | Stamens position: 1. exserted, 2. included | Style position: 1. exserted, 2. included | Nutlet margin: 1. type I unwinged, 2. type II winged | Disc of nutlet: 1. smooth, 2. smooth or sparsely echinulate 3. densely or sparsely glochidiate-aculeate | Sepal indumentum: 1. sparsely hairy, 2. softly tomentose or pilose, 3. glabrous |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Mattiastrum luristanicum* | 4.5–5 | 1–1.6 | 6–6.7 | 2 | 7 | 1 | 5 | 8.6–8.8 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. turcomanicum* | 3.5–3.9 | 1–1.5 | 4.7–4.8 | 2 | 7 | 1 | 5 | 8–8.5 | 3 | 2 | 2 | 2 | 2 | 1 |
| *M. densum* | 7.3–7.5 | 2–2.4 | 8–8.5 | 2 | 7 | 1 | 5 | 9.5–10 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. dielsii* | 5.3–5.5 | 2–2.8 | 11–11.5 | 2 | 7 | 1 | 5 | 10.3–10.7 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. polyanthum* | 1.5–2 | 0.5–0.8 | 4–4.3 | 2 | 7 | 1 | 5 | 3.4–3.9 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. heratense* | 2.4–2.4 | 0.5–0.8 | 4–4.5 | 2 | 7 | 1 | 5 | 6.3–6.7 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. pygmaeum* | 2.3–2.7 | 2–2.5 | 3–3.2 | 2 | 7 | 1 | 5 | 5–5.5 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. cristatum* | 5.3–5.5 | 1–1.4 | 5.5–5.6 | 2 | 7 | 1 | 5 | 10–10.5 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. honigbergeri* | 7.2–7.6 | 2–2.3 | 8–8.2 | 2 | 7 | 1 | 5 | 8.5–9.1 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. crista–galli* | 2.2–2.6 | 0.5–1 | 3–3.2 | 2 | 7 | 1 | 5 | 9.1–9.5 | 1 | 2 | 2 | 2 | 2 | 1 |
| *M. modestum* | 3–3.5 | 2–2.1 | 6.5–6.6 | 2 | 7 | 2 | 5 | 6.5–7 | 1 | 2 | 2 | 2 | 2 | 1 |

Table 2. Continued.

| Taxa | Calyx length (mm) | Calyx width (mm) | Corolla length (mm) | Corolla shape: 1. funnel-shaped, 2. cylindrical-campanulate, 3. campanulate to infundibular, 4. cylindrical to infundibular, 5. cylindrical | Corolla color: 1. dark purple, 2. pink or whitish, 3. dark maroon to deep pink,4. pale yellow, 5. bluish-purple, 6. red or reddish-purple, 7. violet-purple | Faucal appendages (scales): 1. in throat, 2. in tube | Nutlet shape: 1. sub-orbicular – rarely ovate, 2. ovate, 3. ovate-orbicular, 4. orbicular, 5. sub-orbicular | Nutlet length (mm) | Nutlet surface ornamentation: 1. tuberculate, 2. granulate, 3. tabular papillae | Stamens position: 1. exserted, 2. included | Style position: 1. exserted, 2. included | Nutlet margin: 1. type I unwinged, 2. type II winged | Disc of nutlet: 1. smooth, 2. smooth or sparsely echinulate 3. densely or sparsely glochidiate-aculeate | Sepal indumentum: 1. sparsely hairy, 2. softly tomentose or pilose, 3. glabrous |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *M. leptophyllum* | 5.2–5.3 | 2–2.5 | 6–6.1 | 2 | 7 | 1 | 5 | 9.5–10 | 1 | 2 | 2 | 2 | 2 | 1 |
| *Cynoglossum creticum* | 5.7–6.7 | 3–3.2 | 7–7.9 | 3 | 1 | 1 | 3 | 5.1–6.5 | 1 | 2 | 1 | 1 | 3 | 1 |
| *C. teheranicum* | 4.3–4.5 | 2–2.3 | 7–7.3 | 4 | 1 | 1 | 2 | 5.6–6.7 | 1 | 2 | 1 | 1 | 3 | 1 |
| *C. officinale* | 4.7–4.8 | 2–2.6 | 7.9–8 | 3 | 1 | 1 | 2 | 7.3–7.5 | 1 | 2 | 1 | 1 | 3 | 1 |
| *Solenanthus circinatus* | 4.5–4.7 | 1.5–1.9 | 5–6.4 | 4 | 2 | 2 | 2 | 5.5–5.6 | 1 | 1 | 1 | 1 | 3 | 1 |
| *S. stamineus* | 5.3–5.5 | 1–1.5 | 6.5–6.6 | 4 | 2 | 2 | 2 | 5.3–6.5 | 1 | 1 | 1 | 1 | 3 | 1 |
| *Rindera lanata* | 6.1–6.5 | 3–3.2 | 10–10.5 | 2 | 3 | 2 | 4 | 22.3–22.8 | 3 | 2 | 1 | 2 | 1 | 2 |
| *R. cyclodonta* | 5.8–6 | 3–3.7 | 7.9–8 | 2 | 3 | 1 | 4 | 15.3–15.8 | 3 | 2 | 1 | 2 | 1 | 2 |
| *R. albida* | 5–6.6 | 1–1.4 | 7–8.5 | 2 | 4 | 2 | 4 | 15.7–15.9 | 3 | 1 | 1 | 2 | 1 | 2 |
| *R. bungei* | 3.6–7 | 1–1.8 | 4.4–7 | 2 | 5 | 2 | 4 | 7.4–7.8 | 3 | 1 | 1 | 2 | 1 | 2 |
| *Trachelanthus cerinthoides* | 4.5–4.7 | 2–2.6 | 7.6–9.7 | 5 | 6 | 1 | 2 | 7–7.5 | 1 | 2 | 1 | 1 | 3 | 3 |

Table 2. Continued.

| **Taxa** | Calyx length (mm) | Calyx width (mm) | Corolla length (mm) | Corolla shape: 1. funnel-shaped, 2. cylindrical-campanulate, 3. campanulate to infundibular, 4. cylindrical to infundibular, 5. cylindrical | Corolla color: 1. dark purple, 2. pink or whitish, 3. dark maroon to deep pink,4. pale yellow, 5. bluish-purple, 6. red or reddish-purple, 7. violet-purple | Faucal appendages (scales): 1. in throat, 2. in tube | Nutlet shape: 1. sub-orbicular – rarely ovate, 2. ovate, 3. ovate-orbicular, 4. orbicular, 5. sub-orbicular | Nutlet length (mm) | Nutlet surface ornamentation: 1. tuberculate, 2. granulate, 3. tabular papillae | Stamens position: 1. exserted, 2. included | Style position: 1. exserted, 2. included | Nutlet margin: 1. type I unwinged, 2. type II winged | Disc of nutlet: 1. smooth, 2. smooth or sparsely echinulate 3. densely or sparsely glochidiate-aculeate | Sepal indumentum: 1. sparsely hairy, 2. softly tomentose or pilose, 3. glabrous |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lindelofia kandavanensis* | 7.1–7.3 | 2–2.5 | 7–7.5 | 1 | 6 | 1 | 4 | 7.1–7.3 | 1 | 2 | 1 | 1 | 3 | 1 |
| *Microparacaryum bungei* | 1.4–2 | 0.4–0.6 | 2–2.2 | 2 | 7 | 1 | 2 | 3.2–3.5 | 2 | 2 | 2 | 2 | 2 | 1 |
| *M. salsum* | 1.2–2 | 0.5–0.7 | 2.5–2.6 | 2 | 7 | 1 | 2 | 4.1–4.5 | 2 | 2 | 2 | 2 | 2 | 1 |
| *M. intermedium* f. *stellatum* | 1–1.7 | 0.5–0.6 | 1.9–2.1 | 2 | 7 | 1 | 2 | 3.5–4.5 | 2 | 2 | 2 | 2 | 2 | 1 |

# RESULTS

## Floral structure

Floral morphology and micro-morphological features extensively varied among the studied taxa. For example, regarding the floral characteristics, the calyx length varied between 1 mm (*Paracaryum stellatum H. Riedl*) to 7.6 mm (*Mattiastrum honigbergeri* Rech.f.), and calyx lobes width varied from 0.5 mm (*Microparacaryum salsum* (Boiss.) Hilger & Podlech, *M. intermedium* f. *stellatum*, *M. bungei* (Boiss.) Khatamsaz) to 3.7 mm (*Rindera lanata* Bongei and *R. cyclodonta* Bongei).

The corolla length varied between 2 mm (*Microparacaryum salsum*, *M. intermedium* f. *stellatum*, *M. bungei*) to 12 mm (*Mattiastrum dielsii* Bornm.). Dominant corolla color is in the range of red, violet, purple, and there are other colors like dark purple (*Cynoglossum officinale*) (fig. 1, a3), pink or whitish (*Cynoglossum creticum* Miller, *R. lanata* ), (fig. 1, a2, d1), dark maroon to deep pink (*Cynoglossum teheranicum* Riedl), (fig. 1, a1), pale yellow (*Trachelanthus cerinthoides* Kunze), (fig. 1, c), bluish-purple (*Solenanthus circinatus* Ledebour), (fig. 1, e1), red or reddish-purple (*Solenanthus stamineus* Macbride, *Rindera albida* Kusn.) (fig. 1, e2, d2), violet-purple (*Paracaryum cyclhymenium* (Boiss.) Riedl, *P. rugulosum* Boissier, *P. persicum* (Boiss.) Boiss. subsp. *persicum*, *P. persicum* subsp. *Macrocarpum* Riedl, *P. platycalyx* Riedl, *P. sintenisii* Hausskn. ex Bornm., *P. intermedium* Lipsky, *P. strictum* Boiss., *P. undulatum* Boiss., *Microparacaryum salsum*, *M. intermedium* f. *stellatum*, *M. bungei*, and *Mattiastrum species*) (fig. 1, f1, g1-g7, h1).

Corolla shape also revealed high variability among the studied species, for example, funnel-shape in *Lindelofia kandavanensis* Bornm. & Gauba (fig. 1, b), cylindrical-campanulate in *Cynoglossum officinale, Rindera lanata, R. cyclodonta, R. albida, R. bungei, Paracaryum cyclhymenium, P. rugulosum, P. persicum* subsp. *persicum, P. persicum* subsp. *macrocarpum, P. platycalyx, P. intermedium, P. sintenisii, P. strictum, P. undulatum, Microparacaryum salsum, M. intermedium* f. *stellatum, M. bungei*, and *Mattiastrum* taxa (fig. 1, a3, d1-d4, f1, g1-g7, h1), shortly campanulate to infundibular (*Cynoglossum creticum*) (fig. 1, a2), cylindrical to infundibular (*Cynoglossum tehranicum, Solenanthus circinatus, S. stamineus*) (fig.1, a1, e1-e2), cylindrical (*Trachelanthus cerinthoides*) (fig. 1, c). Stamens long exserted from corolla in *Solenanthus circinatus, S. stamineus, R. bungee;* short exserted in *Rindera albida, Paracaryum cyclhymenium, P. persicum* subsp. *persicum* and *P. persicum* subsp. *macrocarpum*; stamens included in the corolla, in *Cynoglossum creticum, C. tehranicum, C. officinale, Rindera lanata, R. cyclodonta, Paracaryum rugulosum, P. intermedium, P. platycalyx, P. sintenisii, P. strictum, P. undulatum, Microparacaryum salsum, M. intermedium* f. *stellatum, M. bungei* and *Mattiastrum* taxa, *Lindelofia kandavanensis* and *Trachelanthus cerinthoides.*

The style was exserted in *Rindera*, *Cynoglossum*, *Solenanthus*, *Trachelanthus*, and *Lindelofia* or included in *Paracaryum*, *Mattiastrum*, *Microparacaryum*.

## Nutlet morphology

Some differences in the size of nutlets were observed. *Rindera lanata* has the largest nutlets (22.3-22.8 mm diam.) whereas *Microparacaryum bungei and M. intermedium* f. *stellatum* have the smallest nutlets (3.2 - 4.5 mm diameter) (table 2). Nutlet shape shows some differences, sub-orbicular, rarely ovate in *Paracaryum* taxa (fig. 2, g1-g8), ovate in *Microparacaryum*, *Cynoglossum*, *Trachelanthus*, and *Solenanthus* (fig. 2, a1-a2, c, d1-d2, f1-f2; fig. 2, f3-f4), orbicular in *Rindera* and *Lindelofia* (fig. 2, c, e1-e4), and sub-orbicular in *Mattiastrum* (fig. 2, h1-h12).

The nutlets surfaces also have different ornamentations which are described in more detail in table 2. Two types of nutlet surface ornamentation were recognized:

-The glochidiate surface was found in *Cynoglossum*, *Lindelofia*, and *Solenanthus*, (fig. 2, a1-a2, b, d1-d2).

This type is the most common in the studied species.

-The tuberculate surface was found in *Trachelanthus*, *Rindera*, and *Mattiastrum turcomanicum* (Bornm. & Sint.) Brand (fig. 2, c, e1-e2, h10).

Based on the nutlet margin, two types are recognized:

**Type I (unwinged nutlets)**

Nutlets densely glochidiate, without a wing, was observed in *Cynoglossum*, *Solenanthus*, *Trachelanthus*, and *Lindelofia* (figs. 3, a1-a2, b, c, d1-d2). In these genera, there are some similarities and some differences. In *Cynoglossum*, nutlet length varies from 5.1-6.7 mm (*C. creticum*, *C. teheranicum*) to 7 mm (*C. officinale*). Their nutlets are ovate to ovate-orbicular, dorsal surface (disc) convex or depressed, with or without raised margin, glochidiate surface. In *Cynoglossum officinale* disc is sparsely glochidiate, ventral and lateral surfaces densely glochidiate, in *C. creticum* densely and evenly glochidiate, with tubercles between glochids on the dorsal surface, in *C. teheranicum* dorsal surface flat to convex, less glochidiate than lateral and ventral surfaces, with or without scattered small tubercles between glochids.

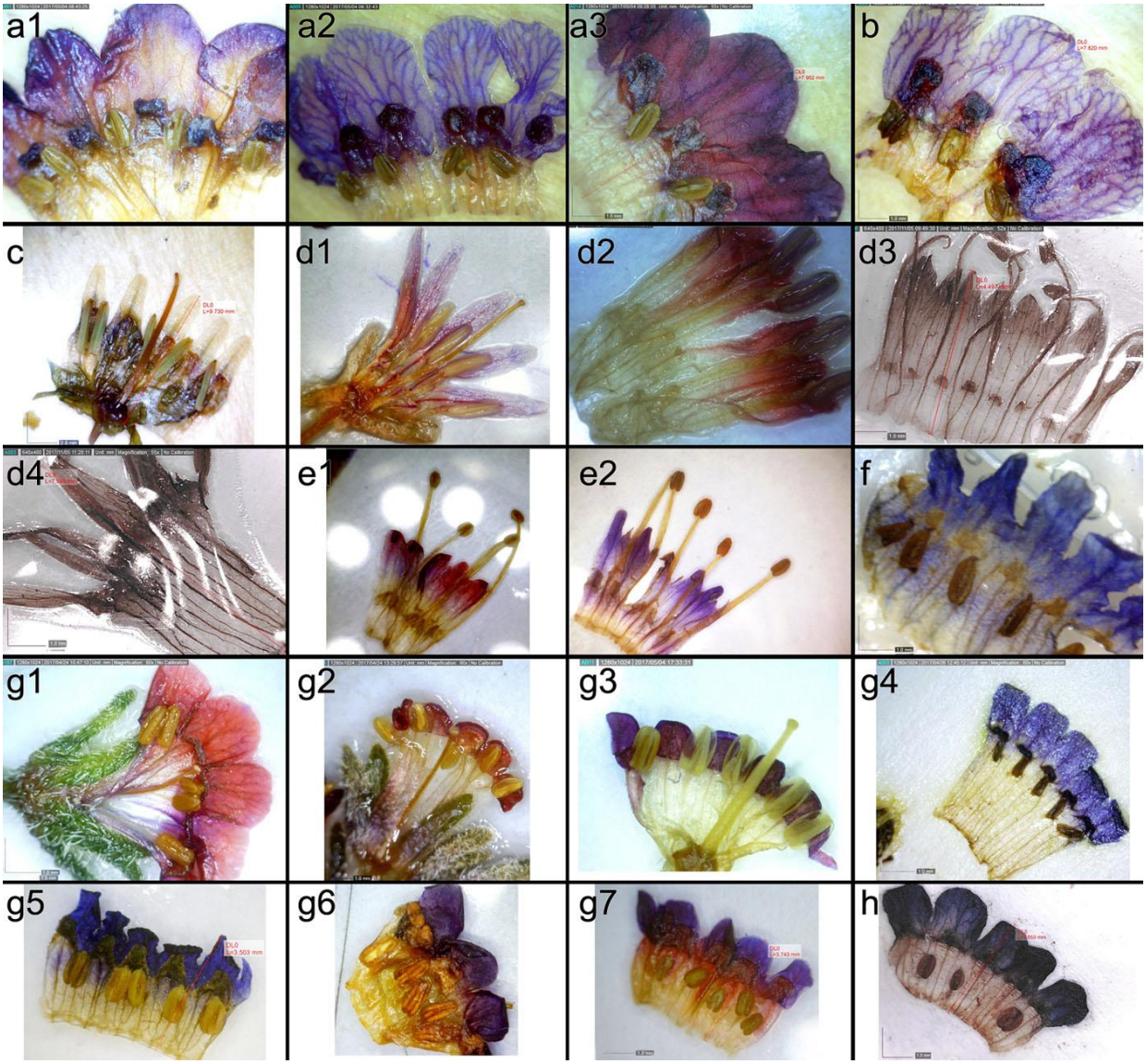


Fig. 1. Petal shapes of the studied species by stereomicroscope. a1: *Cynoglossum teheranicum*, a2: *Cynoglossum creticum*, a3: *Cynoglossum officinale*, b: *Lindelofia kandavanensis*, c: *Trachelanthus cerinthoides*, d1: *Rindera lanata*, d2: *Rindera albida*, d3: *Rindera bungei*, d4: *Rindera cyclodonta*, e1: *Solenanthus circinatus*, e2: *Solenanthus stamineus*, f1: *Paracaryum stellatum*, g1: *Paracaryum cyclhymenium*, g2: *Paracaryum persicum* subsp. *macrocarpum*, g3*: Paracaryum persicum* subsp. *persicum*, g4: *Paracaryum rugulosum*, g5: *Paracaryum sintenisii*, g6: *Paracaryum strictum*, g7: *Paracaryum undulatum*, h1: *Mattiastrum crista-galli*

In *Solenanthus circinatus* nutlets are brown, 5.5-5.6 mm width, ovate, densely glochidiate, marginal glochids much longer than those of dorsal surface; tubercles on dorsal surface inconspicuous or absent but in *S. stamineus* nutlet length is 5.3-6.5 mm, sparsely glochidiate on dorsal surface; margin elevated, densely glochidiate; tubercles between glochids few or absent. In *Trachelanthus cerinthoides* nutlets are broadly ovate, 7-7.5 mm, densely glochidiate-aculeate on sides and ventral surface, disc smooth with few long erect glochids, and in *Lindelofia* nutlets are dorsoventrally compressed, orbicular, ca. 7 mm, abaxially discoid glochidiate.

**Type II (winged nutlets)**

Characteristics of winged nutlets were found in all examined species of *Paracaryum*, *Mattiastrum*, *Microparacaryum*, *Rindera*. This type can be divided into 2 subtypes:

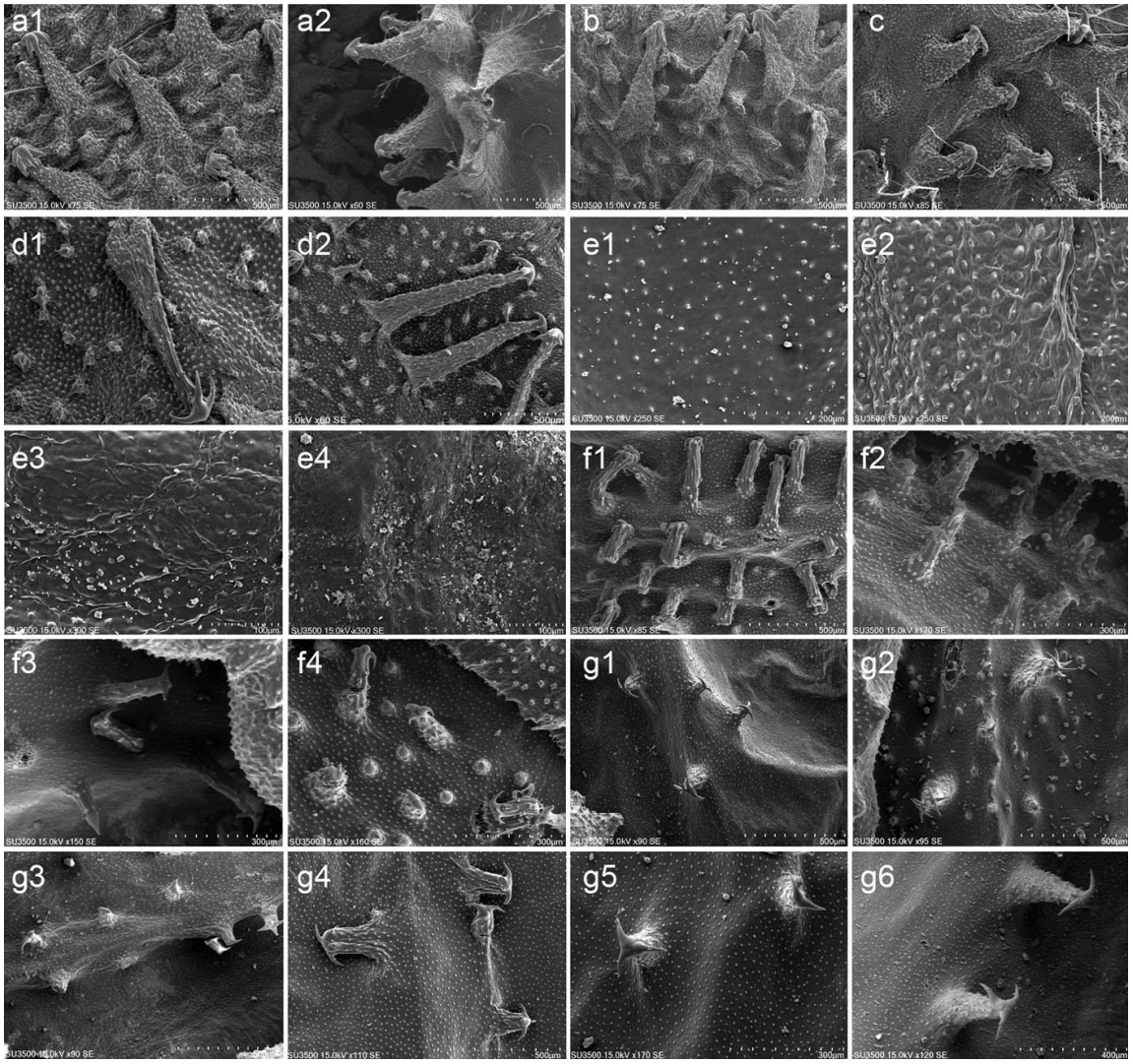


Fig. 2. Electron micrographs (SEM) of ornamentations of nutlet surface. a1, *Cynoglossum teheranicum*; a2, *C. creticum*; b, *Lindelofia kandavanensis*; c, *Trachelanthus cerinthoides*; d1, *Solenanthus circinatus*; d2, *S. stamineus;* e1, *Rindera lanata;* e2, *R. albida*; e3, *R. bungei;* e4, *R. cyclodonta*; f1, *Microparacaryum bungei*; f2, *Paracaryum intermedium*; f3, *Microparacaryum salsum*; f4, *M. intermedium* f. *stellatum*; g1, *Paracaryum cyclhymenium*; g2, *P. persicum* subsp. *Macrocarpum*; g3, *P. persicum* subsp. *Persicum*; g4, *P. platycalyx*; g5, *P. rugulosum*; g6, *P. sintenisii.*

**Subtype A**:

Wing of nutlets is incurved in *Paracaryum* and *Microparacaryum* (fig. 2, f1-f4, g1-g8).

In *Paracaryum*, nutlets are 4, sub-orbicular, rarely ovate, dorsal surface glabrous or with protuberances, incurved wings, membranous, partly covered the disc, and its internal margin is distinctly denticulate only in *P. strictum*, and indistinctly denticulate in other species. In *Paracaryum sintenisii* Hausskn. ex Bornm. nutlets are 5-8 mm diam; disc smooth or with a few spinules on keel; wing broad, incurved, with denticulate margin, teeth in 2 rows. In *P. strictum* nutlets are 3.6-3.9 mm diam, disc echinulate, the margin of wing incurved, teeth in 3 rows. In *P. rugulosum* nutlets are 4-6 mm, disc smooth or sparsely echinulate; margin of wing incurved, rugulose, entire or irregularly denticulate (fig. 4, g5). In *Microparacaryum* nutlets are ovate, 3-4 mm broad, margin broadly winged with a small to large aperture, papillose, inner margin lobed denticulate, middle dorsal area smooth or glochidiate.

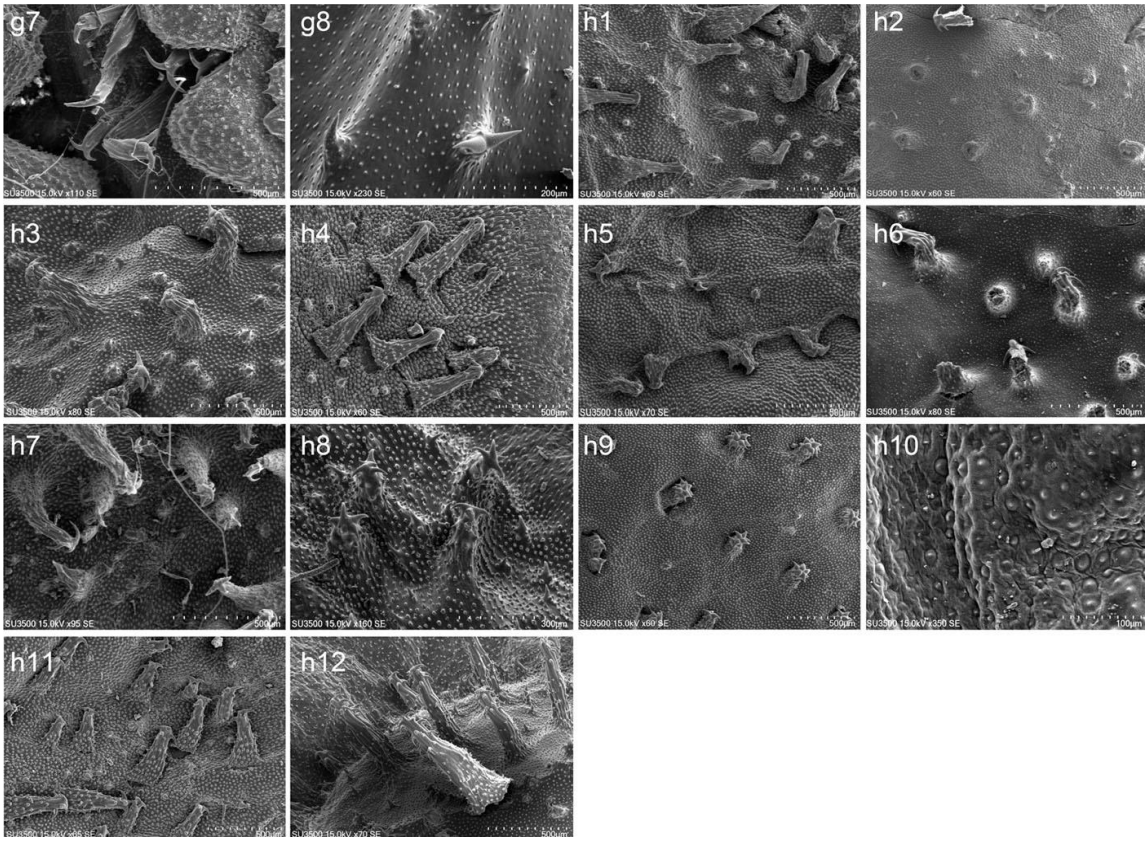


Fig. 2 continued. g7, *Paracaryum strictum*; g8, *P. undulatum*; h1, *Mattiastrum cristatum*; h2, *M. densum*; h3, *M. dielsii*; h4, *M. heratense*; h5, *M. luristanicum*; h6, *M. honigbergeri*; h7, *M. leptophyllum*; h8, *M. polyanthum*; h9, *M. odestum*; h10, *M. turcomanicum*; h11, *M. crista-galli*; h12, *M. pygmaeum.*

**Subtype B**:

In *Rindera* and *Mattiastrum* taxa, the wing of nutlets is flat and does not cover the disc (fig. 3, e1-e4, h1-h12).*Paracaryum* is similar to *Mattiastrum* but differs from it by having incurved wing nutlets. Based on the external margin of the wings, the following three shapes are recognized: 1. entire in *M. densum*, *M. dielsii*, and *M. turcomanicum* (fig. 3, h2, h3, h10); 2. denticulate in *M. luristanicum* , *M. pygmaeum*, *M. modestum*, and *M. crista-galli* & *M. pygmaeum* (fig. 3, h5, h9, h11-h12) and 3. denticulate-glochidiate in *M. cristatum*, *M. heratense*, *M. polyanthum*, *M. honigbergeri* and *M. leptophyllum* (fig. 3, h1, h4, h6-7).

In *Rindera albida* and *R. bungei* nutlets are 7-15 mm, two-winged; outer wing 3 mm broad, margin undulate, inner 2 mm broad, incurved, margin cristate-dentate, glochids absent, while in *R. lanata* and *R. cyclodonta* nutlets are 15-22 mm, smooth, the wing with smooth or undulate and often blue margin, without glochids.

**Multivariate analysis**

**Cluster analysis**

The relationship among the studied species was explored, based on flower and nutlet morphology, using UPGMA. The dendrogram constructed using UPGMA, clustering separated the species within the studied genera into distinct clusters (fig. 6).

Two major clusters are noticeable containing the species of *Paracaryum*, *Microparacaryum*, *Mattiastrum*, and *Rindera* in the first major cluster, and *Trachelanthus cerinthoides*, *Solenanthus*, *Lindelofia kandavanensis*, *Cynoglossum* species in the second major cluster.


Fig. 3. Nutlet micrographs of studied species. a1, *Cynoglossum teheranicum;* a2, *C. creticum;* b, *Lindelofia kandavanensis;* c, *Trachelanthus cerinthoides;* d1, *Solenanthus circinatus;* d2, *S. stamineus;* e1, *Rindera lanata*; e2, *R. albida*; e3 *R. bungei*; e4, *R. cyclodonta*; f1, *Microparacaryum bungei*; f2, *Paracaryum intermedium*; f3, *Microparacaryum salsum*; f4, *M. intermedium* f. *stellatum;* g1, *Paracaryum cyclhymenium*; g2, *P. persicum* subsp. *macrocarpum*; g3, *P. persicum* subsp. *Persicum;* g4, *P. platycalyx*; g5, *P. rugulosum*; g6, *P. sintenisii.*

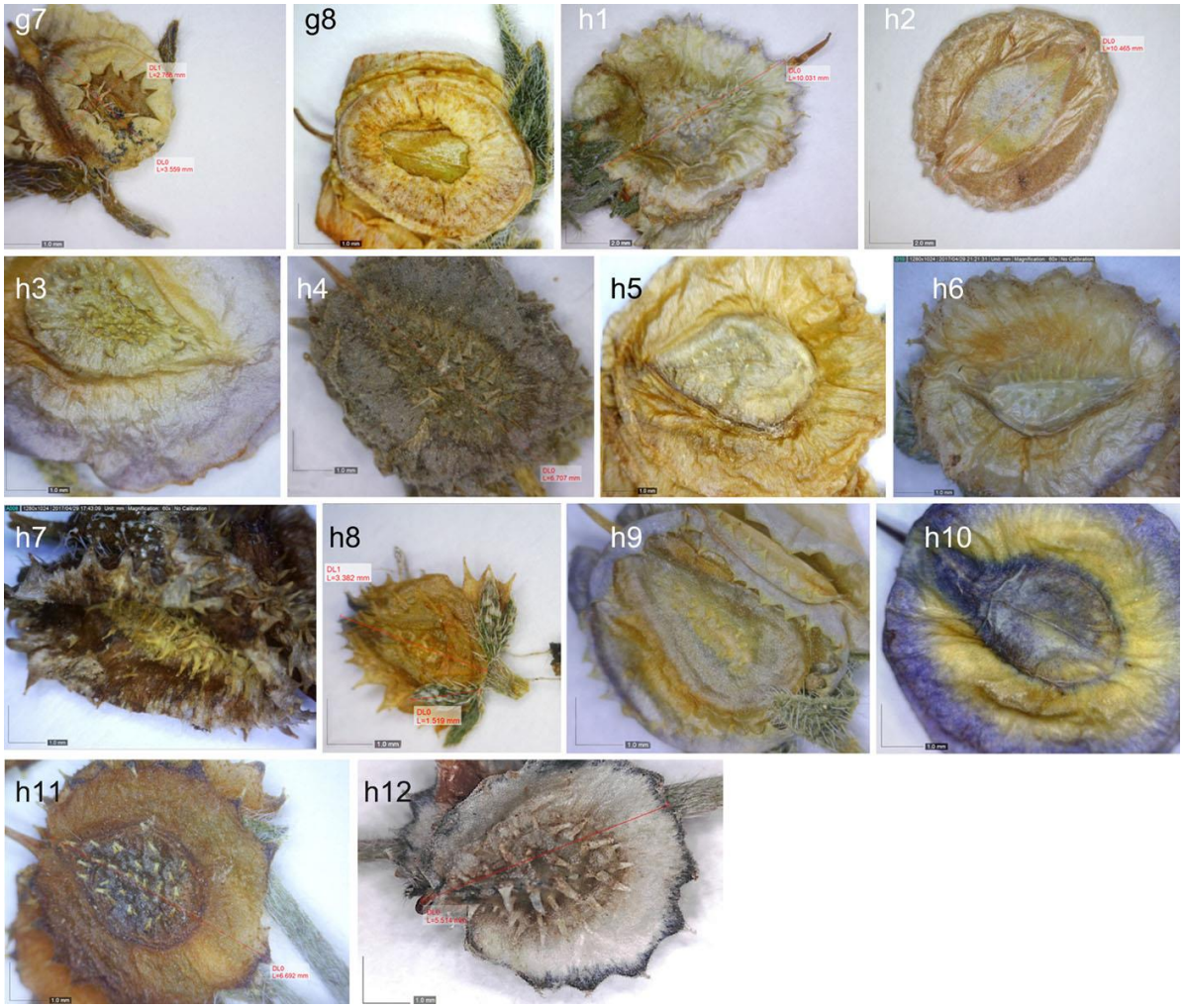


Fig. 3. Continued. g7, *Paracaryum strictum*; g8, *P. undulatum*; h1, *Mattiastrum cristatum*; h2, *M. densum*; h3, *M. dielsii*; h4, *M. heratense*; h5, *M. luristanicum*; h6, *M. honigbergeri*; h7, *M. leptophyllum*; h8, *M. polyanthum*; h9, *M. moddestum*; h10, *M. turcomanicum*; h11, *M. crista-galli*; h12, *M. pygmaeum*.

The first major cluster contained two sub-clusters: the species of *Paracaryum*, *Microparacaryum*, *Mattiastrum*, and *Rindera* were placed close to each other due to morphological similarity. They were characterized by winged nutlets. *Microparacaryum* and *Paracaryum* species in the first sub-clusters are characterized by the strongly incurved nutlet wing and *Microparacaryum* species have the smallest nutlet and flower. The second sub-cluster was formed by *Mattiastrum* and *Rindera* species. These species are characterized by having a flat nutlet wing and absence of covering disc.

The second major cluster contains the species of *Cynoglossum, Solenanthus, Lindelofia kandavanensis*, and *Trachelanthus cerinthoides* which are characterized by spiny-glochidiate, without wing nutlets.

**Principal component analysis (PCA)**

To determine the most variable characters among the studied species, factor analysis based on PCA was performed revealing that the first three factors comprise about 80% of total variation (fig. 7). In the first PCA axis with 57% of the total variance, characteristics such as corolla shape, corolla color, nutlet shape, nutlet length, nutlet surface ornamentation had the highest correlation (≥0.7). In the second factor with about 16 % of total variation stamens position, style position, nutlet margin, nutlet disc, sepal hairs possessed the highest correlations. Therefore, these are the most variable morphological characters among the studied genera and can be used in taxonomical investigations.

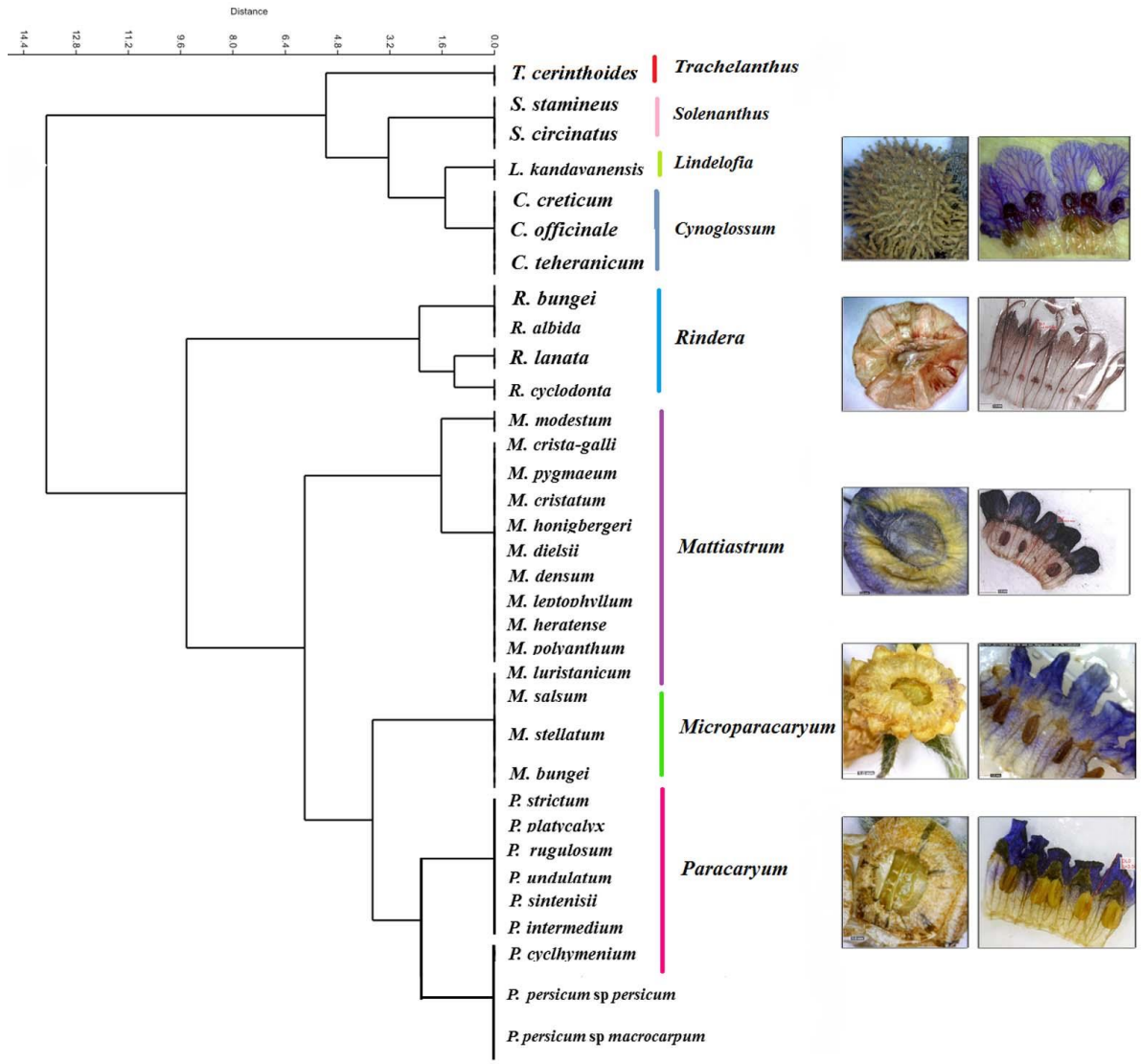


Fig. 4. UPGMA clustering based on nutlet and flower characteristics.

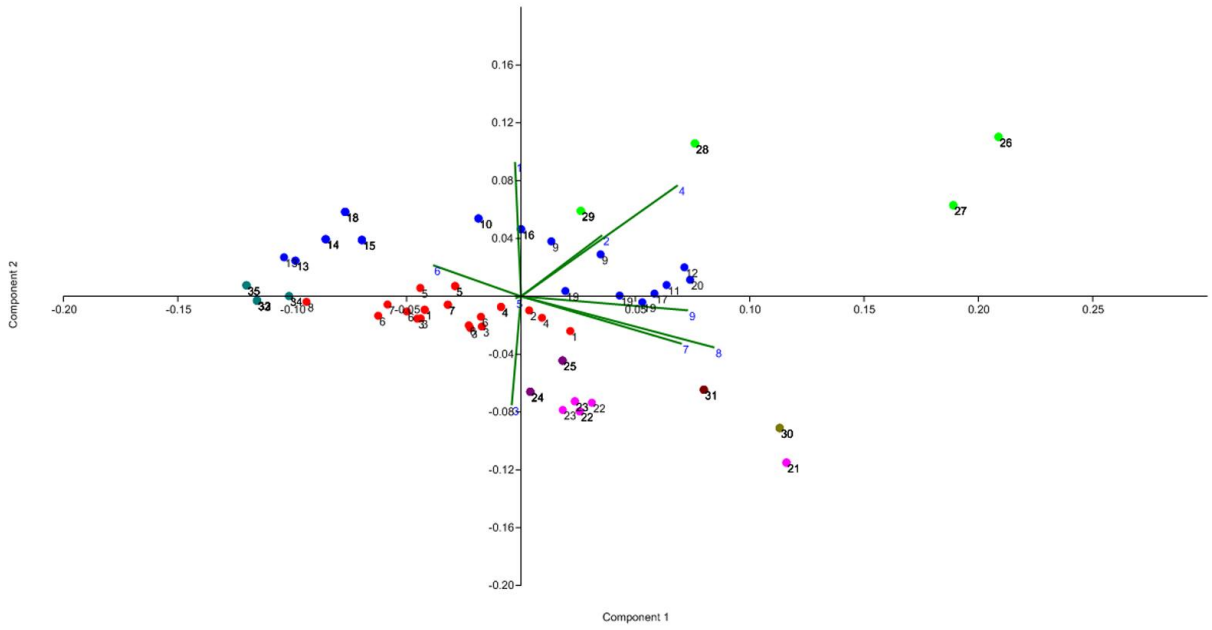


Fig. 5. PCA scatter diagram based on morphological characters revealing species delimitation (the numbers correspond to the species given in table 1).

## DISCUSSION

In previous works, the micro-morphology of seed and fruit was studied in several species and their importance in plant taxonomy was emphasized (Olgun and Beyazoglu 1997; Coskuncelebi & al. 2000; Khalik & al. 2008; Arabi & al. 2017; Hoseini & al. 2017).

According to Chacón & al. (2016), the phylogenetic analyses based on sequences from three cpDNA regions successfully resolved some major issues on the monophyly of the main tribes of Boraginaceae and provided more detailed insights into the evolution of the Cynoglosseae s.l.

Detailed taxonomic and phylogenetic studies of the subtribe Cynoglossinae are required to resolve this complex group (Chacón & al. 2016). However, there is a whole range of segregate genera that have been proposed for *Cynoglossum* and their phylogenetic relationships are not at all resolved. Some of them may be monophyletic, but at present, all of them appear to be nested in *Cynoglossum* based on Chacón & al. (2016).

*Omphalodes* Moench and *Cynoglossum* were retrieved as either poly or paraphyletic, showing that the morphological characters used in traditional taxonomic classifications are highly homoplasious (Weigend & al. 2013). On the other hand, nutlet characters together with nutlet orientation and sculpturing tend to circumscribe the natural groups. Although the polytomies obtained in Weigend & al. (2013) are largely resolved, most nodes remain unsupported, and *Lindelofia*, *Mattiastrum*, *Microparacaryum*, *Paracaryum*, *Pardoglossum*, *Rindera*, *Solenanthus*, and *Trachelanthus* are retrieved as either para-, or polyphyletic and/or nested in *Cynoglossum* s.str. as they also suggested in Selvi & al. (2011).

All recent studies show that there is still a lot of confusion with regards to the infra-familial classification of Boraginaceae and that, the various partial classifications published in the last decades have largely created unnatural units. However, the present study indicates that the nutlet characters, especially surface ornamentation, could be used to classify the species of the complex genera in the tribe Cynoglosseae.

**Identification key to the genera of Cynoglosseae**

A key was developed to differentiate the species of the tribe Cynoglosseae based on the morphological characters of nutlets and flowers, to facilitate the identification.

1: Nutlets winged..... 2.
-Nutlets unwinged ..... 5
2. Nutlets 3-4 mm in diameter; corolla 2-2.5 mm long..... *Microparacaryum*

-Nutlets and corolla longer..... 3.
3. Wing of nutlet strongly incurved, partly covering central disc..... *Paracaryum*
-Wing of nutlet flat, not covering central disc ..... 4.
4. Stamens included in corolla tube ..... *Mattiastrum*
-Stamens included or long exserted..... *Rindera*
5. Calyx glabrous; corolla lobes narrow, as long as tube ..... *Trachelanthus*
-Calyx hairy-tomentose; corolla lobes oblong, shorter than tube or rarely equaling the tube..... 6
6. Stamens long exserted (6-8 mm) ..... *Solenanthus*
-Stamens short, included in corolla..... 7
7. Appendage of throat flat or slightly concave; anthers 1.3 mm long; filaments 2 mm long.....*Lindelofia*
-Appendage of throat inflated; anthers 1 mm long; filaments 1.5 mm long .....*Cynoglossum*

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