NUTLET AND FLOWER MORPHOLOGICAL AND MICROMORPHOLOGICAL STUDIES ON ONOSMA L. (BORAGINACEAE) IN IRAN

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The morphology and micromorphology of flower and nutlet in 29 Iranian *Onosma* species were examinated comparatively using SEM method in order to evaluate and clarify their taxonomic values and specific emphasis was on their role in classification. Our study highlights the importance of the floral and nutlet characters in relation to identification of most of the studied species based on the PCoA and cluster analysis. Besides, Palynological, morphological and molecular studies confirmed our results. Although there are some taxa which cannot be determined solely using the mentioned characters, but these characters accompanied with other important characters are useful tools in distinguishing many taxa and solving some complexities in *Onosma*. Moreover if molecular evidence (chloroplast and nuclear sequences) confirm our results, the divergence of *Onosma orientalis* and *Onosma rostellata* from the genus *Onosma* is expexcted, then these taxa may be transferred to different genera. On the other hand, combinations of these characters along with morphological and molecular evidence provide a perfect view for taxonomy and comphrensive taxonomical revision of *Onosma*.

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KEY WORD: Boraginaceae; *Onosma;* morphology*;* micromorphology*;* taxonomy; SEM; Iran

مطالعات ریخت شناسی و ریز ریخت شناسی گل و فندقه برخی از گونه های (Onosma L. (Boraginaceae در ایران

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ریخت شناسی و ریز ریخت شناسی گل و بذر در ۲۹ گونه ایرانی Onosma به صورت مقایسه‌ای با استفاده از میکروسکپ الکترونی نگاره برای ارزیابی و تبیین ارزش های تاکسونومیکی آن ها با تاکید ویژه بر نقش آن ها در رده بندی صورت گرفته است. مطالعه ما سبب برجسته نمودن اهمیت صفات گل و فندقه در ارتباط با شناسایی غالب گونه های مطالعه شده بر اساس آنالیز تجزیه به مولفه های اصلی و آنالیز خوشه ای شده است. به علاوه، مطالعات گرده شناسی، ریخت شناسی و مولکولی نتایج ما را تایید نموده اند. اگرچه تاکسون هایی وجود دارند که به تنهایی براساس صفات ذکر شده قابل شناسایی نیستند، اما این صفات به همراهی با صفات با اهمیت دیگر، ابزار مهمی در تشخیص و برطرف نمودن پیچیدگی های تاکسونومیکی جنس Onosma می باشند. اگر شواهد مولکولی (توالی های هسته ای و کلروپلاستی) نتایج ما را درباره واگرایی Onosma orientalis و Onosma rostellata نسبت به جنس Onosma تایید نمایند، این تاکسون ها می توانند به جنس های دیگری منتقل شوند. از سوی دیگر، ترکیب صفات بذر و گل همراه با شواهد ریخت شناختی و مولکولی نگرش جامعی پیرامون بازبینی تاکسونومیکیOnosma فراهم می آورند.

INTRODUCTION

Onosma L. is a genus of the tribe *Lithospermeae* Dumort. belonging to the large family Boraginaceae, comprising ca. 150 species (Mehrabian et al. 2012; Kolarčik 2010; Cecchi & Selvi 2009) distributed mainly in western, central Asia and in the

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Mediterranean area (Jávorka 1906; Meusel et al. 1978). These taxa are considered as a taxonomically difficult group.Taxonomic treatments within the genus are highly controversial and many closely related taxa were described based on minor morphological differences. Due to the similarities among the *Onosma* taxa, there are many problems in their identification (Binzet & Akcin 200 9). Moreover, the species are distinguished on the basis of indumentum leading to several mistakes in taxonomy of the genus in the past (Ball 1972; Maggi 2008). Due to mentioned complexities, Riedl (1978) emphasized to providing the useful evidence in a new classification. Khatoon et al (1994) did the first study of the ultra-morphology of Boraginaceae including *Onosma*. Moreover many authors have used trichome micro-morphology (Khatamsaz 2002) and Palynology (Binzet 2011; Qureshi & Qaiser 1987; Binzet & Orcan 2003a & b; Mehrabian et al. 2012) for character evaluation in *Onosma.* Moreover corolla epidermis ornamentations appear to be of great value in some flowering plants i.e. Fabales (Christensen & Hansen 1998; Hammett et al. 1994), *Rubus* (Sharifnia & Shakib 2012) and *Onosma* (Riedl 1978).

Nutlet morphology has provided useful evidence on evolutionary classification and systematics of flowering plants (Corner 1976). Besides, they have been used as the most important character in Boraginaceae (Baillon 1888; Gurke 1893; Hilger 1985; Al-Shehbaz 1991; Riedl 1997). Mentioned characters are valuable evidence that can be used for delimitation of taxa in Boraginaceae (Gray 1884; Langstrom & Chase 2002) and specially in *Onosma* (Akcin 2009; Binzet & Akcin 2009).

A comprehensive study on morphological and micro- morphological characters in *Onosma* is lacking. Furthermore, the taxonomic values of these characters is rather ambiguous. On the other hand, it is important to understand the diversity that exists and the prominent characteristics of the nutlet and flower when identifying *Onosma* species. The objective of the present study is to clarify the taxonomic values of mentioned characters and an specific emphasis was on their role in classification and determination of *Onosma* at infrageneric and interspecific levels. In addition, attempts were made to identify unique characters for each taxon and solving some taxonomic complexities.

MATERIALS AND METHODS

A-Micromorphological assessment

In our research, nutlet and petal morphology and micro-morphology of 29 species of the genus *Onosma* from Iran was studied on the basis of SEM and LM microscopy techniques. Voucher data are presented in table 1. Materials used for this study were taken from wild populations as well as samples in HSBU, W and TARI (herbarium abbreviations according to Thiers 2008).

A total of 1-3 populations for each taxon were studied on petal and nutlet morphology and micro-morphology. From each population, 1-3 plant specimens were used and from each plant specimen at least 3-5 petals and 5-10 nutlets were investigated and prepared for scanning electron microscopy (SEM) and light microscopy (LM).

For SEM studies, nutlets and petals were mounted on stubs using double-sided adhesive tape. Samples were coated with 12.5-15 nm of gold. Afterward, coated samples were examined and photographed with Cam Scan- MV 2300 Electron Microscope (fig.1).

The measurements taken from the petal (table 2) and nutlet (table 3) were made by Image Tolls version 3. The general terminology follows Riedl (1967); Davis (1978) and Harris & Harris (2001). The measurements taken from the flowers were 4 quantitative and 11 qualitative characters (table 2) as follows: calyx length, calyx lobe width, calyx accrescent, calyx sections, calyx trichome color, corolla lobe length, corolla length, corolla shape, corolla color, corolla lobe shape, corolla trichome, nectar trichome, exsertion of anther, anthers adherence, corolla epidermis ornamentation and nutlet measurements are including 5 quantitative and 4 qualitative characters including nutlet orientation, nutlet shape, nutlet ventral keel, nutlet length, nutlet width, nutlet epidermis ornamentation, nutlet beak length/ beak length, nutlet length and nutlet length/nutlet width.

B-Statistical analysis

For grouping of the studied species on the basis of 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. Mentioned data were used for cluster analysis, including UPGMA (Unweighted Paired Group Using Average Method) and principal coordinate analysis (PCoA), (Rholf 1972) and SPSS ver. 9 software were used for statistical analysis. (PCoA polts can be found in figs 3, 6 and 8 and cluster diagrams are in figs 2, 5, 7).

RESULTS

A-Flower

Floral morphology and micro-morphology showed high variability among the mentioned taxa. The flower characters are discussed below:

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Table 1. List of species used in this study.

Species	Locality	Collector & Voucher
Onosma cornuta Riedl	Kordestan, bijar, 1931m	Mehrabian, HSBU-2010274
Onosma cornuta Riedl	Kermanshah, Between Eslam Abad and Ivan, 1420m	Mozaffarian, TARI-74735
Onosma straussii Riedl	Markazi, Arak, Gavar, 1990m	Mehrabian, HSBU-2010212
Onosma rascheyana Boiss.	Zanjan, Mahneshan, Belghe Mt, 1298m	Mehrabian, HSBU-281
Onosma rascheyana Boiss.	Azarbayjan, Oromieh, 1750m	Zehzad, HSBU-2010310
Onosma alborosea Fisch.	Khoram Abad, 1650m	Riedl & Laraze, W-103
Onosma microcarpa DC.	Azarbayjan,Khalkhal, 1793m	Mehrabian, HSBU-2010252
Onosma microcarpa DC.	Azarbaijan, Khoy to Ghotor, 1700m	Mehrabian, HSBU-2010242
Onosma microcarpa DC.	Tehran, Dizin, 2316m	Mehrabian, HSBU-2010244
Onosma kurdica Teppner	Kordestan , Hamze Arab Mt, 2510m	Lamond&Termeh, WU-06112
*Onosma kilouyensis*Boiss.	Kermanshah,Gahvare to kuzaran, 1469m	Mehrabian&Mohamadi, HSBU-260
Onosma sericea willd.	Kermanshah,Gardaneh-e-Ghalajeh, 1923m	Mehrabian, HSBU-265
Onosma sericea willd.	Kermanshah, Bijar, 1700m	Mehrabian, HSBU-2011276
Onosma nervosa Riedl	Kordestan,15 Km Marivan to sanandaj, 1878m	Mozaffarian, TARI-74656
Onosma dasytricha Boiss.	30 Km Noor Abad, near Robat, 1600m	Assadi &Abuhamze, TARI-38361
Onosma rostellata Lehm.	Kermanshah ,Pave, bayangan, 1421m	Mehrabian, HSBU-2010225
Onosma stenosiphon Boiss.	Tehran, 13km Firuzkuh toward Semnan, 2000m	Assadi& Mozaffrian, TARI-35237
Onosma stenosiphon Boiss.	Kerman , Bijar Mt, 1857m	Kanani, HSBU-2011-121
Onosma elwendica wettst.	Kermanshah, Ghardaneh-e-Ghalajeh, 1923m	Mehrabian, HSBU-268
Onosma dichroanta Boiss.	Golestan, Golestan National Park, 1500m	Heidari, et al. HSBU-2007300
Onosma sabalanica Ponert	Azarbayjan, Meshkin Shahr, Sabalan Mt, 4762m	Mehrabian, HSBU-2010-201
Onosma sabalanica Ponert	Azarbaijan, Arasbaran Protected Area,2000m	Zehzad, HSBU-83898
Onosma olivieri Boiss.	Kermanshah, 2103m	Zargani, IRAN-2901
Onosma olivieri Boiss.	Kermashah, Nosoud to nodesheh, 1800m	Mehrabian, HSBU-2010900
Onosma longiloba Bge.	Semnan, 20km Mohamad abad from Firuzkuh,22.5.2012, Pahlevani, 2100m	Iranshahr, HSBU-2010100
Onosma pachypoda Boiss.	Azarabijan, Tabriz, Mishodagh Mt. 1800m	Mehrabian, HSBU-2010602
Onosma bulbotrica DC.	Zanjan,nik Pey to Mahneshan, 1608m	Mehrabian, HSBU-2010290
Onosma bulbotrica DC.	Kermanshah, Gilane-Gharb, Ghalajeh, 1630m	Mehrabian, HSBU-2010263
Onosma bulbotrica DC.	Ghazvin, Takestan to Abhar, 1400m	Mehrabian, HSBU-2010254
Onosma kotschyi Boiss.	Esfahan, Semirom Padena, 2397m	Mehrabian &Mostafavi, HSBU-123
Onosma orientalis L.	Fars between Shiraz and Kazerun, 2162m	Ghorbani&Habibi, HSBU-2010225
Onosma orientalis L.	Bushehr, Borazjan, Dalaki, Tang-e Eram,	Mozaffarian, TARI-74152
Onosma subsericea Freyn	Azarbayjan, between kiwi to Khalkhal, 1920m	Rechinger, W-07869
Onosma subsericea Freyn	Azarbaijan, Khalkhal to Givi, 1800m	Wendelbo& Assadi, SBU-2010-232
Onosma hebebulba DC.	Kermanshah, Eslam Abad to Ilam, 1437m	Sharif, TARI-5329
Onosma asperrima Bornm.	Fars , Abade, 2007m	Behbudi, W-7148
Onosma asperrima Bornm.	Norabad, Doshman ziari, 2500m	Mehrabian, TARI-45722
Onosma bodeana Bornm.	Tehran , Sohanak, 1832m	Mehrabian, HSBU-272
Onosma gaubae Bornm.	Semnan,Shahmirzad,nizva Mt, 2115m	Zargani, TARI-2794
Onosma gaubae Bornm.	Alborz, Near the Amirkabir Dam, 1900m	Mehrabian, HSBU-
Onosma armena DC.	Zanjan, Mahneshan, 1296m	Mehrabian, HSBU-2010654
Onosma platyphylla Riedl	Lorestan, Aligudarz, Ghalikoh, 3121m	Rechinger, W-05714
Onosma platyphylla Riedl	Lorestan, 45km Dorood, 1600m	Assadi& Mozaffarian, TARI-37007
Onosma macrophylla Bornm.	Kurdestan, Baneh, 2200m	Rechinger, W-05718

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Calyx

Calyx length is varied between 6mm (*O. rostellata*) to 22.5mm (*O. kurdica*). Calyx lobe width: 0.74mm (*O. rostellata*) to 6.42 mm (*O. straussii*). Calyx with non-accrescent strucure is most common, but some showed accrescent structure as follow : *O. pachypoda, O. cornuta, O. dichroantha.* Calyx usually is sectioned at the base, but *O. cornuta* and *O. dichroantha* lack this trait and are divided near to base.

Corolla morphology

Corolla length showed variation between 10 mm (*O. kotschyi*) to 33 mm (*O. dichroantha*). But dominantly it is between 15-24 mm. Corolla lobe length is ranged between 0.8 mm (*O. dasytricha, O. rostellata*) to 6 mm (*O. longiloba*). Moreover a wide range of taxa showed wide corolla lobe, but linear (long) lobes were only found in *O. rostellata* and *O. platyphylla*. Corolla dominantly show yellow color, but there are other colors that includ: white (*O. bulbotricha, O. Gaubae*), red-pink (*O. rascheyana, O. straussii)*, blue-violet (*O. dichroantha, O. dasytricha*). Corolla shape shows high variability among studied species: tubular (*O. rostellata, O. orientalis, O. sabalanica*), campanulate (*O. olivieri , O. bulbotricha*), tubular-campanulate (*O. kilouyensis. O. platyphylla*), tubular-clavate (*O. Gaubae, O. alborosea*), funnel-campanulate (only *O. starussii*), clavate (*O. sericea, O. rascheyana),* Moreover two types of nectary were observed: most species showed glaber nectar, but *O. bulbotricha* and *O. stenosiphon* are with tomentose trichomes and *O. cornuta,* and *O. orientalis* by villous trichomes. Furthermore corolla epidermis represent glaber (*O. sabalanica, O. Gaubae*) to tomentose (*O. dichroantha, O. bodeana*) structure.

Anthers are in 3 groups: they are predominantly connected to the base. Other patterns are connected over the stamen (only in *O. rostellata*) and free (*O. asperrima* and *O. kotschyi*).

Data on type of nectary, corolla trichomes and continuity of anthers have not been recognized previously in the treatments of the Iranian *Onosma* species.

Corolla micromorphology

Five different patterns of petal epidermis ornamentations are observed in studied *Onosma* species as below:

Type 1: This type is characterized by rugose ornamentation that is covered with coarse reticulate lines. Mentioned type is seen in 3 subsections as follows: sect. *Asterotricha* (*O. hebebulba*), sect. *Haplotricha (O. bodeana*) *and sect. Heterotricha* (*O. nervosa*) (figs.1.1-3).

Type 2: This type is characterized by farinaceous ornamentations that is covered with small granules and is finely mealy. This type was observed in *O. longiloba* (fig.1.10) and *O. kurdica* (fig. 1.11).

Type 3: This type is characterized by Papilllate (verrucate/tuberculate) ornamentations, having minute, rounded protuberances. This type is observed in *O. rascheyana* (fig1.12) *and O. sabalanica* (figs. 1.13,14).

Type 4: This type is characterized by aculeate (prickly) ornamentations, with sharp prickles, no spine, and no thorny appendages. *Onosma platyphylla* (figs.1.15,16) is the only species that shows mentioned characters.

Type 5: This type is characterized by scurfy ornamentations, having small granules and is covered by tomentose trichomes. A wide range of taxa are classified in this type *O. sericea* (fig.1.17), *O. Gaubae* (fig.1.18), *O. rostellata* (fig.1.19), *O. orientalis* (fig.1.20) *O. asperrima* (fig.1.21), *O. cornuta* (fig.1.22), *O. kilouyensise* (fig1.23), *O. pachypoda* (fig1.24), *O. macrophyla* (fig1.25), *O. straussii* (fig.1.26), *O. albo-rosea* (fig.1.27) and *O. armena* (fig.1.28). Petal epidermis ornamentation is reported here for the first time on Iranian *Onosma*.

The UPGMA trees of flower characters (fig. 2) showed a relatively high cophenetic correlation (r >0.80). In general, two main clusters are observed. PCA and Cluster analysis didn't show clearly sectional divisions.

B- Nutlet morphology

Nutlts Size varies greatly in studied taxa. Nutlet length varies from 1.71 mm (*O. rascheyana*) to 8.18 mm (*O. alborosea*). But dominant range is 4-5.5 mm. Nutlet width showed variation between 2 mm (*O. orientalis*) to 5.5 mm (*O. olivieri*). Nutlet beak length varies from 0.8 mm (*O. orientalis*) to 3.08 mm (*O. rascheyana*). Moreover beak length/nutlet length varies between 0.12 mm (*O. olivieri*) to 0.43 mm (*O. rostellata*) and nutlet length/nutlet width varies between 0.59 mm (*O. rascheyana*) to 2.08 mm (*O. sabalanica*).

Nutlet shapes are broad obvoate in *O. nervosa* (fig. 4.3), *O. cornuta* (fig.4.21) *O. rostellata* (fig.4.31), *O. kilouyense* (fig.4.15), *O. kotschyi* (fig.4.39), *O. microcarpa* (fig.4.11), *O. albo-rosea* (fig.4.9), *O. elwendica* (fig.4.41), *O. olivieri* (fig.4.17) ovate in *O. dichroantha* (fig. 4. 5), *O. orientalis* (fig.4.33), *O. sabalanica* (fig.4.23), *O. sericea* (fig.4.29), *O. stenosiphon* (fig.4.25), *O. rascheyana* (fig.4.1), *O. dasytricha* (fig.4.19), *O. longiloba* (fig4.37), pyramid in *O. bulbotricha* (fig.4.27) and *O. straussii* (fig. 4. 7), and rhombic in *O. kurdica* (Fig. 4.13). Broad ovate is the most common shape in the studied species. Moreover nutlet orientation is incurved (only in O. orientalis),(fig. 4.33) and straight (in other species).

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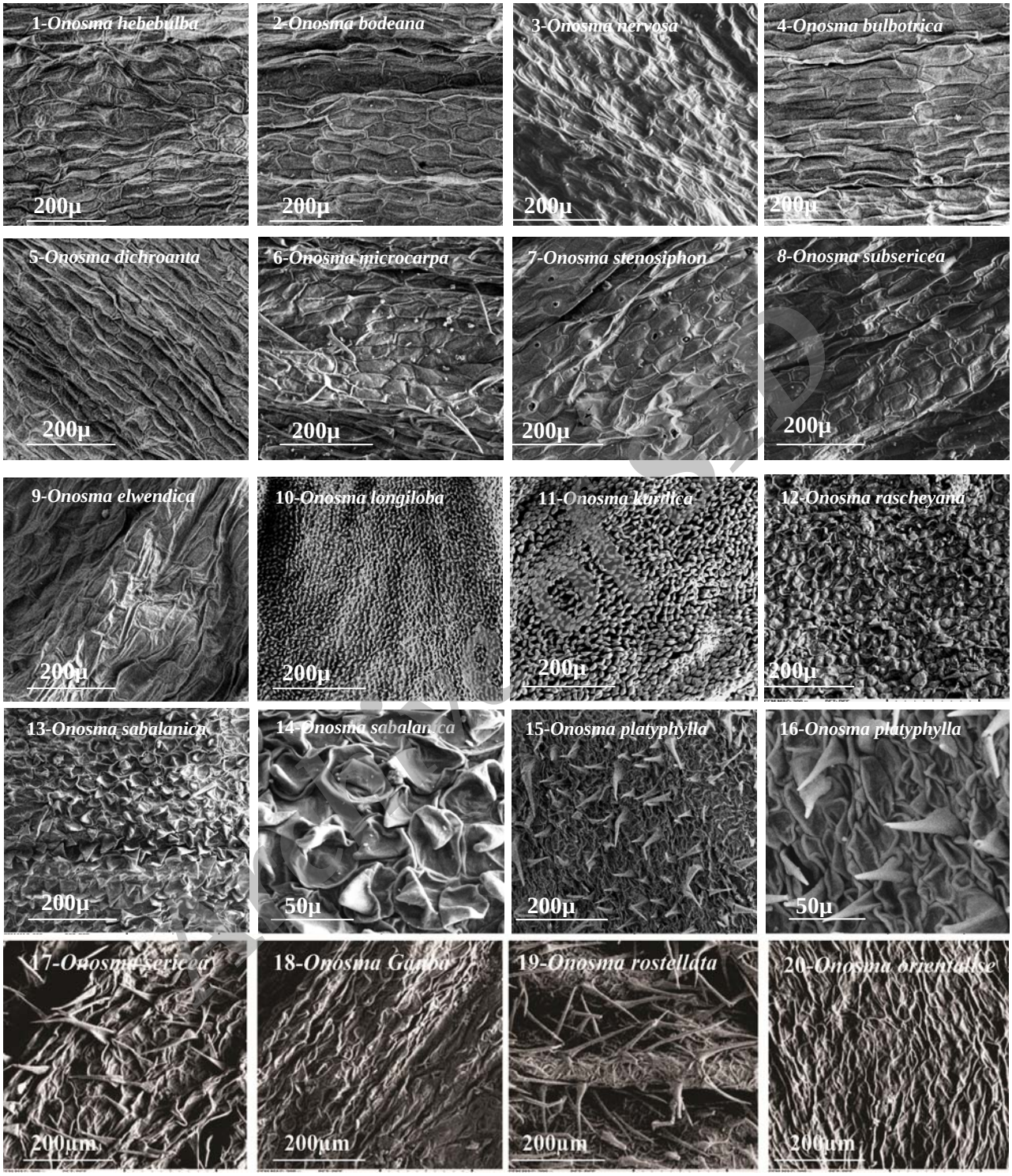

Fig. 1: Corolla epidermis ornamentation (1-28) and nectar trichomes (29-32). 1, *O. hebebulba*; 2, *O. bodeana*; 3-*O. nervosa*; 4, *O. bulbotrica*; 5, *O. dichroanta*; 6, *O. microcarpa*; 7, *O. stenosiphon*; *8*, *O. subsericea*; 9,*O. elwendica;* 10, *O. longiloba*; 11, *O. kurdica*; 12, *O. rascheyana*;13-14, *O. sabalanica*; 15-16, *O. platyphylla*; 17, *O. sericea*; 18,*O. gaubae*; 19, *O. rostellata*; 20, *O. orientalis.*

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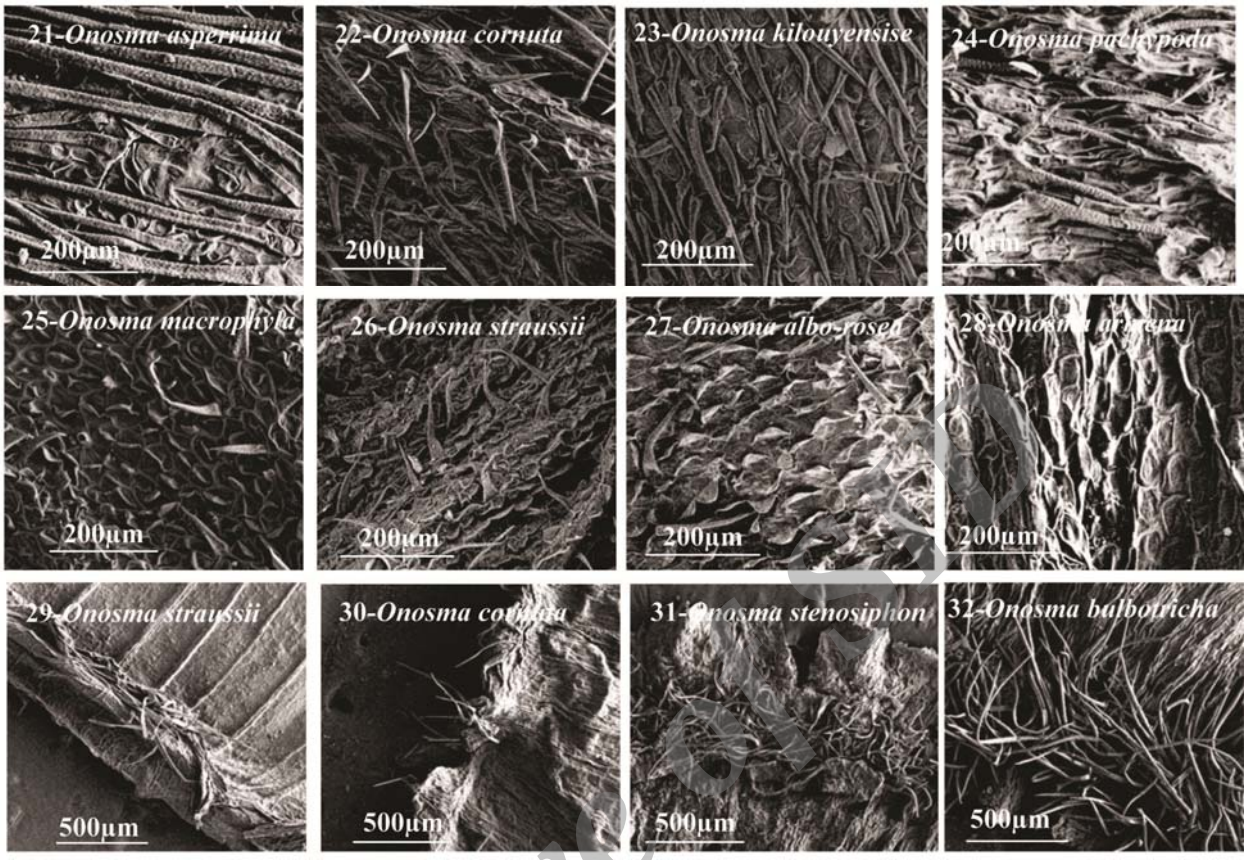

Fig. 1: Continued Corolla epidermis ornamentation (21-28) and nectar trichomes (29-32). 21, *O. asperrima*; 22,*O. cornuta*; 23, *O. kilouyensis*; 24,*O. pachypoda*; 25, *O. macrophyla*; 26, *O.* straussii; 27, *O. albo-rosea*; 28, *O. armena*; 29, *O. straussii*; 30,*O. cornuta*; 31, *O. stenosiphon*; 32,*O. bulbotricha*.

Furthermore nutlet ventral keel can be found in *O. rascheyana* (fig. 4.1), *O. kurdica* (fig.4.13), *O. elwendica* (fig.4.41), *O. nervosa* (fig4.31).

Nutlet micromorphology

Six types of nutlet epidermis ornamentations are defined for the studied taxa as follows:

Type I: Epidermal layer is characterized by rugose or bullate shape covered with coarse reticulate lines. This type can be divided to two subtypes: first subtype is seen in . *rascheyana* (fig.4. 2), *O. nervosa* (fig. 4.4), *O. dichroanta* (fig.4.6), *O. straussii* (fig.4.8), *O. albo-rosea* (fig.4.10), *O. microcarpa* (fig.4.12), *O. kurdica* (fig.4.14), *O. kilouyense* (fig.4.16), *O. olivieri* (fig.4.18), and second one only was observed *in O. dasytricha* (fig.4.20).

Type II: Epidermal layer is wrinkled. It is observed in *O. cornut* (fig.4.22) and *O. sabalanica* (fig.4.24).

Type III: Epidermal layer is rugose-striate including parallel coarse reticulate lines. Mentioned type is observed *O. stenosiphon* (fig.4.26), *O. bulbotricha* (fig.4.28) and *O. sericea* (fig.4.30).

Type IV: Epidermal layer is farinaceous (granular) that is finely mealy and covered with small granules. *Onosma rostellata* (fig.4.32) shows mentioned structure.

Type V: Epidermal layer is papillate (tuberculate or verrucate) having minute, rounded protuberances. Onosma *orientalis* (fig. 4.34) is classified in this type.

Type VI: Epidermal layer is relatively to completely smooth, *O. elwendica* (fig.4.42), *O. longiloba* (fig.4.38), *O. pachypoda* (fig.4.36) and *O. kotschyi* (fig.4.40) are in this type.

The UPGMA trees of nutlet evidence (fig. 5) showed a relatively high cophenetic correlation (r >0.80). In general, three main clusters can be shown. PCoA an Cluster analysis showed a relatively acceptable sepration at sectional level.

DISCUSSION

Measuring the size of plant organs and parts is important in description and identification. Within each taxon, the size of the flower parts is very variable. Calyx length and width were used in some references

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Table 2. Measurements taken from the flower characters. Calyx accrescent (ab: absence, ex: exsistence), sectioned calyx, (1: in base, 2: close to base); corolla color (1: yellow, 2: white, 3: red-pink, 4: blue-violet), corolla shape (1: tubular, 2: campanualte, 3: tubular campanulate, 4: tubular-clavate, 5: funnel-campanulate, 6: clavate), corolla lobe (1: broad, 2: linear), corolla epidermis ornamentation (I:reticulate, II:farinaceous, III:papillate, IV:aculeate, V:scurfy); anther exsertion (1: inside, 2: ouside), anther adhrence(1: free, 2: at base, 3: along side); nectar trichome (ab: absence, ex: exsistence).

Characters / Species	Calyx length (mm) (A)	Calyx lobe width (mm) (B)	Calyx accrescent (C)	Calyx section (D)	Calyx trichome color (O)	Corolla lobe length (mm) (E)	Corolla length (mm) (F)	Corolla shape (G)	Corolla color (H)	Corolla lobe shape (M)	Anther exsertion (I)	corolla epidermis ornamentation (J)	Anthers Adherence (k)	Corolla trichome (L)	Nectar trichome (N)
O. rostellata	6 5-8.5	0.74 0.6-0.9	ab	1	0	0.8±0.2	10 7-11	1	3	1	2	V	3	ex	ab
O. orientalis	10 6-12	0.8 0.7-1	ab	1	0	2.5±0.5	12 10-13	1	4	1	2	V	2	ab	ex
O. asperrima	8.5 6-12	1.14 1-1.3	ab	1	0	1±0.2	18 16-22	3	1	1	1	V	1	ex	ab
O. bodeana	20.5 18-22	2.16 2-2.5	ab	1	0	1.8±0.2	25 19-27.5	3	1	1	1	I	2	ab	ab
O. bulbotrica	17.5 12.2-18.9	1.5 1.2-2	ab	1	0	2±0.35	20 17-23	1	2	1	1	I	2	ab	ex
O. cornuta	11 9-14	4.71 4-7	ex	2	0	1.7±0.3	14.5 14-16	3	1	1	1	V	2	ex	ex
O. dichroanta	20 18-25	2.86 2.5-3	ex	2	0	2±0.35	33 21-38	3	4	1	1	I	2	ab	ab
O. Gaubae	13.5 12-15	1.1 0.9-1.3	ab	1	0	2±0.35	17 15-18	4	1	1	1	V	2	ex	ab
O. kilouyensis	15 14-17	5.85 5-8	ab	1	0	1.8±0.3	20 18-21	3	3	1	1	V	1	ex	ab
O. kotschyi	8.5 7-11	1.2 1.1-1.3	ab	1	0	2±0.35	9 7-10	1	1	1	1	–	1	ex	ab
O. longiloba	11 10-13.5	3.85 3-5	ab	1	0	6±0.6	9 8-12.5	1	4	2	1	II	1	ab	ab
O. microcarpa	10 8-14	1.07 1-1.3	ab	1	1	1.1±0.2	18.5 15-25	3	1	1	1	I	2	ex	ab
O. pachypoda	17 14-18	0.81 0.7-1	ex	1	0	1.5±0.2	26 24.5-29	6	4	1	1	V	1	ex	ab
O. platyphylla	10 8-13	0.97 0.8-1.2	ab	1	0	3±0.4	13 12-15	3	1	2	2	IV	1	ex	ab

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Table 2. Continued.

O. sabalanica	16-15	4.85 2-7	ab	1	1	2.8±0.4	17 15-20	2	1	1	1	III	2	ex	ab
O. sericea	14 12-16	7.6 7-9	ab	1	0	1.9±0.3	17.5 15-20	6	1	1	1	V	2	ex	ab
O. stenosiphon	11.2 7.3-14.2	1.44 1-2	ab	1	0	1±0.2	12 7.3-14	1	4	1	1	I	2	ab	ex
O. subsericea	20.8 18.3-25	2.61 2.3-3	ab	1	0	1±0.2	26.5 22-28	2	1	1	1	I	3	ex	ab
O. elwendica	16 14-17	5.85 5-8	ex	2	0	2±0.35	19 18-20.5	3	4	1	1	I	2	ab	ab
O. macrophyllla	11 10-13.5	4.71 4-6	ab	1	0	1.5±0.2	18 17-19	2	1	1	1	V	2	ex	ab
O. nervos	11 10-13	1.7 0.8-2	ex	1	1	1.3±0.2	13 12-15	2	3	1	1	I	2	ab	ab
O. olivieri	15.5 13-16.6	2.5 2-2.9	ab	1	0	1.2±0.2	21 20-23	2	1	1	1	_	2	ex	ab
O. straussii	9.5 7-10	6.42 5-7	ab	1	0	2±0.3	17.5 16-20	5	3	1	2	V	2	ex	ab
O. albo-rosea	15 14-17	8.8 5-11	ab	1	0	2±0.4	24 22-27	4	3	1	1	V	2	ex	ab
O. armena	9.5 9-10	0.85 0.8-0.9	ab	1	0	1.8±0.2	18 16-20	1	1	1	1	V	2	ab	ab
O. dasytricha	18 16-20	3.94 2-4.7	ab	1	0	0.8±0.2	24 20-28	4	4	1	1	_	2	ex	ab
O. hebebulba	16 15-18	1.6 1.2-1.9	ab	1	0	1.8±0.2	15 14-17	3	1	1	1	I	2	ab	ab
O. kurdica	22.5 20-23.5	0.9 0.8-1	ab	1	0	0.8±0.2	25 23-27	6	3	1	2	II	2	ab	ab
O.rascheyana	13.5 12-16.5	1.5 1-2.1	ab	1	0	1±0.2	22.5 20-25.2	6	3	1	1	III	1	ab	ab

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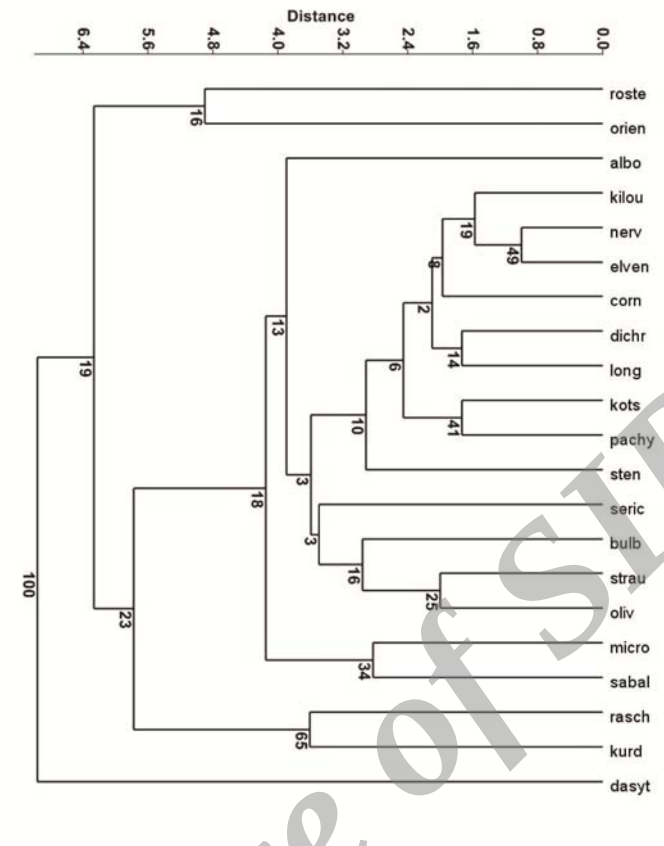

Fig 2. UPGMA tree of studied taxa based on flower character.

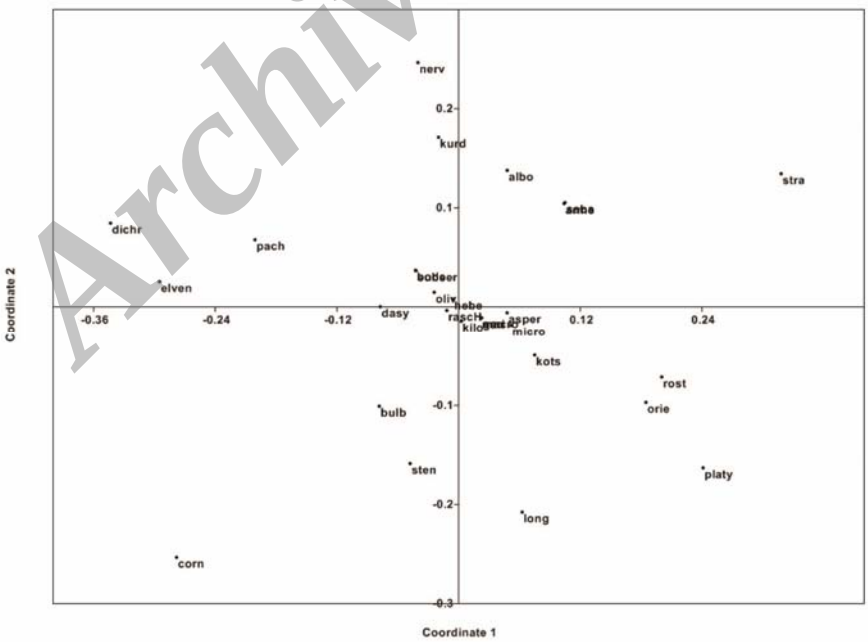

Fig 3. PCoA plot of studied taxa based on flower evidence.

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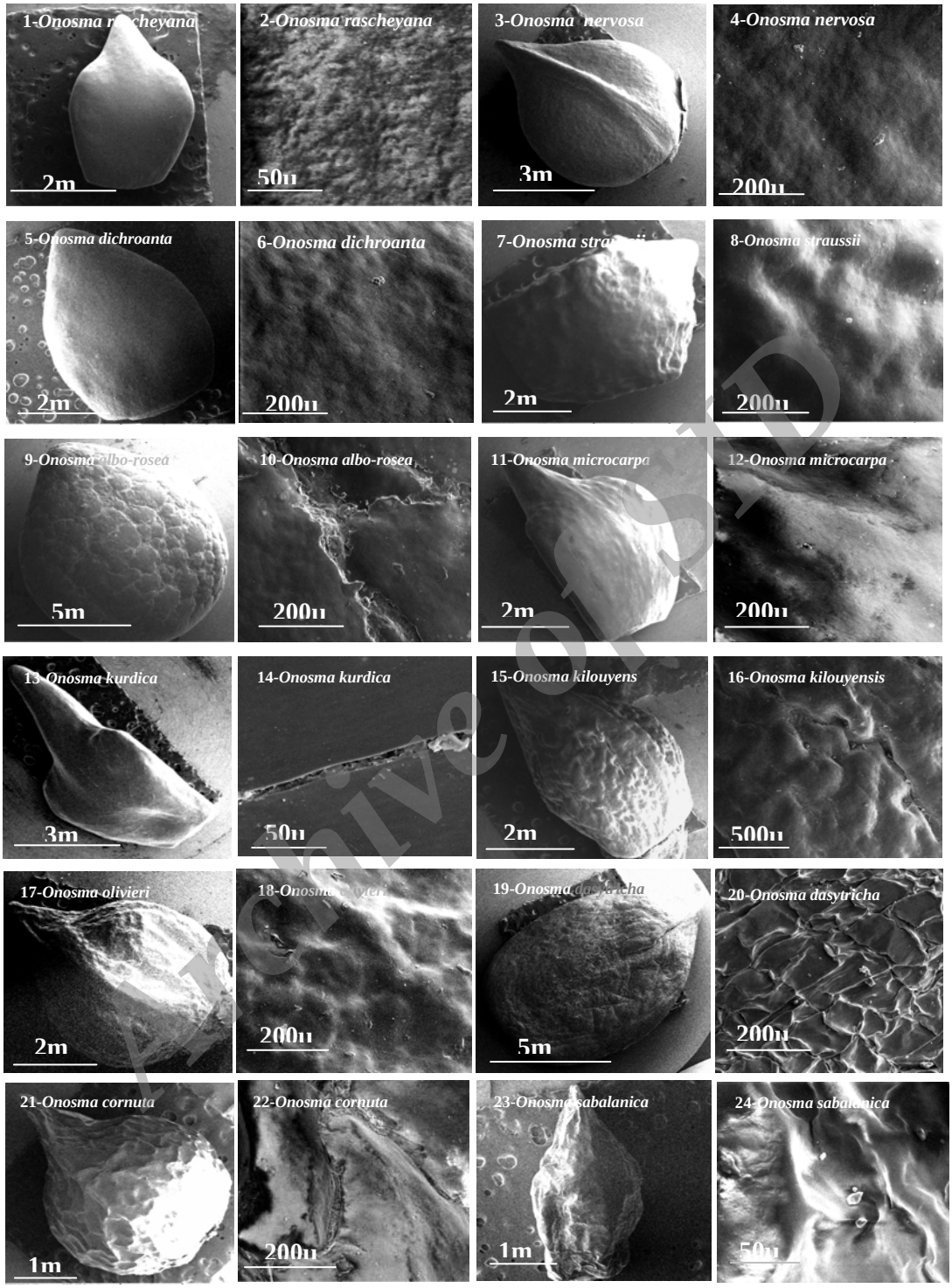

Fig. 4: nutlet micromorphology of studied species. 1-2, *Onosma rascheyana*; 3-4 *O. nervosa*; 5-6, *O. dichroanta*; 7-8, *O. straussii*; 9-10, *O. albo-rosea*;11-12, *O. microcarpa;* 13-14, *O. kurdica*; 15-16 *O. kilouyensis*; 17-18, *O. olivieri*; 19-20, *O. dasytricha*; 21-22, *O. cornuta*; 23-24, *O. sabalanica*.

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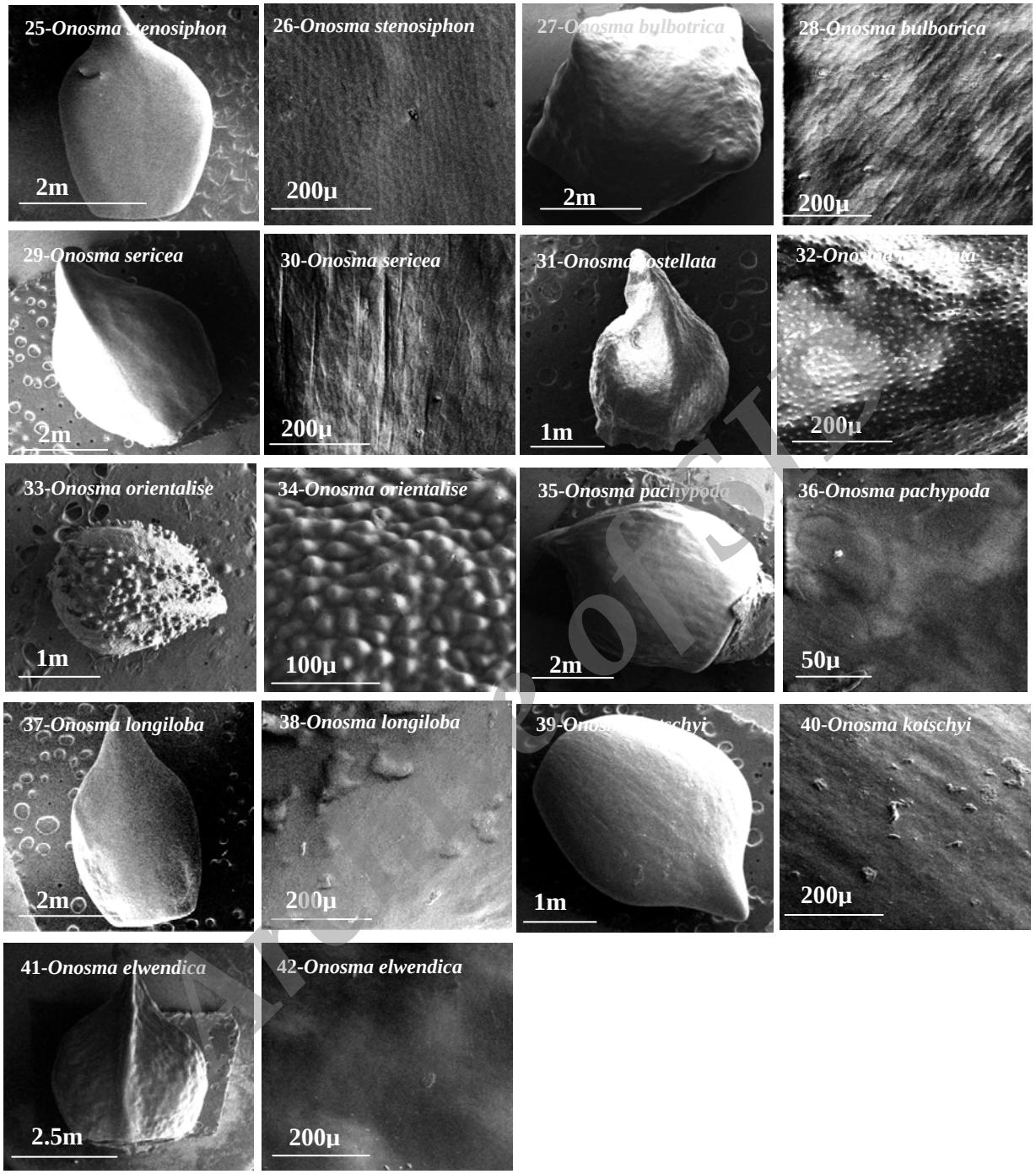

Fig. 4: Continued. nutlet micromorphology of studied species. 25-26, *O. stenosiphon*; 27-28, *O. bulbotrica*; 29-30, *O. sericea*; 31-32, *O. rostellata*; 33-34, *O. orientalis*; 35-36, *O. pachypoda*; 37-38, *O. longiloba*; 39-40, *O. kotschyi*; 41-42, *O. elwendica*.

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Table 3. Measurements taken from the nutlet characters. Nutlet orientation (1:straight, 2: incurved), nutlet shape (ov: ovate, wo: wide ovate, py: Pyramid:, rh: rhombic), nutlet ventral keel (ex: exsistence, A. absence, M: middle), nutlet epidermis ornamentation (typeI: rugose, typeII: wrinkled, type3: rugose-striate, type4: farinaceous, type5: papillae, type 6: smooth).

Character / ecies	**Nutlet orientation (A)**	**Nutlet shape (B)**	**Nutlet ventral keel (C)**	**Nutlet epidermis ornamentation (D)**	**Nutlet width (mm) (E)**	**Nutlet length (mm) (F)**	**Nutlet beak length (mm) (G)**	**Beak length/nutlet length (H)**	**Nutlet length/nutlet width (I)**
O. rostellata Lehm.	1	wo	ex	IV	3.37 3-3.5	3.38 3-3.6	1.43±0.2	0.43 0.39-0.47	1.00 0.85-1.2
O. orientalis	2	ov	ab	V	2 1.5-1.8	2.25 2.3-2.5	0.8±0.2	0.44 0.32-0.34	1.55 1.27-1.66
O. bulbotrica	1	py	m	III	4.47 4-4.9	4.67 4-5	0.86±0.2	0.18 0.17-0.21	0.92 0.81-1.25
O. cornuta	1	Wo	ex	II	2.8 2.6-3	4.15 4-4.3	1±0.2	0.26 0.23-0.27	1.27 1.15-1.72
O. dichroanta	1	ov	ab	I	2.97 2.9-3	4.33 4-5	1.23±0.2	0.28 0.24-0.30	1.46 1.33-1.72
O. kilouyensis	1	wo	ex	I	3.83 3.5-4	5.28 5-6	1±0.2	0.20 0.16-0.21	1.29 1.25-1.71
O. longiloba	1	ov	ab	VI	2.23 2-2.5	3.46 3.2-3.6	0.87±0.2	0.27 0.24-0.27	1.46 1.28-1.80
O. kotschyi	1	wo	ab	VI	2.11 1.5-2.5	3.77 3.5-4	1.82±0.2	0.49 0.44-0.52	1.82 1.4-2.6
O. microcarpa	1	wo	ex	I	2 1.7-2.5	2.57 2.5-3	0.9±0.2	0.34 0.3-0.36	1.19 1-1.76
O. pachypoda	1	wo	ab	VI	3.35 3.2-3.5	5.5 5.3-5.7	1.6±0.2	0.29 0.28-0.30	1.64 1.51-1.78
O. sabalanica	1	ov	m	II	2 1.8-2.1	4.06 3.7-4.3	1±0.2	0.27 0.23-0.27	2.08 1.76-2.38
O. sericea	1	ov	ex	III	3.64 3-4	4.8 4.4-5	1.62±0.2	0.34 0.32-0.36	1.33 1.1-1.66
O. stenosiphon	1	ov	ab	III	2.06 2-2.2	4.08 4-4.2	0.83±0.2	0.20 0.19-0.20	1.95 1.81-2.10
O. straussii	1	py	m	I	4.3 4.1-4.5	5.47 5.3-5.7	1.2±0.2	0.22 0.21-0.22	1.36 1.20-1.33
O. albo-rosea	1	wo	ab	I	5.78 5.5-6	8.18 7.8-8.3	0.93±0.2	0.12 0.11-0.12	1.37 1.30-1.50
O. dasytricha	1	ov	m	I	5.21 5-5.4	6.63 6-7	2.72±0.3	0.40 0.38-0.45	1.44 1.11-1.5
O. rascheyana	1	ov	ab	I	2.88 2.8-3	1.71 1.5-2	3.07±0.4	1.67 1.53-2.04	0.59 0.50-0.71
O. kurdicua	1	rh	ex	I	4 3.8-4.2	8 7.5-8.3	3.2±0.4	0.4 0.38-0.42	0.8 1.78-2.18
O. elwendica	1	wo	ex	VI	3.86 3.5-4	5.28 5-6	1.36±0.2	0.18 0.22-0.27	1.36 1.25-1.71
O. nervosa	1	wo	ex	I	3.8 3.7-4	4.04 3.9-4.3	1.31±0.2	0.32 0.30-0.33	1.06 0.97-1.16
O. olivieri	1	wo	m	I	5.5 5.3-5.7	7.5 7.3-7.7	2±0.3	0.26 0.25-0.27	1.36 1.28-1.45

(Shishkin 1953) as a weak-effect character, but along with other diagnostic characters, they can be used in determination of *Onosma* taxa. Our study revealed taxonomic significance of the corolla length in some *Onosma* taxa, for example *O. kotschyi* (corolla length ca. 9mm) and *O. asperrima* (corolla length ca. 25mm) as closely related species, can be distinguished based on this character. Moreover Riedl (1967) referred to it as a valuable character. Furthermore Peruzzi & Passalacqua. (2008) and Mehrabian et al. (2012) used corolla length for interpretation of population variability in *Onosma*.

Six kinds of corolla shapes can be distinguished in studied taxa. In our study it appeared as an important diagnostic character at species level in some taxa and as a clear character is able to distinguish some groups that faced taxonomical complexities. On the basis of this character *O. sericea, O. cornuta* and *O. elwendica* as a complex taxa are determinable. Moreover Riedl (1967) and Davis (1978) confirmed the importance of corolla shape in taxonomy of the genus.

Wodehouse (1935) and Lee (1979) have reported a direct correlation between corolla and pollen size. But Binzet (2011) in *Onosma* proved that there was no correlation between corolla size and pollen size. Mehrabian et al. (2012) proved Some species with large corolla i.e. *O. alborosea* and *O. bulbotrichum* have longer pollens (18-21µm and 15-18 µm

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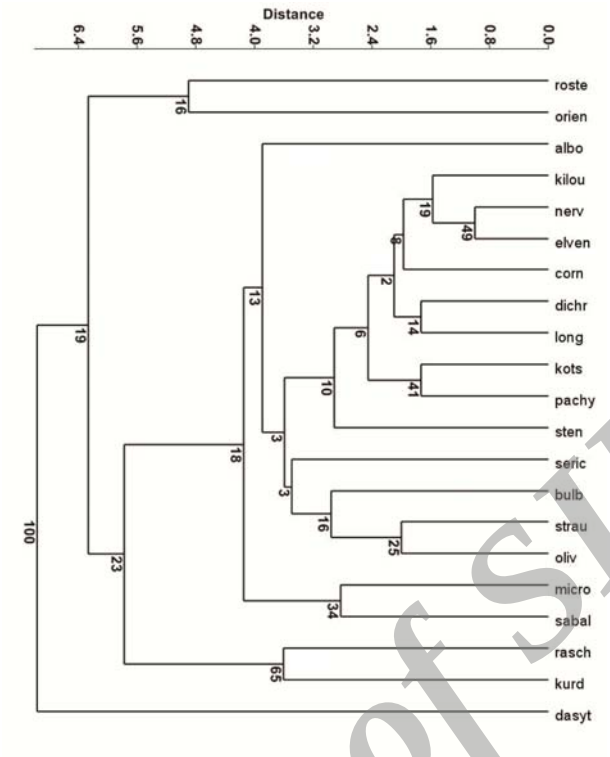

Fig 5. UPGMA tree based on nutlet character.

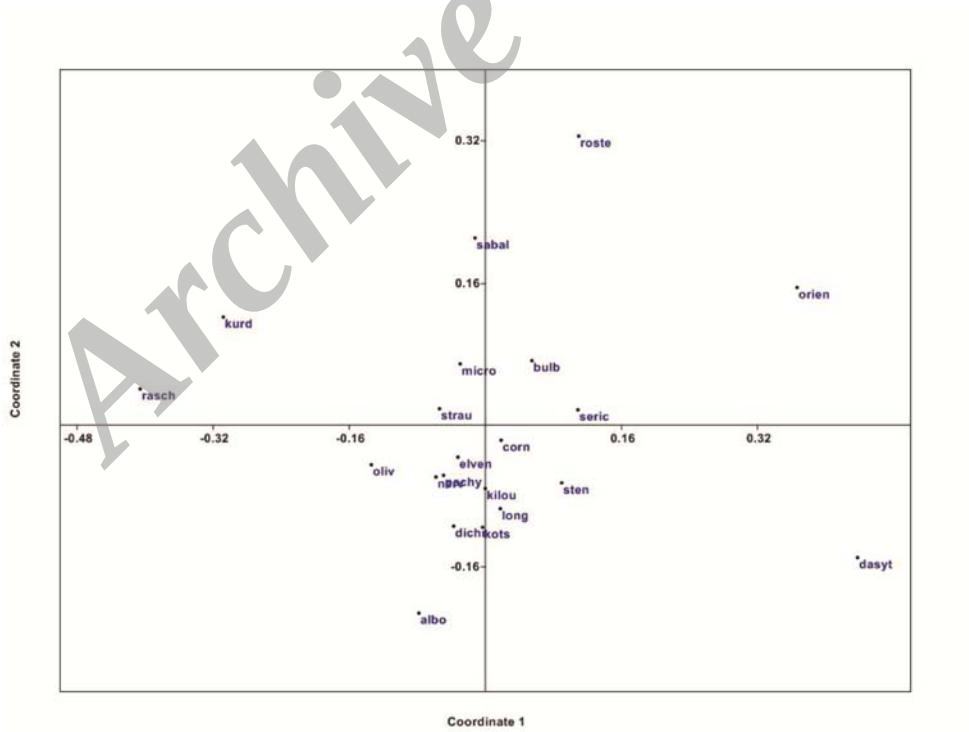

Fig. 6. PCoA plot of studied taxa based on nutlet character.

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respectively) but some taxa , i.e. *O. microcarpa* and *O. rostellata* with shorter corolla showed larger pollen (16-19µm and 16-22 µm respectively). Our results confirm those provided by Binzet (2011).

Corolla color varies considerably. Besides it showed some variations in diverse ecological (especially soil and climate) conditions (Mehrabian 2011) in different populations. For instance *O. pachypoda* and *O. bodeana* showed variability in diverse ecological habitats. Furthermore, several species showed similarity in corolla color. This character is hardly reliable but as a subsidiary diagnostic character along with other important characters can be used to distinguish *Onosma* taxa.

In the present study, the nectary trichomes of the studied taxa appeared to be diagnostic in restricted taxa i.e. in *O. straussii* (fig.1.29), *O. cornuta* (fig.1.30), *O. stenosiphon* (fig.1.31) of the sect. *Onosma* and *O. orientalis* (fig.1.32) of the sect. *Protonosma.* Therefore as a weak delimitation character can be used along with other characters for definition of limited taxa.

Corolla lobe is hardly reliable, nevertheless, *O. longiloba* (ca. 6mm) and *O. paltyphylla* (ca. 3.5mm) are distinguished from other taxa by short corolla lobe (less than 2.8 mm). Calyx accrescent was observed in two subsections for example *O. cornutua*, *O. dichroantha, O. pachypoda* belonging to subsect. *Haplotricha*, *O. elwendica* and *O. nervosa* from subsect. *Heterotricha*. Hence it can be used as a diagnostic character for delimitation of some closely and hardly distinguishable taxa. For instance based on calyx accrescent *O. elwendica* can easily be distinguished from its close species *O. olivieri*. Calyx division did not show taxonomic importance, most species of *Onosma* have shown division at the base but only *O. cornuta*, *O. dichroantha* belonging to subsect. *Haplotricha* and *O. elwendica* from subsect. *Heterotrica* represent calyx division near the base. Also Calyx trichome color showed weak valuae in taxonomy of *Onosma*, because only *O. sabalanica* (subsect. *Haplotricha*) , *O. elwendica* and *O. nervosa* (subsect. *Asterotricha*) showed this properties. Riedl (1968); Davis (1978); Shishkin (1953); Akcin (2009) used mentioned features as a diagnostic characters in *Onosma* .

Anther features have proved their abilities for delimitation of *Onosma* species. Anthers are connected along side in sect. *Podonosma*, but they are free to connected at the base in other sections. Nevertheless, they are diagnostic characters as follow : *O. asperrima* with free anthers is distinguishable from very closely relatad species *O. kotschyi* with connected anthers at base and *O. pachypoda* with free anthers is diffreable from *O. bodeana* with connected anthers at base. *Onosoma rostellata* of sect. *Protonosma* with connected anthers in along sides, ellipsodal pollen is different from the other *Onosma* taxa which have prolate or subprolate pollen shape (Mehrabian et al. 2012) differences in molecular characters (Mehrabian et al. 2011) and the granulate ornamentation in nutlet surface are the diagnostic characteristics that brings the idea of transfering this onosma species to a different genus. Moreover *Onosma orientalis* (section. *Podonosma* Boiss.) with divergence in morphology, differences in molecular (Mehrabian et al. 2011), trichome characters and tuberculate ornamentation of nutlet surface shows differences from the other *Onosma* taxa. Complementary studeis are needed to prove its taxonomic status within Boraginaceae, i.e. phylogenetic stdies besed on chloroplast and nuclear genome sequences.

Corolla epidermis showed five ornamental patterns. These detailed patterns showed clearly boundaries of species. Some types are unique characteristcs for special species. Type IV was only observed in *O. platyphylla* (subsect. *Haplotricha*) and type I was observed only in sect. *Onosma*. Moreover type III and type II can be seen in sect. *Onosma*. On this baisis, these features accompained by other morphological characters are useful for species determination and solving some taxonomical complexities in *Onosma*. Akcin (2009) have used petal morphology as a valuable character.

Some nutlet studies in Boraginaceae (Selvi et al. 2006) proved nutlet morphology to be important for differentiation of these taxa at the infrageneric categories.

Nutlet orientation follows two patterns: straight, and incurved. *Onosma orientalis* (sect.*Podonosma*) showed high divergence compared to other *onosma* sections, therefore as a unique chracter it is valuable for infrageneric delimitation in *Onosma.*

Nutlet size and witdh in some cases appeared to be important among studeid species. *Onosma kurdica*, *O. bulbotricha* and *O. starussii* showed specific shapes that help to distinguish them from the other species. Moreover some species showed very different size for example *O. alborosea* that is differable from its closely related taxon *O. dasytricha.* Nutlet ventral keel showed diversity in studied species, due to its appearance in all sections and subsections, this character is not useful for delimitation of species but as a valuable character showed diagnostic ability to define closely related species in complicated species groups. For example *O. kurdiua* (by ventral Keel) and *O. rascheyana* (no ventral keel) are distinguished.

Nutlet ornamentation represent five patterns in studied taxa. Each section have specific pattern as

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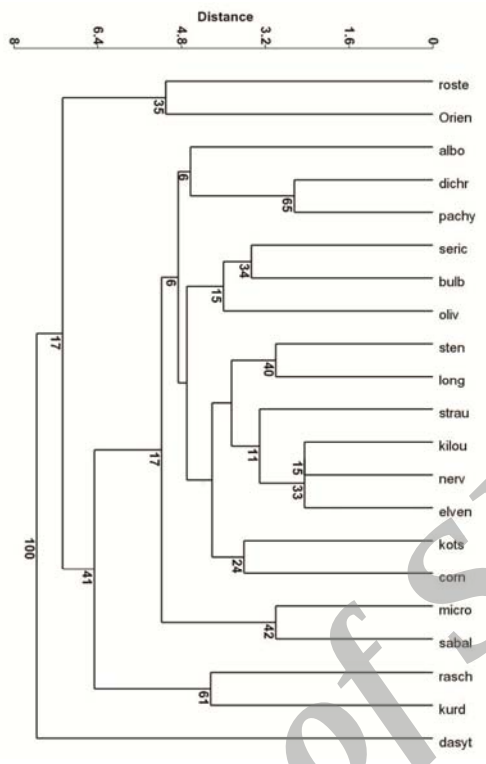

Fig. 7: Combined UPGMA tree based on nutlet and flower characters.

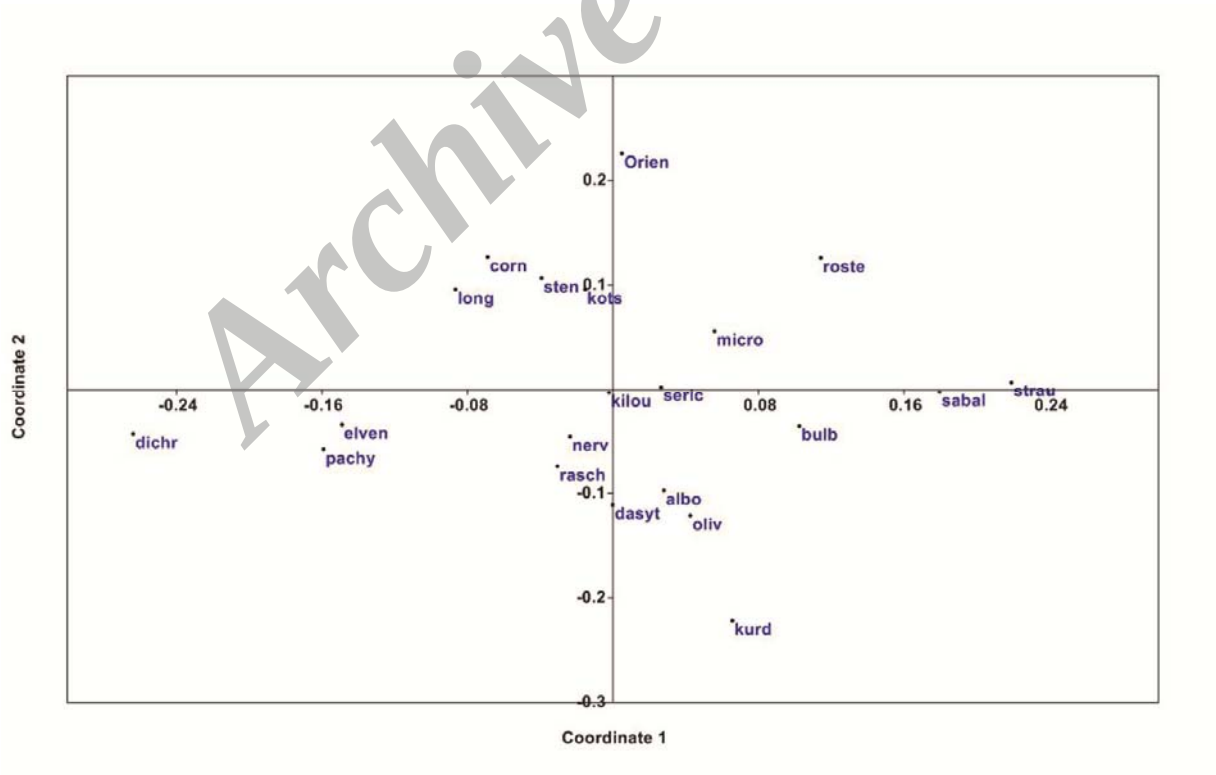

Fig. 8. Combined PCoA plot based on nutlet and flower characters.

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follow: *O. orientalis* (Sect. *Podonosma*) by Papillate pattern and *O. rostellata* (Sect. *Protonosma*) with farinaceous pattern. Furthermore there are rugose (*O.rascheyana, O. nervosa*), rugose-striate (*O. pachypoda*, *O. longiloba* and smooth pattern (*O. bulbotricha, O. stenosiphon*) in sect.*Onosma.* Accordingly, this character is useful in recognition of *Onosma* at sectional level, and as a valuable character in species delimitation. Moreover some complex species groups (groups with many overlapping morphological features) such as *O. bulbotricha, O. straussii, O. cornuta* and *O. sericea* are distinguisable based on mentioned patterns. Besides, some closely related taxa like *O. rascheyana* and *O. kurdica* can be sepretaed by this character. PCoA and cluster analysis showed their efficiency for sectional delimitation, but not useful to seprate the subsections. Binzet and Akcin (2009) and Akcin & Binzet (2011) had the same results.

Cluster analysis based on palynological (Mehrabian et al. 2012), morphological and molecular (Mehrabian et al. 2012) evidence confirmed our logic on diagnosis value of studied charcters at sectional and species level. The results of the present study highlight the importance of the morphology and micro-morphology of floral and nutlet characters in relation to identification and delimitation of most of the studied species and improvement of taxonomic knowledege about *Onosma.* Although there are some taxa which cannot be determined solely using the mentioned characters, but these characters are useful tools in distinguishing many taxa and solving some complexities in *Onosma*. On the other hand the combination of these characters along with morphological, palynological and molecular evidence provide a perfect view for taxonomy and careful taxonomical revision of *Onosma*.

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