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# POLLEN MORPHOLOGY AND SYSTEMATIC SIGNIFICANCE OF SOME *ONOSMA* L. SPECIES (BORAGINACEAE) DISTRIBUTED IN PAN HIMALAYAN REGIONS

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## Abstract

The pollen morphology of 15 *Onosma* L. species has been examined by SEM for the first time from Pan Himalayan regions of Pakistan and China.

Generally, the pollen grains are single, isocolpate, radially symmetrical, isopolar or heteropolar, 3-colporate or 3-syncolporate, small to medium in size. The mean polar axis ranged from 10.00-25.43 µm long while the equatorial diameter ranged from 8.68-16.93 µm. The mean P/E ratio generally ranged from 1.04-1.56 µm. On the basis of P/E ratio, three pollen shapes have been recognized (prolate spheroidal, subprolate and prolate). Ornamented aperture membrane and lolongate ora uniformly observed among all studied taxa. However, the results show great diversity within species in regard of polarity, apertures and exine ornamentation (spinulose, rugulate, rugulate-echinate, rugulate to granulate). On the basis of aperture type, two basic pollen types have been recognized. Type-I 3-syncolporate observed in five species (*O. confertum, O. dicroanthum, O. hispida, O. limitaneum and O. paniculatum*) and Type-II 3-colporate in 10 species (*O. exertum, O. fistulosum, O. glomeratum, O. hookeri* var. *hirsutum, O. hookeri* var. *longiflorum, O. maaikangense, O. multiramosum, O. sinicum, O. waddellii,* and *O. waltonii*).

On the basis of 10 different pollen characters (seven qualitative and three quantitative) and 24 characters states two types of numerical analyses (PCA and CA) have been carried out in order to determine the potential of the pollen morphological characteristics for determination of the species relationships. The present palynological data therefore provides new information about pollen morphology of all *Onosma* L. species especially *O. glomeratum* and *O. multiramosum, O. hookeri* var. *hirsutum, O. hookeri* var. *longiflorum.* The current study can be helpful to analyze the pollen variation within *Onosma* L. species. It also highlights the significance of pollen characters used to determine intraspecific relationships as well as correlation of pollen characters with the taxonomy of the genus.

**Key words:** Pollen morphology, SEM, *Onosma,* Lithospermeae, Boraginaceae, Pan Himalayan, Numerical analysis, Systematic significance.

## Introduction

The Boraginaceae Juss family is a cosmopolitan family that belongs to the order Lamiales and widely distributed in the tropical, subtropical, and temperate regions of the world. The centers of the highest diversity in the North Temperate Zone are the Irano-Turanian and Mediterranean regions and in the tropics, the centers of the highest diversity are Central America and Northern and Central South America (Al-Shehbaz, 1991; APG IV, 2016).

*Onosma* L. is a large genus of about 150 species belonging to the tribe Lithospermeae which is commonly spread in the temperate and subtropical regions of the world (Liu *et al.,* 2010). However, genus *Onosma* has great diversity in Europe as well as Asia (including Pakistan, India, Afghanistan, Iran, China), Turkey and Himalayan regions like Kashmir and Tibet etc. Many species of the *Onosma* L. have been used in ethno-pharmacology and the ethanol extract of some species has been reported to have anti-inflammatory and analgesic properties. Some are used for other health remedies, treatment of wounds, as herb, traditional medicine and dyes (Özgen *et al*., 2003; 2004; Naz *et al*., 2006; Tosun *et al*., 2008, Kumar *et al.,* 2013; Daironas *et al*., 2014; Imran *et al.,* 2018). The floral parts of some species are edible and taken as vegetables (Öztürk & Özçelik, 1991).

The genus *Onosma* is a natural and fairly a homogeneous taxon with little morphological variation and due to the similarities among the *Onosma* taxa, there are many problems in their identification. Hence, systematically and taxonomically, it is considered a difficult genus (Tutin *et al.,* 1972; Qureshi & Qaiser, 1987; Binzet & Akcin, 2009; Binzet, 2011). Taxonomic treatments within the genus are highly controversial and many closely related taxa described on the basis of minor morphological differences leading to several mistakes in the taxonomy of the genus in past (Ball, 1972; Maggi *et al.,* 2008; Arabameri, 2014). Moreover, most of the species are distinguished based on indumentum characteristics and the within the genus different sections have been proposed by various taxonomists Boissier, (1875), Popov & Shishkin (1974) and Riedl, (1978) etc. However, due to above mentioned complexities, Riedl, (1978) considered this classification partly artificial and suggested re-investigation of taxa by different taxonomic approaches (palynological and karyological) in order to provide some useful evidence in a new classification.

Palynology is considered as an additional source for solving taxonomic problems at different levels like generic and specific. Palynological data can be used for placement of taxonomically controversial taxa by re-arrangement, withdrawals and separations, as well as corroborating other lines of evidence (Davis & Heywood, 1963). Palynology has been proved significantly important in delimitation of genera of Boraginaceae and in understating of evolutionary trends within the entire family (Clarke *et al.,* 1977; Al Shehbaz, 1991). Such generic level studies have contributed a lot for determination of species relationships within genera and has been proved useful for identification of taxonomically problematic taxa. The contributions to the pollen morphology of different Boraginacecae genera includes pollen studies on *Cordia* L. Nowicke & Ridgway (1973); *Tournefortia* L. (Nowicke & Skvarla, 1974); *Arnebia*

Frossk. (Qureshi *et al.,* 1989); *Heliotropium* L. (Qureshi, 1979; 1985); *Onosma* L. (Qureshi & Qaiser, 1987); *Microula* Benth. and allied taxa Ning *et al.,* (1993); *Anchusa* L. (Diez, 1994); *Echiochilon* (Osaloo & Khatamsaz, 1994); *Lobostemon* Lehm. *Echiostayhs* Levyns and *Echium* L. (Retief & Van Wyk, 1997); *Cordia* L. (Liu *et al.,* 2001); *Buglosssoides* (Retief & Van Wyk, 2002); *Cryptantha* Lehmann ex G. Don (Hargrove & Simpson, 2003); *Anchusa* L. and *Nonea* Medik. (Jamalou *et al.,* 2006; *Nonea* Medik. (Falatoury *et al.,* 2011); *Omphalodes* Mill. (Coutinho *et al.,* 2012); *Mertensia* Roth; *s. lat.* (Fukuda & Ikeda, 2012) and *Eritrichium* Schard. (Mazari *et al.,* 2018) etc.

Different studies related to morphological, micromorphological, anatomical, ecological, karyological, molecular and palynological characteristics of *Onosma* species have been carried out (Teppener, 1971; 1972; 1991; Azizian *et al.*, 2000; Vauillamoz, 2001; Bigazzi & Selvi, 2000; Akçin & Engin, 2001; 2005; Akçin, 2004; 2007a; 2007b; 2009; Martonfi *et al.*, 2008; Peruzzi & Passalacqua, 2008; Kolarcik *et al.*, 2010; Akcin & Binzet, 2010; 2011; Binzet & Akçin, 2009; 2012; Binzet & Orcan, 2003a; 2003b; 2009; Binzet, 2011; Rajanbar & Almasi, 2013; Arabmeri *et al*., 2014; Daironas *et al*., 2014; Kolarcik *et al.*, 2014; Mehrabian *et al*., 2014; Almasi & Rajanbar, 2015; Teke & Binzet, 2014; 2017; Mehrabian *et al.*, 2017 and Binzet *et al.*, 2018). However, the systematics of *Onosma* still has not been fully resolved and needs more attention. Studies on the *Onosma* genus are limited especially the palynological studies (Maggi *et al*., 2008). However, Johnston, (1954) studied pollen grains of different genera of Lithospermae by light microscopy, including 45 species of *Onosma.* Similarly, Liu *et al.*, (2010) also investigated pollen characteristics of different genera of the tribe Lithospermeae including five *Onosma* species. Qureshi & Qaiser, (1987) reported the pollen features of 9 *Onosma* species. Perveen *et al.,* (1995) investigated five *Onosma* species while studying 49 species from 20 genera of Boraginaceae. A comparative study of *Onosma* and *Maharanga* from China has been provided by Ning *et al*., (1995). Khatamsaz, (2001) examined pollen morphology of 42 species, belonging to 32 genera of Boraginaceae including three *Onosma* species. Other literature regarding pollen characters of *Onosma* species include studies by Maggi *et al.,* (2008); Binzet, (2011), Mehrabian *et al.,* (2012), Binzet & Ozler, (2014) and Binzet *et al.*, (2018). Such studies are totally lacking from Pan Himalayan and most of the *Onosma* taxa studied for pollen morphology from Turkey and Iran are endemic to these regions. Furthermore, the taxa included in this study are rarely studied in previous palynological studies.

The aim of this research is to provide some new information about the pollen characters of *Onosma* and to evaluate systematic significance of pollen characters in taxonomy. The studied pollen characters can be helpful for identification and delimitation of *Onosma* species as well as for correlation with taxonomic characters of the genus.

## Material and Methods

### Sampling and scanning electron microscopy (SEM)

**Pollen samples:** Fresh pollen samples were collected from Pan Himalayan regions of China and Pakistan (Fig. 1) during 2015-2017. The plants were dried and deposited in the Herbarium of Beijing Normal University (BNU). Pollen samples were collected from the specimens and some of the pollen samples were collected from Herbarium of Center for Plant Conservation, University of Karachi, Pakistan (KUH) and Chinese National Herbarium, Beijing China (PE). The list of examined *Onosma* L. species and their voucher information is provided in Table 1.

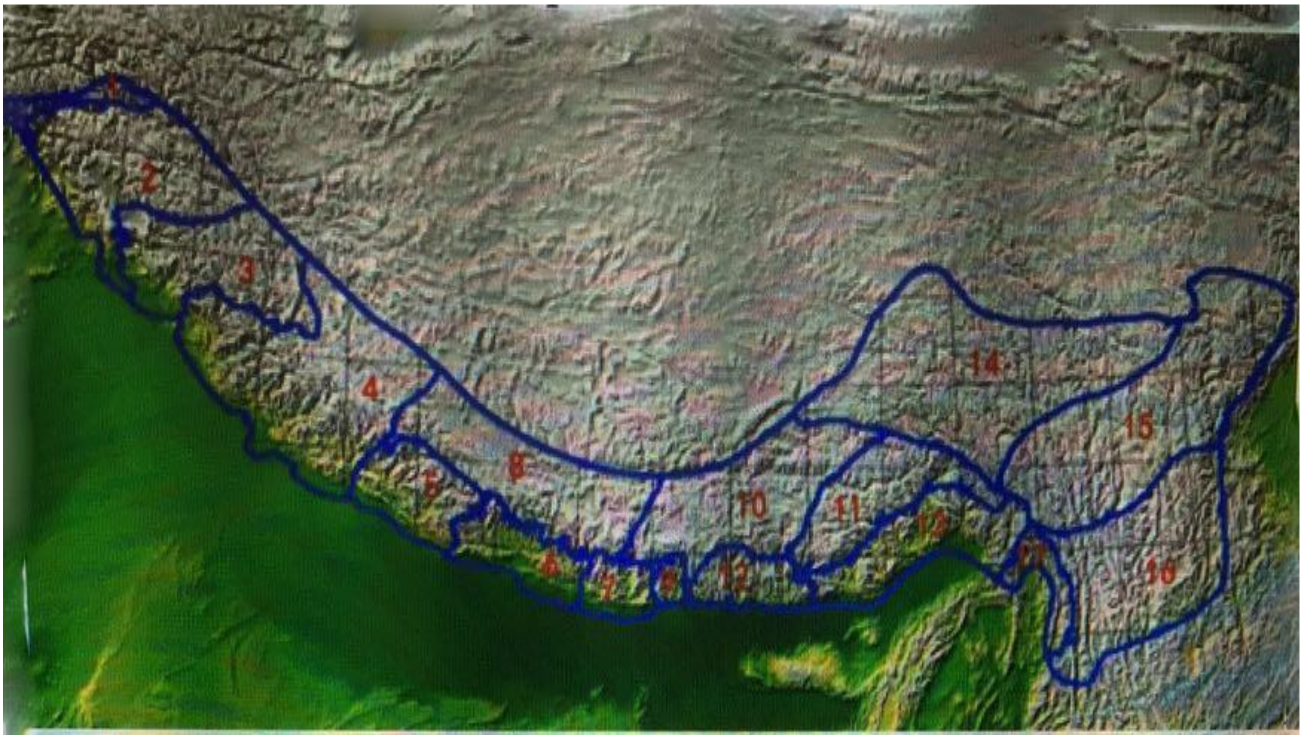

Fig. 1. Sketch Map showing 17 different geographical subdivisions of Pan Himalaya.

**Table 1. List of the studied *Onosma* taxa and the voucher information.**

| Name of taxa | Collectors & vouchers | Locality |
|---|---|---|
| *Onosma confertum* W. W. Smith | Wu Kai, J. C. Hao, YN02, (BNU) | China: Yunnan |
| *O. dicroanthum* Boiss. | Y. Nasir & Khan, 18576, (KUH) | Pakistan: Malakand |
| *O. exsertum* Hemsl. | He Shiyuan, (BNU) | China: Yunnan |
| *O. fistulosum* I. M. Jhonst. | Ke Cheng-Sheng Pl. Exped. 12961 (PE) | China: Sichuan |
| *O. glomeratum* Y. L. Liu | Yu Sheng-Xiang et.al 5557 (PE) | China: Tibet |
| *O. hispida* Wall ex G. Don | Ahmad L., LA 07 (BNU) | Pakistan: Muzaffarabad |
| *O. hookeri* var. *hirsutum* Y. L. Liu | Qinghai-Xizang Exped. 74-3618 (PE) | China: Qinghai Xizang |
| *O. hookeri* var. *longiflorum* (Duthie) Duthie ex Stapf | Xizang Med. Pl. Exped. 732 (PE) | China: Tibet |
| *O. limitaneum* I. M. Johnst. | Ahmad L., LA 25, (BNU) | Pakistan: Dir |
| *O. maaikangense* W. T. Wang ex Y. L. Liu | Wu Kai, XMLY12033 (BNU) | China: Sichuan |
| *O. multiramosum* Hand. -Mazz. | J. C. Hao 15576 (BNU) | China: Tibet |
| *O. paniculatum* Bur. et Franch. | Wu Kai, J.C. Hao, YN021, (BNU) | China: Yunnan |
| *O. sinicum* Diels. | Wang Zuo-Bin, 14081 (PE) | China: Gansu |
| *O. waddellii* Duthie | He Yi, XZ2017326, (BNU) | China: Tibet |
| *O. waltonii* Duthie | Wei Lai, He Yi, XZ2016382(BNU) | China: Southern Tibet |

## Results

**Pollen morphological analysis:** The pollen grains of genus *Onosma* L. has been examined by scanning electron microscopy (SEM) for the first time from Pan Himalayan regions of China and Pakistan. Generally, the pollen grains are single and radially symmetric, isocolpate, isopolar or heteropolar, small to medium in size. Further detailed information obtained from SEM of pollen grains is described below and also presented in Table 2.

**Pollen size:** Aaccording to Erdtman (1952) classification of pollen sizes, the size of pollen grains of studied *Onosma* L. species ranged from small to medium (Fig. 2). In all the species the size of the pollen grains was small (less than 10 µm) except one species *O. multiramosum* (Fig. 5 a-c) that possess medium sized pollen grains with 25.43 µm long polar axis and 16.93 µm equatorial diameter. However, the smallest polar axis is observed in *O. waltonii* (Fig. 5 m-o) i.e., 10.00 µm while the smallest equatorial diameter (Fig. 2) i.e., 6.68 µm is recorded in *O. confertum* (Fig. 3 a-c).

**Polarity:** The polarity also differed greatly among the studied species of *Onosma* L. In 9 species (*O. hispida* (Fig. 4 a-c), *O. paniculatum* (Fig. 5 d-f), *O. fistulosum* (Fig. 3 j-l), *O. glomeratum,* (Fig. 3 m-o), *O. hookeri* var. *hirsutum* (Fig. 4 d-f) *O. hookeri* var. *longiflorum* (Fig. 4 g-i)*, O. maaikangense, O. multiramosum, O. sinicum* (Fig. 5 a-c and g-i), isopolar pollen grains were recorded, 4 species *O. confertum, O. dicroanthum* (Fig. 3 a-c & d-f)*, O. waddellii,* and *O. waltonii* (Fig. 5 j-l & m-o), were heteropolar while 2 species *O. exertum* (Fig. 3 g-i) *O. limitaneum* (Fig. 4 j-l) were isopolar to slightly heteropolar.

**Pollen shape:** On the basis of P/E ratio (Fig. 2) (fallowing by Ertdman, 1952) three pollen shapes have been recognized.

**Type I:** Prolate spheroidal (P/E ratio 1.00 – 1.14 µm): This type comprised two species *O. confertum* (Fig. 3 a-c)*,* with P/E ratio 1.04 µm and *O. waltonii* (Fig. 5 m-o), with P/ E ratio 1. 11 µm.

**Type II:** Subprolate (P/E ratio 1.15-1.33 µm): This type comprised seven species *O. dicroanthum, O. fistulosum, O. glomeratum,* (Fig. 3 d-f, j-l & m-o) *O. hispida,* (Fig 4 a-c) *O. hookeri* var. *hirsutum, O. maaikangense* (Fig. 4 d-f & m-o) and *O. sinicum* (Fig. 5 g-i) with a range of P/E ratio from 1.15 µm to 1.31 µm.

**Type III:** Prolate: This type comprised of six species *O. exertum* (Fig. 3 g-i)*, O. hookeri* var. *longiflorum, O. limitaneum* (Fig. 4 j-l) *O. multiramosum, O. paniculatum and O. waddellii* (Fig. 5 a-c, d-f & j-i) with P/E ratio range from 1.35 µm to 1.56 µm.

**Aperture type:** Within studied *Onosma* L. species two basic types of apertures have been recognized.

**Type-I** 3-syncolporate: 3-syncolporate pollen grains have been observed in five species i.e., *O. confertum, O. dicroanthum* (Fig. 3 a-c & d-f)*, O. hispida, O. limitaneum,* (Fig. 4 a-c & j-l) *O. paniculatum* (Fig. 5 d-f).

**Type-II** 3-colporate: This type has been found in 10 species i.e. *O. exertum, O. fistulosum, O. glomeratum* (Fig. 3 g-i, j-l & m-o), *O. hookeri* var. *hirsutum, O. hookeri* var. *longiflorum, O. maaikangense* (Fig. 4 d-f, g-I & m-o), *O. multiramosum, O. sinicum, O. waddellii,* and *O. waltonii* (Fig. a-c, g-i, j-l & m-o).

**Ora and Exine ornamentation (Tectum):** Although there was less diversity in ora i.e., lolongate in all the studied species. However, a great diversity in exine ornamentation and four tectum types was observed.

**Type I** Spinulose: The spinulose tectum was found in five species i.e., *O. confertum, O. dicroanthum, O. fistulosum* (Fig. 3 a-c, d-f & j-l)*, O. limitaneum* (Fig. 4 j-o), *O. paniculatum* (Fig. 5 d-f).

**Table 2. Qualitative and quantitative pollen characters of *Onosma* L. species (Scanning electron microscopy).**

| Taxa | Pollen type | Polarity | Polar view | Equatorial view | (P) mean (min-max) | (E) mean (min-max) | P/E ratio | Shape | Size | Tectum | Ora |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Onosma confertum* W. W. Smith | 3-syncolporate | Heteropolar | Circular | Ovate | 10.2 (9.50-10.90) | 8.68 (7.80-9.56) | 1.04 | Prolate-spheroidal | Small | Spinulose | Lolongate |
| *O. dicroanthum* Boiss. | 3-syncolporate | Heteropolar | Triangular | Ovate | 14.33 (13.30-15.37) | 10.86 (8.55-13.18) | 1.31 | Subprolate | Small | Spinulose | Lolongate |
| *O. exsertum* Hemsl. | 3-colporate | Isopolar-heteropolar | Rounded triangular-circular | Oblong | 16.00 (14.68-17.33) | 10.81 (9.34-12.28) | 1.48 | Prolate | Small | Rugulate-echinate | Lolongate |
| *O. fistulosum* I. M. Jhonst. | 3-colporate | Isopolar | Circular | Oval-spheroidal | 14.88 (13.11-16.55) | 12.85 (12.07-13.64) | 1.15 | Subprolate | Small | Spinulose | Lolongate |
| *O. glomeratum* Y. L. Liu | 3-colporate | Isopolar | Rounded triangular-circular | Oval-oblong | 18.27 (17.40-19.40) | 14.58 (13.40-15.76 | 1.24 | Subprolate | Small | Rugulate-granulate | Lolongate |
| *O. hispida* Wall ex G. Don | 3-syncolporate | Isopolar | Rounded triangular-circular | Ovate | 13.49 (11.98-15.00) | 10.62 (9.12-12.13) | 1.27 | Subprolate | Small | Rugulate-granulate | Lolongate |
| *O. hookeri* var. *hirsutum* Y. L. Liu | 3-colporate | Isopolar | Rounded triangular-circular | Oval-oblong | 19.06 (15.91-20.85) | 14.57 (13.76-15.38) | 1.30 | Subprolate | Small | Rugulate-granulate | Lolongate |
| *O. hookeri* var. *longiflorum* (Duthie) Duthie ex Stapf | 3-colporate | Isopolar | Rounded triangular-circular | Oval-oblong | 19.59 (17.90-21.28) | 14.50 (13.50-15.51) | 1.35 | Prolate | Small | Rugulate-granulate | Lolongate |
| *O. limitaneum* I. M. Johnst. | 3-syncolporate | Isopolar-heteropolar | Rounded triangular-circular | Ovate | 14.52 (12.80-16.90 | 10.54 (10.00-12.50) | 1.37 | Prolate | Small | Spinulose | Lolongate |
| *O. maaikangense* W.T. Wang ex Y. L. Liu | 3-colporate | Isopolar | Rounded triangular-circular | Oblong | 17.21 (15.8-18.62) | 14.14 (11.78-16.50) | 1.21 | Subprolate | Small | Rugulate-granulate | Lolongate |
| *O. multiramosum* Hand. -Mazz. | 3-colporate | Heteropolar | Rounded triangular-circular | Oblong | 25.43 (13.79-37.07) | 16.93 (8.83-25.04) | 1.50 | Prolate | Medium | Rugulate | Lolongate |
| *O. paniculatum* Bur. et Franch. | 3-syncolporate | Isopolar | Rounded triangular-circular | Ovate | 24.00 (13.00-35.00) | 16.90 (8.90-25.00) | 1.42 | Prolate | Small | Spinulose | Lolongate |
| *O. sinicum* Diels. | 3-colporate | Isopolar | Circular | Oval-oblong | 15.01 (14.16-15.86) | 12.59 (11.89-13.30) | 1.19 | Subprolate | Small | Rugulate-granulate | Lolongate |
| *O. waddellii* Duthie | 3-colporate | Heteropolar | Triangular | Oblong | 20.51 (18.30- 22.73) | 13.01 (11.40-14.80) | 1.56 | Prolate | Small | Rugulate-granulate | Lolongate |
| *O. waltonii* Duthie | 3-colporate | Isopolar | Rounded triangular-circular | Oval-oblong | 10.00 (9.62-10.38) | 8.96 (7.92-10.00) | 1.11 | Prolate-spheroidal | Small | Rugulate-granulate | Lolongate |

Note: All the measurements are in µm. P= Polar length Mean (min-ax.), E= Equatorial diameter Mean (min-max.), P/E= Mean of P/mean of E

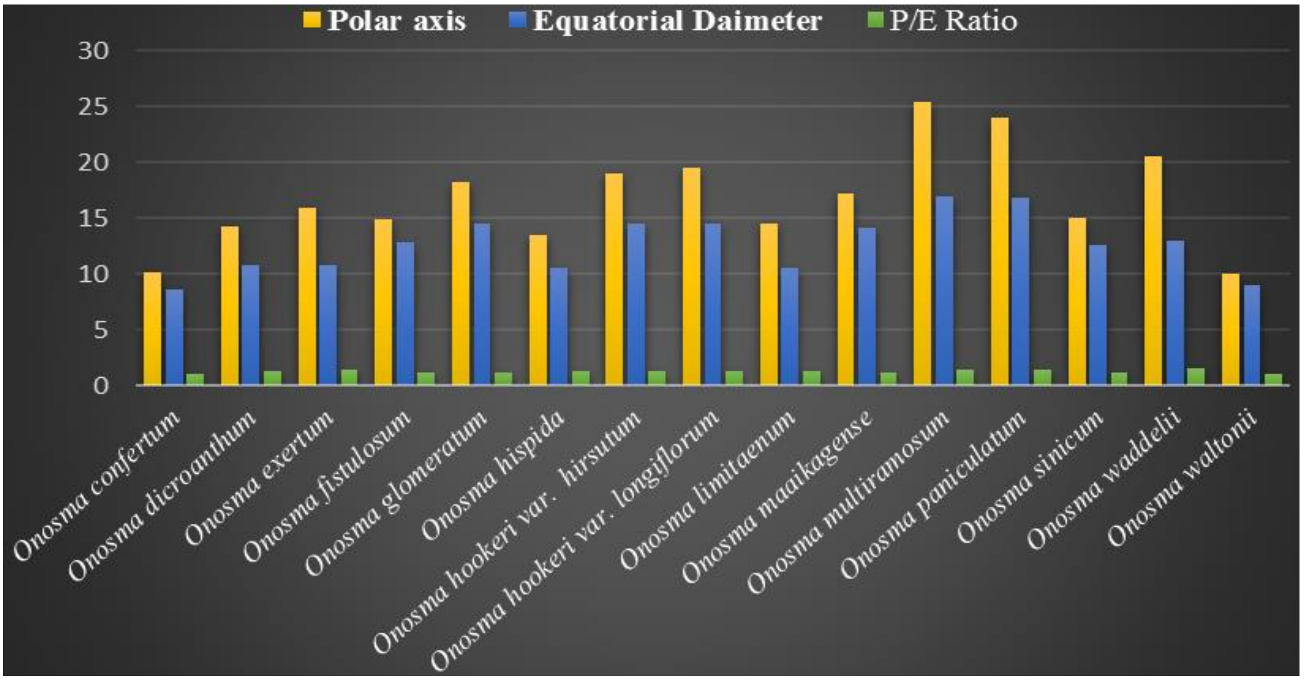


Fig. 2. Graph showing range of Polar axis (P), Equatorial axis (E) and P/E ratio of the studied *Onosma* taxa.

**Type II** Rugulate: This type of tectum was only found in *O. multiramosum* (Fig. 5 a-c).

**Type III** Rugulate-echinate: This type of tectum was observed in only *O. exsertum* (Fig. 3 g-i).

**Type IV** Rugulate to granulate: This type of tectum was recorded in 8 species *O. glomeratum* (Fig. 3 m-o), *O. hispida, O. hookeri* var. *hirsutum, O. hookeri* var. *longiflorum, O. maaikangense* (Fig. 4 a-c, d-f, g-i & m-o) *O. sinicum, O. waddellii,* and *O. waltonii* (Fig. 5 g-i, j-l & m-o).

**Numerical analysis:** In this study, 10 different qualitative and quantitative characters and 20-character states (Table 3) of 15 *Onosma* species (Table 1) have been investigated numerically. Two types of analyses, cluster (CA) and principal component analysis (PCA) for the ordination of the taxa were carried out.

**Cluster analysis:** The resulting UPGMA dendrogram (Fig. 6) grouped all 15 species into two main clusters named cluster "A" and cluster "B" respectively. The first cluster "A" consisted of two species *O. multiramosum* and *O. paniculatum,* while the second main cluster "B" comprised of *O. waltonii* and was further subdivided into two sub clusters "B1" comprised of *O. confertum* while the sub cluster "B2" further splited into 2 subgroups comprised *O. glomeratum, O. hookeri* var. *hirsutum, O. hookeri* var. *longiflorum, O. maaikangense, O. waddelii,* and *O. dichroanthum, O. limitaneum, O. hispida, O. exsertum, O. fistulosum, O. sinicum* respectively. Moreover, the cluster analysis clearly separated *Onosma waltonii* from *O. multiramosum* and *O. paniculatum* into two different clusters and rest of the species were groupedin two sub clusters.

**Principal component analysis** (**PCA**)**:** In order to know the contribution of each trait for total variation among the examined species, PCA was carried out. It was observed that the most of the traits contributed significantly and were important in explaining the total variation. The results of the variables and loading scores for each of the principal components for 15 studied *Onosma* taxa and eigenvalue and % variance obtained from the correlation matrix based on 10 characters are presented in Tables 5 and 6. The results revealed that the three components explain 76.52% of the total variance in the pollen morphological character. PC1 accounted for 35.9% of variance, PC2 accounted for 30.82% and PC3 for 9.72% of variance (Table 6). The resulted PCA scatter (Fig. 7) supported the separation of the taxa. For examples *O. waltonii* on the positive side of the scale at PC1 was clearly separated by the rest of the species. *O. fistulosum* and *O. sinicum* are also found on the same side and both species showing a close relationship on both cluster and PCA analysis. Similarly, *O. multiramosum* was found on the positive side of the scale at PC2. However, as compared to cluster analysis, PCA analysis partially supported the closeness of *O. paniculatum* and *O. multiramosum*. Overall, separation of *O. confertum* and relationship of other species is supported by PCA.

## Discussion

Pollen data is now is widely used to recognize morphotypes (Huysmans *et al.,* 1994 Bigazzi & Selvi, 1998). It has been proved to be valuable in delimitation of taxa in several recent pollen morphological studies (Binzet *et al.,* 2010; Tukkmen *et al.,* 2010; Mehrabian *et al.,* 2012; Faghir *et al.,* 2015; Elkorady & Faried, 2017; Magda *et al.,* 2017; Rashid *et al.,* 2017 and Umdale *et al.,* 2017, Binzet *et al.,* 2018) and others. Moreover, numerical analysis is one of the widely used approach in field of palynology. Binzet *et al.,* 2018 highlighted the importance of numerical taxonomy verses classical taxonomy and suggested the use of numerical taxonomy for morphologically similar taxa (like *Onosma*). They emphasized that the numerical taxonomy is the best way to establish the morphological relationships and their identities in the genus *Onosma*. Numerical analysis covers several methods but PCA and CA have been widely used. PCA is used to evaluate most suitable traits for classification and allows multicollinear data used to determine the traits. However, CA helps to analyze qualitative and quantitative features in which each trait is used as an independent entry. In the current study overall results obtained from the numerical analysis both cluster analysis (UPGMA) and principal component analysis (PCA) (Figs. 6 & 7) showed good diversity and variation among the studied *Onosma* species.

Boraginaceae is considered an exclusively eurypalynous and their pollen characters used to recognize large number of species (Clarke, 1977; Diez, 1984; Diez & Valdes, 1991; Perveen *et al.,* 1995). The presence of isocolpate and heterocolpate pollen grains is a fundamental and diagnostic pollen character for Boraginaceae taxa (Diez & Valdes, 1991; Perveen *et al.*, 1995; Bigazzi & Selvi, 1998; Hagroove & Simpson, 2003). Isocolpate pollen grains are characteristically found in Lithospermeae and according to Cohen, (2014) the tribe Lithospermeae is the most diverse regarding pollen shape. In previous studies on pollen morphology of *Onosma* (Qureshi & Qaiser, 1987; Ning *et al.*, 1995, Perveen *et al.*, 1995, Liu *et al.*, 2010; Maggi *et al.*, 2008; Mehrabian *et al.*, 2012; Binzet *et al.*, 2014 subprolate, prolate, ellipsoidal, prolate-spheroidal and spheroidal pollen shapes have been reported. In the present study three pollen shapes have been observed viz., Prolate, subprolate and prolate spheroidal. Liu *et al*., (2010) also reported the pollen diversity within the tribe Lithospermeae and *Onosma* species in regard of shape and other palynological features. They reported five different pollen shapes i.e., subprolate, subcircular, prolate, dumbbell, ovoid, and cocoon shaped, in different *Onosma* species. In addition, the aperture type (3-colporate and 3-syncolporate) and polarity (isopolar or heteropolar) pollen grains in *Onosma* are also important pollen features and are reported and discussed in previous palynological studies of *Onosma* (Qureshi & Qaiser, 1987, Huynh, 1972, Perveen *et al.,* 1995, Maggi *et al.,* 2008, Binzet, 2011, Mehrabian *et al.,* 2012 and Binzet & Ozler, 2014 and Binzet *et al.,* 2018). In the present study 10 species were observed with 3-colporate aperture type and five possessed 3-syncolporate pollen types. Exine ornamentation plays a significant role in systematics. Ning *et al.,* (1995) separated the genus *Onosma* and *Maharanga* based on pollen characters especially the exine ornamentation. On other hand different exine types microechinate, scrabate, foveolate, rugulate-granulate, rugulate-tuberculate, are reported in studies of Ning *et al.*, 1995; Perveen *et al.*, 1995; Maggi *et al.,* 2008; Liu *et al.,* 2010; Binzet *et al.,* 2014 and others. Furthermore, Perveen *et al.,* (1995) has reported the subspilate tectum in *O. dicroanthum,* scabrate in *O. limitaneum* and foveolate in *O. hispida.* However, in our study it was revealed that there were four tectum types are present in *Onosma* species viz., rugulate, spinulose, rugulate-granulate and rugulate-granulate.

**Table 3. Pollen characters and character states of 15 *Onosma* species used for numerical analysis.**

| Pollen characters and character states used for numerical analysis | Abbreviations |
|---|---|
| 1. Mean of the length of polar axis | **P** |
| 2. Mean of equatorial diameter | **E** |
| 3. Polar axis/Equatorial axis (P/E) | **PE** |
| 4. Size range of grain: Small (0), Medium (1) | **S** |
| 5. Polarity: Isopolar (0) Heteropolar (1) Isopolar-heteropolar (2) | **POL** |
| 6. Outline in Polar View (AMB): Circular (0), Rounded Triangular-Circular (1) Triangular (2) | **AMB** |
| 7. Outline in Equatorial view: Ovate (0), Oblong (1), Oval-oblong (2), Oval-spheroidal (3) | **EO** |
| 8. Aperture type: 3-syncolporate (0) 3-colporate (1) | **AP** |
| 9. Pollen shape: Prolate-spheroidal (0), Subprolate (1), Prolate (2) | **SH** |
| 10. Exine: Spinulose (0), Rugulate-echinate (1), Rugulate-granulate (2), Rugulate (3). | **EX** |

**Table 4. Data matrix used for multivariate analysis of studied *Onosma* L. species.**

| Taxa | P | E | P/E | S | POL | AMB | EO | AP | SH | EX |
|---|---|---|---|---|---|---|---|---|---|---|
| *O. confertum* | 10.2 | 8 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *O. dicroanthum* | 14.3 | 10.8 | 1.3 | 0 | 1 | 2 | 0 | 0 | 1 | 0 |
| *O. exertum* | 16 | 10.8 | 1.4 | 0 | 2 | 1 | 1 | 1 | 2 | 1 |
| *O. fistulosum* | 14.8 | 12.8 | 1.1 | 0 | 0 | 0 | 3 | 1 | 1 | 0 |
| *O. glomeratum* | 18.2 | 14.5 | 1.2 | 0 | 0 | 1 | 2 | 1 | 1 | 2 |
| *O. hispida* | 13.4 | 10.6 | 1.2 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| *O. hookeri* var. *hirsutum* | 19 | 14.5 | 1.3 | 0 | 0 | 1 | 2 | 1 | 1 | 2 |
| *O. hookeri* var. *longiflorum* | 19.5 | 14.5 | 1.3 | 0 | 0 | 1 | 2 | 1 | 2 | 2 |
| *O. limantaneum* | 14.5 | 10.5 | 1.3 | 0 | 2 | 1 | 0 | 0 | 2 | 0 |
| *O. maikangense* | 17.2 | 14.1 | 1.2 | 0 | 0 | 1 | 1 | 1 | 1 | 2 |
| *O. multiramosum* | 25.4 | 16.9 | 1.5 | 1 | 1 | 1 | 1 | 1 | 2 | 3 |
| *O. paniculatum* | 24 | 16.9 | 1.4 | 0 | 0 | 1 | 0 | 0 | 2 | 0 |
| *O. sinicum* | 15 | 12.5 | 1.1 | 0 | 0 | 0 | 2 | 1 | 1 | 2 |
| *O. waddelii* | 20.5 | 13 | 1.5 | 0 | 1 | 2 | 1 | 1 | 2 | 2 |
| *O. waltonii* | 10 | 18.9 | 1.1 | 0 | 0 | 1 | 3 | 1 | 0 | 2 |

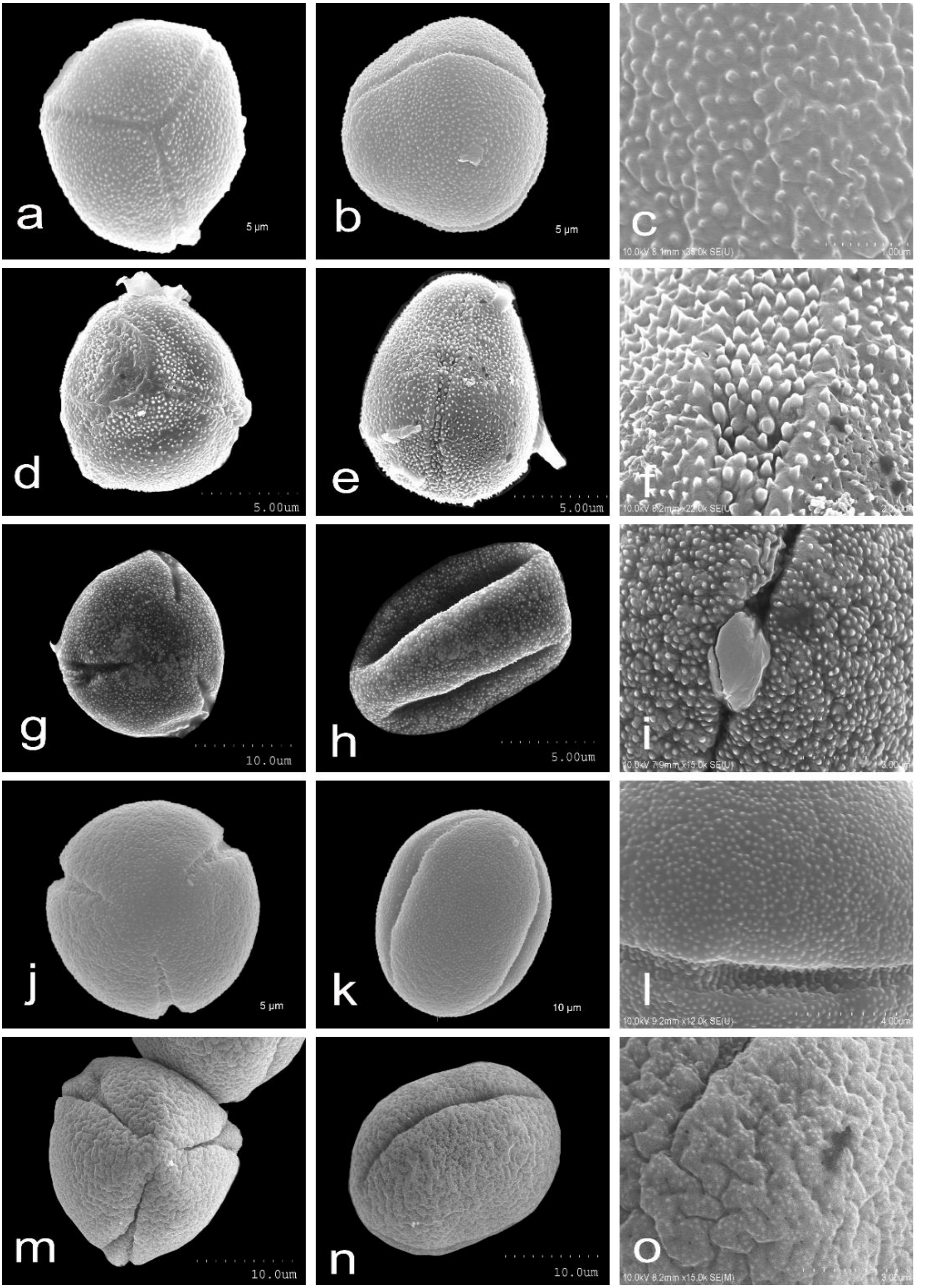


Fig. 3. SEM micrgrpahs of *O. confertum* a-c, *O. dichroanthum* d-f, *O. exsertum* g-i, *O. fistulosum* j-l, *O. glomeratum* m-o.

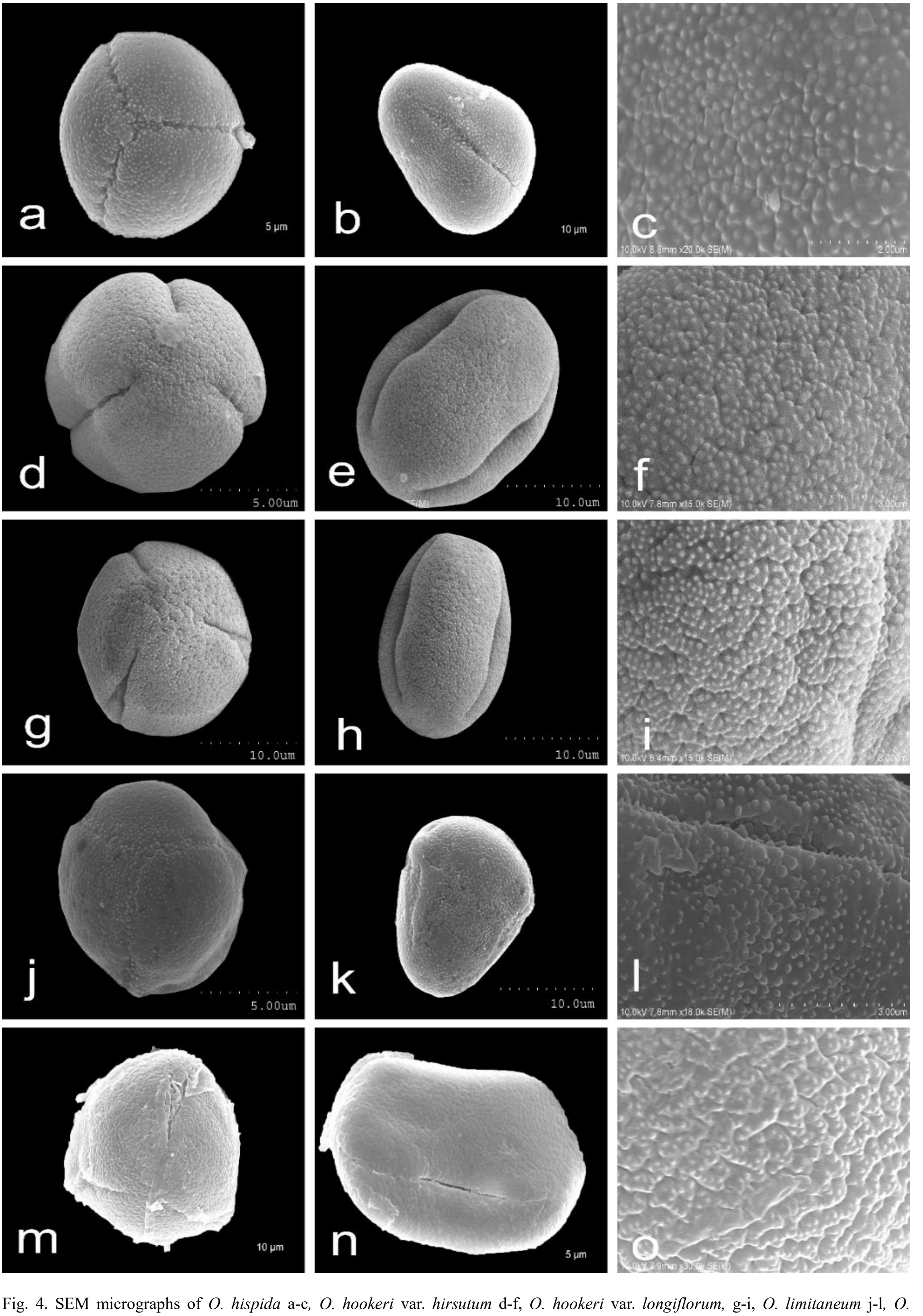


Fig. 4. SEM micrographs of *O. hispida* a-c*, O. hookeri* var. *hirsutum* d-f, *O. hookeri* var. *longiflorum,* g-i, *O. limitaneum* j-l*, O. maaikangense* m-o.

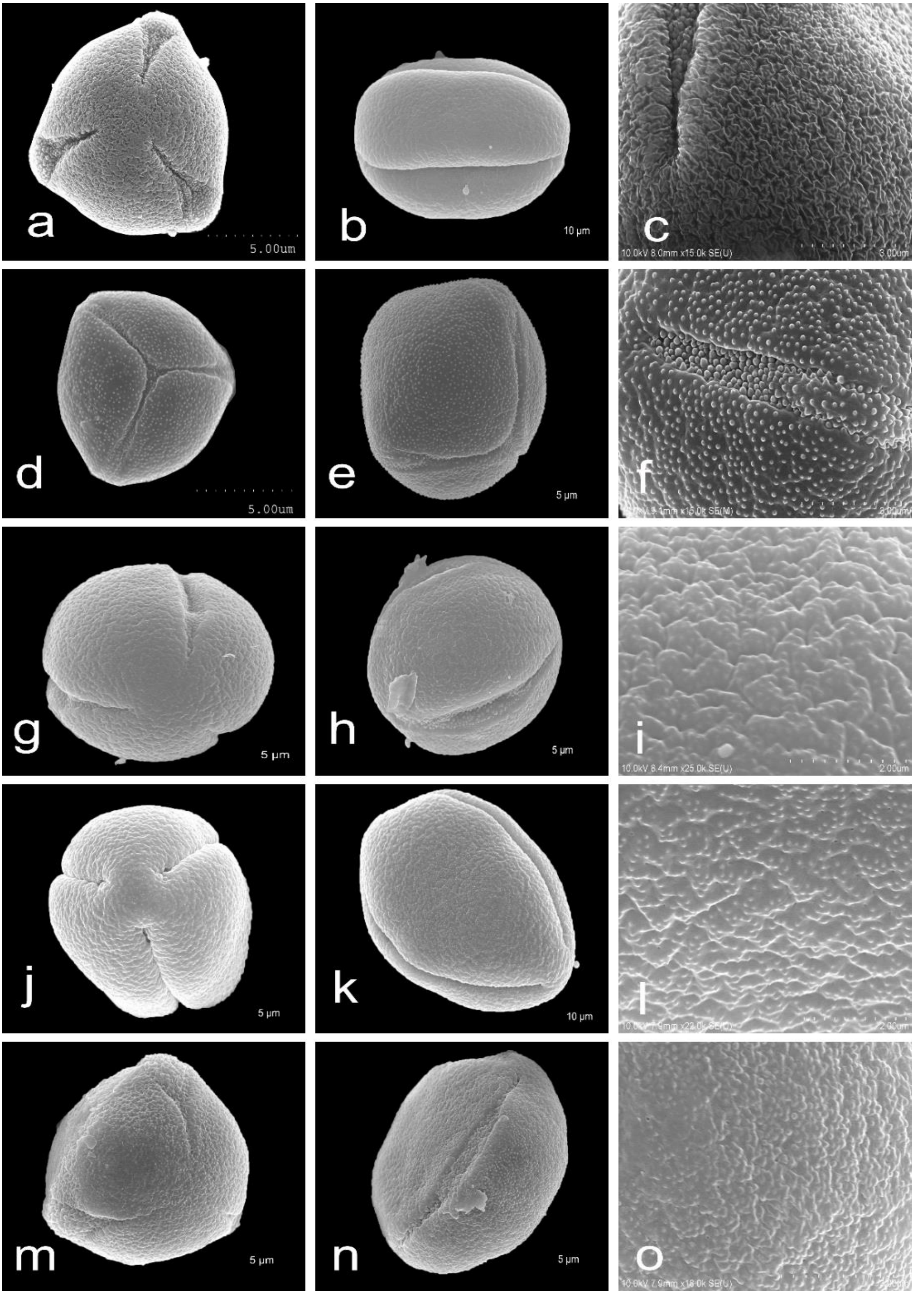


Fig. 5. SEM micrographs of *O. multiramosum* a-c, *O. paniculatum* d-f, *O. sinicum* g-i, *O. waddellii* j-l, *O. waltonii* m-o.

**Table 5. The variables loadings for each of the principal component (PC1, PC2 and PC3) for 15 *Onosma* taxa. (Note: For the abbreviations of the variables, see table 3).**

| Variables | PC-1 | PC-2 | PC-3 |
|---|---|---|---|
| P | 0.889 | -0.039 | 0.008 |
| E | 0.553 | 0.007 | -0.228 |
| P/E | 0.893 | -0.459 | -0.106 |
| S | 0.626 | 0.045 | 0.631 |
| POL | 0.100 | -0.740 | 0.359 |
| AMB | 0.525 | -0.379 | -0.603 |
| EO | 0.012 | 0.889 | -0.011 |
| AP | 0.401 | 0.743 | 0.107 |
| SH | 0.752 | -0.425 | 0.020 |
| EX | 0.543 | -0.576 | 0.075 |

**Table 6. Eigenvalue and % variance obtained from the correlation matrix.**

| Principal components | Eigenvalue | % Variance |
|---|---|---|
| 1 | 22.906 | 35.98 |
| 2 | 6.71056 | 30.82 |
| 3 | 1.018 | 9.72 |
| 4 | 0.655 | 8.60 |
| 5 | 0.46 | 7.40 |
| 6 | 0.21 | 4.56 |
| 7 | 0.099 | 1.52 |
| 8 | 0.25 | 0.92 |
| 9 | 0.0080 | 0.28 |
| 10 | 0.00071 | 1.58 |

Wodehouse, (1965) has reported correlation between flower and pollen size. However, Pandey (1971) demonstrated in the genus *Nicotiana* that there was no correlation between flower and pollen size and the species with the largest flowers had small pollen grains. Binzet *et al.,* (2014) also discussed the correlation of flower and pollen size and their results were concordant with the results of Wodehouse, (1965) and Pandey, (1971). In current study, we found that the pollen grains of *O. waltonii* were smaller but flowers were quite bigger, whereas pollen of *O. multiramosum* were relatively bigger with smaller flowers. Our results are in agreement with the results Pandey, (1971) that there is no correlation of pollen size with flower size. However, pollen characters may help in the identification of species and/or to find level of correlation of pollen characters with taxonomic characters of the *Onosma* taxa.

Although palynology is a relatively a recent branch of plant sciences, but it has contributed a lot by providing useful information for phylogenetic analysis (Perveen, 2000). All pollen characters play vital role in systematics. Generally, the classification of pollen grains depends on different pollen characters like; size, shape, polarity, symmetry, aperture type, and exine ornamentation. Walker & Doyle, (1975) discussed the importance of different pollen characters and the evolutionary tendencies of pollen grains within angiosperms and emphasized that some pollen characters are more important from phylogenetic and evolutionary point of view like pollen polarity, symmetry, aperture types. Aperture morphology actually plays a vital role in phylogenetic-evolutionary context specially the number and position of apertures is of paramount importance. The pollen polarity is also important because it is largely determined by the aperture condition. However, pollen size is undoubtedly being a more or less revisable character and the determination of primitive pollen size class for pollen of any particular taxon must be correlated with other characters of taxon. Similarly, the evolution of pollen wall architecture offers great potential as a source of important phylogenetic information, but the exine ornamentation is also undoubtedly is a more or less revisable character and must be interpreted as individual correlations within taxa. The evolution of pollen grains in angiosperms discussed by Walker & Doyle, (1975) suggested that the primitive pollen grains character in terms of size are large pollen grains, in terms of polarity heteropolar from which there is evolution of basic isopolar pollen type through apolar inaperturate pollen and the evolution of apertures in angiosperms starts with inaperturate pollen grains to mono-aperturate and then to multi-aperturate pollen grains. The evolutionary tendencies based on pollen apertures showed that among angiosperms as well as in Boraginaceae, 3-colpate pollen are the primitive type of and other types are derived from it (Nair, 1964; Ning, 1991; Ahn & Lee, 1986; Khatamsaz, 2001). On the basis of evolutionary trends of pollen grains, it is observed that the family Boraginaceae has various evolutionary tendencies which can be clearly observed especially from the evolution of pollen apertures. Based on aperture evolution it is noted that 3-colporate pollen aperture type is more primitive condition in the Lithospermeae than 3-syncolporate pollen grains and then may be leading to multi-aperturate pollen grains. However, on the basis of polarity, heteropolar pollen grains are more advanced than isopolar or iso-heteropolar. Similarly, exine ornamentation was found very diverse either rugulate, spinulose, echinate-rugulate and echinate-granulate. These characters of exine are more advanced than the smooth tectum reported in other members of Lithospermeae for example *Lithospermum* species. These results are in consistence with Liu *et al.,* 2010. Further based on present literature it is noted that *Onosma* (rugulate, spinulose, echinate-rugulate and echinate-granulate) is closely related to some genera of Lithospermeae like *Arnebia* and *Echium* than to other genera like *Lithospermum* that possess smooth tectum. However, from evolutionary point of view based on apertures, *Echium* and *Onosma* (with 3-colporate and 3-syncolporate apertures) are more primitive than *Lithospermum* and *Arnebia* (with 4-8 colporate and 4-7-syncolporate apertures respectively). The evolutionary trends of pollen grains also provide an insight for understanding generic relationships based on pollen traits within the tribe Lithospermeae.

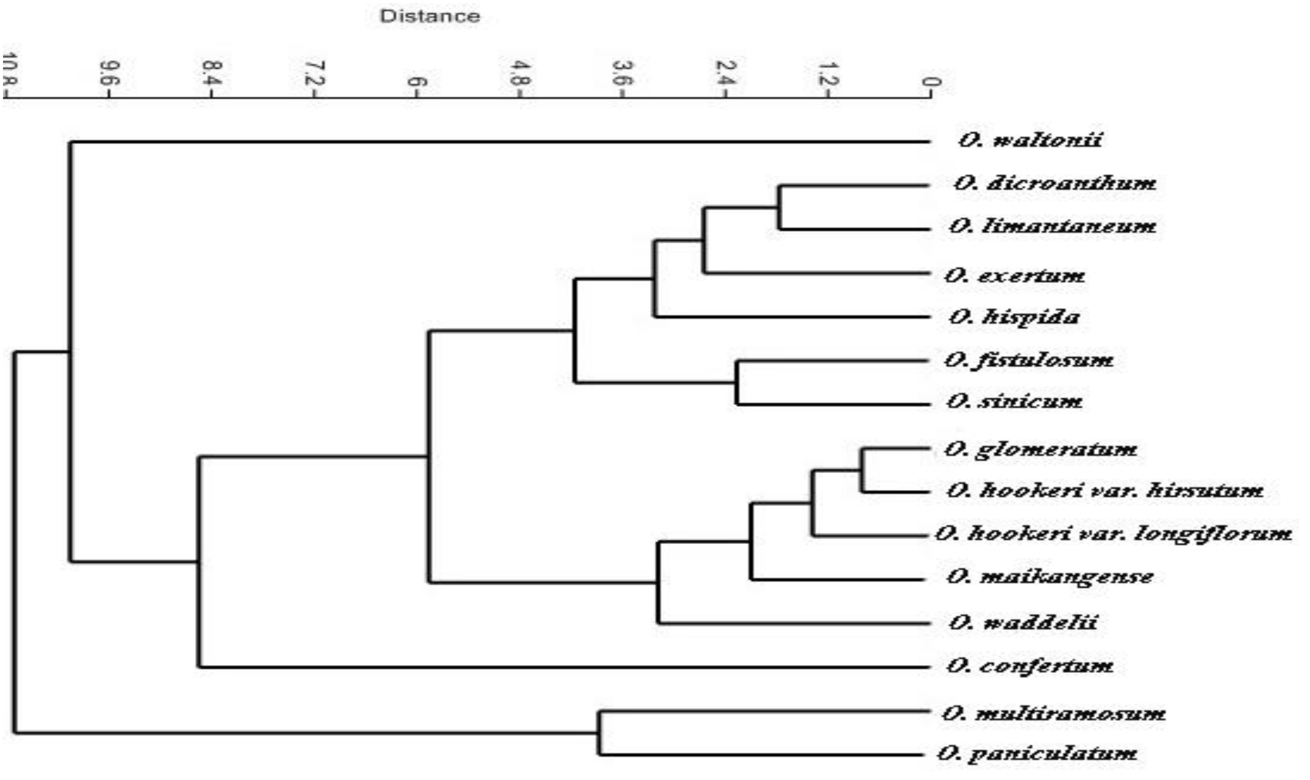


Fig. 6. UPGMA dendrogram showing clustering of 15 *Onosma* species based on palynological characters.

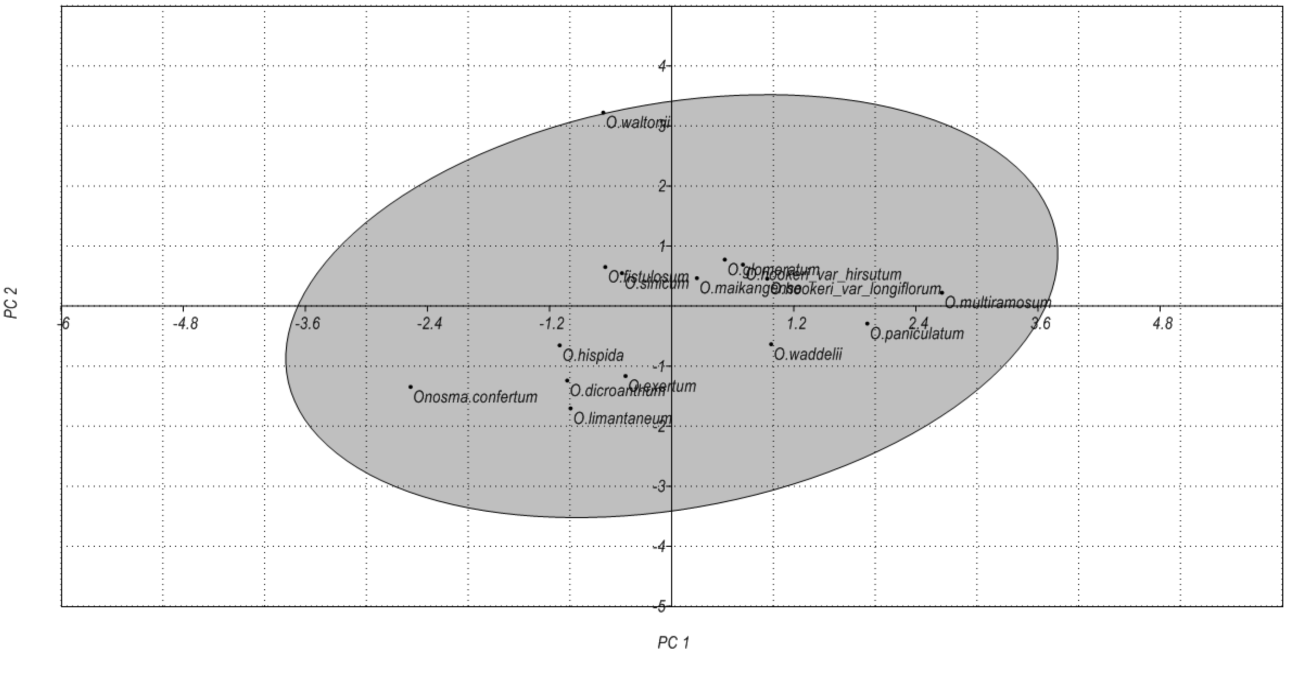


Fig. 7. Scatter plot obtained from Principal Component analysis (PCA) based on correlation method.

## Conclusion

The present palynological data provides new information about pollen morphology of taxonomically complex taxa *Onosma*, especially *O. glomeratum*, *O. multiramosum, O. hookeri* var. *hirsutum, and O. hookeri* var. *longiflorum,* which are studied for the first time for pollen characters. The study revealed that there was great pollen diversity within *Onosma* L. species. Three types of pollen (Prolate, subprolate and prolate-spheroidal) were observed within studied taxa. The numerical analysis showed interesting results in UPGMA dendrogram with two main clades and subclades, showing the relatedness of the taxa. This study can be helpful in the identification of *Onosma* species as it provides some new information regarding pollen features of this genus and can be used for future comparative studies. The relationships among the studied species and systematics significance of pollen characters within *Onosma* are well understood.

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