Morphology and Palynology Systematic Studies of The Genus Bellevalia Lapeyr. (Asparagaceae) in Kurdistan-Iraq

ABSTRACT

Morphological and palynological systematic studies for the 10 species of genus Bellevalia Lapeyr. (Asparagaceae) in Kurdistan-Iraq conducted during the growing seasons (2015-2016) to determine the species distribution and recording new localities.

The morphological investigation was dealt with the characteristics of the bulbs, stems, leaves, flowers, inflorescences, fruits and seeds and it was indicated through discussing the characters diversity that the flowers characters are taxonomically more important than vegetative characters to isolate the species.

The palynological study is first conducted on Iraqi botanical specimens and indicated that pollen grains of all studied taxa of the genus Bellevalia, monads and monosulcate with variations in other characters of pollen grains which give a taxonomic importance.

The species (B. macrobotrys, B. longipes, B. glauca, B. saviczii, B. olivieri, B. mosheovi, B. kurdistanica and B. pycnantha) were previously recorded and two species are new for Iraq (Bellevalia fominii and B. longistyla).

INTRODUCTION:

Plant Taxonomy (Systematic) was begun as trials to understand and sort the surrounding requirement by the human, nominating them and explaining their diversity depending on morphological characters (Pandy, 2005; Potter et al., 2007), Recently plant taxonomy became more progressing and accurate in using other plant characters such as cytology, anatomy, ecology, phytogeography, other biological sciences and non-biological sciences such as geology and chemistry (NasirAlla, 2007) and it is regarded as a principle for the other sciences which they depend on it so as they could not be isolated from each other (Abdul-Mohsen, 2012), and new technologies and instruments such as Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) have attributed in developing the taxonomic studies (Abdul-Razaq, 2008; Mohamad, 2010). The plants are regarded as an important wealth in Iraq which includes about 141 vascular plant families with 860 genera and more than 2500 species. For instance, Lily family contains important medical and ornamental plants (Yıldırım et al., 2015). The authors have different ideas about the number of genera and species of the family Liliaceae, Hooker (1894) mentioned 187 genera with 2500 spp, Lawrence (1951) indicated about 240 genera and 4000 species, Rendle (1953) referred to that the genus Bellevalia is included within the family Liliaceae which involving 200

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genera with 2600 species, Al-Musawi (1987) mentioned 250 genera with 3500 species belong to the family Liliaceae in the world, while Al-Katib (1988) indicated 250 genera with 4000 species in the world and more than 100 wild species and 18 cultivated species in Iraq. Townsend and Guest (1985) confirmed that the genus *Bellevalia* Lapeyr. belongs to the family Liliaceae with 21 genera in Iraq and out of 75 species of the genus *Bellevalia* in the world, 10 species are existed in Kurdistan of Iraq. Singh (2004) divided the Liliaceae family on 11 genera and 545 species in the world.

The importance of pollen is obvious in many botanical studies based on morphological characters in solving a lot of problems for taxonomic studies. Hyde and Wiliams (1945) were the first used the term “Palynology” which means science of pollen or spores, while Lindley (1840) was the first used the pollen study successfully in isolating the genera of family Orchidaceae and Fritszche (1832) and Mohl (1835) contributed insubstantial coverage of the information in the field of morphology of the pollen. Taxonomic value of the pollen grains is determined by several important characteristics such as the size of the grain, shape and colour and the type of sculpture and the presence of furrows or apertures or both, and their numbers and shapes and dimensions of the grains, which is one of the micro morphological characters (NasirAlla, 2007). The study of the wall of pollen and sculpture has expanded through using each of the Scanning Electron Microscope and Transmission Electron Microscope, the exine thickness in Liliaceae is 1 mm with reticulate surface (Erdtman et al., 1961, Abdul-Mohsen, 2012).

Pollen morphology due to the remarkably symmetrical structure and surface patterns, pollen grains morphology has long been recognized as an important criterion in determining the nature relationship of plant genera and families. The structure of the pollen grain wall characters are used in species identification. On the other hand, there are cases in which pollen grains of very like structure occur in quite unrelated plant families (Salih, 2002). Franchi and Pacini (2000) referred to that the pollen grains of the family Liliaceae are monads but not mentioned about genus *Bellevalia*. Due to the lack of taxonomic details on the genus *Bellevalia* and unavailability of the information about the taxonomy position, the aim of this study is to focus on the morphological characters and to compare the pollen morphology of the all taxa of the genus *Bellevalia* distributed in Kurdistan of Iraq, using Light microscopy (LM) and Scanning Electron Microscopy (SEM).

**MATERIALS AND METHODS**

More than 900 specimens were collected during 30 field trips covered more than 80 localities of Iraqi Kurdistan districts (Amadiyah District MAM, Rawanduz District MRO, Sulaimani District MSU, Erbil District FAR, Persian district PFP and Kirkuk District FKI) from March 2015 to May 2016 in general survey in which were visited twice or more. The specimen was collected from March to June and investigated in vegetative growth stage, flowering and fruiting stage. Each specimen was labeling by the information of scientific name, habit, altitude by Global Positioning System (GPS) of the type (Garmin Rino110), collection date, collector name, ecological notes.

**MORPHOLOGY**

All plant parts (Roots, bulbs, scapes, leafs, inflorescences, flowers, fruit and seed) were studied in detail by Dissecting Microscope and the key of some floras [Flora of Syria, Palastine and Sinai (Post, 1933), Flora of lowland Iraq (Richinger, 1964), Flora of Europe (Tutin et al., 1980), Flora of Turkey (Davis, 1984), Flora of Iraq (Townsend and Guest, 1985), Flora of Kuwait (Al-Rawi, 1987), Flora Iranica (Rechinger, 1964, 1990)] were used to identify the species. The specimens were photographed in the field, environment in which the plants live in addition to their feature.

**PALYN/OLOGY**

**Pollen preparation:**

**Light Microscopy (LM).** Figure 6,7

Pollens samples of examined species were obtained from fresh specimens. Pollen grains were prepared for light microscopy (LM) by using standard methods (Erdtman, 1969; Al-Mayah, 1983;
Salih, 2002). Pollens mounted on glycerin jelly onto glass slides and stained with Safranin. Finally, the observations were made with Olympus microscope under E 40 with 10X eyepiece 40X of a compound light microscope (Olympus). Pollen diameter in polar view (P) and equatorial view (E) were measured for 20-25 pollen of each species, all of which were photographed and exine thickness were measured and range value was recorded for each measurement using the Ocular micrometer.

**Scanning Electron Microscopy (SEM).** { Figure 8}

The technique developed by Erdtman (1969) and modified by Takahashi (1987) was used to prepare the pollen samples for SEM. The anthers were soaked overnight in acetic acid for softening in 2 ml polyethylene centrifuge tube and were crushed prior to acetolysis. The utmost care was taken to remove the debris and/or unwanted material e.g., fractions of floral parts or anthers, filament. The acetic acid was then decanted and acetolysis mixture (9 ml acetic anhydride: 1 ml conc. sulphuric acid) was added to the centrifuge tube. The acetolysis took place at 100°C for 3 – 5 min. A glass rod was inserted into each tube to stir the pollen sample within acetolysis mixture for the completion acetolysis process evenly. After acetolysis, grains became yellow-brown to brown in color. A sizeable sample of taxa was subjected to Scanning Electron Microscopy. The acetolysed pollen grains were sputter-coated with gold in a JFC 1600 Auto fine Coater for the (SEM) studies, and SEM micrographs taken using JEOL-JSM 6390 LA instrument. Nair’s terminology (Nair, 1971) was used to analyze the palynological characters.

**RESULTS AND DISCUSSION:**

**MORPHOLOGY**

**Habit and duration:**

All species herbaceous plants, perennial, erect, were spread by bulbs and seeds. Often, they grow and found in different regions of mountain, rocky slopes, foothills, valleys, plains, road sides, near springs and streams, forests, fields, shad and wet places. Also, it was remarked that all under studying species were grow in various soils as clay soils, rock soils, mestone and gravelly clay. The range of plant length was between 6 to 70 cm. Adventitious roots, arises from the base of the bulb, unbranched, shallow and spread horizontally; roots were ranging from 1-4mm. in diameters.

**Stem:**

**Subterranean Stem (Bulb):**

A bulb was a modified stem containing a complete miniature plant, including embryonic leaf, stem, and flower parts, and surrounded by fleshy scales which provide food for the young plant and a basal plate which produces roots. The outer layers of scales become dry and papery, forming the tunic, which protect the bulb from the outer environmental conditions, insect and mechanical injury. The bulbs consist of a much-compressed, fleshy stems. The results seemed presence of differences among species bulbs in dimensions, shape and color.

The dimensions of bulbs of the studied taxa shown variations as average among them, length ranged 2 cm. in *B. mosheovii* and 5 cm. in *B. longistyla*; and width ranged 1.8 cm. in *B. mosheovii* and 4 cm. in *B. longistyla*. The rest of species are located between the two values. According to the bulb shapes all species can be divided into three groups as followes:-

1. Globose bulbs are in *B. macrobotrys, B. saviczii* and *B. mosheovii*.
2. Broadly ovoid bulbs are in *B. glauca* and *B. olivieri*.
3. Ovoid shape bulbs are in remaining under studied species.

The color of bulbs had taxonomic value in isolation the species. Depending on bulbs colors species can be divided into the following groups:

1. Black color as in *B. longipes*.
2. Creamy color in *B. saviczii* and *B. mosheovii*.
3. Brownish- creamy in *B. glauca* and *B. fominii*.
4. Brownish color in remaining under studying species.
Plate 1: Qualitative and Quantitative characteristics of bulbs.

**Arial stem (Scape):**

Morphological study of the scape revealed that the scape is a stalk springing from the root which bears the flower and fruit but not the leaves in all studied species, simple, stout, erect and smooth with taxonomic values. The results showed that the characters of colour, length, number and thickness of scape have taxonomic values.

The length of scapes averaged between 9 cm. in *B. mosheovii* and 45 cm. in *B. macrobotrys*, Diameter of scape averaged between 2.0 mm. in *B. mosheovii* and 5.0 mm. in *B. longipes*.

Another matter useful in isolating the species was scape section (scape shape) being rounded to quarterly in *B. macrobotrys, B. glauca, B. olivieri* and *B. fominii* and rounded in shape in other under
studying species. Number of Scape was one (solitary) in *B. longipes*, *B. pycnanth* and *B. fominii* and 1-2(3) scape in all remineds species. The colors of scape were varied among investigated species as followes:
1. Green to violet in *B. olivieri*.
2. Green violet punctate in *B. longipes*, *B. pycnantha* and *B. fominii*.
3. Violet or green violet punctuate in *B. longistyla*.
4. Green in all reminders species.

![Plate 2: Qualitative and Quantitative characteristics of scapes and inflorescences of studied species](image)

**Leaves:**
Leaves in all studying species were radical (Basal attachment), 2-7 number, simple, parallel-veined, blade base was rounded in shape, the apex of leaves acuminate except in *B. olivieri*, *B. saviczii* and *B. kurdistanica* which was acute, and with aciliate margin, which have taxonomical value. It was found in this study that the mode of shape, dimensions, number and margin of leaf are the characters with taxonomic values.
The leaf’s dimensions were the most variable characters, Length was averaged between 12.5 cm. in *B. mosheovii* and 33.0 cm. in *B. macrobotrys*; width averaged between 10 mm. in *B. pycnantha* and 50 mm. in *B. olivieri*. Concerning the leaf shape were lanceolate to lorate (canaliculate) as in *B. longipes* and *B. pycnantha*; and lanceolate to ellipticas in *B. glauca*, *B. olivieri* and *B. longistylya*; and lorate-linear in *B. Kurdistanica* and *B. fominii*; linear in other species. The results also showed that the shapes are somewhat undulate in *B. galuca*, *B. mosheovii* and *B. Kurdistanica*, convolute in *B. macrobotrys*. The number of leaves, were 2-3 in the species *B. macrobotrys* and *B. pycnantha* and 5-7 in *B. olivieri* and *B. Kurdistanica*, while the rest species had the leaves number between the two values.

According to margin, the species can divided in to the following groups:
1. Minutely ciliate leaf margin in *B. macrobotrys*, *B. mosheovii* and *B. fominii*.
2. Densely long ciliate leaf margin in *B. glauca* and *B. olivieri*.
3. Ciliate to scab rid rarely entire leaf margin in *B. saviczii* and *B. longipes*.
4. Shortly ciliate and glaucos in *B. Kurdistanica*.
5. Entireor long ciliate in *B. longistylya*.
6. Entireas in *B. pycnantha*.

Moreover, the species *B. longipes* and *B. fominii* are with falcately recurved leaf apex.

**Plate 3:** leave shapes of the studied species

**Inflorescences:**
Racemose inflorescences (indeterminate), conical outline, cylindrical, ovoid, rarely spike-like, often with sterile upper flowers. Usually numerous flowers are located on the axils, flowers arrangements usually are alternate often spirally alternate, bracts membranous, very small and grow terminally on smooth cylindrical pedicel. The flowering raceme in all species of the genus *Bellevalia* are taxonomically important. The dimensions of inflorescence are varied in all studied taxa, as the minimum length average was 3.5 cm. in *B. mosheovii* and the maximum average was 17.5 cm. in *B. macrobotrys*, and the minimum width was 2.25 cm. in *B. kurzistanica* and the maximum was 7 cm. in *B. longipes*, while the rest of taxa under study were between the two values.
The current study has indicated that the inflorescences or flowering raceme of this genus has the following shapes:

1. Cylindrical in *B. macrobotrys* and *B. fominii*, axis usually reddish-violet above.
2. Conical in both species *B. longipes* and *B. longistyla*.
3. Conical or oblong-ovoid in *B. glauca*.
4. Broadly ovoid with densein *B. olivieri*.
5. Ellipsoid to cylindrical, sparse in *B. kurdistanica*.
6. Broadly ovoid to ellipsoid, densein *B. pycnantha*.
7. Ovoidin other species.

Variations were found among the studied species in the flower number of inflorescences, number of flowers in one inflorescence was less than 50 flowers in *B. macrobotrys, B. longipes, B. olivieri, B. mosheovii* and *B. kurdistanica*; and with more than 50 flowers in other species. Pedicels length average was between 3.5mm. in *B. pycnantha* to 21mm. in *B. saviczii*, while the other species were located between the two values.

Pedicel shape differs among species as follows:

1. Arcuate (recurved) in *B. macrobotrys, B. pycnantha* and *B. fominii*.
2. Ascending in *B. savizii* and *B. mosheovii*.
3. Ascending in first, then arcuate (recurved) in other species.

The pedicels colors are green in all species except the species *B. saviczii, B. mosheovii* and *B. Pycnantha* which have violet colors.

**Flowers (Florets):**

Flowers of all studying taxa of this genus were perfect, actinomorphic symmetry, usually were bright, perigon with six tepals. Six identically shaped bracts were 1/3-1/2 their length and deformed tubular, bell-shaped or funnel-shaped in form. The dimensions of perigon of the studying taxa showed a variation among those species, as the minimum length of perigonwas 5.5mm. as average in *B. pycnantha* and the maximum length of perigonwas 11.5mm. in *B. olivierias* average and the perigon dimensions of other taxa were located between the two values and the minimum width of perigonwas 2.5mm. as average in *B. longipes* and the maximum width of perigonis 3.8mm. in the *B. olivierias* average and the other taxa were located between the two values. The perigon consist of two parts; tube and teeth(lobe), both of which part were unequal in dimension also has taxonomic value which can divided into the following groups:

1. Lobes length to tube length 1/2-1/3 in *B. macrobotrys, B. saviczii* and *B. longistyla*.
2. Lobes length equal to tube length in *B. longipes*.
3. Lobes length 1/3 as tube length in *B. olivieri* and *B. pycnantha*.
4. Lobes length 1/2 as tube length in the other species.

Perigon shapes were tubular to campanulate in all species except *B. longipes, B. glauca* and *B. longistyla* were campanulate.

According to color the flowers, the species can be divided into the following groups:

1. Olive-green, violet base and turning livid in *B. macrobotrys*.
2. Purplish or dirty purple with paler or greenish lobes in *B. longipes*.
3. Purplish in bud, becoming purplish-green with white, green-nerved lobes during flowering in *B. glauca*.
4. Greenish-white in bud, perigon brownish with green nerves in *B. olivieri*.
5. Perigon before flowering greenish-white and finally pale purplish, soon becoming brownish in *B. saviczii*.
6. Perigon before flowering white or White to Pale blue, becoming pale lurid or brown in *B. mosheovii*.
7. Perigon at flowering greenish-white, with purplish tinge, becoming pale gray-brown in *B. kurdistanica*.
8. Perigonat flowering very dark dull blue-violet or dirty violet-blue in *B. pycnantha*.
9. Perigonat flowering purplish to blue, becoming violet in *B. fominii*. 
10. Perigonat flowering purplish tube with green-veined lobes, finally becoming brown in *B. longistyla*.

**Androecium:**

Androecium with six fertile stamens, each stamen usually consists of filament and anther.

**Filament:**

Filament six, flattened and narrowly triangular or cylindrical, often connate at the base of the perigon-lobes, basifixed innation, green or glaucos color. Which they varied in the dimensions of their filaments, as the minimum length of filament was 4.3 mm. as average in *B. saviczii* and the maximum length of filament was 9.8 mm. as average in the *B. olivieri* and the rest taxa were between the two values.

**Anther:**

Anthers six, bilobed, longitude in aldehiscense, light purplish color in *B. longipes*; violet or somewhat lilac in *B. glauca*; dark violet in *B. olivieri*; lilac in *B. mosheovii*; yellow in both taxa *B. pycnantha* and *B. fominii*, while the other studied species with violet color. The average length of anther was between 1.1 mm. in *B. mosheovii* to 2.0 mm. in *B. longipes* and the rest of taxa were located between the two values.

**Gynoecium:**

Gynoecium polycarpous, bipistil, each with ovary, style and stigma.

**Ovary:**

Ovary superior, Tri-angled, 3-2ovuled, ovoid, sessile with axil placentation in all species. Length averaged between 2.5 mm. in *B. saviczii* to 4.4 mm. in *B. olivarii* and the other species were located between the two values. Ovary width averaged between 0.90 mm. in *B. mosheovii* and 2.8 mm. in *B. olivieri*, and the other studied species were between the two values. Ovary colors differ among studied species as follows:

1. Blue in *B. longipes*.
2. Green to yellow in *B. olivieri*.
3. Yellow in *B. kurdistanica*.

**Style:**

Single, elongate with same color of ovary and the dimensions are varied, the minimum average. Length of style was 3.4 mm. in *B. saviczii* and the maximum average length of style was 6.5 mm. in *B. kurdistanica*.

**Stigma:**

Capitate, obtuse, three lobes, yellow in all species.

**Fruit:**

The shape and dimensions of fruiting raceme are characters with taxonomical values. Fruiting racemes length averaged 5 cm. in *B. mosheovii* to 25 cm. in *B. longipes*, while the others species were located between the two values, and width average between 3 cm. in *B. pycnanthato* 12.5 cm. in *B. olivieri* and *B. saviczii*; while the rest species were between the two values. Fruiting raceme shape’s as following:

1. Cylindrical in the species *B. macrobotrys, B. kurdistanica* and *B. fominii*.
2. Broad at base, conical in *B. longipes* and *B. olivieri*.
3. Broadly ovate in *B. saviczii*.
4. Ovate in *B. mosheovii*.
5. Conical in other species.
**Fruits shapes as follows:**

1. Broadly ovoid or almost round, retuse at apex in *B. macrobotrys*.
2. Ovate-oblone, retuse at apex in *B. longipes*.
3. Obovate, triquetrous in *B. glauca*.
4. Ellipsoid-ovoid, emarginated at the apex in *B. olivieri*.
5. Sub-spherical to broadly ovoid or broadly ellipsoid, emarginated at the apex in *B. saviczii*.
6. Broadly ovoid with rounded apex in *B. mosheovii*.
7. Sub-spherical to broadly ovoid, retuse at apex in *B. kurdistanica*.
8. Spherical, retuse at apex in the *B. pycnantha*.
9. Broadly ovoid, retuse at apex in *B. fominii*.
10. Broadly ovoid in the *B. longistyla*.

Color of fruits  green or dark green in all species. Diameter averaged between 7.5 mm. in *B. pycnantha* to 18.5 mm. in *B. olivieri* and the others species were between the two values. Shown in plate 4.

**Plate 4:** Qualitative and Quantitative characters of Fruits of studies species
Seed:

Seeds shapes were ovoid in all species except in *B. pycnantha* is ovoid-oblong; seed color of all species were black except in *B. pycnantha* is brown. Diameter average of seed was between 1.6 mm. in *B. longistyla* and 2.10mm. in *B. saviczii* and the other species were ranged between the two values as shown in Plate.5.

Plate 5: Qualitative and Quantitative characteristics of seed.

Results and Discussion:

**PALYNOLOGY** { Plate 6, 7, 8}

The present results showed that the pollen grains were monads, monosulcate, and ellipsoidal asymmetry. According to SEM and LM studies, the pollen grains of *Bellevalia* were only monosolcate. The monosulcate pollen type occurs widely in monocotyledons, which is interpreted to be a distinguishable trait among seed plants(Erdtman, 1971; Özler and Pehlivan, 2007). Polar view of examined species was medium in size except (*B. longipes, B. olivierii* and *B. pycnantha*) with small size. While the Equatorial (E) view of the studied taxa can be divided in to three groups:

1. Pollen grain with 22.5 μm in size(diameter) in *B. fominii*.
2. Pollen grain with 50-60 μm in size in *B. longipes, B. glauca, B. saviczii* and *B. kurdistanica*.
3. All other species with 40-50 μm in size.

Pollens were prolate to prolate-spherical and the exine structure was semi-tectate in the studied species. The sulcus extends from distal to proximal in all species. Sulcus surface were reticulate, regulate or granulate. Surface was reticulate to sub-reticules .The reticulate become smaller towards the edge of the sulcus and larger at the lateral surface in all species. The sulcus extends
between two exterm sides of pollen and the sulcus ends were narrowed and acute. The exine ornamentation of the proximal surface was reticulate-perforate in all the taxa under study.

According to outline of pollen grains in polar view (P), the studied species can divide into two groups, group with ellipsoid pollen grains as in *B. macrobotrys*, *B. mosheovii* and *B. longistyla* and groups with spherical pollen, in reminder species. Shapes of Equatorial view (E), also can divided species into two groups, group with ovoid pollens in *B. mosheovii*, *B. kurdistanica* and *B. fominii*; and the other with ellipsoid shape in other species. The exine thickness should be another important palynological trait among the examined species of pollen grains were ranged between 1.4μm as minimum in species *B. macrobotrys* to 2.8μm as maximum in *B. longistyla*. The result of pollen morphology agrees with results of each Erdtman (1971,1969), Murry and El-Ghazly (1990), Pehlivan & Özler (2003), Nwachukwu *et al.* (2008) and Borzatti Von Loewenstern, Giordani *et al.* (2013).

![Plate 6: Pollen grains of polar view photographs by LM of studied species](image-url)
Plate 7: Pollen grains of equatorial view photographs by LM of studied species
plate 8: Pollens photographs by SEM of studied species
REFERENCES:


دراسة تصنيفية مظهرية وحبوب اللقاح للجنس Bellevalia Lapeyr في كوردستان العراق

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المستخلص

أجريت دراسة تصنيفية للصفات المظهرية وحبوب اللقاح للجنس Bellevalia Lapeyr التابع للعائلة الهليونية Asparagaceae في كوردستان العراق خلال موسمي النمو (2015-2016) لإيجاد إنتشار الأنواع وتسجيل أنواع محلية جديدة. تناولت الدراسة المظهرية صفات الأغلال والسيقان والأوراق والرخاء الزهراء والثمار والبذور. وقد تم تقدير من خلال مناقشة تتنوع الصفات أن صفات الأزهار لها أهمية تصنيفية أكثر مقارنة بالصفات الأخرى لغرض عزل الأنواع. وأُجريت دراسة حبوب اللقاح لأول مرة على العينات النباتية العراقية والتي أظهرت أن حبوب اللقاح لكافة المرئيات المدرجة للجنس Bellevalia تكون بصورة متغيرة ذات فتحة واحدة بينما تمتلك الصفات الأخرى تغييرات لها أهمية تصنيفية خاصة. و إن الأنواع (Bellevalia. macrobotrys, B. longipes, B. glauca, B. saviczii, B. olivierii, B. mosheovii kurdistanica, B. longistyla و Bellevalia fominii) تم تسجيلها سابقاً ويبيندا النوعان B. pycnantha و Bellevalia chrisii تم تسجيلها في الشرق الأوراسي وشرق أوروبا.

الكلمات المفتاحية: Palynology, Morphology, family (Asparagaceae), Bellevalia.