

## Morphoanatomical study of some species from Liliaceae Juss. Family in Ghurfa-Adhaim district

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#### Introduction

Liliaceae is a monocot family plants, with 250 genera and more than 3,500 species distribute in southern hemisphere (Africa, South America, Australia, and India) and in East Asia [1]. More than 460 species belong to 36 genera present in Turkey [2]; 21 genera represented in Iraq [3], with high variation in morphological characters regardless genomic correlation between species [4]. The family characterized by bulb or rhizome root, tepals (not distinguish calyx from corolla) colour which grouped in two cycle, superior ovary and fruits capsule [5]. Different Liliaceae species are cultivated and using as food such as onion *Allium sativum* L. and garlic *Allium cepa* L., other beautiful ornamental species are cultivated all over the world, but many species are wild plants. Several Liliaceae plants used as traditional medicinal uses such as bulbs of *Fritillaria roylei* Hook. used in tuberculosis [6], and *Hosta ventricosa* used as mastitis, otitis media, folliculitis and snake bites [7]. The genus *Gagea* Salisb. comprise 200 species distributed in Mediterranean region [8], 9 species in Iraq are found: *G. confuse* A., *G. anisanthos* C.Loch., *G. dubia* A.Terr., *G. linearifolia* A. Terr., *G. commutate* C.Koch., *G. reticulata* (Pall.) J. Aet J., *G. chloranthus* (M.B.) J. A. etJ. H. Shultes, *G. uliginosa* Siche et Pascher and *G. gageoides* (Zucc.)Vved. [3]. Taxonomically, the genus *Gagea* L consisted of two subgenera: *Gagea*

#### Abstract

Four species from Liliaceae family were studied: *Bellevalia glauca* (Lindl.) Kunth., *Bellevalia kurdistanica* Feinbrum, *Gagea reticulata* Salisb. and *Ornithogalum brachystachys* C.Koch, morphologically and anatomically. Plants were collected from Gurfa-Adhim district between March and May, 2016. Bulb, scape, stem leaves, inflorescence and flowers were examined and variations in size, shape and dimensions were recorded. The results revealed close morphological relationship between *Bellevalia galuca* and *B. kurdistanica* in characters: bulbs, stem leaves and stamens, absence of cambium and xylem fibers in outer part of vascular bundle; meanwhile, the two *Bellevalia* species are varied from *Gagea reticulata* and *Ornithoigalium brachystachys* in morphological and anatomical features.

(*Eugagea* Pascher) with spherical seed, and subgenera *Hornungia* (Bern.) distinguished by triangular seed shape [9]. Many morphological characters were used to identify plant species of lily family, like outer and inner tepals of *Lloydia serotina* (L.) Salsb. ex. Reich. [10]; bulb size, bracts and pedicel of *Gagea* Salisb. [11]; gynoeceum character is anatropous bitegmic of *Gagea villosa* (M.Bieb.) Duby. [2]; pollen morphology characters includes size and exine sculpture of *Erythronium caucasicum*, *E. gegantum* and *E. sibiricum* [12]; pollen shape monosulcate heteropolar subprolate of *Fritillaria mughlae* M.Tesen & Aytac [13]. Anatomical characters also used in Liliaceae species that phloem elements below the endodermis layer are arranged in single layer alternating with pericycle cells of *Lilium candidum* L. [14]; root and leaf sections were used in identification of *Hyacinthella glabrescens* (K.Koch) Schur. [15]. Embryonic feature is trilocular ovary with anatropous ovule in each and cellular characters of plastids of *Gagea bohimica* (Zauschn.) Schult.& Schult.F. [16]. Some rare species of Liliaceae such as *Tulipa akamasica* Christodoulou, Hand & Charalambous exists in small area in Cyprus which morphologically characters are illustrated as globose-elongate bulb 17-25 × 12-18 mm in dimension and red bright distinct tepals of solitary flowers [17]; chromosome number is hexaploid of sterile plant and the reproductive

(flowering) depends on bulbils of *Gagea lutea* (L.) Ker Gawl and *Gagea spathacea* (Hayne) Salisb [18]; systematic revision of *Gagea villosa* [19]; phylogenetic study based on plastid DNA of 7 *Gagea* species in Germany revealed close related between species rather than diversification [20].

The genus *Bellevalia* Lapeyr. is a member of subfamilies Scilloideae (Engler, 1887) which is closely related to the genus *Muscari* Mill. within the family Liliaceae [21], 6 species of *Bellevalia* were used to investigate their relationship based on karyotypic and was found both diploid and tetraploid variation between the studied species [22] and [23]. Recently, *Bellevalia* considered from Hyacinthaceae/Asparagaceae family [24]. The genus *Bellevalia* of about 65 species, 10 species found in Iraq [3].

The genus *Ornithogalum* L. consists of 150 species, only 8 species in Iraq [3]. Several studies dealt with *Ornithogalum* species and described morphological features such as seed micromorphology of *Ornithogalum* species [25]; ploidy levels of chromosome number of *Ornithogalum umbellatum* [26]; 31 morphological analysis of *Ornithogalum* using dichotomous key [27]; as well as study of cytotoxic effect of *Ornithogalum cuspidatum* Bertol. on Prostate Cancer [28], and antioxidant action of phenolic and flavonoid compounds of bulb and vegetative part extracted from *Ornithogalum sintenisii* L. [29]. However, wild species from

Liliaceae family in DGA (Ghurfa-Adhaim district) in Iraq are not examined; the aims of this study are to understand the variations, diversity, species limitation and relationships based on morphological and anatomical attributes of species included in the this study.

### Materials and methods

Plant samples were collected from Ghurfa-Adhaim district, particularly from West of Amirli town, and in Adhaim dam near Tigris River at altitude of 110-160 m with limestone soil, no dense vegetation and absence of tree species. The collected plants were dried by using newspaper. Measurements were recorded after two weeks. The changes in the colour of leaves and bulbs were noticed after collecting, morphological characters were observed using dissecting binocular microscope type Stereo TS-70 made in Germany. For anatomical study, cross sections of leaves and stem were made [30] by razor blade, the sections were placed in Ethanol 90% for 5 minutes, then washed in D.W., they stained with safranin stain was used, then mounted with 50% Aqueous Glycerine, coverslip were placed, the measurements by micrometres were taken using Altay binocular microscope made in Italy, and photos were taken by digital camera type Sony. Stomatal index and stomatal frequency were measured as follow:

$$\text{Stomata index} = \frac{\text{Number of stomata}}{\text{Number of stomata} + \text{Number of ordinary epidermal cells}} \times 100$$

Stomatal frequency = Number of stomata in one microscope field  $\times 40$

### Results

The taxonomic descriptions of the studied taxa as follows (Table 1, 2 & Figs: 1-3):

***Bellevalia glauca*** (Lindl.) Kunth.: plant 18-40 cm high. Bulb 2-2.7 cm diam. Leaves 5-6 not longer than inflorescence, 21-24 cm length, 8-10 mm width, lanceolate-linear, smooth margin, basal leaves a ciliate margin. Scape 10-20 (-30) cm including the raceme. Raceme cylindrical long carrying 20-26 flowered. Flower pedicel 7-8.1 and up to 10 mm long. Perianth 9-10 mm long, campanulate, purplish when buds become purplish-green after maturity. Stamen 6, 3 mm long, anther 1 mm long black purplish, basifixed, longitudinal dehiscence. Pistil 5 mm long, 2-2.6 mm width.

***Bellevalia kurdistanica*** Feinbrum: plant 22-28 cm high. Bulb 2.5-4.5 cm diam. Leaves 5-6, (6-)16-21 cm length, 3-3.5 mm width, broadly lanceolate, ciliate margin. Scape 6-7.5 cm long including the raceme. Raceme 3-4.5 cm long, cylindrical carrying 9-15 lilac flowers. Pedicel 2-4 mm long becoming curved in flowering. Perianth 3-5 mm long, campanulate, lilac. Flower raceme many flowered, 3.6-5 cm long, 1.3-1.6 cm broad, with lilac become flowers. Pedicel 3.2-4 mm long soon becoming elongated. Tepals 3-6 mm long, 2.1-3 mm width, with

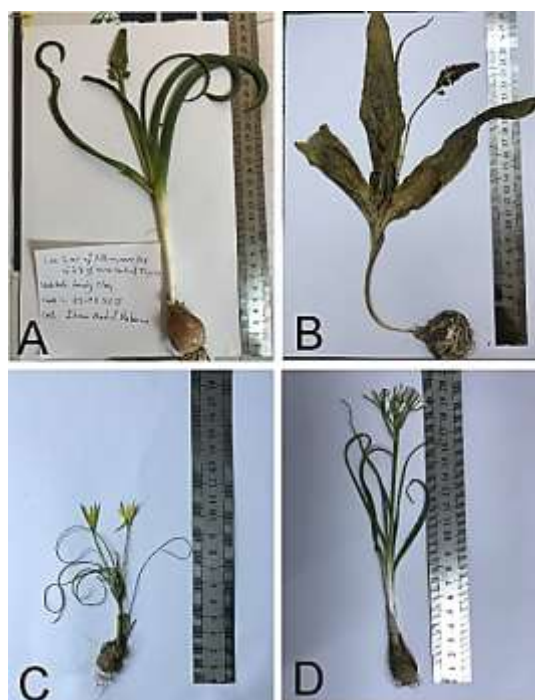
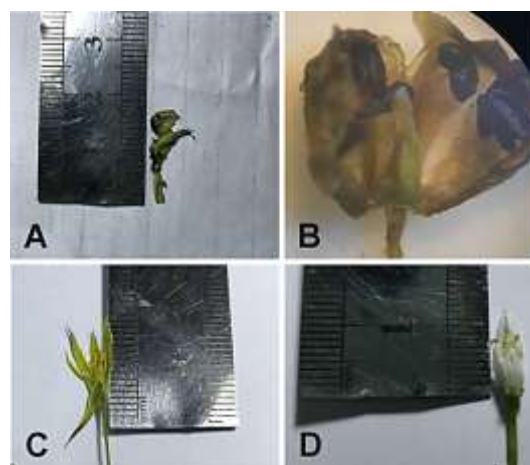
lobes c.½ as long as the tube, ovate-oblong. Stamens 6, 5 mm long, anthers 1-1.5 mm long, blue, basifixed. Pistil 3 mm long, 1.5 mm width.

***Gagea reticulata*** Salisb.: plant (6-)12-20 cm in high. Bulb 12 mm diam. Leaves 6-8, linear exceeding the inflorescence, 4-10 cm long, 1.9-3 mm width, semicylindric, canaliculate, pubescent, ciliate margin. Scape 9mm exceeding inflorescence, linear. Inflorescence umbiliform, 1-3 flowers. Pedicels 3-5 cm long. Perianth 6-15 mm long, segments, glabrous, yellowish green. Tepals 14 mm long, 4.6-5.1 mm width, bract 7 mm long sheathing, yellowish green. Stamens 6, 4-4.5 mm long, anther 1.5 mm long, yellowish green, basifixed. Pistil of 4.8-5 mm long, 1-1.5 mm width.

***Ornithogalum brachystachys*** C. Koch: plant 10-15 (30) cm high. Bulb 18-25 mm diam. Leaves 4-6, not exceeding the inflorescence, 12-15 cm long, canaliculate. Scape 17 cm including the raceme, ciliate margin. Leaf 10-14 cm long, 1.9- 3 mm in width. Bract 1.8-2.1 cm long, sheathing. Pedicels 6-9 cm long. Perianth 12-15 mm long, white with green midrib. Flower 1.5 cm long. Tepals 10-14 mm long, 5-5.1 mm width, white with green midrib on adaxial surface, elliptic-oblong. Stamens 6, 6-6.2 mm long, anther 2 mm in long, white. Pistil 5-5.1 mm long, 1-1.5 width.

**Table 1: Morphological characters of the studied taxa**

Character	<i>Bellevalia glauca</i>	<i>Bellevalia kurdistanica</i>	<i>Gagea reticulata</i>	<i>Ornithogalum brachystachys</i>
Bulb	2×2.7 mm dimension	2.5×4.5 mm dimension	6×12 mm dimension	18×25 mm dimension
Scape	10-20 (-30) mm length	6-7.5 mm length	9 mm length	17 cm length
Stem leaves	21-24 cm length, 8-10 mm width, lanceolate-linear	(6-)16-21 cm length, 3-3.5 mm width, broad lanceolate	4-10 mm long, 1.9-3 mm width, semicylindric, canaliculate	12-15 mm long, canaliculate
Perianth	9-10 mm long, campanulate, purplish	3-5 mm long, campanulate, lilac	6-15 mm long, segments, glabrous, yellowish green	12-15 mm long, white with green midrib
Stamens	6, 3 mm long, anther 1 mm long black purplish	6, 5 mm long, anthers 1-1.5 mm long, blue	6, 4-4.5 mm long, anther 1.5 mm long, yellowish green	6, 6-6.2 mm long, anther 2 mm in long, white
Pistil	5 mm long, 2-2.6 mm ovary width	3 mm long, 1.5 mm width ovary	4.8-5 mm long, 1-1.5 mm ovary width	5-5.1 mm long, 1-1.5 ovary width

**Fig.1. Flowers: A) *B. glauca*; B) *B. kurdistanica*; C) *Gagea reticulata*; D) *Ornithogalum brachystachys*.****Fig.2. Bulbs: A) *B. kurdistanica*; B) *B. glauca*; C) *Ornithogalum brachystachys*; D) *Gagea reticulata*.****Fig.3. Plant specimens: A) *B. glauca*; B) *B. kurdistanica*; C) *Gagea reticulata*; D) *Ornithogalum brachystachys*.****Anatomical descriptions:**

***Bellevalia glauca*:** the cross section of scape show presence of thick cuticle layer of unicellular layer of epidermal cell, no hairs are presented. Then multiple layers of hypodermis of rounded cell type arranged under the epidermis. The main characteristic of the stem is the presence of lacunar collenchyma that responsible for mechanical support to the stem in most herbaceous plant scape stem. The distribution of parenchyma tissue as a ground tissue with distribution of vascular bundles characterized of absence of cambium; and the sclerenchyma tissue presence in outer part of the vascular bundle, and xylem parenchyma and tracheids in the inner of vascular bundles. Phloem tissue consists of phloem sclerenchyma in outer part, with phloem parenchyma and companion cells. The leaf anatomy shows that there is parenchymous palisade layer under tow layers of mesophile; in both abaxial and adaxial surfaces there are anomocytic type stomata, but more on upper surface, stomatal index 24 and stomata frequency 3.9%. The vascular bundle consists of xylem and phloem surrounded by a single distinct layer of bundle sheath.

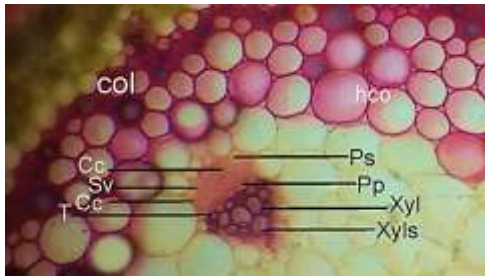


Fig. 4. Cross sections of stem of *B. glauca*: col: Collenchyma, hco: Hypodermis, Cc: Companion cell, Sv: Sieve tube, Xyl: Xylem, Xyls: Xylem sclerenchyma, Ps: Phloem sclerenchyma, Pp: Phloem parenchyma, T: Tracheids.

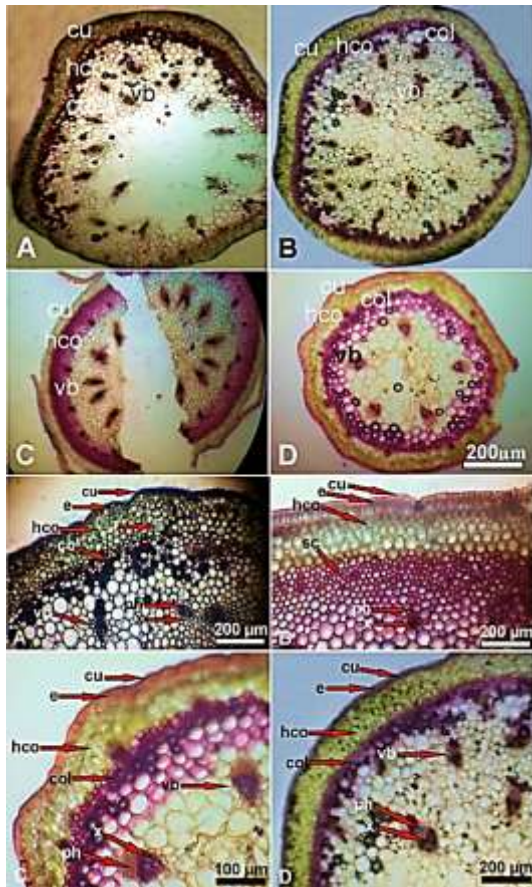


Fig. 5. Cross sections of scape: A) *B. glauca*; B) *B. kurdistanica*; C) *Gagea reticulata*; D) *Ornithogalum brachystachys*. cu: cuticle, hco: hypodermis, vb: vascular bundle, col: collenchyma, x: xylem. Ph: phloem.

***Bellevalia kurdistanica*:** this species demonstrate similarities in anatomical characters with the *B. glauca*, but only with thinner collenchyma tissue below the hypodermis layer cells which 3-layers cells arranged. The leaf anatomy shows similarities of that lower surface (abaxial surface) with anomocytic type of stomata complex in both adaxial and abaxial surfaces, stomatal index 24 and stomata frequency 3.9%, with two of mesophyll layer. The vascular bundle in a single row with uniseriate of upper epidermis, and the cell wall with less cuticle thickness.

***Gagea reticulata*:** the anatomical characters of this species shows similarities with the *B. glauca*, only with thinner collenchyma tissue below the hypodermis layer cells which 4-layers cells arranged. Stem contain vascular bundles distributed in the ground tissue, in the inner parts the xylem parenchyma and tracheids, in the outer parts the phloem tissue consists of phloem sclerenchyma, phloem parenchyma and companion cells. The cross section of leaf demonstrate that both abaxial and adaxial surfaces with anomocytic type of stomata complex, stomatal index 34 and stomata frequency 8.8%, with two of mesophyll layer. The vascular bundle in a single row, and with uniseriate of upper epidermis contain less thick cuticle.

***Ornithogalum brachystachys*:** the anatomical characters of this species shows similarities with the *B. glauca*, only with thinner collenchyma tissue below the hypodermis layer cells which 4-layers cells arranged. The cross section of stem reveals that vascular bundles distributed in the ground tissue randomly, the xylem parenchyma and tracheids in inner part of the bundle, the phloem tissue consists of phloem sclerenchyma, phloem parenchyma and companion cells in the outer part of the bundle. The cross section of leaf demonstrate that both abaxial and adaxial surfaces with anomocytic type of stomata complex, stomatal index 31 and stomata frequency 7.6%, with two of mesophyll layer, and the vascular bundle in a single row, and with uniseriate upper epidermis contain thick cuticle.

#### Discussion

The studied species of Liliaceae from monocotyledons are flowering between March and April; in a lower mountain slopes habit that no trees present and rocky clay soil and moister habitat or more rainfall locations at altitude 500-800 (1000) meter. The morphological characters illustrations in the current study are in major congruent with the description in Flora of Iraq [3]. However, morphological characters of bulb, scape, stem leaves, and inflorescence are revealed variations in size and shape from Flora of Iraq, also androecium characters are mentioned for the first time. From table 1 & Fig. 2, the bulb size and shape show variations, *B. glauca* has and elongate ovoidei smallest bulb (2-2.7 cm), but ovoidei biggest bulb (18-25) cm for *O. brachystachys*. Scape of *B. kurdistanica* is solitary small 6-7.5 cm (including the raceme) glabrous, meanwhile two plant species show close relation: *B. glauca* and *O. brachystachys*, which reflects the converged species boundaries in Liliaceae family species [33]. Stem leaves are small in *G. reticulata* 4-10×1.9-3 mm, in *B. glauca* has 21-24×8-10 mm stem leaves, the reason of this variation in morphological character is ecological factors and growing in height levels [34]. The leaf margin of *B. kurdistanica* is revolute with spiny colourless shiny hairs as described by [35]. Stamen in all studied species is 6 numbers, but vary in length (Table 1): the short

stamens and anther in *B. glauca* (3 mm length, 1 mm width), in *O. brachystachys* 6-6.2 mm length and 2 mm anther length; also variations in stamens colour revealed from black purplish in *B. glauca* to white in *O. brachystachys*. Pistil is small  $3 \times 1.5$  mm, but bigger in *O. brachystachys*  $5 \times 2-2.6$  mm. The anatomical characters reveals variation between the studied taxa (Figs. 3; 4 & 5), however the most important anatomical character in most Liliaceae family species in monocotyledon plant is the presence of stomata in both upper and lower surfaces, with more stomata dense in lower surfaces [36].

Thick cuticle layer in *B. glauca* and *O. brachystachys*, but in *B. kurdistanica* and *G.*

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*reticulata* with thin cuticle, this character considered significance anatomical distinguish feature among the species [37] & [38]. The hypodermis is distinct multilayer in *B. glauca*, *G. reticulata* and *O. brachystachys*, only *B. kurdistanica* is varied with only 3 layers of hypodermist. Thick layer of collenchyma tissue present in *B. glauca*, meanwhile thinner collenchyma is present in both *G. reticulata* and *O. brachystachys*, the significance character is recorded for the first time in this study in *B. kurdistanica* with sclerenchyma tissue replaced collenchyma tissue, this could be high variation feature due to ecological effect and isolation [39].

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## دراسة مظهرية تشريحية لبعض الانواع من العائلة الزنبقية المنتشرة في مقاطعة غرفة عظيم

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### الملخص

تمت دراسة اربعة انواع مختلفة من العائلة الزنبقية: *Bellevalia kurdistanica* Feinbrun، *Bellevalia glauca* (Lindl.) Kunth. و *Gagea reticulata* Salisb و *Ornithogalum brachystachys* C.Koch، بالاعتماد على الصفات المظهرية والتشريحية. تم جمع العينات النباتية من مقاطعة غرفة عظيم بين شهري آذار ونيسان من سنة 2016. الصفات: الابصال، الاوراق القاعدية، السيقان الورقية، النورات الزهرية والازهار عبر قياس الابعاد والاحجام. اوضحت النتائج وجود تشابه في الصفات المظهرية بين النوعين *Bellevalia galuca* و *Bellevalia kurdistanica* في صفات الابصال، السيقان الورقية والاسدية فضلاً عن الصفات التشريحية للكامبيوم الوعائي وتوزيع الخشب والسكليريدات. بينما نواع الجنس *Bellevalia* كانت مختلفة عن النوعين *Gagea reticulata* و *Ornithogalum brachystachys* في مجمل الصفات المظهرية والتشريحية.