

**SYSTEMATICS OF *SISYRINCHIUM ANGUSTIFOLIUM* MILL. (IRIDACEAE),
A NEWLY RECORDED SPECIES FROM TURKEY**

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Abstract

Sisyrinchium angustifolium Mill. (Iridoideae, Sisyrinchieae) has been reported as a new record for the flora of Turkey from NE Anatolia, and taxonomic and conservation status of this species are evaluated. Detailed morphological description, leaf and stem anatomical properties, and chromosome numbers are provided. The present chromosome count is the first tetraploid level for this species reported from Eastern Anatolia, Turkey.

Introduction

Sisyrinchium L. (Iridoideae, Sisyrinchieae) includes approximately 200 species (Rudall *et al.*, 1986) and occurs in wet to dry grasslands, rocky or sandy soils, and also in highly transformed areas, such as roadsides or grazed and burned fields at elevations of up to 800 m (Bicknell, 1899). During fieldwork in Artvin Province (Turkey) some interesting specimens belonging to Iridaceae were collected. After critical study and consultation with relevant literature (Fedchenko, 1968; Davis, 1984, 1988; Güner *et al.*, 2000; Özhatay *et al.*, 2011), the specimens have been identified as *Sisyrinchium angustifolium* Mill. The species was not reported earlier from Turkey (Özhatay *et al.*, 2011; Eminağaoğlu *et al.*, 2012; Eminağaoğlu and Özcan, 2013) and hence reported as a new record for the flora of Turkey. *S. angustifolium* is the first species of the genus *Sisyrinchium* to have been reported for Turkey. The number of genera of Iridaceae in the flora of Turkey is increased to 6 with addition of *Sisyrinchium*. In this study, we aim to describe detailed morphological characters of *Sisyrinchium angustifolium*, to determine its conservation status, and distribution along with exploring the anatomical and cytological properties of the newly recorded species.

Materials and Methods

Morphological analysis: Plant materials were collected from distinct parts of Artvin, Turkey, at different altitudes in May and June, 2013. The collected materials were critically studied. The voucher specimens have been deposited at the Herbarium of Artvin Coruh University (ARTH), Artvin, Turkey.

Anatomical preparation: Anatomical observations were performed in stem and leaf from living specimens. Plant samples were stored in 70% alcohol for anatomical studies. Transverse sections of stem and leaf, and peripheral sections of upper and lower epidermis of leaves were taken by hand using commercial razor blades and stained in Haematoxylin for about 15 min. To remove the excess stain, sections were washed in water several times (Algan, 1981). Semi-permanent slides were mounted in glycerin or permanent slides were covered with glycerin-gelatin

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(Vardar, 1987). Well stained sections were examined under a light microscope and photographed using an Olympus BX-53 microscope with digital camera attachment DP 73.

Chromosome count: For mitotic chromosome observation root tips growing in pots were cut off and pretreated with 0.05% colchicine at room temperature for 4.0-4.5 h (Ozcan *et al.*, 2008), then fixed in fresh Carnoy absolute alcohol-glacial acetic acid (3:1) for 24 h at 4°C. For chromosome counts, root tips were hydrolyzed in 5N HCl for 10-12 min at room temperature and then rinsed with distilled water for 2-3 min. Staining was carried out in lacto-propionic orcein at least for 2 h at room temperature. Permanent slides were prepared from at least ten well-spread cells. The best metaphase plates were photographed with Olympus BX-53 microscope with digital camera attachment DP 73.

Results and Discussion

Sisyrinchium angustifolium Mill. Gard. Dict., ed. 8. n. 2 (1768). *Sisyrinchium angustifolium* f. *album* J.K. Sim & Y.S. Kim, Korean J. Pl. Taxon. 22: 3 (1992); *Sisyrinchium hibernicum* Å. Löve & D. Löve, Bot. Not. 114: 37 (1961); *Sisyrinchium membranaceum* E.P. Bicknell, Bull. Torrey Bot. Club 26: 612 (1899); *Bermudiana homomalla* (Klatt) Kuntze, Revis. Gen. Pl. 2: 700 (1891); *Sisyrinchium homomallum* Klatt, Abh. Naturf. Ges. Halle 15: 378 (1882); *Bermudiana graminea* Gaertn., Fruct. Sem. Pl. 1: 32 (1788); *Sisyrinchium gramineum* Lam., Encycl. 1: 408 (1785).

(Fig. 1).

Caespitose, perennial herb, up to 40 cm tall. Stem erect or ascending, branched, conspicuously winged towards base, the wing 3-5 mm broad; with 1-2 nodes, 2.3-5.0 mm wide, glabrous, margin often minutely denticulate especially basally. Leaves radical, linear-ensiform, 3-5 mm broad, leaf blades glabrous. Flowers 1-4 in inflorescence, c. 15 mm in diameter; spathes usually green, wider than supporting branch, glabrous, keels denticulate to entire; outer 18-38 mm long, 2.0-9.5 mm longer than inner, usually tapering evenly towards apex, margin basally connate 4-6 mm; inner with keel evenly curved or straight, hyaline, margin 0.1-0.3 mm wide, apex acuminate to acute, ending 0.2-0.7 mm proximal to green apex. Flowers pale blue to violet, occasionally white, bases yellow; outer tepals 7.7-12.5 mm long, apex rounded or emarginate, aristate. Stamens inserted at the base of perianth, the filaments usually more or less adnate to the tube, filaments connate ± entirely, stipitate-glandular basally; anthers erect or ascending. Ovary spherical or shortly oblong, 3-celled, with numerous ovules in each cell; style filiform, short or exceeding the stamens, the tips of style branches acting as receptive stigmatic surfaces; ovary similar in colour to foliage. Capsules dark brown or black, sometimes with purplish tinge, ± globose, 4-7 mm long; fruiting pedicel spreading or ascending. Seeds globose to obconic, lacking obvious depression, 0.5-1.2 mm long, rugulose.

Flowering period: March - July; *fruiting period:* June - August.

Specimens examined: **Turkey:** A8 Artvin, Hopa, Kemalpaşa, damp roadside, grassland, 20 m, 8 June 2013, 41°30'30"N, 41°32'16"E, Ö. Emin. 15855 (ARTH 5248); Artvin, Hopa, Hopa to Borçka roadside, gravelly soil, 270 m, 21 June 2013, 41°23'42"N, 41°29'54"E, Ö. Emin. 16948 (ARTH 5249); Artvin, Hopa, Kemalpaşa, damp roadside, grassland, 3 m, 8 May 2014, 41°30'16"N, 41°32'07"E, Ö. Emin. 19361 (ARTH 5266).

Distribution: Austria, Brazil, Canada, Colombia, Czechoslovakia, Germany, Great Britain, Mexico, United States, Russia and Georgia. New to Turkey.

Ecology: *Sisyrinchium angustifolium* grows on damp, humid and gravelly soil including roadsides and forest sides at 20-300 m with *Plantago major* L., *Polygonum aviculare* L., *Oxalis corniculata* L., *Equisetum fluviatile* L., and *Juncus effusus* L.

Conservation status: *Sisyrrinchium angustifolium* is only known from two localities in Artvin district. The natural habitat of the species is under threat due to animal grazing and road construction. In this state, according to the IUCN (2013), the species is considered to be vulnerable (VU B2b(ii) - the estimated area of occupancy is less than 2.000 km², and the field observations indicate a continuing decline of habitat quality). Therefore, *S. angustifolium* should be included in the List of Threatened Species of Turkey's Flora.

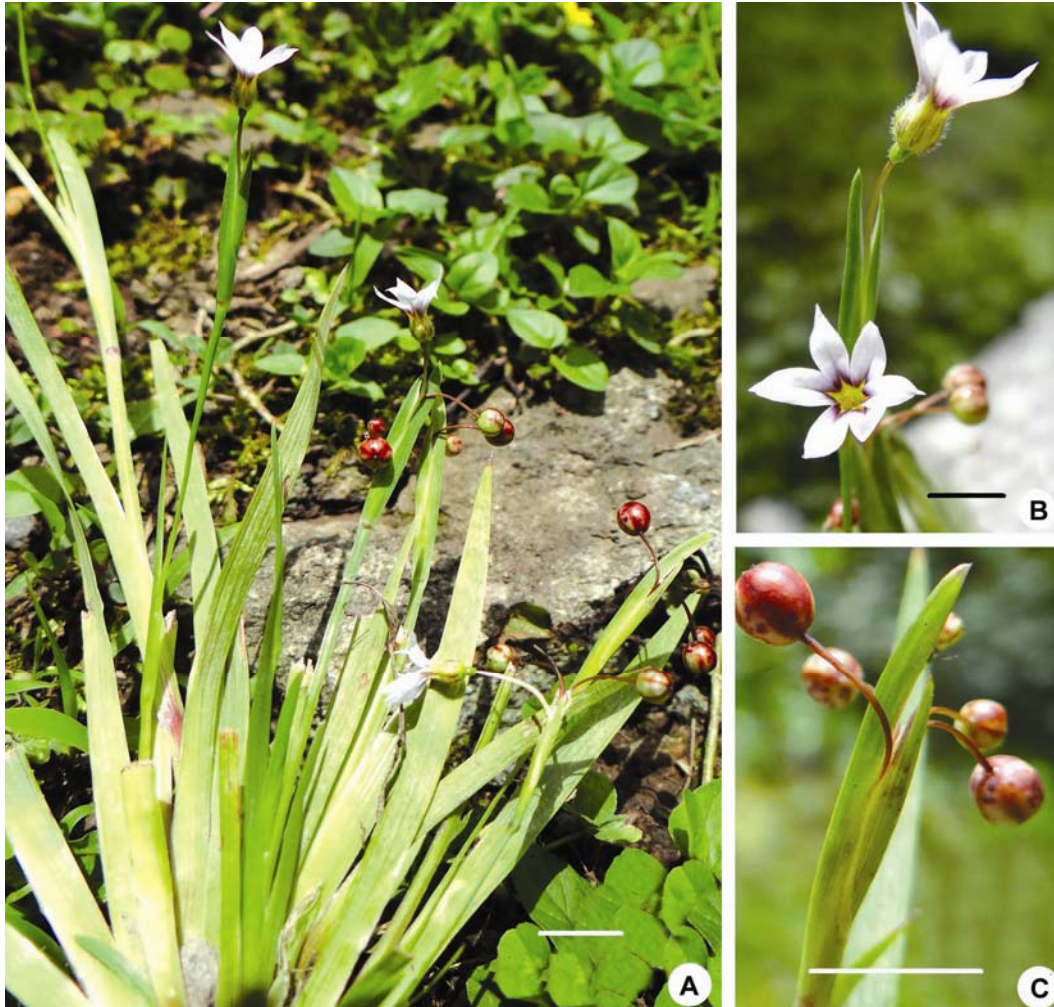


Fig. 1. *Sisyrrinchium angustifolium*; A. Habitat; B. Front and lateral view of the flowers; C. Capsules. Scale bars: A, B = 1 cm; C = 0.5 cm

Anatomical characteristics:

Anatomical studies reveal that stem is ancipital in two directions. The epidermis contains a single layer of cells. Cuticle is very thick and cell walls of the epidermis are prominently thickened. There is no papilla above the epidermis. Several sunken stomata are observed in this

layer. Mechanical tissue does not develop. Collateral vascular bundles in two circles and surrounded by sclerenchymatic ring. The larger bundles are present near to the centre (Fig. 2).

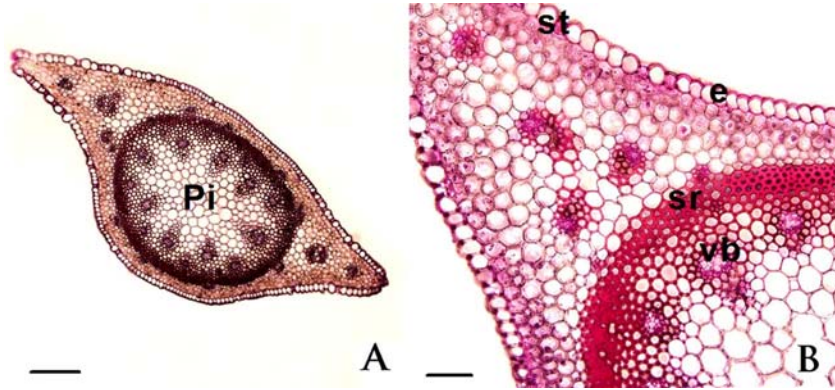


Fig. 2. Stem anatomy of *Sisyrinchium angustifolium*; A. Cross section of stem; B. Vascular bundles in stem. e: epidermis, pi: pith, sr: sclerenchymatic ring, st: stomata, vb: vascular bundle. Scale bars: A = 200 μ m; B = 50 μ m.

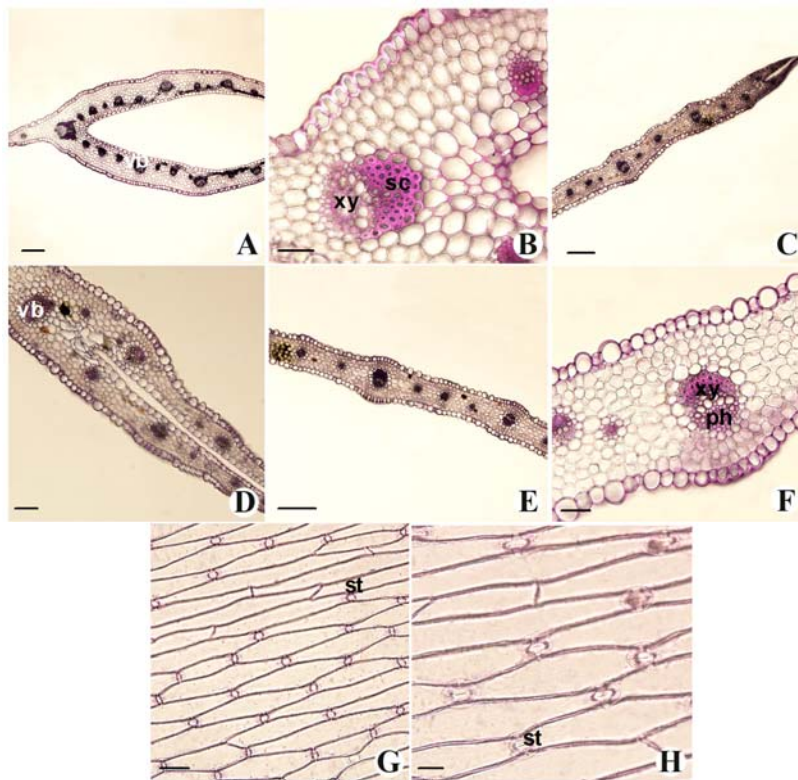


Fig. 3. Leaf anatomy of *Sisyrinchium angustifolium*. A-F. Cross section of leaf; G,H. Peripheral section. ph: phloem, sc: sclerenchymatic cap, st: stomata, xy: xylem, Scale bars: A,C,E = 200 μ m; D = 100 μ m; B,F,G = 50 μ m; H = 20 μ m.

The innermost part of the cylinder is occupied by a pith with thick-walled parenchyma. In some cases, hollow areas were observed instead of these cells in the center. The sheathing base is unifacial with both abaxial and adaxial epidermis, and leaf blade is unifacial, with an abaxial epidermis only. Surfaces do not contain any trichomes. The cuticle is smooth, epidermal cells of both surfaces are arranged in a single layer. Adaxial epidermal cells are thin-walled and stomata are absent in this surface, whereas abaxial epidermal cells are thick-walled, longer and narrower over veins than in intercostals areas (Fig. 3A-F). In the leaf blade, mesophyll is almost entirely chlorenchymatic. Vascular bundles with different sizes are collateral and surrounded by parenchymatous sheath cells, and they are arranged in a single row. Sclerenchymatic caps are observed in xylem pole. In peripheral sections, cells next to the stomata sometimes with slightly protruding over guard cells. Stomata are anomocytic in abaxial parts and sunken (Fig. 3G, H).

Mechanical tissue does not exist in the stems of examined samples. This feature has been previously reported in *Sisyrrinchium* by Holm (1908). Leaf blade are longitudinally furrowed and vascular bundles with sclerenchymatic cap at the xylem poles. The study also reveals that sunken stomata are only found in abaxial surface. Rudall *et al.* (1986) investigated anatomical and chromosomal characteristics of 11 taxa and reported in these characteristics in the section of *Bermudiana* in *Sisyrrinchium* which is supported by the present study.

Cytological investigation:

In the present study the somatic chromosome number in *Sisyrrinchium angustifolium* has been determined as $2n = 4x = 32$ (Fig. 4).

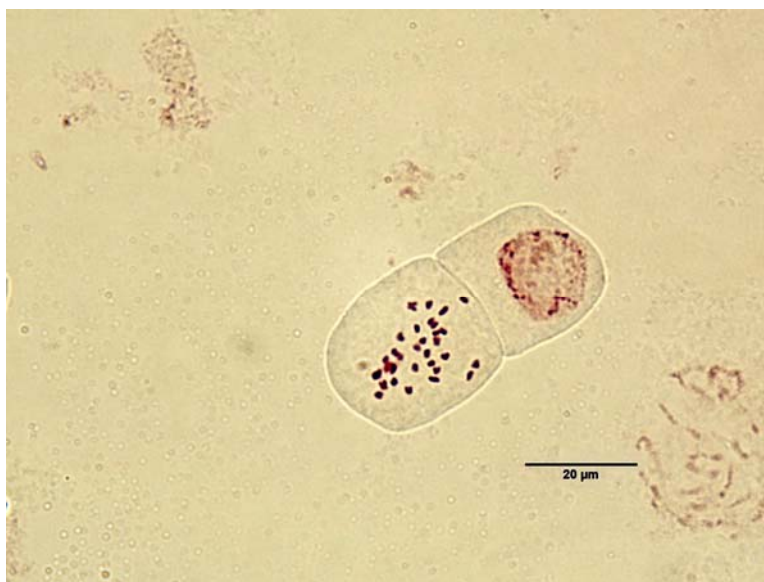


Fig. 4. Somatic metaphase of *Sisyrrinchium angustifolium*. $2n = 32$

Basic chromosome number of the genus *Sisyrrinchium* has been reported by Oliver and Lewis (1962) as $x = 8, 9$ and 17 . In addition, Rudal *et al.* (1986) reported that basic chromosome number of the section of *Bermudiana* as $x = 8$ in the genus. This species belongs to the section *Bermudiana*. Our chromosome count from Eastern Anatolia is the first tetraploid level in *S.*

angustifolium. Till now, only one previous record in a dodecaploid level ($2n = 12x = 96$) for this species has been reported by Murin and Majovsky (1976).

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