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Morpho-anatomical and karyological studies on *Iris pumila* (*Iridaceae*)

Abstract

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Iris pumila L (subgen. *Iris* sect. *Iris*) is widely distributed in southeastern Europe and a member of the dwarf bearded group. The morphological features (leaf, rhizome, root, pollen), along with the karyological observations ($2n = 36$), showed that the investigated specimens belong to *Iris pumila* var *azurea*, which is a hybrid of two dwarf taxa of the section *Pogoniris*.

Introduction

I. pumila L. is a dwarf bearded species, member of a group that was referred to the section *Pogoniris* by Dykes (1913) and to the subgenus *Iris* section *Iris* by Mathew (1981). This is the best known of the dwarf pogon irises and is much-used in the breeding of a new race of dwarf bearded cultivars, as parental form of miniature dwarf, standard dwarf and intermediate hybrids, all of which are important garden series (Warburton & Hamblen 1978). *I. pumila* is a variable plant and it has been the subject of many morphological (Luscombe 1972, 1973, 1974) and cytological (Simonet 1932, 1934; Rondolph & Heing 1951; Mitra 1956; Rondolph & Mitra 1959, 1961) studies. Luscombe (1973) states that "*I. pumila* is not a single, clearly definable entity, but a community or complex of many components, and this may include various sub-species, varieties and forms. The full scope of morphologically distinct botanical units and the entire geographical range is not yet fully determined". In many instances, the flowers smell like heliotrope, vanilla, wild orchids, violets or honeysuckle, while others appear to have no fragrance at all (Luscombe 1972; Dykes 1913; Warburton & Hamblen 1978). The character of the habitat is usually phrygana or quite frequently steppe and as a rule it prefers mountains, hills and slopes of hillsides, even steppe slopes in barren areas, growing usually on south and west aspects with free exposure to sunshine (Luscombe 1973). The natural geographical range of *I. pumila* is central and northern Yugoslavia and Austria, eastwards through Bulgaria, Czechoslovakia, Hungary and Romania to Russia, where it reaches up to the Urals (Mathew 1981).

In former publications, *I. pumila* had been reported to grow in Turkey (Rechinger 1938; Werckmeister 1967; Luscombe 1972), however, recent studies have demonstrated that this plant does not exist there (Mathew 1981, 1984; Koca 1982), Determined specimens of this plant have been obtained from the Paris Botanical Garden in order to compare them with

the dwarf group of section *Pogoniris*, growing in Turkey. The present investigation deals with morpho-anatomical and karyological analyses of the *I. pumila* samples mentioned above.

Materials and Methods

I. pumila specimens were obtained from the Paris Botanical Garden in 1976 (coming originally from Moravia) and cultivated in the greenhouse of the Faculty of Pharmacy of Istanbul University. Descriptions are based on living plants. Voucher specimens have been placed in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE).

In the anatomical study, the plant material used, was fixed in 70% alcohol. Cross and surface sections of the plant leaf, rhizome and root were made by hand and stained with Sartur solution (Baytop 1972). Additional rhizome sections were cleared in 10% sodium hydroxide, stained with phloroglucinol and hydrochloric acid and mounted in gelatin-glycerin (Vardar 1962; Yakar-Olgun 1960). Carrying out 50 counts, the stomatal number was calculated by counting the average number of stomata per square milimeter of epidermis in the blade and sheath of leaves. In addition, starch grains' sizes were determined, taking 30 measurements. The anatomical terms are used according to Esau (1965) and Cutler (1978).

Pollen samples were taken from dried plants and were prepared for light microscopy using the Wodehouse method (Aytuğ 1967). However, pilot experiments showed that it was not useful for determining the shapes of pollen grains and for conducting measurements, since gelatin-glycerin, used in this method, caused an extreme swelling and deformation of the grains. Therefore, oil immersion was used as medium. The sizes of 30 grains from three individuals were measured.

The karyological study was carried out by means of root-tip squashes. The root-tips were pre-treated in a saturated solution of alpha-bromonaphthalene (ABN) for 24 hours at a temperature of 3-4 °C or in equal proportions of an 0,002 M aqueous solution of 8-hydroxyquinoline (8-OHQ) and ABN for 5 hours at room temperature. They were then fixed in 1:3 acetic alcohol and stained using the Feulgen technique. Permanent slides were made by the liquid CO_2 method and stored. The centromeric positions were determined according to Levan & al (1964). Anatomical and karyological drawings were made using an SM-LUX Leitz drawing tube. The photograph of the pollen grain was taken using scanning electron microscope (SEM) at the Department of Histology and Embryology, Faculty of Medicine, İstanbul University. Measurements were obtained with an SM-LUX Leitz microscope (objective 40, ocular 10, 1 mark 2,63 µm).

Morphological observations

***Iris pumila* L., Sp. Pl. 38 (1753).**

Plant 10-14,5 cm high. Rhizome 11-14 mm in width, branched, whitish cream inside. Leaves four in number, equitant, 50-87 x 6-8,5 mm at anthesis, ensiform, green, apex acute. Stem 1,3-2 cm long, concealed inside the leaves. Spathe valves are on different nodes and shorter or equalling to the perianth tube. Outer valves 60-70 x 11-20 mm,

slightly inflated, green, sometimes of flesh colour at the top, lanceolate, sometimes ± linear, keeled, apex acute. Inner valves 60-76 x 11-18 mm, green or pale green; apex pale brown, scarious closely wrapping the tube, rarely not so, ± linear or linear-oblanceolate, rounded and inflated on the back; apex obtuse, sometimes acute. Flowers solitary, blue, fragrant. Ovary 14-20 x 5,2-6 mm with pedicel 2-2,5 mm, cylindrical with six longitudinal grooves at equal intervals. Perianth tube 4-5,2 cm with three purple stripes in the line of the standards, expanded at top. Outer segments (falls) 45-56 x 19-22 mm, oblanceolate-spathulate, apex rounded and margin slightly undulate, irregularly toothed. Beard hairs white in front and tipped with yellow at the base, haft is veined with brown-purple on a white ground, blade is veined with grey-purple. Inner segments (standards) 44-54 x 23-30 mm, oblong, apex rounded and retuse in the middle, margins undulate, unguiculate, blade blue with dark blue veins narrowing suddenly to the half, which is veined with brown-purple. Stamens 19-22 mm, anthers 9-10,5 x 1.6-1,9 mm, linear, apex obtuse or retuse, narrow angulate at the base, creamy yellow, margins of thecae blue. Pollen cream, pale blue. Filaments 10-12 x 1,2-1,8 mm, slightly triangular and wide at the base, pale purple. Style branches 38-41 x 9,5-12 mm, oblanceolate or oblong-lanceolate, purple at the edges with a blue keel, crests 8,5-10 x 7-7,5 mm, oblong-triangular, margins irregulary toothed, blue. Stigma emarginate or rounded, truncate, purple in colour (Fig. 1).

Flowering time: March-April.

Anatomical observations

Leaf

The epidermic cells are rectangular in the transverse sections taken from the blade and the sheath of the leaf. The cells on the internal surface of the sheath are bigger than those on the outer surface. The cells are extended in length and have simple pits on their walls in surface section. Stomata and papillae have been detected on both sides of the blade and also on the outer side of sheath. The stomata are sunken and have no subsidiary cells. The average number of stomata per mm^2 in the blade and in the sheath are 66 (min. 40 - max. 113) and 20,5 (min. 0 - max. 42), respectively.

In the blade region, the mesophyll has an isolateral structure (in the sense that both sides of the blade are similar in regard to uniform mesophyll and inverted bundles, although there is no palisade tissue). It contains compressed or circular cells that have intercellular spaces. These bear abundant chloroplasts under the epidermis. The cells in the medial area are bigger and carry either less or no chloroplasts at all. The sheath mesophyll is dorsiventral. The cells under the outer epidermis are small and contain chloroplasts, whereas the ones under the inner epidermis are bigger in diameter, have wide intercellular spaces and contain no chloroplasts (water storing parenchyma). Both mesophylls have idioblasts, containing prismatic crystals. In the blade area, there are collateral vascular bundles in two rows near the epidermis. While phloem is directed towards the epidermis, xylem is oriented towards the central section of the blade. There is a single row of vascular bundles in the sheath area, and the xylem is against the epidermis on the internal side. Phloem fibers are strongly developed and lignified. A parenchymatic sheath surrounds all

vascular bundles. The margins of the blade and sheath and also the backside of the latter are supported by sclerenchymatic cell groups (Fig. 2-4).

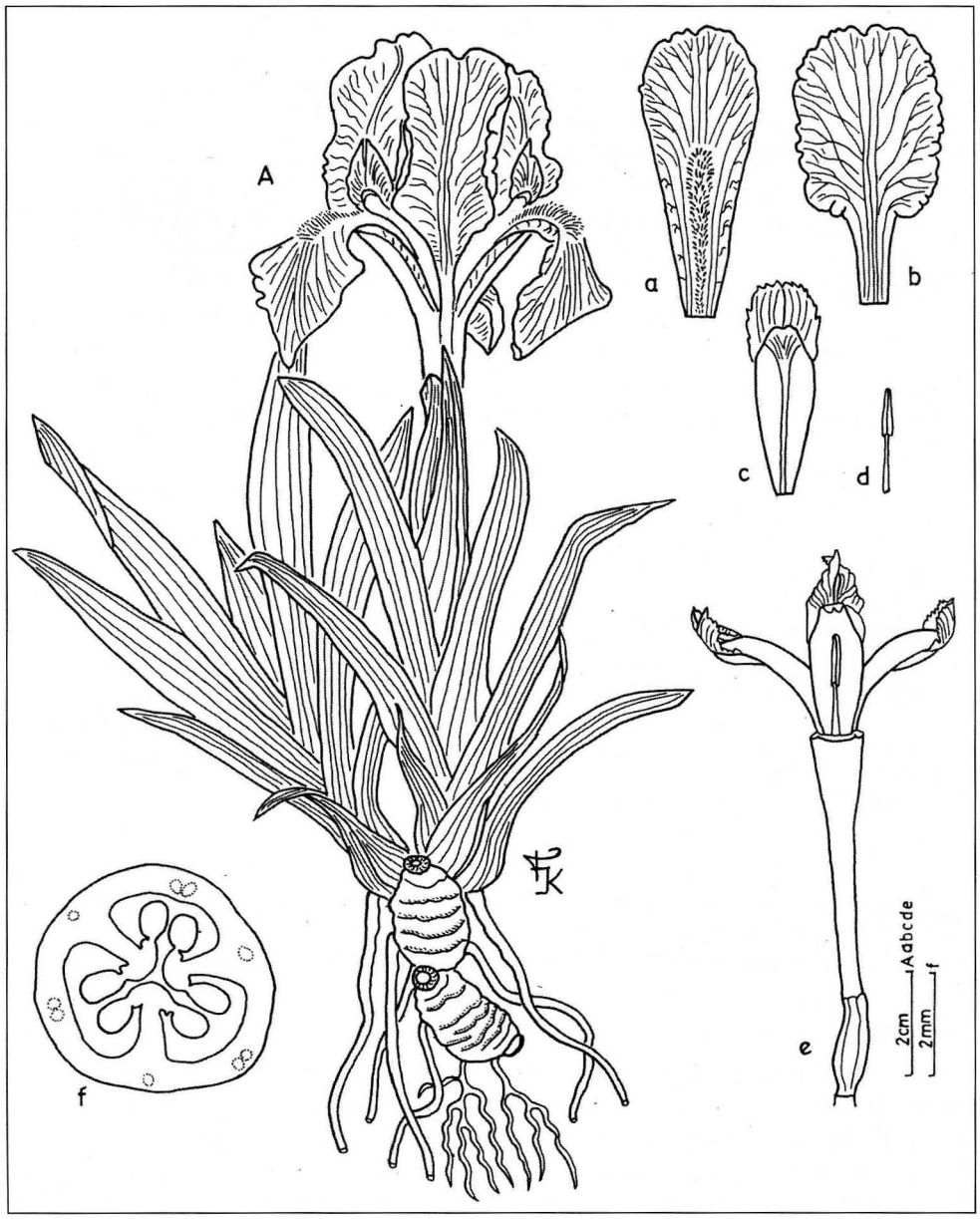

Fig.1. *Iris pumila* (ISTE 49188): A. habit; a. outer perianth segment (fall); b. inner perianth segment (standard); c. stylus branch; d. stamen; e. pistil and stamens; f. cross section of ovary.

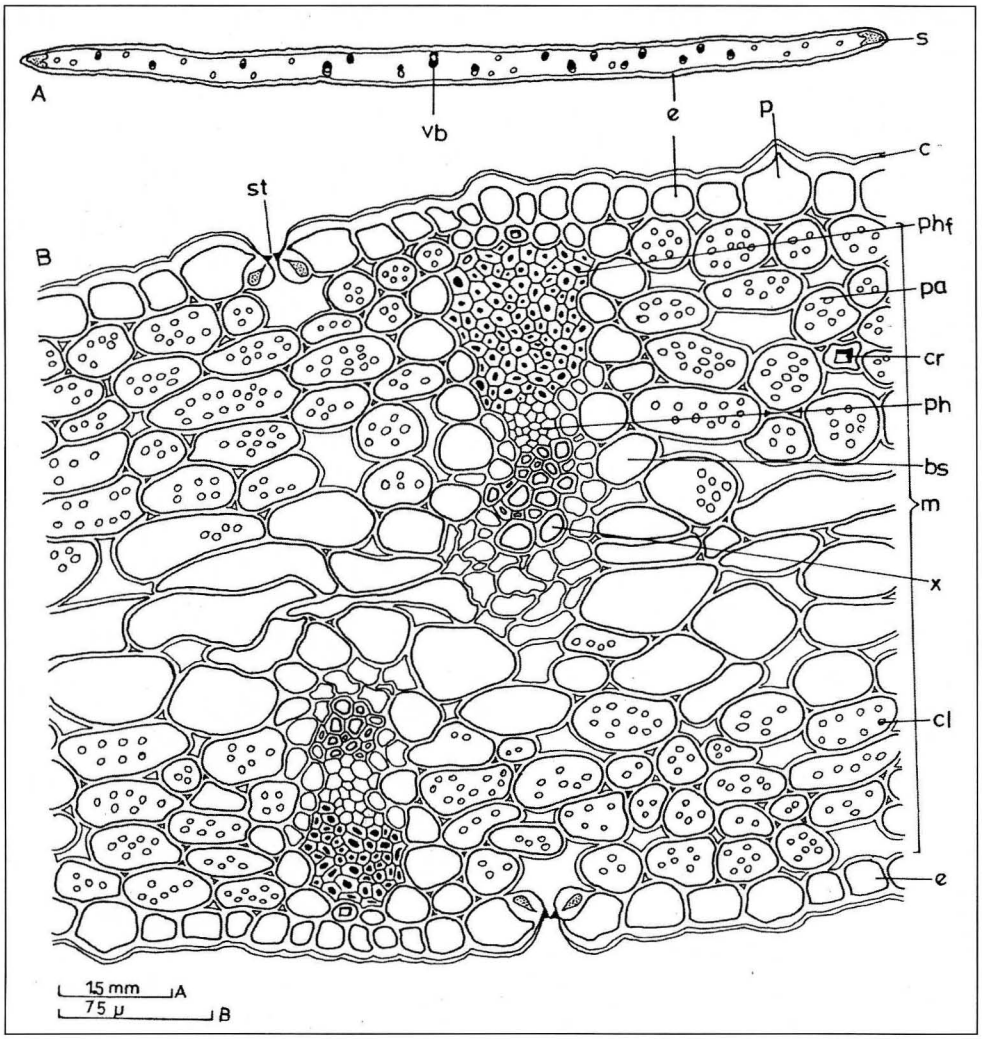

Fig. 2. *Iris pumila* (ISTE 49188), AB. cross sections of leaf blade: e. epidermis; vb. vascular bundle; s. sclerenchyma; p. papilla; st. stoma; c. cuticle; pa. parenchyma; cl. chloroplast; cr. crystal; m. mesophyll; x. xylem; ph. phloem; bs. bundle seath; phf. phloem fibers.

RHIZOME

A wide periderma is visible on the transverse section obtained from rhizomes. There is a parenchymatic ground tissue in the entire stem. Its cells are polygonal or circular. The tissue has abundant intercellular spaces and simple pits on the walls and is covered with starch grains and long prismatic crystals. Starch grains are of oval, spherical or oblong form. The long grains are flat on one end and rounded on the other, or rounded on both ends. Their length and width are 3-24,5 µm and 3-15 µm, respectively. Vascular bundles are scattered throughout the vascular cylinder. They are dense at the perimeter of the

vascular cylinder but sparse towards the center. Xylem surrounds phloem either completely (amphivasal) or U shaped (Fig. 5B, 6).

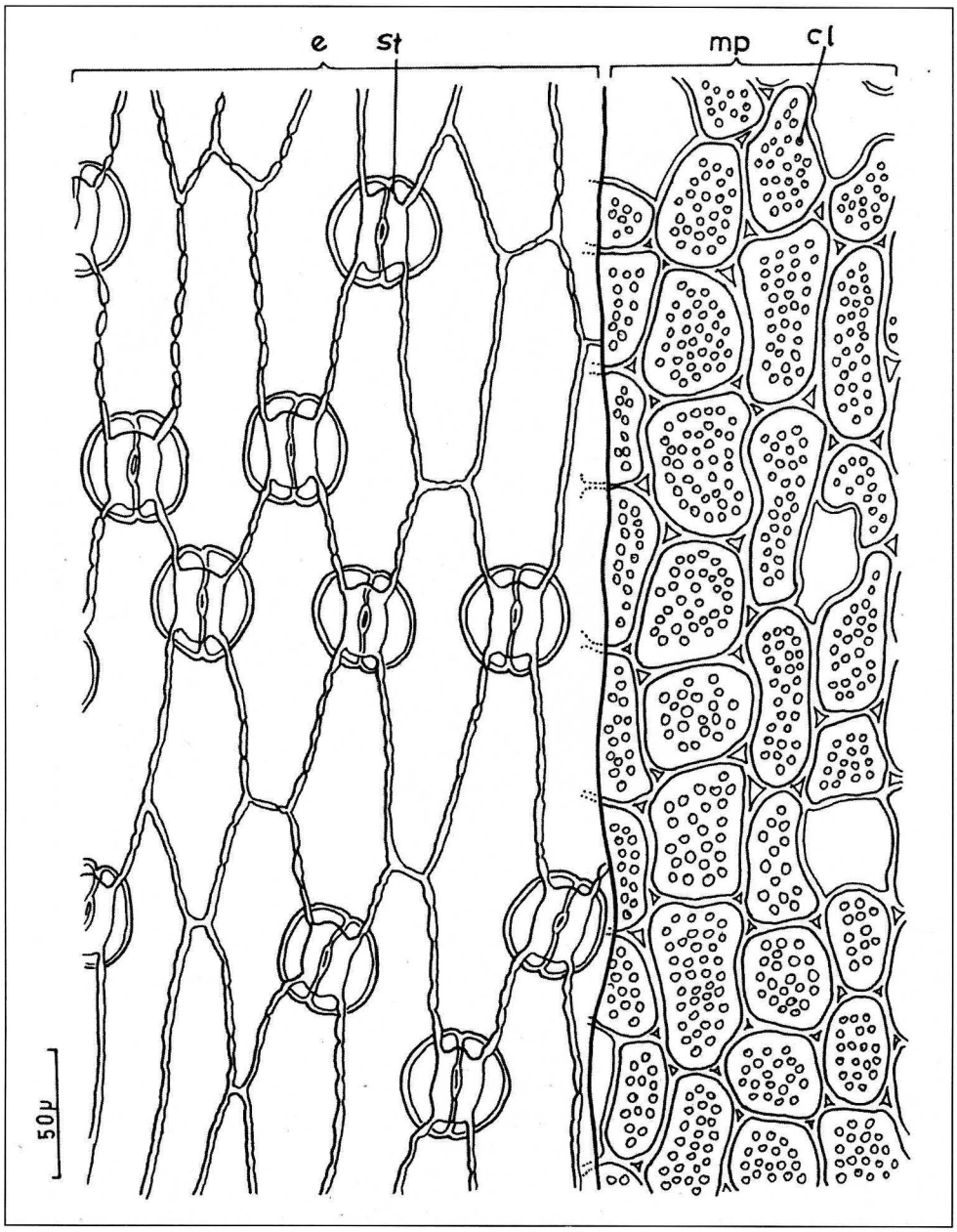

Fig. 3. *Iris pumila* (ISTE 49188), surface section of leaf blade: e. epidermis; st. stoma; mp. mesophyll parenchyma; cl. chloroplast.

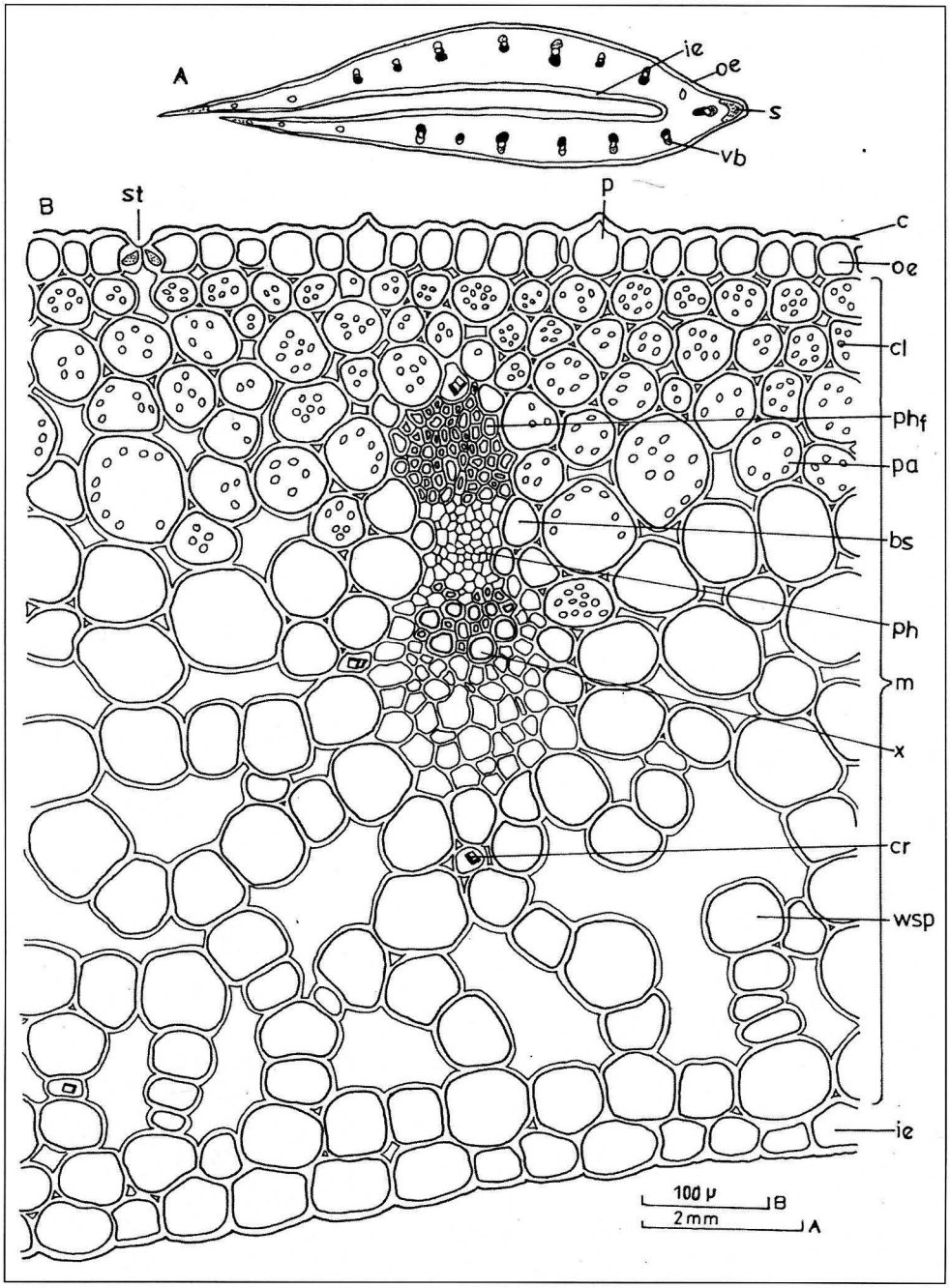

Fig. 4. *Iris pumila* (ISTE 49188), AB. cross sections of leaf sheath: ie. inner epidermis; vb. vascular bundle; s. sclerenchyma; oe. outer epidermis; c. cuticle; p. papilla; cl. chloroplast; pa. parenchyma; bs. bundle sheath; phf. phloem fibers; ph. phloem; m. mesophyll; x. xylem; cr. crystal; wsp. water storing parenchyma; st. stoma.

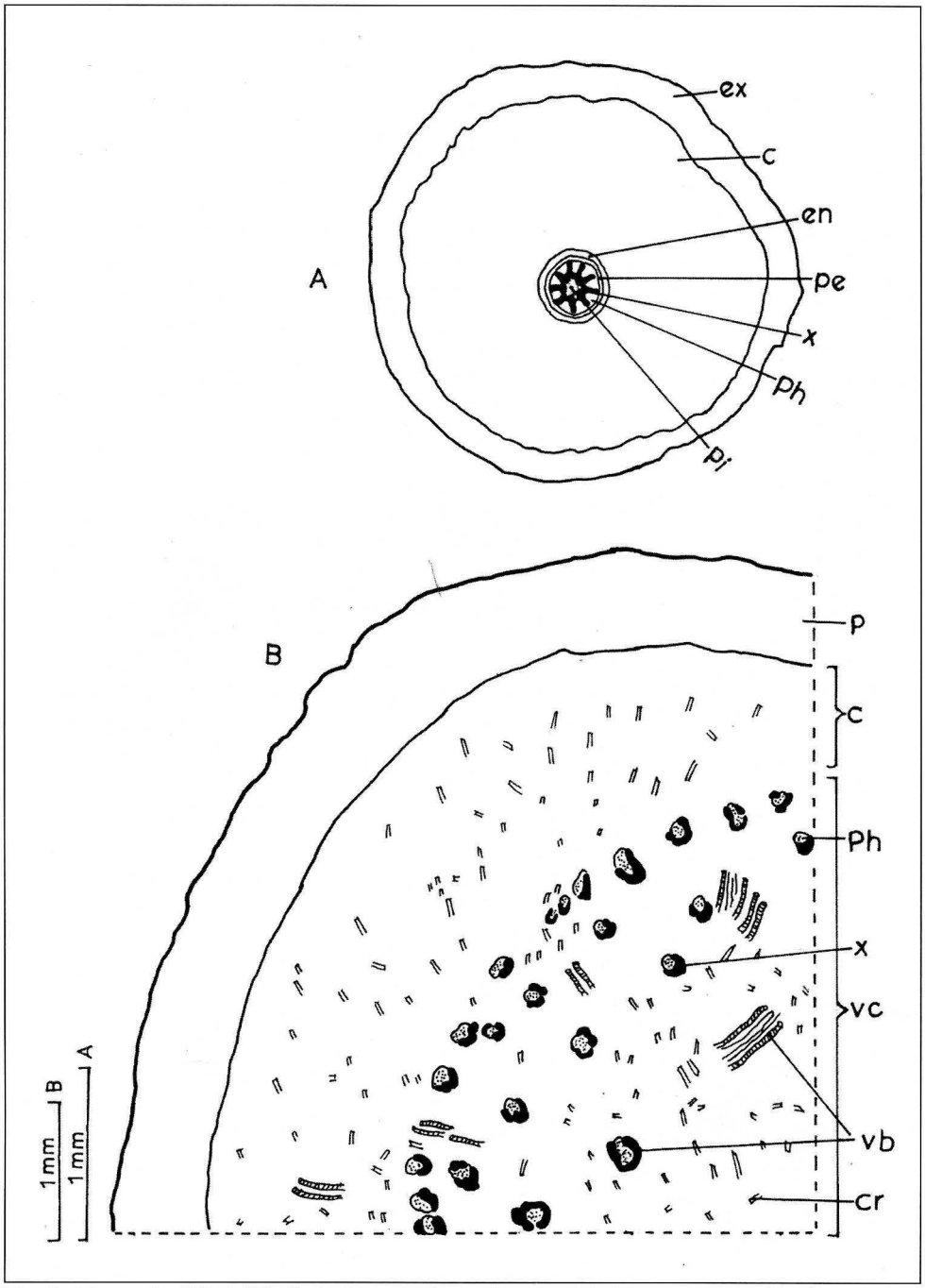

Fig. 5. *Iris pumila* (ISTE 49188): A. cross sections of root and B. rhizome: ex. exodermis; c. cortex; en. endodermis; pe. pericycle; x. xylem; ph. phloem; pi. pith; p. periderm; vb. vascular bundles; cr. crystal; vc. vascular cylinder.

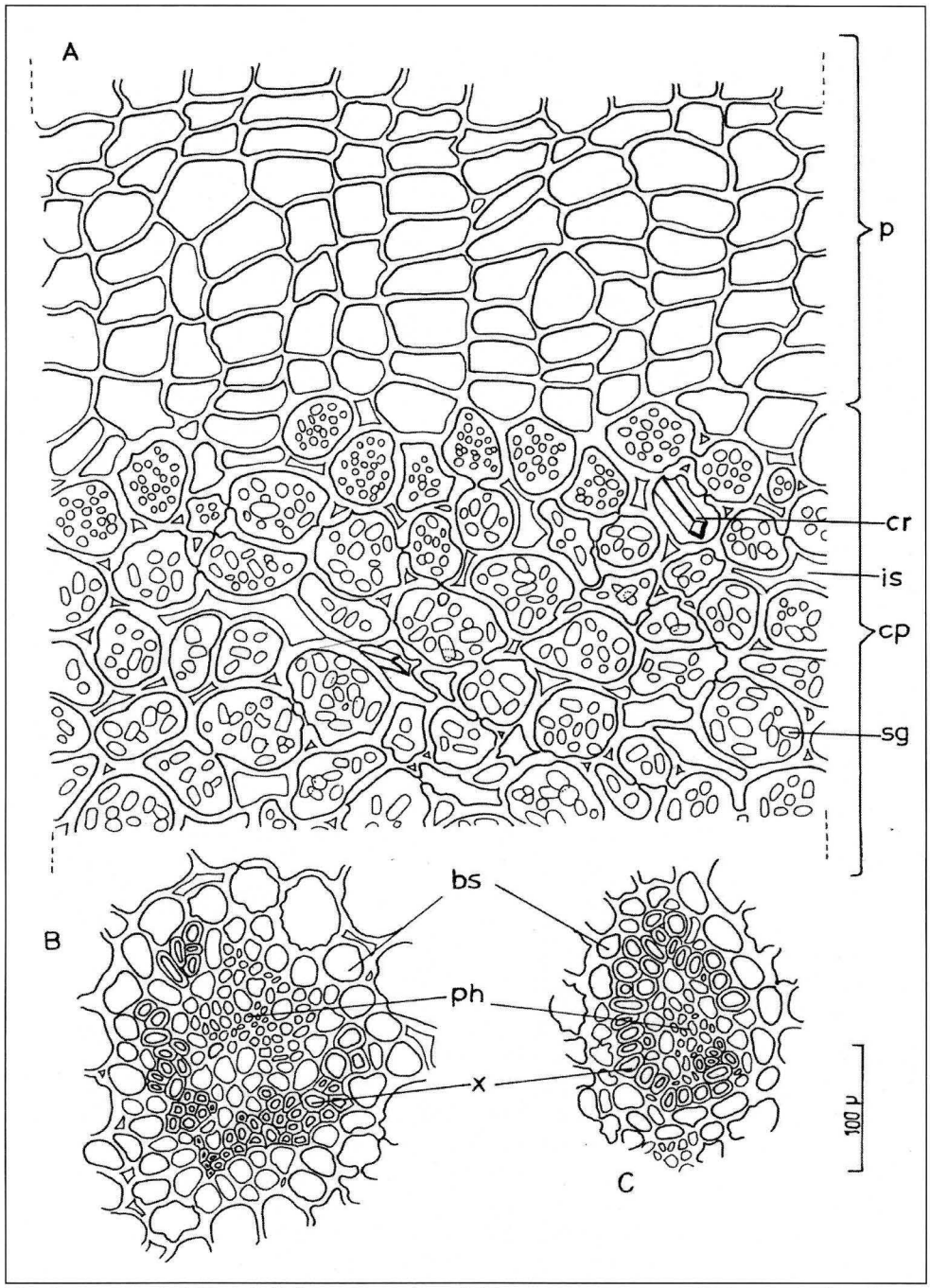

Fig. 6. *Iris pumila* (ISTE 49188), cross section of rhizome: A. outer zone; BC. vascular bundles; p. periderm; sg. starch grain; is. interselular space; cp. cortical parenchyma; cr. crystal; bs. bundle sheath; ph. phloem; x. xylem.

ROOT

In transverse sections, the root has epidermis on the tip zone and exodermis in 3-4 layers at the upper areas, exodermic cells are polygonal. The cortex consists of parenchymatic cells that are polygonal or circular with a thick wall. It contains abundant intercellular spaces and bears simple pits on the walls. In some cells, starch and prismatic crystals have been observed. The endodermis consists of a single layer of prominent cells whose radial and inner tangential walls are thickened. A single layer of cells with thin walls is positioned in pericycle. Vascular bundles are in radial rows, the xylem has 9-10 strands and the pith is sclerenchymatic (Fig. 5A, 7)

POLLEN

Besides the normally shaped, very big and very small pollen grains, malformed pollen grains have also been detected under microscopic observation. Normally shaped pollens are 20-115 µm, long and 15-105 µm wide. Their shapes are wide ellipsoid, longish ovoid or sometimes spherical, monocolpate. The colpus lies along the long axis and is depressed on one end (Fig. 8).

Karyological observations

The chromosome number of the samples that we examined was $2n = 36$. It has been observed that 4 long metacentric chromosomes were generally accompanied by a small metacentric chromosome, the remaining chromosomes being submetacentric, subtelocentric and telocentric. The number of chromosomes belonging to each of the above types was variable in the karyotype, and was found as follows: submetacentrics 2-6; subtelocentrics 11, 15, 16, 18, 20; telocentrics 8, 10, 11, 13, 15; 1 or 4-6 satellites have been seen on the short arms of subtelocentric and telocentric chromosomes. Only in one case a satellite on the short arm of a submetacentric chromosome was observed. Sometimes, a secondary constriction was observed on the long arm of one telocentric chromosome (Fig. 9).

I. pumila has been reported as having $2n = 32, 36$ by Simonet (1932), $2n = 32$ by Mitra (1956), Lovka & al. (1971) and Popova & Ceschmedjiev (1975), as well as $2n = 30, 31+f, 32$ by Randolph & Mitra (1959, 1961).

Discussion and Conclusion

According to Dykes (1913), Luscombe (1972, 1973, 1974), and Mathew (1981), *I. pumila* is a variable species. A lot of variant names in different taxonomic levels were mentioned in Luscombe's (1974) study. Our morphological findings are compatible with the results of the aforementioned studies. The morphological characteristics of the plant are: very short stem; ensiform leaves; green and keeled outer spathe; light green inner spathe: dry, scarious and tan colour at the top; it is rounded on the back and closely wraps the perigon tube; the stem has only one flower. The anatomy of its leaves is characterized by some xeromorphic properties such as its leaves being unifacial, the stomata being sunken, the presence of trichomes, the isolateral organization of the mesophyll, the

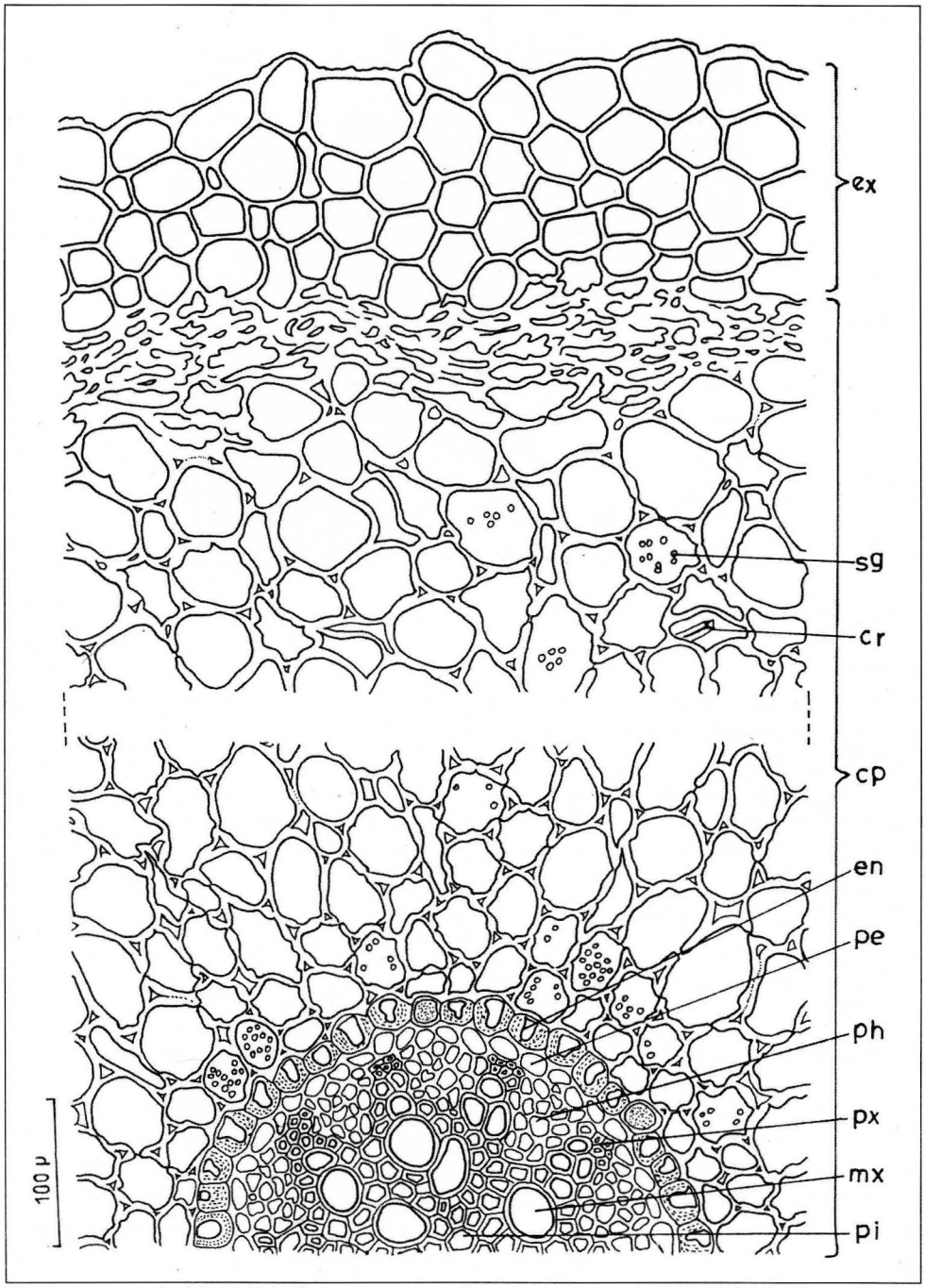

Fig. 7. *Iris pumila* (ISTE 49188), cross section of root: ex. exodermis; cp. cortical parenchyma; cr. crystal; sg. starch grain; en. endodermis; pe. pericycle; ph. phloem; px. protoxylem; mx. metaxylem; pi. pith.

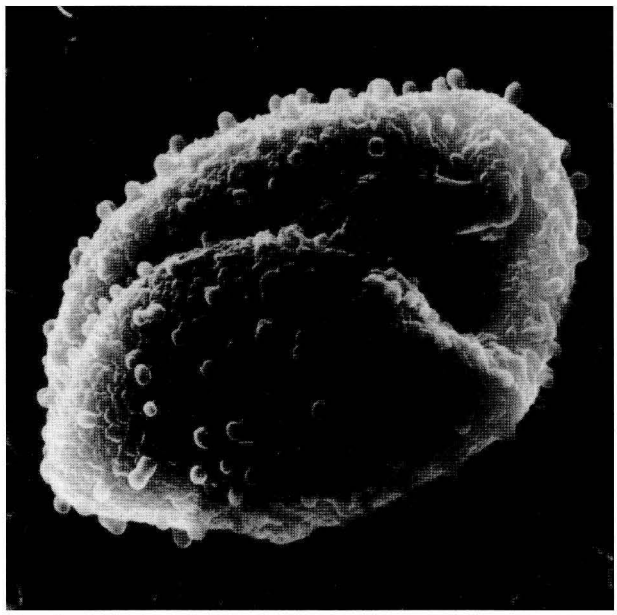

Fig. 8. Scanning electron micrograph of pollen grain of *Iris pumila* (x1800, ISTE 49188).

existence of water storing parenchyma and the development of strong fibres on vascular bundle, all of which are completely in accordance with the natural habitat of *I. pumila*. The root, rhizome and leaf anatomical characteristics of the samples, that we examined, are generally in agreement with the anatomical findings of Irises that belong to the same group (Kasaplıgil 1961; Koca 1982, 1996).

The karyotypic analysis of the samples showed that the number of the chromosomes was $2n = 36$ and the karyotype was determined to be heteromorphic. The number of submetacentric, subtelocentric and telocentric chromosomes was variable in the karyotype with the exception of 4 long metacentric chromosomes. This kind of variation has also been detected in other *Iris* species that the author has previously examined (Koca 1985, 1989). It is obvious that the differential shortening of the arms during the preparation has been caused the chromosomal variation in the karyotype. The metaphase chromosomes at the root tip that was pretreated with alpha-bromonaphthalene shrunk more and only one satellite could be observed in the karyotype (Fig. 9B). On the contrary, chromosome shortenings were not so intense and the satellites could be observed better, following pretreatment with a mixture of 8- hydroxyquinoline and alpha-bromonaphthalene in equal proportions (Fig. 9A).

The pollen features (malformed pollen grains together with normally shaped, very big and very small pollen grains) of the samples that we studied are those characterizing hybrid pollens (Aytuğ 1967). Karyotype results also support these findings.

Mitra (1956) conducted karyological studies on the samples that they collected from natural habitats, and stated that true *I. pumila* ($2n = 32$) was an amphidiploid of *I. attica* Boiss. & Heldr. ($2n = 16$) and *I. pseudopumila* Tineo. ($2n = 16$). Our karyotype analysis is

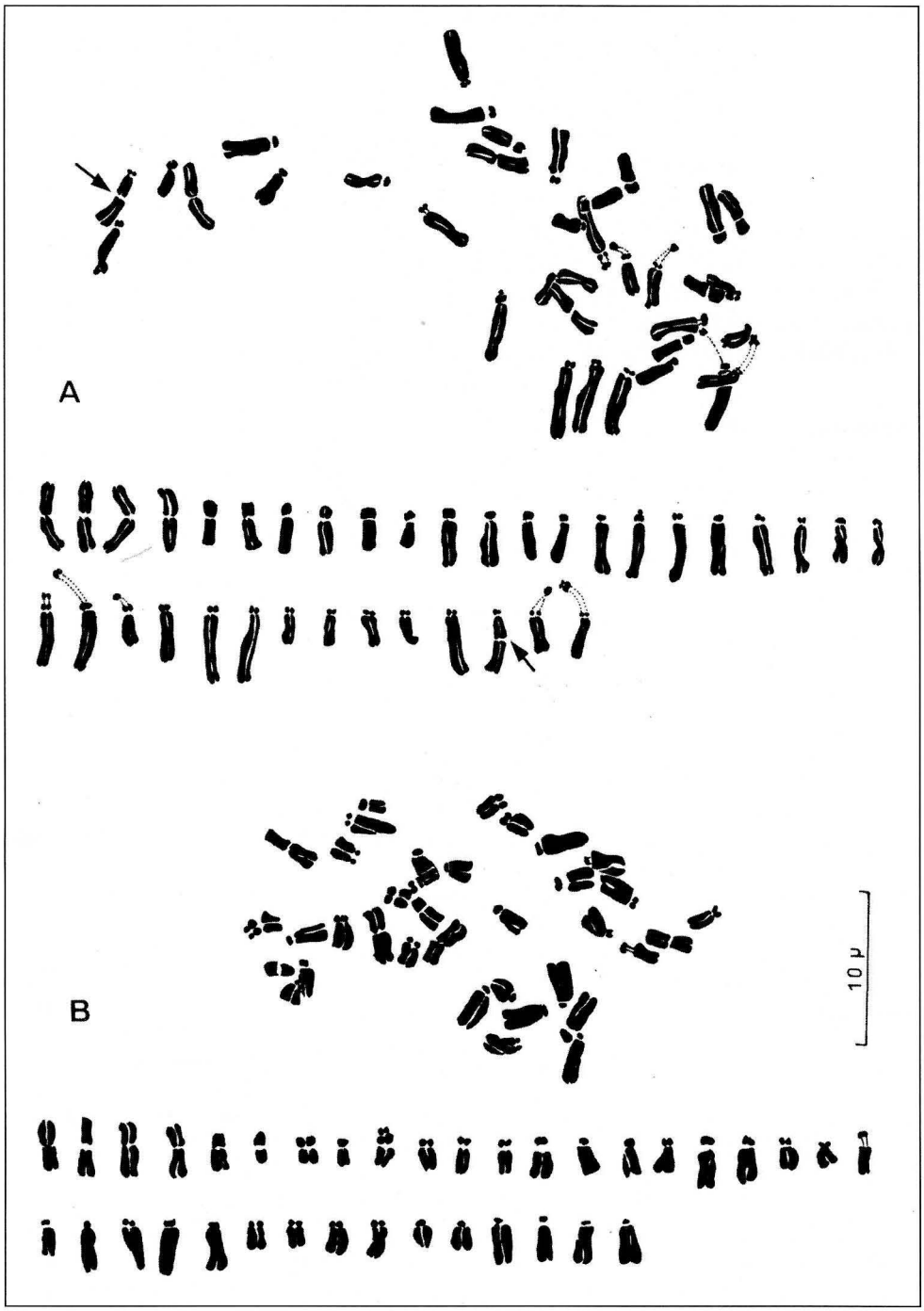

Fig. 9. A, B. katyotypes and karyograms of somatic metaphase of *Iris pumila* (ISTE 49188), $2n = 36$. - Arrow indicate secondary constriction.

not concordant with the results that Mitra (l.c.) reached. However, cytological studies on *I. pumila* var. *azurea* Hort, by Simonet (1932) identified the chromosome number as $2n = 36$, and also showed 4 V and 6 satellited chromosomes. These data are in accordance with our findings. Wanting to find the origin of this plant due to the fact that he had come across abnormal chromosome matching during metaphase, Simonet (1934) proceeded by crossbreeding *I. pumila* L. ($n = 16$) and *I. chamaeiris* Bert. ($n = 20$). By inspecting the hybrid offspring, he found the chromosome number as $2n = 36$, similar to *I. pumila* var. *azurea*. Based on these observations, the author asserts that *I. pumila* var. *azurea* is a hybrid of two dwarf taxa of the section *Pogoniris*.

What can be concluded from our study is that the samples that we analyzed belong to *I. pumila* var. *azurea,* which is a natural hybrid (Dykes 1913; Simonet 1932; Luscombe 1972; Warburton & Hamblen 1978) and quite prevalent in commercial culture.

Acknowledgements

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