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Morphological, anatomical and karyological investigations of the Turkish endemic species *Lathyrus woronowii* Bornm. (Fabaceae)

Fatma Güneş[1], Çiler Meriç[2]

[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, Trakya University, Edirne 22100, Turkey.
[2]Department of Biology, Faculty of Sciences, Trakya University, Edirne 22100, Turkey

Abstract – *Lathyrus woronowii* Bornm., an endemic species of Turkey, is threatened with extinction due to dam construction. It exists only in the Çoruh valley, Artvin. This annual species is in the critically endangered (CR) category according to the International Union of Conservation of Nature (IUCN) criteria. Its morphology, anatomy and karyology are studied here for the first time. A detailed description is given and the general appearance of the species has been drawn; cross sections from the stem and leaf have been taken and examined; and the diploid chromosome number (2n = 14) has been reported and illustrated for the first time.

Key words: anatomy, endemic, karyology, *Lathyrus*, morphology, Turkey.

Introduction

The genus *Lathyrus* L. (Fabaceae) is represented by 13 sections and about 187 subspecies worldwide (Allkin et al. 1983), most of which are annual or perennial plants, predominantly centred in the Mediterranean region (Kupicha 1983). In Turkey, *Lathyrus* is divided into 10 sections with 76 species, 26 of which are endemic (Davis 1970, Davis et al. 1988, Güneş and Özhatay 2000, Genç and Şahin 2011, Güneş and Çırpıcı 2012, Güneş 2014).

Lathyrus woronowii Bornm. belongs to the section *Orobastrum*, which contains eight species, two of which (*L. woronowii* and *L. tauricola* P. H. Davis) are endemic to Turkey. The main characteristics of this section are that they are annual; are procumbent to climbing; have angled or sometimes very narrowly winged stems; have tendrilous or aristate leaves with single to several-paired, linear to elliptic, parallel-veined leaflets; have peduncles that are single-flowered; and have legumes that are glabrous or pubescent, with the upper suture not winged. Only *L. saxatilis* (Vent.) Vis., *L. vinealis* Boiss. & Noë and *L. woronowii* have more than one pair of 2 or 3 leaflets, whereas all the other species in the section have only one pair. Leaves of *L. saxatilis* and *L. woronowii* are mucronate and shortly aristate, respectively, whereas all other species have leaves with simple tendrils, at least on the upper parts of the plant. The flower colour is creamy to yellow in *L. saxatilis* and *L. woronowii*; red in *L. vinealis*, *L. sphaericus* Retz. and *L. setifolius* L.; and lavender in *L. inconspicuus* L. and *L. tauricola*. Pods are hairy in *L. inconspicuus* and *L. setifolius* but glabrous in other *Lathyrus* species (Davis 1970).

Pollen morphology and seed morphology of the *Orobastrum* section have been investigated by Chernoff et al. (1992), Pastor-Cavada et al. (2011), Güneş (2011a, 2012, 2013) and Güneş and Çırpıcı (2011). It was previously reported that the pollen type is 3-zonocolporate and pollen shape is prolate in *L. woronowii* and that the shape is subprolate or spheroidal in eight other studied taxa. The pollen structure of all studied taxa has a tectate infrastructure and reticulate ornamentation; the seed shape is spheroidal in *L. woronowii* and subprolate in *L. vinealis* and *L. sphaericus*; and the seed surface is mostly smooth.

Some karyology studies were previously conducted within this genus, including the studies of Senn (1938), Ress and Hazarika (1969), Fouzdar and Tandon (1975), Yamamoto and Fujiwara (1984), Kuriyan and Narayan (1988), Kumar and Sinha (1989), Kakoli and Sumitra (1991), Şahin and Babaç (1995), Şahin et al. (2000), Klamt and Schifino-Wittmann (2000), Seijo and Fernandez (2003), Ayaz and Ertekin (2008), and Güneş (2011b). These studies indicated that the genus *Lathyrus* generally has a diploid chromosome number of 2n = 14, as reported in approximately 95 *Lathyrus* species worldwide. Tetraploid counts of 2n = 28 have been reported in *L. pratensis* L. (Darlington and Wylie 1955), *L. venosus* Sweet. (Başaran et al. 2007) and *L. brachypterus* (Güneş 2011b). Hexaploid counts of 2n = 42 have been reported in *L. palustris* L. subsp. *palustris* (Ball 1968, Güneş and Çırpıcı 2008). Several

* Corresponding author, e-mail: drgunes@gmail.com

studies were conducted on the species of the *Orobastrum* section in the Elâzığ area by Şahin and Altan (1990), in the Black Sea region by Özcan et al. (2006) and in the European region of Turkey by Güneş and Çırpıcı (2008). Karyotype analysis is an important tool for distinguishing controversial species, especially when there are very similar morphological characters, such as those in *L. spatulatus* Čel. and *L. elongatus* (Bornm.) Sirj. (Güneş 2011b).

There are several anatomical studies on *Lathyrus* species. *Lathyrus latifolius* L. was investigated for its morphological, anatomical and physiology characteristics by Krstic et al. (2002). The study by Çildir (2011) of the morphology, anatomy and systematics of the 25 taxa belonging to the genus *Lathyrus* in central Anatolia (Turkey) has been presented. In addition, there are a few studies on the anatomy of Turkish *Lathyrus* (Mantar et al. 2002, Mantar et al. 2003, Celep et al. 2011, Kahraman et al. 2014). Since there have been no previous detailed morphological, anatomical or karyological studies on the Turkish endemic *L. woronowii*, the goal of this study is to determine the morphological, anatomical and karyological properties of *L. woronowii*, which is under threat of extinction due to several dam construction projects in the Çoruh valley, and to use this information to aid in identification of the species and raise awareness about its threatened status.

Materials and methods

In 2009, 72 specimens of *Lathyrus woronowii* were collected from four different localities in Artvin Province of north-eastern Turkey during their flowering time between April and May. The specimens were deposited in the Herbarium of the Faculty of Pharmacy. Morphological observations and biometric measurements were made on fresh material and herbarium specimens. They were examined and drawn using an Olympus SZX7 microscope.

For anatomical studies, the material was stored in 70% alcohol. Transverse sections of the stems and leaves and the surface sections of leaves from fixed samples were made with a razor blade. Some sections were stained with alcian blue (Sigma) for pectic substances and others with safranin (Sigma) for lignin, using 20 µL alcian blue + 20 µL safranin in 10 mL of 25% glycerine for 4 hours. Stained and unstained sections were mounted in glycerine–gelatin to preserve the preparations, according to the methods of Jensen (1962). Slides were examined using an Olympus BH2 microscope fitted with a digital camera. Images were taken with a ProgRes C12 Plus digital camera.

For karyological studies, germinating root tips were collected and pre-treated in a saturated solution of α-bromonaphthalene for 24 hours at 4 °C and then fixed in Carnoy's solution. The root tips were hydrolysed in 1 N HCl for 10–20 minutes at 60 °C and stained with Feulgen solution. Semi-permanent slides were examined with a light microscope. Mitotic chromosomes were photographed on a Nikon E600 trinocular photomicroscope using a 100 × magnification oil immersion objective. Chromosome measurements were made using 4000 × magnified photographs of five well-spread metaphase plates. Short arm lengths (S) and long arm lengths (L) were measured, and the total length of the chromosomes (C), arm ratio (R = L/S), centromeric index (I) and relative length (R) were calculated. The classification of chromosomes by centromeric indices followed the methods of Levan et al. (1964).

Results

The habitat (Fig. 1) is given, while Tab. 1 shows the morphological features of *L. woronowii*. Previously, seed and pollen properties were studied by Güneş (2012, 2013). The general appearance and flower parts have been drawn (Fig. 2). Three-paired leaflets were observed in some specimens of *L. woronowii* in this study. Although peduncles were reported as being single-flowered in the Flora of Turkey (Davis 1970), 2-flowered peduncles have also been found here. The standard colour of *L. woronowii* is yellow when it is fresh, turning violet when pressed in herbarium specimens.

Stems are covered with a single-layered epidermis, with a 5–6-layered parenchymatic cortex underneath. The endodermis is not distinct. The central cylinder contains four units of collateral vascular bundles. The bundles have a sclerenchyma cap above the phloem. The vascular cambium is not distinct between the phloem and xylem. The pith is filled with parenchymatous cells (Fig. 3a). The leaves are covered with a single layer of epidermis. The mesophyll is bifacial and differentiated as palisade and spongy parenchyma. Un-

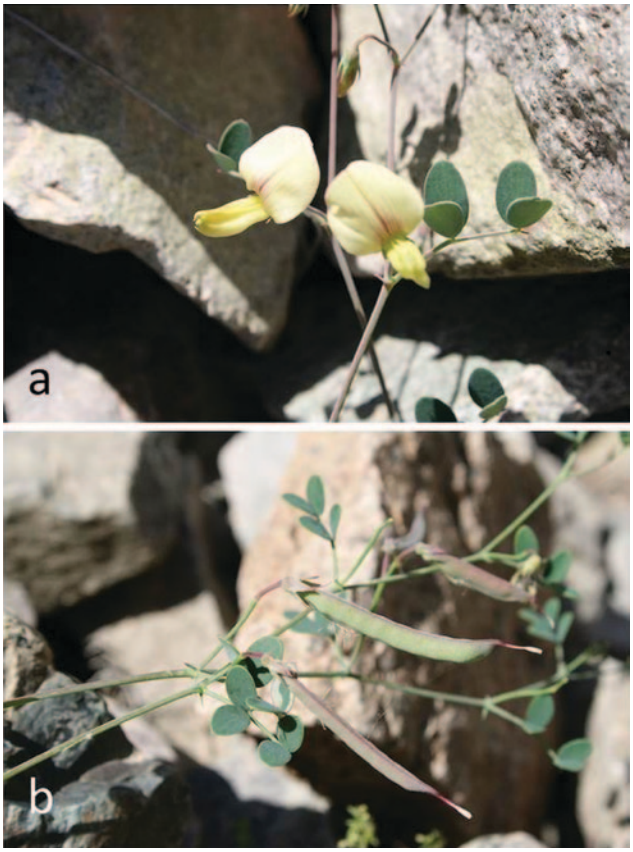

Fig. 1. Habit and general view of *Lathyrus woronowii*, (a) flower, and (b) pod (Güneş 2190).

Tab. 1. Morphological features of *Lathyrus woronowii.* P – polar axis, E – eguatorial diameter, M – median point, m – median region, sm – submedian region, st – subterminal region.

Features	P. H. Davis 1970	Our results
Habit	low, slender annual and glabrous	low, slender annual and glabrous
Stem	stems are ascending or procumbent	stems are ascending or procumbent
Stem length (cm)	8–18	8–57; some stems drying blackish
Stem width (mm)	not winged	0.5–1.5; angled
Leaves apex (mm)	shortly aristate	shortly aristate, 1–2
Leaflets, length and width (mm)	ovate-elliptic, the lowest often with 1-paired leaflets the remainder 2-paired, c. 12 × 5	ovate-elliptic, the lowest with 1-paired leaflets, the remainder 2 or 3-paired, 3-12 × 1–6
Vein type	unspecified	5–7 parallel-veined
Petiol length (mm)	unspecified	3–15
Stipules, length × width (mm)	lanceolate-subulate, semi-sagittate	lanceolate-subulate, semi-sagittate, 1–5 × 0.1
Peduncles (mm)	1-flowered, shorter than leaf	1 (–2) flowered, 4–15
Pedicel (mm)	unspecified	3–4
Calyx (mm)	c. 5 mm, glabrous	5–6 (–7) glabrous
Calyx tube (mm)	unspecified	2–2.5 (–3)
Calyx teeth (mm)	subequal, lanceolate, a little longer than the tube	subequal, lanceolate, upper teeth 3–4, the lowest 4–5
Flowers length (mm)	unspecified	8–14
Standard colour and length (mm)	standard violet	standard yellow with red veins, drying violet or purple, 9–14 × 8–12
Wings (mm)	wings cream	yellow, 11–13 × 3–5
Keel (mm)	unspecified	cream, 11–14 × 4–6
Style (mm)	unspecified	5–6 × c.1
Stigma (mm)	unspecified	hairy, enlarged
Legume, length × width (mm)	3.5 × 0.6 cm Altan (2001)	glabrous, reticulate nerved, upper sture not winged, 18–28 × (3–)4–4.5 (–5), with 6–10 seeds
Seeds, length × width [min. value (Mean) max. value (mm)], color and surface structure	unspecified	spheroidal (P/E = 1.04), smooth, pressed, 1.71 (2.21) 2.66 × 1.69 (2.13) 2.58, yellow-green tones and speckled (Güneş 2013)
Hilum length × width [min. value (Mean) max. value (mm)]	unspecified	0.32 (0.44) 0.55 × 0.18 (0.24) 0.33 (Güneş 2013)
Pollen shape length × width (µm)	unspecified	prolate (P/E = 1.36), P × E = 35.78 x 26.31 (Güneş 2012)
Chromosome number and type	unspecified	2n = 14; m, m, sm-st, M, sm, m-sm, m^{sat}
Flowering time	May	April – May
Habitat	schictone screes	schictone screes

Tab. 2. Chromosome characters of *Lathyrus woronowii.* MC – mean chromosome, MR – mean ratio, M – median point, m – median region, sm – submedian region, st – subterminal region

Chromosome pairs	Total length (C, µm)	Long arm (L, µm)	Short arm (S, µm)	Arm ratio R=L/S	Centromeric index (I)	Relative length (R)	Chromosome type
I	2.56	1.31	1.25	1.05	48.79	8.83	m
II	2.25	1.38	0.88	1.57	38.88	7.76	m
III	2.00	1.50	0.50	3.00	25.00	6.90	sm-st
IV	2.00	1.00	1.00	1.00	50.00	6.90	M
V	2.00	1.38	0.63	2.2	31.25	6.90	sm
VI	2.00	1.25	0.75	1.67	37.50	6.90	m-sm
VII	1.44	0.75	0.69	1.09	47.92	4.97	m
Average	MC=2.04			MR=1.65			

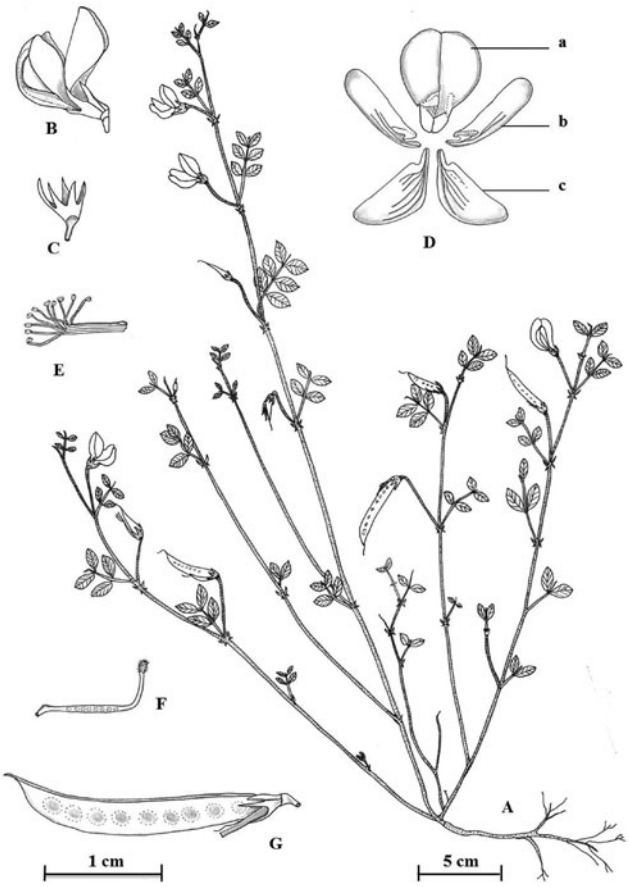

Fig. 2. *Lathyrus woronowii* schematic view: A) general habit, B) flower, C) calyx, D) corolla (a – standard, b – wing, c – keel), E) androecium, F) gynoecium and G) fruit. Author: F. Güneş (Güneş 2190, Güneş 2244).

der the upper epidermis, the mesophyll contains two layers of palisade cells, which are elongated cells. Under the lower epidermis, spongy parenchyma is composed of two layers of oval-shaped cells with intercellular spaces. The vascular bundles of leaves are collateral (Fig. 3b). The leaves are amphistomatic, and stomata are found along the upper and lower epidermis. The stomata index is 23.38 for the upper epidermis and 28.02 for the lower epidermis (Figs. 3c, d).

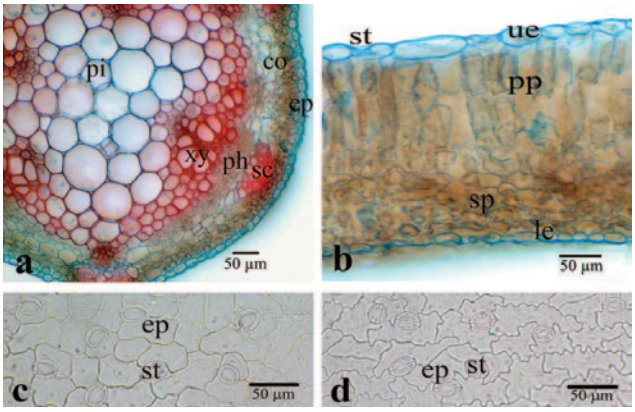

Fig. 3. Anatomical sections of *Lathyrus woronowii*. a) cross-section of stem; b) cross-section of leaf; c) upper surface section of leaf; d) lower surface section of leaf. (co – cortex, ep – epidermis, le – lower epidermis, ph – phloem, pi – pith, pp – palisade parenchyma, sc – sclerenchyma, sp – spongy parenchyma, st – stomata, ue – upper epidermis, xy – xylem.

The chromosome number was determined to be 2n = 14, and the detailed characteristics of chromosomes are shown in Tab. 2 and Fig. 4.

Discussion

Lathyrus woronowii is a critically endangered endemic species [CR C2a(i)] (IUCN 2001), restricted to the province of Artvin (A8 Çoruh valley) in north-eastern Turkey (Ekim et al. 2013). It is faced with the threat of flooding due to 15 dams having been planned along the Çoruh river. Its distribution area is approximately 30 km in diameter. Specimens of living plants and seeds are preserved in the Nezahat Gökyiğit Botanical Garden (NGBB) in İstanbul.

Altan (2001) reported *L. woronowii* stem length (60 cm) and pod size (3.5 × 0.6 cm). Our results supported the stem length (8–57 cm; Tab. 1) but not the pod size (18–28 × (3–) 4–4.5 (–5) mm).

The anatomical features of *L. woronowii* are reported for the first time in this study. Metcalfe and Chalk (1950) suggested that the presence or absence and structure of the

Table 3. Chromosome number and chromosome type of *Orobastrum*. M – median point, m – median region, sm – submedian region, st – subterminal region

Taxa	Chromosome number (2n)	Chromosome types	Total length of largest and smallest chromosome (µm)	Reference
L. saxatilis (Vent.) Vis.	14	sm,st^{sat},sm,sm,st,st,M	4.89 and 2.86	(Şahin and Altan 1990)
L. vinealis Boiss. & Noe	14	m^{sat},sm,sm,sm,sm,m,M	4.66 and 2.66	(Şahin and Altan 1990)
L. sphaericus Retz.	14	m,m,m,sm,m,m,m	6.92 and 4.68	(Genç and Şahin 2001)
		m,m^{sat},sm,m,m,m,sm	3.96 and 2.72	(Özcan et al. 2006)
		M,m,sm,m,sm,m,M	4.50 and 2.83	(Güneş and Çırpıcı 2008)
L. inconspicuus L.	14	M^{sat},sm,sm,m,sm,sm,m	4.44 and 2.64	(Şahin and Altan 1990)
		m^{sat},m,sm,m,sm,m,sm	4.66 and 3.28	(Genç and Şahin 2001)
L. tauricola P. H. Davis	14	m^{sat}, sm,m,sm,sm,m,m	6.06 and 3.98	(Genç and Şahin 2001)
L. setifolius L.	14	m^{sat},sm,st,sm,sm,m,M	6.87 and 2.66	(Şahin and Altan 1990)
		m^{sat},sm,sm,sm,sm,m,m	7.16 and 3.46	(Genç and Şahin 2001)
		m^{sat},sm,st,sm,sm,m,M	5.25 and 3.00	(Güneş and Çırpıcı 2008)

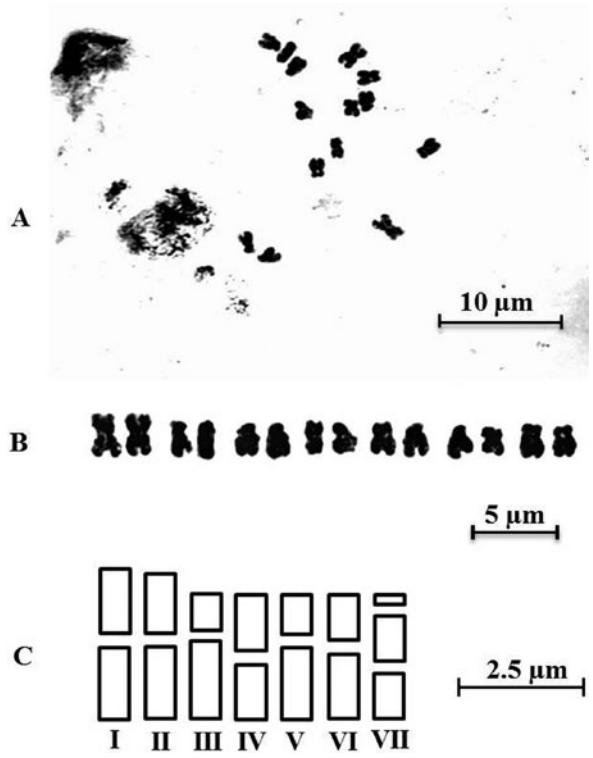

Fig. 4. Mitotic metaphase chromosomes (a), karyogram (b), and idiogram (c) of *Lathyrus woronowii* (Güneş 2244).

wings in the stem are taxonomically important in the distinction of *Lathyrus* species. The stem of *L. woronowii* has no wings like those of *L. cilicicus* Hayek & Siehe (Celep et al. 2011); *L. inconspicuus* L.; *L. vinealis* (Mantar et al. 2002); and *L. nissolia* L. (Kahraman et al. 2014). Stems of *L. maritimus* Bigel, *L. pratensis*, *L. sylvestris* L. (Metcalfe and Chalk 1950), *L. latifolius* L. (Krstic et al. 2002), *L. hirsutus* L. and *L. sativus* L. (Mantar et al. 2003) contain wings. Çildir (2011) reported that *L. incurvus* (Roth.) Willd., *L. cassius* Boiss., *L. cicera* L. and *L. chloranthus* Boiss. have winged stems, while stems of *L. aureus* (Steven) Brandza, *L. brachypterus*, *L. haussknechtii* Širj., *L. armenus* (Boiss & Huet) Širj., *L. digitatus* (M. Bieb.) Fiori, *L. tukhtensis* Czeczott, *L. spathulatus* Čelak., *L. pratensis*, *L. laxiflorus* (Desf.) O. Kuntze subsp. *laxiflorus*, *L. czeczottianus* Bässler, *L. roseus* Steven subsp. *roseus*, *L. tuberosus* L., *L. saxatilis*, *L. sphaericus*, *L. aphaca* L. var. *biflorus* Post and *L. aphaca* L. var. *affinis* (Guss.) Arc. contain no wings.

The pith is composed of large parenchyma cells in *L. woronowii.*, *L. aureus*, *L. armenus*, *L. laxiflorus* subsp. *laxiflorus*, *L. roseus* subsp. *roseus*, *L. cilicicus*, *L. maritimus* and *L. pratensis*, while *L. latifolius* stems are hollow at the centre. However, the stems of *L. incurvus*, *L. brachypterus*, *L. haussknechtii*, *L. digitatus*, *L. tukhtensis*, *L. spathulatus*, *L. pratensis*, *L. czeczottianus*, *L. tuberosus*, *L. cassius*, *L. hirsutus*, *L. chloranthus*, *L. sativus*, *L. cicera*, *L. saxatilis*, *L. inconspicuus* var. *inconspicuus*, *L. sphaericus*, *L. nissolia*, *L. aphaca* var. *biflorus* and *L. aphaca* var. *affinis* are hollow (Metcalfe and Chalk 1950, Krstic et al. 2002, Mantar et al. 2002, 2003, Celep et al. 2011, Çildir 2011, Kahraman et al. 2014). *L. woronowii* has amphistomatic leaflets, like those of *L. incurvus*, *L. pratensis*, *L. czeczottianus*, *L. sphaericus*, *L. cassius*, *L. cicera*, *L. latifolius* and *L. cilicicus*, whereas *L. roseus* subsp. *roseus* and *L. laxiflorus* subsp. *laxiflorus* have hypostomatic leaves (Krstic et al. 2002, Celep et al. 2011, Çildir 2011).

The chromosome measurements of *Lathyrus* species belonging to section *Orobastrum* are shown in Tab. 3. The largest and smallest chromosomes of *L. woronowii* were measured as 2.56 and 1.44 µm, respectively. The total lengths of the largest and smallest chromosomes of other species belonging to the *Orobastrum* section were stated by the authors. In this section, *L. woronowii* has the smallest chromosome set. The largest set belongs to *L. setifolius*, and the chromosomal numbers of *Lathyrus* species in the present study (Tab. 3) agree with previous records (Şahin and Altan 1990, Genç and Şahin 2001, Özcan et al. 2006, Güneş and Çırpıcı 2008). According to Ress and Hazarika (1969); Yamamoto et al. (1984); and Güneş and Çırpıcı (2008), the perennial species of the genus *Lathyrus* have larger chromosomes in comparison with those of the annual species.

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