



# Use of morphological and anatomical characters to delimit varieties of *Paliurus spina-christi* Mill. (*Rhamnaceae*)

Uso de caracteres morfológicos y anatómicos para delimitar variedades de *Paliurus spina-christi* Mill. (*Rhamnaceae*)

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## SCIENTIFIC RESEARCH

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*Paliurus spina-christi* L. var. *spina-christi*, *Paliurus spina-christi* var. *macrocarpa* Beck., Isobilateral mesophyll, leaf anatomy, Petiole anatomy, Cuticular structure.

## ABSTRACT

**Introduction:** *Paliurus spina-christi* Mill is a species with two varieties namely *Paliurus spina-christi* L. var. *spina-christi* and *Paliurus spina-christi* var. *macrocarpa* Beck native to mountains of Kurdistan, rarely growing in the upper plains of northern Iraq. **Materials and Methods:** A total of 15 plants from different parts of Kurdistan region were sampled. 30 measurements for each characters of Leaf, Inflorescence, Flowers, Fruit, seeds, and leaf anatomical characters were measured for comparison between the two varieties. **Results and Discussion:** Fruit diameter of var. *macrocarpa* is significantly larger than fruit diameter of var. *spina-christi*. Most mean flower parts especially the ovary, in addition to seed size is larger in var. *macrocarpa*. Upper epidermal layer is always thicker than the lower epidermal layer for the same blade. The isobilateral mesophyll includes 2 layers of long palisade cells adaxially and 2-3 layers of short cells abaxially. Many vascular bundles are seen equally spaced in the mesophyll tissue. With the exception of the presence of simple hairs on the petiole adaxial side of the var. *spina-christi* and the absence of these hairs from the same site of var. *macrocarpa*, all other anatomical features are considered to be of no taxonomic application. Stomatal density of the adaxial leaf side is up to 19 stomata per mm<sup>2</sup> in var. *spina-christi* while it is up to 38 stomata per mm<sup>2</sup> in var. *macrocarpa*. **Conclusions:** Both fruit diameter and the stomatal density of the adaxial leaf side constitute diagnostic characters for separating between the two varieties of *Paliurus spina-christi* Mill.

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## INTRODUCTION

*Paliurus* Mill., according to Townsend and Guest <sup>(1)</sup> is a genus of 6 species; only *Paliurus spina-chriti* Mill. is present natively to Kurdistan region. The species is so widely distributed in different physiographical region of northern Iraq as to recognize two varieties namely *Paliurus spina-chriti* var. *spina-chriti* with the fruits, as indicated by Townsend and Guest <sup>(1)</sup> less than 2.5mm in diameter and *P. spina-chriti* var. *macrocarpa* Beck. With the fruits diameter between 2.5-3.2 cm. The extant genus *Paliurus* from the Miocene of East China is divided by Xiangchuan et al., <sup>(2)</sup> into *P. ramosissimus* and *P. spina-christi* groups based on analyses of the present and previous fossil fruit records. According to the researchers, diversification of *Paliurus* had begun in northern transpacific regions by the Late Eocene.

The Germplasm Resources Information Network (GRIN) recognizes 9 species. In their revision of Rhamnaceae, <sup>(3)</sup> indicated that the genus of *Paliurus* in China consist of: *P. hirsutus*, *P. ramosissimus* (Lour.) Poir., *P. spina-christi* Mill., *P. orientalis* (Franch.) Hemsl., and *P. hemsleyanus* Reh. ex Schir. and Ola. For Basinger and Dilcher <sup>(4)</sup>, the Rhamnaceae appears to be an old lineage because a rhamnaceous flower has been found dating back to 94-96 million years ago. For Manchester <sup>(5)</sup>, the fossil records of *Paliurus*, based mostly on leaves and fruit characters, are very soundly identified in tertiary flora, and are significant in understanding the biogeographic affinities of North American tertiary flora.

The *Paliurus* flowers, like other Rhamnaceae flowers, are antipetalous (obhaplostemony) and possess tendency towards xeromorphism. Obhaplostemony is considered a relatively rare character in angiosperms, and this has resulted in associating Rhamnaceae with other families such as Vitaceae and Cornaceae which exhibit the same arrangement. The xeromorphic of adaptations to aridity exhibited by some members

of the Rhamnaceae consist of reduction or absence of leaves, crowding of leaves, shortening of branch axes, presence of thorns or spines, and a low, shrubby habit.

Literature on the leaf anatomy of our native species *P. spina-christi* is not available on the level of Iraq, and is rare even universally. Wood anatomy has been more investigated <sup>(6)</sup>. On the other hand, Literature is too much on the leaf anatomy of the closely related species of the genus *Zizyphus* Mill. Investigation of *Z. nummularia* (Burm.F.) and *Z. spina-christi* L. leaves shows anatomical characters of xerophyte plants such as high frequently of papilla on both leaf surfaces, epidermis with thick cuticle and stomata sunken in crypts <sup>(7)</sup>. In contrary, *Z. jujube* Mill has superficial stomata and simple papilla which is rarely observed on the abaxial surface of the leaf midrib, moreover, epidermis consists of one layer of large cells. Stomata are anisocytic. Leaf isobilateral mesophyll includes 3-4 layers of long palisade cells adaxially and 2-3 layers of short cells on abaxial surface. Each vascular bundle is collateral and surrounded by parenchymatous bundle sheath <sup>(8)</sup>.

*Paliurus spina-chriti* is commonly distributed in mountains of Kurdistan, very rarely in the upper plains of northern Iraq. Often grows in the lower forest zone, in degraded woodland, mostly near streams and other water sources, up to 1500 m altitude <sup>(1)</sup>. Plants are widely used for hedging. In traditional medicine, the plant is usually effective as a hyposcholesterolemic, antihypertensive, astringent, and tonic, diuretic.

The aim of this study is to provide a detailed description and to present more accurate and distinct diagnostic characters for distinguishing the two infra specific taxa of *C. tournefortii* var. *spina-christi* and *C. tournefortii* var. *macrocarpa* using morphological and anatomical traits.

## MATERIALS AND METHOD

Field expeditions were started in 2014, continued during 2015 with the aim of collecting *Paliurus spina-christi* throughout Kurdistan region. Selection was preliminary based on previous literature (1,9), herbarium specimens deposited in different Iraqi herbaria, indigenous information and the conspicuous presence of the target species. 10 field trips were conducted each more lasted from 1-5 days. A total of 15 plants from different parts of Kurdistan region were sampled. An equal number of herbarium specimens were prepared and deposited in the herbarium of the College of Agriculture/ University of Duhok (DPUH) (table 1). Thirty measurements were taken for each of the following characters:

### Leaf:

Blade length, Blade width, Blade length/Blade width,

Petiole length, Shape of blade apex, Shape of blade base. Petiole length, diameter, color, shape, leaf indumentum.

### Inflorescence:

Inflorescence type, length, width, length/width ratio, color, number of flowers/inflorescence, peduncle length, peduncle width (at the mid-point).

### Flowers:

Calyx, corolla, stamens (number, shape, color, dimensions, indumentum); pistile: ovary, style, stigma (shape, color, dimension, number, indumentums), pedicel length, receptacle shape and dimension.

### Fruit and seeds:

Fruit length, width, length/ width ratio, thickness. Seed length, width, thickness.

**Table 1-a.** Specimens from Iraq Herbaria Used in the Study, Abbreviation According to Holmgren and Keuken (10).

Taxon	Herbarium	Collected position	Collector	Altitude (m)	Date of Collection	Specimen Number
<i>Paliurus spina-christi</i> var. <i>spina-christi</i>	BAG	Khurda Look village (Dukan)	A. Rawi	740	14/6/1957	21788
	BAG	Bikhair mountain	A. Rawi	800	27/5/1957	22966
	BAG	Armotah village (Koi Sangak)	S. Omer & F. Karim	775	17/4/1970	37254
	BAG	Shaqlawa	F. Karim		13/5/1970	37516
	BAG	Aqra	A. Rawi	1000	31/5/1948	11373
	BAG	Sarchinar	J. B. Gillett	800	16/4/1947	7687
	BAG	Tagaran Stream		866	9/9/2014	14-2559
	SUAH	Hawar	Saman A.	922	21/5/2012	12-555
<i>Paliurus spina-christi</i> var. <i>microcarpa</i>	BAG	Between Shaqlawa & Harir	S. Omer		11/6/1970	37623
	BAG	Ain Terma (Shaqlawa)	Sahira		17/7/1970	37573
	BAG	Jindian	Evan Guest	700	24/8/1930	13003
	BAG	Hauraman mountain	Chak, Rawi and Nuri		26/6/1961	19716
	BAG	Safin Dgh	F. Karim		11/7/1972	39392

National Herbarium of Iraq - Baghdad (BAG).

Sulaimaniy University Agriculture Herbarium (SUAH).

**Table 1-b.** Herbarium specimens deposited in Duhok University Herbarium (DPUH).

Taxon	Collected position	Altitude (m)	Longitude	Latitude	Date of Collection	Specimen Number
<i>Paliurus spina-christi</i> <i>var. spina-christi</i>	Zawita	972	43.136941 E	36.889674 N	14/5/2014	3592
	Gali Ali Beg	570	44.451229 E	36.626912 N	3/5/2015	3594
	Zarda Bi village	856	45.528717 E	35.689578 N	4/10/2015	3593
<i>Paliurus spina-christi</i> <i>var. macrocarpa</i>	Zawita	955	43.136492 E	36.889566 N	23/5/2015	3599
	Bana Nok (Shaqlawa)	968	44.353415 E	36.386672 N	5/10/2015	3600

### Leaf anatomy

Mature leaves were collected from naturally growing plants of *Paliurus spina-christii*. Five leaves from each 15 trees were obtained. Samples for anatomical investigation were prepared according to Fathoulaah (11) and Saeed (12):

1. Killing and Fixation using FAA solution.
2. Washing and Dehydration by ascending concentration of ethanol.
3. Clearing and Infiltration by placing samples in a mixture of absolute alcohol and xylene and then in a mixture of melted paraffin and xylene.
4. Embedding and Mounting.
5. Removing of wax and staining
6. Staining Procedure.

### Cuticular Structure

Mature leaves from trees or shrubs were selected for light microscope measurements. Samples were dehydrated using ethyl alcohol 90%, then stored in 70% ethanol until to uses, and subsequently leaves of each taxon were sampled randomly from ethanol, washed in distilled water, dried, then immersed in glacial acetic acid and hydrogen peroxide (1:1 volumes), left in oven at 60 °C for 20-40 hours, depending on the species. Adaxial and abaxial peelings from the macerated leaves were stained with safranin–glycerin jelly, mounted on microscopic slides, covered by slides. The following measurements were recorded, average of 25 observations for each:

1. Abaxial and adaxial epidermal cell dimensions.

2. Epidermal cell density = number of epidermal cells / mm<sup>2</sup>.
3. Stomatal dimensions.
4. Stomatal density = number of stomata / mm<sup>2</sup>.
5. Stomatal index % = {stomata density / (stomata density + epidermal cell density)} \*100.
6. Summary statistics (mean, range, and standard deviation) were calculated for each character.

## RESULTS AND DISCUSSION

*Paliurus spina-christi* Mill., Gard. Divt. Ed. 8 (176); Nab. in Publ. Fac. Sci. Univ. Masaryk. 35: 63 (1923); Fl. Pal. Ed. 2, 1: 288(1932); Guest in Dep. Agr. Iraq Bull. 27: 70 1933); Bornm. In Beih. Bot. Centralbl. 57B: 249(137); Blackklock in Kew Bull. 3: 410(1948); Grubov in Fl. U. R. S.S. 14: 636 (1949); Zoh. in Dep. Agr. Iraq Bull. 31: 103(1950); Rawi in Dep. Agr. Iraq Tech. Bull. 4: 42 (1964); Fl. Turkey 2:523 (1967); Nouv. Fl. Syr. 2: 554 (1970).

*Rhamnus paliurus* L. Sp. Pl. ed. 1: 194 (1753).

*Paliurus australis* Gaertn., Fruct. 1: 203, t. 43, f. 5 (1758).

*Paliurus aculeatus* Lam., Tabl. Encycl. III. 2: 347, no. 611, t 210, f. 2 (1793); Fl. Orient. 2: 12 (1872).

Plants are shrubs or small trees. Branches zigzagged. Leaves are alternate, somewhat distichous, leathery, ovate, and 3-nerved from the base, entire, weakly serrulate or crenate margin, petiolate. Stipules are spinous, dimorphic. Flowers are hermaphrodite, in axillary cymes. Calyx 4-5(6-), with a poculiform tube and deltoid lobes, deciduous. Petals 4-5, inserted with the stamens on the disk. Ovary half immersed in the disk, 3 locular; styles 2-3. Fruits dray woody

drupe centered in a circular wing, up to 12.08- 36.17 mm.

*Paliurus spina-christi* L. **var.** *spina-christi*

Deciduous shrubs or small trees 2.5-4 m height, diameter at ground level 1.75-6.20 cm, width 1-2.65 m. Lower bark smooth, dark brown sometime gray. Twigs are slender, zigzagged or flexuose, reddish-brown, smooth, spreading. Crown is open. Buds are small 1.10-2.51 mm length, 1.04-2.31mm width, globular, brown, puberulent, densely wooly, bud scale shape ovate, outer surface hairy, inner side hairy or glabrous. Leaves are 0.801-7.20cm long, and 0.501-4.50cm wide, texture sub-coriaceous. Leaf shape is ovate to obliquely ovate, ad axially glossy green, abaxially dark green. Blade apex is obtuse, acute or acuminate; base oblique-rounded' margins entire, weakly serrulate or crenate; blade length/ blade width ratio 0.577 – 4.28. Venation is 3-nerved from the base. Leaf blade is 0.650-6.85 cm long, 0.501 - 4.50 cm wide. Petiole length is 0.150-1.80cm, rounded. Leaf length / Petiole length ratio is 3.12 - 19.20. Spine is straight, 0.639-1.569cm long, recurve, 0.316-0.670cm wide, dark brown, 2 on the outside of each knot, dimorphic, one straight and the other recurve and hooked. Inflorescences are yellowish cymes, born laterally, 0.801-2.21 cm long, and 1.20-2.31 cm wide. Flowers are actinomorphic, bisexual, small yellowish. Peduncle is 0.114-0.314 cm length and 0.057-0.085 cm diameter. Pedicel with 0.228-0.957 cm length, 0.028-0.057 cm width. Ovary with 0.065-0.146 cm length, 0.051-0.123 cm width, greenish-yellow, half-immersed in the disk, 3-locular. Style is 0.061-0.115 cm long, 0.015-0.025 cm wide, greenish-yellow, 2-3 lingual elongate with structure bubbly form. Stigma with 0.015-0.038 cm diameter, greenish-yellow, globular. Petal is 4-5, 0.095-0.193 cm long, 0.061-0.115 cm wide, yellow, ovate-spathulate more delicate, inserted with the stamens on the disk. Sepal is 4-5(-6), 0.153-0.264 cm long, and 0.143-0.261 cm wide, greenish-yellow, broadly deltoid-ovate, glabrous, firm. Filament is 0.085-0.153 cm long, and 0.015-0.023 cm wide, and greenish-yellow, oblate. Another is 0.045-0.053 cm long and 0.030-0.045 cm wide, yellow, oblate. Bract is 0.115-0.385 cm long and 0.021-0.096 cm wide, greenish, deltoid, hairy tapering to a point, margin serrate. Number of stamens is 4-6 free. Fruits are dry, indehiscent, depressed subglobose drupe with broad circular wing, light brown, glabrous, 12.08-24.32 mm diameter.

Peduncle with 2.21-8.06 mm length and 0.481-1.05 mm diameter. Pedicel with 12.09-19.16 mm length and 0.414-0.585 mm width. Stone (endocarp) with 6.56-8.85 mm length and 5.74-7.98 mm width, and brown, globular, smooth. Seed with 3.81-5.48 mm length and 3.34-4.83 mm width, 1.80-2.23 mm thickness, and flattened, dark brown, smooth. (Figure 1).

*Paliurus spina-christi* **var.** *macrocarpa* Beck. in Fedde Rep. 17:451(192).

Deciduous shrubs or small trees 2.5-3.7 m height, diameter at ground level 1.90-6.52 cm, width 1-2.5 m. Lower bark smooth, dark brown sometime gray. Twigs are slender, zigzagged or flexuose, reddish-brown, smooth, spreading. Crown is open. Buds are small 1.51-3.59 mm long and 1.38-3.51 mm wide, globular, brown, puberulent, and densely wooly, bud scale shape ovate, outer surface hairy, inner side hairy or glabrous, and sharpened. Leaves with 3.01 - 7.23 cm length, and 1.20-4.40 cm width, texture sub-coriaceous. Leaf shape ovate to obliquely ovate, adaxial leaf color glossy green, abaxial leaf color dark green, leaf blade apex obtuse, acute or acuminate, leaf blade base oblique-rounded, leaf blade entire, weakly serrulate or crenate; blade length/blade width ratio is 0.577 – 4.28 . Venation is 3-nerved from the base. Leaf blade is 2.40 - 6.50 cm long and 1.20- 4.40 cm wide. Petiole length is 0.501 - 1.82 cm, rounded. Leaf length / Petiole length ratio is 3.12 - 19.20. Spines are 2 on the outside of each knot, dimorphic, one is straight, 0.710-2.06 cm long, and the other is recurve and hooked, 0.310-0.804 cm long. Inflorescences are yellowish cymes, born laterally, 1.12-2.42cm long and 1.31-2.63 cm wide. Flowers are actinomorphic, bisexual, small yellowish. Peduncle is 0.214-0.471 cm length and 0.071-0.131 cm diameter. Pedicel is with 0.285-1.18 cm length and 0.043-0.071 cm width. Ovary is 0.116-0.177 cm long and 0.101-0.176 cm width, greenish-yellow, half-immersed in the disk, 3-locular. Style is 0.077-0.123 cm long and 0.030-0.046 cm wide, greenish-yellow, 2-3 lingual elongate with structure bubbly form. Stigma is 0.034-0.048 cm diameter, greenish-yellow, globular. Petal is 4-5, 0.192-0.246 cm long and 0.107-0.138 cm wide, yellow, ovate-spathulate more delicate, inserted with the stamens on the disk. Sepals are 4-5(-6) in number, 0.207-0.276 cm long and 0.207-0.272 cm wide, greenish-yellow, broadly deltoid-ovate, glabrous, firm. Filament is 0.131-0.192 cm long and 0.015-0.038 cm wide, greenish-yellow, oblate. Anther is 0.053-0.069 cm long and 0.038-0.057 cm wide, yellow, oblate. Bract is 0.181-0.231

cm long and 0.038-0.092 cm wide, greenish, deltoid, hairy, tapering to a point, margin serrate. Stamens are free, 4-6 in number. Fruits are dry, indehiscent, depressed subglobose drupe with broad circular wing, light brown, glabrous, with 25.29-36.17 mm diameter. Peduncle is 1.13-7.12 mm long and 0.562-1.13 mm diameter. Pedicel is 12.06-18.11 mm long and 0.571-0.785 mm wide. Stone (endocarp) is 8.32-10.48 mm long and 7.62-9.31 mm wide, brown, globular, smooth. Seed is 5.01-5.37 mm long, 3.95-4.60 mm wide, and 2.07-2.25 mm thick, flattened, dark brown, smooth. (Figure 2).

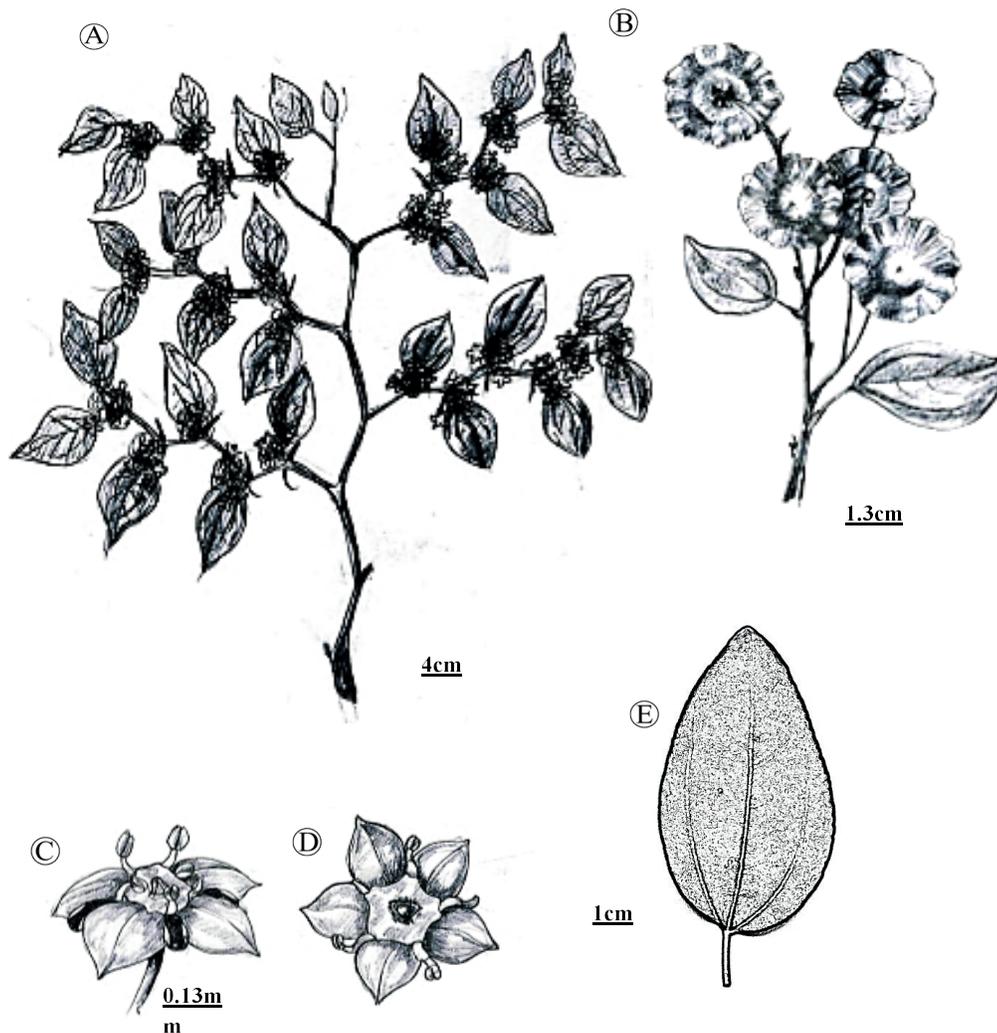
**Key to the varieties based on fruits and seeds and leaves:**

*P. spina-christi* var. *macrocarpa*: Fruits 2.52 - 3.61 cm in diameter, seed mean size up to 5.2 x 4.16 x 2.15mm.  
*P. spina-christi* var. *spina-christi*: Fruits 1.20 - 2.43 cm in diameter, seed mean size up to 4.57 x 3.97 x 2.02 mm x 6.85 cm.

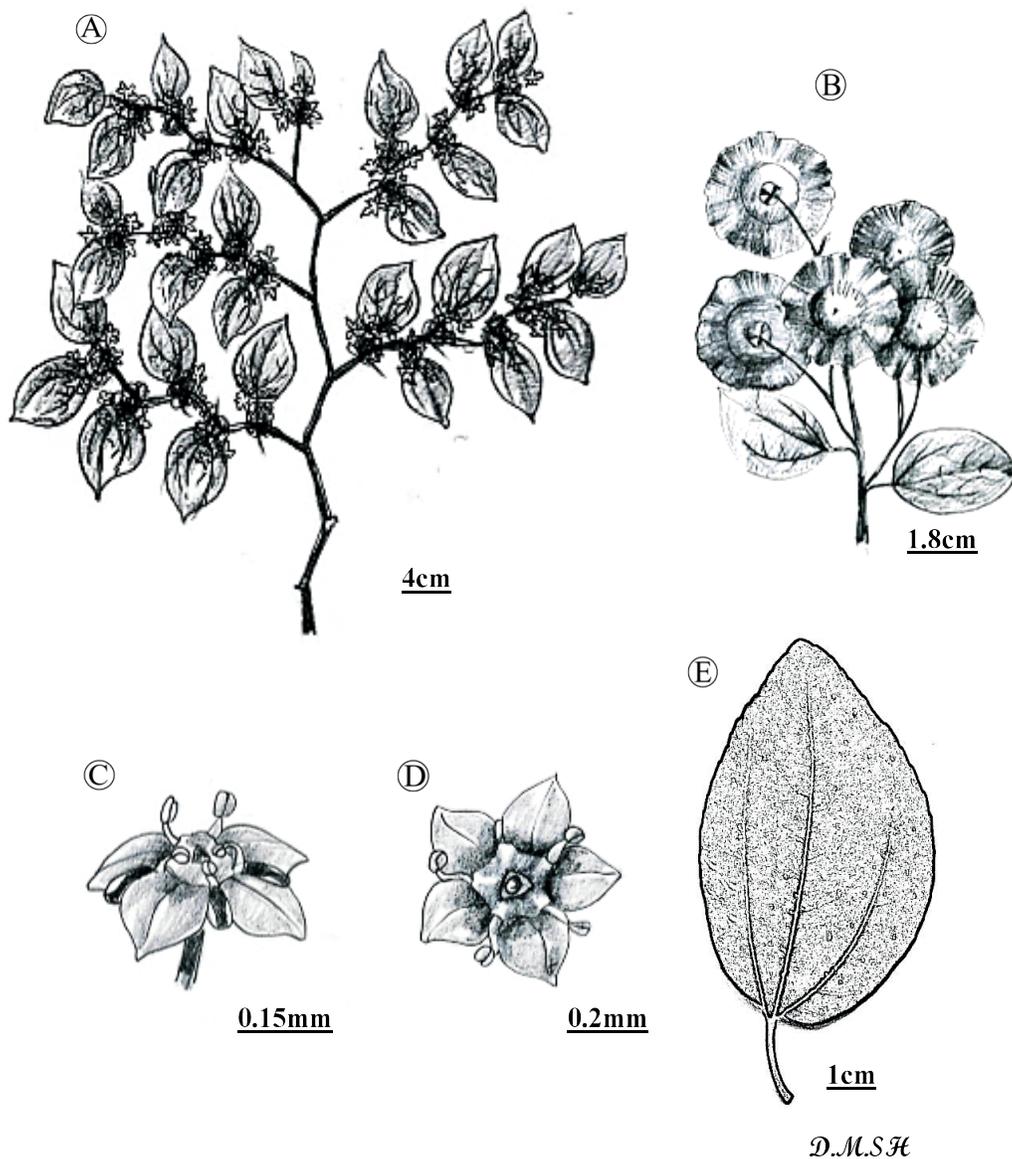
**Key to the varieties based on flower parts:**

*P. spina-christi* var. *macrocarpa*: Ovaries relatively large up to 0.76 x 0.77 cm in size, styles up to 0.123 cm long, and filaments up to 0.192 cm long.

*P. spina-christi* var. *spina-christi*: Ovaries relatively small up to 0.123 x 0.146 cm in size, styles up to 0.115 cm long, and filaments up to 0.153 cm long.



**Figure 1.** *Paliurus spina-christi* var. *spina-christi*: (A) Flowering twig, (B) Fruiting twig, (C, D) Bisexual flower, (E) Leaf.



**Figure 2.** *Paliurus spina-christi* var. *macrocarpa*: (A) Flowering twig, (B) Fruiting twig, (C, D) Bisexual flower, (E) Leaf.

### Anatomical characteristics

#### *Paliurus spina-christi*:

Epidermis consists of one layer of highly variable large cells, polygonal or circular in shape with thick outer wall and thick cuticle on both abaxial and adaxial surfaces. Stomata as seen in cross section are in the same level of the epidermis or slightly sunken. Upper epidermal layer is always thicker than the lower epidermal layer for the same blade. Leaves are isobilateral or unifacial (having the same structure on both sides of the leaf). Isobilateral mesophyll in-

cludes 2 layers of long palisade cells adaxially and 2-3 layers of short cells on abaxial surface. Many vascular bundles are seen equally spaced in the mesophyll tissue (Figures 3).

Midrib is little raised from the adaxial side, semicircle in the abaxial side. The vascular bundle is surrounded by a distinct parenchyma bundle sheath (border parenchyma). Two distinct thick patches of collenchyma just beneath the epidermis (26.53 – 43.64µm), one is at the adaxial and the other at the abaxial face leaving the two sides of the blade without the collen-

chyma tissue. Vascular bundles are collateral cupped by the sclerenchyma and arranged in u-shape with the xylem facing the adaxial side and the phloem the abaxial side (Figure 4).

Secretory canals are common feature in the parenchymatous tissue of midribs (Figures 3, 4, 5), main leaf vein and petioles of this species showing great fluctuation in number and size, thus providing insignificant taxonomic value. For Liu *et al.*,<sup>(13)</sup> secretory structures in plants decrease in density with development and growth of stems and leaves.

Petiole epidermis also includes one layer of cells covered by cuticle. Outline at the midpoint is semi-spherical in shape convexes in abaxial side, flat or slightly concaves in adaxial side, becomes more or less elliptic at places nearer to leaf base (Figure 5). Cortex includes layers of sub epidermal collenchyma (61.24 -101.22 $\mu$ m) followed by parenchyma layer tissue of variable width (95.78 - 158.48 $\mu$ m) (Figure 2). Secretory canals are distinct and distributed in the cortical parenchyma of the petiole. The anatomical characteristics of petiole in this species go along with their midrib and the main veins characteristics. Like midrib the vascular bundles are also u-shaped with the xylem towards adaxial and the phloem towards the abaxial face and the latter is strengthened by a well-developed sclerenchyma tissue in the same direction.

Druses are the only crystals found present in the mesophyll of *P. spina-christi* leaves.

*Paliurus spina-christi* var. *spina-christi* and *P. spina-christi* var. *macrocarpa*

Leaf anatomical features show no considerable differences between the two varieties of *P. spina-christi*. Data shown in table (2) reveals high overlapping in measured quantitative characters, no spacial distance occurs. Therefore characters are considered merely variation within the species of no taxonomic importance. The presence of simple non glandular hairs concentrated on the adaxial side of the *P. spina-christi* var. *spina-christi* petiole and the absence of these hairs from the same site of *P. spina-christi* var. *macrocarpa* petiole may represent the only character of taxo-

nomie application.

### Key to the varieties based on the petiole indumentum:

*P. spina-christi* var. *spina-christi*: Simple non glandular hairs present on the adaxial side of the petiole.

*P. spina-christi* var. *macrocarpa*: Simple non glandular hairs not present on the adaxial side of the petiole.

### Cuticular structure

#### Characteristics of the two varieties

Differences between var. *spina-christi* and var. *macrocarpa* in shape and anticlinal walls of normal epidermal cells are not distinguishable. Data displayed in table (3) shows no significant differences in cell sizes between the two varieties in both the adaxial and abaxial faces. Normal epidermal cells are significantly higher in density at the abaxial side than cells at the adaxial side, but these differences between the two varieties are insignificant because of the high data overlapping and the lack of spatial distance.

As a mean value, stomata of var. *macrocarpa* are considerably larger in size than stomata of var. *spina-christi* (table 4), but no differences are evident in stomata shape and subsidiary cells arrangement at both the abaxial and adaxial sides. Stomatal density, especially at the adaxial face may provide an important diagnostic character for separating var. *spina-christi* from var. *macrocarpa*. Number of stomata per mm<sup>2</sup> is much higher in leaves of var. *macrocarpa* than in var. *spina-christi*.

### Key to the varieties based on stomatal density and size:

*P. spina-christi* var. *spina-christi*: Stomatal density of the adaxial leaf side up to 19 stomata per mm<sup>2</sup>, adaxial and abaxial stomatal size 9.91– 24.20 x 8.20–25.79  $\mu$ m are smaller.

*P. spina-christi* var. *macrocarpa*: Stomatal density of the adaxial leaf side up to 38 stomata per mm<sup>2</sup>, adaxial and abaxial stomatal size 13.19– 34.45 x 9.54 –31.09  $\mu$ m are larger.

**Table 2.** Quantitative Characters of Cells and Tissues in Cross Section of Leaf Blade, Mid-rib and Leaf Petiole of *Paliurus spina-christi* ( $\mu\text{m}$ ).

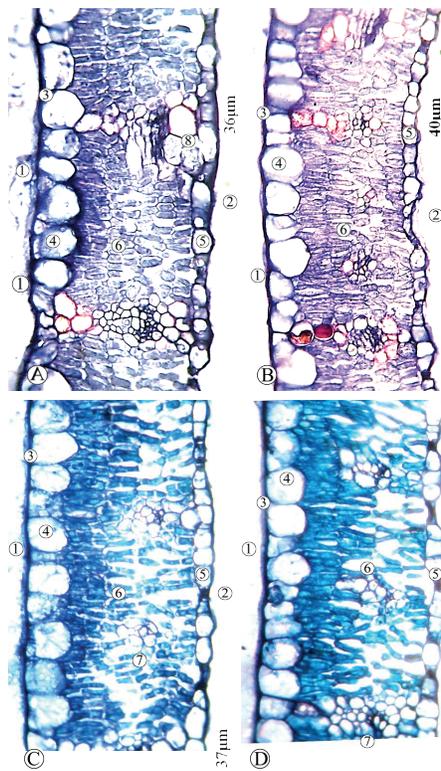
Character		<i>Paliurus spina-christi</i> var. <i>spina-christi</i>	<i>Paliurus spina-christi</i> var. <i>macrocarpa</i>
Leaf thickness	Mean	162.78	163.25
	Range	153.17 – 175.18	143.11 – 188.87
	STD	6.80	14.58
Upper cuticle thickness	Mean	2.44	2.86
	Range	1.98 – 3.02	1.55 – 3.96
	STD	0.333	0.561
Lower cuticle thickness	Mean	1.71	2.01
	Range	1.15 – 2.41	1.06 – 2.78
	STD	0.376	0.491
Upper epidermis thickness	Mean	29.86	26.84
	Range	25.86 – 33.50	25.40 – 28.14
	STD	2.61	1.02
Lower epidermis thickness	Mean	15.72	15.21
	Range	14.31 – 17.66	12.03 -17.55
	STD	0.914	1.80
Palisade parenchyma height, first layer	Mean	28.40	29.96
	Range	23.61 - 37.80	21.46 – 37.21
	STD	3.37	4.50
Palisade parenchyma, height second layer	Mean	23.27	25.67
	Range	18.38 - 29.02	19.73 – 30.51
	STD	2.54	3.38
Palisade parenchyma, height last layer	Mean	15.44	17.04
	Range	11.35 -20.06	14.64 – 21.25
	STD	2.08	1.96
Palisade parenchyma one cell width	Mean	5.15	5.10
	Range	3.79 – 6.34	3.03 – 7.94
	STD	0.665	0.924
<b>Mid-rib</b>			
Epidermis thickness	Mean	7.74	6.37
	Range	6.72 – 8.44	5.60 – 7.12
	STD	0.491	0.412
Cortex thickness	Mean	125.83	134.85
	Range	96.41 – 158.49	102.30 – 166.92
	STD	20.49	20.18
Collenchyma thickness	Mean	34.85	37.15
	Range	26.53 – 43.64	31.37 – 42.49
	STD	5.83	3.64
Parenchyma Thickness	Mean	81.63	86.75
	Range	62.41 – 105.97	57.12 – 115.73

**Continue Table 2.** Quantitative Characters of Cells and Tissues in Cross Section of Leaf Blade, Mid-rib and Leaf Petiole of *Paliurus spina-christi* ( $\mu\text{m}$ ).

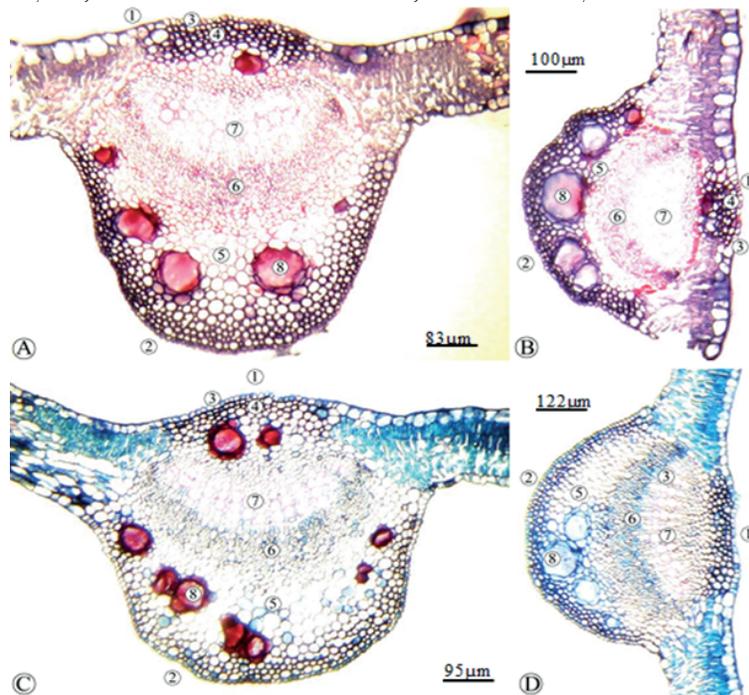
Character	<i>Paliurus spina-christi</i> var. <i>spina-christi</i>		<i>Paliurus spina-christi</i> var. <i>macrocarpa</i>	
	Mean	Range	Mean	Range
Fiber layer thickness	Mean	37.09	37.55	
	Range	30.65 – 43.32	32.36 – 41.13	
	STD	3.91	2.80	
Sclerenchyma Thickness	Mean	68.16	70.25	
	Range	61.58 – 74.46	59.71 – 78.91	
	STD	4.44	5.90	
vascular tissue thickness	Mean	107.63	125.29	
	Range	84.23 – 126.49	108.47 – 156.66	
	STD	14.93	14.12	
Pith thickness	Mean	33.36	44.65	
	Range	25.32 – 40.79	24.19 – 62.98	
	STD	5.21	10.64	
<b>Petiole</b>				
Epidermis Thickness	Mean	8.81	7.97	
	Range	4.83 – 11.42	6.07 – 10.95	
	STD	1.51	1.11	
Cortex thickness	Mean	186.17	228.86	
	Range	162.16 – 225.63	208.17 – 279.12	
	STD	17.86	21.82	
Collenchyma thickness	Mean	68.55	77.81	
	Range	61.24 – 76.93	71.03 – 87.39	
	STD	4.58	5.70	
Parenchyma Thickness	Mean	108.57	127.75	
	Range	95.78 – 135.74	112.05 – 158.48	
	STD	12.94	14.08	
Fiber layer thickness	Mean	47.75	44.26	
	Range	41.75 – 55.18	38.62 – 53.47	
	STD	4.13	2.89	
Sclerenchyma thickness	Mean	60.56	61.28	
	Range	47.51 – 74.47	50.15 – 77.51	
	STD	8.11	8.12	
Vascular tissue thickness	Mean	122.13	108.77	
	Range	101.16 – 139.69	88.11 – 122.41	
	STD	10.85	11.80	
Pith thickness	Mean	105.57	97.61	
	Range	79.60 – 131.37	90.17 – 102.45	
	STD	18.70	4.71	

**Table 3.** Dimension of the Epidermal Cells ( $\mu\text{m}$ ), Stomatal density (stomata per  $\text{mm}^2$ ), and stomatal index.

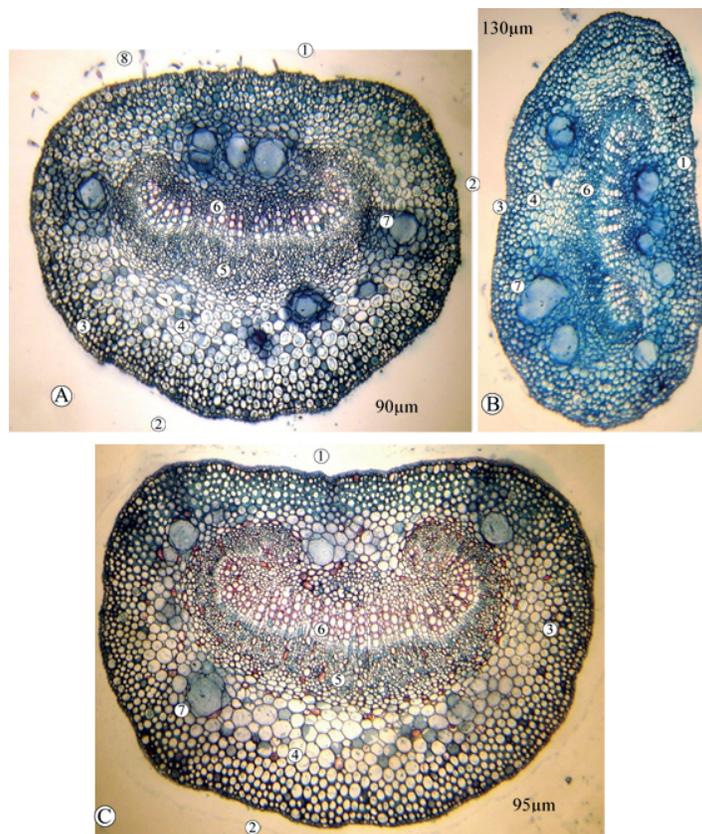
Character			<i>Paliurus spina-christi</i> var. <i>spina-christi</i>	<i>Paliurus spina-christi</i> var. <i>macrocarpa</i>	
Adaxial	Epidermis cell	Mean	33.71	29.43	
		Length	Range	21.33 – 51.80	14.31 – 42.02
			STD	6.58	6.66
			Mean	22.46	18.20
		Width	Range	12.58 – 33.13	12.40 – 24.19
			STD	4.24	2.78
			Mean	1843.16	1854.08
		Density/ $\text{mm}^2$	Range	1684 – 1981	1696 – 1984
			STD	76.54	83.10
	Mean		21.10	25.92	
	Stomata	Length	Range	16.68 – 24.20	21.49 – 34.45
			STD	2.61	3.43
			Mean	14.02	18.45
		Width	Range	9.91 – 17.71	13.19 – 25.41
			STD	2.31	2.71
			Mean	9.28	24.96
		Density/ $\text{mm}^2$	Range	4 – 19	17 – 38
			STD	4.08	6.49
			Mean	0.501	1.33
	Index	Range	0.207 – 1.06	0.849 – 2.16	
		STD	0.222	0.359	
Abaxial	Epidermis cell	Mean	24.26	26.42	
		Length	Range	17.25 – 32.07	16.48 – 35.14
			STD	4.02	4.71
			Mean	16.10	15.89
		Width	Range	9.24 – 24.88	11.22 – 21.82
			STD	2.61	2.82
			Mean	2556.56	2464.44
		Density/ $\text{mm}^2$	Range	2375 – 2756	2286 – 2734
			STD	100.62	139.52
	Mean		19.38	24.87	
	Stomata	Length	Range	11.32 – 25.79	15.36 – 31.09
			STD	3.15	3.48
			Mean	12.20	12.81
		Width	Range	8.20 – 17.48	9.54 – 18.81
			STD	2.33	1.94
			Mean	296.56	340.88
		Density/ $\text{mm}^2$	Range	241 – 375	283 – 394
			STD	38.71	31.37
			Mean	10.38	12.14
	Index	Range	8.90 – 13.43	10.71 – 13.44	
		STD	1.25	0.779	



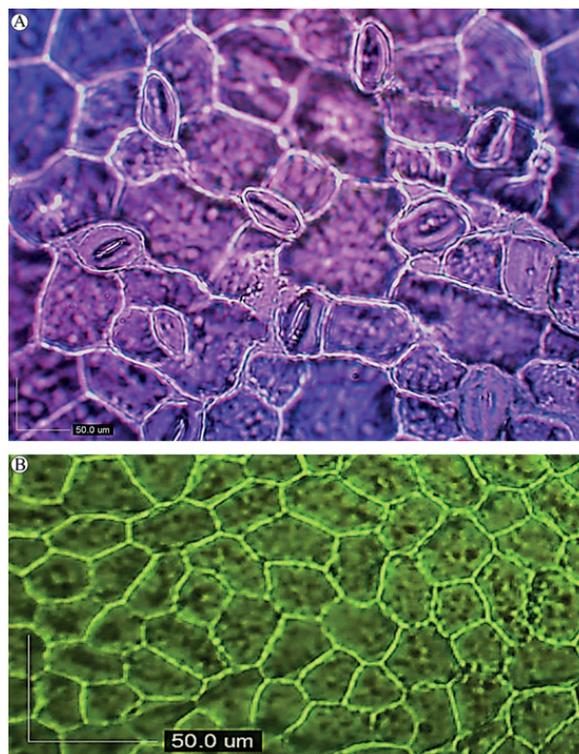
**Figure 3.** Leaf blade cross section. (A-B) *Paliurus spina-christi* var. *spina-christi*, (C-D) *Paliurus spina-christi* var. *macrocarpa*: 1. Adaxial face, 2. Abaxial face, 3. Cuticle layer, 4. Upper epidermal layer, 5. Lower epidermal layer, 6. Palisade parenchyma, 7. Veinlet vascular bundle, 8. Druses crystal.



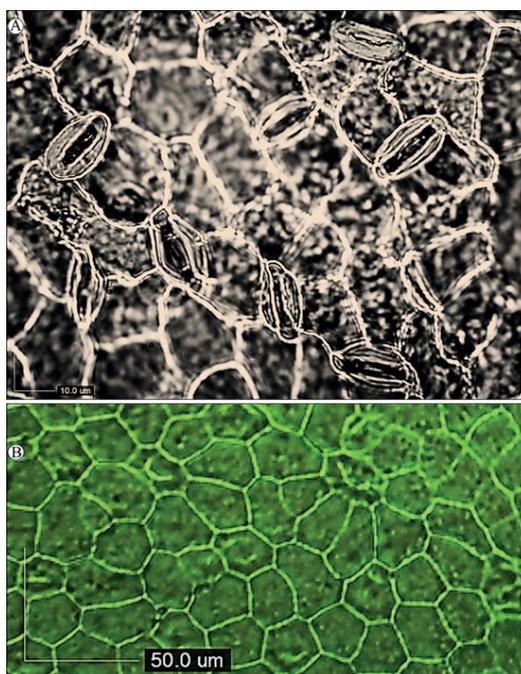
**Figure 4.** (A-B) *Paliurus spina-christi* var. *spina-christi*, (A) Midrib cross section (B) Main vein cross section, (C-D) *Paliurus spina-christi* var. *ls.*



**Figure 5.** (A-B) *Paliurus spina-christi* var. *spina-christi*, (A) Petiole cross section (B) Petiole cross section near leaf base, (C) Leaf petiole cross section of *Paliurus spina-christi* var. *macrocarpa*: 1. Adaxial face, 2. Abaxial face, 3. Collenchyma, 4. Parenchyma, 5. Sclerenchyma, 6. Vascular bundle, 7. Secretory canals, 8. Unicellular hair.



**Figure 6.** Leaf cuticular structure of *Paliurus spina-christi* var. *spina-christi*: (A) Abaxial epidermis, (B) Adaxial epidermis.



**Figure 7.** Leaf cuticular structure of *Paliurus Spina-christi* var. *macrocarpa*: (C) Abaxial epidermis, (D) Adaxial epidermis.

## REFERENCES

1. Townsend C C, and Guest E (Eds.). Flora of Iraq. Ministry of Agriculture and Agrarian Reform, Baghdad-Iraq. 1980, Vol.4.
2. Xiangchuan L I, Wang H., Leng Q., Xiao L., Guo J., Wenlong H E. *Paliurus* (Paliureae, Rhamnaceae) from the Miocene of East China and its Macrofossil-based Phylogenetic and Phytogeographical History. *Acta Geologica Sinica (English Edition)* 2014; 88, (5): 1364-1377. <https://doi.org/10.1111/1755-6724.12304>
3. Chen Yi-ling and Chou Pan-kai. Rhamnaceae. In: Chen Yi-ling, ed., Fl. Reipubl. *Popularis Sin.* 1982; 48(1): i–vi, 1–169.
4. Basinger J, and Dilcher D. Ancient bisexual flowers. *Science* 1984; 224: 511–513. <https://doi.org/10.1126/science.224.4648.511>
5. Manchester S R. (1999). Biogeographical relationships of North American Tertiary floras. *Ann Mo Bot Gard* 1999; 86:472-522. <https://www.jstor.org/stable/2666183>. <https://doi.org/10.2307/2666183>
6. Merev N, Gerçek Z, and Serdar B. Wood anatomy of some Turkish plants with special reference to perforated ray cells. *Turkish Journal of Botany* 2005; 29: 269–281.
7. Zarinkamar F. Comparative foliar anatomy of five xerophyte species from Iran. *Iranian Journal of Botany* 1993; 6: 153–168.
8. Dinarvand M, and Zarinkamar F. Anatomy-taxonomy of the genus *Ziziphus* in Iran. *Iranian Journal of Botany* 2006; 12: 36–41.
9. Raeder-Roitzsch J E. Forest trees in Iraq. *Bab. Fac. of Agriculture*, University of Mosul, Iraq, 1969.
10. Holmgren K P, and Keuken W. Intermountain Flora: Vascular plants of the intermountain west, U.S.A. 1989; 3B: 1–279. New York Botanical Garden, Bronx, New York.
11. Fathoulaah Ch N. Biological and Anatomical study of different *Cuscuta* species. Msc. Thesis, Salahaddin Univ., Coll. Of Sci.: P: 19. features. *Ann. Missouri Bot. Garden* 2005; 73: 228-275.
12. Saeed, J. F. Systematic study of the genus *Campanula* L. (Campanulaceae) in Iraq Ph.D. Thesis, Educ. Coll. Salahaddin Univ. Erbil. 2003, (In Arabic).
13. Liu Z, Carpenter S B, Bourgeois W J, Yu Y, Constantin R J, Falcon M J, and Adams J C. Variation in the secondary metabolite camptothecin in relation to tissue age and season in *Camptotheca acuminata* (Nyssaceae). *Tree Physiol*, 1998; 18: 265-270. <https://doi.org/10.1093/treephys/18.4.265>