

## Taxonomic Studies in Tribe Loteae (Fabaceae) in Egypt. I: Subtribe Anthyllidinae (*Anthyllis*, *Hymenocarpus* and *Tripodion*)

Zaki A. Turki, Faiza A. Shehata<sup>#</sup>, Esam M. Aqlan

Botany and Microbiology Department, Faculty of Science, Menoufia University, Shebein El-Koom, Egypt.

**T**HE PLANT morphology, anatomical characters of stem and leaves, SEM of seed coat surfaces were used to reassess the conflicted taxonomic relationships between the genera *Anthyllis*, *Hymenocarpus* and *Tripodion*. Previous studies treated the genera *Hymenocarpus* and *Tripodion* as synonyms to *Anthyllis*. The present study clearly indicated considerably differences between the three genera. The most important characters used to distinguish the studied genera are habit, inflorescence type, bract shape, calyx, pod shape and seed coat pattern. On the bases of morphology, anatomy and seed characters, three distinct genera, *Anthyllis*, *Hymenocarpus* and *Tripodion* are represented in the Egyptian flora, each with only one species.

**Keywords:** *Anthyllis*, *Hymenocarpus*, *Tripodion*, Morphological, Anatomy, SEM seed.

### Introduction

The genus *Anthyllis* L. and *Hymenocarpus* Savi. are two genera of the family Fabaceae, belonging to the subfamily Faboideae Rudd., tribe Loteae DC., subtribe Anthyllidinae W.D.J. Koch. The two genera distributed in Mediterranean regions, with some taxa extending into northern Europe, the Atlantic islands, Northern and Eastern Africa, and Western Asia (Polhill, 1981; Castroviejo, 2000 and Sokoloff, 2003 a).

Tournefort (1719) was the first who gave a more or less exact vision of the genus *Anthyllis* (syn. *Vulneraria*), recognizing five species. Linnaeus (1753) included 10 species under the genus *Anthyllis*, of which *A. tetraphylla* and *A. vulneraria*, and treated *Hymenocarpus circinnatus* as *Medicago circinnata*. Medikus (1787) separated *Anthyllis tetraphylla* as genus *Tripodion*, including one species; *T. tetraphylla*. Savi (1798) separated *Medicago circinnata* as genus *Hymenocarpus*, including one species; *H. circinnatus*. He also reported *Anthyllis* with two species; *A. tetraphylla* and *A. vulneraria*.

Moench (1794) and Gussone (1827/28) included *Anthyllis tetraphylla* as synonymy to *Vulneraria tetraphylla*. Boissier (1838, 1839-1845) separated *Anthyllis tetraphylla* as genus *Physanthyllis*, including one species; *P. tetraphylla*. Boissier (1872) recognized *Anthyllis* into *A. vulneraria*, *H. circinnatus*, *H. nummularius* and *P. tetraphylla*. Taubert (1894) included

*Hymenocarpus* as synonymy to *Circinus*. Lassen (1986, 1987) has shown that *Tripodion* is an earlier name for *Physanthyllis* and recognized three species in *Tripodion*. Akulova (1985, 1986) and Sokoloff (2003 a, b) included *Hymenocarpus* as synonymy to *Anthyllis*.

In Egypt, Täckholm (1974) recognized genus *Anthyllis* into *A. tetraphylla* and *A. vulneraria* and genus *Hymenocarpus* into *H. circinnatus* and *H. nummularius*. El-Hadidi & Fayed (1994/95) and Boulos (1995) recognized *Tripodion tetraphyllum* as a separate genus and treated *A. tetraphylla* as a synonym. Boulos (1999, 2009) treated *Tripodion tetraphyllum* as a synonym to *A. tetraphylla*.

The present study aimed to revise critically the taxonomic relationships between the species of the related genera; *Anthyllis*, *Hymenocarpus* and *Tripodion* in Egypt.

### Materials and Methods

The present study is based on available fresh material collected from their natural habitats in Egypt in addition to collections kept in Menoufia University Herbarium (MNF). Seeds of *A. vulneraria* and *Tripodion tetraphyllum* are obtained from Institute of Plant Genetics and Crop Research (IPK), Western Regional Plant Introduction Station– Washington State University and Desert Legume Program– The University of Arizona (Table 1). The seeds were cultivated in plastic house, till fruiting stage.

<sup>#</sup>Corresponding author email: faizashehata@yahoo.com

DOI: 10.21608/ejbo.2019.5705.1234

Edited by: Prof. Dr. Fco Martin Huerta-Martinez, University of Guadalajara, Mexico

©2019 National Information and Documentation Center (NIDOC)

**TABLE 1. Plant names, collection details and sources of seeds included in the present study.**

Taxa	Source of seeds	Localities	Geographical coordinates	Date of collection
<i>A. vulneraria</i>	PI 311348	-	-	-
<i>Hymenocarpus circinnatus</i>	-	Al-Mathani Al-Bahria - Marsa Matrouh.	31° 27.963 <sup>\</sup> N 26° 45.287 <sup>\</sup> E	3,4 / 2014-2018
	-	Wadi Umm El-Rakham – Marsa Matrouh.	31° 24.076 <sup>\</sup> N 27° 01.704 <sup>\</sup> E	
<i>Tripodion tetraphyllum</i>	Akz-Nr: ANTHY 14 (IPK)	-	-	-

Samples for anatomy of the stem and mature leaves were chosen from fresh materials. All assessment was made on all plants at similar developmental stages (fruiting stage) and in comparable positions of each plant. Fresh material was fixed in F.A.A. (5:5:90). After fixation stems and leaves were transformed in ethyl alcohol series and then embedded in paraffin wax. The stems and leaves were sectioned at 10-15 $\mu$ m; sections were dehydrated in alcohol-xylol series. Sections were stained in safranin and light green according to Sass (1961). The transverse sections were examined and photographed by Zeiss research microscope. A planimeter was used for estimation of the width of each tissue in the section. Terminology followed Abd El-Rahman et al. (1976), Pandey (1982) and Abd El-Gawad et al. (1989).

SEM study of the investigated seeds was carried out by mounting mature seeds on brass stubs and coated with a thin layer of gold using JEOL JSM 530P SEM at the electron microscopic unit, Faculty of Science, Alexandria University. Terminology followed Lersten (1981), Brochmann (1992), Stearn (1992) and Kirkbride et al. (2003).

## Results

### Macromorphological studies (Table 2 and Fig. 1)

*Anthyllis vulneraria* L., Sp. Pl. 719 (1753). subsp. *maura* (Beck) Maire, Bull. Soc. Hist. Nat. Afr. Nord 20: 20 (1929).

Syn. *Anthyllis maura* Beck, Ann. K. K. Naturhist. Hofmus. 11: 64 (1896).

Perennial herb, 35-45cm height, stem decumbent-erect, branched at base, tomentose, cylindrical, whitish green, internodes up to 1mm

long in decumbent stem while 3-3.5cm long in erect branches. Lower leaves simple, while upper leaves imparipinnate, all leaves alternate, whitish green, petiolate or sessile; petiolate leaves with petioles 1.7-3.7cm long, puberulent; lamina in simple leaf 4.2-4.5  $\times$  0.8-0.9cm, narrow elliptic, acuminate apex, entire margin, glabrous in upper surface and tomentulose in lower surface; imparipinnate leaves opposite-alternate with 3-9 leaflets, petiolules 0.5-1mm long; lamina in terminal leaflets 3-5.5  $\times$  0.9-1.5cm while in lateral leaflets 0.5-3  $\times$  0.3-0.7cm, lateral leaflets unequal, narrow elliptic-elliptic acuminate-acute apex, entire margin, glabrous in upper surface and tomentose in lower surface; leaf rachis (4-) 14-25mm long, stipules filiform. Inflorescence head, 11-23 flowers; peduncle cylindrical, tomentulose, 10-12cm long. Bracts palmatisect, 11-17mm long, whitish green, pubescent in upper surface but tomentose in lower surface; lower bracts 5-7 lobes while upper bracts 3 lobes, lobes elliptic-lanceolate-oblong, 5-13  $\times$  2-4mm, acute apex, entire margin. Flowers 14.5-15mm long, pedicel 0.5-1mm long, tomentose; calyx tubular, with oblique mouth, white with violet at apex, tube 7-9mm long, tomentose in outer surface, teeth lanceolate-triangular, 1-2.5 mm long, unequal, acute apex, entire margin, tomentose in outer surface; standard purple white, lamina 13-14  $\times$  4.5-5mm, auriculate, entire margin, obtuse apex, claw 6-6.5 mm long, veins violet, 2-3mm long, glabrous; wings purple greenish white, lamina 12-13  $\times$  2-2.5mm, ovate, entire margin, rounded apex, claw 8-8.5 mm long, auriculate 0.3-0.5 mm long, truncate apex; keel greenish white, lamina 12-13  $\times$  1.5-2mm, straight apex, beak obtuse, dark violet, claw 8-8.5 mm long. Androecium monadelphous, the united filament with the free parts 2-2.5mm long, united part 9-10mm long, filament dilated above, anther uniform. Gynoecium stipitate,

9.5-12.5mm long, gynophore 1.5-2.5mm long, glabrous; ovary  $2 \times 1$ mm, ellipsoid, glabrous, style 7-8mm long, lower part thin and purple; stigma capitates. Pod ellipsoid,  $4-5 \times 2.5-3$ mm, pale brown-creamy, reticulate venation, included

within the calyx, beak 0.5-1mm long, seeds 1-2. Seeds ovoid with rounded poles,  $2-3 \times 1-1.5$ mm, brown or yellowish with terminal part pale green, hilum subapical.

TABLE 2. Morphological characters of the studied taxa.

Character		<i>Anthyllis vulneraria</i>	<i>Hymenocarpus circinnatus</i>	<i>Tripodion tetraphyllum</i>
		Herb	Herb	Herb
Plant	Habit	Perennial	Annual	Annual
		Decumbent-erect	Decumbent	Decumbent
	Height (cm)	35-45	35-60	20-35
	Colour	Whitish green	Whitish green	Yellowish green
Stem	Shape	Cylindrical	Cylindrical	Cylindrical
	Surface	Tomentose	Pilose	Pilose
	Colour	Whitish green	Whitish green	Reddish brown
	Branched	At base	At base	At base
	Internode length (cm)	Up to 1 mm in decumbent stem 3-3.5 in erect stem	3-6	1-2
Stipule	Shape	Filiform	Absent	Absent
	Length (mm)	Up to 0.5	-	-
Leaf	Type	First - Lower Simple Upper Imparipinnate	2 Simple Imparipinnate	2 Simple Imparipinnate
	Arrangement	Alternate	Alternate	Alternate
	Colour	Whitish green	Whitish green	Yellowish green
	Leaf rachis	Length (mm)	(4-) 14-25	5-15
Limb simple leaf	Shape	Narrow elliptic	Obovate	Elliptic
	Size L $\times$ W (cm)	4.2-4.5 $\times$ 0.8-0.9	4-5.2 $\times$ 1.4-1.7	1.5-2 $\times$ 0.6-0.7
	Apex	Acuminate	Acute-rounded	Acute
	Margin	Entire	Entire	Entire
	Surface	Glabrous in upper Tomentulose in lower	Pilose in both	Pilose in both
Leaflet	No.	3-9	2-7	2-5
	Petiolule	0.5-1 mm	Sessile	Up to 0.5 mm
		Opposite-alternate	Opposite-alternate	Alternate
Limb leaflet	Shape	Narrow elliptic-elliptic	Elliptic-obovate	Elliptic-obovate
	Size L $\times$ W (cm)	Terminal 3-5.5 $\times$ 0.9-1.5 Lateral 0.5-3 $\times$ 0.3-0.7	Terminal 3-6 $\times$ 1-2.3 Lateral 0.7-2.6 $\times$ 0.3-1.2	Terminal 1.2-2.5 $\times$ 0.7-1.2 Lateral 0.3-1.2 $\times$ 0.1-0.6
	Apex	Acuminate-acute	Obtuse-acute	Terminal rounded-truncate Lateral acuminate-acute
	Margin	Entire	Entire	Entire
	Surface	Glabrous in upper Tomentulose in lower	Pilose in both	Pubescent in upper Tomentulose in lower

TABLE 2. Cont.

Character		<i>Anthyllis vulneraria</i>	<i>Hymenocarpus circinnatus</i>	<i>Tripodion tetraphyllum</i>
Petiole leaf	Length (cm)	1.7-3.7 or sessile	1.5-2	Up to 0.5
	Surface	Puberulent	Pilose	Tomentulose
Peduncle	Length (cm)	10-12	1-2	Sessile
	Surface	Tomentulose	Pilose	-
Inflorescence	Type	Head	Umbellate	Cluster
No. of flowers		11-23	2-3	4-6
Bract	No.	1	1	1
	Type	Palmatisect	Simple	Simple
	Shape	5-7 lobes and 3 lobes, elliptic-lanceolate-oblong	Elliptic	Elliptic
	Length (mm)	11-17	8.5-14 × 4-7	4.5-5.5 × 2-2.5
	Lobe length (mm)	5-13 × 2-4	-	-
	Colour	Whitish green	Whitish green	Green
	Apex	Acute	Acute	Acute
	Margin	Entire	Entire	Entire
	Surface	Pubescent in upper Tomentose in lower	Pilose in both	Pubescent in both
	Flower	Length (mm)	14.5-15	6-7
Pedicel	Length (mm)	0.5-1	0.5-1	0.5-1
	Surface	Tomentose	Pilose	Tomentose
Calyx	Colour	White with violet apex	Whitish green	Greenish white with red vein
	Tube shape	Tubular, with oblique mouth	Campanulate	Tubular
	Tube length (mm)	7-9	1-1.5	8-10
	Tube surface	Tomentose in outer	Pilose in outer	Tomentose in outer
	Teeth shape	Lanceolate-Triangular	Linear	Lanceolate
	Teeth length (mm)	1-2.5	3-3.5	2.5-3
	Teeth apex	Acute	Acute	Acute
	Teeth margin	Entire	Entire	Entire
	Teeth surface	Tomentose in outer	Pilose in outer	Tomentose in outer and inner
	Standard	Shape	Auriculate	Circular
Colour		Purple white	Orangish yellow	Greenish white
Lamina L × W (mm)		13-14 × 4.5-5	4-4.5 × 3.5-4	14.5-17 × 4.5-5.5
Claw length (mm)		6-6.5	0.5-1	8-10
Vein colour		Violet	Absent	Crimson
Vein length (mm)		2-3	-	5-6
Margin		Entire	Entire	Entire
Apex		Obtuse	Rounded	Rounded
Surface		Glabrous	Glabrous	Tomentose in outer

TABLE 2. Cont.

Character	<i>Anthyllis vulneraria</i>	<i>Hymenocarpus circinnatus</i>	<i>Tripodion tetraphyllum</i>	
Wings	Shape	Ovate	Oblong	Ovate
	Colour	Purple greenish white	Yellow	Greenish yellow
	Lamina L × W (mm)	12-13 × 2-2.5	4-4.5 × 1.5-2	12.5-15 × 2-2.5
	Claw length (mm)	8-8.5	1-1.2	8-9
	Apex	Rounded	Rounded	Rounded
	Margin	Entire	Entire	Entire
	Surface	Glabrous	Glabrous	Glabrous
	Auricule length (mm)	0.3-0.5	0.4-0.6	0.5-0.8
	Auricule apex	Truncate	Rounded	Truncate
Keel	Colour	Greenish white	Shiny yellow	Greenish white
	Lamina L × W (mm)	12-13 × 1.5-2	4.5-6 × 1.5-2	12-14 × 1.5-2
	Claw length (mm)	8-8.5	1-1.5	9-9.5
	Apex	Straight beak, obtuse	Straight beak, obtuse	Straight beak, acute
	Surface	Glabrous	Glabrous	Glabrous
	Beak colour	Dark violet	Yellow	Dark violet
Androecium	Type	Monadelphous	Diadelphous	Diadelphous
Stamens	Free length (mm)	-	3.5-4	10-13
	Filaments united (free parts length mm)	2-2.5	5 = 3-3.5 4 = 2-2.5	1-2
	Filaments united (united parts length mm)	9-10	2-2.5	9-11
		Filament dilated above	Filament dilated above	Filament dilated above
	Anthers	Uniform	Uniform	Uniform
Ovary	Shape	Ellipsoid	Narrow oblong	Narrow oblong
	Size L × W	2 × 1	1.2-1.5 × 0.5	3 × 0.5
	Surface	Glabrous	Puberulent	Puberulent in apex
	Colour	Green	Green	Light green
Gynophores	Length (mm)	1.5-2.5	0.2-0.3	1.5-2
	Surface	Glabrous	Glabrous	Puberulent
Style	Length (mm)	7-8	4-4.5	6-8
		Lower part thin and purple	Lower part thin	Lower part thin
Stigma	Shape	Capitates	Ellipsoid	Capitates
	Shape	Ellipsoid	Orbicular- reniform	Ellipsoid - cylindrical
Pod	Size L × W (mm)	4-5 × 2.5-3	12-18 × 11-16	4-5.5 × 3-3.5
	Colour	Pale brown-creamy	Blackish brown-pale brown-brown	Pale brown-creamy
	Surface	Reticulate	Reticulate, appressed hairy	Reticulate, pubescent
	Margin	-	Margins denticulate, membranous wing	-
	Constriction	-	-	Constricted between the seeds
		Included within the calyx	-	Included within the calyx
	Beak length (mm)	0.5-1	-	2-3
Seed No.	1-2	2	1-2	



**Fig. 1. Habit of the studied taxa: A. *Anthyllis vulneraria*; B. *Hymenocarpus circinnatus*; C. *Tripodion tetraphyllum*.**

*Hymenocarpus circinnatus* (L.) Savi, Fl. Pis. 2:205 (1798).

Syns. *Medicago circinnata* L., Sp. Pl., ed. 1, 778 (1753). *Medicago nummularia* DC., Cat. Hort. Monsp. 124 (1813). *Hymenocarpus nummularis* (DC.) G. Don, Gen. Hist. 2:173 (1832). *Hymenocarpus nummularius* (DC.) Boiss., Fl. Orient. 2:160 (1872). *Cornicina circinnata* (L.) Boiss., Voy. Bot. Midi Esp. 2:163 (1839). *Anthyllis circinnata* (L.) D.D. Sokoloff, Byull. Moskovsk. Obshch. Isp. Prir., 108 (3):46 (2003).

Annual herb, 35-60cm height, stem decumbent, branched at base, cylindrical, pilose, whitish green, internodes 3-6mm long. The first two leaves simple, alternate, whitish green, petiolate, petioles 1.5-2cm long, pilose, lamina 4-5.2 × 1.4-1.7cm, obovate, acute-rounded apex, entire margin, pilose in both surfaces; leaves imparipinnate, exstipulate, sessile, with 3-7 leaflets; leaflets opposite-alternate, petiolate- sessile, elliptic-obovate, obtuse-acute apex, entire margin, pilose in both surfaces, unequal; lamina in terminal leaflet 3-6 × 1-2.3cm while in lateral leaflet 0.7-2.6 × 0.3-1.2cm, leaf rachis 5-15mm long. Inflorescence umbellate, 2-3 flowers; peduncle 1-2cm long, cylindrical, pilose. Bracts simple, elliptic, 8.5-14 × 4-7mm, whitish green, pilose in both surfaces, acute apex, entire margin. Flowers 6-7mm long, pedicel 0.5-1mm long, pilose; calyx whitish green, campanulate, tube 1-1.5mm long, pilose in outer surface, teeth linear, 3-3.5mm long, equal, acute apex, entire margin, pilose in outer surface; standard orange-

yellow, lamina 4-4.5 × 3.5-4mm, circular, entire margin, rounded apex, claw veinless, glabrous 0.5-1 mm long; wings yellow, lamina 4-4.5 × 1.5-2mm, oblong, entire margin, rounded apex, claw 1-1.2mm long, auricule 0.4-0.6mm long, rounded apex; keel shiny yellow, lamina 4.5-6 × 1.5-2mm, straight apex, beak obtuse, claw 1-1.5mm long. Androecium diadelphous, free filament 3.5-4mm long, the other united filament with the free parts have two lengths, five filaments 3-3.5mm long, four filaments 2-2.5mm long, united part 2-2.5mm long, filament dilated above, anther uniform. Gynoecium stipitate, 5.4-6.3mm long, gynophore 0.2-0.3mm long, glabrous; ovary 1.2-1.5 × 0.5mm, narrow oblong, puberulent, style 4-4.5mm long, lower part thin; stigma ellipsoid. Pod orbicular-reniform, 12-18 × 11-16mm, blackish brown-pale brown, reticulate venation, appressed hairy, margins denticulate, membranous wing, with two seeds. Seeds reniform, 3-3.5 × 2-2.5mm, yellowish-creamy-pale brown, with rounded poles, hilum blackish brown and lateral.

*Tripodion tetraphyllum* (L.) Fourr., Ann. Soc. Linn. Lyon, ser. 2, 16:359 (1868).

Syns. *Anthyllis tetraphylla* L., Sp. Pl. 719 (1735). *Vulneraria tetraphylla* (L.) Guss., Fl. Sicul. Prodr. 2:395 (1828-32). *Physanthyllis tetraphylla* (L.) Boiss., Voy. Bot. Espagne 2:162 (1840).'

Annual herb, 20-35cm height, stem decumbent, branched at base, cylindrical, pilose, reddish brown, internodes 1-2mm long. The first two

leaves simple, alternate, yellowish green, petiolate, petioles up to 0.5cm long, tomentulose; lamina 1.5-2 × 0.6-0.7cm, elliptic, acute apex, entire margin, pilose in both surfaces; leaves imparipinnate, exstipulate, alternate, yellowish green, petiolate, petioles up to 0.5 cm long, tomentulose; leaf 3-5 leaflets, petiolules up to 0.5 mm long, lamina in terminal leaflet 1.2-2.5 × 0.7-1.2cm while in lateral leaflet 0.3-1.2 × 0.1-0.6cm, leaflets unequal, apex elliptic-obovate, rounded-truncate in terminal leaflet while acuminate-acute in lateral leaflet, entire margin, pubescent in upper surface while tomentulose in lower surface; leaf rachis 3-5mm long. Inflorescence axillary clusters, 4-6 flowers. Bracts simple, elliptic, 4.5-5.5 × 2-2.5mm, green, pubescent in both surfaces, acute apex, entire margin. Flowers 14-18mm long, pedicel 0.5-1 mm long, tomentose; calyx tubular, greenish white with reddish at apex and veined, tube 8-10mm long, tomentose in outer surface, teeth lanceolate, 2.5-3mm long, equal, acute apex, entire margin, tomentose in both surfaces; standard greenish white, lamina 14.5-17 × 4.5-5.5mm, pandurate, entire margin, rounded apex, claw 8-10mm long, veins crimson, 5-6 mm long, tomentose in lower surface; wings greenish white, lamina 12.5-15 × 2-2.5mm, ovate, entire margin, rounded apex, claw 8-9mm long, auricle 0.5-0.8mm long, truncate apex; keel greenish white, lamina 12-14 × 1.5-2mm, straight apex, beak acute, dark violet, claw 9-9.5mm long. Androecium diadelphous, free filament 10-13 mm long, the other united filament with the free parts 1-2 mm long, united part 9-11mm long, filament dilated above, anther uniform. Gynoecium stipitate, 10.5-13mm long, gynophore 1.5-2 mm long, puberulent; ovary 3 × 0.5mm, narrow oblong, puberulent in apex, style 6-8 mm long, lower part thin; stigma capitate. Pod ellipsoid-cylindrical, 4.5-5 × 3-3.5mm, pale brown-creamy, reticulate venation, pubescent, constricted between the seeds, included within the calyx, beak 2-3mm long, with 1-2 seeds. Seeds oblong-ellipsoid crisate, 3-3.5 × 2-2.5mm, brown with black spots, with rounded- truncate poles, hilum lateral.

#### *Anatomical studies*

The anatomical investigations of the stem and leaf of the studied taxa is represented in Tables 3, 4 and Fig. 2, 3.

#### *Stem anatomy*

##### *Anthyllis vulneraria*

Stem circular, 1.2-1.3mm diameter; epidermal

layer 12.5-15µm thickness covered with 2.5-5µm cuticle thickness. Cortex 7-8 parenchymatous layers, 100-137.5µm thickness. Vascular cylinder 15-16 bundles, each bundle 150-200µm long, pericyclic fibers 3-4 layers, 37.5-55µm thickness. Phloem 3-4 layers, 20-30µm thickness. Cambium 2 layers, 10-12.5µm thickness. Xylem 2-7 arches, 4-5 vessels per arch, 100-160µm length. Pith cells parenchymatous, 520-530µm diameter.

##### *Hymenocarpos circinnatus*

Stem circular, 1.9-2mm diameter; epidermal layer 12.5-20µm thickness covered with 2.5-5µm cuticle thickness. Cortex 5-6 layers parenchymatous; 112.5-175µm thickness. Vascular cylinder 16-17 bundles, each bundle 220-300µm long, pericyclic fibers 4-5 layers, 50-75µm thickness. Phloem 4-5 layers, 35-50µm thickness. Cambium 2 layers, 10-12.5µm thickness. Xylem 2-5 arches, 3-4 vessels per arch, 100-150µm length. Pith cells parenchymatous, 1000-1025µm diameter.

##### *Tripodion tetraphyllum*

Stem circular, 1.9-2.1mm diameter; epidermal layer 12.5-22.5µm thickness covered with 2.5-5µm cuticle thickness. Cortex 7-9 layers parenchymatous; 200-370µm thickness, with solitary crystals. Vascular cylinder 17-19 bundles, each bundle 140-270µm wide, pericyclic fibers 4-6 layers, 40-62.5µm thickness. Phloem 3-4 layers, 25-37.5µm thickness. Cambium 2 layers, 12.5-15µm thickness. Xylem 2-8 arches, 3-8 vessels per arch, 60-150µm length. Pith cells parenchymatous, 950-975µm diameter, with solitary crystals. Tannin cells few in pith.

#### *Leaf anatomy*

##### *Anthyllis vulneraria*

The leaf in midrib region is v-shaped, 400-420µm thickness, upper and lower epidermis uniseriate, 12.5-25µm thickness, covered with 2.5µm cuticle thickness. In midrib region, parenchyma 2-3 layers, isodiametric- flattened, 87.5-100µm thickness. Xylem 4-6 arches, 2-6 vessels per arch, xylem arch 75-82.5µm length. Phloem 3-4 layers, 20-25µm thickness. Phloem fibers at midrib region below the main vascular bundle, 3-4 layers, 25-37.5µm thickness. Below the main vascular bundles 3-4 layers of parenchyma cells, 100-107.5µm thickness. The wings 300-350µm thickness, mesophyll tissue chlorophyllous with wide air spaces, 5-6 rows, 250-315µm thickness, with solitary crystals.

TABLE 3. Anatomical characters of stem in the studied taxa.

Stem character		<i>Anthyllis vulneraria</i>	<i>Hymenocarpus circinnatus</i>	<i>Tripodion tetraphyllum</i>
Outline		Circular	Circular	Circular
Diameter ( $\mu\text{m}$ )		1250-1275	1925-1970	1925-2025
Cuticle thickness ( $\mu\text{m}$ )		2.5-5	2.5-5	2.5-5
Epidermal cell	Shape	Isodiametric-radially elongated	Isodiametric-radially elongated	Isodiametric-radially elongated
	Width ( $\mu\text{m}$ )	12.5-15	12.5-20	12.5-22.5
Cortex	Parenchyma number of layers	7-8	5-6	7-9
	Parenchyma	Width ( $\mu\text{m}$ )	100-137.5	112.5-175
Vascular bundles number	15-16	16-17	17-19	
Vascular bundles width ( $\mu\text{m}$ )		150-200	220-300	140-270
Pericyclic fiber number of layers		3-4	4-5	4-6
Pericyclic width ( $\mu\text{m}$ )		37.5-55	50-75	40-62.5
Phloem number of layers		3-4	4-5	3-4
Phloem width ( $\mu\text{m}$ )		20-30	35-50	25-37.5
Cambium number of layers		2	2	2
Cambium width ( $\mu\text{m}$ )		10-12.5	10-12.5	12.5-15
Xylem number of arches		2-7	2-5	2-8
Xylem number of layers vessels		4-5	3-4	3-8
Xylem width ( $\mu\text{m}$ )		100-160	100-150	60-150
Pith	Cell shape	Isodiametric-tangentially elongated	Isodiametric-flattened	Isodiametric-flattened
	Diameter ( $\mu\text{m}$ )	520-530	1000-1025	950-975
Crystals	In cortex	Absent	Absent	Solitary
	In pith	Absent	Absent	Solitary
Tannin cells	In cortex	Absent	Absent	Absent
	In pith	Absent	Absent	Few

*Hymenocarpus circinnatus*

The leaf in midrib region is u-shaped, 450-500 $\mu\text{m}$  thickness, upper and lower epidermis uniseriate, 12.5-37.5 $\mu\text{m}$  thickness, covered with 2.5  $\mu\text{m}$  cuticle thickness. In midrib region, parenchyma 3-4 layers, angular-irregular, 120-132.5 $\mu\text{m}$  thickness. Xylem 3-4 arches, 3-5 vessels per arch, xylem arch 87.5-107.5 $\mu\text{m}$  length. Phloem 3-4 layers, 25-30 $\mu\text{m}$  thickness. Phloem fibers 3-4 layers, 25-37.5 $\mu\text{m}$  thickness. Below the main vascular bundles 3-4 layers of parenchyma cells, 112.5-125 $\mu\text{m}$  thickness. The wings 280-310 $\mu\text{m}$  thickness, mesophyll tissue chlorophyllous with wide air spaces, 5-6 rows, 210-275 $\mu\text{m}$  thickness.

*Tripodion tetraphyllum*

The leaf in midrib region is u-shaped, 480-500 $\mu\text{m}$  thickness, Upper and lower epidermis uniseriate, 15-40 $\mu\text{m}$  thickness, covered with 2.5 $\mu\text{m}$  cuticle thickness. In midrib region, parenchyma 3-4 layers, flattened-tangentially elongated, 112.5-125 $\mu\text{m}$  thickness. Xylem 5-6 arches, 2-3 vessels per arch, xylem arch 55-62.5 $\mu\text{m}$  length. Phloem 3-4 layers, 30-32.5 $\mu\text{m}$  thickness. Phloem fibers 2-3 layers, 12.5-25 $\mu\text{m}$  thickness. Below the main vascular bundles 4-5 layers of parenchyma cells, 187.5-200 $\mu\text{m}$  thickness, have 2-3 tannin cells. The wings 220-230 $\mu\text{m}$  thickness Mesophyll tissue consists of palisade and spongy tissues. Palisade tissue 2-3 rows, 62.5-85 $\mu\text{m}$  thickness, spongy tissue 3-4 rows, 70-87.5 $\mu\text{m}$  thickness.



TABLE 4. Anatomical characters of leaf in the studied taxa.

Character		<i>Anthyllis vulneraria</i>	<i>Hymenocarpus circinnatus</i>	<i>Tripodion tetraphyllum</i>	
Shape		V	U	U	
Midrib thickness (µm)		400-420	450-500	480-500	
Cuticle thickness (µm)		2.5	2.5	2.5	
Leaflet midrib	Epidermal cells	Thickness (µm)	12.5-25 up. 12.5-20 lo.	20-37.5 up. 12.5-22.5 lo.	25-40 up. 15-20 lo.
		Shape	Isodiametric-tangentially elongated	Tubular-tangentially elongated	Isodiametric-tangentially elongated
	Xylem number of arches		4-6	3-4	5-6
	Xylem thickness (µm)		75-82.5	87.5-107.5	55-62.5
	Xylem number of vessels in arch		2-6	3-5	2-3
	Phloem number of layers		3-4	3-4	3-4
	Phloem thickness (µm)		20-25	25-30	30-32.5
	Parenchyma number of layers		2-3 up. 3-4 lo.	3-4 up. 3-4 lo.	3-4 up. 4-5 lo.
	Parenchyma	Thickness (µm)	87.5-100 up. 100-107.5 lo.	120-132.5 up. 112.5-125 lo.	112.5-125 up. 187.5-200 lo.
		Cell shape	Isodiametric- flattened	Angular-irregular	Flattened-tangentially elongated
Crystals		Absent	Absent	Absent	
fiber number of layers		3-4	3-4	2-3	
Fiber thickness (µm)		25-37.5	25-37.5	12.5-25	
Tannin cells	Upper vascular bundle	Absent	Absent	2-3	
	Lower vascular bundle	Absent	Absent	Absent	
Thickness (µm)		300-350	280-310	220-230	
Mesophyll with interspace widely	Number of rows	5-6	5-6	-	
	Thickness (µm)	250-315	210-275	-	
Leaflet lamina	Palisade layer	Number of rows	-	2-3	
		Thickness (µm)	-	62.5-85	
	Spongy layer	Number of rows	-	-	3-4
		Thickness (µm)	-	-	70-87.5
Tannin cells		Absent	Absent	Absent	
Crystals		Solitary	Absent	Absent	

*SEM of the spermoderm (Table 5 and Fig. 4)*

SEM of the epidermal cells clarifies the texture and reticulation of their anticlinal (radial) walls, the appearance of the outer periclinal walls and the persistency of the primary cell walls.

*Anthyllis vulneraria*

Seed ovoid with rounded poles, 2-3 × 1-1.5mm

size, brown or yellowish with terminal part pale green in colour, covered with wax, hilum circular, 83.87 × 80.65µm size, subapical in position, rim aril raised, micropyle obtriangular, and 7.8 × 7.8µm size. Seed coat pattern irregularly reticulate, anticlinal wall wavy, relief of cell boundary slightly channeled, thickness of cell boundary moderate, curvature of outer periclinal wall smooth and concave.

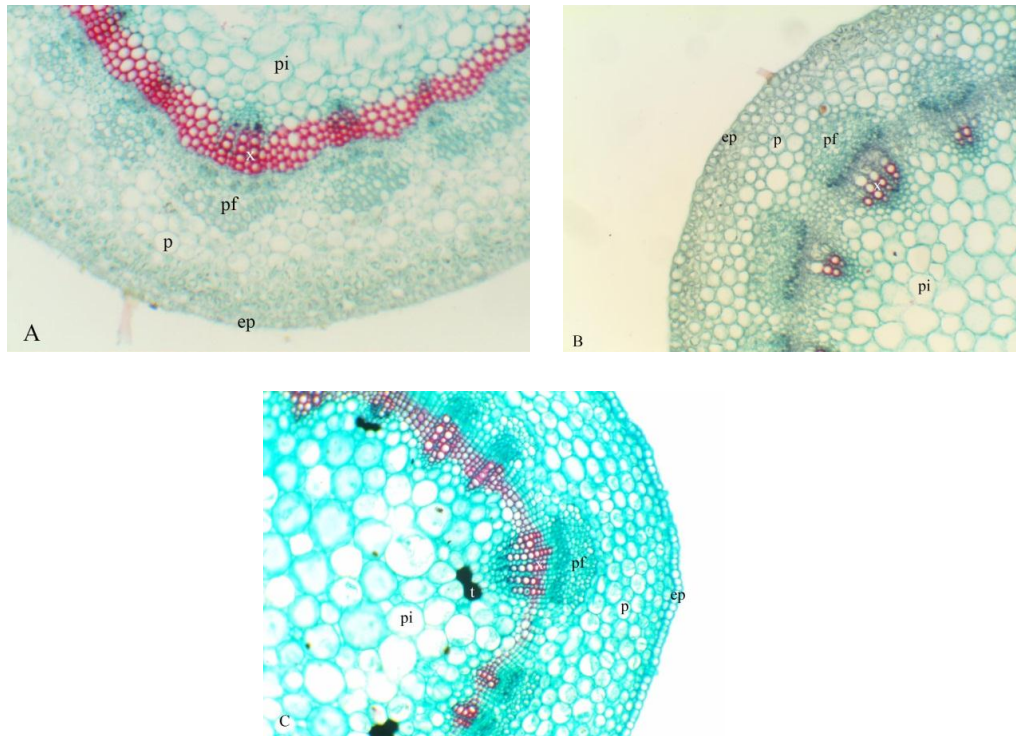


Fig. 2. Stem anatomy of the studied taxa; (A) *Anthyllis vulneraria*, (B) *Hymenocarpos circinnatus*, (C) *Tripodion tetraphyllum* [ep= Epidermis, pi= Pith, x= Xylem, p= Parenchyma, pf= Phloem fiber, t= Tannin cell (x 100)].

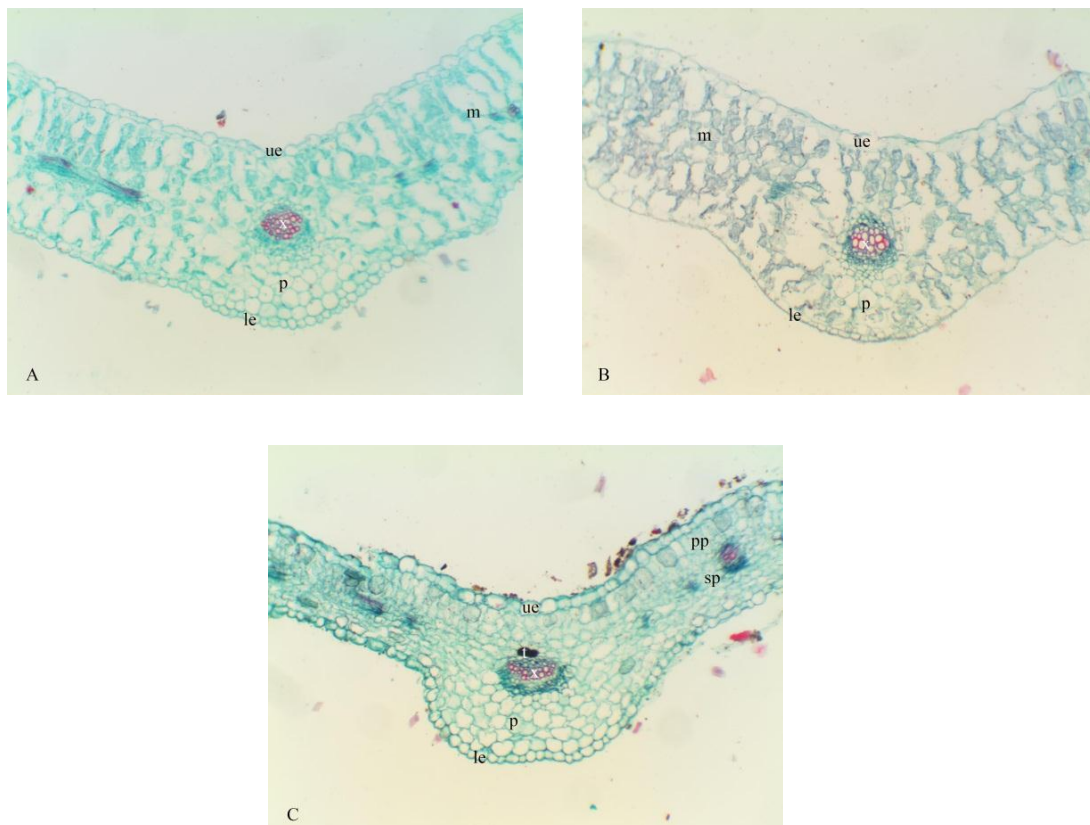


Fig. 3. Leaf anatomy of the studied taxa, (A) *Anthyllis vulneraria*, (B) *Hymenocarpos circinnatus*, (C) *Tripodion tetraphyllum* [ue= Upper epidermis, x= Xylem, p= Parenchyma; m= Mesophyll, pp= Palisade parenchyma, sp= Spongy parenchyma, le= Lower epidermis, t= Tannin cell (x 50)].

TABLE 5. The morphological aspect of the spermoderm of the studied taxa.

Character		<i>Anthyllis vulneraria</i>	<i>Hymenocarpus circinnatus</i>	<i>Tripodion tetraphyllum</i>
Seed colour		Yellowish with green in terminal part - brown	Yellowish – creamy – pale brown, hilum blackish brown	Brown with black spots
Seed size	L × W(mm)	2-3 × 1-1.5	3-3.5 × 2-2.5	3-3.5 × 2-2.5
	L/W ratio	2	1.4	1.4
Seed pole		Rounded	Rounded	Rounded- truncate
Seed shape		Ovoid	Reniform	Oblong – Ellipsoid Crispate
Seed coat pattern		Reticulate	Micropappilate in rows	Papillae
Hilum	Position	Subapical	Lateral	Lateral
	Shape	Circular	Oblate	Ovate
	L × W(μm)	83.87 × 80.65	75.81 × 88.71	83.87 × 77.42
Rim aril		Raised	Raised	Raised
Micropyle	Shape	Obtriangular	Narrow oblong	Narrow oblong with one open end
	L × W(μm)	7.8 × 7.8	7.8 × 1.56	9.36 × 1.56
Outline of cells		Reticulate-irregular	Oblong-radial elongated	Tetra-hexagonal
Anticlinal wall		Wavy	Wavy	Lobbed
Relief of cell boundary		Slightly channeled	Slightly channeled	Channeled
Thickness of cell boundary		Moderately	Moderately	Very thick
Curvature of outer periclinal wall		Concave-smooth surface	Concave-smooth surface	Concave- irregular
Wax		Present	Present	Present

*Hymenocarpus circinnatus*

Seed reniform, 3-3.5 × 2-2.5mm size, yellowish-creamy-pale brown in colour with hilum blackish, seed poles rounded. Seed coat pattern micropappilate in rows, outline of cells oblong-radial elongated, anticlinal wall wavy, relief of cell boundary slightly channeled, thickness of cell boundary moderately, curvature of outer periclinal wall concave, smooth surface. Hilum oblate, 75.81 × 88.71μm size, lateral in position. Rim aril raised. Micropyle narrow oblong, 7.8 × 1.56μm size. Wax present.

*Tripodion tetraphyllum*

Seed oblong-ellipsoid crispate, 3-3.5 × 2-2.5mm size, brown with black spots in colour, seed poles rounded-truncate. Seed coat pattern papillae in rows, outline of cells tetra-hexagonal, anticlinal wall lobbed, relief of cell boundary channeled, thickness of cell boundary very thick, curvature of outer periclinal wall concave irregular. Hilum ovate, 83.87 × 77.42μm size, lateral in position. Rim aril raised. Micropyle narrow oblong with one open end, 9.36 × 1.56μm size. Wax present.

**Discussion**

Several attempts have been made in

distinguishing and identification of the genera *Anthyllis*, *Hymenocarpus* and *Tripodion*. Taubert (1894) included *Hymenocarpus* as synonym to *Circinas*. Sokoloff (2003 a) have treated *Hymenocarpus* as synonym to *Anthyllis* (subgenus *Cornicina*, section *Hymenocarpus*). Tikhomirov & Sokoloff (1996), Benedi (2000) and Sokoloff (2003 a) have treated *Anthyllis tetraphylla* as genus *Tripodion*.

The morphological characteristics of the studied taxa revealed that they varied from each other and can be distinguished depending on the habit, characteristics of leaves, inflorescence, flowers, pods, and seeds.

The use of anatomical characters in taxonomic investigation is becoming of increasing interest. Anatomical structure is most likely providing evidence helping to establish the affinities of genera of uncertain taxonomic status, yet in the same time proves very helpful for individual identification (Welkie & Caldwell, 1970 and Osmond et al., 1980). Turki (2007) and Kasem (2016) reported the importance of anatomical differences in the distinction among species.

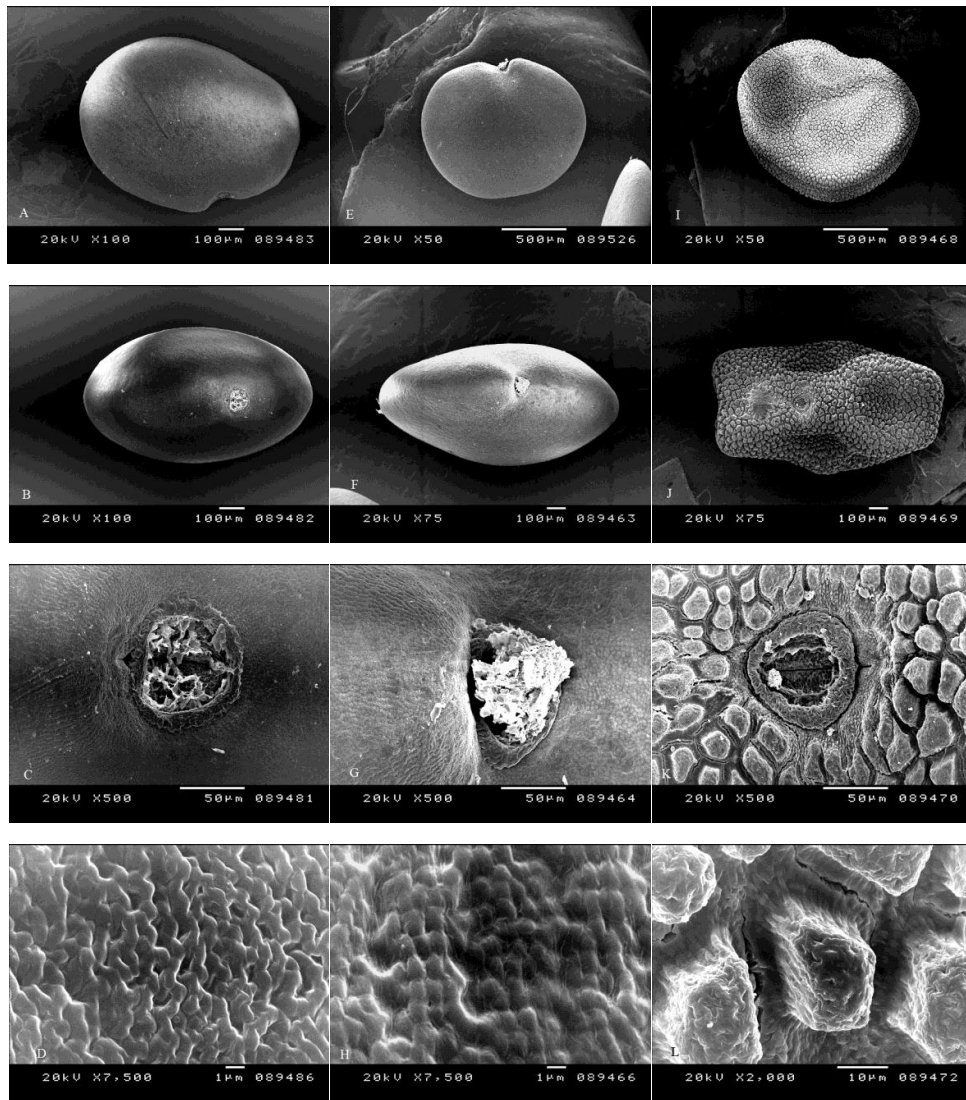


Fig. 4. SEM micrograph of spermoderm surface of the studied taxa; (A-D) *Anthyllis vulneraria*, (E-H) *Hymenocarpus circinnatus*, (I-L) *Tripodion tetraphyllum* [A, E, I: Seed morphology; B, F, J: Hilum position; C, G, K: Hilum shape; D, H, L: Spermoderm surface].

The studied taxa displayed remarkable differences in the anatomical investigations of their stems and leaflets. Comparison of the internal structure of the stems revealed differences in cortex, number of vascular bundles, xylem, pith diameter, crystals and tannin cells. Comparison of the internal structure of the leaflets revealed differences in shape and thickness of midrib and xylem, lamina thickness, and crystals and tannin cells.

Anticlinal undulations and characters of cell boundaries in the seed exine are of high taxonomic significance and often characterize between the species and genus level (Barthlott & Voit, 1979 and Barthlott, 1981). Kaplan et al. (2007) and

Fawzi et al. (2010) reported seed coat characters are successfully employed in the identification and classification of taxa.

The results of spermoderm SEM revealed differences in seed coat pattern, outline cells, relief and thickness of cell boundary, curvature of outer periclinal wall.

The present study, depending on the results of macromorphological and anatomical investigations, SEM studies on seeds clearly indicates considerable differences between the three studied genera *Anthyllis*, *Hymenocarpus* and *Tripodion* and support the treatment of them as different genera. The results agree with Medikus

(1787) in that *Tripodion* treated as a separate genus.

#### Key to the genera

- 1- Perennial herb, inflorescence head, bract palmatisect, the leaf in midrib region is v-shaped, hilum subapical in position, seed coat pattern irregularly reticulate.. *Anthyllis*
- Annual herb, inflorescence umbellate or cluster, bract simple, the leaf in midrib region is u-shaped, hilum lateral in position, seed coat pattern otherwise .....2
- 2- Flower 6-7mm long, calyx campanulate, pod orbicular-reniform, cortex up to 6 layers, seed coat pattern micropapilate.....*Hymenocarpos*
- Flower 14-18mm long, calyx tubular, pod ellipsoid- cylindrical, cortex 7-9 layers, seed coat pattern papillae..... *Tripodion*

#### References

- Abd El-Gawad, M.A., Salem, M.O. and Hegazi, A.M. (1989) Anatomy of Alfalfa leaflets as affected by NPK-fertilization and saline irrigation. *Ann. Agric. Sci. Moshtohor*; **27**(3), 1439-1447.
- Abd El-Rahman, A.A., Ibrahim, A.A. and Hassan, H.T. (1976) Contribution to the anatomical characters of some xerophytes. *Bull. Fac. Sci. Cairo Uni.* **49**, 139-162.
- Akulova, Z.V. (1985) To the question on ways of morphological evolution in the genus *Anthyllis* L. In: Anonymous (Ed.), *Proceedings of 7<sup>th</sup> Conference of Young Scientists of V.L. Komarov Botanical Institute*. VINITI, Moscow, pp. 29–36.
- Akulova, Z.V. (1986) The Genus *Anthyllis* L. in the Flora of USSR: Systematics, Morphology, and Usage. *Ph.D. Thesis*, Leningrad (in Russian).
- Barthlott, W. (1981) Epidermal and seed surface characters of plants. Systematic applicability and some evolutionary aspect. *Nord. J. Bot.* **1**, 345-355.
- Barthlott, W. and Voit, G. (1979) Mikromorphologie der Samenschalen und Taxonomie der *Cactaceae*: Ein rasterelektronen-mikroskopischer überblick. *Plant Syst. Evol.* **132**, 205-229.
- Benedi, C. (2000) *Anthyllis, Hymenocarpos*. In: "*Flora Iberica*" Talavera, S. et al. (Eds.), pp. 829–862 and 868–873. 7(2). Real Jardín Botánico, Madrid.
- Boissier, P.É. (1838) "*Elenchus Plantarum Novarum...*", Genève.
- Boissier, P.É. (1839-1845) "*Voyage Botanique dans le Midi de l'Espagne*". Vol. 2: 158-164. Paris.
- Boissier, P.É. (1872) "*Flora Orientalis*". Vol. 2:156-158. Basilea, Genève, Lyon.
- Boulos, L. (1995) "*Flora of Egypt Checklist*". Al-Hadara Publishing, Cairo.
- Boulos, L. (1999) "*Flora of Egypt*". Vol. 1. Al-Hadara Publishing, Cairo.
- Boulos, L. (2009) "*Flora of Egypt Checklist*", Revised annotated edition. Al-Hadara Publishing, Cairo.
- Brochmann, C. (1992) Pollen and seed anatomy of Nordic *Draba* (Brassicaceae) phylogenetic and ecological implications. *Nordic J. Botany*, **12**(6), 657-673.
- Castroviejo, S. (ed.) (2000) "*Flora Iberica*". Vol VII (II) Leguminosae (Partim). Real Jardín Botánico (CSIC), Madrid, Spain.
- El-Hadidi, M.N. and Fayed, A.A. (1994/95) Material for Excursion Flora of Egypt - *Taeckholmia*, **15**, 1-223.
- Fawzi, N.M., Fawzy A.M. and Mohamed, A.A.H.A. (2010) Seed morphological studies on some species of *Silene* L. (Caryophyllaceae). *Inter. J. Bot.* **6**(3), 287-292.
- Gussone, G. (1827/28) "*Florae Siculae Prodrromus*". Regia Typographia. Neapoli.
- Kaplan, A., Hasanoğlu, A. and Ince, I.A. (2007) Morphological, anatomical and palynological properties of some Turkish *Veronica* L. species (Scrophulariaceae). *Inter. J. Bot.* **3**(1), 23-32.
- Kasem, W. (2016) Anatomical, pollen grains and seed exomorphic studies on five species of *Cleome* L (Cleomaceae Bercht. & Presl) collected from South West of Saudi Arabia. *J. Plant Sciences*, **4**, 29-36.
- Kirkbride, J.H., Gunn, C.R. and Weitzman, A.L. (2003) *Fruits and seeds of genera in the subfamily*

- Faboideae (Fabaceae)*. United States Department of Agriculture, Technical Bulletin 1208 No. 1890.
- Lassen, P. (1986) In: "*Hymenocarpus* and *Tripodion*". W. Greuter and T. Raus (Eds.), 16, pp. 111-112. Med-Checklist notulae, 13. *Willdenowia*.
- Lassen, P. (1987) In: W. Greuter & T. Raus (eds.), Med-Checklist notulae, 14. *Willdenowia* **16**: 443.
- Lersten, N.R. (1981) Testa topography in Leguminosae subfamily Papilionoideae. *Proc. Iowa Acad. Sci.* **88(4)**, 180-191.
- Linnaeus, C. (1753) *Species plantarum*, Vol II. Stockholm, Sweden.
- Medikus, F.K. (1787) Neue methode bei Pflanzen zu ordnen. *Vorlesungen der Churpf lizischen physicalisch-conomischen Gesellschaft*, **2**, 354-382.
- Moench, C. (1794) A staminum situ describendi. *Methodus Plantas Horti Botanici et Agri Marburgensis*, **1**, 1-368.
- Osmond, C.B., Bjorkman and Anderson, D.J. (1980) Physiological process in plant Ecology towards a synthesis with *Atriplex*. – Berlin.
- Pandey, B.P. (1982) "*Plant Anatomy*". New Delhi.
- Polhill, R.M. (1981) Papilionoideae. In: "*Advances Legume Systematics*", R.M. Polhill and P.H. Raven (Eds.), part 1, pp. 191-208. Royal Botanic Gardens, Kew.
- Sass, J.E. (1961) "*Botanical Microtechnique*", 3<sup>rd</sup> ed. – Amsterdam.
- Savi, G. (1798) "*Flora Pisana*", Vol. 1-2. Pietro
- Giacomelli Editore, Pisa.
- Sokoloff, D.D. (2003 a) On system and phylogeny of the tribe Loteae DC. (Leguminosae) [in Russian]. *Bulletin of Moscow Society of Naturalists, Biological Series*, **108(3)**, 35-48.
- Sokoloff, D.D. (2003 b) Morphology and Classification of the Tribe Loteae DC. of the Family Leguminosae. *Ph.D. Thesis*, Moscow (in Russian).
- Stearn, W.T. (1992) "*Botanical Latin*", 4<sup>th</sup> ed. Timber Press, Oregon, USA.
- Täckholm, V. (1974) "*Students' Flora of Egypt*". 2<sup>nd</sup> ed., Cairo University, Egypt.
- Taubert, P. (1894) In: "*Leguminosae*". Engler, A., Prantl, K., Die natürlichen Pflanzenfamilien 3:W. Engelmann, Leipzig, pp. 70-385.
- Tikhomirov, V.N. and Sokoloff, D.D. (1996) On the division of the genus *Anthyllis* L. (Papilionaceae, Loteae) into subgenera and sections. *Bulletin of Moscow Society of Naturalists, Biological Series*, **101**, part 1, 61-73 (in Russian).
- Tournefort, J. (1719) *Institutione rei herbariae*, 3rd ed., Paris.
- Turki, Z.A. (2007) The genus *Ammannia* L. (Lythraceae) in Egypt. *Flora Mediterranea*, **17**, 97-114.
- Welkie, J.T. and Caldwell, M. (1970) Leaf anatomy of species in some dicotyledon. Families as related to the C3 and C4 pathways of Carbon fixation. *Canad. J. Bot.* **48**, 2135-2146.

(Received 24/10/2018;  
accepted 27/3 /2019)

### دراسات تصنيفية لعشيرة Loteae (الفصيلة الفولية) في مصر. تحت العشيرة Anthyllidinae (*Tripodion* و *Hymenocarpus* و *Anthyllis*)

زكي عبد الحميد تركي، فائزة عبد الموجود شحاتة، عصام محمد عقلمان  
قسم النبات والميكروبيولوجي - كلية العلوم - جامعة المنوفية - شبين الكوم - مصر.

تهدف الدراسة إعادة تقييم العلاقات التصنيفية المتداخلة بين أجناس *Anthyllis* و *Hymenocarpus* و *Tripodion* على أساس دراسة تفصيلية لكل من الشكل الظاهري للنبات والصفات التشريحية للساق والأوراق، ودراسة قصرة البذور باستخدام المجهر الإلكتروني الماسح. تشير النتائج إلى وجود اختلافات كبيرة بين الأجناس المدروسة وتدعم معاملتها كأجناس مختلفة وليس كما أشار بعض الباحثين أن جنس *Hymenocarpus* و جنس *Tripodion* أسماء مرادفة لجنس *Anthyllis* يمثل في مصر كل جنس من *Anthyllis* و *Hymenocarpus* و *Tripodion* بنوع واحد.