Korean J. Pl. Taxon. 49(1): 13–27 (2019)
https://doi.org/10.11110/kjpt.2019.49.1.13
RESEARCH ARTICLE

pISSN 1225-8318
eISSN 2466-1546
Korean Journal of
Plant Taxonomy

Taxonomic implications of multivariate analyses of Egyptian *Ononis* L. (Fabaceae) based on morphological traits

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(Received 30 October 2018; Revised 18 March 2019; Accepted 26 March 2019)

ABSTRACT: Numerical taxonomy is employed to determine the phenetic proximity of the Egyptian taxa belonging to the genus *Ononis* L. A classical clustering analysis and a principal component analysis (PCA) were used to separate 57 macro- and micromorphological characters in order to circumscribe 11 taxa of *Ononis*. A clustering analysis using the unweighted pair-group method with the arithmetic means (UPGMA) method gives the highest co-phenetic correlation. Results from clustering and PCA revealed the segregation of five groups. Our results are in line, to some certain degree, with the traditional sub-sectional concept, as can be seen in the grouping of the representative members of the subsections *Diffusae* and *Mittisimae* together and the representative members of the subsections *Viscosae* and *Natrix*. The phenetic uniqueness of *Ononis variegata* and *O. reclinata* subsp. *mollis* was formally established. However, our findings contradict the classic sectional concept; this opinion was suggested earlier in previous phylogenetic circumscriptions of the genus. The most useful characters that provide taxonomic clarity were discussed.

Keywords: Fabaceae, numerical taxonomy, *Ononis*, PCA, Trifolieae, UPGMA

Ononis L., or commonly the restharrow, is a large genus in the subfamily Faboideae (Fabaceae). The genus was first recognized by Linnaeus (1753, 1754) who described 17 species. Currently, it compromises as many as 86 species (Turini et al., 2010). Most of them are common herbs in the temperate Mediterranean regions; few are sub-shrubs and rarely shrubs. Its members are famous with their sticky hairs, glandular or eglandular. Their papilionaceous flowers with yellow, white, pink or violet corolla are often strongly scented. *Ononis* along with another ten genera namely: *Cicer*, *Galega*, *Lathyrus*, *Medicago*, *Melilotus*, *Parochetus*, *Pisum*, *Trifolium*, *Trigonella*, and *Vicia* are outlining a monophyletic group named "the Vicioid group," according to a phylogenetic study based on the plastidial gene matK (Wojciechowski et al., 2000; Steele and Wojciechowski, 2003; Wojciechowski et al., 2004).

Many previous authors had reported the paramount taxonomic significance of macro-morphology in taxonomy of *Ononis*, both the vegetative and reproductive characters, among them Linnaeus (1753), De Candolle (1825), Willkomm (1877), Battandier and Trabut (1889), Ascherson and Graebner (1907), Širjaev (1932), Hutchinson (1964), and Turini et al. (2010). The taxonomic significance of epidermal characters and trichome diversity for tribe Trifolieae, in general, and for genus *Ononis* particularly was a topic for many studies such as Gupta and Murty (1977) and Taia (2004). *Ononis* is famous by its clothing glandular trichomes which give the plant its shinny and often sticky appearance, Metcalfe and Chalk (1979) recorded two types of trichomes on *Ononis*; non-glandular uniseriate with equal-sized cells and glandular club-shaped with long stalks.

Traditionally, *Ononis* was often treated under tribe Trifolieae (Bentham & Hooker, 1865) along with *Trifolium*, *Medicago*, *Melilotus*, *Trigonella*, and *Parochetus*; all these genera share one character: the trifoliate leaf. Many authors adopted this

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tribal position (Boissier, 1872; Taubert, 1891; Meikle, 1977; Polhill and Raven, 1981). However, *Ononis* differs from these genera by its monadelphous androecium and beaked keel. Therefore, Hutchinson (1964) separated *Ononis* and its small segregate *Passaea* into tribe Ononideae, and some authors followed this classification such as (Huber-Morath, 1970; Zohary, 1972; Townsend, 1974; Rechinger, 1984).

A monographic revision of the genus of *Ononis* was presented by Širjaev (1932) based intrinsically on the morphology, he subdivided the genus into 2 sections and 22 subsections. Then, the monophyletic nature of the genus *Ononis* was supported based on both morphology and phylogenetic data obtained from the plastid trnL-F and the nuclear internal transcribed spacer (ITS) DNA (Turini et al., 2010). However, the molecular evidence did not support the traditional sectional concept established by Širjaev (1932), instead five major lineages were suggested.

In Egypt, *Ononis* is represented by ten species and one subspecific taxon (Fayed et al., in press); six of which are located in clade III *sensu* Turini et al. (2010), and the remaining five taxa are located in clade V (see Table 1). According to an assumption made by Turini et al. (2010), clade V contains taxa that are adapted to aridity with an annual habit; this assumption goes well with the fact that Egypt is dominated by semi- to hyper-arid climate.

Phenetic studies of exomorphological characters can generate some accurate estimations of relationships between taxa (El-Hadidy et al., 2018). In a recent phenetic study on family Leguminosae, subfamily Papilionoideae in Egypt, all Egyptian species of *Ononis* were grouped together in one phenon along with some species of each *Medicago*, *Trifolium* and *Lathyrus* (El-Gazzar et al., 2013).

The premise of this study is to inspect the phenetic relationships between *Ononis* taxa in Egypt; to assess the diversity of trichomes and the epidermal characteristics of these taxa and determine its taxonomic significance and finally, to critically relate our phenetic findings to the previous phylogenetic studies of *Ononis*.

Materials and Methods

Plant materials

This phenetic analysis was based on herbarium specimens collected from different habitats of Egypt and preserved in four Egyptian herbaria (ASTU, CAI, CAIM and Loutfy Boulos), acronyms *sensu* Thiers (2017), number of specimens studied for each taxon ranged from 1 to 10. The identification decision for each specimen was done with use of the floras of Egypt and the adjacent countries (Huber-Morath, 1970; Zohary, 1972; Jafri, 1980; Boulos, 1999; Collenette, 1999).

Trichome diversity assessment

For assessing the trichome types and other epidermal characters (Table 2), different small plant segments were mounted onto clean stubs using double-sided cello tape. Vouchers used in SEM analysis are listed in Appendix 1. The stubs were coated with gold in a JEOL JFC 1100E ion sputtering device then examined with JEOL JSM 5400LV scanning electron microscopy (Jeol, Tokyo, Japan) that is operated at accelerated voltage of 15 kv at the Electron Microscopy Unit (EMU) in Assiut University, Egypt.

Table 1. Comparison between the traditional classification and the phylogenetic classification of *Ononis* taxa in Egypt.

Taxa	Širjaev (1932)		Turini et al. (2010)	
	Section	Sub-section	Clade	Sub-clade
Ononis diffusa	Ononis	Diffusae	V	C
O. serrata	Ononis	Diffusae	V	C
O. mitissima	Ononis	Mitissimae	V	C
O. variegata	Ononis	Variegatae	V	B
O. natrix subsp. *natrix*	Natrix	Natrix	III	A
O. natrix subsp. *stenophylla*	Natrix	Natrix	III	A
O. vaginalis	Natrix	Natrix	III	A
O. reclinata subsp. *mollis*	Natrix	Reclinatae	V	B
O. pubescens	Natrix	Viscosae	III	A
O. sicula	Natrix	Viscosae	III	A
O. viscosa subsp. *breviflora*	Natrix	Viscosae	III	A

Table 2. Epidermal characters and trichome diversity for the studied taxa.

Taxa	Epidermal cell of adaxial surface of leaves			Stomatal type	Trichome type(s)
	Anticlinal walls	Periclinal walls	Size (μm)		
Ononis diffusa	Raised, straight	Tabular	45–75 ×20–27	Anomocytic	GTM, NCT^{ii}
O. serrata	Raised, straight	Concave	72–80 × 40–70	Anomocytic	GTU^{ii}, GTM, NCT^{ii}
O. mitissima	Raised, straight	Concave	26–39 × 13–39	Diacytic	GTM
O. variegata	Raised, straight	Tabular	22–27 × 23–25	Anomocytic	NCT^{i}
O. natrix subsp. *natrix*	Raised, sinuate	Tabular	31–54 × 28–49	Diacytic	GTM, GTU^{ii}, NCT^{ii}
O. natrix subsp. *stenophylla*	Raised, straight	Convex	47–62 × 20–33	Diacytic	GTM, NCT^{ii}
O. vaginalis	Raised, straight	Convex	70–80 × 20–28	Diacytic	GTM, GTU^{i}, NCT^{ii}
O. reclinata subsp. *mollis*	Raised, straight	Tabular	33–44 ×15–18	Diacytic	GTM, NCT^{ii}
O. pubescens	Raised, straight	Tabular	45–54 × 144–153	Diacytic	GTM
O. sicula	Raised, sinuate	Tabular	64–77 × 30–40	Anomocytic	GTM, NCT^{ii}
O. viscosa subsp. *breviflora*	Raised, sinuate	Tabular	35–45 × 26–30	Anomocytic	GTM, NCT^{ii}, GTU^{ii}

Note: for trichome abbreviations see the synopsis of trichomes in the discussion section.

Data collection and analysis

Fifty-seven morphological traits divided to 147 character states were analysed and scored (Table 3). Data matrix was constructed of 11 OTUs × 50 binary and multistate characters (Table 4). Cluster and ordination analyses were used to asses the phenetic relationships between taxa using PAST 2.17c (PAlaeontological STatistics) software package (Hammer et al., 2001).

A similarity matrix was calculated without data standardization using the Manhattan distance measure in three different clustering algorithms: single linkage, ward's method and un-weighted pair-group method with arithmetic means (UPGMA) to generate phenograms. The co-phenetic correlations were then calculated in the three different cases between the tree matrix and the similarity matrix. High co-phenetic correlation coefficient (between 0.8 and 0.9) indicates that the hierarchic classification obtained by the clustering method is a reasonably faithful representation of the original resemblance matrix (Sokal, 1986). The phenogram with the highest co-phenetic correlation coefficient is the best. In order to check the repeatability of our phenetic data in grouping taxa, ordination analysis was then carried out using the principal component analysis method (PCA).

Results

Morphological characters

The most correlated characters with PC 1 and PC 2 that can be used for delimitation Egyptian taxa of *Ononis* are: habit, shape of the leaflet blade, leaflets symmetry, petiole length, number of teeth on leaflet margins, stipules, type of inflorescence, length of pedicel, wither peduncle elongate to produce arista, colour of flowers, hairs on sepals, length of calyx tube in relation to calyx teeth, length of calyx in relation to length of corolla, standard hairiness, presence or absence of conjunctive teeth on the wing petals, legume shape, number of seeds per pod, seed colour and shape (Figs. 1–3).

Epidermal characters and different types of trichomes found on Egyptian *Ononis*

1. Non-glandular compound trihomes (NCT), further devided into two subtypes

- (NCT^{i}) composed of 2–4 uniformly elongated cells, 150–170 μm long, the apical cell with hook- like ending, the basal cell often enlarged in bulbus-like sturcture. This type is uniquely recorded in *O. variegata* and it can be used to diffrentiate this taxon from the remaining taxa (Fig. 4E).

- (NCT^{ii}) composed of 3 to 9 cells, 360–400 μm long, apical cell is acute, accuminate, or obtuse, basal cell often elongated (Fig. 4F). This is a universal type in all studied taxa, however its more recognized on calices of *O. reclinata*, *O. sicula*, and *O. viscosa* and where it is very long (up to 2.0 mm) and intermixed with shorther glandular trichomes (Fig. 4B, D, F).

2. Glandular trichomes with unicellular head (GTU), further devided into two sub-types

- (GTU^{i}) with unicellular stalks 50–60 μm long and unicellular head, it was recorded on *O. diffusa* and *O. serrata.*

- (GTU^{ii}) with bicelled (or sometimes 3-celled) stalks 300–350 μm long and unicellular head it was recorded on *O. diffusa*, *O. natrix*, and *O. serrata* (Fig. 4C, F).

Table 3. Studied morphological characters, character states and their taxonomic codes.

Code	Morphological characters	Character states and taxonomic codes	Code	Morphological characters	Character states and taxonomic codes
1	Habit	Herb (1), subshrub (2)	17	Stipules shape	Ovate (1), oblong (2), obovate (3), linear- lanceolate (4), deltoid (5), rhombic (6)
2	Plant height (cm)	<40 cm (1), >40 cm (2)	18	Stipules margin	Entire (1), denticulate (2)
3	Leaves	Mostly trifoliate (1), mostly unifoliate (2)	19	Stipule apex	Acute (1), subulate (2)
4	Leaflets blade	Obovate (1), oblong (2), ovate (3), linear-lanceolate (4), deltoid (5)	20	Number of main veins on stipules	3–5 nerved (1), many nerves (2)
5	Leaflets symmetry	Median 1.25 folds larger than lateral (1), median 1.5–2 folds larger than lateral (2), NA (3)	21	Type of inflorescence	Solitary flowers (1), loose raceme (2); dense raceme or dense spike (3)
6	Leaflet length	Small ranges 1.0-6.0 mm (1), large up to 25.0 mm (2)	22	Colour of flower	Whitish to pink (1), yellow (2)
7	Leaflet width	Narrow with maximum width not exceeding 2.5 mm (1), width up to 12.0 mm (2)	23	Aristum	Present (1), absent (2)
8	Leaflet base	Attenuate (1), cuneate (2)	24	Length of peduncle	Sessile or extremely short ranges 0.0–1.5 mm (1), medium-length 1.5–3.5 mm (2), well-developed 5.0-30.0 (3)
9	Petiole length	Sessile (1), short 2.0–8.0 mm (2), long 9.0-20.0 mm (3)	25	Sepal symmetry	Equal sepals (1), unequal sepals (2)
10	Petiolule of median leaflet	Sessile (1), short < 1.0 mm (2), long > 1.0 mm (3), NA (4)	26	Shape of sepals	Linear (1), lanceolate (2), oblong (3), ovate (4)
11	Teeth pairs on leaflet margin	More than 9 pairs (1), less than 9 pairs (2)	27	Lengths of calyx tube in relation to calyx teeth (lobes)	Tube > teeth (1), teeth subequaling or Slightly > tube (2), teeth = 1.5- to 2.5-folds tube (3), teeth = 3- to -5-folds or more tube (4)
12	Hairs on leaves	Glandular (1), glandular and non-glandular (2)			
13	Base of stipules	Sheathing (1), not sheathing (2)	28	Hairs on sepals	Glandular (1), glandular and non-glandular (2), glabrous (3)
14	Type of stipules	Membranous (1), green (2)	29	Length of corolla in relation to length of calyx	Corolla obviously shorter than calyx (1), corolla sub-equaling calyx, slightly longer or slightly shorter than calyx (2), corolla longer than calyx (3)
15	Hairs on stipules	Glandular (1), glabrous (2)			
16	Stipules length	Max. ≤ 5.0 mm (1), max > 5.0 mm (2)			

Table 3. Continued.

Code	Morphological characters	Character states and taxonomic codes	Code	Morphological characters	Character states and taxonomic codes
30	Shape of standard	Orbicular (1), elliptic (2), obovate (3)	45	Legume shape	Globose (1), subglobose (2), ovoid (3), elliptic (4), linear (5)
31	Length of standard	<10.0 mm (1), >10.0 mm (2)	46	Legume beak	Present (1), absent (2)
32	Length of standard's claw	Short < 1.0 mm (1), medium 1.0–1.5 mm (2); Subulate up to 3.0 mm (3)	47	Fruiting calyx	Accrescent (1), not accrescent (2)
33	Hairs on standard	Glandular (1), glabrous (2)	48	Number of seeds per pod	Few seeds 2–6 (1), many seeded up to 25 (2)
34	Apex of standard	Acute (1), subobtuse (2), obtuse (3), retuse (4), mucronate (5)	49	Seed shape	Orbicular (1), sub-orbicular (2), elliptic (3), oblong (4), reniform (5);
35	Standard margins	Entire (1), sinuate (2)	50	Seed colour	Brown (1), blackish (2), dark green (3), pale green (4)
36	Wing length	<10.0 mm (1), >10.0 mm (2)	51	Non-glandular compound trihomes with acute or obtuse apex	Present (1), absent (2)
37	Wing auricles	Minute 0.1 mm to 0.4 mm (1), small 0.5 mm to 0.75 mm (2), large 1.0-1.5 mm (3)	52	Non-glandular compound trichomes with hooked apex	Present (1), absent (2)
38	Conjunctive teeth	Present (1), absent (2)			
39	Keel length	<10.0 mm (1), >10.0 mm (2)	53	Glandular trichomes with unicellular head	Present (1), absent (2)
40	Androecium	Filaments fused to its full-length (1), fused to half-length (2), fused to 3/4-length of the stamina tube (3)			
41	Style	deflexed (1), not deflexed (2)	54	Glandular trichomes with multicellular heads	Present (1), absent (2)
42	Length of style	<5.0 mm (1), >5.0 mm (2)			
43	Hairs on base of style	Present (1), absent (2)	56	Epidermal anticlinal walls	Raised and straight (1), raised and sinuate (2)
44	Legume	Pendent (1), not pendent (2)	57	Epidermal periclinal walls	Concave (1), convex (2), tabular (3)

Table 4. Data matrix obtained from coding of 57 characters (see Table 3).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
O. diffusa	1	2	1	1	1	2	2	1	2	3	1	1	2	2	1	1	1	2	1	1	3	1	2	1	2	4	3	1	3
O. serrata	1	2	1	2	1	2	2	1	2	2	2	1	2	2	1	1	1	2	1	1	2	1	2	1	1	1	2	1	3
O. mitissima	1	2	1	1	2	2	2	2	2	3	1	1	2	1	2	2	3	1	1	2	3	1	2	1	1	2	1	1	2
O. variegata	1	1	2	3	3	1	1	2	1	4	2	1	1	2	2	1	6	2	1	2	1	2	2	2	1	4	2	3	3
O. natrix subsp. *natrix*	2	2	1	2	2	2	2	2	3	3	1	2	2	2	1	2	4	1	2	1	2	2	1	2	1	1	3	2	3
O. natrix subsp. *stenophylla*	2	2	1	4	2	1	1	2	2	2	2	2	2	2	1	1	2	1	1	1	2	2	1	2	1	1	3	2	3
O. vaginalis	2	2	1	3	2	1	1	1	1	1	2	2	1	2	1	2	6	2	1	2	1	2	1	2	1	2	3	2	3
O. reclinata subsp. *mollis*	1	1	1	5	2	2	2	1	2	2	2	2	2	2	1	1	3	2	1	1	3	2	2	1	1	3	4	2	2
O. pubescens	1	1	1	2	1	2	2	2	3	3	1	1	2	2	1	2	1	1	1	1	1	1	2	1	1	1	4	1	1
O. sicula	1	1	1	4	2	2	1	2	2	3	2	2	2	2	1	2	4	1	2	1	1	2	1	2	1	2	4	2	1
O. viscosa subsp. *breviflora*	1	1	1	3	1	2	2	2	2	3	2	2	2	2	1	2	5	1	1	1	1	2	1	2	1	1	4	2	2

Table 4. Continued.

Character coding	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
O. diffusa	1	2	3	1	5	1	3	2	1	2	3	2	2	2	2	2	1	1	1	2	1	1	2	2	1	1	1	3
O. serrata	2	1	3	1	1	1	1	1	1	1	2	2	2	2	3	2	1	1	1	1	1	1	2	1	1	1	1	1
O. mitissima	1	2	3	2	3	1	1	2	1	1	3	2	2	2	4	1	1	2	1	1	1	2	2	2	1	2	1	1
O. variegata	3	2	3	1	3	1	2	1	2	2	1	2	1	2	4	2	1	2	1	5	1	2	1	2	2	1	1	3
O. natrix subsp. *natrix*	1	1	3	2	3	2	3	1	2	2	3	2	2	1	5	1	1	2	2	5	2	2	2	1	1	2	2	3
O. natrix subsp. *stenophylla*	1	1	1	2	3	2	2	1	2	1	3	2	2	1	5	1	1	2	2	5	2	2	2	2	1	2	1	2
O. vaginalis	1	1	2	2	3	2	3	1	2	2	3	2	2	1	5	2	1	2	2	4	3	2	2	1	1	2	1	2
O. reclinata subsp. *mollis*	1	2	1	2	1	1	1	1	1	1	2	2	1	2	1	1	2	1	2	2	1	1	2	2	1	2	1	3
O. pubescens	2	2	1	2	3	2	2	1	2	2	2	1	2	1	5	2	1	2	2	2	1	2	2	2	1	2	1	3
O. sicula	2	1	2	2	3	1	1	1	2	1	3	1	2	1	5	1	1	2	2	5	1	1	2	2	1	1	2	3
O. viscosa subsp. *breviflora*	3	1	2	2	4	1	2	1	2	1	3	1	2	1	5	1	1	2	2	4	4	1	2	1	1	1	2	3

Table 5. Correlation between the morphological characters and the first two principal components PC1 and PC2.

Code	Morphological trait	PC 1	PC 2	Code	Morphological trait	PC 1	PC 2
1	Habit	0.073643	-0.0535	30	Shape of standard	0.084387	0.071358
2	Plant height	-0.04648	-0.03391	31	Length of standard	-0.08966	0.10593
3	Leaves	0.026011	0.10722	32	Length of standard's claw[2]	-0.04836	0.16594
4	Leaflets blade[a]	0.1869	0.05428	33	Hairs on standard	0.061651	-0.12213
5	Leaflets symmetry[b]	0.097326	0.18297	34	Apex of standard	0.078137	-0.0313
6	Leaflet length	-0.07664	-0.08135	35	Standard margins	0.057666	-0.11525
7	Leaflet width	-0.10399	-0.06609	36	Wing length	0.085165	-0.00651
8	Leaflet base	0.076759	-0.05927	37	Wing auricles	-0.07646	0.027849
9	Petiole length[b]	-0.05312	-0.20836	38	Conjunctive teeth[1]	0.14336	-0.05221
10	Petiolule of median leaflet	0.008145	0.096978	39	Keel length	0.023195	0.052745
11	Teeth pairs on leaflet margin[b]	0.069416	0.061539	40	Androecium[2]	0.030858	-0.17705
12	Hairs on leaves	0.11008	-0.06507	41	Style	-0.04371	0.10592
13	Base of stipules	-0.06015	-0.11897	42	Length of style[2]	-0.00277	-0.13983
14	Type of stipules	0.032457	-0.00469	43	Hairs on base of style	-0.11735	0.15943
15	Hairs on stipules	-0.00645	0.11191	44	Legume[1,2]	0.31872	-0.29532
16	Stipules length	0.068406	-0.11712	45	Legume shape	-0.04349	0.072129
17	Stipules shape[a,b]	0.48628	0.38752	46	Legume beak	-0.02324	0.03261
18	Stipules margin[b]	-0.05075	0.16649	47	Fruiting calyx	0.1109	-0.04752
19	Stipule apex	0.050372	-0.0429	48	Number of seeds per pod	0.094108	-0.12682
20	Number of main veins on stipules	0.027697	0.12366	49	Seed shape[1]	0.48766	0.049355
21	Type of inflorescence[a]	-0.20355	0.047407	50	Seed colour[1]	0.20476	-0.12847
22	Colour of flower[a]	0.1361	0.042155	51	Non-glandular compound trichomes with acute or obtuse apex	0.05122	-0.00334
23	Aristum[a]	-0.13332	0.097675	52	Non-glandular compound trichomes with hooked apex	-0.02601	-0.10722
24	Length of peduncle[a]	0.15934	0.009546	53	Glandular trichomes with unicellular head	-0.045818	0.053045
25	Sepal symmetry	-0.044	0.023159	54	Glandular trichomes with multicellular heads	0.026011	0.10722
26	Shape of sepals[b]	-0.0714	0.45754	55	Type of stomata	0.00197	-0.07796
27	Lengths of calyx tube in relation to calyx teeth[b]	0.10303	-0.18166	56	Epidermal anticlinal walls	0.082697	-0.07181
28	Hairs on sepals[a,b]	0.16211	0.14938	57	Epidermal anticlinal walls	0.082222	0.050339
29	Length of corolla in relation to length of calyx	0.000611	0.14564				

[a]Traits with high scores in PC1. [b]Traits with high scores in PC2.

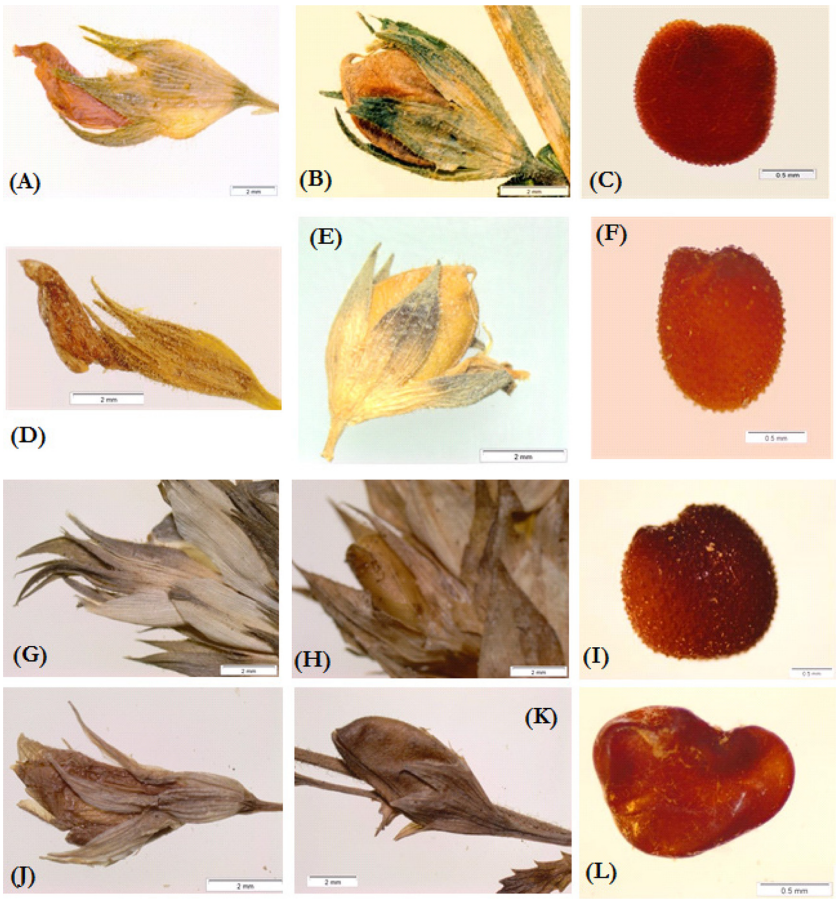

Fig. 1. Flower, pod and seed morphology of *Ononis diffusa* (**A**–**C**), *O. serrata* (**D**–**F**), *O. mitissima* (**G**–**I**), and *O. variegata* (**J**–**L**). Scale bar = 2 mm for flowers and pods, 0.5 mm for seeds.

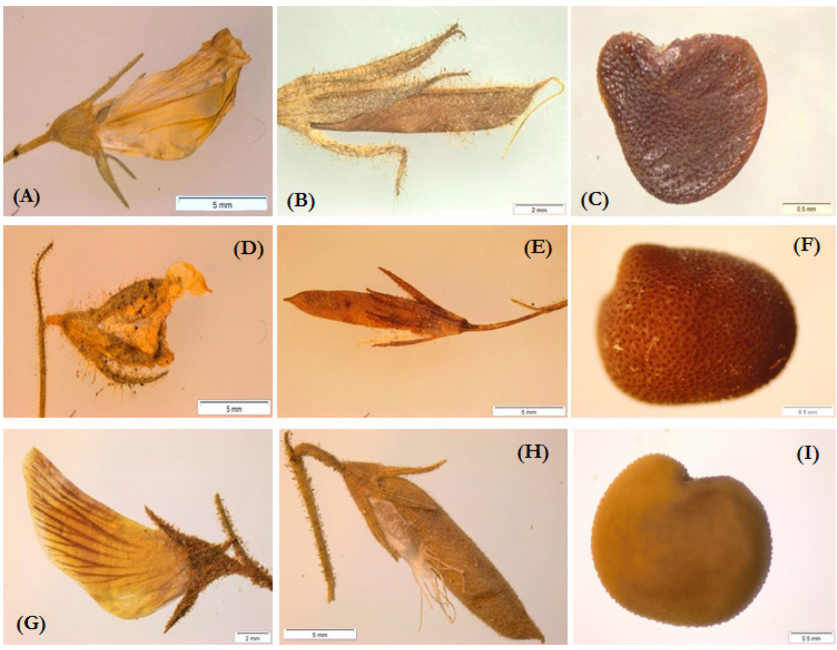

Fig. 2. Flower, pod and seed morphology of *Ononis natrix* L. subsp. *natrix* (**A**–**C**), *O. natrix*. subsp. *stenophylla* (**D**–**F**), and *O. vaginalis* (**G**–**I**). Scale bar = 5 mm for flowers and pods except for B, G (2 mm) and 0.5 mm for seeds.

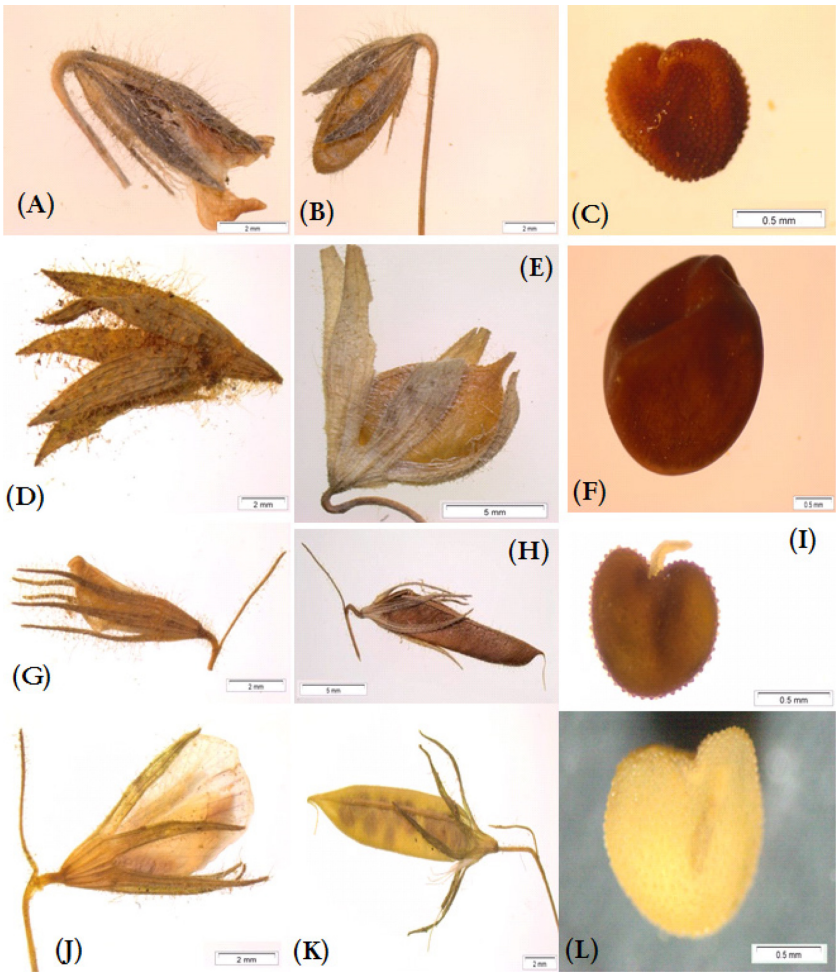

Fig. 3. Flower, pod and seed morphology of *Ononis reclinata* subsp. *mollis* (**A**–**C**), *O. pubescens* (**D**–**F**), *O. sicula* (**G**–**I**), and *O. viscosa* subsp. *breviflora* (**J**–**L**). Scale bar = 2 mm for flowers and pods except for E, H (5 mm) and 0.5 mm for seeds.

3. Glandular trichomes with clavate multicellular heads (GTM) and uniseriate multicellular stalks of 450–600 μm long, the glandular head compose of 3–6 cells divided longitudinally and/or tranversally, this type is dominant in all Egyptian *Ononis* (Fig. 5A, C, E) except for *O. variegata*.

In general, stomata are very variable in Papilionoideae, no single type being present throughout any one of the tribes. For Egyptian *Ononis*, two types were recorded (Table 2), the diacytic (the Rubiaceous type) in *O. diffusa* (Fig. 6A), *O. serrata* (Fig. 6B), *O. variegata* (Fig. 6D), *O. sicula* (Fig. 7D), and *O. viscosa* subsp. *breviflora* (Fig. 7E); and the anomocytic (the Ranunculaceous type) in *O. natrix* (Fig. 6E, F), *O. vaginalis* (Fig. 7A), *O. reclinata* (Fig. 7B), *O. pubescens* (Fig. 7C), and *O. mitissima* (Fig. 6C).

Ultrastructure of foliar epidermal cells

These characters can also be used as good tools for delimitation of some closely allied taxa, for instance, *O. diffusa* and *O. serrata* are very similar species; however, the periclinal walls of the epidermal cells is tabular in the former and concave in the latter (Fig. 6). Another example, the two subspecific taxa of *Ononis natrix* have different foliar epidermal cells ultrastructure; in *O. natrix* subsp. *natrix* the anticlinal walls are sinnuate and the periclinal walls are tabular, while in *O. natrix* subsp. *stenophylla* the anticlinal walls are straight and the periclinal walls are convex (Fig. 6).

The cluster analysis

The chosen phenogram is the one calculated by UPGMA (Fig. 8), because it obtained the highest co-phenetic correlation coefficient (0.8898) which indicates a good fit between the phenogram and the distance matrix. Single linkage and ward's method of clustering obtained co-phenetic correlation coefficients of 0.862 and 0.7747, respectively. At dissimilarity

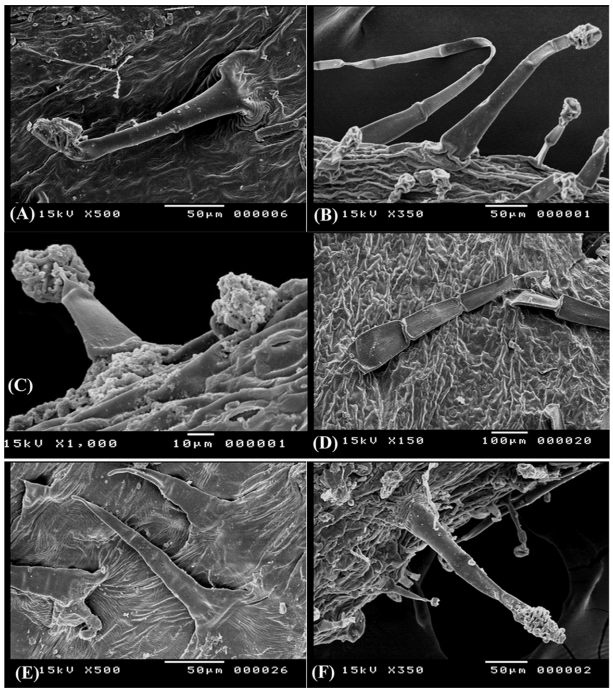

Fig. 4. Trichome diversity in *Ononis diffusa* on calyx lobe (**A**), *O. serrata* on calyx lobe (**B**) and adaxial surface of leaf (**C**), *O. mitissima* on abaxial surface of leaf (**D**), *O. variegata* on calyx lobe (**E**), and *O. natrix* subsp. *natrix* on calyx lobe (**F**).

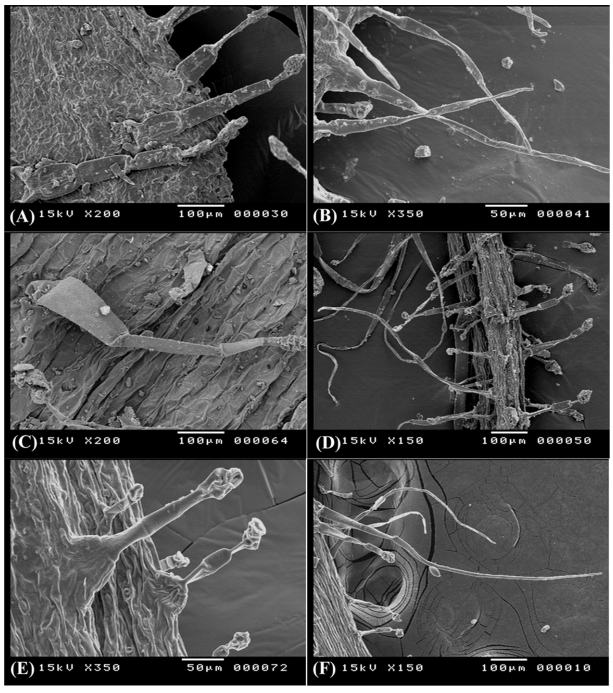

Fig. 5. Trichome diversity in *Ononis natrix* subsp. *natrix* on abaxial surface of leaf (**A**), *O. vaginalis* on abaxial surface of leaf (**B**), *O. pubescens* on abaxial surface of leaf (**C**), *O. sicula* on calyx lobe (**D**), and *O. viscosa* subsp. *breviflora* on adaxial surface of leaf (**E**) and on calyx lobe (**F**).

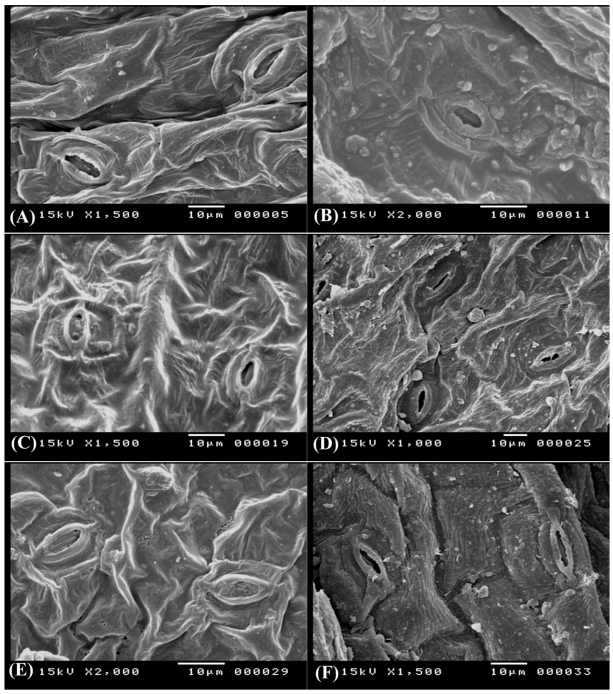

Fig. 6. Epidermal cells and stomata on adaxial surface of leaves in *Ononis diffusa* (**A**), *O. serrata* (**B**), *O. mitissima* (**C**), *O. variegata* (**D**), *O. natrix* subsp. *natrix* (**E**), and *O. natrix* subsp. *stenophylla* (**F**).

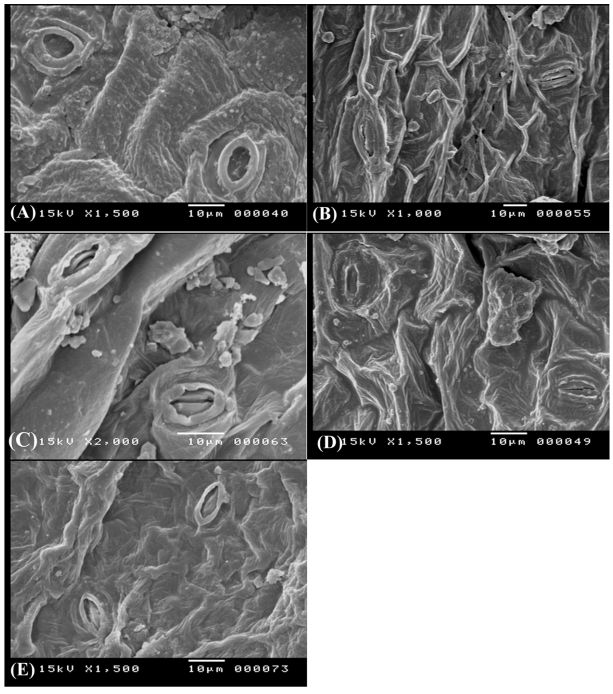

Fig. 7. Epidermal cells and stomata on adaxial surface of leaves in *Ononis vaginalis* (**A**), *O. reclinata* (**B**), *O. pubescens* (**C**), *O. sicula* (**D**), and *O. viscosa* subsp. *breviflora* (**E**).

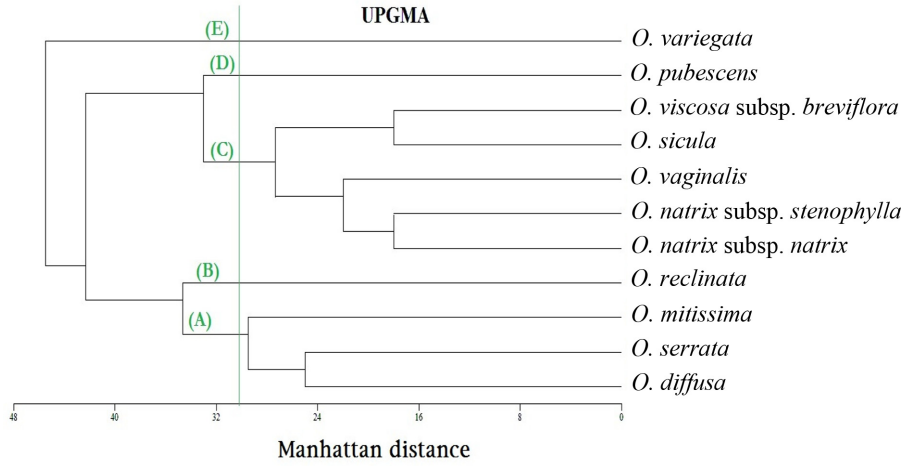

Fig. 8. Un-weighted pair-group method with arithmetic means (UPGMA) dendrogram of the 11 taxa of *Ononis* in Egypt using the Manhattan distance measure based on 57 morphological characters.

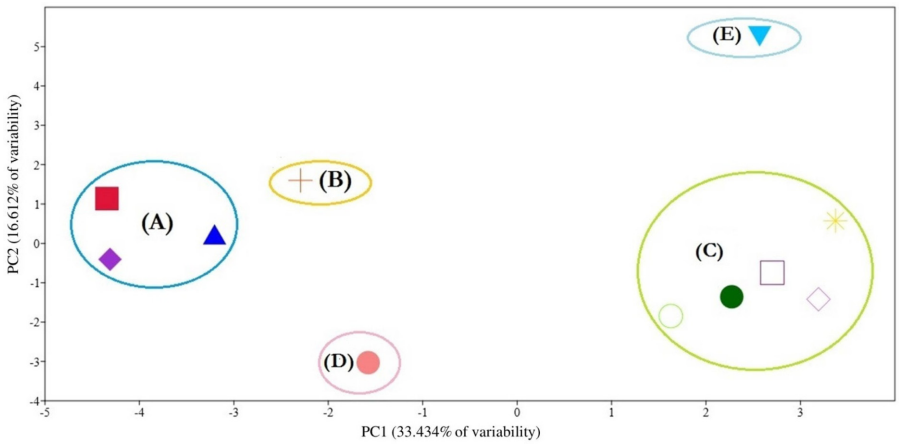

Fig. 9. Scatter plot of the first two principal components based on 57 morphological characters. Symbols represents species: (■)*Ononis diffusa*, (▲) *O. mitissima*, (●) *O. natrix* subsp. *natrix*, (○) *O. natrix* subsp. *stenophylla*, (●) *O. pubescens*, (+) *O. reclinata*, (♦) *O. serrata*, (□) *O. sicula*, (✱) *O. vaginalis*, (▼) *O. variegata*, (◊) *O. viscosa* subsp. *breviflora*

level of 30.5, five groups are separated:

- Group (A): consists of two sub-groups separated at dissimilarity level of 29.5, the basal one comprises *Ononis diffusa* and *O. serrata*, the other contains *O. mitissima*.

- Group (B): comprises only *O. reclinata* subsp. *mollis* (separated at dissimil. 34.67).

- Group (C): contains two sub-groups separated at dissimilarity level of 27.33. The first consists of three taxa: *O. vaginalis* (separated at dissimil. 22.0) and the two subspecies of *O. natrix* (subsp. *natrix* and subsp. *stenophylla*, separated at dissimil. 18.00). The second sub-groub contains two taxa, namely: *O. sicula* and *O. viscosa* subsp. *breviflora* (separated at dissimil. 18.0).

- Group (D): includes only *O. pubescens* (separated at dissimil. 33.0).

- Group (E): contains only *O. variegata* (separated at dissimil. 45.5).

Principal component analysis

The PCA provided the numerical values corresponding to the morphological attributres used in our taxonomic analysis. The first 10 principal components are accounted for 100% of the variation. The PCA scater plot (Fig. 9) gives the same five groups separated by the UPGMA clustering (Fig. 8).

Discussion

Multivariate statistical analyses are good tools to impartially

compare among numerous characters (Barrington et al., 1989). Claiming cluster analysis produces poor representation of the more distant relationships among OTUs, and on the contrary the ordination produces poor representation of the closer relationships (Sneath, 1976), therefore we decided to use both methods of analyses and compare their results. In the present study, the UPGMA cluster analysis (Fig. 8) and PCA analysis (Fig. 9) gave the same five segregates, which indicate unbiased phenetic delimitation of taxa. The first two principal components are responsible for 50.046% of the variability.

Group A: the *Ononis* group

Its members are characterized by sessile to sub-sessile flowers (pedicel length ranges from 0.0–1.5 mm); flowers are arranged on raceme or spike-like inflorescences; the floral peduncles don't elongate forming arista; calyx shoter than corolla; corolla white with pink or purple lines on standard, wing petals with distinct conjunctive teeth; legume not pendent, globose to ovoid or elliptic, few seeded; seeds are dark coloured and tuberculate (Fig. 1).

This group is further sub-divided into:

1. Sub-group A1

This sub-group is characterized by glandular green stipules; the calyx teeh are longer than calyx tube (1.5×–2.5×); floral standard covered with glandular hairs; plants are covered with heterotrichous indumentum (consists of glandular and non-glandular trichomes); leaves have annomocytic stomata (Figs. 4, 6).

2. Sub-group A2

This sub-group is characterized by glabrous membranous stipules; the calyx teeth is shorter than its tube; glabrous standard; plants are covered with homotrichous indumentum (consists of glandular trichomes only); leaves have diacytic stomata (Figs. 4, 6).

Group B: The *Reclinata* group

This taxon is a very small annual herb, ranging from 3.0–20.0 (–30.0) cm long, characterized by deltoid cuneate leaflets with only about 2–3 pair of teeth on leaflet's margins; flowers are solitary, deflexed; floral pedicel is 5.0–15.0 mm long; floral peduncle does not elongate to arista; calyx longer than or subequals corolla; calyx teeth much longer that its tube (3×–5×); corolla white with pink glabrous standard; wing petals without conjunctive teeth; legume pendent, linear, many seeded; legume apex rounded (not beaked); the plant covered with heterotrichous indumentum, leaves have diacytic stomata (Figs. 3, 5, 7).

Group C: the *Natrix* group

This group is characterized by trifoliate leaves as the dominant type of leaves; stipules are large, green and glandular, flowers are yellow, standard glabrous with stripped by reddish veins; floral pedicels are long; peduncles elongate to produce arista; legume is linear, pendent and multi-seeded; the plants are covered with heterotrichous indumentum consists of glandular and non-glandular trichomes (Figs. 2–5).

The group is further subdivided into two sub-groups:

1. Sub-group C1

It comprises the annual herbs, its members are characterized with short corolla, shorter than or sub-equals the calyx; the length of calyx teeth is about 1.5×–2.5× the length of calyx tube; leaves have anomocytic stomata (Fig. 7).

2. Sub-group C2

It comprises the sub-shrubby taxa; they are characterized by long corolla, much longer than calyx; the length of calyx teeth is about 3× to 5× of the length of calyx tube; leaves have diacytic stomata (Fig. 6).

Group D: the *Pubescens* group

This taxon is characterized by homotrichous indumentum densely covering the whole plant, consisting of multicellular glandular trichomes; the leaves are of two type (trifoliate and simple); leaflets are oblong; stipules very large (9.0–18 × 2.0–4.0 mm); flowers are arranged on dense racemes, pedicels are long; peduncles not elongate into arista; flowers yellow; calyx sub-equals corolla; calyx teeth much longer than calyx tube; wing petals with distinct conjunctive teeth; base of style hairy; legume not pendent, subglobose, few seeded; seeds are large and smooth; leaves have diacytic stomata (Figs. 3, 5, 7).

Group E: the *Variegata* group

This group is characterized by simple leaves as the dominant type of leaves; stipules are small, green and glabrous, floral pedicels are long; peduncles don't elongate to produce arista; flowers are white, calyx glabrous, shorter than corolla; standard hairy with stripped by purple veins; base of style hairy; legume is oblong, pendent and multi-seeded; seeds are reniform and dark coloured; the plants are covered with homotrichous indumentum consists of non-glandular trichomes with the apical cells with hook-like ending and the basal cells often enlarged in bulbus-like structure; leaves have diacytic stomata (Figs. 1, 4, 6).

In conclusion, the present study suggests a new circumscription for the taxonomy of the genus *Ononis* in Egypt. Our results are, in some certain degree, in line with the

traditional sub-generic classification of Širjaev (1932), this fact is clear in grouping the representative members of sub-sections *Diffusae* (in a sub-group under group A) and *Mittisimae* (in the other sub-groups under the same group), the same goes for Group C which encloses the representative members of sub-sections *Viscosae* (in sub-group) and *Natrix* (in the other). However, the segregation of *Ononis reclinata* subsp. *mollis*, *O. pubescens* and *O. variegata* cannot be explained in the view point of the traditional sub-generic classification made by Širjaev (1932).

Although, our rudimentary phenetic data cannot yet reflect the true evolutionary history and the phylogeny among *Ononis* species in Egypt, however, our findings are with some accordance with the molecular circumscriptions described by previous authors such as (Turini et al., 2010). The phylogenetic results obtained by Turini et al. (2010) using the trnL-F and ITS DNA proved the closeness of *Ononis diffusa*, *O. serrata*, and *O. mitissima*, as they were all grouped in Clade V - sub-clade C (here are comprised in group A) which are morphologically sharing the annual habit; reduced floral peduncles without arista; whitish-pink flowers and globose, sub-globose to elliptic few-seeded pods (2–6 seeds/pod). Also, the molecular phylogeny proved the closeness of *O. visccosa*, *O. sicula*, *O. natrix*, and *O. vaginalis*, all grouped together in Clade III – sub-clade A (here in group C), these species are phenetically sharing the well-developed floral peduncles with distinct arista; yellow flowers and linear many-seeded pods (up to 25 seeds/pod).

Turini et al. (2010) discussed the polyphyly of sub-sect. *Reclinatae*, and they recommended reassigning some taxa out of this sub-section. Here, the phenetic uniqueness of *Ononis reclinata* subsp. *mollis* is formally established.

It was clearly nested in a separate group (group B), it exhibits an intermediate position between the two large groups A and C, sharing some characters from both groups, it shares the pinkish flowers and the ex-aristate peduncles with group A and it has the long pedicels and linear many-seeded pods in common with group C.

Moreover, our phenetic observations contradict with the classic sectional concept (dividing the genus into two sections namely, sect. *Ononis* and sect. *Natrix*, *sensu* Širjaev 1932). This opinion was also adopted by Turini et al. (2010). A case in point is *Ononis variegata*, a species was once classified by Širjaev (1932) to be included in sect. *Ononis*, sub-sect. *Variegatae*, however, the molecular phylogenetic proved that, sub-section *Variegatae* was unnaturally placed, and it is more closely related to members of Clade V (sub-clade B) away from section *Ononis* sensu Širjaev (1932). From our phenetic point of view, *O. variegata* is phenetically separated (in group E) away from the remaining representatives of section *Ononis* (*O. diffusa*, *O. serrata* and *O. mitissima*).

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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Appendix 1. Voucher specimens used for the scanning electron microscopy

Taxa	Voucher
O. diffusa	EGYPT. Rafah, Apr 1921, *Hefnawy s.n.* (CAIM)
O. serrata	EGYPT. El-Garawla, 15 km before Marsa Matruh, 31°15'34"N, 27°22'12"E, elev. 11 m, 7 Apr 2015, *Faried, A., Olwey, A. and Hassan, M. s.n.* (ASTU)
O. mitissima	EGYPT Shubra, on wayside of fields, 10 Apr 1940, *Shabetai, J. R. z6248* (CAIM)
O. variegate	ALGÉRIE. Sable maritime à Sidi – Ferruch, 18 Apr 1953, *D'Alleizette, Ch. s.n.* (CAI)
O. natrix subsp. natrix	LIBYA. Wadi Derna, 14 Mar 1968, *Boulos, L. 2435* (CAI)
O. natrix subsp. stenophylla	EGYPT. Rafah; desert sands of north the coast, 4 Jun 1934, *Shabetai, J. R. z6032* (CAIM)
O. vaginalis	EGYPT. Al-Dakhla, Ras El-Hekma, 31.45741N,26.79281E, elev. 5 m, 1 Jun 2011, *Zareh, M. and Faried, A. s.n.* (ASTU)
O. pubescens	JORDON. 7 km east of Al-Hemma, 13 May 1976, *Täckholm, V., El-Hadidi, M. N., Lahham, J. and Boulos, L. 8907* (CAI)
O. reclinata subsp. mollis	EGYPT. Wadi of Rokhama between Cairo and Suez, 15 Apr 1926, *Boulos, L. s.n.* (CAI); West El-Arish, near the town, 22 Apr 1959, *Täckholm, G. s.n.* (CAI)
O. sicula	EGYPT. Sinai, Rod El Ahmer, Wadi Rozneh, 12 May 1927, *Kaiser, A. 720* (CAIM)
O. viscosa subsp. breviflora	EGYPT. Southern Sinai. Wadi Gebal region: Al-Sheq, 28.3226N, 33.5623E, elev. 1,940 m, 13 May 2004, *Fayed, A., El Garf I., Abdel-Khalik, K. and Osman, A. s.n.* (ASTU)