SDS-PAGE of Seed Protein Criteria in Relation to Taxonomy of *Onobrychis* Sect. *Lophobrychis* s. str. and the Egyptian Species

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Summary SDS-PAGE of seed protein bands profile was recorded in 22 individuals of 6 species of *Onobrychis* from 6 populations, including the Egyptian species and most representatives of Sect. *Lophobrychis*. The produced data, in addition to the available morphological, cytological and the geographical characters in the literature were analyzed by the NTSYS program package using the UPGMA clustering method. The interspecific variability within the species studied of the section *Lophobrychis* was discussed; the genetic and morphological differences between the Egyptian species were assessed.

Key words Cytotaxonomy, Electrophoresis, Polyploidy, Protein profiles, UPGMA.

Onobrychis Miller is a forage genus, drought-resistant, nitrogen fixing and has been exploited to maintain soil productivity of wild pasture and as high-protein fodder plant for ruminants and equines (Lane *et al.* 2000). Their species are distributed in the temperate regions of Northern Hemisphere and extends from the Mediterranean region to Caucasia, the Zagros Mountains and central Asia with a centre of diversity in Northwest Asia (Yildiz *et al.* 1999, Aktoklu 2001). The genus belonging to the Tribe Hedysareae, Subfamily Papilionoideae of the Leguminosae comprises about 170 mostly perennial species (Aktoklu 2001), 8 sections and 2 subgenera (Boissier 1872, Sirjaev 1925, Yildiz *et al.* 1999). Only 2 species represent the genus in the Egyptian wild flora, namely *O. crista-galli* (L.) Lam. of Sect. *Lophobrychis* Hand.-Mazz., Subg. *Onobrychis* (Bunge) Grossh and *O. ptolemaica* (Delile) DC. of Sect. *Hymenobrychis* D.C., Subg. *Sisyrosemma* Bunge (Ball 1968, Tackholm 1974, Boulos 1999).

The Sect. *Lophobrychis* has been a subject of much confusion mainly because of comprising the annual species of the genus (Handel-Mazzetti 1910, Sirjaev 1925) as well as the different approaches to species delimitation. Goldblatt (1981) pointed out that, only the species of *Lophobrychis* have $x=8$ whereas those of the whole genus have $x=7$ and considered the section as unspecialized. However, controversial chromosome counts even basic numbers have been reported within most species viz. $2n=2x=14$, $2n=2x=16$ and $2n=4x=28$ in *O. aequidentata*, $2n=2x=14$ and $2n=2x=16$ in *O. caput-galli* and $2n=2x=14$ and $2n=2x=16$ in the Egyptian *O. crista-galli*. Abou-El-Enain (2002) pointed out that the interspecific variability based on karyological criteria demonstrates a complexity of the evolutionary patterns of these species. Ashurmetov and Normatov (1998) reported interspecific variability in the embryological features of some *Lophobrychis* species that are only found in a comparatively small number of angiosperms. On the other hand, Magulaev (1995) and Yildiz *et al.* (1999) reported a close phylogenetic relationship between the Sect. *Lophobrychis* and its related Sect. *Hymenobrychis* based on cytological and fruit morphologi-

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cal characters, respectively.

Polyacrylamide gel electrophoresis technique in the presence of sodium dodecyl sulfate (SDS-PAGE) of seed proteins has a useful role in the identification and classification of various taxa (Paino *et al.* 1990, Ahmad and Slinkard 1992, Nath *et al.* 1997, Abou-El-Enain and Loutfy 1999, Badr *et al.* 2000). Except those of Cenci and Mizianty (1997) and Cenci *et al.* (2000), no studies have been made on the impact of seed protein electrophoretic criteria on the interspecific relationships in the genus *Onobrychis*. None of these studies have concentrated either on the Egyptian species or those of Sect. *Lophobrychis*. Using the seed protein electrophoretic criteria, the present work aimed at demonstrating the genetic and morphological differences between the Egyptian species, thereby establishing the taxonomic relationships between the species studied. In this analysis, available data on morphological, cytological characters as well as the geographical distribution are referred in considering numerical methods.

Materials and methods

In the present study, seeds of 22 individuals representing 6 species from 6 populations including the Egyptian species and most representatives of Sect. *Lophobrychis* were examined. The studied materials and their source, origin, geographical distribution and sectional delimitation (Yildiz *et al.* 1999) are given in Table 1. Taxonomic identification and nomenclature of the Egyptian species follow Tackholm (1974) and Boulos (1999). Voucher specimens are kept at the Herbarium of Biological Sciences and Geology Department, Faculty of Education, Ain Shams University, Egypt.

For extracting and analysing the total seed protein, SDS-PAGE technique was used as described by Badr *et al.* (2000). For creating the data matrix for computation, the distinguishable seed protein bands of the present study and the other criteria compiled from the literature *i.e.* morphological (Ball 1968, Tackholm 1974, Boulos 1999, Yildiz *et al.* 1999), geographical distribution (Table 1) and cytological (Abou-El-Enain 2002) were selected, scored and coded as indicated in Table 3. The relationship between the species studied was measured by calculating their average taxonomic distance (dissimilarity) and presented as phenograms based on the analysis for each of the morphological, seed protein and the all combined data sets (Figs. 2–4, respectively). These analyses were performed using the NTSYS program package for IBM-pc as described by Rohlf (1993). In the computer analyses, the species are numbered as indicated in Table 1.

Results and discussion

The micrograph revealing the produced SDS-PAGE banding patterns of seed protein analysis in the species studied is shown in Fig. 1, whereas the produced bands and their molecular mass (MW) are presented in Table 2. The selected 118 characters (40 SDS-PAGE) of this study and those obtained from the literatures (50 morphological, 26 geographical, 2 cytological) and their codes are given in Table 3. The produced phenograms illustrating the relationships (classifications) of the species studied are presented in Figs. 2–4, in which the characters analyses of the morphological data, seed protein data and the all combined data sets are presented, using the UPGMA clustering method.

The produced classification of the species studied based on the character analysis of the all combined data sets (Fig. 4) reveals a clear distinction and considerable linked characteristics among the species studied of the Sect. *Lophobrychis*, *i.e.* *O. aequidentata* (1), *O. caput-galli* (3), *O. cristagalli* (4) and *O. pulchella* (6). They are clustered together and distinguished from those of Sect. *Hymenobrychis* *i.e.* *O. bobrovii* (2) and *O. ptolemaica* (5) due to the presence of bands of numbers 14, 21 and 33 with molecular mass of 43.0, 32.3 and 20.5 kD, respectively, and absence of the bands of numbers 13, 19 and 35 with molecular mass of 47.7, 36.0 and 17.2 kD, respectively (Table 2). Such

Table 1. The examined species; their source; origin; geographical distribution and sectional delimitation

Species	Source	Origin	Geographical distribution	Section
1) <i>O. aequidentata</i> (Smith) d'Urv. ^{1,2} (= <i>Hedysarum aequidentatum</i> Smith ¹ , <i>Onobrychis aequidentata</i> (Smith) d'Urv. subsp. <i>foveolata</i> (DC.) Sirj. ¹ , <i>O. cretica</i> Desv. ² , <i>O.</i> <i>foveolata</i> DC. ¹)	IPK	France	Asia: Iran ³ , Iraq ⁴ Europe: Albania ^{1,2} , Bulgaria ^{1,2,5} , Crete ^{1,2} , former Yugoslavia ^{1,2} , France ¹ , Greece ^{1,2} , Italy ^{1,2,6} , Sicily ¹ , Spain ^{1,2} , Turkey in Europe ^{1,2,7} , Middle East: Cyprus ¹ , Greece ¹ , Turkey in Asia ¹	Lophobrychis
2) <i>O. bobrovii</i> Grossh. ^{8,9} (= <i>Xanthobrychis bobrovii</i> (Grossh.) Galushko. ⁸)	IPK	Daghestan	Asia: USSR ⁹	Hymenobrychis
3) <i>O. caput-galli</i> (L.) Lam. ^{1,2,7} (= <i>Hedysarum caput-galli</i> L. ¹)	IPK	France	Africa: Algeria ¹ , Libya ¹ , Morocco ¹ , Tunisia ¹ Asia: Iran ^{3,4} , Iraq ⁴ Europe: Albania ^{1,2} , Bulgaria ^{1,2,5} , Crete ^{1,2} , former Yugoslavia ^{1,2} , France ^{1,2} , Greece ^{1,2} , Italy ^{1,2,6} , Sicily ¹ , Spain ^{1,2,10} , Turkey ^{1,2,7} , Middle East: Cyprus ^{1,11} , Greece ¹ , Jordan ¹² , Syria ¹ , Turkey ^{1,7}	Lophobrychis
4) <i>O. crista-galli</i> (L.) Lam. ^{2,13} (= <i>Hedysarum crista-galli</i> L. ¹ , <i>O.</i> <i>armatus</i> Pampan. ¹⁴ , <i>O. ligulifera</i> Pau ¹ , <i>O. squarrosa</i> Viv. ¹⁵)	Wild	Egypt, Borg-Elarab, Bahieg	Africa: Algeria ¹ , Egypt ¹ , Libya ¹ , Morocco ¹ , Tunisia ¹ Asia: Iran ³ , Iraq ⁴ Europe: France ^{1,2} Middle East: Cyprus ¹ , Greece ¹ , Jordan ¹ , Syria ¹ , Sinai ¹ , Turkey ¹	Lophobrychis
5) <i>Onobrychis ptolemaica</i> (Del.) DC. ¹ (= <i>Hedysarum ptolemaicum</i> Delile ¹³ , <i>Onobrychis ptolemaica</i> (Del.) DC. subsp. <i>macroptera</i> C. Towns. ¹ , <i>O.</i> <i>lanata</i> Boiss. ¹³ , <i>O. olivieri</i> Boiss. ¹³)	Wild	Egypt, K. 65th Cairo-Suez Road	Africa: Egypt ^{1,13} Middle East: Palestine ¹ , Lebanon ¹ , Turkey ¹	Hymenobrychis
6) <i>Onobrychis pulchella</i> Schrenk ^{3,7} (= <i>Onobrychis alba</i> (Waldst. & Kit.) Desv. subsp. <i>laconica</i> (Orph. ex Boiss.) Hayek ²)	IPK	Turkmenistan	Asia: Afghanistan ¹⁶ , Iran ¹⁷ , USSR ⁹	Lophobrychis

(1) Greuter, W. *et al.* (eds.) 1989. *Mediterranean flora checklist*. Willdenowia 19: 27–48; (2) Ball, P. W. 1968. (3) Rechinger, K. H. 1984. *Flora Iranica*. Papilionaceae II, Graz Academy of Science; (4) Townsend, C. and Guest, E. 1974. *Flora of Iraq*, Vol. 3, Ministry of Agriculture, Baghdad; (5) Jordanov, D. (ed.) 1976. *Flora Reipublicae Popularis Bulgari-cae*, 6 [1]–590; (6) Pignatti, S. 1982. *Flora Italica*. Edagricole, Bologna; (7) Davis, P. H. 1970. *Flora of Turkey & the East Aegean Islands*, Vol. 3, Edinburgh University Press, Edinburgh, 560–589; (8) Czerepanov, S. K. 1981. *Plantae Vasculares*, Sosudistye Rasteniia SSSR. Leningrad; (9) Komarov, V. L. (ed.) 1948. *Flora of the USSR*. Vol. xiii, [1]–588, Izdatel'stvo Akademii 'Nauka' SSSR, Leningrad; (10) Bolos, O. and Vigo, J. 1984. *Flora de les Paisos Catalans*. Vol. 1, Editorial barci-no, Barcelopa, [1]–736; (11) Meikle, R. D. 1977. *Flora of Cyprus*. Vol. 1, Bentham Moxon Trust, Royal Botanic Gardens, Kew, 1: 1–832; (12) Zohary, M. and Feinbrun-Dothan, N. 1972. *Flora of Palestina*, Part 2. The Israel Academy of Sciences and Humanities, Jerusalem; (13) Boulos, L. 1999. see References (14) Jafri, S. M. 1980. *Fabaceae*. In: *Flora of Libya*, Pt. 86: 1–147. Al Faateh University. Tripoli; (15) Tackholm, V. 1974. see References (16) Kitamura, S. 1960. *Flora of Afghanistan*. Research of Kyoto University Expedition. Vol. 2; (17) Parsa, A. 1948. *Flore de l'Iran*. Vol. 2. Teheran: Im-primerie Mazaheri. IPK=The Institute of Plant Genetic & Horticulture gene bank, Gatersleben, Germany.

relationships are confirmed by the character analyses of all the used data sets (Figs. 2–4). Morphologically, they are characterized as follows: appressed-hairy surface; stems erect or ascending; stipules 0.4–0.6 cm, acuminate, scarious with a green median nerve; leaflets 5–9 pairs, 0.6–1.2×0.1–0.3 cm, short-petiololate, oblong to linear, the apex truncate-mucronate; peduncles as long as the subtending leaf; racemes 2–10-flowered; calyx teeth 0.5–0.6 cm; corolla as long as the calyx, pink or white; pod shows ventral and dorsal asymmetry, 1–1.2×0.8–1 cm, oblong to hemispherical, compressed, with dorsally crest, setae, spines; valves pubescent; seeds 0.2, 0.3×0.3 cm, compressed, smooth, brown (Ball 1968, Boulos 1999, Yeldiz *et al.* 1999). Embryologically, they are characterized by the anomalous microsporogenesis and pollen grain development and the formation of the embryosac long before anthesis and formation of endosperm haustoria (Ashurmetov and Normatov 1998).

On the other hand, the seed protein data (Figs. 1 and 3; Table 2) of the species studied of the section *Lophobrychis* revealed heterogeneity in the band distribution in the protein profiles. The diploid and polyploid species are distinguished each other at relatively small values of dissimilarity levels and could be characterized one another by a unique band in their profiles. *O. aequidentata* ($2n=2x=16$), *O. caput-galli* ($2n=4x=28$) and *O. crista-galli* ($2n=4x=32$) are characterized by presence of a protein band of the number 28, 34, and 26 with molecular mass of 25.3, 18.5 and 27.0 kD, respectively, whereas *O. pulchella* ($2n=4x=32$) is characterized by absence of the protein band number 4 with molecular mass of 69.5 kD. Thus, the produced protein banding patterns are species specific and are compatible with those of other lines of taxonomic evidence in demonstrating the interspecific variability among the species studied of Sect. *Lophobrychis*.

Investigation of the geographical distribution data of the species studied of the section *Lophobrychis* (Table 1) in relation to their chromosome counts (Abou-El-Enain 2002) revealed that they are distributed in about 26 different coun-

Fig. 1. Banding patterns of seed protein analysis using SDS-PAGE technique (0, Marker; 1, *O. aequidentata*; 2, *O. bobrovii*; 3, *O. caput-galli*; 4, *O. crista-galli*; 5, *O. ptolemaica*; 6, *O. pulchella*).

(Fig. 2)

(Fig. 3)

(Fig. 4)

Figs. 2–4. The phenograms illustrating the relationships between the species studied based on the characters analyses of morphological data set (Fig. 2), SDS-PAGE of seed proteins data set (Fig. 3) and all combined data sets (Fig. 4) using UPGMA clustering method (1, *O. aequidentata*; 2, *O. bobrovii*; 3, *O. caput-galli*; 4, *O. crista-galli*; 5, *O. ptolemaica*; 6, *O. pulchella*).

Table 2. The produced banding patterns in the seed protein electrophoretic profiles of the species studied as scored from Fig. 1

Band No.	MW (kD)	1	2	3	4	5	6
		<i>O. aequidentata</i>	<i>O. bobrovii</i>	<i>O. caput-galli</i>	<i>O. crista-galli</i>	<i>O. ptolemaica</i>	<i>O. pulchella</i>
1	94.0	+-	+-	---	---	---	---
2	78.8			---	---		---
3	73.4	---	+-		---	---	---
4	69.5	+++		---	+-		
5	67.0		+-	+++	---	---	---
6	63.4	---	---		+++		---
7	61.0	+++		+++	---	+++	+-
8	59.5		---	---	---	---	---
9	57.2	+-		---			---
10	55.2			+++	+++		---
11	53.5	+++	---		---	+++	---
12	51.2	+++		---	---	---	---
13	47.7		+++			+++	
14	43.0	+-		+++	+++		---
15	42.3	---	---		---	+++	+-
16	40.5		---	---	+++	---	
17	38.5	+++		---			+-
18	37.2		---				
19	36.0		---			---	
20	35.2					---	
21	32.3	---		+-	---		---
22	31.0	---	---			---	
23	30.0	---	---			---	
24	29.0					---	---
25	28.6		---	---		+-	
26	27.0				+++		
27	26.4	+-			---		
28	25.3	---					
29	24.0		---	---		+-	
30	23.0	---	+-		---	---	---
31	22.0	+++	---		+++	---	---
32	21.0		---	+-	---	+++	+++
33	20.5	---		---	+++		---
34	18.5			---			
35	17.2		---			---	
36	16.2	+-	---	---		---	
37	15.5	---			---		---
38	14.4		---		---	---	---
39	13.0	+-		---			
Total band no.		22	21	20	23	23	22

MW=Molecular mass, kD=kilo Dalton, Blank=absent, +++=deep dark, +- =dark to medium, ---=faint.

tries as native or introduced plants. All of them are recorded in Iran, Iraq and Turkey where the centre of diversity is found in Northwest Asia. They extend regularly as far as Mediterranean countries either in East Europe or in North Africa where they are recorded as introduced plants (Ashurmetov and Normatov 1998). The most frequent species is *O. caput-galli* ($2n=4x=28$; 22 countries), followed by *O. aequidentata* ($2n=2x=16$; 15 countries), *O. crista-galli* ($2n=4x=32$; 15 countries) and the least common one is *O. pulchella* ($2n=4x=32$; 3 countries). These data besides the recorded role of the aneuploidy in the *Lophobrychis* evolution and the presence of polyploidy in

Table 3. The 118 characters used in the present study and their codes for the numerical analyses

I. Morphological characters:	
Whole Plant: (01) Habit: perennial=0, annual=1; (02) Height: <30 cm=0, 30–40 cm=1, 40–50 cm=2, 50–60 cm=3, >60 cm=4;	
Stem: (03) Branched base: absent=0, present=1; (04) Surface: glabrous=0, pubescent=1, appressed-hairy=2; (05) Woody base: absent=0, present=1.	
Stipules: (06) Length: <0.4 cm=0, 0.4–0.6 cm=1, 0.6–0.8 cm=2, >0.9 cm=3; (07) Shape: triangular=0, triangular acuminate=1; (08) Surface: glabrous=0, pubescent=1, scarious with a green median nerve=2.	
Leaves: (09) Petiole length: <0.8 cm=0, about 1.2 cm=1; (10) Diameter: 4–12 cm=0, 6–14 cm=1.	
Leaflets: (11) Pairs number: less than 8=0, more than 8=1; (12) Diameters: >0.7–1.8×0.3–0.8 cm=0, about 0.7–1.8×0.3–0.8 cm=1; <0.8–1.8×0.3–0.8 cm=2; (13) Shape: oblong-elliptical=0, elliptical to linear=1; (14) Apex shape: acute or obtuse=0, truncate-mucronate=1; (15) Upper surface: glabrous=0, patent hairs=1; (16) Lower surface: hairy=0, villous=1.	
Peduncles: (17) Length: shorter than subtending leaves in flower=0, equaling the subtending leaves=1, longer than the subtending leaves=2, much longer than the subtending leaves=3; (18) Elongation in fruiting: absent=0, present=1.	
Racemes: (19) Flowers number: many-flowered=0, 2–3 flowered=1.	
Bracts: (20) Length: <0.5 cm=0, about 0.5 cm=1; >0.5 cm=2.	
Pedicels: (21) Length: <0.25 cm=0, 0.26–0.35 cm=1, 0.36–0.45 cm=2, >0.45 cm=3.	
Calyx: (22) Length: <0.6 cm=0, 0.6–0.65 cm=1, 0.65–0.75 cm=2, >0.75 cm=3, (23) Surface: glabrous=0, pubescent=1, hairy=2; (24) Teeth length: shorter than the tube=0, as long as the tube=1, longer than the tube=2; (25) Teeth shape: lanceolate=0, narrowly lanceolate=1, triangular=2.	
Corolla: (26) Color: white=0, yellow with brown veins=1, pink=2, reddish-purple=3; (27) Length: <0.7 cm=0, 0.7–0.9 cm=1, 0.9–1.1 cm=2, 1.1–1.3 cm=3, >1.3 cm=4.	
Ovary: (28) Stalk: sessile=0, stalked=1; (29) Length: shorter than the calyx=0, as long as the calyx=1.	
Pod: (30) Diameter: <1–1.2×0.8–1 cm=0, >1–1.2×0.8–1 cm=1; (31) Shape: orbicular=0, oblong to hemispherical=1; (32) Compression: absent=0, present=1; (33) Valves surface: densely villous=0, hairy=1; (34) Segment number: one=0, two or more=1; (35) Faveole: partly ordered at least 3–4 rows=0, ordered=1; (36) Number of first faveole row: with 2 faveoles=0, with 3 or more faveoles=1; (37) Number of large faveole on the first row: with 1 large faveole=0, with 2 large faveoles=1; (38) Ventral spine of large faveole: absent=0, present=1; (39) Faveolate crest of fruit: absent=0, present=1; (40) Border of fruit: absent=0, present=1; (41) Subcanaliculate margin of fruit: absent=0, present=1; (42) Spines or setae on the margin: Spines present as 1 row=0, Spines or setae present as 2 rows symmetrically (on upper and under sides)=1; (43) Marginal vein and spines border length: the same=0, Variable=1; (44) Structure of the marginal spines or setae: Spine or setae absent or setae present and setae not widened=0, Spine present and quite widened at the base=1; (45) Curvation of fruit: ventral suture straight=0, ventral suture fully curved=1.	
Seeds: (46) Diameter: ≤0.3×0.3 cm=0, >0.3×0.3 cm=1; (47) Compression: absent=0, present=1; (48) Surface: smooth=0, rough=1; (49) Color: yellowish brown=0, brown=1, dark brown=2.	
Ovule: (50) Number: one=0, two or more=1.	

II. Seed protein characters: The characters from 51 to 89 and 90 represents the recorded bands and their total numbers, respectively as indicated in Table 1. These are coded as follows: absent=0, deep dark=1, dark to medium=2, faint=3; total number of bands: 20=0, 21=1, 22=2, 23=3.

III. Geographical distribution: The absence or presence of the species in each of the following country is coded 1 or 0, respectively: (91) Algeria; (92) Egypt; (93) Libya; (94) Morocco; (95) Tunisia; (96) Afghanistan; (97) Iran; (98) Iraq; (99) USSR; (100) Albania; (101) Bulgaria; (102) Crete; (103) France; (104) Greece; (105) Italy; (106) Sicily; (107) Spain; (108) Turkey in Europe; (109) Yugoslavia (former); (110) Cyprus; (111) Jordan; (112) Lebanon; (113) Palestine; (114) Balkan Peninsula; (115) Syria; (116) Turkey in Asia.

IV. Cytological characters: (117) Basic chromosome number (x): $x=7=0$, $x=8=1$; (118) Diploid chromosome number ($2n$): $2n=16=0$, $2n=28=1$, $2n=32=2$.

a percentage of more than 60% among the whole genus (Abou-El-Enain 2002) confirm the comparatively highly derived organization of the species studied of Sect. *Lophobrychis*.

Comparison of the produced classifications (Figs. 2–4) revealed a considerable consistency in separation of the two Egyptian species *O. crista-galli* (No. 4) and *O. ptolemaica* (No. 5) among the two different groups of the species studied. The seed protein data revealed that the Egyptian species

have 13 bands in common, each has 10 different bands and the similarity between the two species is about 56%. *O. crista-galli* differs from *O. ptolemaica* in the presence of the bands numbered 2, 4, 5, 10, 14, 21, 26, 27, 33 and 37, with molecular mass of 78.8, 69.5, 63.4, 55.2, 43.0, 32.3, 27.0, 26.4, 20.5 and 15.5 kD, respectively. *O. ptolemaica* differs from *O. crista-galli* in the presence of the bands numbered 13, 19, 20, 22, 23, 24, 25, 29, 35 and 36 with molecular mass of 47.7, 36.0, 35.2, 31.0, 30.0, 29.0, 28.6, 24.0, 17.2 and 16.2 kD, respectively.

Morphologically, *O. ptolemaica* is characterized by racemes 10–15 cm, many-flowered, pod orbicular, flattened, densely villous, whereas *O. crista-galli* is characterized by racemes 2–4 cm, 2–5-flowered and pod oblong to hemispherical, compressed, with a crest of irregular spinose lobes 0.2–0.5 cm. Geographically, *O. ptolemaica* is infrequent in the Sinai Peninsula (*i.e.* the West Asian part of Egypt) and the Desert East of the Nile, while *O. crista-galli* is frequent in the Mediterranean coastal strip from Port Said to the border with Libya near El-Sollum of the Egyptian territories. Cytologically, *O. ptolemaica* is characterized by its diploid chromosome count of $2n=2x=16$ and the xerophytic nature. On the other hand, *O. crista-galli* is characterized by its annual habit, a tetraploid chromosome count of $2n=4x=32$, and a mesophytic nature. These data support the taxonomic delimitation of *O. crista-galli* and *O. ptolemaica* in the two Sections Lophobrychis and Hymenobrychis, respectively, that previously inferred by the systematic studies based on the embryological (Ashurmetov and Normatov 1998), fruit morphological (Yeldiz *et al.* 1999) and cytological criteria (Heyn 1962, Abou-El-Enain 2002).

The previous studies on the genus *Onobrychis* revealed that *O. caput-galli* exhibits common morphological, anatomical and cytological characters with the two Egyptian species. These characters are the measurements of fruit in length, width, thickness and number of thorns as well as the anatomical characteristics of the seed coats (Cenci *et al.* 2000) and the 2 different cytotypes regarding the chromosome numbers *i.e.* $2n=14$, 16 based on $x=7$ and 8, respectively, (Slavivk *et al.* 1993, Abou-El-Enain 2002) in addition to a high ecological amplitude as revealed by the wide range of distribution in different habitats (Ashurmetov and Normatov 1998). This reveals the possibility of considering *O. caput-galli* as evolutionary linkage in the diversity of the two Egyptian species.

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