Studies in the Maloideae (Rosaceae)
1- *Chaenomeles* Lindley and *Cydonia* Miller

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Macromorphological characters, SEM of seed coat surface criteria, seed coat anatomy and seed protein electrophoresis aspects, were used to re-assess the taxonomic relationships between the genera *Chaenomeles* and *Cydonia*. Characters were analyzed by the NTsys-pc. program package, using the UPGMA clustering method. The dendrograms produced were discused and showed a close relationship between *Chaenomeles sinensis* Koehne and *Cydonia oblonga* Miller. The result gives support to the merging of the former taxon in the genus *Cydonia* as presented in Mabberley (1997).

Key words**:** *Chaenomeles* – *Cydonia* – Electrophoresis – Maloideae – Rosaceae - Systematics

Introduction

The **Maloideae** (**Pyroideae**) is a natural sub-family of the **Rosaceae**, with 23-28 genera and 940-1110 species (Phipps *et al.,* 1990 and Robertson *et al*., 1991). It stands apart from all the rest of the family by several aspects as :- Basic chromosome numbers of 17 (except in some American species of *Crataegus*), unique fruit structure (pome), narrow medullary rays and a well developed seed testa (Stebbins 1950, Mclean & Cook 1956, Corner 1976 and Heywood 1993). As it is the case with most natural groups, the differences between the genera of the Maloideae are slight, generic limits cannot be drawn sharply between many of the apparent genera (Fernald 1947 and Bailey 1949 b). Inconsistency of the main generic characters has generated a great deal of disagreement in the taxonomic treatment of the group (Aldasoro *et al.,* 1998). The genera *Chaenomeles* and *Cydonia* (Tribe Sorbeae) are closely related, being differentiated from each other by few morphological and floral characters (Bailey 1949 a, Eames 1961 and Mabberley 1997). The two genera hybridize well with each other and between their species and numerous cultivars are now available (Hillier 1981 and Beckett 1983). Bailey & Bailey (1976) and Mabberley (1997) stated that *Chaenomeles* contains (3-4) species: *C. cathayensis* (Hemsl) Schneid., *C. japonica* (Thumb). Lindl. and *C. speciosa* (Sweet) Nakai, while *Cydonia* contains two species: *C. oblonga* Mill. and *C. sinensis* Thouin. However, controversies still exist, as to the delimitation of the two genera or at the sub-species level.

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Several studies were made on the two genera and the subfamily **Maloideae** using different morphological and molecular criteria. Weber (1964) gave a detailed account on the genus *Chaenomeles* and its relations with other genera of the Maloideae. He reported that *Cydonia*, *Malus* and *Pyrus* could hybridize among themselves. Sterling (1966) and Kalkman (1988) proposed that *Pyrus* may have branched from the ancestor of *Cydonia* before the latter acquired the pluriovulate carpels. Phipps *et al*., (1990) proposed a checklist of the genera of the Maloideae. According to them, the genus *Chaenomeles* contains 4 species. Phipps *et al.,* (1991) and Robertson *et al.,* (1991) discussed the phylogeny of the Maloideae and presented a synopsis of genera in the sub-family in an attempt to aid in their delimitation. Rohrer *et al.*, (1991) showed that the fruit morphology supports a close relationship between the *Chaenomeles* species. Robertson *et al*., (1992) stated that *Pseudocydonia* (C*ydonia sinensis*) differed from all the other genera of the Maloideae, in possessing leaves with cylindrical gland tipped teeth. Rohrer *et al*., (1994) stated that *Cydonia* and *Pyrus* are sister groups according to a cladistic analysis utilizing some morphological aspects. The same result was arrived at by Campbell *et al.*, (1995) utilizing molecular criteria and Aldasoro *et al*., (1998) by studying the pome anatomy.

The significance of seed structure in taxonomic and phylogenetic studies has been emphasized by many authors (Netolitzky 1926, Martin 1946, Duke 1961, Corner 1976 & 1992, Rezk 1980 & 1987). SEM of seed coat surface is useful in the identification and classification of various taxa (Stant 1973, Brisson & Peterson 1976, Barthlott 1981 and Boesewinkel & Bouman 1984). A comparison of surface scan patterns of the seed coat has efficiently been used in studying species of some genera including *Vigna* (Kumar *et al*., 1984), the **Abutileae** (Khushk & Vaughan 1986), the **Vicieae** (Chernoff *et al*., 1992) and *Ranunculus* (Xuhan & Van-Lammeren, 1994). As far as the literature cited, no attempt has been made for studying the seeds of *Chaenomeles* & *Cydonia* in particular, except Corner (1976) who studied the seed coat anatomy of *Chaenomeles japonica* Lindl. & *Cydonia oblonga* Mill. According to him, the former species differs from the latter in that the testa is scarcely mucilaginous and the cells scarcely radially elongate. Rudenko & Rotaru (1988) studied the variation in seed anatomy, especially the epidermis and testa structures in diploid varieties of *Cydonia oblonga* and *Chaenomeles japonica* ($2n = 34$), allotetraploid F_2 *Cydonia* and *Malus* hybrids ($2n = 68$) and *Pyrus and Cydonia* hybrids ($2n = 34$). Differences were found between the species and their hybrids. As to the Maloideae in general, one would refer to the works of Pechoutre (1902), Netolitzky (1926) and Sterling (1966).

On the other hand, seed proteins are highly stable, being unaffected by environmental conditions Haborne & Turner (1984). Thus electrophoretic patterns of total seed protein (protein profiles) as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) have provided a valid source of taxonomic evidence and were used to address taxonomic relationships at the generic and specific levels, for example *Vigna* (Paino *et al*., 1993), *Phaseolus* (Schmit *et al*., 1996) *Sesbania* (Badr *et al*., 1998) & *Nigella* (Jensen 1984).

The present study aims at using seed characters (macro and micromorphological) including seed coat anatomy and SEM of seed coat surface, together with characters from vegetative morphology, seed storage protein profiles and numerical taxonomic methods to help in clarifying and delimiting the two genera studied.

Materials and Methods

Seeds of the examined species and their sources are listed in Table (1). Macromorphological aspects were collected from relevant literature (Makins 1948, Bailey 1949 a, Bean 1950, Bailey & Bailey 1976, Hillier 1981, Beckett 1983 and Mabberley 1997).

Table (1): Sources of the studied Taxa.

Taxon	English name	Source	Country of origin	Distribution in Egypt
1) *Chaenomeles japonica* (Lindl.) Spach syn: *Chaenomeles Maulei* Schneid. syn: *Cydonia Maulei* Moore syn: *Pyrus japonica* Thunb.	Dwarf Japanese quince	TMPRS	Japan	------
2) *Chaenomeles sinensis* Koehne. syn: *Cydonia sinensis* Thouin syn: *Pseudocydonia sinensis* Schneid.	Chinese quince	TMPRS	China	Rare, some specimens were planted in Bircher's Garden at El Saff
3) *Chaenomeles speciosa* Nakai. *syn: Chaenomeles lagenaria* Koidz. syn: *Cydonia lagenaria* Loisel *syn: Chaenomeles japonica* Hort. syn: *Cydonia japonica* Pers. syn: *Pyrus japonica* Sims.	Japanese quince	TMPRS	China & Japan	Rare, some specimens were planted in Bircher's Garden at El Saff (Bircher 1960)
4) Cydonia oblonga Mill. syn: *Cydonia vulgaris* Pers. syn: *Pyrus cydonia* L.	Edible quince	OBS	Unknown, cultivated & naturalized in many places of the Old World	Cultivated on a small scale for its edible fruits. It is also used as a stock for grafting pears and apples. (Bircher 1960)

TMPRS = Tsukuba Medicinal Plant Research Station, National institute of health sciences, Tsukuba, Japan.

OBS = Orto Botani Dell' Universita di Siena, Italy.

For study of seed coat surface using SEM, two seeds were mounted with colloidal silver on copper stubs and coated with a thin layer of gold in Polaron E 5000. The epidermal seed coat was photographed by a JEOL- Scanning Microscope at the central lab. of Faculty of Science – Alexandria University. The terminology of Stearn (1966), Barthlott (1981) and Boesewinkel & Bouman (1984) has been used to describe the characteristics of the seed coat.

In addition, transverse sections were made in the seed coats of the studied taxa by hand microtome at 15-20 µ at the Faculty of Science – Ain shams University. Sections were photographed using Carl-Zeiss photomicroscope III at a magnification of x=200, 256 & 320 at the Faculty of Education – Ain shams University. Description and terminology presented by Corner (1976) has been used to describe the anatomical features of the seed coat.

For SDS-PAGE electrophoresis, three replicates of 0.1 gm of seeds were mixed, each with an equal weight of pure, clean, sterile fine sand and powdered using mortar and pestle. Extraction of proteins was carried out using four buffers; Tris-Glycine (pH 8.2), Tris-EDTA (pH 8.8), Tris-HCl (8.0) in the presence of 2-mercaptoethanol (under reducing

condition) and Tris-HCl (pH 8.0) without 2 ME (under non-reducing condition). The powder was homogenized with 1 ml of each buffer for 2 hr. at 20 ºC. SDS-polyacrylamide gel electrophoresis was carried out in 12.5 % acrylamide gels in Tris-Glycine running buffer (pH 8.3) at 150 V for 3 hr. using a low molecular weight protein of Sigma as a marker in each run. Gels were then stained in Comassie brilliant blue R-250 for 30 min., destained, photographed and molecular weight values for subunits were determined by comparison with standard proteins as described by Matta *et al.* (1981). Analysis was carried out using pro-analyzer version 2.0.

For the data analysis, the total number of the recorded characters (178) in each taxon, were scored, combined together in four sets of data & coded for creating the data matrix of computation:

a) Morphological characters of whole plant.
b) Anatomical characters of seed coat (LM).
c) Morphological characters of seed coat (SEM).
d) SDS-PAGE characters.
e) All characters combined.

The presence or absence of each 178 different characters was treated as a binary character in a data matrix i.e. coded 1 and 0 respectively (Table 2).

The relationships between the taxa studied, expressed by average taxonomic distance (dissimilarity), have been demonstrated as phenograms, based on the analysis of the recorded characters using the NTsys program package for IBM-pc as described by Rohlf (1989).

Results

I. Selected macromorphological features of the studied taxa (After Makins 1948, Bailey 1949 a, Bean 1950, Bailey & Bailey 1976, Hillier 1981, Beckett 1983 and Mabberley 1997) are summarized below:

(1). ***Chaenomeles japonica*** (Thumb.) Lindl.

A thorny semi-deciduous sub-shrub 90 cm high. Leaves simple, leaf base with large stipules, blade margin serrate to crenate and apex accuminate to obtuse. Young branchlets and leaves downy. Flowers 3 cm wide, orange red to scarlet, in clusters on previous year's wood, styles joined at base. Fruit 3 cm wide and yellowish red. Seeds globose to pear shaped and dark brown.

(2). ***Chaenomeles sinensis*** Koehne.
(=***Cydonia sinensis*** Thouin., ***Pseudocydonia sinensis*** Schneider)

Spineless semi deciduous shrub 3-6 m high. Leaves simple. Leaf base with small stipules. Petiole short and pubescent with glandular teeth. Blade elliptical to obovate. Margin serrate. Apex acuminate. Flowers solitary, pale pink, sepals reflexed and serrulate, styles joined at base. Fruit 15 cm wide, oblong, yellow and fragrant. Seed pear shaped and dark brown.

(3). ***Chaenomeles speciosa*** (Sweet) Nakai

Thorny shrub 3 m high. Semi deciduous. Leaves glabrous and simple 3-4 cm. Leaf base with large stipules. Blade oblong to ovate. Margin serrate and apex acuminate. Flowers in small clusters, scarlet red, styles joined at base. Fruit globose to ovoid, medium sized and green yellow. Seeds pear shaped and dark brown.

(4). ***Cydonia oblonga*** Miller

Spineless shrub 4-6 m high. Deciduous. Leaves simple with small stipules, petiole short and downy. Blade ovate, margin entire and apex acute. Flowers solitary, white to pale pink. Styles free . Fruit pyriform large and fragrant. Calyx persistent. Seeds pear shaped and slightly curved.

II. Anatomical aspects of the examined seed coat by LM (Terminology after Corner 1976) are summarized as follows:

(1). ***Chaenomeles japonica*** (Thunb.) Lindl.

Testa: Outer epidermal cells illdefined. Mesophyll: Several layers of highly lignified cells. The outer ones narrow and irregularly rectangular and the inner ones irregular hexagonal.
Tegmen: 3-4 layers of cells, outer ones crushed, inner ones narrow rectangular with a dark brown pigmentation.
Endosperm: Two layers thick.

(2). ***Chaenomeles sinensis*** Koehne.
(= ***Cydonia sinensis*** Thouin., ***Pseudocydonia sinensis*** Schneider)

Testa: Outer epidermal cells illdefined, some possess long pointed hairs. Mesophyll: Several layers of irregularly hexagonal highly lignified cells, the inner ones crushed.
Tegmen: 2-3 layers of narrow irregular rectangular cells with brown pigmentation.
Endosperm: 4-6 layers thick.

(3). ***Chaenomeles speciosa*** (Sweet) Nakai.

Testa: Outer epidermal cells illdefined. Mesophyll: Several layers of highly lignified irregular cells.
Tegmen: Illdefined.
Endosperm: 4-5 layers thick, some vacuoles or air lacunae are seen.

(4). ***Cydonia oblonga*** Miller.

Testa: Outer epidermal cells illdefined. Many possess very long narrow pointed hairs. Mesophyll: Composed of several layers of highly lignified irregular cells, the inner ones crushed.

Tegmen: Outer layers crushed, the inner layers irregularly rectangular with dark pigmentation.
Endosperm: 8-10 layers.

III. Description and terminology of examined seed coat by SEM (after Stearn 1966, Barthlott 1981 and Boesewinkel & Bouman 1984) are summerized as follows:

(1). *Chaenomeles japonica* (Thunb.) Lindl.

Spermoderm reticulate to colliculate. Epidermal cells monomorphic, isodiametric and irregularly pentagonal to hexagonal in shape. Anticlinal walls straight, thin, slightly raised and highly striated. Striations occasionally radiating, forming short ridges. Periclinal walls slightly convex, generally smooth with few striations.

(2). *Chaenomeles sinensis* Koehne.
(*Cydonia sinensis* Thouin., ***Pseudocydonia* sinensis** Schneider**)**

Spermoderm reticulate to colliculate. Epidermal cells rounded and monomorphic. Anticlinal walls curved, very thin, flat and striated. Periclinal walls flat to slightly convex and highly striated.

(3). *Chaenomeles speciosa* (Sweet) Nakai.

Spermoderm reticulate. Epidermal cells monomorphic, pentagonal to round. Anticlinal walls curved, very thick and highly raised. Periclinal walls flat to slightly concave, rough and slightly tuberculate.

(4). *Cydonia oblonga* Miller.

Spermoderm irregularly reticulate. Epidermal cells monomorphic and irregularly hexagonal. Anticlinal walls wavy, slightly thick, slightly raised and highly striated. Periclinal walls flat and highly striated. Long narrow hair-like structure or protrusions appear all over the cell wall.

IV. The electrophoretic banding patterns of the different buffers extracted proteins are shown in Fig. 3 (a-d). The distribution of protein bands in the different taxa based on their molecular weight is shown in Table (2). *Chaenomeles japonica* was found to have the highest number of band (20), whereas the lowest number (12) was found in *Cydonia oblonga*. While the highest molecular weight protein (103 KD) was found in *Chaenomeles speciosa*, the lowest molecular weight (3 KD) was found in *Chaenomeles speciosa* and *Cydonia oblonga*.

V. The numerical analysis of the recorded characters are summarized as follow;
The phenogram produced by cluster analysis based on 89 morphological characters clearly divided the four species into two groups (*Chaenomeles japonica* & *Chaenomeles speciosa*) and (*Chaenomeles sinensis* & *Cydonia oblonga*) at 1.47

level. The first two species are separated at 1.30 average taxonomic distance and the other two species are separated at 1.31 average taxonomic distance (Fig. 4-a).

Table (2): Characters used in the numerical analysis and their codes.
0 = absent; 1 = present; A= *Chaenomeles japonica*; B= *Chaenomeles sinensis*; C= *Chaenomeles speciosa*;D= *Cydonia oblonga*

No	I- Morphological characters:							
	Character				A	B	C	D
1	General Characteristics	Habit		Sub-shrub.	1	0	0	0
2				Shrub.	0	1	1	1
3		Height		90 cm.	1	0	0	0
4				3-6 m.	0	1	0	0
5				3 m.	0	0	1	0
6				4.5-6 m.	0	0	0	1
7		Texture		Thomy.	1	0	1	0
8				Spineless.	0	1	0	1
9		Leaf duration		Deciduous.	0	0	0	1
10				Semi-deciduous.	1	1	1	0
11		Origin		China.	0	1	1	0
12				Japan.	1	0	0	0
13				Iran to Turkistan.	0	0	0	1
14	Leaf Characteristics	Leaf base	Stipules	Very large.	1	0	1	0
15				Small.	0	1	0	1
16		Petiole	Gland	Present or absent	0	1	0	0
17			Length	Short.	0	1	0	1
18				Varying.	1	0	1	0
19			Texture	Pubescent.	1	1	0	1
20				Glabrous.	0	0	1	0
21		Leaf blade	Type	Simple.	1	1	1	1
22			Shape	Oblong to ovate.	0	0	1	0
23				Ovate.	0	0	0	1
24				Ovate to obovate.	1	0	0	0
25				Ellipt. to obovate.	0	1	0	0
26		Leaf Margin		Entire.	0	0	0	1
27				Crenate to serrate	1	0	0	0
28				Sharp serrate.	0	1	1	0
29	Leaf Characteristics	Leaf Apex		Acute to obtuse.	1	0	0	0
30				Acuminate.	0	1	1	0
31				Acute.	0	0	0	1
32		Leaf texture		Pubescent.	1	1	0	1
33				Glabrous.	0	0	1	0
34		Leaf length		3-4 cm.	1	0	0	0
35				5 cm.	0	1	0	0
36				5-9 cm.	0	0	1	0
37				5-10 cm.	0	0	0	1

Table (2): Cont.

No	Character				A	B	C	D
38	Floral Characteristics	Flower size		4 cm.	1	1	0	0
39				5 cm.	0	0	1	1
40		Flower colour		Light pink.	0	1	0	0
41				White to pink.	0	0	0	1
42				Orange scarlet.	1	0	0	0
43				Red scarlet.	0	0	1	0
44		sepals	Number	5	1	1	1	1
45			Shape.	Erect.	1	0	1	0
46				Reflex.	0	1	0	1
47				Entire.	1	0	1	1
48				Serrulate.	0	1	0	0
49		Petals	Number	5	1	1	1	1
50			Texture.	Waxy.	0	0	1	0
51				Not waxy.	1	0	0	0
52		Stamens number		More than 25	1	1	1	0
53				20-25	0	0	0	1
54		Ovary	Type	Inferior.	1	1	1	1
55			Carpels	5 united.	1	1	1	1
56			Placenta	Axile.	1	1	1	1
57			Styles	Free.	0	0	0	1
58				Joined at base.	1	1	1	0
59		Ovu.	Number	Many.	1	1	1	1
60			type	Anatropous.	1	1	1	1
61		Inflorescence.		Solitary.	0	1	0	1
62				In small cluster.	1	0	1	0
63	Fruit Characters	Shape		Glubose.	1	0	0	0
64				Oblong.	0	1	0	0
65				Glubose to ovoid.	0	0	1	0
66				Pyriform.	0	0	0	1
67		Type		Pomme.	1	1	1	1
68		size		Large.	0	1	0	1
69				Medium.	0	0	1	0
70				Small.	1	0	0	0
71	Fruit Characters	colour		Yellow.	0	1	0	1
72				Yellow red.	1	0	0	0
73				Green yellow.	0	0	1	0
74		Specific features		Fragrant.	0	1	0	1
75				Naked cav.	0	1	0	0
76				Calyx persist.	0	0	0	1

Table (2) Cont.

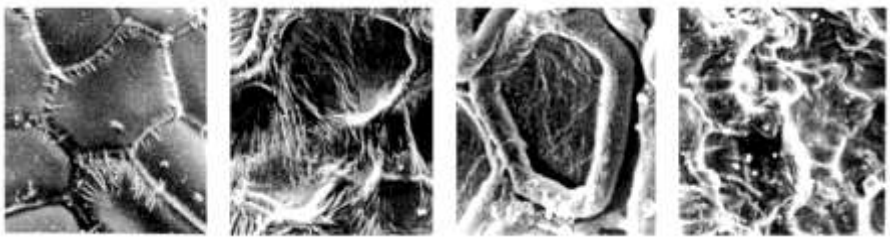
No	Character				A	B	C	D
77	Macromorphological Seed Aspects	shape		Glubose to pear.	1	0	0	0
78				Pear shaped.	0	1	1	0
79				Slightly curved.	0	0	0	1
80		colour		Dark brown.	1	1	1	1
81		Size		4x3x2	1	0	0	0
82				8x4x0.5	0	1	0	0
83				8x5x2	0	0	1	0
84				7x3x1	0	0	0	1
85		Texture		Rough.	0	1	0	1
86				Smooth.	1	0	1	0
87		Mucilage layer		Thick.	0	0	0	1
88				Moderate.	0	1	0	0
89				Thin.	1	0	1	0
II- Seed coat anatomy (T.S.).								
90	Testa	Outer epid. hairs		Present.	0	1	0	1
91				Absent.	1	0	1	0
92		Mesophyll	Outer layer	Irregular hexagonal.	0	1	0	0
93				Irregular rectangular.	1	0	0	0
94				Irregular.	0	0	1	1
95			Inner layer	Irregular hexagonal.	1	1	0	0
96				Irregular.	0	0	1	0
97				Crushed.	0	0	0	1
98	Tegmen	Shape	Number of layers	2-3	1	0	0	0
99				3-4	0	1	0	0
100				4-5	0	0	0	0
101				Illdefined.	0	0	1	1
102			Outer layer	Crushed.	1	0	0	1
103				Irregular rectangular.	0	1	0	0
104				Illdefined.	0	0	0	0
105			Inner layer	Rectangular.	1	0	0	0
106				Irregular rectangular.	0	1	0	1
107				Illdefined.	0	0	0	0
108	Endosperm	Number of layers		2	1	0	0	0
109				4-5	0	1	1	0
110				8-10	0	0	0	1
111		vacules		Present.	0	0	1	0

Table (2): Cont.

III- Seed coat structure (SEM)							
No	Characters			A	B	C	D
112	Over all seed coat pattern		Reticulate to colliculate.	1	1	0	0
113			Reticulate.	0	0	1	0
114			Irregular reticulate.	0	0	0	1
115	Epidermal cell	Shape	Irregular penta. to hexa.	1	0	0	0
116			Rounded.	0	1	0	0
117			Pentagonal to rounded.	0	0	1	0
118			Irregular hexagonal.	0	0	0	1
119		Size	Monomorphic.	1	1	1	1
120	Hairs		Present.	0	1	0	1
121			Absent.	0	0	0	0
122	Anticlinal walls	Undulation	Straight.	1	0	0	0
123			Wavy.	0	0	0	1
124			Curved.	0	1	1	0
125		Thickness	Very thin.	0	1	0	0
126			Thin.	1	0	0	0
127			Slightly thick.	0	0	0	1
128			Very thick.	0	0	1	0
129		Texture	Rough.	1	1	1	1
130			Smooth.	0	0	0	0
131			Highly striated.	1	1	0	0
132			Striated.	0	0	0	1
133			Not striated.	0	0	1	0
134		Hight	Flat.	1	1	0	0
135			Slightly raised.	0	0	0	1
136			Highly raised.	0	0	1	0
137	Periclinal wall	Surface	Flat.	0	0	0	1
138			Flat to slightly concave.	0	0	1	0
139			Slightly convex.	1	0	0	0
140			Flat to slightly convex.	0	1	0	0
141		Texture	Highly striated.	0	1	0	1
142			Smooth with few striations	1	0	0	0
143			Rough & sligh. tuberculate	0	0	1	0

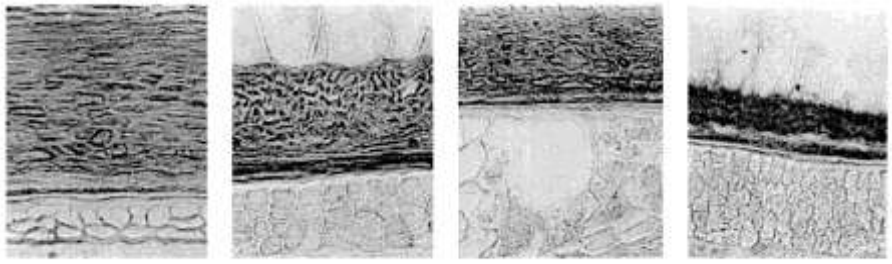
Table (2): Continued

		IV- Seed protein electrophoresis			
No.	Mol.wt. (KD)	A	B	C	D
144	103	0	0	1	0
145	102	1	0	0	0
146	97	0	0	0	1
147	92	1	1	0	0
148	90	1	0	1	1
149	80	1	1	0	0
150	69	1	0	1	1
151	63	0	1	0	0
152	61	1	0	1	0
153	59	1	1	1	1
154	52	1	1	1	1
155	50	0	1	1	0
156	48	0	0	1	0
157	54	1	1	0	1
158	42	1	1	1	0
159	41	1	1	0	1
160	40	1	0	0	0
161	38	1	0	1	0
162	36	0	1	0	0
163	34	1	0	1	0
164	32	0	1	0	1
165	30	0	1	0	0
166	28	1	0	0	0
167	25	1	1	1	1
168	21	1	0	0	0
169	20	1	0	1	0
170	18	0	1	0	0
171	14	0	1	0	0
172	11	0	0	0	1
173	9	0	0	1	0
174	8	1	1	1	0
175	6	0	0	1	0
176	5	0	1	0	0
177	4	1	0	0	1
178	3	0	0	1	1
Total	no. of bands	20	17	17	12



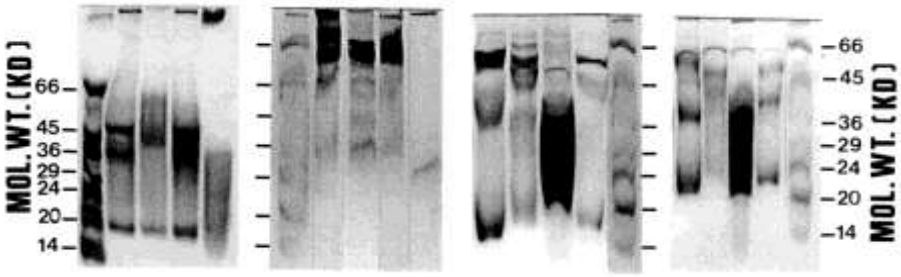
A B C D

Fig. (1): Scanning electron micrographs of the studied taxa.
(A) at x = 1500 (B & D) at x = 1000 (C) at x = 2000



A B C D

Fig. (2): T.S. in seed coats of the studied taxa.
(A) at x = 320 (B & D) at x = 256 (C) at x = 200



A B C D

Fig. (3): Electrophoretic banding profiles of seed protein extracted in
(a) Tris-HCl (under reducing condition)
(b) Tris-HCl (under non-reducing condition)
(c) Tris-EDTA
(d) Tris-Glycine buffers of the studied taxa

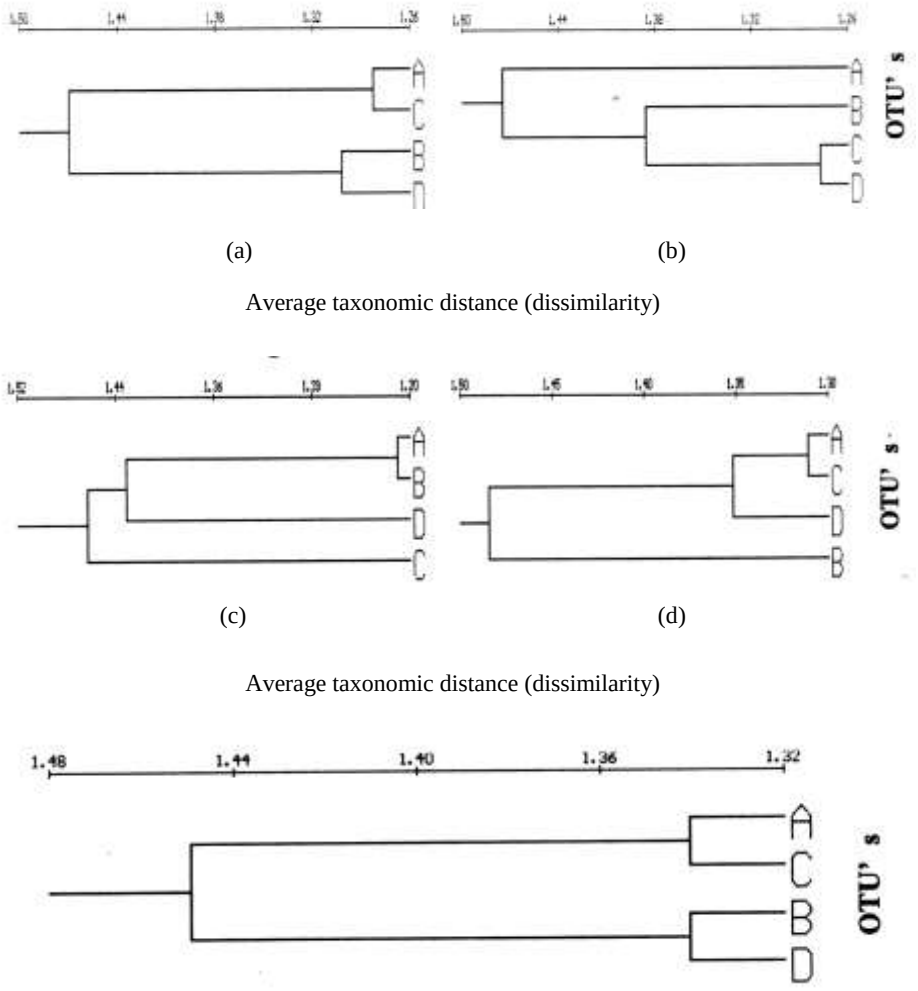


Fig. (4): UPGMA-phenogram based on:

(a) 89 morphological characters,
(b) 22 anatomical characters of seed coat T.S.,
(c) 32 attributes of seed coats scanning,
(d) 35 attributes obtained from SDS-PAGE profiles of seed proteins,
(e) 178 attributes (all characters), illustrating average taxonomic distance (dissimilarity) between the studied taxa.

In the phenogram produced based on 22 anatomical characters of seed coats, *Chaenomeles japonica* is split off at 1.47, then *Chaenomeles sinensis* is split off at 1.38. *Chaenomeles speciosa* and *Cydonia oblonga* are grouped together and then divided at the dissimilarity level of 1.28 (Fig. 4-b).

In the phenogram produced based on 32 attributes of seed coats scanning *Chaenomeles speciosa* is split off at 1.47, then *Cydonia oblonga* is split at 1.43 average taxonomic distance. *Chaenomeles japonica* and *Chaenomeles sinensis* are grouped together and separated at 1.21 dissimilarity level (Fig. 4-c).

In the phenogram produced based on 35 attributes obtained from seed protein electrophoresis *Chaenomeles sinensis* is split off at the dissimilarity level of 1.48 and then *Cydonia oblonga* is split off at 1.38 level. The two species (*Chaenomeles japonica* and *Chaenomeles speciosa*) are grouped together and then separated from each other at 1.31 level of average taxonomic distance (Fig. 4-d).

The last phenogram produced based on all the studied characters clearly divided the four species into two groups (Fig. 4-e) at 1.45 level of dissimilarity, the first group included *Chaenomeles japonica* and *Chaenomeles speciosa* and the other one included *Chaenomeles sinensis* and *Cydonia oblonga*. The two species of the first group were separated from each other at 1.34 average taxonomic distance and at the same level the two species of the second group were separated from each other.

Discussion

The genera *Chaenomeles* Lindl. and *Cydonia* Miller have long been the subject of controversy among different authors (Table 3). Donoghue & Sanderson (1992) have stressed on the importance of utilizing different criteria, both morphological and molecular, in reconstructing plant phylogeny and in the re-assessment of relationships between taxa. In their opinion, using only one or few criteria can be misleading.

In the present study, the phenogram constructed according to the analysis of 89 macromorphological characters showed a closer relation between *Chaenomeles japonica* and *Chaenomeles speciosa* on one hand and between *Chaenomeles sinensis* and *Cydonia oblonga* on the other hand. The former two taxa clustered at the dissimilarity level of 1.30 and the latter at 1.31. This result was in accordance with Bailey & Bailey (1976) and Mabberley (1997) who transferred *Chaenomeles sinensis* to *Cydonia* to become *Cydonia sinensis*. *Chaenomeles japonica* and *Chaenomeles speciosa* were shown to be closely related by Rohrer *et al*. (1991), from studying the fruit structure, morphologically and anatomically.

In the phenogram constructed according to the analysis of 22 anatomical characters of seed coats, the results were generally inconclusive. Here, *Chaenomeles japonica* was split off from the other three taxa at a high dissimilarity level of 1.47, while *Chaenomeles speciosa* was grouped with *Cydonia oblonga* at a low dissimilarity level of 1.28. This result was contradictory to the relevant literature that stated the close relation between *Chaenomeles japonica* and *Chaenomeles speciosa* as they readily hybridize among themselves (Mabberley, 1997). These results may be due to the analyzing of only 22 characters. It is worth mentioning that Corner (1976) stated that in the **Maloideae** in general, their seeds, offer no striking microscopic structure except their well developed testa, suggesting their close relationships and recent common ancestry.

Table (3): A survey of the taxa recorded in *Chaenomeles* and *Cydonia* showing the controversies encountering their delimitation.

Taxon no.	2n	Taxon status
1		*Chaenomeles cathayensis* (Hemsley) Schneid.
		(Schneider, 1912; Bailey & Bailey, 1976; Hillier, 1981 and Beckett 1983)
		Chaenomeles lagenaria var. *cathayensis* Rehd.
		(Rehder, 1927 and Bailey, 1949)
		Cydonia cathayensis Hemsley
	34	(Hemsley, 1873; Makins, 1948 and Bean, 1950)
2	34	*Chaenomeles lagenaria Koidz*. (Bailey, 1949)
	34	=*Chaenomeles speciosa* (Sweet) Nakai
		(Bailey & Bailey, 1976; Hillier, 1981 and Mabberley, 1997)
		Cydonia lagenaria Loisel
		(Makins, 1948 and Bean, 1950)
3		*Chaenomeles japonica* Lindley
		(Bailey, 1949; Bailey & Bailey, 1976; Hillier, 1981; Beckett, 1983 and Mabberley, 1997)
		Cydonia japonica Lindley.
	34	(Makins, 1948 and Bean, 1950)
4	34	*Chaenomeles sinensis* Koehne
		(Koehne, 1893 and Bailey, 1949)
	32	*Cydonia sinensis* Thouin
		(Makins, 1948; Bean, 1950; Bailey & Bailey, 1976 and Mabberley, 1997)
		Pseudocydonia sinensis Schneid.
		(Schneider, 1912; Hillier, 1981 and Robertson *et. al*., 1992)
5	34	*Cydonia oblonga* Mill.
		(Agreed about its status among different authorities)

2*n* = Basic chromosome number as reported in Fedorov 1969.

The phenogram constructed according to the analysis of 32 attributes of seed coat scan showed the grouping of *Chaenomeles japonica* and *Chaenomeles sinensis* at a 1.21 dissimilarity level. This suggested a close relation between the two taxa and so the retaining of *Chaenomeles sinensis* in the genus *Chaenomeles* as proposed by (Koehne, 1890; Bailey 1949 a and Mabberley, 1987). The splitting of *Chaenomeles speciosa* from the other three taxa at dissimilarity level of 1.47, then *Cydonia oblonga* at 1.43 followed by the other two taxa, gives an indication that generic limits cannot be drawn sharply between the two genera. A fact that was stated by Fernald (1947), Bailey (1949) and Aldasoro *et al*. (1998) for the **Rosaceae** in general and the **Maloideae** in particular.

The phenogram constructed according to the analysis of 35 attributes obtained from seed protein electrophoresis showed that *Chaenomeles sinensis* is split off from the remaining taxa at the high dissimilarity level of 1.48. This result is in accordance with

Bean 1950 and Robertson *et al.* (1992), who stated that this taxon differed from all the other genera of the Maloideae in possessing leaves with cylindrical gland tipped teeth. The latter author placed it in the monotypic genus *Pseudocydonia*, as was proposed earlier by Schneider (1912) and Hillier (1981). The two species *Chaenomeles japonica* and *Chaenomeles speciosa* clustered together at the dissimilarity level 1.31. This result is in agreement with Bailey (1949a), Bean (1950), Weber (1964), Bailey & Bailey (1976), Hillier (1981), Beckett (1983) and Mabberley (1987 &1997).

The last phenogram based on all characters studied, delimited the taxa in the two genera *Chaenomeles* Lindl. and *Cydonia* Miller as presented in Bailey & Bailey (1976) and Mabberley (1997), giving further support to the merging of *Chaenomeles sinensis* with the genus *Cydonia*, as *Cydonia sinensis* Thouin and as presented earlier in Bean (1950).

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References

Aldasoro, J. J., Aedo, C. and Navarro, C. (1998): Pome Anatomy of Rosaceae Sub-fam. Maloideae, with special reference to *Pyrus*. *Ann. Missouri. Bot. Gard*. **85**: 518-527.

Badr, A., Abou-El-Enain, M. M. and El-Shazly, H. H. (1998): Variation in seed protein electrophoretic pattern and species relationships in *Sesbania*. *Proceedings of the 6th Egyptian Conference of Plant Sciences, Cairo University* (24-26 November, 1998). Vol. **III**: 493-501.

Bailey, L. H. (1949): *Manual of Cultivated Plants*. *Macmillan Publishing Comp*., New York.

…….. (1949 b): The Pyrus-Malus puzzle. Gentes. Herb. **8**: 40-43.

……...…… and Bailey, E. Z. (1976): **"***Hortus Third. A Concise Dictionary of Plants Cultivated in the U.S. & Canada***". "**Revised by staff of the L.H. Bailey Hortorium**".** *Macmillan Publ. Co. New York***.**

Barthlott, W. (1981): Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nord. J. Bot*. **1**: 345-355.

Bean, W. J. (1950): *Trees & Shrubs Hardy in the British Isles*. Vol. **1**: From A-E, 568-572. *John Murray, London.*

Beckett, K. A. (1983): *The Concise Encyclopedia of Garden Plants*. *Orbis Ltd. London*.

Bircher, W. (1960): *Gardens of The Hesperides*. Anglo-Egyption Bookshop Cairo, pp. 321.

Boesewinkel, F. D. & Bouman, F. (1984): The seed structure. *In "Embryology of Angiosperms*" B. M. Johri (Ed.). *Springer-Verlage, Berlin, Heidelberg, New York, Tokyo*. 638-681.

Brisson, J.D. and Peterson, R.I. (1976): A critical review of the use of scanning electron microscopy in the study of the seed coat. *Scan. Elect. Mic* **2**: 477-495.

Campbell, C. S., Donoghue, M. J., Baldwin, B. G. and Wojeiechowski, M. F. (1995): Phylogenetic relationships in Maloideae (Rosaceae): Evidence from sequences of the internal transcribed spacers of nuclear ribosomal DNA and its congruence with morphology. *Amer. J. Bot.* **82**: 903-918.

Chernoff, M., Plitmann, U. and Kislev, M. E. (1992): Seed characters and testa texture in species of the Vicieae, their taxonomic significance. *Isr. J. Bot*., **41**: 167-186.

Corner, E. J. H. (1976): The Seeds of Dicotyledons. Vol. (**1 &2**): *Cambridge Univ. Press*.

…… (1992): The pachychalaza in dicotyledons: primitive or advanced. *Bot. J. Linn. Soc*. **108**: 15-19.

Donoghue, M. J. and Sanderson, M. J. (1992): The Suitability of Molecular and Morphological Evidence in Reconstructing Plant Phylogeny. In "*Molecular Systematics of Plants*." Soltis, P.S., Soltis, D.E. and Doyle, J.J. (Eds.). *Chapman & Hall-New York.*

Duke, J. A. (1961): Preliminary revision of the genus *Drymaria*. *Ann. Missouri. Bot. Gard*. **48**: 173.

Eames, A. J. (1961): *Morphology of the Angiosperms*. *McGraw-Hill Book Company. New York.*

Fedorov, A. A. (1969) (Ed.): *Chromosome Number of Flowering Plants*. Leningrad.

Fernald, M. L. (1947): Minor transfers in *Pyrus* (reasons for uniting *Malus, Sorbus* and *Aronia* with *Pyrus*). *Rhodora* **49**: 229-233.

Haborne, J. B. and Turner, B. L. (1984): *Plant Chemo-systematics*. *Academic Press, New York.*

Hemsley, W. B. (1873): *Handbook of Hardy Trees, Shrubs and Herbaceous Plants*. London.

Heywood, V. H. (1993): *Flowering Plants of the World*. *Andromeda, Oxford Ltd*.

Hillier, H. G. (1981): *Hilliers Manual of Trees and Shrubs* (5 th Edition). *Van Nostrand Reinhold Company. New York-London.*

Jensen, U. (1984): Legumin-like and Vicilin-like storage proteins *in Nigella damascena* (Ranunculaceae) and six other dicotyledonous species. *J. Plant Physiol*. **115**: 161-170.

Kalkman, C. (1988): The phylogeny of the Rosaceae. *Bot. Jour. Linn. Soc*. **98**: 37-59.

Khushk, M. T. and Vaughan, J. G. (1986): Seed structure in relation to taxonomy of the Abutileae. *Pak. J. Bot.* **18** (**10**): 191-197.

Koehne, E. (1890): *Die Gattungen der Pomaceen. R. Gaertners*. *Verlags Buchhandlung Hermann Heyfelder. Berlin.*

Kumar, D., Rangaswamy, N. S. & Dinesh, K., (1984): SEM studies on seed surface of wild and cultivated species of *Vigna* Savi. Proc. *Ind. Acad. Pl. Sc*., **93**: 35-42.

Mabberley, D. J. (1987): *The Plant Book: A Portable Dictionary of the Higher Plants*. *Cambridge University Press New York*.

…….. (1997): *The Plant-Book, A Portable Dictionary of the Vascular Plants*. *Cambridge Univ. Press*.

Makins, F. k. (1948): *The Idntification of Trees & Shrubs*. *J. M. Dent & Sons LTD. London.*

Martin, A. C. (1946): The comparative internal morphology of seeds. *Am. Midl. Nat.* **36**: 513-660.

Matta, N. K., Gatehouse, J. A. and Boulter, D. (1981): The structure of legumin of *Vicia faba* L. a reappraisal. J. *Exp. Bot*. **32**: 183-197.

McLean, R. C. and Ivimey-Cook, W. K. (1956): *Textbook of Theoretical Botany*. Vol. II Page 1651. Longmans & Green, London.

Netolitzky, F. (1926): *Anatomie der Angiospermen Samen*. (Cited from Corner E. J. H. 1976, *The Seeds of Dicotyledons. Cambridge Univ. Press*).

Paino, D., Urzo, M., Pedalino, M., Grillo, S., Rao, R., Tucci, M., Urzo-M.P., Ng-NQ & Monti, L. M. (1990): Variability in major seed proteins in different *Vigna* species. *Cowpea genetic resources*, **1**: 90-110 (Niger).

Pechoutre, F. (1902): Contribution a l'etude du developpement de l'ovule et de la graine des Rosacees. *Annls Sci. nat. (Bot.) Ser*. **8**, 16, 1-158.

Phipps, J. B., Smith, P. G. and Rohrer, J. R. (1990): A checklist of the subfamily Maloideae (Rosaceae). *Canad. Journ. Bot.* **68**: 2209-2269.

Phipps, J. B., Robertson K. R., Rohrer, J. R. and Smith, P. G. (1991): Origins and evolution of the subfamily Maloideae (Rosaceae). *Syst. Bot*. **16**: 376-394.

Rehder, A. (1927): *Manual of Cultivated Trees and Shrubs*. *New York*.

Rezk, M. R. (1980): Seed structure as a phylogenetic criterion. A case of *Plantago* seed. *Egypt. J. Bot*. **23**: 51-62.

Rezk, M. R. (1987): Variation in seed coat microsculpture in five species of *Plantago*. Alex. Sci. Exch. 8 (3).

Robertson, K. R., Phipps, J. B., Rohrer, J. R. and Smith, P. G. (1991): A synopsis of genera in Maloideae (Rosaceae). *Syst. Bot.* **16**: 376-394.

……..., Phipps, J. B. and Rohrer, J. R. (1992): Summary of leaves in the genera of the Maloideae. *Ann. Missouri. Bot. Gard*. **79**: 81-94.

Rohrer, J. R., Robertson, K. R. and Phipps, J. B. (1991): Variation in structure among fruits of Maloideae (Rosaceae). *Amer. J. Bot*. **78** (**12**): 1617-1635.

……., Robertson, K. R. and Phipps, J. B. (1994): Floral morphology of Maloideae (Rosaceae) and its systematic relevance. *Amer. J. Bot*. **81**: 574-584.

Rohlf, F.J. (1989): NTSYS-pc, Numerical taxonomy and multivariate analysis system. *New York. Exeter Publ., Ltd.*

Rudenko, I. S. and Rotaru, G. I. (1988): Morphological and anatomical features of the seeds of hybrid forms of apple, pear and quince. *Izvestiya-Akademii-Nauk-Moldavskoi-SSR. – Bioloicheskie-I-Khimicheskie-Nauki*. No. **5**, 15-20.

Schmit, V., Debouck, S. G. and Baudoin, J. P. (1996): Biogeographical and molecular observations of *Phaseolus glabellus* (Fabaceae: Phaseolinae) and its taxonomic status. *Taxon* **45**: 493-501.

Schneider, C. K. (1912): *Illustriertes Handbuch de Laubholzkunde* 2 vols. Cited from Bean W. J. (1950). *Trees and Shrubs Hardy in the British Isles*. *John Murray. London.*

Stant, M. T. (1973): The role of the scanning electron microscope in plant. *Kew Bull*., **28**: 105-115.

Stearn, W. T. (1966): *Botanical Latin*. *Nelson & Sons. London*. Pages 506-507.

Stebbins, G. L. (1950): *Variation and Evolution in Plants*. *Oxford Univ. Press. London*.

Sterling 1966: Comparative morphology of the carpel in the Rosaceae. VII. Pomoideae: *Chaenomeles, Cydonia, Docynia*. *Amer. J. Bot*. **53**: 225-231.

Weber, C. (1964): The genus *Chaenomeles* (Rosaceae). *J. Arnold Arboretum*. **45**: 161-205.

Xuhan, X. and Van-Lammeren, A. A. M. (1994): The ultrastructure of seed coat development in *Ranunculus sceleratus*. *Acta-Botanica-Neerlandica*. **43** (**1**): 27-37.