Taxonomic relationships in *Veronica* sect. *Beccabunga* (*Plantaginaceae* s.l.) of Egypt: evidences from morphometric and molecular analyses

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Abstract. There has been a great deal of controversy regarding the taxa and their taxonomic status of *Veronica* sect. *Beccabunga* in Egypt. The present study aimed at critical reappraisal of the taxonomy of *Veronica* sect. *Beccabunga* in Egypt by numerical and molecular approaches. Representative specimens were collected from their natural habitats and subjected to morphological description and RAPD amplification, using five random primers. Cluster analysis was applied to morphological and RAPD data separately. Numerical analysis of morphological and molecular data has led to discrimination of *V. anagallis-aquatica* var. *anagallis-aquatica* and *V. anagallis-aquatica* var. *nilotica*. Three subspecies of *V. anagalloides* were recognized: *V. anagalloides* subsp. *taeckholmiorum*, *V. anagalloides* subsp. *anagalloides* and *V. anagalloides* subsp. *heureka*. *Veronica catenata* can be represented by *V. catenata* subsp. *pseudocatenata* and *V. catenata* var. *catenata*. The identification of *V. scardica* subsp. *africana* was confirmed, and *V. beccabunga* was recorded, which is mainly confined to the Mediterranean region. Specimens collected from the Fayoum depression, tentatively identified as *V. kaiseri* – a species considered endemic to the Sinai Peninsula – show distinct RAPD patterns.

Key words: Egypt, morphological traits, numerical taxonomy, *Plantaginaceae* s.l., RAPD, *Scrophulariaceae*, *Veronica*

Introduction

Veronica is the largest genus of the *Plantaginaceae* s.l. according to APG (1998), APG II (2003) having been formerly placed in *Scrophulariaceae*, with about 400–500 species. The genus comprises annual and perennial herbs which are distributed mainly in the temperate regions of both hemispheres (Albach & al. 2004a). The taxonomic history of *Veronica* shows that the genus includes some generally derived characters, in which many species have developed character states otherwise considered plesiomorphic, detected not only in the flower but also in inflorescence morphology, embryology and karyology (Albach & al. 2004b).

In Egypt, Boulos (2002) recognized 11 species of *Veronica* belonging to subgenus *Beccabunga*. This subgenus is divided into two sections (Albach & al. 2004a): sect. *Beccabunga* (including *V. anagallis-aquatica*, *V. catenata* subsp. *pseudocatenata*, *V. anagalloides* subsp. *taeckholmiorum*, *V. kaiseri* (= *musa*) and *V. scardica* subsp. *africana*), and sect. *Acinifolia*. Section *Beccabunga* comprises two subsections: subsect. *Beccabunga* and subsect. *Anagallis* (= *V. anagallis-aquatica* aggregate). *Veronica anagallis-aquatica* aggregate is an extremely confusing complex of several species and subspecies closely connected to each other (Chrtek & Osbornová-Kosinová 1981; Öztürk & Fischer 1982; Saeidi & Kharabian 2005). Members of *Veronica* sect. *Beccabunga* in Egypt have been treated differently by several au-

thors (Täckholm 1956, 1974; Chrtek & Osbornová-Kosinová 1981; Boulos 1995, 2002; El Hadidi & Fayed 1994/1995; El Hadidi & al. 1999). The controversy extended to the identification and number of taxa. These studies were based on morphological characters, including leaf shape, density of inflorescence, length of peduncle and pedicel, shape of bract, shape of capsule, and relation between length of calyx and capsule. Recently, Abd El-Ghani & al. (2010) published a taxonomic revision of *Veronica* sect. *Beccabunga* and confirmed the record of *V. anagalloides* subsp. *anagalloides*, *V. anagalloides* subsp. *heureka* and *V. catenata* var. *catenata* as new additions to the flora of Egypt. In the meantime, the occurrence of *V. anagallis-aquatica* var. *anagallis-aquatica* was also confirmed by the same authors. The occurrence of *V. kaiseri* (endemic to Sinai) in the Fayoum region needs further investigation.

In a polymorphic genus like *Veronica*, randomly amplified polymorphic DNA is among the techniques that have been developed as molecular markers for visualizing DNA sequence polymorphism (Williams & al. 1990). Among the advantages of RAPDs are suitability for work on anonymous genomes, applicability of available limited DNA, efficiency and low expense functional in distinguishing individuals, cultivars or accessions (Karp & al. 1996). Molecular systematic and phylogenetic analyses have been applied to *Veronica* and related genera using three different DNA regions: the nuclear ribosomal ITS region (Wagstaff & Garnock-Jones 1998; Albach & Chase 2001), the plastid trnL-F region (Albach & al. 2004b), and the plastid rps16 intron (Albach & Chase 2004). Recently, Albach & Meudt (2010) reconstructed the phylogeny of *Veronica* using six DNA markers to address the subgeneric relationships, topological incongruence in the earlier studies, and relationships among the main species groups in the Southern Hemisphere.

The purposes of this study are to examine the patterns of phenetic structure and the levels of genetic variations within and among the species of *Veronica* sect. *Beccabunga* in Egypt by using random amplified polymorphic DNA (RAPD) markers and morphological characters.

Material and methods

Plant material

The present study was based on collecting fresh materials from their natural habitats during the growing seasons of 2007 and 2008 (Table 1). More than 800 specimens were collected and examined with a MS003A binocular head zoom stereo microscope. In addition, morphological data of the Egyptian taxa were also based on herbarium collections kept in the major Egyptian herbaria: Cairo University (CAI), Agricultural Museum (CAIM), and National Research Centre (CAIRC) (for codes see Holmgren & Holmgren 1998), and were examined for variation in morphological characters. For taxonomy (and nomenclature), literature was also consulted, e.g., Täckholm (1974), Chrtek & Osbornová-Kosinová (1981), Fischer (1981), El Hadidi & Fayed (1994/1995), Boulos (1995, 2002), El Hadidi & al. (1999). The classification of the studied taxa can be summarized as follows:

V. subsect. *Beccabunga*
- *V. beccabunga*

V. subsect. *Anagallis*
- *V. anagallis-aquatica*
  - var. *anagallis-aquatica*
  - var. *nilotica*
- *V. scardica* subsp. *africana*
- *V. kaiseri*
- *V. catenata*
  - subsp. *pseudocatenata*
  - var. *catenata*
- *V. anagalloides*
  - subsp. *heureka*
  - subsp. *taeckholmiorum*
  - subsp. *anagalloides*

Morphological analysis

Forty-six representatives of each of the ten collected taxa were used as Operational Taxonomic Units (OTU's). A total of 29 characters (12 quantitative and 17 qualitative) were measured for each specimen (Table 2). Only mature plants were chosen for morphometric analysis on the basis of differences among species in the vegetative and reproductive parts. The criteria mainly represent leaf structure, floral and fruit morphology. Thirteen qualitative characters were scored as binary, and the rest were scored as multi-state characters. The measurements of all specimens of a taxon were averaged into one OTU score for each of the characters. OTU scores for quantitative characters were the average measurements of at least 10 specimens (wherever possible). Bearing in mind that herbarium specimens cannot

Table 1. List of localities, sampling sites and dates of the collected species. Species abbreviations: *Q* = *V. anagallis-aquatica* var. *anagallis-aquatica*, *AN* = *V. anagallis-aquatica* var. *nilotica*, *B* = *V. beccabunga*, *S* = *V. scardica* subsp. *africana*, *K* = *V. kaiseri*, *C* = *V. catenata* var. *catenata*, *Pc* = *V. catenata* subsp. *pseudocatenata*, *H* = *V. anagalloides* subsp. *heureka*, *T* = *V. anagalloides* subsp. *taeckholmiorum*, and *AG* = *V. anagalloides* subsp. *anagalloides*.

Species abb.	Locality and coordinates	Sampling site and date
Q	1. Borg El-Arab (30°54'51" N, 29°41'49" E) 2. Abo-Qier (31°14'10" N, 29°59'15" E) 3. Rashid (31°23'29" N, 30°25'26" E) 4. El Beheira Province (31°10'47" N, 30°31'48" E) 5. Cultivated land of Fayoum (29°18'35" N, 30°50'23" E)	1. New Borg El-Arab (1.6.2007) 2. El Siouf-Ele'waayd (1.6.2007) 3. Domain of El salts (1.6.2007) 4. Mahmudiya (1.6.2007) 5.1. Fayoum: Menshat Abd-alla (21.3.2007); Azbet Safer (5.5.2007); Elmoror (2.4.2008); Howaret Adlan (23.2.2008) 5.2. Sinnures: Azbet Abd-El Azem (Menshat Beni Othman, Menshat Tantway) (10.3.2007); Domain El Sheikh Abead (5.2.2007); Mtrtaars (7.2.2007) 5.3. Etsa: El-Gharaq (23.2.2007, 13.4.2007, 29.2.2008)
AN	Cultivated land of Fayoum	Etsa: El-Gharaq (3.2.2007, 13.4.2007)
B	1. Abo-Qier 2. Rashid	1. El Siouf-Ele'waayd (1.6.2007) 2. Domain of El salts (1.6.2007)
S	1. Borg El-Arab 2. Abo-Qier 3. Rashid 4. Cultivated land of Fayoum	1. New Borg El-Arab (1.6.2007) 2. El Siouf-Ele'waayd (1.6.2007) 3. Domain of El salts (1.6.2007) 4. Ebshawai: Zmam El khold (5.2.2007)
K	Cultivated land of Fayoum	1. Fayoum: Azbet Safer (5.5.2007); Elmoror (2.4.2008) 2. Sinnures: Azbet Abd-El Azem, Menshat Tantway (10.3.2007) 3. Tamya: El Mhsara (1.6.2008) 4. Etsa: El-Gharaq) 3.4.2007)
C	Cultivated land of Fayoum	1. Fayoum: Manthat Abd-alla (21.3.2007); Azbet Safer (5.5.2007); Elmoror (2.4.2008); Howaret Adlan (23.2.2008) 2. Sinnures: Azbet Abd-El Azem (Menshat Beni Othman, Menshat Tantway) (10.3.2007); Domain El Sheikh Abead (5.2.2007); Mtrtaars (7.2.2007) 3. Etsa: El-Gharaq) 23.2.2007, 13.4.2007, 29.2.2008)
Pc	1. Abo-Qier 2. Rashid 3. Cultivated land of Fayoum	1. El Siouf-Ele'waayd (1.6.2007) 2. Domain of El salts (1.6.2007) 3.1. Fayoum: Manthat Abd-allah (21.3.2007); Azbet Safer (5.5.2007); Elmoror (2.4.2008); Howaret Adlan (23.2.2008) 3.2. Sinnures: Azbet Abd-El Azem, Menshat Beni Othman, Menshat Tantway (10.3.2007); Domain El Sheikh Abead (5.2.2007); Mtrtaars 7.2.2007) 3.3. Etsa: El-Gharaq) 23.2.2007, 13.4.2007, 29.2.2008)
H	Cultivated land of Fayoum	1. Fayou m: Azbet Safer (5.5.2007); El-Zawaia (14.4.2008); El-Edwa (1.6.2008) 2. Tamya: El Mhsara (1.6.2008) 3. Etsa: El-Garq (3.4.2007, 29.2.2008)
T	1. Cultivated land of Beni Suef (29°4'47" N, 31°5'23" E) 2. Cultivated land of Fayoum	1. El Dwaaldda 2. Fayoum: El-Zawaia (14.4.2008)
AG	Rashid	Domain of El salts (1.6.2007)

be regarded as a random sample of the species, we followed Wieringa (1999) by calculating the mean value of the minimum and maximum measurements. The complete data matrix is available on request from the Botany Department, Faculty of Science, Fayoum University, Egypt.

In order to test for differences between the species, a multivariate analysis of variance (MANOVA), including all quantitative characters, was performed, followed by univariate ANOVAs for each character by SPSS 10.0 software (SPSS Inc., Chicago, IL). Variability of the morphological characters within each species was measured by calculating the coefficient of variance (CV) (Sokal & Rohlf 1995). Analysis of morphological data was conducted using NTSYS-pc version 2.01a (Rohlf 1998). For cluster analysis, standardized data were used to compute a distance matrix based on the average taxonomic distances, Manhattan distances, and Euclidean distances with the SIMINT function, prior to being subjected to the unweighted pair-group method with an arithmetic averages (UPGMA) clustering algorithm with SAHN function. In order to estimate how well the phenogram represents its corresponding pair-wise distance matrix, the co-phenetic correlation coefficients with a Mantel test (Mantel 1967) were calculated for

each phenogram and data matrix pair. The phenogram with the highest co-phenetic correlation coefficient (r) was reported here. This was done with the help of COPH and MXCOMP functions of NTSYS-pc (Rohlf 1998). For ordination, Principal Coordinate Analysis (PCoA: Gower 1966) was performed using the product-moment correlation as a coefficient. The procedure SIMINT was used to calculate the distance matrix based on STAND data, while the procedures EIGEN, PROJ and MXPLOT were used to perform the PCoA.

Table 2. List of 29 characters and states used in the morphological analysis of *Veronica* section *Beccabunga* in Egypt.

Characters	Abbreviation	State
Duration	DU	Annual (0), perennial (1)
Stem habit	STH	Erect (0), procumbent (1)
Stem height (cm)	STL	Number (five categories)
Stem texture	STT	Glabrous (0), hairy (1)
Stem branching	STB	Absent (0), present (1)
Leaf insertion	LFI	Cauline (0), cauline and ramal (1)
Leaf petiole	LFP	Sessile (0), petiolate (1)
Sessile position at leaf	SPAL	Lower leaves (0), upper leaves (1)
Petiole position at leaf	PPAL	Upper leaves (0), lower leaves (1)
Leaf blade shape	LFBS	Ovate-lanceolate (0), elliptical-lanceolate (1), linear-lanceolate to (2), oblanceolate to lanceolate (3), ovate (4), rhombic (5), elliptic-ovate (6)
Leaf blade margin	LFBM	Entire (0), serrate (1), serrate to subentire (2) sub-entire to crenate (3), denticulate (4)
Leaf blade length	LFBL	Number (two categories)
Leaf blade width	LFBW	Number (two categories)
Inflorescence position	INP	Terminal (0), axillary (1)
Inflorescence arrangement	INAR	Alternate (0), opposite (1)
Number of lateral racemes	NLR	Number (two categories)
Density of inflorescence	IND	Dense (0), lax (1)
Length of inflorescence (mm)	IFL	Number (two categories)
Length of peduncle (mm)	PDL	Number (two categories)
Length of pedicel at flowering time (mm)	PDLFL	Number (two categories)
Length of pedicel at fruiting time (mm)	PDLFR	Number (two categories)
Length of bract	BRL	Number (two categories)
Shape of bract	BRS	Linear –lanceolate to lanceolate (0), linear (1), oblong to elliptic (2)
Length of sepals (mm)	CXL	Number (two categories)
Length of capsule	CPL	Number (two categories)
Length of style	STYL	Number (two categories)
Shape of capsule	CPS	Orbicular (0), elliptic (1)
Apex of capsule	CPA	Rounded (0), acute (1)
Surface of capsule	CPSR	Glabrous (0), hairy (1)

In the classification output, differences among the main groups of species (second and third levels) in relation to the 29 measured morphological characters were evaluated simultaneously by Kruskal-Wallis one-way analysis of variance. Differences in the morphological characters between the two main groups at the first level of classification were tested by the Mann-Whitney test. All statistical techniques were according to SPSS 10.0 software (SPSS Inc., Chicago, IL).

Molecular analysis

Genomic DNA samples were extracted from 5 mg of germinated seed of a representative sample of each taxon, according to Porebsky & al. (1997). Five random primers termed A-01 (5'CAGGCCCTTC3'), A-02 (5'TGCCGAGCTG3'), B-01 (5'GTTTCGCTCC3'), B-02 (5'TGATCCCTGG3'), and B-03 (5'CATCCCCCTG3') were used for RAPD reactions. The mix for a 50 µl reaction comprised 50 µg genomic DNA, 1 µM primer, 1.0 µM dNTP (supplied as a 50X deoxynucleoside triphosphate Mastermix; Bioline USA, Inc.), 5 mM $MgCl_2$ (supplied as a 50 mM stock; Bioline), 10 mM 10X Taq reaction buffer (Bioline), and 1 U BIOTAQ DNA polymerase (Bioline). Amplifications were performed in the DNA Thermal Cycler (Hybaid Ltd.) programmed for initial 6 min denaturation at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 46 °C and 1 min at 72 °C. PCR products were separated in a 1.5 % agarose gel containing 0.2 pg/ml of ethidium bromide. Molecular weight of the PCR products was estimated by using a 100-bp ladder (Amersham-Pharmacia-Biotech, Uppsala, Sweden) as a molecular size standard.

A data matrix was created from photographs of gels by using a gel documentation system (AAB Advanced American Biotechnology 1166 E, Valencia Dr. Unit 6 C, Fullerton CA 92631). RAPD bands were scored as present (1) or absent (0), and a pair-wise correlation distance matrix was obtained. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering by using the unweighted pair group method with arithmetic mean (UPGMA) method was then performed, and a phenogram was generated, as described by Sneath & Sokal (1973) using NTSYS-pc version 2.01a (Rohlf 1998).

Results

Morphological data

Normality of distribution of characters was assessed for the 12 variables, and square root transformation was carried out for peduncle length (PDCL), capsule length (CPL) and style length (STYL). Univariate ANOVAs revealed significant differences for nine of the 12 quantitative examined characters (Table 3). The constructed phenogram by taxonomic average distances showed the least distortion with a co-phenetic correlation coefficient r = 0.854 (Mantel test = p <0.002), indicating a good fit of the phenogram to the distance matrix (Rohlf 1998). In Euclidean distances, r = 0.853; and in Manhattan distances, r = 0.828. Fig. 1 showed the UPGMA phenogram based on the analysis of 29 morphological characters, which separated the ten taxa used in this study at distance 0.65.

Results of the statistical evaluation of difference at each level, represented by P-values of the Mann-Whitney test at the first level, and Kruskal-Wallis test at the second and third levels, were displayed in Table 4. Four species-specific clusters can be distinguished at 65% similarity level. The first level of the phenogram separates *V. beccabunga* from all other species on the basis of stem height, leaf blade margin, number of lateral racemes, density of inflorescence, length of inflorescence, length of bract, and length of peduncle. At the second level of hierarchy, two main subgroups were distinguished; the most conspicuous included *V. anagalloides* subsp. *anagalloides* and *V. anagalloides* subsp. *taeckholmiorum*, in relation to duration, stem height, stem

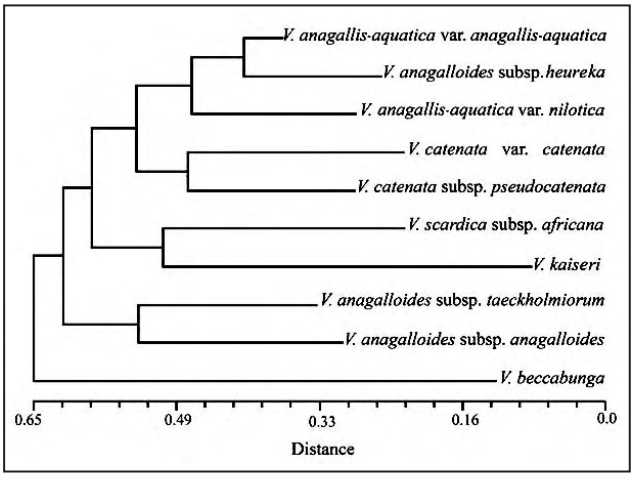

Fig. 1. The UPGMA phenogram based on the morphological data of *Veronica* (sect. *Beccabunga*).

Table 3. Means, coefficients of variation (%, in parentheses) and F statistics for 12 quantitative morphological characters. F = significant at 1% (**) and at 5% (*), d.f. = degrees of freedom. For species abbreviations see Table 1. For character abbreviations, see Table 2.

Character	Species F (d.f.= 9)	Mean values									
		Q	*AG*	*AN*	*B*	*C*	*H*	*K*	*Pc*	*S*	*T*
STL (cm)	6.02**	42.7 (25.1)	72.0 (3.9)	24.0 (57.9)	49.3 (40.2)	25.3 (29.2)	35.5 (16.6)	29.2 (28.0)	32.0 20.5)	22.0 (13.6)	33.7 (18.4)
LFBL (mm)	5.64**	32.0 (23.6)	42.0 (16.8)	12.0 (30.0)	37.7 (7.7)	22.0 (44.6)	17.0 (17.1)	15.0 (42.2)	34.2 (30.1)	20.7 (32.2)	37.4 (35.6)
LFBW (mm)	1.84	1.10 (15.7)	1.60 (30.0)	1.0 (14.8)	1.80 (8.3)	0.98 (37.2)	0.97 (21.6)	0.90 (41.6)	1.60 (58.8)	1.07 (39.0)	0.82 (19.2)
NLR (no.)	3.31**	4.3 (35.2)	10.0 (14.1)	4.3 (53.3)	8.0 (33.1)	5.3 (57.5)	8.5 (12.4)	3.0 (62.4)	6.20 (37.4)	3.7 (78.7)	7.7 (9.9)
IFLAF (mm)	2.06	5.00 (87.2)	13.50 ((36.7)	5.00 (20.0)	13.3 (15.6)	7.00 (29.7)	9.20 (34.4)	8.00 (55.9)	8.1 (43.0)	7.70 (27.1)	6.20 (21.1)
PDCL (mm)	4.52**	20.00 (15.0)	27.0 (10.5)	15.3 (7.5)	23.7 (12.9)	17.9 (31.7)	15.0 (10.7)	14.4 (18.1)	25.2 (28.1)	14.3 (24.5)	19.3 (12.1)
PDLFL (mm)	9.13**	2.00 (50.0)	2.00 (0.1)	2.7 (21.6)	6.0 (33.3)	2.14 (32.2)	2.25 (26.7)	1.40 (39.1)	1.50 (44.9)	1.50 (44.9)	1.75 (20.0)
PDLFR (mm)	3.05**	3.00 (57.7)	6.50 (10.9)	3.33 (69.3)	7.67 (32.8)	4.00 (62.9)	2.25 (16.8)	2.00 (0.1)	5.20 (50.8)	3.00 (33.3)	2.7 (20.2)
BRL (mm)	13.70**	2.33 (65.5)	8.50 (8.3)	2.33 (24.7)	1.00 (0.1)	2.60 (80.5)	1.25 (15.9)	2.80 (16.0)	6.42 (26.1)	1.33 (43.3)	5.25 (62.4)
CXL (mm)	2.12*	1.67 (69.3)	5.50 (12.9)	2.67 (10.8)	5.00 (40.0)	4.43 (71.2)	2.75 (34.5)	3.00 (0.1)	4.50 (50.6)	1.67 (69.3)	1.25 (12.6)
CPL (mm)	2.08*	1.33 (43.3)	2.50 (28.3)	1.00 (0.1)	2.33 (24.7)	2.86 (37.4)	1.50 (19.1)	1.60 (55.9)	1.75 (77.5)	1.00 (0.1)	1.00 (17.5)
STYL (mm)	1.94	1.67 (34.6)	2.00 (0.1)	2.00 (50.0)	2.00 (0.1)	2.00 (28.9)	2.00 (49.3)	2.20 (20.3)	2.17 (26.6)	1.00 (0.1)	1.00 (21.2)

branching, leaf insertion, leaf blade shape, length of leaf blade, position of inflorescence, number of lateral racemes, density of inflorescence, length of bract, and apex, shape and surface of capsule, and style length. At the third level of classification, a group including *V. kaiseri* and *V. scardica* subsp. *africana* was split from the others on the basis of duration, leaf blade shape, length of leaf blade, position of inflorescence, number of lateral racemes, length of peduncle, length of pedicel at flowering, length of pedicel at fruiting, and bract shape. Finally, *V. catenata* var. *catenata* and *V. catenata* subsp. *pseudocatenata*, and *V. anagallis-aquatica* var. *nilotica*, *V. anagalloides* subsp. *heureka*, and *V. anagallis-aquatica* var. *anagallis-aquatica* were separated from each other on the basis of duration, leaf blade shape, position of inflorescence, density of inflorescence, length of peduncle, length and surface of bract, length of sepal and apex of capsule. However, *V. anagallis-aquatica* var. *nilotica* was separated at a distance 0.47 in a distinct cluster.

Table 4. Significance values (P-values) of Mann-Whitney and Kruskal-Wallis tests for statistical evaluation of the differences in mean characters in relation to cluster groups. **P> 0.01, *P>0.05 (For character abbreviations, see Table 2).

Character	Level of hierarchy			
	1st	2nd	3rd	4th
	Mann–Whitney		Kruskal–Wallis	
DU	3.01	7.13**	8.68**	16.93**
STH	2.98	1.09	0.68	0.76
STL	4.45*	4.59**	2.32	0.65
STT	3.34	2.09	3.65	0.83
STB	2.49	7.83**	1.32	0.79
LFI	2.49	7.83**	1.32	0.79
LFP	0.34	1.45	0.41	0.70
SPAL	2.90	2.77	1.34	3.09
PPAL	3.67	0.99	3.84	2.05
LFBS	0.16	7.27**	5.70*	16.72**
LFBM	13.60**	1.60	0.02	0.30
LFBL	2.39	6.74**	6.37*	3.64
LFBW	3.30	0.63	0.86	0.38
INP	3.68	8.90*	8.41**	12.90**
INAR	4.17	3.69	3.78	0.98
NLR	11.53**	0.11	0.46	5.73*
IND	10.84**	25.73**	3.34	6.14*
IFL	7.01*	0.41	0.005	1.04
PDL	8.52**	0.45	3.81*	3.64
PDLFL	66.0**	0.04	5.74*	4.38*
PDLFR	3.67	4.34	5.73*	4.03
BRL	3.93*	5.97*	2.61	8.31**
BRS	1.49	3.54	4.47*	6.32*
CXL	1.48	1.36	0.84	5.13*
CPL	0.94	0.18	1.35	2.62
STYL	0.07	4.38*	0.36	1.18
CPS	0.46	42.0**	1.42	0.06
CPA	2.89	17.6**	0.91	11.08**
CPSR	5.54	20.1**	1.15	1.90

The 3-D plot of the 46 OTU's on the first three axes of principal coordinate analysis (PCoA) was shown in Fig. 2, which explained 45.28 % of the total observed variation. Along the first axis (33.05 % of the total variation), segregation between two groups was demonstrated: (1) the group of *V. beccabunga*, *V. anagallis-aquatica*, *V. catenata* var. *catenata,* and *V. catenata* subsp. *Pseudocatenata,* and (2) the group of *V. anagallis-aquatica* var. *nilotica, V. scardica* subsp. *africana, V. kaiseri*, *V. anagalloides* subsp. *heureka*, *V. anagalloides* subsp. *taeckholmiorum* and *V. anagalloides* subsp. *anagalloides*. The main characters explaining this segregation (characters with high factor loading >0.6) were stem height, length of petiole, density of inflorescence, length of peduncle, length of pedicel at flowering, length of pedicel at fruiting, length of sepals, length of style, and shape of capsule (Table 5). The second axis (6.82 % of the total variation) revealed a split between (1) *V. catenata* subsp. *pseudocatenata,* and *V. beccabunga* and (2) *V. scardica* subsp. *africana*. This split was based mainly on the length of petiole, leaf blade shape, position of inflorescence, number of lateral racemes, and shape of capsule. Along the third axis (5.41 % of the total variation), three groups can be separated: (1) *V. anagallis-aquatica* var.

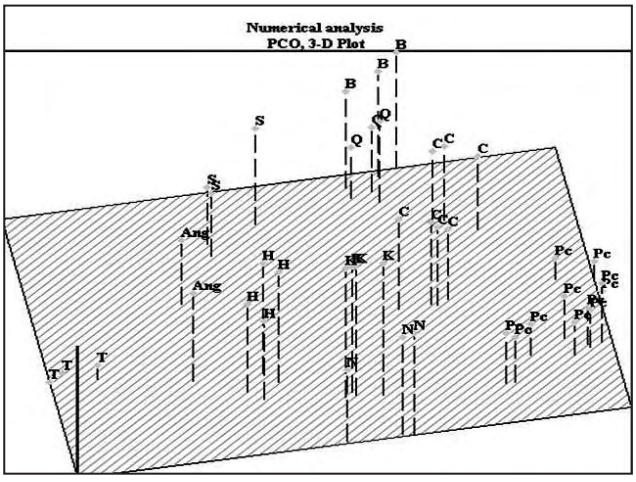

Fig. 2. 3-D plot of 46 OTU's on the first three principal coordinate axes. **Q** = *V. anagallis-aquatica* var. *anagallis-aquatica,* **N** = *V. anagallis-aquatica* var. *nilotica*, **B** = *V. beccabunga*, **S** = *V. scardica* subsp. *africana*, **K** = *V. kaiseri,* **C** = *V. catenata* var. *catenata*, **Pc** = *V. catenata* subsp. *pseudocatenata*, **H** = *V. anagalloides* subsp. *heureka*, **T** = *V. anagalloides* subsp. *taeckholmiorum,* and **Ang** = *V. anagalloides* subsp. *anagalloides.*

Table 5. Morphological characters showing the highest factor loadings on the first three principal coordinate axes.

Characters	Principal coordinates		
	1	2	3
	Factor loadings		
Duration	1.36	-1.31	4.32
Stem habit	3.88	-3.37	1.07
Stem height	-8.31	2.57	7.59
Stem texture	3.88	-3.37	1.07
Stem branching	3.16	2.50	7.80
Leaf insertion	-5.66	-2.81	-5.54
length of petiole	-7.96	7.20	2.37
Sessile position at leaf	1.14	1.49	4.32
Petiole position at leaf	-1.04	1.74	-5.12
Leaf blade shape	2.54	-9.06	-2.17
Leaf blade margin	-1.27	4.66	-3.92
Leaf blade length	-2.65	1.37	-3.92
Leaf blade width	4.68	2.22	1.33
Inflorescence position	4.96	7.82	-5.31
Inflorescence arrangement	3.78	-3.87	1.98
Number of lateral racemes	-1.88	-9.57	-2.89
Density of inflorescence	7.31	-1.71	2.36
Length of inflorescence	-1.14	3.37	3.27
Length of peduncle	7.18	2.42	-5.27
Length of pedicel at flowering time	9.24	2.70	2.36
Length of pedicel at fruiting time	7.58	-1.07	4.43
Length of bract	3.99	-2.69	-5.99
Shape of bract	2.49	-1.31	-8.19
Length of sepals	-8.53	-2.51	9.60
Length of capsule	1.20	4.68	2.07
Length of style	6.26	-1.41	4.14
Shape of capsule	-8.61	-7.66	-2.48
Apex of capsule	-1.14	-3.25	-5.86
Surface of capsule	-1.39	-3.58	-6.6
Percentage per PCoA	33.05	6.82	5.41
Percentage total variation for the first three principal coordinate axes amounts to = 45.28 %			

anagallis-aquatica and *V. catenata* var. *catenata*, (2) *V. anagallis-aquatica* var. *nilotica*, *V. kaiseri* and *V. anagalloides* subsp. *heureka*, and (3) *V. anagalloides* subsp. *taeckholmiorum* and *V. anagalloides* subsp. *anagalloides*. This separation was based mainly on stem height, stem branching, shape of bract, length of sepal, and surface of capsule (Table 5).

In conclusion, on the basis of factor loading of the first axis (15.83 %), the ten studied taxa were separated into two groups. The first group comprised the taxa with positive factor loadings (*V. anagallis-aquatica* subsp. *anagallis-aquatica*, *V. anagallis-aquatica* var. *nolotica*, *V. beccabunga* and *V. scardica* subsp. *africana*). The other group constituted the other species (*V. kaiseri, V. catenata* var. *catenata, V. catenata* subsp. *pseudocatenata*, *V. anagalloides* subsp. *heureka*, *V. anagalloides* subsp. *taeckholmiorum, V. anagalloides* subsp. *anagalloides*) with negative factor loadings (Table 6). On the other hand, the second axis (15.25 %) separated *V. anagallis-aquatica* and *V. scardica* with factor loading > 0.6; *V. beccabunga* with factor loading -1.25, *V. anagallis-aquatica* var. *nilotica* with factor loading -2.02, while *V. catenata* var. *catenata* was separated from the remaining taxa with factor loading 5.24. The third axis (13.98 %) segregated *V. kaiseri* and *V. anagalloides* subsp. *heureka* with factor loadings between -2.20, -2.81, respectively; *V. catenata* var. *catenata* and *V. catenata* subsp. *pseudocatenata* in another subgroup, with factor loadings between -7.27, -4.73, respectively; and *V. anagalloides* subsp. *taeckholmiorum* and *V. anagalloides* subsp. *anagalloides* in the third subgroup with factor loadings between 6.06, 7.05, respectively.

Table 6. Factor loadings of *Veronica* species on the first three PCoA axes.

Species	Principal coordinates		
	1	2	3
V. anagallis-aquatica var. *anagallis-aquatica*	6.64	7.19	1.58
V. anagallis-aquatica var. *nilotica*	2.98	-2.02	7.07
V. beccabunga	7.84	-1.25	-5.22
V. scardica subsp. *africana*	8.31	6.93	-1.52
V. kaiseri	-6.67	-4.66	-2.20
V. catenata var. *catenata*	-4.39	5.24	-7.27
V. catenata subsp. *pseudocatenata*	-5.40	-2.36	-4.73
V. anagalloides subsp. *heureka*	-7.33	-1.39	-2.81
V. anagalloides subsp. *taeckholmiorum*	-4.58	1.20	6.06
V. anagalloides subsp. *anagalloides*	-4.01	-2.80	7.05
Percentage per PCO Axis	15.83 %	15.25 %	13.98 %
Percentage total variation for the first three principal coordinates amounts to = 45.06%			

RAPD data

The five random primers applied for screening the studied ten *Veronica* taxa revealed that of a total of 279 bands, 206 RAPD fragments were polymorphic (73.83 %). Of the recorded RAPD fragments, a total of 31 fragments were specific for the studied samples of *V. anagallis aquatica* var. *anagallis aquatica*, 28 for *V. anagallis aquatica* var. *nilotica*, 37 for *V. beccabunga*, 34 for *V. scardica* subsp. *africana*, 9 for *V. kaiseri*, 34 for *V. catenata* var. *catenata*, 25 *V. catenata* subsp. *pseudocatenata*, 29 *V. anagalloides* subsp. *heureka*, and 26 for *V. anagalloides* subsp. *taeckholmiorum* and *V. anagalloides* subsp. *anagalloides.* A RAPD polymorphism profile generat-

ed from five decamer primers was shown in Fig. 3. The number of loci amplified by each primer was clearly different. The maximum number of bands (55) was produced by the primer B-01, whereas the least number of bands (29) was produced by the primer B-02 (Table 7).

The UPGMA dendrogram obtained from RAPD results of the ten taxa of sect. *Beccabunga* is displayed in Fig. 4. The studied *Veronica* taxa can be distinguished into two main clusters at a distance of 0.50. While *V. beccabunga* occupied one cluster, all other taxa were included in the second. At a distance of 0.475, *V. scardica* subsp. *africana* and *V. anagallis-aquatica* constituted one group. The remaining taxa were separated at a distance of 0.465 in such a way that *V. catenata* var. *catenata* occupied a distinctive group. At a distance of 0.425, *V. anagalloides* subsp. *taeckholmiorum, V. anagalloides* subsp. *anagalloides* and *V. anagallis-aquatica* var. *nilotica* were distinguished as separate clusters, while *V. anagalloides* subsp. *heureka, V. catenata* subsp. *pseudocatenata* and *V. kaiseri* were grouped into a separate cluster. The dendrogram represented the close distances between the species occurring in adjacent tips of the classification, according to numerical taxonomy (Sneath & Sokal 1973).

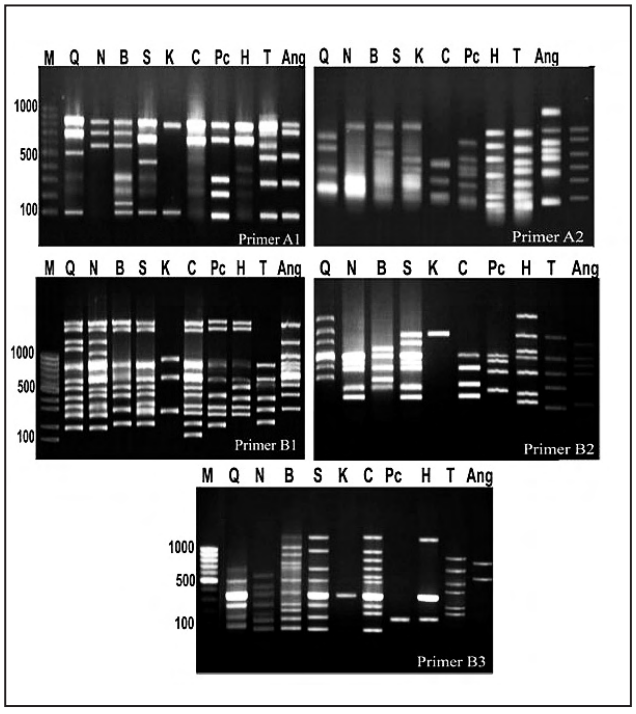

Fig. 3. RAPD fingerprinting patterns of the 10 taxa of *Veronica* (sect. *Beccabunga*) generated by five primers. For species abbreviations, see Fig. 2.

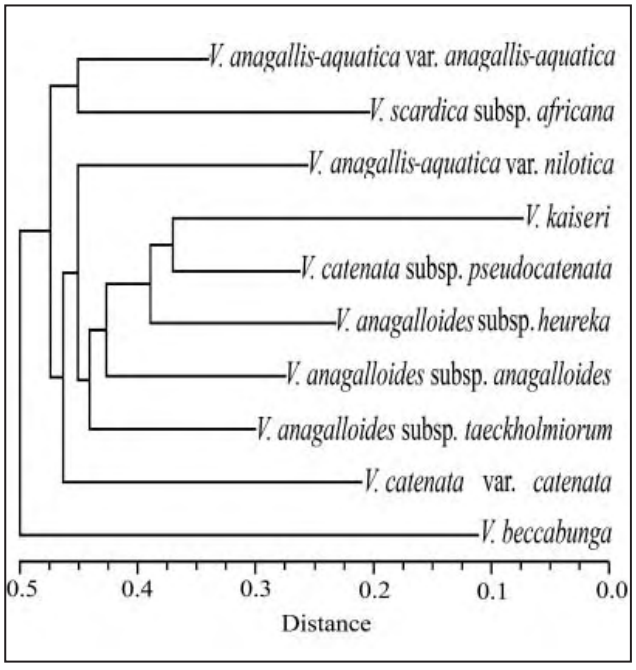

Fig. 4. Phenogram of the UPGMA cluster using molecular data (RAPD) comprising all studied taxa.

Table 7. Sequences, size, number of polymorphic bands and percentages as generated by five arbitrary primers.

Primer code	Nucleotide sequence (5'–3')	Size (bp) min–max	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands
A-01	CAG GCC CTT C⁻	285–880	49	37	75.51
A-02	TGC CGA GCT G	300–962	60	43	71.67
B-01	GTT TCG CTC C	262–1600	84	55	65.48
B-02	TGA TCC CTG G	110–1000	37	29	78.38
B-03	CAT CCC CCT G⁻	126–1178	49	42	85.71
Total			279	206	73.83
Mean			55.8	41.2	73.83

Discussion

In the present study, *V. beccabunga* was segregated into a separate cluster, after morphological and RAPD data were considered. Distinctness of *V. beccabunga* agrees with the classification of sect. *Beccabunga* into two subsections: subsect. *Beccabunga* including *V. beccabunga,* and subsect. *Anagallis* including the other nine taxa (Chrtek & Osbornová-Kosinová 1981; Öztürk & Fischer 1982). Täckholm (1974) recorded the occurrence of *V. beccabunga* var. *beccabunga* in addition to *V. beccabunga* var. *aegyptiaca*. On the other hand, Chrtek & Osbornová-Kosinová (1981), El Hadidi & al. (1999) and

Boulos (2002) suspected the occurrence of these taxa, considering them as *V. scardica* subsp. *africana, V. catenata* subsp. *pseudocatenata,* or *V. kaiseri.* The cluster analysis based on either morphological or RAPD data obviously revealed the distinctness of *V. scardica* subsp. *africana* and *V. kaiseri*. Besides, *V. beccabunga* showed 37 RAPD fragments, while *V. scardica* subsp. *africana* had 34 RAPD fragments, sharing only four RAPD fragments (A-01 323bp, A-02 800bp, 388bp, B-01 780bp). These results were in favor of the view of Täckholm (1974) about the occurrence of *V. beccabunga* in Egypt, distinct from *V. scardica* subsp. *africana,* or *V. kaiseri*. It was reported by Chrtek & Osbornová-Kosinová (1981) that *V. scardica* had often been misidentified as *V. beccabunga.* However, the present investigation may provide a means for discriminating the two species at the molecular level by RAPD analysis.

Veronica kaiseri has been considered an endemic species to Egypt, with its distribution limits in the Sinai region (Täckholm 1956, 1974; Chrtek & Osbornová-Kosinová 1981; El Hadidi & Fayed 1994/1995; Boulos 1995, 2002; El Hadidi & al. 1999). However, we have collected materials from the Nile Delta (Fayoum Area) resembling *V. kaiseri,* having alternate, lax inflorescence with few flowers and capsules with acute apex. Our specimens differ from the holotype kept in CAIM herbarium in leaf morphology (elliptic-ovate) and petiole length of the lower leaves (1 mm long). These specimens showed a very characteristic RAPD pattern different from the other studied taxa (only nine RAPD fragments sharing the RAPD fragment B-01, 780bp) with *V. beccabunga*, *V. scardica* subsp. *africana, V. catenata* var. *catenata* and *V. catenata* subsp. *pseudocatenata*. In personal communications with Prof. L. Boulos and Prof. Dirk C. Albach, they both agreed with the identification of specimens from the Nile Delta as *V. kaiseri*. However, it would take further investigations to elucidate the identification of these samples, and to compare *V. kaiseri* from Sinai with our collected samples by chromosome number and DNA markers (ITS, AFLP, SSR).

In the present study, *V. anagallis-aquatica* var. *anagallis-aquatica* and *V. anagallis-aquatica* var. *nilotica* were recognized on the basis of differences in leaf morphology, inflorescence and capsule. Cluster analysis of morphological and molecular data has clearly divided the two taxa into two distinctive groups. Besides, RAPD analysis revealed 31 RAPD fragments in *V. anagallis-aquatica* var. *anagallis-aquatica,* while *V. anagallis-aquatica* var. *nilotica* exhibited 28 RAPD fragments, sharing only one band (B-02 387bp). Earlier studies reported that Egyptian specimens of *V. anagallis-aquatica* var. *anagallis-aquatica* may be identified as *V. anagallis-aquatica* var. *nilotica* (Chrtek & Osbornová-Kosinová 1981; El Hadidi & al. 1999). On the other hand, Täckholm (1956, 1974), El Hadidi & Fayed (1994/1995) and Boulos (1995, 2002) treated this taxon as *V. anagallis-aquatica*. However, the data of this study, as well as the distance between the two taxa based on RAPD analysis (0.5) indicated the occurrence of *V. anagallis-aquatica* var. *anagallis-aquatica,* as well as of *V. anagallis-aquatica* var. *nilotica*.

Chrtek & Osbornová-Kosinová (1981) examined the specimens previously identified as *V. anagallis-aquatica* or *V. anagallis-aquatica* var. *aquatica* and concluded that these materials had to be identified as *V. catenata.* The differences between the Egyptian material and typical *V. catenata* (cf. Burnett 1950) have prompted them to give the Egyptian material subspecific rank, naming it *V. catenata* subsp. *pseudocatenata*. However, among the collected taxa in the present study, there were specimens identified as *V. catenata* subsp. *pseudocatenata* and others identified as *V. catenata* var. *catenata*. The two taxa differed morphologically in the shape of the bract and in relation between the length of bract and pedicel. On the other hand, the two taxa differed markedly in the number of RAPD fragments (34 and 25 respectively, with only six common bands: A-01, 753bp, A-02, 408bp; B-01, 1467bp, 1000bp, 780bp, 500bp) and were clustered into separate groups, according to UPGMA clustering analysis of morphological and molecular data. These observations, along with the distance between the two taxa (0.47), suggested the occurrence of *V. catenata* subsp. *pseudocatenata* in addition to the newly recorded *V. catenata* var. *catenata*.

Studies dealing with *V. anagalloides* in Egypt recorded the occurrence of *V. anagalloides* subsp. *taeckholmiorum* as common and endemic to Egypt (Chrtek & Osbornová-Kosinová 1981; El Hadidi & Fayed 1994/1995; Boulos 1995, 2002; El Hadidi & al. 1999). In the present study, the collected materials can be identified as *V. anagalloides* subsp. *anagalloides* and *V. anagalloides* subsp. *heureka* on the basis of differences in the pedicel to bract ratio, density of inflorescence, capsule and lifeform. The three taxa differ noticeably in the number of RAPD fragments, having in common only one RAPD fragment (A-01, 292bp), which led to their distinction by UPGMA clustering analysis. These findings suggest

the occurrence of *V. anagalloides* subsp. *taeckholmiorum,* as well as of *V. anagalloides* subsp. *heureka* and *V. anagalloides* subsp. *anagalloides* as new records.

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