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MORPHOLOGICAL AND MOLECULAR DIFFERENTIATION BETWEEN EGYPTIAN SPECIES OF *PANCRATIUM* L. (AMARYLLIDACEAE)

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Three problems in the taxonomy of *Pancratium* in Egypt are the lack of publications, a lack of clarity about the relationships between recently distinguished species, and the lack of markers for examining the levels and patterns of variation in rare and endemic species; the latter hinders work in plant conservation genetics. In this study we reassessed the taxonomic status of the *Pancratium* species of Egypt, and examined morphological and genetic variation within and between species, using specimens from different populations collected throughout its distribution range in the country. Our assessment was based on 38 macromorphological characters mainly representing vegetative parts, flowers, fruits and seeds, in addition to RAPD data. The results revealed five morphologically distinguished *Pancratium* species in Egypt, of which *P. trianthum* Herb. is newly recorded. Species identification was confirmed by two phenetic dendrograms generated with 26 quantitative morphological characters and RAPD data, while species delimitation was verified by principal component analysis. The diagnostic floral characters are those of the perianth, corona teeth, pistil, stamens, aerial scape, spathe, and number of flowers. The retrieved RAPD polymorphic bands show better resolution of the morphologically and ecologically closely allied *Pancratium* species (*P. arabicum* and *P. maritimum*), and also confirm the morphological and ecological divergence of *P. tortuosum* from the other studied species. These results are supported by the constructed UPGMA dendrogram.

Key words: Taxonomic revision, morphometrics, RAPD, monocotyledons, UPGMA, reappraisal, species separation.

INTRODUCTION

Pancratium (Asparagales, Amaryllidaceae, Pancratieae; APG III, 2009) is the most widespread of all the genera in the Eurasian clade of Amaryllidaceae. It is a palaeotropical genus comprising 16 species distributed in Macaronesia, the Mediterranean basin, and throughout Africa to tropical Asia, and is also introduced and cultivated in many countries (Mabberley, 1993). *Pancratium* can tolerate extreme climates and inhabits extremely dry and sandy areas. The plants are geophytic monocots, bulbous herbaceous perennials producing showy white fragrant flowers with a straight perianth tube and conspicuous corona formed by the basal connection of the staminal filaments. Owing to a certain similarity in the flowers' "Pancratioid floral morphology" the neotropical genus *Hymenocallis* Salisb. was originally assigned to *Pancratium* (Meerow et al., 2002). However, *Pancratium* differs from the closely similar *Hymenocallis* in its numerous dry seeds with a phytomelan-dark testa. *Pancratium* species are economically important and highly valued medicinally. Several species are cultivated for their ornamental uses, to treat cancer with their unique alkaloids (Ioset et al., 2001), while others are of local importance in traditional medicine or folk practice. *Pancratium tenuifolium* Hochst. ex A. Rich. was used in Botswana in a coming-of-age ceremony (psychoactive properties, *Pancratium* TOW; http://lists.ibiblio.org/pipermail/pbs/2004-March/017456.html). *Pancratium trianthum* is employed as a psychoactive agent by the Bushmen in Dobe, Botswana. The bulb of this species is repeatedly rubbed over incisions on the head to induce visual hallucinations (Golden Guide: Hallucinogenic Plants, http://www.zauberpilz.com/golden/g21-30.html). *Pancratium maritimum* shows anti-fungal activity (Sür-Altiner et al., 1999) and also is used as a biopesticide, emetic, hypotensive, purgative, and for spleen inflammation (Stephen Mifsud/www.MaltaWildPlants.com/Malta).

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TABLE 1. Locations, habitats and abbreviations of the examined *Pancratium* specimens. *– newly recorded

Species	Species abbreviation	Location	Habitat
Pancratium maritimum L.	*Pm*	Sidi Barani, NW Mediterranean coast	Sandy maritime seashores
P. maritimum L.	*Pm*	Mersa Matrûh, NW Mediterranean coast	Sandy maritime seashores
P. maritimum L.	*Pm*	Ras El Hekma, NW Mediterranean coast	Sand dunes, sandy maritime seashores
P. maritimum L.	*Pm*	Burg El Arab, NW Mediterranean coast	Sand dunes
P. sickenbergeri Asch. & Schweinf. ex Barb.-Boiss. & Barbey	*Ps*	Bir El Abd, NE Mediterranean coast	Sandy desert wadis, roadsides, sandy or calcareous hill slopes, gravelly plains
P. arabicum Sickenb.	*Pa*	El Arish, Sheikh Zuwayed, Rafah, NE Mediterranean coast	Sandy maritime seashores and sand dunes
**P. trianthum* Herb.	*Ptr*	Gebel Elba area	In pockets of light soil among rocks
P. tortuosum Herb.	*Pto*	Wadi Yahameib	Red Sea sandy plains, and adjacent wadis

Habitat loss is currently the greatest threat to *Pancratium*; many species are endangered, vulnerable or nearly threatened. *Pancratium maritimum* is endangered in its original range, the sandy coasts of the Mediterranean exposed to the sea (Grassi et al., 2005). Other species such as *P. sickenbergeri* are subjected to heavy grazing (Saltz and Ward, 2000). When aboveground biomass is lacking, gazelles dig for underground parts of *P. sickenbergeri* and may consume all or part of the bulb, which constitutes most of the plant's volume.

Like many monocotyledons groups, *Pancratium* includes species that are difficult to distinguish due to shortcomings of publications on the genus, the lack of studies of wild populations, and problems in distinguishing floral characters in herbarium specimens. During this study we realized that morphological variability has led to much confusion about the exact current number of *Pancratium* species, especially in light of the many diagnostic characters proposed by different authors (Baker, 1898; Dinsmore, 1933; Andrews, 1956; Maire, 1959; Morton, 1965; Björnstad, 1973; El Gadi, 1978; Meikle, 1985; Feinbrun-Dothan, 1986; Heller and Heyn, 1991; Boulos, 2005). In Egypt, Täckholm and Drar (1954) recorded four *Pancratium* species: *P. arabicum*, *P. maritimum*, *P. sickenbergeri* and *P. tortuosum*. Their identification of taxa relied on a few gross morphological characters.

In addition to macromorphological characters, additional genotypic information is required to differentiate the studied species and to support taxonomic methods of identification. Sufficient genotypic data may be easily obtained using random amplified polymorphic DNA (RAPD), the simplest and the most economical technique. RAPD analysis detects DNA polymorphisms using arbitrary single-primer polymerase chain reactions (PCR) (Karb et al., 1996). The technique has been successfully employed as a molecular marker for estimating the genetic, taxonomic and phylogenetic relationships of plants, especially when combined with morphology (Williams et al., 1990; Kresovich et al., 1992; Wachira et al., 1995). The application of RAPD technique is very limited in the genus *Pancratium*, restricted to population genetics, especially that of *Pancratium maritimum*. Zahreddine et al. (2004) surveyed its ecogeographic range and population dynamics in Lebanon. Grassi et al. (2005) evaluated genetic distance among its populations from different locations and examined conservation strategies.

To our knowledge, no attempt has been made to integrate morphological and molecular data in the genus *Pancratium*. In this work we reassessed the taxonomic status of the Egyptian species and examined morphological and genetic variation within and between species, using specimens from different populations collected throughout its distribution range in the country.

MATERIAL AND METHODS

PLANT MATERIAL

The present study was based on examination of *Pancratium* species kept in the major Egyptian herbaria [Cairo University (CAI), Agricultural Museum (CAIM), National Research Centre (CAIRC)] and authentic specimens kept in the herbarium of

the Royal Botanic Gardens at Kew (K); acronyms follow Holmgren et al. (1990). Examined living materials included those collected during field trips and accessions grown in the greenhouse of Cairo University. Specimens were identified according to Täckholm and Drar (1954), Morton (1965), Björnstad (1973), Täckholm (1974), Feinbrun-Dothan (1986) and Boulos (2005). Abbreviations of authors' names follow Brummitt and Powel (1992).

FIELD STUDIES

During a 4-year period (2002–2006), fresh samples of the studied *Pancratium* species were collected, the vegetative characters were examined, and juvenile leaves were excised and kept at -20°C for molecular investigations. Ecological observations of natural populations were made in different localities belonging to three phytogeographic territories of Egypt (Tab. 1). Seven populations are included in this study: four populations of *P. maritimum* from the western Mediterranean coast, one of *P. tortuosum* from Gebel Elba (GE) at the Egyptian-Sudanese border, and one each of *P. sickenbergeri* and *P. arabicum* from the Mediterranean coast of Sinai Peninsula. For *P. trianthum* only herbarium specimens were available. In each of the studied populations about 20 individuals were randomly collected. Within each population the distance between collected plants was at least 10 m, in order to increase the chance of detecting potential variation. Morphometric measurements were performed at flowering time and directly in the field in order to avoid destructive sampling. Voucher specimens are deposited in CAI.

MORPHOLOGICAL ANALYSIS

The examined morphometric characters were chosen on the basis of differences between species in vegetative and reproductive parts. Only mature plants were measured; 38 macromorphological characters, 26 quantitative and 12 qualitative, were measured for each of the 50 sampled specimens (Tab. 2). The criteria mainly represent leaf structure and floral and fruit morphology. Qualitative characters were scored 0 for absence and 1 for presence. OTU scores for quantitative characters were averages of measurements from at least 10 specimens when possible. Bearing in mind that herbarium specimens cannot be regarded as random samples of the species, we followed Wieringa (1999) by calculating the mean value of the minimum and maximum measurements. Fresh material of *Pancratium trianthum* was not found in Egypt; our herbarium specimens were in flower and the capsule was not seen, so capsule size was obtained from the type specimen (*Heudelot* 542; K – holotype; P – isotype).

MOLECULAR ANALYSIS

Genomic DNA was extracted from 50 mg of juvenile leaf tissues in each sample according to Porebsky et al. (1997) using the modified CTAB protocol (Doyle and Doyle, 1990). RNA was eliminated by adding 0.7 units of RNAase to each sample. Polymorphism of DNA in each sample was detected using standard DNA primers (eleven primers of 10 bases each; Operon Technologies Inc., Alameda CA). PCRs were done in a total volume of 50 µl in a Perkin Elmer Cetus DNA Thermal Cycler (Perkin Elmer-Cetus, Norwalk, U.S.A.). The developed bands were stained with ethidium bromide, visualized under UV, photographed and scored. *Pancratium trianthum* was not collected during this study so it was excluded from molecular analysis.

DATA ANALYSIS

Differences between species were tested by multivariate analysis of variance (MANOVA) including all quantitative characters, performed, followed by univariate ANOVA for each character, using SPSS 10.0 software (SPSS Inc., Chicago IL). Morphological variability within each species was measured by calculating the coefficient of variance (CV) (Sokal and Rohlf, 1995). Quantitative morphological data were converted to a similarity matrix using Euclidean distance with the SIMINT function. A dendrogram was generated from the dissimilarity matrix by the unweighted pair group method with arithmetic mean (UPGMA) (Sokal and Michener, 1958) with the SAHN function. The cophenetic correlation coefficient was calculated with the Mantel test (Mantel, 1967) by comparing the matrix of cophenetic values with the similarity matrix in order to estimate how well the dendrogram represents its corresponding pairwise distance matrix. This was done with the COPH and MXCOMP modules of NTSYS-pc. Principal component analysis (PCA) was performed for the studied 50 samples based on 12 significantly differing quantitative characters using the Multivariate Statistics Package (MVSP) ver. 3.13 for Windows (Kovach, 1999).

RAPD polymorphic bands were scored as present (1) or absent (0), and genetic distance values were calculated based on Jaccard's coefficient (Anderberg, 1973) using the formula

$$D_{ij}=1-(2B_{ij}/M_{ij}),$$

where D_{ij} is the distance between genotypes i and j, B_{ij} is the number of bands common to i and j, and M_{ij} is the total number of bands scored in i and j. The expected genetic distance ranged from 0 to 1 depending on the degree of similarity between the genome of the studied specimens. Sequential agglomerative hierarchical non-overlapping (SAHN)

TABLE 2. Characteristics and their categories used in morphological analysis of *Pancratium*

	Character	Abbreviation	States
			Tunica
01	Tunica	TUN	Brown (0); dark to blackish or greyish brown (1)
			Leaf
02	Leaf base	LFB	Truncate (0); long attenuate (1)
03	Leaf sheath	LFS	Absent (0); present (1)
04	Leaf habit	LFH	Decumbent and spirally twisted (0); not decumbent and spirally twisted (1); not decumbent and straight (2)
05	Leaf broadest part	LFBP	At the middle or above (0); at the middle or below (1)
06	Leaf width	LFW	Narrow, 1.0 cm or less (0); broader, 1.0 cm or more (1)
			Scape
07	Aerial scape width	ASW	1.0 cm or less (0); 1.0 cm or more (1)
08	Aerial scape dilation	ASD	Dilated at base (0); dilated at tip (1)
09	Aerial scape number	ASN	1 (0); 1–2 (1)
			Spathe
10	Spathe number	SPN	1 (0); 2 (1)
11	Spathe apex	SPA	Bifid (0); entire (1)
12	Spathe base	SPB	Contorted (0); imbricate (1)
13	Spathe length	SPL	3–5 cm (0); 4–9 cm (1)
14	Spathe length/width	SPL/W	L=2–2.5xW (0); L=at least 3xW (1)
			Flower
15	Flower length	FLL	7–10 cm (0); 10–18 cm (1); 18–24 cm (2)
16	Flower/spathe	FL/SP	1.5–2.0x spathe (0); 2.0–3.0x spathe (1); 3.0–4.0x spathe (2); 4.0-6.0x spathe (3)
17	Flower number	FLN	2–5 (0); 6–14 (1)
18	Flower pedicel length	FLPL	<1.0 cm (0); > 1.0 cm (1)
19	Flower perianth length	FLPRL	6–16 cm (0); 16–22 cm (1)
20	Flower perianth tube length	FLPRTL	2.0–4.0 cm (0); 5.0–8.0 cm (1); 8.0–11.0 cm (2);11.0–16.0 cm (3)
21	Flower perianth segment length	FLPRSGL	3.0–5.0 cm (0); 5.0–7.0 cm (1)
22	Flower perianth tube/ perianth segments	FLPRT/PRSG	≤perianth segments (0); 1.5–2.0x perianth segments (1); 2.0–3.0x perianth segments (2)
23	Flower perianth segments/corona	FLPRSG/C	1.1–1.5x corona (0); 1.5–2.0x corona (1)
24	Corona teeth apex	CTA	Acute (0); acuminate (1)
25	Corona teeth length	CTL	4.0–8.0 mm (0); 7.0–11.0 mm (1)
26	Anther length	AL	5.0–10.0 mm (0); 10.0–17.0 mm (1)
27	Free filament length	FFL	3.0–5.0 mm (0); 5.0–10.0 mm (1); 10.0–15.0 mm (2)
28	Ovary length	OVL	1.0–2.0 cm (0); 2.0–3.0 cm (1)
29	Ovary length/width	OVL/W	L=2–3xW (0); L=3–5xW (1)
30	Style color	STC	White (0); glaucous (1)
31	Style length	STL	6.0–8.0 cm (0); 8.0–16.0 cm (1); 16.0–22.0 cm (2)
			Fruit
32	Fruit width	FRW	<2.0 cm (0); >2.0 cm (1)
33	Fruit length	FRL	1.5–3.0 cm (0); 3.0–5.0 cm (1)
34	Fruit length/width	FRL/W	L=1.0–1.5xW (0); L=1.5–2.0xW (1)
35	Mature valve apex	MVA	Retuse or emarginate (0); subrotundate (1); obtuse (2); abruptly attenuate (3)
36	Mature valve base	MVB	Subrotundate (0); abruptly attenuate (1); cordate (2); obtuse or truncate (3)
			Seed
37	Seed length	SDL	4.0–6.0 mm (0); 6.0–9.0 mm (1); 10.0–14.0 mm (2)
38	Seed width	SDW	<7.0 mm (0); >7.0 mm (1)

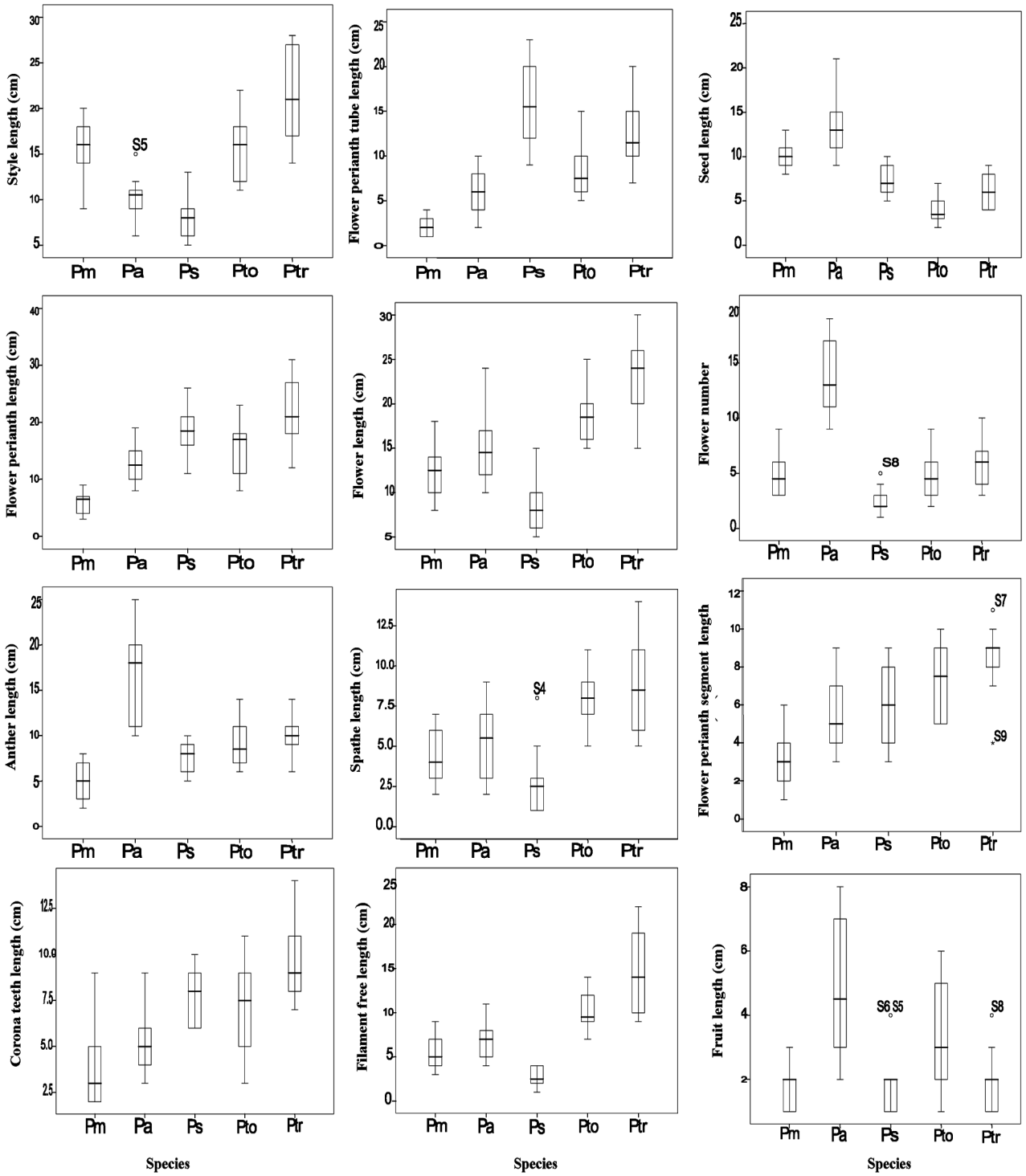

Fig. 1. Comparison of *Pancratium* species using boxplots of 12 of the quantitative morphological characters examined. Pm – *Pancratium maritimum*; Pa – *P. arabicum*; Ps – *P. sickenbergeri*; Pto – *P. tortuosum*; Ptr – *P. trianthum*; – median; *outlier; box – 90th percentile; lines – minimum-maximum.

clustering using UPGMA was then performed, and a dendrogram was generated as described by Sneath and Sokal (1973) using NTSYS-pc ver. 2.0. Nei's genetic distance (Nei, 1978) was calculated to examine the patterns of genetic differentiation among the studied species.

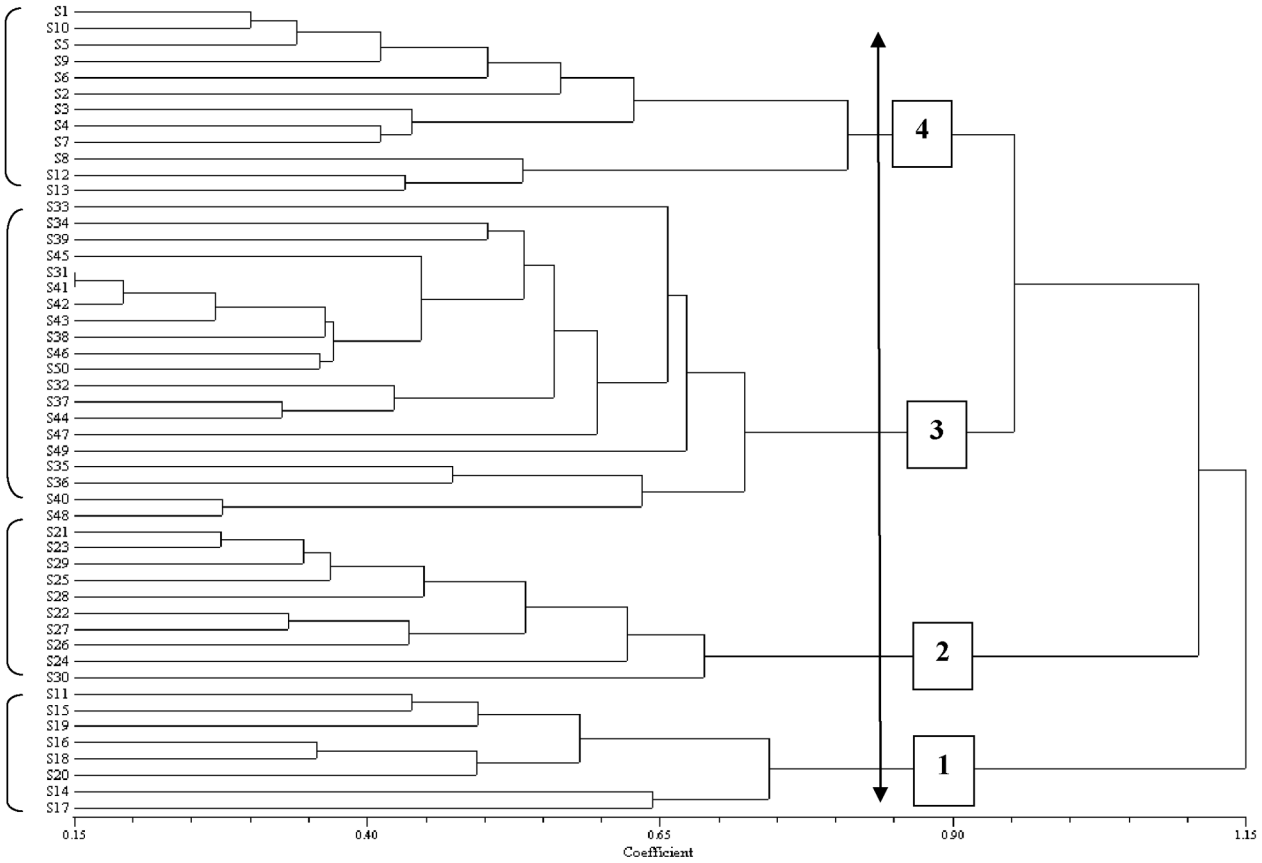

Fig. 2. UPGMA dendrogram based on 12 quantitative morphological characters of *Pancratium*. 1, 2, 3, 4 – four distinguished groups; S – sample.

RESULTS

MORPHOLOGICAL TRAITS

All characters showed normal distributions except free filament length (FFL), flower number (FLN), perianth tube length (FLPRTL) and fruit length (FRL), which were highly asymmetric. Normality for these four traits was obtained after square root transformation. Univariate ANOVA revealed significant differences for 12 of the 26 quantitative examined characters. Figure 1 shows boxplots for variation of the quantitative characters, highlighting species polymorphism and overlap of variation ranges between species.

Figure 2 gives the UPGMA phenogram of the studied species. The cophenetic correlation of the distance matrix and tree matrix was 0.90 (Mantel test, $p<0.0002$), indicating good fit of the phenogram to the distance matrix (Rohlf, 1998). Four species-specific clusters were distinguished at 80% similarity level. The first cluster includes all specimens belonging to *Pancratium arabicum* with long anthers, large seeds and several flower numbers. The second cluster contains all specimens of *P. sickenbergeri* characterized by short flower perianth tube, few flowers, and short spathe, flowers, free filaments, styles, fruits and seeds. The third cluster comprises all specimens of *Pancratium tortuosum* and *P. trianthum* with long spathe, flowers, free filaments, flower perianth segments, corona teeth and styles. The fourth cluster, which includes specimens of *P. maritimum*, did not show morphological variability. Separation of means by the Tukey test showed that no single character strictly discriminated one species from all others. The numbers of significant differences between the five studied species based on quantitative morphological

TABLE 3. Significant differences between the five species of *Pancratium* for the 12 studied quantitative characters, by the Tukey test. For species abbreviations see Table 1

	Pa	*Pm*	*Ps*	*Pto*
Pm	7			
Ps	8	6		
Pto	5	8	7	
Ptr	12	10	6	5

TABLE 4. Means, coefficients of variation (%) and F statistics for 12 quantitative characters giving a significant result by ANOVA. F – significant at 1% (**); d.f. – degrees of freedom. For character abbreviations see Table 2

Character	Species F (d.f.= 4)	Mean values				
		Pa	*Pm*	*Ps*	*Pto*	*Ptr*
SPL (cm)	12.34**	5.4 (6.0)	4.2 (3.1)	2.9 (4.8)	8.1 (3.2)	8.8 (8.6)
FLL (cm)	24.4**	14.9 (17.7)	12.5 (10.5)	8.4 (9.4)	18.6 (8.9)	23.4 (20.9)
FLN (no.)	33.6**	13.9 (13.0)	4.9 (3.9)	2.5 (1.6)	4.7 (4.7)	5.9 (5.4)
FLPRL (cm)	18.3**	12.9 (12.1)	5.9 (4.3)	18.6 (16.5)	16.1 (24.3)	21.2 (38.4)
FLPRTL (cm)	27.0**	6.1 (6.5)	2.1 (1.0)	16.0 (21.5)	8.3 (9.6)	12.7 (16.5)
FLPRSGL (cm)	11.3**	5.5 (3.8)	3.0 (2.7)	5.9 (4.5)	7.2 (3.5)	8.4 (3.6)
CTL (mm)	10.83**	5.4 (3.6)	3.9 (5.9)	7.7 (2.0)	7.1 (5.6)	9.7 (5.6)
AL (mm)	21.1**	16.9 (26.8)	4.9 (5.4)	7.8 (3.1)	9.0 (6.2)	10.0 (5.3)
FFL (mm)	27.5**	7.2 (4.8)	5.4 (4.3)	2.7 (1.1)	10.1 (5.6)	14.7 (22.7)
STL (cm)	21.1**	10.2 (6.4)	15.6 (13.8)	7.9 (5.0)	15.9 (14.8)	21.1 (24.8)
FRL (cm)	8.4**	4.8 (4.4)	1.7 (0.5)	2.1 (1.2)	3.3 (2.9)	2.0 (0.9)
SDL (mm)	26.7**	13.8 (16.0)	10.3 (2.2)	7.3 (2.6)	3.9 (2.8)	6.3 (3.8)

characters are shown in Table 3. Interestingly, the majority (10–12) of significantly differing morphological traits showed a higher number of significant differences between *P. trianthum* and both *P. arabicum* and *P. maritimum* than between it and the other two species (*P. sickenbergeri* and *P. tortuosum*). *Pancratiumm trianthum* was also distinguished from the other four species by its higher coefficients of variation. The traits that distinguish *P. arabicum* from others, which are flower number (FLN), anther length (AL), fruit length (FRL) and seed length (SDL), are significant (Tab. 4).

PCA based on the 12 significantly differing quantitative characters (Fig. 3) explained 70.18% of the total variation. Axis one explained 33.61%, axis two 21.47%, and axis three 15.09%. Corona teeth length (CTL), flower number (FLN), flower length (FLL), length of flower perianth (FLPRL) and flower perianth tube length (FLPRTL) were more correlated with each other and showed the highest loadings in relation to PC1 (Tabs. 5, 6). Although the lengths of the style (STL), anther (AL), seed (SDL), fruit (FRL), spathe (SPL) and free filament (FFL) were less correlated and contributed weakly to PC1, they had significant loadings in relation to PC2 (Tabs. 5, 6).

MOLECULAR DIFFERENTIATION

Eleven 10-mer random oligonucleotide primers (selected from 28 screening primers) were used to amplify the genomic DNA of *Pancratium*. A total of 140 RAPD markers were produced and 111 (74.13%) were polymorphic at species level; the data are outlined in Figure 4. This indicates that plants of the genus *Pancratium* show high genetic diversity and distinct genetic variation both between and within species. The number of loci amplified by each primer clearly differed (17 bands by OPC-08, only one by OPB-15; Tab. 7).

The developed polymorphic bands distinguished *P. arabicum* from other species (characteristic bands at, e.g., 830 and 407 bp for OPA-02, 1129 bp for OPC-03, 2113 bp for OPC-04). The bands at 764 bp (OPA-02), 954 and 680 bp (OPC-03), and 1520 and 685 bp (OPC-04) differentiated *P. maritimum*. *P. sickenbergeri* is differentiated by the bands at 340 bp (OPA-02), 1000 and 584 bp (OPA-11), and others (Fig. 4). These species-specific RAPD markers are potentially useful for identifying

TABLE 5. Loadings of 12 quantitative morphological characters on the first three PCA axes. Figures in boldface are the highest loadings. For character abbreviations see Table 2

Components	PC1	PC2	PC3
Eigen values	0.337	0.278	0.121
AL	-0.1412	**0.7092**	-0.3418
CTL	**0.5829**	-0.2207	0.2418
FFL	-0.2274	**-0.5794**	-0.381
FLL	**-0.4949**	-0.5259	**-0.3945**
FLN	**-0.5496**	**0.7214**	-0.17
FLPRL	**0.8107**	0.0733	-0.3234
FLPRSL	0.4486	-0.3105	0.0561
FLPRTL	**0.8799**	0.0213	0.3222
FRL	-0.0844	0.292	0.2487
SDL	-0.475	**0.6072**	**0.4979**
SPL	-0.1948	-0.5535	-0.1287
STL	-0.4903	**-0.6864**	0.3685

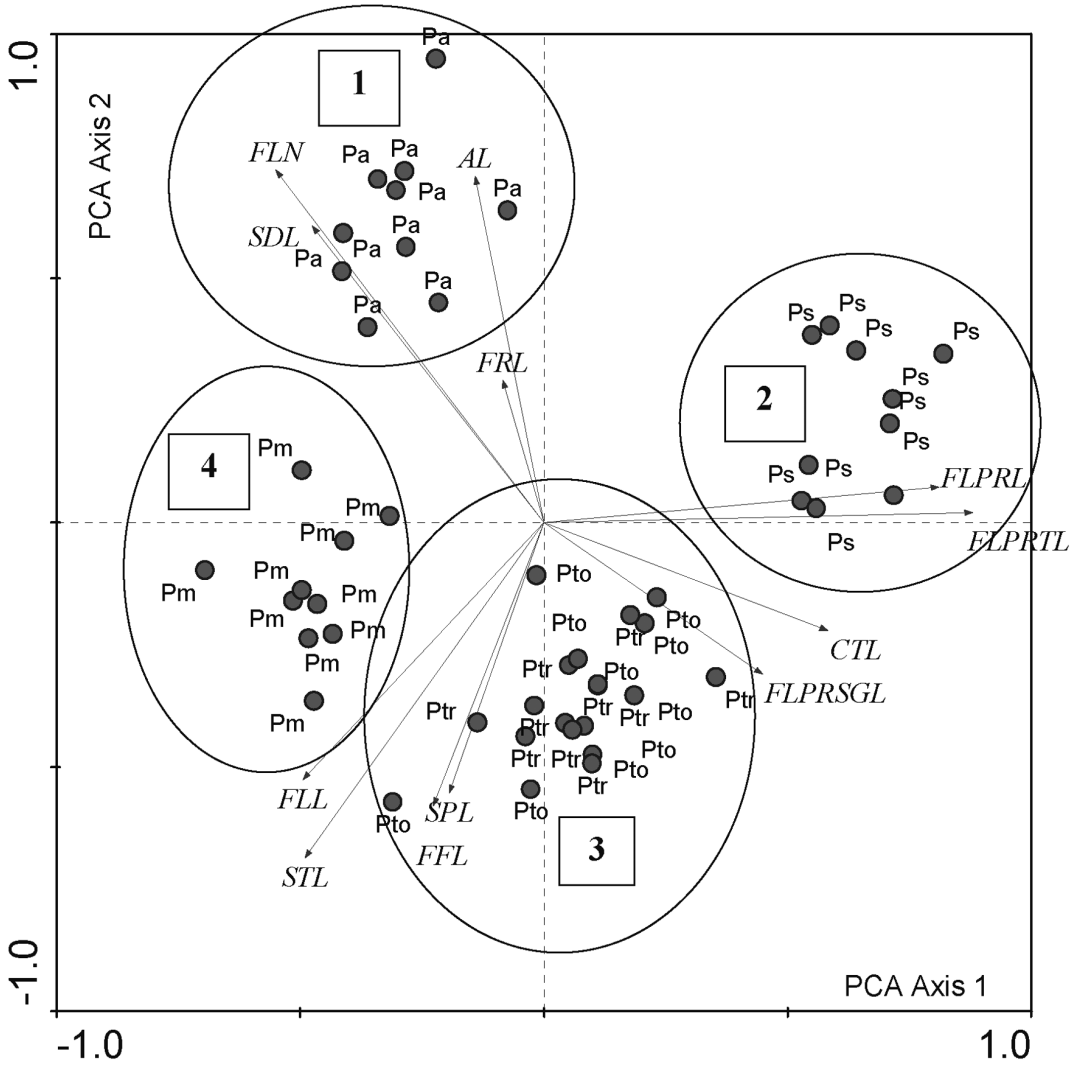

Fig. 3. Principal component analysis (PCA) of the examined specimens based on 12 quantitative morphological characters (arrows), together with their clusters (1, 2, 3, 4). For species see Table 1; for character abbreviations see Table 2.

Pancratium species within any mixed population. A similar approach has been used successfully for molecular diagnosis of species and cultivars by many workers (Sosinski and Doucher, 1996; Yamamoto and Duich, 1994).

Further analysis of these RAPD profiles for band similarity indices clearly differentiated all the species of *Pancratium*. Table 8 presents the similarity matrix obtained after multivariate analysis using Nei's genetic distance (Nei, 1978). It shows that *P. tortuosum* has about 56%, 51% and 49% similarity with *P. arabicum*, *P. maritimum* and *P. sickenbergeri* respectively. All the species share more than 15% similarity. The UPGMA dendrogram (Fig. 5) obtained from RAPD results fitted the calculated genetic distance, and grouped the four *Pancratium* species into three main clusters; the first cluster includes *P. tortuosum*, the second *P. sickenbergeri*, and the third the closely allied species *P. arabicum* and *P. maritimum*.

DISCUSSION

MORPHOLOGICAL TRAITS

Our data indicate that five morphologically distinguished *Pancratium* species occur in Egypt, of which *P. arabicum* is endemic and *P. trianthum* Herb. is newly recorded (El-Hadidy et al., 2011).

The bulbs of the different species vary in size but are uniform in general characters. In *Pancratium maritimum* the bulbs are smooth-

TABLE 6. Coefficients of correlation between the quantitative characters. For character abbreviations see Table 2; * – P<0.05, ** – P<0.01

	AL	CTL	FFL	FLL	FLN	FLPRL	FLPRSL	FLPRTL	FRL	SDL	SPL
AL											
CTL	0.315										
FFL	0.277	0.193									
FLL	0.228	0.25	0.77**								
FLN	0.504**	-0.213	0.305	0.347							
FLPRL	0.174	0.565**	0.184	0.327	-0.05						
FLPRSL	0.288	0.402*	0.361	0.581**	0.032	0.529**					
FLPRTL	0.155	0.616**	0.025	0.084	-0.371	0.64**	0.483*				
FRL	0.426*	-0.013	0.139	0.104	0.38*	-0.03	0.199	-0.123			
SDL	0.044	-0.377	-0.388	-0.288	0.427**	-0.389	-0.445	-0.434	0.083		
SPL	0.259	0.432*	0.635**	0.715**	0.257	0.255	0.46*	0.083	0.076	-0.333	
STL	-0.065	0.164	0.576**	0.588**	-0.004	-0.026	0.347	-0.06	-0.087	-0.297	0.489*

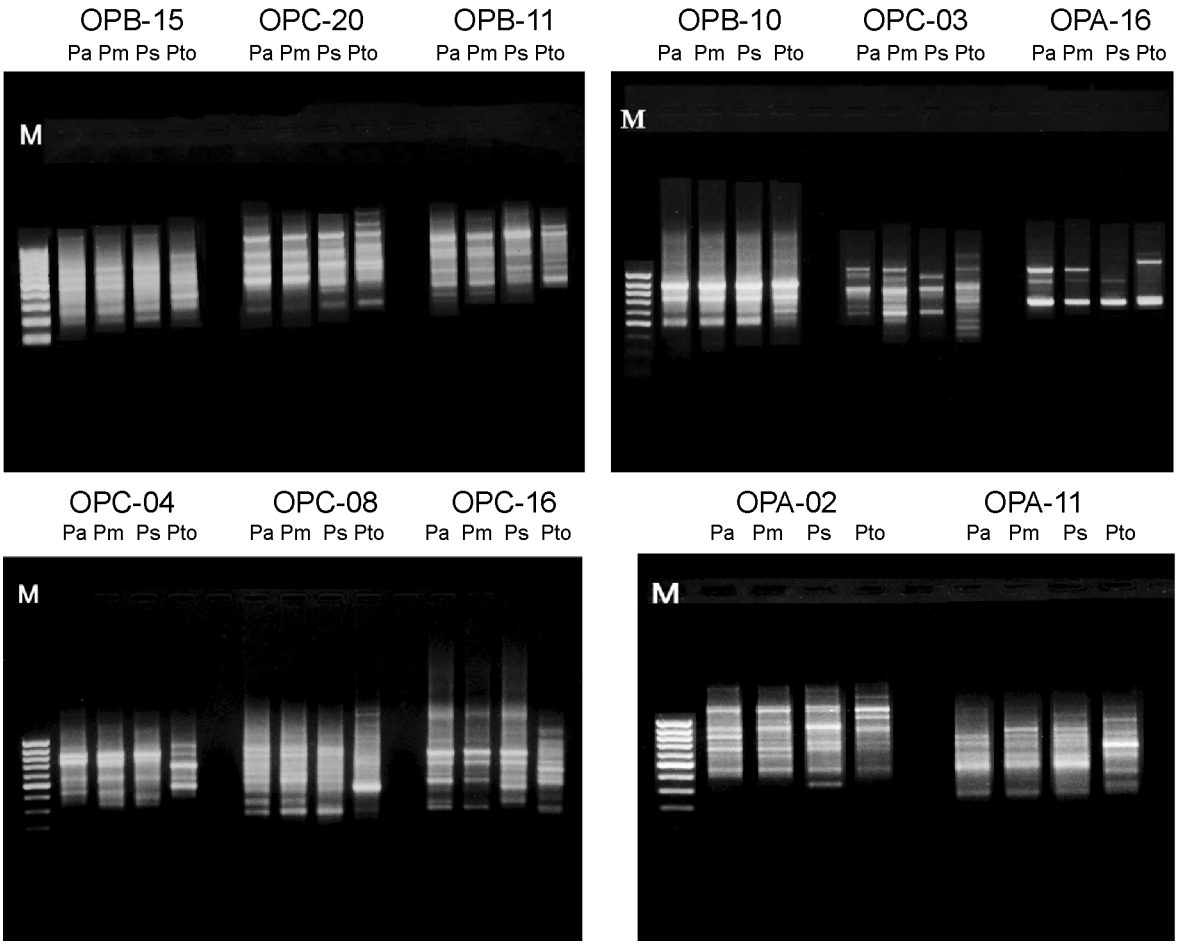

Fig. 4. RAPD fingerprinting patterns of four species of *Pancratium* generated by 11 primers. PA – *Pancratium arabicum*; PM – *P. maritimum*; PS – *P. sickenbergeri*; PT – *P. tortuosum*; M – 100 kb ladder.

TABLE 7. Sequences, size, number of polymorphic bands and percentages as generated by 11 arbitrary primers

Primer code	Nucleotide sequence (5'–3')	Size (bp) min - max	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands
OPA-02	TGC CGA GCT G	340-1797	18	14	77.77
OPA-11	CAA TCG CCG T	272-1096	14	12	85.71
OPA-16	AGC CAG CGA A	652-1337	6	5	83.33
OPB-10	CTG CTG GGA C	506-900	5	2	40.00
OPB-11	GTA GAC CCG T	210-1760	12	10	83.33
OPB-15	GGA GGG TGT T	189-1565	8	1	12.50
OPC-03	GGG GGC GTT T	410-1337	13	12	92.30
OPC-04	GGG AAT TCG G	300-2113	14	12	85.71
OPC-08	TGG ACC GGT G	197-2379	18	17	94.44
OPC-16	CAC ACT CCA G	170-1992	18	16	88.88
OPC-20	ACT TCG CCA C	210-2225	14	10	71.42
Total			140	111	74.13
Mean			12.72	10.09	74.13

TABLE 8. Genetic distance between four studied Pancratium species based on Nei's index. *Pa – Pancratium arabicum, Pm – P. maritimum, Ps – P. sickenbergeri, Pto – P. tortuosum*

Species	*Pa*	*Pm*	*Ps*	*Pto*
Pa				
Pm	0.15			
Ps	0.37	0.32		
Pto	0.56	051	0.49	

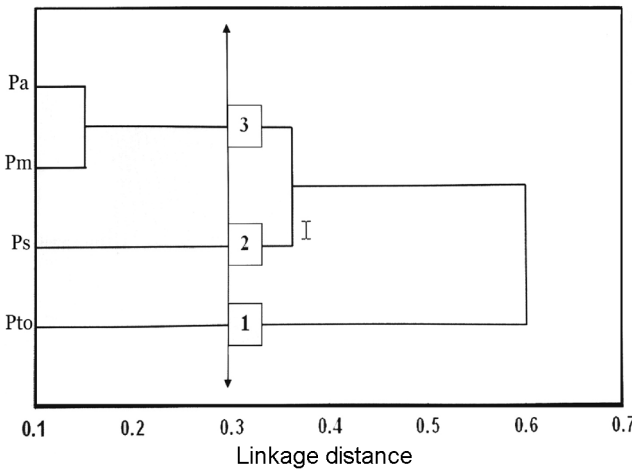

Fig. 5. Dendrogram of cluster analysis of RAPD markers, illustrating the genetic relationships among four species of *Pancratium*. 1, 2, 3 – three distinguished clusters. For species abbreviations see Table 8 and Figure 4.

skinned and with brownish tunics, and in the others are rough and dark to blackish or greyishbrown. Tunics sometimes show characters correlated with ecological conditions. Thus, species confined to desert habitats often have darker tunics.

Although variable within species, leaf length may be sorted into two categories. The first has small to medium-sized leaves 10–40 cm long; the leaf blade is narrow (0.3–1.0 cm diam) and gradually tapers to an acute apex. Three species belong to this category: *Pancratium tortuosum*, *P. tortuosum* and *P. trianthum*. In the second category, two species have large leaves: *P. maritimum* (up to 60 cm long) and *P. arabicum* (up to 80 cm long). The large leaves are broader [1.0–2.5(–3.0) cm diam] and narrow abruptly to an obtuse to subacute apex. In this study the width of leaves and the leaf apex proved useful in separating species of the same category into the two leaf-size categories, while leaf base and curvature were important in distinguishing species within the same category. *Pancratium trianthum* is unique in its long attenuate leaf base, while *P. arabicum* and *P. tortuosum* possess a scarious-membranous sheath. In the others, the base is truncate and the sheath is absent. Leaf blades are spirally twisted in *P. sickenbergeri* and *P. tortuosum*, slightly twisted near the apex in *P. maritimum*, *P. arabicum* and *P. trianthum*. There is inter- and intraspecific variation in number of leaves, limiting its diagnostic value.

Different characters of the spathe (number, shape, size and flower/spathe length ratio) are of taxonomic significance in recognizing certain species and in separating closely allied species. The spathe ranges from 3 to 9 cm long. The spathes are short (3–5 cm long) in *Pancratium tortuosum* and longer [(4–)5–9 cm long] in most of the studied species; the

spathe is narrow (1–2 cm diam.) in *P. tortuosum*, *P. tortuosum* and *P. maritimum*, broader (2–3 cm diam.) in *P. arabicum*, and broadest (up to 4 cm diam.) in *P. trianthum*. Flower/spathe length ratio differentiates two groups: the first with a ratio of 3 or more in (*P. tortuosum*, *P. trianthum*), and the others with a ratio less than three.

The number of flowers, which varies from 2 to 14, is often a taxonomically useful character. Few-flowered species (2–5) can be distinguished from many-flowered species (5–14). The few-flowered species are *P. sickenbergeri*, *P. tortuosum* and *P. trianthum*; the many-flowered species are *P. maritimum* and *P. arabicum*. Reduction of flower number is usually considered the derived state in Amaryllidaceae (Traub, 1963).

The perianth of *Pancratium* consists of six tepals in two whorls, basally connate into a tube of varying length. In large flowers the perianth is long (16–22 cm long), with a well developed perianth tube (11–16 cm long) and with long perianth segments (5–7 cm long). Here the length of the perianth tube is 2–3 times longer than the perianth segments. *Pancratium tortuosum* and *P. trianthum* belong to this category. The medium-sized flowers possess a medium perianth [(8–)9–16 cm long] with a less developed tube (5–12 cm long) and with short to long segments (3–7 cm long). Here the length of the tube is 1.5–2 times longer than the segments. *Pancratium maritimum* and *P. arabicum* belong to this category. These two species are closely allied and may be distinguished by their perianth tube and perianth segments (length, diameter of the green midstripe). In *Pancratium maritimum* the perianth tube is 5–8 cm long, the perianth segments are short (3–5 cm long) and with a narrow (2–4 mm diam) green midstripe. In *P. arabicum* the perianth tube is 8–12 cm long and the perianth segments are long (5–7 cm long) with a broad (4–6 mm diam) green mid-stripe. *Pancratium tortuosum*, with the smallest flowers, is characterized by its short perianth (6–9 cm long), short tube (2–4 cm long) and short segments (3–5 cm long). It is unique in possessing a tube that is commonly shorter than or rarely equal in length to the segments.

Style characters (color, length) are useful taxonomic characters which have been ignored by some workers. In Egypt, *Pancratium trianthum* and *P. tortuosum* are unique in possessing a glaucous style. The species may be sorted into three groups on the basis of style length. It is short [6–8 cm long] in *P. sickenbergeri*, medium [(>8–)10–16 cm long] in *P. maritimum* and *P. arabicum*, and long [>16–22 cm long] in *P. tortuosum* and *P. trianthum*.

The relation of the free filament to the anther differentiates two categories: the first has the free part of the filament as long as or commonly longer than the anther (*Pancratium tortuosum*, *P. trianthum*). In the second the free filament is commonly shorter or rarely subequal (*P. maritimum*, *P. arabicum*, *P. sickenbergeri*).

Capsule width is useful in separating certain species. It is less than 2 cm in diameter in *Pancratium tortuosum*, *P. tortuosum* and *P. trianthum*, and more than 2 cm in diameter in *P. maritimum* and *P. arabicum*. The last two species may be separated by capsule length: 1.5–2.5 cm in *P. maritimum*, (2.5–)3–4 cm in *P. arabicum*.

CONVERGENCE BETWEEN MORPHOLOGICAL AND MOLECULAR DIFFERENTIATION

The genetic diversity and interspecific relationships between the studied species are presented in Figure 5. Genetic diversity generally is the result of long-term evolution and represents the evolutionary potential of a species. Surviving in a harsh environment, a species is subject to change in some aspects and accumulates more genetic variation in order to adapt itself to various environmental pressures (Li et al., 1999). Genetic diversity in this study was high, and exceeded 70% both between and within *Pancratium* species, indicating both high genetic diversity and abundant genetic variation in the genus.

RAPD data in this study gave better resolution of *Pancratium arabicum* and *P. maritimum*, which show morphologically and ecologically related characters. A number of polymorphic bands appeared with 6 of the 11 used primers, separating *P. arabicum* from the other studied species. The RAPD primers confirmed the morphological and ecological divergence of *P. tortuosum* from the other studied species; it had the largest number of polymorphic bands (35 out of 82; Fig. 4). The constructed dendrogram (Fig. 5) shows the close affinity between *P. arabicum* and *P. maritimum*, while *P. tortuosum* is the most distant species on the dendrogram. The overall data from molecular markers support those revealed by morphology.

REFERENCES

Anderberg MR. 1973. *Cluster Analysis for Applications. Monographs and Textbooks on Probability and Mathematical Statistics*. Academic Press Inc., New York.

Andrews FW. 1956. *The Flowering Plants of the Sudan*, vol. 3. Arbroath, Scotland.

Apg III. 2009. An update of the Angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.

Baker JG. 1898. *Pancratium* L. In: Thistelton-Dyer WT [ed.], *Flora of Tropical Africa*, vol. 7, 406–407. L. Reeve and Co. Ltd., Ashford, Kent.

Björnstad IN. 1973. A revision of the genus *Pancratium* in Africa south of the Sahara. *Norwegian Journal of Botany* 20 (4): 281–291.

Boulos L. 2005. *Flora of Egypt. Alismataceae to Orchidaceae*, vol. 4. Al Hadara Publishing, Cairo, Egypt.

Brummitt RK, and Powel CE. 2001. *Authors of Plant Names*. Royal Botanic Gardens, Kew.

Dinsmore JE. 1933. In: Post GE [ed.]. *Flora of Syria, Palestine and Sinai*. 2nd ed., vol. 2. American Press, Beirut.

Doyle JJ, and Doyle JL. 1990. Isolation of plant DNA from fresh tissues. *Focus* 12: 13–15.

El-Gadi A. 1978. Amaryllidaceae In: Jafri SMH, and EL-Gadia [eds.], *Flora of Libya*, vol. 51, 1–5. Al Faateh University, Flora Committee, Tripoli.

El-Hadidy A, Abd El-Ghani M, Amer W, and Hassan R. 2011. Systematic revision of the genus *Pancratium* L. (*Amaryllidaceae*) in Egypt with a new addition. *Notulae Scientia Biologicae* 3(2): 24–38.

Feinbrun-Dothan N. 1986. In: Zohary M [ed.], *Flora Palaestina*, vol. 4. Israel Academy of Sciences and Humanities, Jerusalem.

Grassi F, Cazzaniga E, Minuto L, Peccenini S, BARBERIS G, and BASSO B. 2005. Evaluation of biodiversity and conservation strategies in *Pancratium maritimum* L. for the Northern Tyrrhenian Sea. *Biodiversity and Conservation* 14 (9): 2159–2169.

Heller D, and Heyn CC. 1991. An Annotated catalogue of the Flora of the Middle East. *Conspectus Florae Orientalis* 6: 53. Israel Academy of Sciences and Humanities, Jerusalem.

Holmgren PK, Holmgren NH, and Barnett LC 1990. *Index Herbariorum*. 8th ed., vol. 1. New York Botanical Gardens, New York, U.S.A.

Ioset JR, Marston A, Mahabir, PG, and Hostettmann K. 2001. A methylflavan with free radical scavenging properties from *Pancratium littorale*. *Fitoterapia* 72: 35–39.

Karb A, Seberg O, and Buiatti M. 1996. Molecular techniques in the assessment of botanical diversity. *Annals of Botany* 78: 143–149.

Kovach WL. 1999. *MVSP – A Multivariate Statistical Package for Windows*, version 3.1. Kovach Computing Services, Pentraeth, Wales, U.K.

Kresovich S, Williams JGK, McFerson JR, Routman EJ, and Schaal BA. 1992. Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. *Theoretical and Applied Genetics* 85: 190–196.

Li YC, Fahima T, Beiles A, Korol AB, and Nevo E. 1999. Microclimatic stress differentiation in wild emmer wheat, *Triticum dicoccoides*. *Theoretical and Applied Genetics* 98: 873–883.

Mabberley DJ. 1993. *The Plant Book*. Cambridge University Press, Cambridge.

Meerow AW, Guy CL, Li Q-B, and Clayton JR. 2002. Phylogeny of the tribe Hymenocallideae (Amaryllidaceae) based on morphology and molecular characters. *Annals of Missouri Botanical Gardens* 89(3): 400–413.

Maire R. 1959. *Flore de l'Afrique du Nord. Monocotyledonae*, vol. 6, 33–41. Lechevalier, Paris.

Mantel N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Research* 27: 209–220.

Meikle RD. 1985. *Flora of Cyprus*, vol. 2. Bentham-Moxon Thrust, Royal Botanic Gardens, Kew.

Morton JK. 1965. The experimental taxonomy of the West African species of *Pancratium* L. (*Amaryllidaceae*). *Kew Bulletin* 19 (2): 337–347.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

Porebsky S, Grant-Bailey L, and Baum BR. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter* 15: 8–15.

Rohlf FJ. 1998. NTSYS-pc. *Numerical taxonomy and multivariate analysis system*, version 2.0. Applied Biosystematics Inc., Exeter Software, New York.

Saltz D, and Ward D. 2000. Responding to a three-pronged attack: desert lilies subject to herbivory by *Dorcas gazelles*. *Plant Ecology* 148: 127–138.

Sneath PHA, and Sokal RR. 1973. *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. W.H. Freeman Press, San Francisco.

Sokal RR, and Michener CD. 1958. A statistical method for evaluating systematic relationships. *University of Kansas Bulletin* 38: 1409–1438.

Sokal RR, and Rohlf FJ. 1995. *Biometry*. 3rd ed. W. H. Freeman and Company, New York.

Sosinski B, and Douches DS. 1996. Using polymerase chain reaction based DNA amplification to fingerprint North American potato cultivars. *Horticultural Science* 31:130–133.

Sür-Altiner D, Gürkan E, Mutlu G, Tuzlaci E, and Ang Ö. 1999. The antifungal activity of *Pancratium maritimum*. *Fitoterapia* 70: 187–189.

Täckholm V. 1974. *Students' Flora of Egypt*. 2nd ed. Cairo University Press, Beirut.

Täckholm V, and Drar M. 1954. *Flora of Egypt*, vol. 3, Bulletin of the Faculty of Science of Cairo University 30: 1–638.

Traub HP. 1963. *The Genera of the Amaryllidaceae*. The American Plant Life Society, La Jolla, California.

Wachira FN, Waugh R, Hackett CC, and Powell W. 1995. Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome* 38: 201–210.

Wieringa JJ. 1999. Monopetalanthus exit. A systematic study of *Aphanocalyx, Bikinia, Icuria, Michelsonia*, and *Tetraberlinia* (*Leguminosae, Caesalpinioideae*). Wageningen Agriculture University Papers 99 (4): 115–186.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, and Tinge SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531–6535.

Yamamoto I, and Duich JM. 1994. Electrophoretic identification of cross-pollinated bent grass species and cultivars. *Crop Science* 34: 792–798.

Zahreddine H, Clubbe C, Baalbaki R, Ghalayini A, and Talhouk SN. 2004. Status of native species in threatened Mediterranean habitats: the case of *Pancratium maritimum* L. (sea daffodil) in Lebanon. *Biological Conservation* 120: 11–18.