

Authentication of Three Endemic Species of the Caryophyllaceae from Sinai Peninsula Using DNA Barcoding

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THE CARYOPHYLLACEAE are one of the most represented families with endemic species in Sinai Peninsula, Egypt. rbcL-based DNA barcoding sequences for three species of Caryophyllaceae endemic to Sinai Peninsula (*Bufonia multiceps*, *Silene leucophylla* and *S. oreosinaica*) were developed for the first time. BLASTN for these sequences reflected 100% Caryophyllaceae hits for rbcL sequences. Phylogenetic tree constructed using the newly developed and mined sequences showed an ambiguous classification at both generic and tribal levels. Results reflected that such species were introduced into Sinai Peninsula through two colonization events. The first introduced *S. leucophylla* while the second introduced a common ancestor for the remaining two species.

Keywords: Endemics, Caryophyllaceae, rbcL, *Bufonia multiceps*, *Silene leucophylla*, *Silene oreosinaica*, Sinai.

Introduction

The term *genres endemiques* (endemic taxa) was coined by De Candolle (1820) to describe species restricted to a particular geographic region. However, the term was not commonly employed before the beginnings of the last century where it appeared regularly in an innumerable scientific publications (Hobohm & Tucker, 2014). Aiming to explain endemism, two different models are accepted. The refuge model (Gankin & Major, 1964) suggests that endemic plants are stress-tolerant taxa confined to stressful environment where interspecific competition is minimized (Meyer et al., 1992). On the other hand, the specialist model suggests adaptation of endemics to the habitats they occupy (Meyer, 1986), but not other habitats (Baskauf & Eickmeier, 1994; Wilson, 1994 and Caley & Munday, 2003).

Today, endemism receives increasing attention and is used for identification of biodiversity hotspots (Orme et al., 2005 and Lamoreux et al., 2006). Firstly, endemics are potentially threatened due to their restricted distribution (Linder, 1995 and Gaston & Blackburn, 1996). Secondly, limited resources hinder humans to protect all

species (Myers et al., 2000). Consequently, centers of endemism are very important for conservation planning due to presence of large numbers of endemics in a relatively small land area (Stattersfield et al., 1998 and Myers et al., 2000).

Sinai Peninsula is a major phytogeographical region in Egypt hosting 30 of the 37 taxa believed to be endemic to Egypt in only 6.1% of the area of Egypt (Hosni et al., 2013). It is a bridge between Africa and Asia and hosts flora influenced by both continental masses. Saint Catherine area encloses most of the mountainous area of South Sinai; it occupies about 4350km² including the country's highest mountain, Mount Catherine (2641m, asl). This area is of great biological interest: it contains about 30% of Egypt's endemic taxa (El Hadidi, 2000). Many of such taxa are important components of the Egyptian genetic, food, heritage, and medicinal capabilities (Carlquist, 1974; Strid, 1986 and Shehata & Kamel, 2007). Caryophyllaceae are one of the most represented families among endemic species in Sinai Peninsula. It contains the most represented genus, *Silene* including six endemic taxa (Hosni et al., 2013).

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Authentication of endemic species is an important base for evolutionary and phylogeographic studies as well as for determination of conservation priorities (Ungricht, 2004; Ferreira & Boldrini, 2011 and Bacchetta et al., 2012). DNA barcoding is an authentic cost-effective rapid tool for species recognition employing DNA sequences as species internal tags (Hebert et al., 2003 and Hebert & Gregory, 2005). Due to its high amplification success rate, *rbcl* (ribulose biphosphate carboxylase large subunit) gene is the most published plastid barcode (Newmaster et al., 2006 and Kang et al., 2017). Unlike morphological identification, DNA barcoding is free from subjective biases and does not require taxonomic experience (Bafeel et al., 2012). In addition, molecular techniques can be used even with unrecognizable plant parts that enable tracing of endangered species in illegal trade affecting endemic as well as non-endemic species (Hobohm & Tucker, 2014).

Therefore, the aim of this investigation is to authenticate three endemic species to Sinai Peninsula: *Bufonia multiceps*, *Silene leucophylla* and *S. oreosinaica* (Caryophyllaceae) using *rbcl*-based DNA barcoding.

Materials and Methods

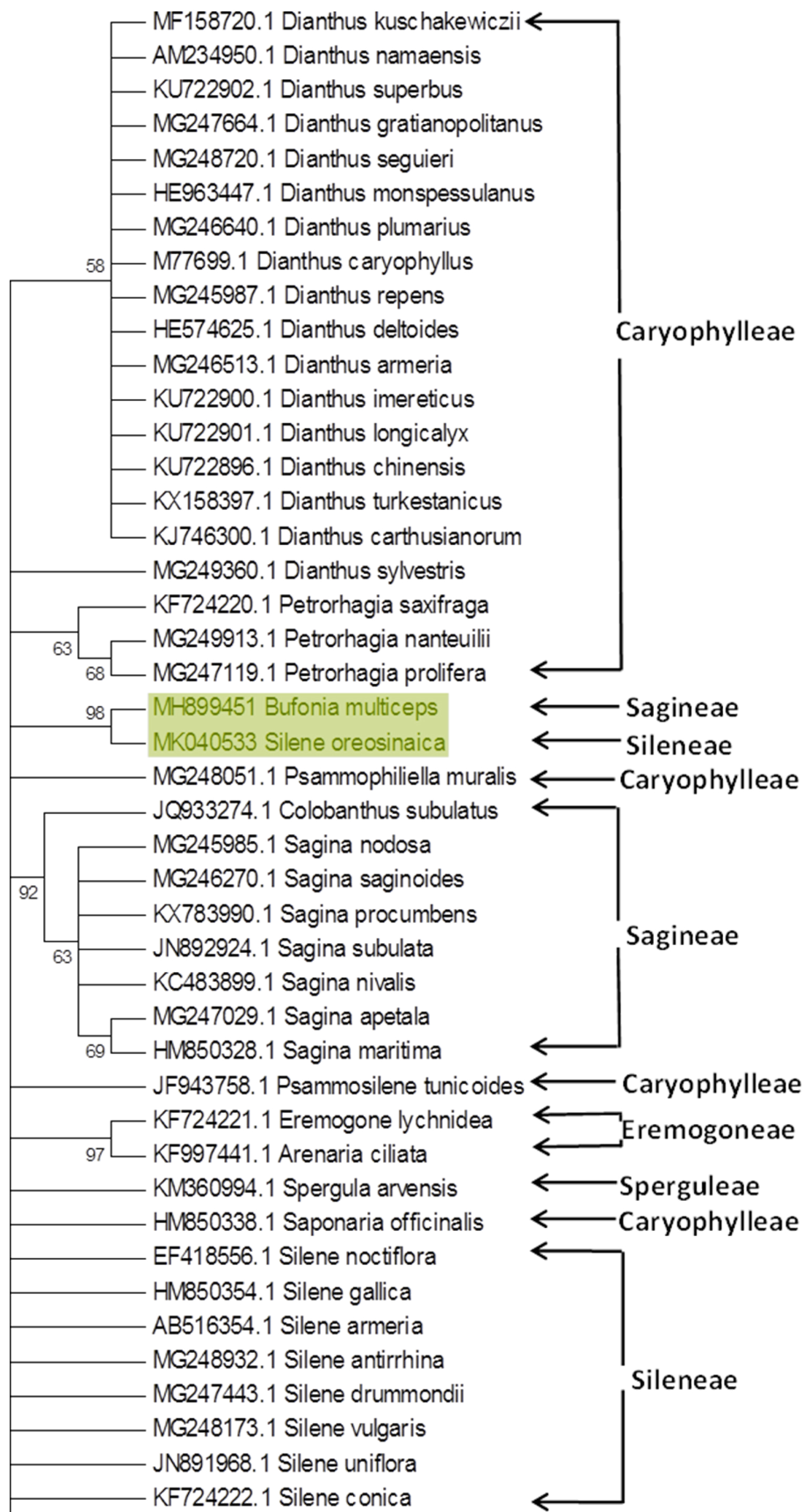
Leaf samples (20mg each) for each species were collected from three herbarium specimens kept in Cairo University Herbarium (CAI) and ground using a mortar and pestle with aid of liquid nitrogen to produce fine powder. Extraction of DNA was carried out using a Qiagen DNeasy kit (Valencia, California, USA) according to manufacturer's protocol with some modifications for herbarium samples. The AP1 buffer was fortified with dithiothreitol (Melford Laboratories, UK) and Proteinase K (Sigma) at 0.12 and 0.04 mg/ml, respectively (de Vere et al., 2012). *rbcl* sequences were amplified in 50 μ l reaction mixture containing 25 μ l PCR Master Mix (Bioline), 20-50ng genomic DNA and 1 μ l of each specific primer (5'-ATGTCACCACAAACAGAAAC-3' and 5'-TCGCATGTACCTGCAGTAGC-3'). The amplification protocol was 95°C for 2min followed by 34 cycles of 94°C for 1min, 55°C for 30sec and 72°C for 1min, then final extension for 7min at 72°C (CBOL Plant Working Group, 2009). PCR products were visualized under UV light on 1.5% (m/v) agarose gel.

After purification step using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), amplification products were sequenced using Big-dye terminator chemistry in 3130xl Genetic Analyzer (Life Technologies, California, USA) by following the standard manufacturer's protocol. The resulted *rbcl* sequences were submitted to the GenBank database under accession numbers MH899451, MH899453 and MK040533 for *Bufonia multiceps*, *Silene leucophylla* and *S. oreosinaica*, respectively. These sequences were blasted against the available ones from GenBank data base; the resulted sequences were used to construct a phylogenetic tree (using the taxonomically distant species *Nicotiana tabacum* as outgroup for best resolution) and pairwise distances calculations using Maximum Likelihood (ML) method and Clustal W in MEGA v. 6 (Tamura et al., 2013) based on the Kimura 2-parameter model (Kimura, 1980) with gamma distribution. Significance was assessed using 1000 bootstrap replications (Felsenstein, 1985).

Results and Discussion

PCR amplifications for *rbcl* regions resulted in 750 bp band in each species (Fig. 1 Suppl.). Generally, a Product of 650-850bp resulted following amplification of such region (Kress et al., 2005 and Bafeel et al., 2011). Sequencing for *rbcl* regions of *Bufonia multiceps*, *Silene leucophylla* and *S. oreosinaica* resulted in 471, 561 and 522 bp sequences, respectively (Fig. 2 Suppl.). BLASTN for these sequences reflected 100% Caryophyllaceae hits for *rbcl* sequences of 484-1408 bp length.

Sequences mined from GenBank included eight tribes represented by 20 genera (Fig. 1), with overall mean interspecific K2P distance of 0.025 and pairwise distances ranged from 0.00 to 0.102. Among mined sequences, estimates of evolutionary divergence showed that *Psammophiliella muralis*, *Sagina nodosa* and *S. procumbens* were the closest taxa for *Bufonia multiceps* with 0.028 intraspecific K2P distance (Table 1). Smaller distance (0.002) was recorded between *Silene leucophylla* and its closest species; *Atocion rupestre*, *Silene pygmaea*, *S. acaulis*, *S. aprica* and *S. antirrhina*. In contrast, *Colobanthus subulatus*, *Sagina nodosa* and *S. procumbens* (belonging to Saginae) were the closest species to the Sileneae taxon, *Silene oreosinaica*, with intraspecific K2P distance of 0.82.



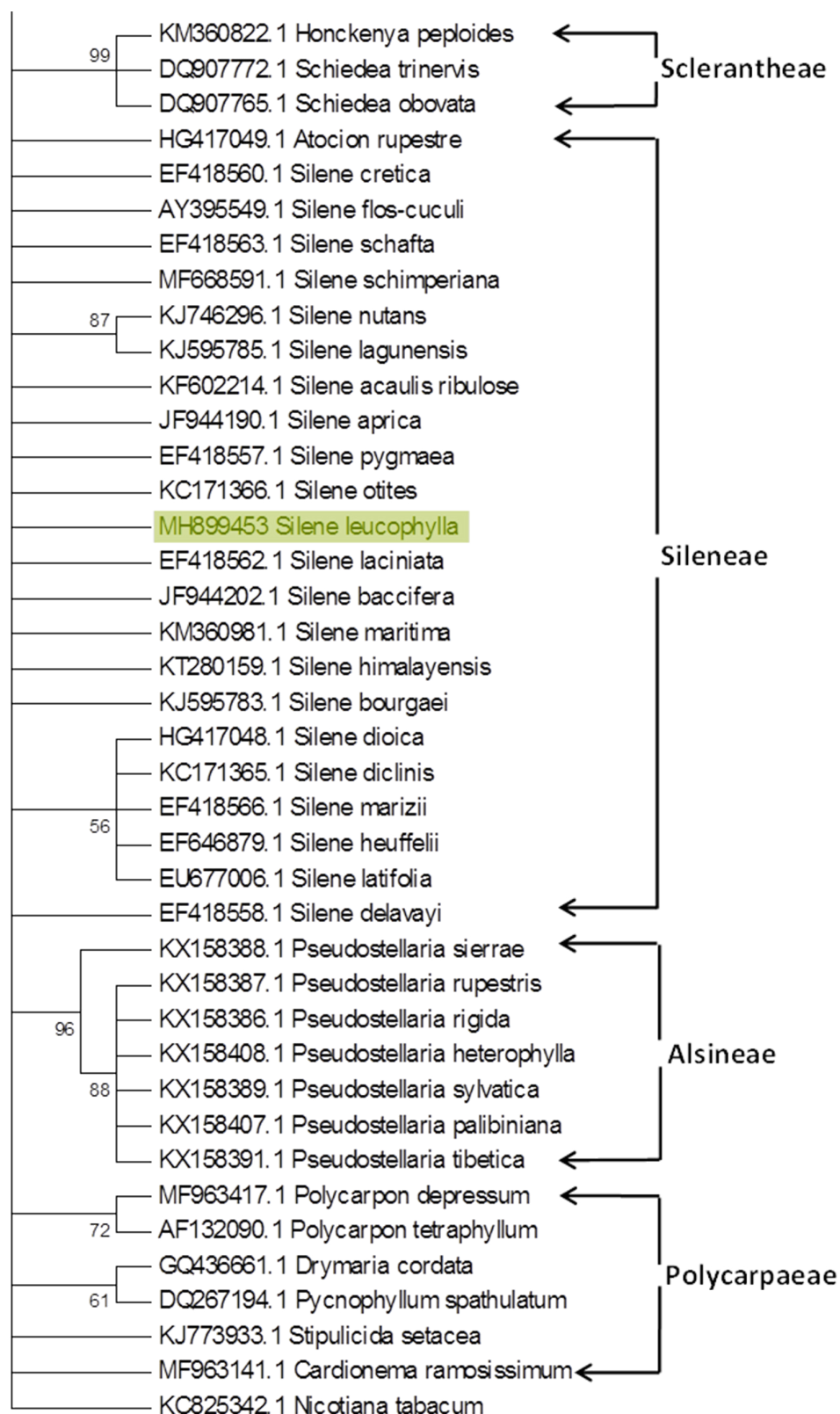


Fig. 1. Phylogenetic tree using Maximum Likelihood method based on Kimura 2-parameter model for *rbcl* sequences. Bootstrap values based on 1000 replications are listed as percentages at branching points [Tribes following Stevens PF (2001)].

TABLE 1. Estimates of evolutionary divergence between rbcL sequences in *B. multiceps*, *S. leucophylla* and *S. oreosinaica* and mined sequences based on the K2P model.

Mined Sequence	<i>Bufonia multiceps</i>	<i>Silene leucophylla</i>	<i>Silene oreosinaica</i>
<i>Bufonia multiceps</i>		0.036	0.060
<i>Silene leucophylla</i>	0.036		0.093
<i>Silene oreosinaica</i>	0.060	0.093	
<i>Dianthus repens</i>	0.033	0.023	0.093
<i>Dianthus deltoides</i>	0.033	0.023	0.093
<i>Dianthus imereticus</i>	0.033	0.023	0.096
<i>Dianthus gratianopolitanus</i>	0.036	0.025	0.093
<i>Dianthus carthusianorum</i>	0.033	0.023	0.093
<i>Dianthus monspessulanus</i>	0.033	0.023	0.093
<i>Dianthus turkestanicus</i>	0.036	0.025	0.093
<i>Dianthus superbus</i>	0.033	0.023	0.093
<i>Dianthus plumarius</i>	0.033	0.023	0.093
<i>Dianthus caryophyllus</i>	0.033	0.023	0.093
<i>Dianthus kuschakewiczii</i>	0.036	0.025	0.093
<i>Dianthus chinensis</i>	0.033	0.023	0.093
<i>Dianthus seguieri</i>	0.033	0.023	0.093
<i>Dianthus armeria</i>	0.033	0.023	0.093
<i>Dianthus longicalyx</i>	0.033	0.023	0.096
<i>Dianthus namaensis</i>	0.038	0.028	0.096
<i>Dianthus sylvestris</i>	0.036	0.025	0.088
<i>Psammophiliella muralis</i>	0.028	0.023	0.093
<i>Petrorragia saxifraga</i>	0.033	0.023	0.090
<i>Petrorragia nanteuillii</i>	0.030	0.020	0.090
<i>Petrorragia prolifera</i>	0.030	0.020	0.099
<i>Honckenya peploides</i>	0.046	0.025	0.102
<i>Schiedea trinervis</i>	0.046	0.025	0.102
<i>Schiedea obovata</i>	0.046	0.025	0.093
<i>Atocion rupestre</i>	0.038	0.002	0.093
<i>Spergula arvensis</i>	0.041	0.028	0.096
<i>Saponaria officinalis</i>	0.041	0.030	0.090
<i>Eremogone lychnidea</i>	0.030	0.025	0.085
<i>Arenaria ciliate</i>	0.030	0.025	0.085
<i>Sagina saginoides</i>	0.031	0.025	0.085
<i>Sagina nivalis</i>	0.033	0.028	0.085
<i>Colobanthus subulatus</i>	0.033	0.028	0.082
<i>Sagina nodosa</i>	0.028	0.023	0.082
<i>Sagina procumbens</i>	0.028	0.023	0.082
<i>Sagina subulata</i>	0.031	0.025	0.085
<i>Sagina apetala</i>	0.031	0.025	0.085
<i>Sagina maritime</i>	0.033	0.028	0.099
<i>Silene nutans</i>	0.041	0.005	0.099
<i>Silene lagunensis</i>	0.041	0.005	0.093

TABLE 1. Cont.

Mined Sequence	<i>Bufonia multiceps</i>	<i>Silene leucophylla</i>	<i>Silene oreosinaica</i>
<i>Silene otites</i>	0.041	0.005	0.093
<i>Silene pygmaea</i>	0.038	0.002	0.093
<i>Silene acaulis</i>	0.038	0.002	0.093
<i>Silene aprica</i>	0.038	0.002	0.090
<i>Silene schafta</i>	0.036	0.005	0.090
<i>Silene schimperiana</i>	0.036	0.005	0.087
<i>Silene antirrhina</i>	0.033	0.002	0.090
<i>Silene cretica</i>	0.044	0.013	0.099
<i>Silene flos-cuculi</i>	0.046	0.015	0.102
<i>Silene gallica</i>	0.041	0.015	0.096
<i>Silene armeria</i>	0.041	0.010	0.093
<i>Silene uniflora</i>	0.033	0.007	0.090
<i>Silene vulgaris</i>	0.036	0.005	0.093
<i>Silene drummondii</i>	0.033	0.005	0.090
<i>Silene laciniata</i>	0.038	0.007	0.093
<i>Silene delavayi</i>	0.041	0.010	0.096
<i>Silene himalayensis</i>	0.038	0.007	0.090
<i>Silene noctiflora</i>	0.046	0.018	0.102
<i>Silene maritima</i>	0.038	0.007	0.093
<i>Silene bourgaei</i>	0.036	0.005	0.093
<i>Silene conica</i>	0.041	0.015	0.096
<i>Silene baccifera</i>	0.041	0.010	0.093
<i>Silene marizii</i>	0.041	0.010	0.096
<i>Silene dioica</i>	0.041	0.010	0.096
<i>Silene diclinis</i>	0.041	0.010	0.093
<i>Silene heuffelii</i>	0.041	0.010	0.096
<i>Silene latifolia</i>	0.038	0.007	0.096
<i>Psammophilene tunicoides</i>	0.038	0.023	0.096
<i>Pseudostellaria sierra</i>	0.044	0.033	0.102
<i>Pseudostellaria heterophylla</i>	0.041	0.030	0.099
<i>Pseudostellaria sylvatica</i>	0.041	0.030	0.099
<i>Pseudostellaria palibiniana</i>	0.041	0.030	0.099
<i>Pseudostellaria rupestris</i>	0.041	0.030	0.099
<i>Pseudostellaria tibetica</i>	0.041	0.030	0.099
<i>Pseudostellaria rigida</i>	0.041	0.030	0.099
<i>Polycarpon depressum</i>	0.038	0.020	0.096
<i>Polycarpon tetraphyllum</i>	0.036	0.018	0.096
<i>Drymaria cordata</i>	0.038	0.023	0.096
<i>Pycnophyllum spathulatum</i>	0.036	0.020	0.096
<i>Stipulicida setacea</i>	0.038	0.023	0.096
<i>Cardionema ramosissimum</i>	0.044	0.028	0.102

In phylogenetic tree (Fig. 1), few *Silene* species were clustered in 2 clades nested in a grade of the remaining *Silene* taxa, including *Silene leucophylla*. The tree showed a generally ambiguous classification at both generic and tribal levels. *Silene* grade was interrupted with *Atocion rupestre* (an allied species in Sileneae) and Scleranthae clade. Similarly, *Silene oreosinaica* and *Bufonia multiceps* formed a clade supported with bootstrap value of 98% nested among Caryophylleae species.

The paraphyly of *Silene* was recorded using different molecular markers including ITS (Oxelman & Lide'n, 1995 and Desfeux & Lejeune, 1996), rps16 (Oxelman et al., 1997), SIX1 and YI (Rautenberg et al., 2008) and matk (Harbaugh et al., 2010). It was also demonstrated by Greenberg & Donoghue (2011) based on analysis of ITS, trnL-trnF, trnQrps16, trnS-trnM, ndhF, and matK sequences. However, the authors observed monophyly of Sileneae using combination of the same sequences. Paraphyly was also observed in other genera of Caryophyllaceae including *Acanthophyllum* (Pirani et al., 2014) and *Arenaria* (Sadeghian et al., 2015) using ITS and plastid rps16 sequences. It may reflect a complex evolutionary history; probably a combination of substitution rate heterogeneity, star-like phylogenetic patterns, and ancient chloroplast recombination (Orthia et al., 2005 and Erixon & Oxelman, 2008).

Appearance of *S. oreosinaica* and *B. multiceps* as sister species on highly bootstrap-supported clade may be explained in light of the findings of Harbaugh et al. (2010) who recorded Sagineae and Sileneae in 2 sister clades. In addition, using few Sagineae taxa in the present tree (due to the insufficient rbcL sequences of this tribe in the GenBank database) may be responsible for the clade carrying *Silene oreosinaica* and *Bufonia multiceps*. Grouping of *Silene* spp. with Caryophyllaceae taxa belonging to other genera was also recorded by Smissen et al. (2002). The authors observed grouping of *Silene antirrhina*, *Spergularia marina* and *Scleranthus biflorus* in *ndhF*-based tree (Smissen et al., 2002).

Based on the present investigation, *S. leucophylla*, *S. oreosinaica* and *B. multiceps* were introduced into Sinai Peninsula through two colonization events. The first introduced *S. leucophylla* while the second introduced a common ancestor for the remaining two species.

Conclusion

Results of this investigation reflect the potential use of DNA barcoding in documentation of endemic endangered species with some issues concerning assigning to the proper taxonomic position. Such issues may be resolved through comprehensive revision of classical taxonomic attributes and filling gaps in present DNA barcoding databases.

References

- Bacchetta, G., Brullo, S., Congiu, A., Fenu, G., Garrido, J.L. and Mattana, E. (2012) A new species of *Aquilegia* (Ranunculaceae) from Sardinia (Italy). *Phytotaxa*, **56**(1), 59-64.
- Bafeel, S.O., Arif, I.A., Bakir, M.A., Khan, H.A., Al Farhan, A.H., Al Homaidan, A.A., Ahamed, A. and Thomas, J. (2011) Comparative evaluation of PCR success with universal primers of maturase K (matK) and ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit (rbcL) for barcoding of some arid plants. *Plant Omics*, **4**(4), 195-198.
- Bafeel, S.O., Arif, I.A., Bakir, M.A., Al Homaidan, A.A., Al Farhan, A.H. and Khan, H.A. (2012) DNA barcoding of arid wild plants using rbcL gene sequences. *Genetics and Molecular Research*, **11**(3), 1934-1941.
- Baskauf, C.J. and Eickmeier, W.G. (1994) Comparative ecophysiology of a rare and widespread species of *Echinacea* (Asteraceae). *American Journal of Botany*, **81**(8), 958-964.
- Caley, M.J. and Munday, P.L. (2003) Growth trades off with habitat specialization. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **270**(2), S175-S177.
- Carlquist, S. (1974) *Island Biology*. Columbia Univ. Press, New York.
- CBOL Plant Working Group (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America*, **106**(31), 12794-12797.
- De Candolle, A.B. (1820) *Essai elementaire de geographie botanique*. In: "*Dictionnaire des Sciences Naturelles*", pp 1-64. Vol. (18), Flevrault, Strasbourg.
- Desfeux, C. and Lejeune, B. (1996) Systematics of

- EuroMediterranean *Silene* (Caryophyllaceae): Evidence from a phylogenetic analysis using ITS sequences. *Sciences de la Vie*, **319**(4), 351-358.
- de Vere, N., Rich, T.C.G., Ford, C.R., Trinder, S.A., Long, C., Moore, C.W., Satterthwaite, D., Davies, H., Allainguillaume, J., Ronca, S., Tatarinova, T., Garbett, H., Walker, K. and Wilkinson, M.J. (2012) DNA barcoding the native flowering plants and conifers of Wales. *PLoS ONE*, **7**(6), e37945.
- El Hadidi, M.N. (2000) Geomorphology, climate and phytogeographic affinities. In: "*Flora Aegyptiaca*", M.N. El Hadidi (Ed.). The Palm Press, Cairo, Egypt.
- Erixon, P. and Oxelman, B. (2008) Reticulate or tree-like chloroplast DNA evolution in Sileneae (Caryophyllaceae)? *Molecular Phylogenetics and Evolution*, **48**(1), 313-325.
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**(4), 783-791.
- Ferreira, P.M.A. and Boldrini, I.I. (2011) Potential reflection on distinct ecological units in plant endemism categories. *Conservation Biology*, **25**(4), 672-679.
- Gankin, R. and Major, J. (1964) *Arctostaphylos myrtifolia*, its biology and relationship to the problem of endemism. *Ecology*, **45**(4), 792-808.
- Gaston, K.J. and Blackburn, T.M. (1996) The spatial distribution of threatened species: Macroscales and New World birds. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **263**(1367), 235-240.
- Greenberg, A.K. and Donoghue, M.J. (2011) Molecular systematics and character evolution in Caryophyllaceae. *Taxon*, **60**(6), 1637-1652.
- Harbaugh, D.T., Nepokroeff, M., Rabeler, R.K., McNeill, J., Zimmer, E.A. and Wagner, W.L. (2010) A new lineage-based tribal classification of the family Caryophyllaceae. *International Journal of Plant Sciences*, **171**(2), 185-198.
- Hebert, P.D.N. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B Biological Sciences*, **270**(1512), 313-321.
- Hebert, P.D.N. and Gregory, T.R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, **54**(5), 852-859.
- Hobohm, C. and Tucker, C.M. (2014) The increasing importance of endemism: responsibility, the media and education. In: "*Endemism in Vascular Plants*". Plant and Vegetation 3-9, C. Hobohm (Ed.). Springer Netherlands, Netherlands.
- Hosni, H., Hosny, A., Shamsou, E. and Hamdy, R. (2013) Endemic and near-endemic taxa in the flora of Egypt. *Egyptian Journal of Botany*, **53**, 357-383.
- Kang, Y., Deng, Z., Zang, R. and Lon, W. (2017) DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. *Scientific Reports*, **7**, 12564.
- Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**(2), 111-120.
- Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A. and Janzen, D.H. (2005) Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences*, **102**(23), 8369-8374.
- Lamoreux, J.F., Morrison, J.C., Ricketts, T.H., Olson, D.M., Dinerstein, E., McKnight, M.W. and Shugart, H.H. (2006) Global tests of biodiversity concordance and the importance of endemism. *Nature*, **440**(7081), 212-214.
- Linder, H.P. (1995) Setting conservation priorities—the importance of endemism and phylogeny in the southern African orchid genus *Herschelia*. *Conservation Biology*, **9**(3), 585-595.
- Meyer, S.E. (1986) The ecology of gypsophile endemism in the eastern Mojave Desert. *Ecology*, **67**(5), 1303-1313.
- Meyer, S.E., García-Moya, E. and Lagunes-Espinoza, L.D.C. (1992) Topographic and soil surface effects on gypsophile plant community patterns in central Mexico. *Journal of Vegetation Science*, **3**(3), 429-438.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A. and Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**(6772), 853-858.
- Egypt. J. Bot. **59**, No.2 (2019)

- Newmaster, S.G., Fazekas, A.J.J. and Ragupathy, S. (2006) DNA barcoding in land plants: Evaluation of rbcL in a multigene tiered approach. *Canadian Journal of Botany*, **84**(3), 335-341.
- Orme, C.D.L., Davies, R.G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V.A., Webster, A.J., Ding, T.S., Rasmussen, P.C., Ridgely, R.S. and Stattersfield, A.J. (2005) Global hotspots of species richness are not congruent with endemism or threat. *Nature*, **436**(7053), 1016.
- Orthia, L.A., Cook, L.G. and Crisp, M.D. (2005) Generic delimitation and phylogenetic uncertainty: An example from a group that has undergone an explosive radiation. *Australian Systematic Botany*, **18**(1), 41-47.
- Oxelman, B. and Lidén, M. (1995) Generic boundaries in the tribe Sileneae (Caryophyllaceae) as inferred from nuclear rDNA sequences. *Taxon*, **44**, 525-542.
- Oxelman, B., Lidén, M. and Berglund, D. (1997) Chloroplast rbcL intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution*, **206**(1-4), 393-410.
- Pirani, A., Zarre, S., Pfeil, B.E., Bertrand, Y.J., Assadi, M. and Oxelman, B. (2014) Molecular phylogeny of *Acanthophyllum* (Caryophyllaceae: Caryophylleae), with emphasis on infrageneric classification. *Taxon*, **63**(3), 592-607.
- Rautenberg, A., Filatov, D., Svennblad, B., Heidari, N. and Oxelman, B. (2008) Conflicting phylogenetic signals in the SIX1/Y1 gene in *Silene*. *BMC Evolutionary Biology*, **8**(1), 299.
- Sadeghian, S., Zarre, S., Rabeler, R.K. and Heubl, G. (2015) Molecular phylogenetic analysis of *Arenaria* (Caryophyllaceae: tribe Arenarieae) and its allies
- inferred from nuclear DNA internal transcribed spacer and plastid DNA rps16 sequences. *Botanical Journal of the Linnean Society*, **178**(4), 648-669.
- Shehata, A.A. and Kamel, W.M. (2007) A contribution to the palynological studies of the endemic flora of Sinai, Egypt. *Roczniki Akademii Rolniczej w Poznaniu. Botanika-Steciana*, **11**.
- Smitsen, R.D., Clement, J.C., Garnock-Jones, P.J. and Chambers, G.K. (2002) Subfamilial relationships within Caryophyllaceae as inferred from 5' ndhF sequences. *American Journal of Botany*, **89**(8), 1336-1341.
- Stattersfield, A.J., Crosby, M.J., Long, A.J. and Wege, M.J. (1998) Endemic bird areas of the world: Priorities for biodiversity conservation. Bird life Conservation Series 7, pp. 13-26. Birdlife International, Cambridge, UK.
- Strid, A. (1986) The mountain flora of Greece with special reference to the Anatolian element. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences*, **89**, 59-68.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30**(12), 2725-2729.
- Ungriht, S. (2004) How many plant species are there? And how many are threatened with extinction? Endemic species in global biodiversity and conservation assessments. *Taxon*, **53**(2), 481-484.
- Wilson, D.S. (1994) On the coexistence of specialists and generalists. *American Naturalist*, **144**(4), 692-707.

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توثيق ثلاثة أنواع من العائلة القرنفلية متوطنة بشبه جزيرة سيناء باستخدام تقنية الباركود للأحماض النووية

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قامت هذه الدراسة بعمل باركود باستخدام الشفرة الوراثية للجين rbcL لثلاثة أنواع متوطنة من العائلة القرنفلية، إحدى أكثر العائلات النباتية تكرارا في فلورة شبه جزيرة سيناء بمصر، (*Silene*, *Bufonia multiceps*) و ذلك للمرة الأولى. بمقارنة تتابعات الشفرات الناتجة لتلك النباتات بتلك المسجلة على قواعد بيانات بنك الجينات ظهر تشابه مع الشفرات المسجلة لجين rbcL لنباتات تنتمي كلها إلى العائلة القرنفلية. أظهرت الشجرة التطورية تصنيفا غامضا على مستوى الجنس والقبيلة. وأظهرت النتائج أن الأنواع الثلاثة قد استوطنوا شبه الجزيرة على مرحلتين، أسفرت الأولى عن ظهور *Silene leucophylla* بينما جاءت الأخرى بسلف مشترك للنوعين الآخرين.