Original article

Molecular characterization of endangered endemic plant *Aloe pseudorubroviolacea* using chloroplast *matK* and plastid *rbcL* gene

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ABSTRACT

An endangered and rare species *Aloe pseudorubroviolacea* from the plant family Asphodelaceae which is presently recorded as endangered in Saudi Arabia collected from Al-Baha region of Saudi Arabia its GPS Latitude and Longitude coordinates 19.8345, 41.5481. The chloroplast *matK* and *rbcL* gene was considered in this study based on molecular identification the size is about 571 and 664 bp respectively. From the sequence analysis the gene *matK* and *rbcL* confirm that this species is very much closely related with *A. rubroviolacea* and also inter related with the species *Astroloba rubriflora, Chrysopogon gryllus, Chortolirion angolense* shows about 98.7% sequence homology. The partial *matK* and *rbcL* gene sequence discriminate *Aloe pseudorubroviolacea* from the closely related plant species, *A. rubroviolacea*. The gene sequence of *rbcL* discriminates the species from *Chrysopogon gryllus* and *Chortolirion angolense*, demonstrates the nucleotide variations in 3 different sites (623C/T; 653C/T; 700C/A). This study showed that *matK* and *rbcL* sequence region of chloroplast gene used to authenticate the samples of *A. pseudorubroviolacea* and which provide to help in correct identification and conservation process of this medicinally valuable endangered plant species.

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1. Introduction

Wood, 1983 described 17 Aloe species from Yemen and recorded three unknown species of Aloe A, B, and C. One of the species, A.inermis has been found outside of Arabian Peninsula. Al-Khulaidi, 2013 counted 33 Aloe species from the flora of Yemen, among 15 species where endemic to Arabia Peninsula, 15 endemic to Yemen, 3 species (Aloe inermis, A. Officinalis and A. Vera) have been found outside Arabian Peninsula. About 50 Aloe species where distributed in the Arabian Peninsula and many of them are endemic to Arabian Peninsula, among the remarkable studies on Aloes of Arabia (Lavranos, 1965, McCoy, 2019). A. pseudorubroviolacea (Arabian Aloe) is a attractive plant and wide rosettes of thick with 2 foot, blue-green leaves that look from bushy stems and 3 to four foot height inflorescences of orange-red flowers late winter into spring. A. rubroviolacea is much like morphologically during winter the foliage takes on pink tones. It was earliest recorded by Sheila et al. (2001). A. pseudorubroviolacea is related to 3 others from Arabia or Eritrea which have comparable inflorescences: A. porphyrostachys (stemless), A. rubroviolacea (smaller, more prolific), and A. schoelleri (yellow to pinkish orange flowers). This plant is similarly related to A. porphyrostachys (Tom et al., 2014) and the resemblance to A. rubroviolacea (Leonard, 2004). Aloe pseudorubroviolacea is one of the 14 endemic Aloes of Saudi Arabia, recorded from Jabal Radhwa, Raidah and near Ajalta (Collenette, 1999), while A. castellorum is recorded from north Abha and Jabal Fayfa (Collenette, 1999). Aloe pseudorubroviolacea is closely contact with local community, two species near the settlement zones such as the endemic A. pseudorubroviolacea and A.castellorum are extremely threatened in

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Albaha, Saudi Arabia. A. pseudorubroviolacea plant as among those endangered plant species in Al-Baha region, Saudi Arabia, with frequency 1.25% and density 1.25 per hectare. (Al-Khulaidi et al., 2018). The genus Aloe are used globally in medicines, especially for the treatment of wound healing, gastrointestinal disorders and microbial infection (Miller and Morris, 2004). Only a very few morphological studies have examined, there is no nucleotide based validation of A. pseudorubroviolacea. Chloroplast gene rbcL, universally used for plant systematics studies (Alaklabi et al., 2014) and DNA molecular tools like RAPD provides easy and fast identification of species with some limits (Hoey et al., 1996). Here, in this study, we described of morphological characters of A. pseudorubroviolacea and determined by matK and rbcL gene sequence based phylogenetic analysis for regionally endangered plant species.

2. Materials and methods

2.1. Plant sampling

Endangered plant specimens A. pseudorubroviolacea were collected from Al-Baha region of Saudi Arabia its GPS Latitude and Longitude coordinate are 19.8345, 41.5481 shown Fig. 1(a,b). It was identified by Taxonomist Abdulwali Al-Khulaidi, at Department of Biology, College of Arts and Sciences, Al - Baha University.

2.2. DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from the leaf specimen using DNeasy plant mini kit (Qiagen). DNA was quantified by using Nanodrop 8000 Spectrophotometer (Thermo Scientific, USA) and it stored at −80oC (Alaklabi et al., 2014). The PCR amplification of the chloroplast genes matK and rbcL was prepared using Gradient master cycler (Eppendorf, Germany). The amplified PCR products were resolved in 1% agarose gel using 0.5 X TBE buffer. The PCR products were sequenced and submitted to GenBank Nucleotide database and its accession number is (MT108300 and MT108301).

2.3. Assignment of taxa

Sequence was searched against with available online databases like BLAST (Basic Local Alignment Search Tool) and BOLD (Barcode of Life Data). Based on homology search related sequences were retrieved from NCBI nucleotide database. Multiple sequence alignment done using ClustalX (Thompson et al., 2003) and phylogenetic analyses were done using MEGA5 (Tamura et al. 2007). Different phylogenetic tree performed by three different methods like maximum likelihood (ML), neighbour joining (NJ) and maximum parsimony (MP) and trees were evaluated by using the methods like bootstrap re-sampling method of Felsenstein (1985) with 1000 replicates. Each sequence was compared by pair-wise alignment comparisons done with BLAST 2 (Tatusova and Madden, 1999).

3. Results

3.1. Species taxonomic description

Morphological features of A.pseudorubroviolacea (Arabian Aloe) are illustrated in Fig. 2A and B. A. pseudorubroviolacea (Arabian Aloe) is a attractive plant and wide rosettes of thick with 2 foot, blue-green leaves that look from bushy stems and 3 to four foot height inflorescences of orange-red flowers late winter into spring. In winter the foliage takes on pink tones much like A. rubroviolacea, (Sheila et al., 2001). This species is related to 3 others from Arabia or Eritrea which have comparable inflorescences: A. porphyrostachys (stemless), A. rubroviolacea (smaller, more prolific), and A. schoelleri (yellow to pinkish orange flowers). This plant is similarly related to A. porphyrostachys (Tom et al., 2014) and the resemblance to A. rubroviolacea (Leonard, 2004).

3.2. Phylogenetic analysis for the gene matK

The PCR product size of matK gene is about 495 (4R1) and 508 bp (4R2) of chloroplast plastid region for the species A. pseudorubroviolacea obtained using respective primers respectively. The produced sequences were searched against with database using BLAST. The specimen, A. pseudorubroviolacea is 100% sequence similarity to A. vera, A. rubriflor, A.variegata and 99% sequence similarities with very close relationship with other plant species of A. foliolosa, G. glauca, H. cooperi, K. disticha, C. gryllus, E. chinensis, C. angolense for matK and rbcL gene respectively. BOLD identification system shows 99.32% homology with different plant species of the genus Aloe (A. vera). BOLD search show 98.1% sequence similarities with A. rubriflora and A.variegata and other plant species of Aloe shows > 98% sequence similarities were
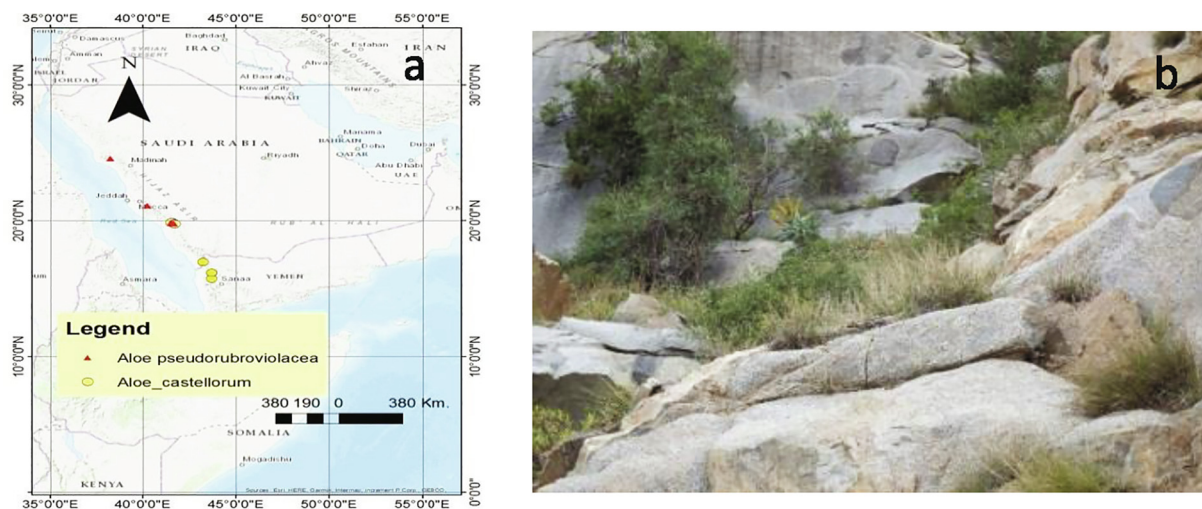

Fig. 1. (a,b) The endangered plant specimens were collected from Al-Baha region, Saudi Arabia (GPS coordinate: 19.8345, 41.5481, Adopted from https://maps.google.com). B. Only remain very few species of A. pseudorubroviolacea on remote an accessible very steep rocky slope. Place South of Al-Baha.

Fig. 2. A Regionally endangered plant *A. pseudorubroviolacea* in the sample collected site (Al-Baha, Saudi Arabia); B. Leaf and stem.

comprised for tree analyses. According to all phylogenetic tree analyses the specimen species *A. pseudorubroviolacea* was very closely similar to that of *A. vera* and also with *Chortolirion angolense* and *Chrysopogon gryllus* respectively using for *matK* gene for phylogenetic study. *A. pseudorubroviolacea* also evolutionarily related with *Aloe variegata* however, revealed a separate lineage from *Kumara disticha* conserved by 92%, 85% and 67% of bootstrap values for ML, NJ and MP trees, respectively shown in Fig. 3A, B and C. Pair-wise sequence similarities of *A. pseudorubroviolacea* were 99.7% with *A. vera* and 99.6% with *Chortolirion angolense* (Table 1). About 99% sequence similarity was observed among 11 plant species under the family Asphodelaceae are used in the tree analyses. Overall Mean distance and Transition/transversion ratios of the eight taxa of Aloe genus for *matK* gene are shown in the Table 3.

3.3. Phylogenetic analysis for the gene rbcL

Using universal primer for plastid *rbcL* gene we obtained PCR products about 648 bp for the species *A. pseudorubroviolacea*. The produced *rbcL* of sequence *A. pseudorubroviolacea* was searched against database using BLAST search which shows is identical 97% sequence similarity to the species *A. variegate* with and similarity with multiple plant species *Astroloba rubriflora, Chrysopogon*

gryllus and *Chortolirion angolense*. By using BOLD identification system showed 87% homology with multiple plant species of the genus Aloe, *Kumara disticha* and *Eremurus chinensis* and 67% with the genus *Kniphofia uvaria*. By using BOLD search show 99.6% resemblances with *A. rubriflora* and *A. variegata*. About 98% sequence similarities was observed using database search. Most of the species showed highly related with each other included in the tree analyses inferred by MP, ML and NJ method shown in the Fig. 4A, B and C. Pair-wise sequence similarities of *A. pseudorubroviolacea* were 99.9% with *Astroloba rubriflora, Aloe variegate* and *Aloe vera* 83.2% with *Gasteria glauca* (Table 2). About 89.4% similarity was observed between 10 plant species under the family Asphodelaceae which are incorporated in the tree analyses. By phylogenetic analysis using *rbcL* gene demonstrated that different plant species *A. pseudorubroviolacea* which is very closely related to the plant *Eremurus chinensis*. Overall Mean distance and Transition/transversion ratios of the eight taxa of Aloe genus for *rbcL* gene are shown in the Table 3.

4. Discussion

In the present study, success rates of amplification in and the correct identification is about 99% and 97% respectively using *matK*
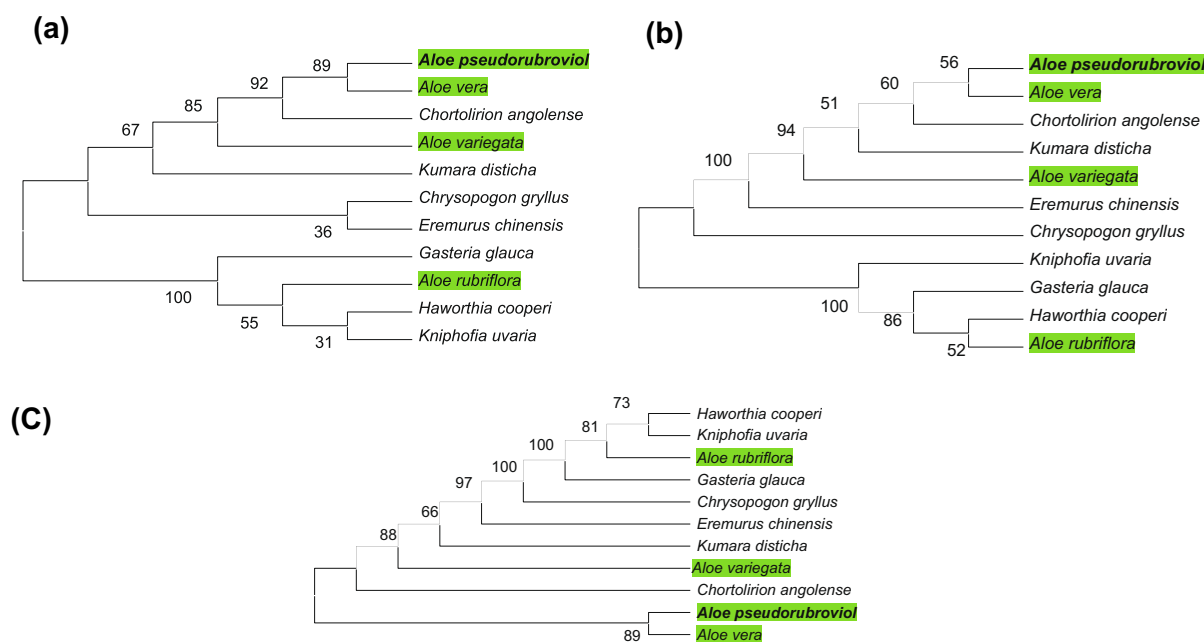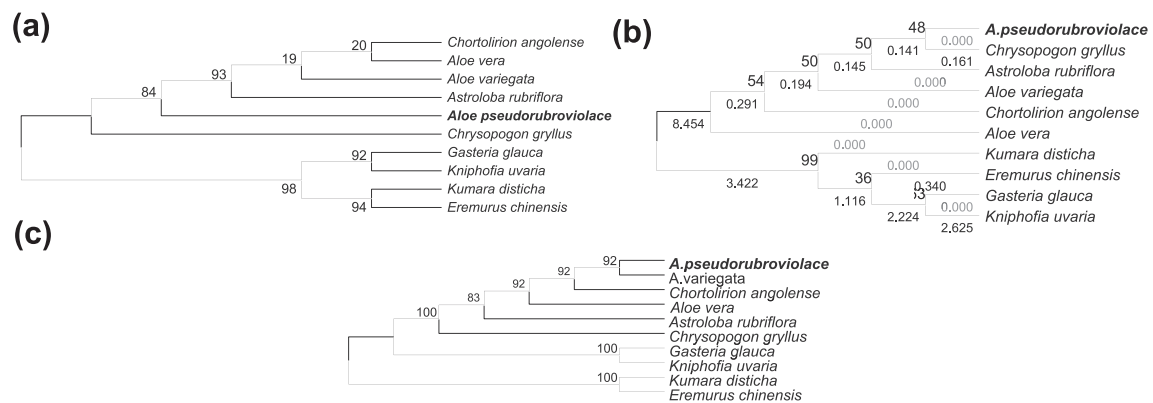

Fig. 3. A. Phylogenetic tree of the *Aloe pseudorubroviolacea* and related 11 taxa were inferred using the Maximum Likelihood method. The bootstrap consensus tree inferred from 1000 replicates. B. Phylogenetic tree of the *Aloe pseudorubroviolacea* and related 11 taxa were inferred using the Neighbour joining method. The bootstrap consensus tree inferred from 1000 replicates. C. Phylogenetic tree of the *Aloe pseudorubroviolacea* and related 11 taxa were inferred using the Maximum Patrimonial method. The bootstrap consensus tree inferred from 1000 replicates.

Table 1

Estimation of pairwise *matK* gene sequence similarities (%) between the genus Aloe species.

Accession number	Species Name	1	2	3	4	5	6	7	8	9	10
JQ024192	*Gasteria glauca*										
KJ557843	*Haworthia cooperi*	99.7									
KC893725	*Kumara disticha*	98.6	99.6								
KJ557835	*Aloe rubriflora*	99.98	99.98	98.62							
JQ024171	*Aloe variegata*	98.67	98.6	99.9	98.6						
KJ557845	*Kniphofia uvaria*	99.9	99.9	98.5	99.9	98.5					
HE967383	*Chrysopogon gryllus*	98	97.8	99.6	97.8	99.6	97.7				
MK435651	*Eremurus chinensis*	98.71	98.6	99.9	98.6	99.9	98.6	99.8			
JX517522	*Chortolirion angolense*	98.6	98.6	99.9	98.6	99.9	98.6	99.6	99.9		
	Aloe pseudorubroviolacea	98.6	98.6	99.9	98.5	99.9	98.5	99.7	99.9	99.9	
KY556640	*Aloe vera*	98.6	98.6	99.9	98.5	99.9	98.5	99.7	99.9	99.9	100

Fig. 4. A. Phylogenetic tree of the *Aloe pseudorubroviolacea* and related 11 taxa were inferred using the Maximum Likelihood method. The bootstrap consensus tree inferred from 1000 replicates. B. Phylogenetic tree of the *Aloe pseudorubroviolacea* and related 11 taxa were inferred using the Neighbour joining method. The bootstrap consensus tree inferred from 1000 replicates. C. Phylogenetic tree of the *A. pseudorubroviolacea* and related 11 taxa were inferred using the Maximum Patrimonial method. The bootstrap consensus tree inferred from 1000 replicates.

Table 2

Estimation of pairwise *rbcL* gene sequence similarities (%) between the genus Aloe species.

Accession number	Species Name	1	2	3	4	5	6	7	8	9	10
JQ024549	*Aloe pseudorubroviolace*										
JQ024549	*Astroloba rubriflora*	99.9									
JQ024543	*Aloe variegata*	99.9	100								
JX572402	*Chortolirion angolense*	99.9	100	100							
KP072718	*Aloe vera*	99.9	100	100	100						
FR865148	*Chrysopogon gryllus*	99.8	99.8	99.8	99.8	99.8					
JQ024565	*Gasteria glauca*	83.2	89.3	89.3	89.3	89.3	86.9				
AJ290267	*Kniphofia uvaria*	76.2	83.2	83.2	83.3	83.3	76.8	99.4			
JQ024531	*Kumara disticha*	89.4	89.4	89.4	89.4	89.4	86.9	97.5	97.2		
HM640526	*Eremurus chinensis*	87	87	87	87	87	83.2	97.5	97.2	99.9	100

Table 3

Transition/transversion ratios of the 8 taxa from Genbank.

Genus	% of Variance of *matK*	% of Variance of *rbcL*	No. of parsimony site	Overall distance mean for *matK*	Overall distance mean for *rbcL*
Aloe	0.587	0.02	3	0.019	0.02
Gasteria	1.143	6.8	3	0.560	10.6
Haworthia	1.143	–	3	0.586	–
Kumara	1.120	10.6	3	0.749	10.6
Kniphofia	1.156	16.8	4	0.777	16.8
Chrysopogon	1.159	0.14	4	1.019	0.14
Eremurus	1.156	13.1	4	0.913	13.1
Chortolirion	1.156	0.02	4	0.891	0.02

and *rbcL* regions at genus level. Kress et al., 2009, reported the *matK* had the lowest overall rate of recovery of 69% at species level. CBOL (2009) conveyed the universal of primers is an important criterion for evaluating the appropriateness of DNA barcoding. The gene *matK* and *rbcL* result in highly conserved and low evolutionary levels for species level of identifications (Kress et al., 2009,

Fazekas et al., 2008, Kang et al., 2017). High identification success rate for both *rbcL* and *matK* at genus level. Therefore, our results suggest that *rbcL* and *matK* used the for identification systems of plants at the genus level for Aloe species.

Hereby phylogenetic evolutionary tree was constructed for Aloe species using *matK* and *rbcL* which is supported by the values of nodes on each branch were higher than 80%, indicating that we obtain highly reliable evolutionary relationships for Aloe. The *matK* and *rbcL* regions are used to construct phylogenetic relationships for tree communities of Borneo (Heckenhauer et al., 2017) and for the order Fabales (Bello et al., 2009). Our results further prove that *matK* and *rbcL* show great efficacy in reconstructing phylogenetic tree for desert plant species. Ever since desert regions were rich in endemic species. Thus phylogenetic tree was constructed by the MEGA for Aloe and its related species respectively.

In this study, very closely related species *Astroloba rubriflora, Aloe variegate, Aloe vera* were assigned based on chloroplast and plastid region *rbcL* and *matK* gene sequences. It seems to be difficult to differentiate nearly identical sequences within these species. The use of *matK* and *rbcL* regions and its variable nucleotides sites in the amplified region of *A. pseudorubroviolacea* to be used as DNA barcode region for accurate identification, ecological management and conservation for this endangered plant.

Acknowledgement

References

Alaklabi, A., Arif, I.A., Bafeel, S.O., Alfarhan, A.H., Ahamed, A., Thomas, J., Bakir, M.A., 2014. Nucleotide based validation of the endangered plant *Diospyros mespiliformis* (Ebenaceae) by evaluating short sequence region of plastid rbcL gene. Plant Omics 7 (2), 102.

Al-Khulaidi, A.A., 2013. Flora of Yemen: a Checklist. Sana'a: The Sustainable Natural Resource Management Project (SNRMP II). EPA and UNDP.

Al-khulaidi, A.W., Al-Sagheer, N.A., Al-turki, T.U., Filimban, F., 2018. Inventory of most rare and endangered plant species in Albaha region Saudi Arabia. IJBPAS 7 (4), 443–460.

Bello, M.A., Bruneau, A., Forest, F., Hawkins, J.A., 2009. Elusive relationships within order.

CBOL Plant Wording Group, 2009. A. DNA barcode for land plants. PNAS 106, 12794–12797.

Collenette S., 1999. Wild Flowers of Saudi Arabia. Riyadh: National Commission for Wild Life Conservation and Development. 4(3).

Fazekas, A.J., Burgess, K.S., Kesanakurti, P.R., Graham, S.W., Newmaster, S.G., Husband, B.C., Percy, D.M., Hajibabaei, M., Barrett, S.C., 2008. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. PloS one 3, (7) e2802.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evol 39 (4), 783–791.

Heckenhauer, J., Abu Salim, K., Chase, M.W., Dexter, K.G., Pennington, R.T., Tan, S., Kaye, M.E., Samuel, R., 2017. Plant DNA barcodes and assessment of phylogenetic community structure of a tropical mixed dipterocarp forest in Brunei Darussalam (Borneo). PLoS ONE 12, (10) e0185861.

Hoey, B.K., Crowe, K.R., Jones, V.M., Polans, N.O., 1996. A phylogenetic analysis of Pisum based on morphological characters, and allozyme and RAPD markers. Theor. Appl. Genet. 92 (1), 92–100.

Kang, Y., Deng, Z., Zang, R., Long, W., 2017. DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. Sci. Rep. 7 (1), 1–9.

Kress, W.J., Erickson, D.L., Jones, F.A., Swenson, N.G., Perez, R., Sanjur, O., Bermingham, E., 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proc. Natl. Acad. Sci. 106 (44), 18621–18626.

Leonard E., 2004. Newton Psudo – false and for the resemblance to Aloe rubroviolacea. Etymological Dictionary of Succulent Plant Names.

Lavranos, J.J., 1965. Notes on the Aloes of Arabia with descriptions of six new species, in Journ. S. Afr. Bot. 31, 55–81.

Miller, A.G., Morris, M., 2004. Ethnoflora of the Soqotra Archipelago. Royal Botanic Garden Edinburgh: UK 776, pages.

McCoy, 2019. The Aloes of Arabia (in press)

Sheila, C., Len, N., Tom McCoy., John, L., John, W., VIPs at Oxford Branch Show, 15 July 2001

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24 (8), 1596–1599.

Tatusova, T.A., Madden, T.L., 1999. BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. FEMS Microbiol. Lett. 174 (2), 247–250.

Thompson, J.D., Gibson, T.J., Higgins, D.G., 2003. Multiple sequence alignment using ClustalW and ClustalX. Curr. Protoc. Bioinformatics 1, 2–3.

Tom, A., McCoy, John J., Lavranos, 2014. Two New Species of Aloe from the Kingdom of Saudi Arabia. Cactus Succulent J. 86 (5), 258–263.

Wood, J.R.I., 1983. An outline of the vegetation of the Yemen Arab Republic. USAID. Corneli Univ, USA.