Saudi Journal of Biological Sciences 28 (2021) 3768–3775

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

The cp genome characterization of Adenium obesum: Gene content, repeat organization and phylogeny

Khalid Mashay Alanazi [a,1], Mohammad Ajmal Ali [b,1,*], Soo-Yong Kim [c], M. Oliur Rahman [d], Mohammad Abul Farah [a], Fahad Alhemaid [b], Meena Elangbam [e], Arun Bahadur Gurung [f,**], Joongku Lee [g]

[a] Genetics Laboratory, Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[b] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[c] International Biological Material Research Center, Korea Research Institute of Bioscience and Biotechnology, 125 Gwahangno, Yuseong-gu, Daejeon 34141, Republic of Korea
[d] Department of Botany, University of Dhaka, Dhaka 1000, Bangladesh
[e] Genetics Laboratory, Centre of Advanced Studies in Life Sciences, Manipur University, Canchipur 795 003, India
[f] Department of Basic Sciences and Social Sciences, North-Eastern Hill University, Shillong – 793022, Meghalaya, India
[g] Department of Environment and Forest Resources, Chungnam National University, Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea

ARTICLE INFO

Article history:
Received 15 February 2021
Revised 15 March 2021
Accepted 16 March 2021
Available online 23 March 2021

Keywords:
Chloroplast genome
Plastome
Poisonous plant
Medicinal plant
Adenium obesum
Apocynaceae

ABSTRACT

Adenium obesum (Forssk.) Roem. & Schult. belonging to the family Apocynaceae, is remarkable for its horticultural and ornamental values, poisonous nature, and medicinal uses. In order to have understanding of cp genome characterization of highly valued medicinal plant, and the evolutionary and systematic relationships, the complete plastome / chloroplast (cp) genome of *A. obesum* was sequenced. The assembled cp genome of *A. obesum* was found to be 154,437 bp, with an overall GC content of 38.1%. A total of 127 unique coding genes were annotated including 96 protein-coding genes, 28 tRNA genes, and 3 rRNA genes. The repeat structures were found to comprise of only mononucleotide repeats. The SSR loci are compososed of only A/T bases. The phylogenetic analysis of cp genomes revealed its proximity with *Nerium oleander*.

1. Introduction

Adenium obesum (Forssk.) Roem. & Schult. (family Apocynaceae), the '*Desert Rose*' is a poisonous, medicinal plant, distributed from Africa to Arabia, and is used traditoinally in the treatment of various ailments e.g. skin diseases, wounds, muscle pain, joint pain, venereal diseases, tooth decay, septic wounds, and nasal infections (Dimmitt and Hanson, 2002; Mouza and Hossain, 2015; Hossain et al., 2017). It is also used as a pesticide (Versiani et al., 2014), arrow poison for hunting in Africa (Oyen, 2008) and fish toxin (Wiseman, 2009). The *A. obesum* plant extract reported to possess cytotoxic (Almehdar et al., 2012), antimicrobial (Hossain et al., 2017) and anti-influenza (Kiyohara et al., 2012) activities. The phytochemical compounds identified from *A. obesum* include cardiac glycosides (cardenolides), pregnanes, triterpenes, flavonoids, and acetyldigitoxigenin (Versiani et al., 2014). The molecular docking of acetyldigitoxigenin elucidates the plausible mechanisms underlying the anticancer properties (Gurung et al., 2020).

The recent development in plastome or chloroplast (cp) genomics due to massive progress in the next-generation sequencing (NGS) platforms (Eid et al., 2009; Rothberg et al., 2011; Pattnaik et al., 2014; Jain et al., 2016; Shendure et al., 2017) and bioinformatics tools (Mavromatis et al., 2007; Knudsen et al., 2010; Huang et al., 2012; McElroy et al., 2012; Shendure and Aiden, 2012; Yang and Rannala, 2012; Caboche et al., 2014; Shcherbina 2014; Kwon et al., 2015; Langmead and Nellore, 2018) have greatly impact on biotechnology application (Spök et al., 2008; Zhang et al., 2015; Daniell et al., 2016). We herein for the first time report the cp genome characterization of highly valued medicinal plant *A.*

* Corresponding author.
** Corresponding author.
E-mail addresses: ajmalpdrc@gmail.com (M.A. Ali), arunbgurung@gmail.com (A.B. Gurung).
[1] The first and second authors provided an equal contribution to this paper.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.03.048
1319-562X/

obesum, and discuss its structure including gene content, repeat organization, and phylogeny.

2. Materials and methods

2.1. DNA sequencing, assembly and annotation

The fresh leaves of *A. obesum* were collected from the wild condition of desert habitat near to Riyadh, Saudi Arabia. The total genomic DNA was isolated using QIAGEN DNeasy DNA extraction kit. The *de novo* sequencing base calling was performed using the Illumina Pipeline 1.3.2 (Nie et al., 2012). The raw reads were filtered using FastQC to obtain the high-quality clean data by removing adaptor sequences using trimmomatic and low-quality reads with Q-value ≤ 20. The filtered reads were assembled using Spades (Bankevich et al., 2012), and annotated using GeSeq (https://chlorobox.mpimp-golm.mpg.de/geseq.html) (Tillich et al., 2017; Hansen et al., 2007). Further downstream analysis from the assembled cp genome included the repeat structure (Benson 1999; Timme et al., 2007) and small inversion (Nagano et al., 1991; Yang et al., 2010; Doorduin et al., 2011; Castro et al., 2013; Beier et al., 2017).

2.2. Comparison of cp genome and phylogenetic analysis

The cp genome of *A. obesum* were plotted using the mVISTA (http://genome.lbl.gov/vista/mvista/submit.shtml) program with a total number of nine complete cp genomes of Apocynaceae [i.e. (1) *Asclepias nivea* Forssk., (2) *Carissa macrocarpa* (Eckl.) A. DC., (3) *Catharanthus roseus* (L.) G. Don, (4) *Cynanchum auriculatum* Buch.-Ham. *ex* Wight, (5) *Echites umbellatus* Jacq., (6) *Nerium oleander* L., (7) *Oncinotis tenuiloba* Stapf, (8) *Pentalinon luteum* (L.) B.F. Hansen & Wunderlin, and (9) *Rhazya stricta* Decne.] (Table 1).

The cp sequences of 48 genes [e.g. ATP synthase genes (*atpA, atpB, atpE, atpF, atpH,* and *atpI*), c-type cytochrome synthesis gene (*ccsA*), envelope membrane protein gene (*cemA*), Maturase gene (*matK*), cytochrome b6/f genes (*petA, petB, petD, petG,* and *petN*), Photosystem I genes (*psaA, psaB, psaC,* and *psaJ*), Photosystem II genes (*psbA, psbC, psbE, psbH, psbI, psbJ, psbK, psbN,* and *psbT*), Rubisco gene (*rbcL*), Large-subunit ribosomal protein genes (*rpl14, rpl2, rpl20, rpl32, rpl33,* and *rpl36*), RNA polymerase subunit genes (*rpoB, rpoC1,* and *rpoC2*), Small-subunit ribosomal protein genes *(rps14, rps15, rps18, rps19, rps2, rps3, rps4, rps7,* and *rps8*), Genes of unknown function (*ycf3,* and *ycf4*)] were retrieved from 19 ingroup taxon comprising 10 species of the family Apocynaceae, the representative of the family Apiaceae, Aquifoliaceae, Apocynaceae, Adoxaceae, Eucommiaceae, Gentianaceae, Icacinaceae, Lamiaceae, Solanaceae, and the outgroup from the family Cornaceae (Table 1), and aligned using Clustal X (Thompson et al., 1994), and the molecular phylogenetic analysis was performed by Maximum Evolution method (Rzhetsky and Nei, 1992) using in MEGA X (Kumar et al., 2018).

3. Results and discussion

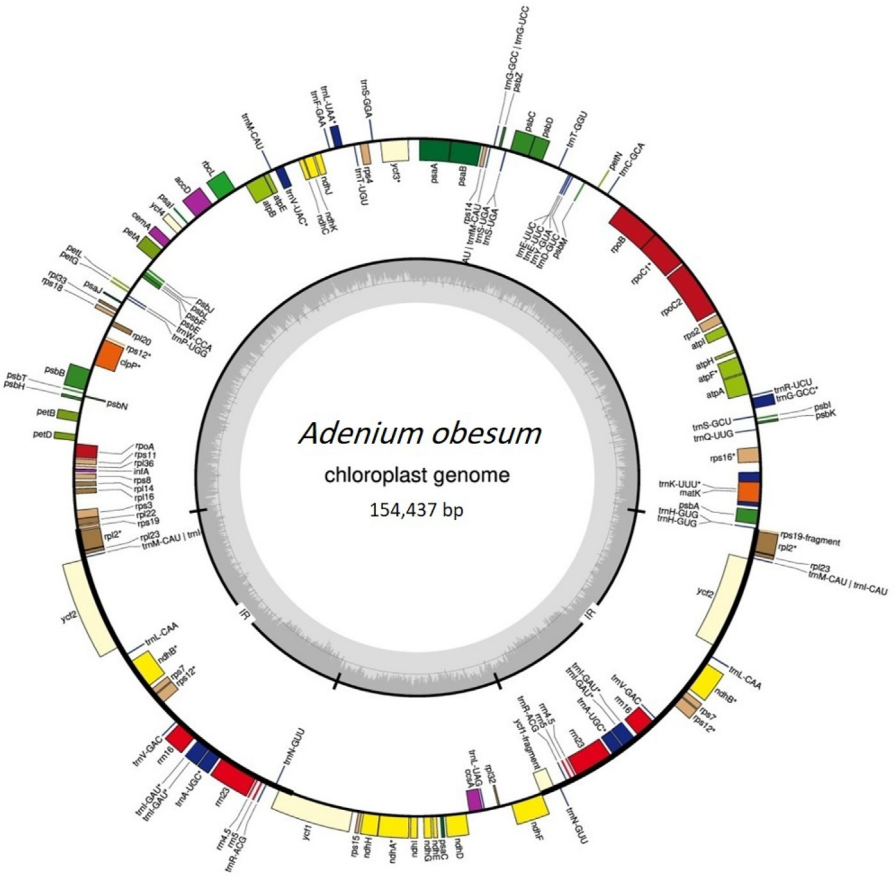
The present study reports assembly of the complete cp genome map as a conserved circular structure comprising a total length of 154,437 bp (including LSC, SSC, IRa, and IRb), with an overall GC content of 38.1% (Fig. 1). The results revealed the gene contents, orientation, and the conservation as well as polymorphisms were found in the chloroplast genome as similar to those of other cp genome of angiosperms (Daniell et al., 2016). A total numner of 127 genes were annotated including 96 protein-coding genes, 28 tRNA genes, and 3 rRNA genes (NCBI GenBank accession number: MN765097).

The sequence identity of *A. obesum* plotted with the nine different complete cp genomes from the family Apocynaceae e.g. *A. nivea, C. macrocarpa, C. roseus, C. auriculatum, E. umbellatus, N. oleander, O. tenuiloba, P. luteum* and *R. stricta* using the mVISTA revealed high similarities amongst them with few regions where the identities was below 90% (Fig. 2).

Moreover, the present study depicted the distribution and location of repeated structures and microsatellites in the cp genome. The microsatellites or simple sequence repeats (SSRs) are tandem repeats which ranges from 1 to 6 bp and are present commonly in cp genomes (Meng et al., 2018). SSRs have been served as an important marker for molecular characterization of plant species. A total of 40 SSRs were predicted in *A. obesum* (Table 2) which were composed of a length of at least 10 bp, all of which were found to be homopolymers containing multiple A or T nucleotides at each locus. These reveal that SSR loci are rich in A–T content in the *A. obesum* cp genome which supports previous chloroplast SSRs

Table 1
The ingroup and outgroup taxon with their classification and GenBank accession number included in the phylogenetic analyses. The GenBank accession number marked with * were included in the mVISTA alignment.

Sl. No.	Taxon	Order	Family	Subfamily	Tribe	Subtribe	GenBank
Ingroup							
1.	*Adenium obesum* (Forssk.) Roem. & Schult.	Gentianales	Apocynaceae	Apocynoideae	Nerieae	Neriinae	MN765097*
2.	*Asclepias nivea* Forssk.	Gentianales	Apocynaceae	Asclepiadoideae	Asclepiadeae	Asclepiadinae	NC_022431.1*
3.	*Cynanchum auriculatum* Buch.-Ham. *ex* Wight	Gentianales	Apocynaceae	Asclepiadoideae	Asclepiadeae	Cynanchinae	NC_029460.1*
4.	*Carissa macrocarpa* (Eckl.) A. DC.	Gentianales	Apocynaceae	Rauvolfioideae	Carisseae		NC_033354.1*
5.	*Catharanthus roseus* (L.) G. Don	Gentianales	Apocynaceae	Rauvolfioideae	Vinceae	Catharanthinae	NC_021423.1*
6.	*Rhazya stricta* Decne.	Gentianales	Apocynaceae	Rauvolfioideae	Amsonieae		NC_024292.1*
7.	*Echites umbellatus* Jacq.	Gentianales	Apocynaceae	Apocynoideae	Echiteae	Echitinae	NC_025655.1*
8.	*Pentalinon luteum* (L.) B.F. Hansen & Wunderlin	Gentianales	Apocynaceae	Apocynoideae	Echiteae	Pentalinoninae	NC_025658.1*
9.	*Nerium oleander* L.	Gentianales	Apocynaceae	Apocynoideae	Nerieae	Neriinae	NC_025656.1*
10.	*Oncinotis tenuiloba* Stapf	Gentianales	Apocynaceae	Apocynoideae	Baisseeae		NC_025657.1*
11.	*Anethum graveolens* L.	Apiales	Apiaceae				NC_029470.1
12.	*Ilex delavayi* Franch.	Aquifoliales	Aquifoliaceae				KX426470.1
13.	*Helianthus annuus* L.	Asterales	Apocynaceae				NC_007977.1
14.	*Viburnum betulifolium* Batalin	Dipsacales	Adoxaceae				NC_037951.1
15.	*Eucommia ulmoides* Oliv.	Garryales	Eucommiaceae				NC_037948.1
16.	*Gentiana tibetica* King *ex* Hook. f.	Gentianales	Gentianaceae				NC_025319.1
17.	*Iodes cirrhosa* Turcz.	Icacinales	Icacinaceae				NC_036254.1
18.	*Premna microphylla* Turcz.	Lamiales	Lamiaceae				NC_026291.1
19.	*Iochroma australe* Griseb.	Solanales	Solanaceae				NC_029833.1
Outgroup							
20.	*Cornus controversa* Hemsl.	Cornales	Cornaceae				MG525004.1



Photosystem I	*psaA,psaB,psaI,psaJ,ycf3,ycf4*
Photosystem II	*psbA, C, D, E, F, H, I, J, K, L, M, N, T*
Cytochrome b6/f	*petA, B, D, G, L, N*
ATP synthase	*atpA, B, E, F , H*
Rubisco	*rbcL*
NADH oxidoreductase	*ndhA, B , C, D, E, F, G, H, I, J, K*
Large subunit ribosomal proteins	*Rpl2,14, 16, 20, 22, 23 c , 32, 33, 36*
Small subunit ribosomal proteins	*Rps2, 3, 4, 7, 8,11,12,14, 15, 16,18,19*
RNAP	*rpoA, rpoB, C1, C2*
Other proteins	*accD, ccsA, cemA, matK, infA*
Proteins of unknown function	*ycf1*
Ribosomal RNAs	*Rrn23,16, 5, 4.5*
Transfer RNAs	*trnA-UGC,trnA-UGC,trnC-GCA,trnD-GUC,trnE-UUC,trnF-GAA,trnfM-CAU,trnG-GCC,trnG-UCC,trnH-GUG,trnI-CAU,trnI-GAU,trnK-UUU,trnL-CAA,trnL-UAA,trnL-UAG,trnM-CAU,trnN-GUU,trnP-UGG,trnQ-UUG,trnR-ACG,trnR-UCU,trnS-GCU,trnS-GGA,trnS-UGA,trnT-GGU,trnT-UGU,trnV-GAC,trnV-UAC,trnW-CCA,trnY-GUA*

Fig. 1. The gene map and genes contained in the cp genome of *A. obesum*.

reports (Li et al., 2017). Among these SSRs, four SSRs were situated in coding regions and 31 were located in the intergenic regions (Table 2). A total number of 19 genes including 11 protein-coding genes and 8 tRNA genes contained one or two introns (Table 3). Furthermore, five SSRs were found in intronic regions. Thus, most of the repeats were situated in the intergenic region. Tandem and dispersed repeats were analyzed for *A. obesum* cp genomes and a total of 25 tandem and 19 dispersed repeats were observed (Fig. 3).

The phylogenetic relationships of a total number of 48 cp genes from the 19 cp genomes including the family Apocynaceae and the representative members of the family Apiaceae (Apiales), Aquifoliaceae (Aquifoliales), Adoxaceae (Dipsacales), Eucommiaceae (Garryales), Gentianaceae (Gentianales), Icacinaceae (Icacinales), Lamiaceae (Lamiales), Solanaceae (Solanales), and the outgroup at the family Cornaceae (Cornales) revealed the proximity of *A. obesum* (Subfamily Apocynoideae, Tribe Nerieae, Subtribe Nerinae) with *Nerium oleander* (Subfamily Apocynoideae, Tribe Ner-

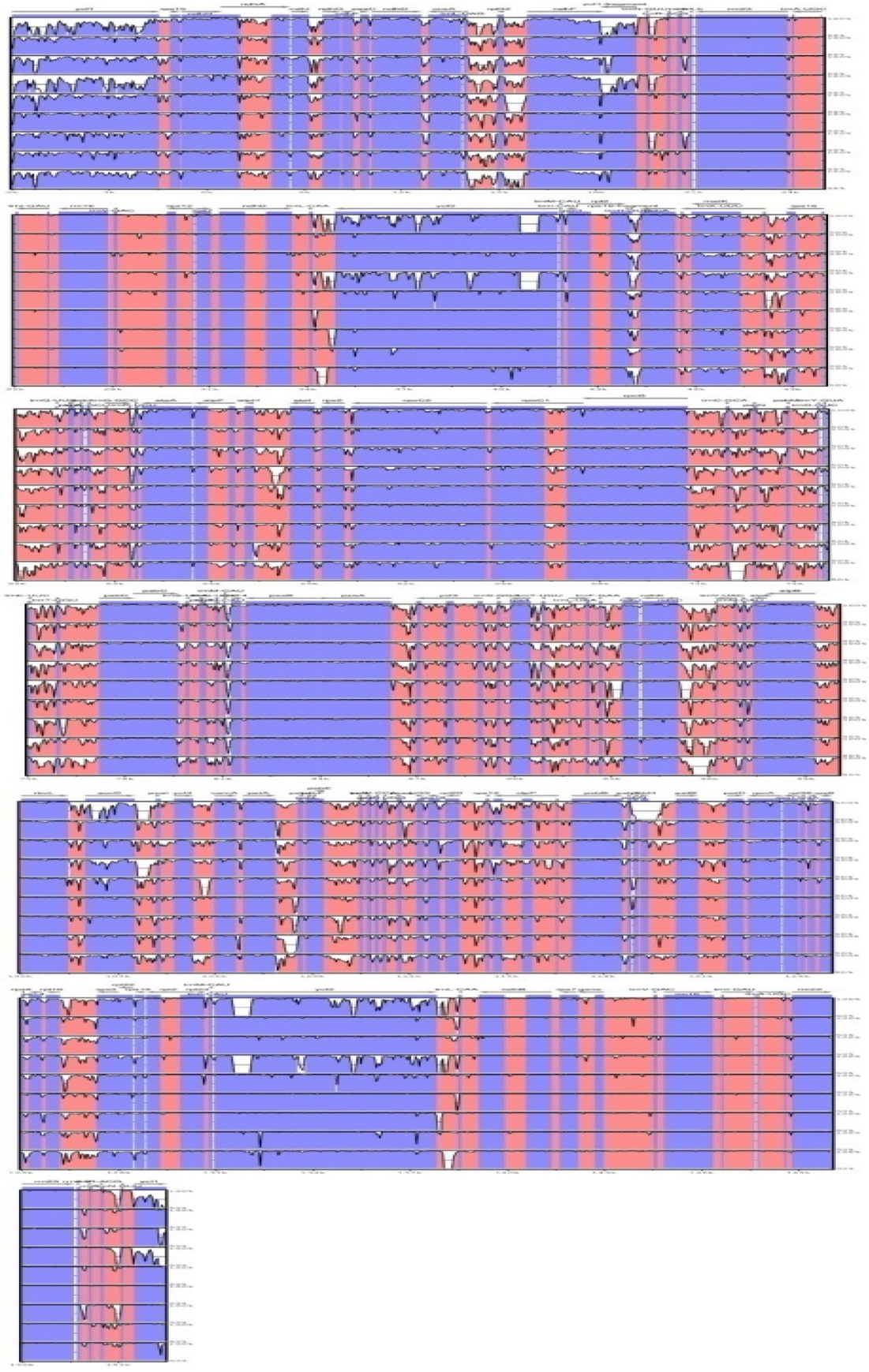


Fig. 2. The percent identity plot for comparison of cp genome of *A. obesum* with the other Apocynaceae genomes. Lane from up to down: *A. nivea, C. macrocarpa, C. roseus, C. auriculatum, E. umbellatus, N. oleander, O. tenuiloba, P. luteum,* and *R. stricta*.

Table 2
The SSR loci in the cp genome of *Adenium obesum*.

Start	End	Repeat	Repeat length of consensus	Locus	Region
2109	2189	(A)10 (A)12	81	*ycf1*	CDS
2914	2925	(A)12	12	*ycf1*	CDS
9557	9566	(T)10	10	*ndhI-ndhG*	intergenic
13,831	13,840	(A)10	10	*ccsA-trnL-UAG*	intergenic
15,378	15,388	(T)11	11	*rpl32-ndhF*	intergenic
15,614	15,624	(A)11	11	*rpl32-ndhF*	intergenic
23,933	23,950	(T)18	18	*rrn23-trnA-UGC*	intergenic
43,878	43,887	(A)10	10	*rpl2-rps19-fragment*	intergenic
49,254	49,266	(T)13	13	*rps16*	intron
52,132	52,141	(A)10	10	*psbI-trnS-GCU*	intergenic
53,347	53,356	(T)10	10	*trnG-GCC*	intron
53,607	53,620	(T)14	14	*trnG-GCC-trnR-UCU*	intergenic
53,763	53,775	(T)13	13	*trnR-UCU-atpA*	intergenic
55,426	55,435	(A)10	10	*atpA-atpF*	intergenic
56,129	56,139	(T)11	11	*atpF*	intron
57,865	57,874	(T)10	10	*atpH-atpI*	intergenic
58,082	58,093	(T)12	12	*atpH-atpI*	intergenic
60,138	60,147	(A)10	10	*rps2-rpoC2*	intergenic
62,367	62,377	(T)11	11	*rpoC2*	CDS
72,576	72,585	(T)10	10	*trnC-GCA-petN*	intergenic
79,736	79,747	(T)12	12	*psbC-trnS-UGA*	intergenic
88,261	88,272	(T)12	12	*ycf3*	intron
95,456	95,465	(T)10	10	*ndhC-trnV-UAC*	intergenic
96,134	96,143	(A)10	10	*ndhC-trnV-UAC*	intergenic
97,206	97,257	(T)12, (T)13	52	*trnM-CAU-atpE*	intergenic
104,329	104,341	(T)13	13	*psaI-ycf4*	intergenic
105,304	105,315	(A)12	12	*ycf4-cemA*	intergenic
105,629	105,642	(T)14	14	*ycf4-cemA*	intergenic
109,707	109,720	(T)14	14	*psbE-petL*	intergenic
109,940	109,949	(A)10	10	*psbE-petL*	intergenic
111,353	111,366	(T)14	14	*trnP-UGG-psaJ*	intergenic
113,000	113,009	(A)10	10	*rps18-rpl20*	intergenic
115,212	115,222	(T)11	11	*clpP*	intron
120,988	120,997	(A)10	10	*petB-petD*	intergenic
122,446	122,455	(A)10	10	*petD-rpoA*	intergenic
125,125	125,136	(T)12	12	*rps8-rpl14*	intergenic
125,645	125,710	(A)10, (T)10	66	*rpl14-rpl16*	intergenic
128,414	128,424	(T)11	11	*rpl22*	CDS
128,795	128,804	(T)10	10	*rps19-rpl2*	intergenic
148,732	148,749	(A)18	18	*trnA-UGC-rrn23*	intergenic

Table 3
The intron containing genes in the cp genome of *Adenium obesum*.

Gene	Location	Exon I bp	Intron I bp	Exon II bp	Intron II bp	Exon III bp
trnA-UGC	IR	35	818	38		
trnI-GAU	IR	35	943	42		
*rps12**	LSC-IR	234	536	25		114
ndhB	IR	777	684	756		
rpl2	IR	391	649	434		
trnK-UUU	LSC	35	2476	37		
rps16	LSC	226	843	41		
trnG-GCC	LSC	23	691	37		
atpF	LSC	411	706	144		
rpoC1	LSC	1613	749	451		
ycf3	LSC	155	773	228	738	124
trnL-UAA	LSC	37	491	50		
trnV-UAC	LSC	37	586	38		
clpP	LSC	229	657	291	746	71
rpl2	IR	434	649	391		
ndhB	IR	756	684	777		
rps12	IR	25	536	234		
trnI-GAU	IR	42	943	35		
trnA-UGC	IR	38	818	35		

**rps12* is *trans*-spliced gene with 5′ end exon located in the LSC region and the duplicated 3′ end exon located in IR regions.

ieae, Subtribe Neriinae) (Fig. 4). The family Apocynaceae is one of the 10 largest angiosperm families with c. 4,500 species under c. 370 genera globally with the greatest diversity in the tropics and subtropics (Stevens, 2001; Endress et al., 2014; APG, 2016). Apart from the large number of molecular phylogenetic studies on the family Apocynaceae (e.g. Liede and Täuber, 2000, 2002; Liede, 2001; Liede and Meve, 2001, 2002; Meve and Liede, 2001, 2002, 2004a,b; Potgieter and Albert, 2001; Liede and Kunze, 2002;

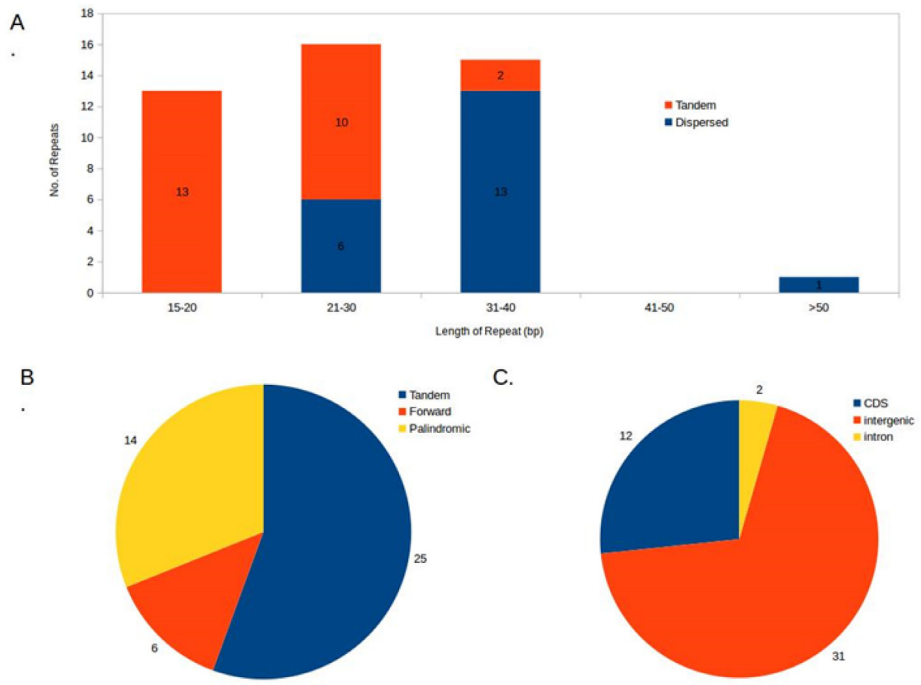


Fig. 3. (A-C). The repeat structure analysis in the cp genome of *A. obesum*. The cutoff value for tandem repeat is 15 bp and 30 bp for dispersed repeat. A. Frequency of repeats by length; B. Repeat type; C. The location distribution of all the repeats.

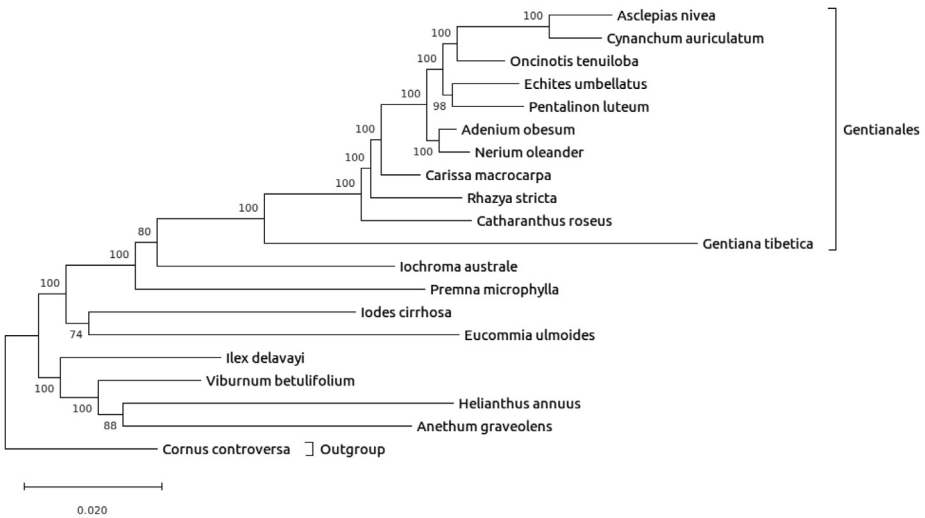


Fig. 4. The maximum likelihood tree inferred from the cp genome of *A. obesum* analyzed together with the members of the family Apocynaceae and Aquifoliaceae, Adoxaceae, Eucommiaceae, Gentianaceae, Icacinaceae, Lamiaceae, and Solanaceae.

Liede et al., 2002a,b; Verhoeven et al., 2003; Rapini et al., 2003, 2004, 2006, 2007; Simões et al., 2004, 2006, 2007; Liede-Schumann et al., 2005; Venter et al., 2006; Endress et al., 2007; Goyder et al., 2007; Ionta and Judd, 2007; Lahaye et al., 2007; Livshultz et al., 2007; Meve and Liede-Schumann, 2007; Wanntorp and Forster, 2007), the family has also been intensely studied for their pollination biology, plant–herbivore interactions, and secondary chemistry (Wyatt and Broyles, 1994; Góngora Castillo et al., 2012; Courdavault et al., 2014; Agrawal et al., 2015). The phylogenetic nesting of the family Asclepiadaceae in Apocynaceae *s.s.* has been demonstrated repeatedly (Wanntorp, 1988; Judd et al., 1994; Sennblad and Bremer, 1996; Potgieter and Albert, 2001). The most recent classification of Apocynaceae (Endress et al., 2014) segregated the family into five subfamilies, two paraphyletic which correspond to the former Apocynaceae *s.s.* (Rauvolfioideae and Apocynoideae) and three monophyletic that relates to the former Asclepiadaceae (Periplocoideae, Secamonoideae, and Asclepiadoideae).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of the research through the research group project #RG-

1438-015. This study was supported by the KRIBB Initiative Program of the Republic of Korea. JL thanks the support from the Chungnam National University, Daejeon, Republic of Korea.

References

Agrawal, A.A., Ali, J.G., Rasmann, S., Fishbein, M., 2015. Macroevolutionary trends in the defense of milkweeds against monarchs: latex, cardenolides, and tolerance of herbivory. In: Oberhauser, K.S., Altizer, S., Nail, K. (Eds.), Monarchs in a changing world: Biology and conservation of an iconic insect. Cornell University Press, Ithaca, NY, USA, pp. 47–59.

Almehdar, H., Abdallah, H.M., Osman, A.M., Abdel-Sattar, E.A., 2012. *In vitro* cytotoxic screening of selected Saudi medicinal plants. J. Nat. Med. 66, 406–412.

APG, IV., 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc. 181, 1–20.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A., Dvorkin, M., Kulikov, A.S., Lesin, V., Nikolenko, S., Pham, S., Prjibelski, A., Pyshkin, A., Sirotkin, A., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (5), 455–477.

Beier, S., Thiel, T., Münch, T., Scholz, U., Mascher, M., 2017. MISA-web: A web server for microsatellite prediction. Bioinform. 33 (16), 2583–2585.

Benson, G., 1999. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 27 (2), 573–580.

Caboche, S., Audebert, C., Lemoine, Y., Hot, D., 2014. Comparison of mapping algorithms used in high-throughput sequencing: application to Ion Torrent data. BMC Genom. 15, 264.

Castro, I., Pinto-Carnide, O., Ortiz, J.M., Martin, J.P., 2013. Chloroplast genome diversity in Portuguese grapevine (*Vitis vinifera* L.) cultivars. Mol. Biotechnol. 54 (2), 528–540.

Courdavault, V.N., Papon, M., Clastre, N., Giglioli Guivarc'h, B., St Pierre, V., Burlat., 2014. A look inside an alkaloid multisite plant: the Catharanthus logistics. Current Opinion in Plant Biol. 19, 43–50.

Daniell, H., Lin, C.S., Yu, M., Chang, W.J., 2016. Chloroplast genomes: diversity, evolution, and applications in genetic engineering. Genome Biol. 17 (1), 134.

Dimmitt, M.A., Hanson, C., 2002. The genus *Adenium* in cultivation. Diplorhynchus Welw Fic & Hiern (Apocynaceae). Mededelingen. Cactus Success J. 63, 223–225.

Doorduin, L., Gravendeel, B., Lammers, Y., Ariyurek, Y., Chin, A.W.T., Vrieling, K., 2011. The complete chloroplast genome of 17 individuals of pest species *Jacobaea vulgaris*: SNPs, microsatellites and barcoding markers for population and phylogenetic studies. DNA Res. 18, 93–105.

Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., Peluso, P., Rank, D., Baybayan, P., Bettman, B., Bibillo, A., Bjornson, K., Chaudhuri, B., Christians, F., Cicero, R., Clark, S., Dalal, R., Dewinter, A., Dixon, J., Foquet, M., Gaertner, A., Hardenbol, P., Heiner, C., Hester, K., Holden, D., Kearns, G., Kong, X., Kuse, R., Lacroix, Y., Lin, S., Lundquist, P., Ma, C., Marks, P., Maxham, M., Murphy, D., Park, I., Pham, T., Phillips, M., Roy, J., Sebra, R., Shen, G., Sorenson, J., Tomaney, A., Travers, K., Trulson, M., Vieceli, J., Wegener, J., Wu, D., Yang, A., Zaccarin, D., Zhao, P., Zhong, F., Korlach, J., Turner, S., 2009. Real-time DNA sequencing from single polymerase molecules. Science 323 (5910), 133–138.

Endress, M.E., Liede-Schumann, S., Meve, U., 2014. An updated classification for Apocynaceae. Phytotaxa 159, 175–194.

Endress, M.E., van der Ham, R.W.J.M., Nilsson, S., Civeyrel, L., Chase, M.W., Sennblad, B., Potgieter, K., Joseph, J., Powell, M., Lorence, D., Zimmerman, Y.-M., Albert, V. A., 2007. A phylogenetic analysis of Alyxieae (Apocynaceae) based on rbcL, matK, trnL intron, trnL-F spacer sequences, and morphological characters. Ann. Miss. Bot. Gard. 94, 1–35.

Góngora Castillo, E., Childs, K.L., Fedewa, G., Hamilton, J.P., Liscombe, D.K., Magallanes Lundback, M., Mandadi, K.K., Nims, E., Runguphan, W., Vaillancourt, B., Varbanova-Herde, M., DellaPenna, D., McKnight, T.D., O'Connor, S., Robin Buell, C., 2012. Development of transcriptomic resources for interrogating the biosynthesis of monoterpene indole alkaloids in medicinal plant species. PLoS ONE 7, e52506.

Goyder, D.J., Nicholas, A., Liede-Schumann, S., 2007. Phylogenetic relationships in subtribe Asclepiadinae (Apocynaceae: Asclepiadoideae). Ann. Miss. Bot. Gard. 94, 423–434.

Gurung, A.B., Ali, M.A., Lee, J., Al-Hemaid, F., Farah, M.A., Al-Anazi, K.M., 2020. Molecular docking elucidates the plausible mechanisms underlying the anticancer properties of acetyldigitoxigenin from *Adenium obesum*. Saudi J. Biological Sciences 27 (7), 1907–1911.

Hansen, D.R., Dastidar, S.G., Cai, Z., Penaflor, C., Kuehl, J.V., Boore, J.L., Jansen, R.K., 2007. Phylogenetic and evolutionary implications of complete chloroplast genome sequences of four early-diverging angiosperms: *Buxus* (Buxaceae), *Chloranthus* (Chloranthaceae), *Dioscorea* (Dioscoreaceae), and *Illicium* (Schisandraceae). Mol. Phylogen. Evol. 45, 547–563.

Hossain, M.A., Akhtar, S.M., Sadri, S.A., 2017. Two new flavonoids from *Adenium obesum* grown in Oman. J. King Saud Univ. Sci. 29, 62–69.

Huang, W., Li, L., Myers, J.R., Marth, G.T., 2012. ART: a next-generation sequencing read simulator. Bioinformatics 28, 593–594.

Ionta, G.M., Judd, W.S., 2007. Phylogenetic relationships in Periplocoideae (Apocynaceae s. l.) and insights into the origin of pollinia. Ann. Miss. Bot. Gard. 94, 360–375.

Jain, M., Olsen, H.E., Paten, B., Akeson, M., 2016. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. Genome Biol. 17, 239.

Judd, W.S., Sanders, R.W., Donoghue, M.J., 1994. Angiosperm family pairs: preliminary phylogenetic analyses. Harvard Papers Bot. 5, 1–51.

Kiyohara, H., Ichino, C., Kawamura, Y., Nagai, T., Sato, N., Yamada, H., Salama, M.M., Abdel-Sattar, E., 2012. *In vitro* anti-influenza virus activity of a cardiotonic glycoside from *Adenium obesum* (Forssk.). Phytomed. 19, 111–114.

Knudsen, B., Forsberg, R., Miyamoto, M.M., 2010. A computer simulator for assessing different challenges and strategies of *de novo* sequence assembly. Genes 1 (2), 263–282.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547–1549.

Kwon, T., Yoo, W.G., Lee, W.-J., Kim, W., Kim, D.-W., 2015. Next-generation sequencing data analysis on cloud computing. Genes Genom. 37, 489–501.

Lahaye, R., Klackenberg, J., Källersjö, M., van Campo, E., Civeyrel, L., 2007. Phylogenetic relationships between derived Apocynaceae *s.l.* and within Secamonoideae based on four chloroplast sequences. Ann. Miss. Bot. Gard. 94, 376–391.

Langmead, B., Nellore, A., 2018. Cloud computing for genomic data analysis and collaboration. Nat. Rev. Genet. 19 (4), 208–219.

Li, B., Lin, F., Huang, P., Guo, W., Zheng, Y., 2017. Complete chloroplast genome sequence of *Decaisnea insignis*: Genome organization, genomic resources and comparative analysis. Scientific Rep. 7, 10073.

Liede, S., 2001. Subtribe Astephaninae (Apocynaceae–Asclepiadoideae) reconsidered: new evidence based on cpDNA spacers. Ann. Miss. Bot. Gard. 88, 657–668.

Liede, S., Kunze, H., 2002. *Cynanchum* and the Cynanchinae (Apocynaceae – Asclepiadoideae) – a molecular, anatomical and latex triterpenoid study. Organisms Diversity & Evol. 2, 239–269.

Liede, S., Meve, U., 2001. New combinations and new names in Malagasy Asclepiadoideae (Apocynaceae). Adansonia, sér.3 23, 347–351.

Liede, S., Meve, U., 2002. Dissolution of *Cynanchum* sect. Macbridea (Apocynaceae – Asclepiadoideae). Nordic J. Bot. 22, 1–13.

Liede, S., Täuber, A., 2000. *Sarcostemma* R. Br. (Apocynaceae–Asclepiadoideae) – a controversial generic circumscription reconsidered: evidence from trnL-F spacers. Plant Syst. Evol. 225, 133–140.

Liede, S., Täuber, A., 2002. Circumscription of the genus *Cynanchum* (Apocynaceae Asclepiadoideae). Syst. Bot. 27, 789–800.

Liede, S., Meve, U., Täuber, A., 2002a. What is the subtribe Glossonematinae (Apocynaceae: Asclepiadoideae)? A phylogenetic study based on cpDNA spacer. Bot. J. Linn. Soc. 139, 145–158.

Liede, S., Täuber, A., Schneidt, J., 2002b. Molecular considerations on the Tylophorinae K. Schum. (Apocynaceae-Asclepiadoideae). Edinburgh J. Bot. 59, 377–403.

Liede-Schumann, S., Rapini, A., Goyder, D.J., Chase, M.W., 2005. Phylogenetics of the New World subtribes of Asclepiadeae (Apocynaceae–Asclepiadoideae): Metastelmatinae, Oxypetalinae, and Gonolobinae. Syst. Bot. 30, 184–195.

Livshultz, T., Middleton, D.J., Endress, M.E., Williams., J.K., 2007. Phylogeny of Apocynoideae and the APSA clade (Apocynaceae *s.l.*). Ann. Miss. Bot. Gard. 94, 324–359.

Mavromatis, K., Ivanova, N., Barry, K., Shapiro, H., Goltsman, E., McHardy, A.C., Rigoutsos, I., Salamov, A., Korzeniewski, F., Land, M., Lapidus, A., Grigoriev, I., Hugenholtz, P., Kyrpides, N.C., 2007. Use of simulated data sets to evaluate the fidelity of metagenomic processing methods. Natural Methods 4, 495–500.

McElroy, K.E., Luciani, F., Thomas, T., 2012. GemSIM: general, error-model based simulator of next-generation sequencing data. BMC Genom. 13, 74.

Meng, J., Li, X., Li, H., Yang, J., Wang, H., He, J., 2018. Comparative analysis of the complete chloroplast genomes of four *Aconitum* medicinal species. Molecules 23, 1015.

Meve, U., Liede, S., 2001. Reconsideration of the status of *Lavrania*, *Larryleachia* and *Notechidnopsis* (Asclepiadoideae-Ceropegieae). S. African J. Bot. 67, 161–168.

Meve, U., Liede, S., 2002. Floristic exchange between mainland Africa and Madagascar: A case study of Apocynaceae-Asclepiadoideae. J. Biogeo. 29, 865–873.

Meve, U., Liede, S., 2004a. Generic delimitations in tuberous Periplocoideae (Apocynaceae) from Africa and Madagascar. Annals Bot. 93, 407–414.

Meve, U., Liede, S., 2004b. Subtribal division of Ceropegieae (Apocynaceae—Asclepiadoideae). Taxon 53, 61–72.

Meve, U., Liede-Schumann, S., 2007. *Ceropegia* (Apocynaceae, Ceropegieae, Stapeliinae): paraphyletic, but still taxonomically sound. Ann. Miss. Bot. Gard. 94, 392–406.

Mouza, K.G., Hossain, M.A., 2015. Determination of total phenolics, flavonoids and antioxidant activity of root crude extracts of *Adenium obesum* traditionally used for the treatment of bone dislocations and rheumatism. Asian Pac. J. Trop. Dis. 5 (Suppl. 1), S155–S158.

Nagano, Y., Ishikawa, H., Matsuno, R., Sasaki, Y., 1991. Nucleotide sequence and expression of the ribosomal protein L2 gene in pea chloroplasts. Plant Mol. Biol. 17, 541–545.

Nie, X., Lv, S., Zhang, Y., Du, X., Wang, L., Biradar, S.S., Tan, X., Wan, F., Weining, S., 2012. Complete chloroplast genome sequence of a major invasive species, crofton weed (Ageratina adenophora). PLoS One 7: e36869.

Oyen, L.P.A., 2008. Adenium obesum (Forssk.) Roem. & Schult. In: Schmelzer, G.H., Gurib-Fakim, A., (Eds.), Plant Resources of Tropical Africa, Backhuys. Wageningen. 11(1), Medicinal Plants 1.

Pattnaik, S., Gupta, S., Rao, A.A., Panda, B., 2014. SInC: an accurate and fast error-model based simulator for SNPs, Indels and CNVs coupled with a read generator for short-read sequence data. BMC Bioinf. 15, 1–9.
Potgieter, K., Albert, V.A., 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. Ann. Miss. Bot. Gard. 88, 523–549.
Rapini, A., Chase, M.W., Goyder, D.J., Griffiths, J., 2003. Asclepiadeae classification: evaluating the phylogenetic relationships of New World Asclepiadoideae (Apocynaceae). Taxon 52, 33–50.
Rapini, A., Fontella-Pereira, J., Lamare, E., Liede-Schumann, S., 2004. Taxonomy of *Peplonia* (including *Gonioanthela*) and a reinterpretation of Orthosieae (Asclepiadoideae, Apocynaceae). Kew Bull. 59, 531–539.
Rapini, A., Chase, M.W., Konno, T.U.P., 2006. Phylogenetics of South American Asclepiadeae (Apocynaceae). Taxon 55, 119–124.
Rapini, A., van den Berg, C., Liede-Schumann, S., 2007. Diversification of Asclepiadoideae (Apocynaceae) in the New World. Ann. Miss. Bot. Gard. 94, 407–422.
Rothberg, J.M., Hinz, W., Rearick, T.M., Schultz, J., Mileski, W., Davey, M., Leamon, J. H., Johnson, K., Milgrew, M.J., Edwards, M., Hoon, J., Simons, J.F., Marran, D., Myers, J.W., Davidson, J.F., Branting, A., Nobile, J.R., Puc, B.P., Light, D., Clark, T.A., Huber, M., Branciforte, J.T., Stoner, I.B., Cawley, S.E., Lyons, M., Fu, Y., Homer, N., Sedova, M., Miao, X., Reed, B., Sabina, J., Feierstein, E., Schorn, M., Alanjary, M., Dimalanta, E., Dressman, D., Kasinskas, R., Sokolsky, T., Fidanza, J.A., Namsaraev, E., McKernan, K.J., Williams, A., Roth, G.T., Bustillo, J., 2011. An integrated semiconductor device enabling non-optical genome sequencing. Nature 475, 348–352.
Rzhetsky, A., Nei, M., 1992. A simple method for estimating and testing minimum evolution trees. Mol. Biol. Evol. 9, 945–967.
Sennblad, B., Bremer, B., 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Plant Syst. Evol. 202, 153–175.
Shcherbina, A., 2014. FASTQSim: platform-independent data characterization and in silico read generation for NGS datasets. BMC Res. Notes 7, 533.
Shendure, J., Aiden, E.L., 2012. The expanding scope of DNA sequencing. Nature Biotech. 30, 1084–1994.
Shendure, J., Balasubramanian, S., Church, G.M., Gilbert, W., Rogers, J., Schloss, J.A., Waterston, R.H., 2017. DNA sequencing at 40: Past, present and future. Nature 550, 345–353.
Simões, A.O., Endress, M.E., van der Niet, T., Conti, E., Kinoshita, L.S., 2004. Tribal and intergeneric relationships of Mesechiteae (Apocynoideae, Apocynaceae): evidence from three noncoding plastid DNA regions and morphology. American J. Bot. 91, 1409–1418.
Simões, A.O., Endress, M.E., van der Niet, T., Kinoshita, L.S., Conti, E., 2006. Is *Mandevilla* (Apocynaceae, Mesechiteae) monophyletic? Evidence from five plastid DNA loci and morphology. American J. Bot. 94, 1409–1418.
Simões, A.O., Livshultz, T., Conti, E., Endress, M.E., 2007. Phylogeny and systematics of the Rauvolfioideae (Apocynaceae) based on molecular and morphological evidence. Ann. Miss. Bot. Gard. 94, 268–297.
Spök, A., Karner, S., Stein, A.J., Rodríguez, C.E., 2008. Plant molecular farming; opportunities and challenges. JRC Scientific Technical Reports.
Stevens, P.F., 2001 onwards. Angiosperm phylogeny website, version 12. http://www.mobot.org/MOBOT/research/APweb/.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Tillich, M., Lehwark, P., Pellizzer, T., Ulbricht-Jones, E.S., Fischer, A., Bock, R., Greiner, S., 2017. GeSeq – versatile and accurate annotation of organelle genomes. Nucleic Acids Res. 45, W6–W11.
Timme, R.E., Kuehl, J.V., Boore, J.L., Jansen, R.K., 2007. A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: Identification of divergent regions and categorization of shared repeats. American J. Bot. 94, 302–312.
Venter, H.J.T., Dold, A.P., Verhoeven, R.L., Ionta, G., 2006. *Kappia lobulata* (Apocynaceae, Periplocoideae), a new genus from South Africa. South African J. Bot. 72, 529–533.
Verhoeven, R.L., Liede, S., Endress, M.E., 2003. The tribal position of *Fockea* and *Cibirhiza* (Apocynaceae: Asclepiadoideae): evidence from pollinium structure and cpDNA sequence data. Grana 42, 70–81.
Versiani, M.A., Ahmed, S.K., Ikram, A., Ali, S.T., Yasmeen, K., Faizi, S., 2014. Chemical constituents and biological activities of *Adenium obesum* (Forsk.). Roem. et Schult. Chem Biodivers. 11 (2), 171–180.
Wanntorp, H.E., 1988. The genus *Microloma* (Asclepiadaceae). Opera Botanica 98, 1–69.
Wanntorp, L., Forster, P.I., 2007. Phylogenetic relationships between *Hoya* and the monotypic genera *Madangia*, *Absolmsia*, and *Micholitzia* (Apocynaceae, Marsdenieae): Insights from flower morphology. Ann. Miss. Bot. Gard. 94, 36–55.
Wiseman, J., 2009. SAS Survival Handbook (Revised Edition). William Morrow Paperbacks, pp. 240.
Wyatt, R., Broyles., S.B., 1994. Ecology and evolution of reproduction in milkweeds. Ann. Rev. Ecol. & Syst. 25, 423– 441.
Yang, M., Zhang, X., Liu, G., Yin, Y., Chen, K., Yun, Q., Zhao, D., Al-Mssallem, I.S., Yu, J., 2010. The complete chloroplast genome sequence of date palm (Phoenix dactylifera L.). PLoS One 5: e12762.
Yang, Z., Rannala, B., 2012. Molecular phylogenetics: Principles and practice. Nat. Rev. Genet. 13 (5), 303–314.
Zhang, J., Khan, S.A., Hasse, C., Ruf, S., Heckel, D.G., Bock, R., 2015. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. Science 347, 991–994.