

The complete plastome of the South African species, *Amaryllis belladonna* L. (Amaryllidaceae)

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ABSTRACT

The complete plastome sequence of *Amaryllis belladonna* L. is assembled and annotated. This is the type species of *Amaryllis* and therefore the type of the family Amaryllidaceae and as such, is important to document the phylogenetic position of the family. The plastome sequence has a length of 158,145 bp, with the large single copy (LSC) regions comprising 85,963 bp, the small single copy (SSC) 18634 bp and two identical inverted repeats (IR) regions each of 26,774 bp. Phylogenetic analysis fully resolved *Amaryllis* in a clade with *Crinum* L. in the Amaryllidoideae, as expected, with the Allioideae as a sister group. *Agapanthus* (Agapanthoideae) is a sister to the other two subfamilies in the Amaryllidaceae. The phylogenetic tree produced corresponds to previous topologies based on plastome molecular markers including *matK*, *ndhF* and *rbcl*. This is the first paper reporting the whole plastome comparison of the type genera of all three subfamilies in the Amaryllidaceae.

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The asparagalean family Amaryllidaceae J. St.-Hil. consists of three subfamilies: Agapanthoideae Endl. (one genus with nine species), Allioideae Herb. (13 genera with 795 species) and Amaryllidoideae (75 genera with ~900 species; Meerow et al. 2020). The family has a cosmopolitan distribution, with centers of diversity for the Amaryllidoideae in the Mediterranean Basin, South Africa, and South America (Rønsted et al. 2012). The relationship between the subfamilies: (Agapanthoideae, Allioideae, Amaryllidoideae) is well-established based on plastid DNA data (Fay et al. 2000; Pires et al. 2006; Seberg et al. 2012; Givnish et al. 2018), however, the relationship between genera within the Amaryllidoideae remains elusive (Meerow et al. 2003; Meerow et al. 2006; Garcia et al. 2014). *Amaryllis belladonna* is the type species of the genus *Amaryllis* L. and consequently the type of the family Amaryllidaceae. *Amaryllis* comprises two species, both native to the Cape Province in South Africa. *Amaryllis belladonna*, also known as the 'belladonna lily', is a widely planted ornamental of economic importance (Tallini et al. 2017) and may have antiplasmodial uses (Cho et al. 2018). *Amaryllis paradisicola* Snijman is a rare species from Richtersveld National Park (Snijman and Williamson 1998) comprising, possibly, around 2000 individuals (Snijman et al. 2016). Here we report the complete plastome sequence of *A. belladonna* to contribute to the bioinformatic and systematic knowledge of the Amaryllidaceae.

Scape tissue was collected in silica gel from *A. belladonna* blooming at the Royal Horticultural Society's Garden, Wisley,

UK (51.312695° N, 0.476724° W). A herbarium voucher was deposited at WSY (WSY0150055; contact: Yvette Harvey, YvetteHarvey@rhs.org.uk). Total genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit (QIAGEN, Manchester, UK). Library development and Illumina HiSeq 150 bp PE sequencing were completed by Novogene Company Limited (Cambridge, UK).

Fast-Plast v1.2.8 (McKain and Wilson 2017) and NovoPlasty v3.7.0 (Dierckxsens et al. 2017) were used to assemble the plastome. The Bowtie reference index was built using Asparagales plastomes included in Fast-Plast. The complete plastome of *Narcissus poeticus* L. (MH706763; Könyves et al. 2018) was used as the starting seed for the NovoPlasty assembly. The memory was limited to 6Gb. The complete plastome was annotated against the published *Narcissus poeticus* L. plastome (MH706763; Könyves et al. 2018) using Geneious Prime (v2020.2.5; <https://www.geneious.com/>). The *A. belladonna* plastome sequence was aligned to 20 published Asparagales plastome sequences including *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. (MN824434, Asparagaceae; Garnett et al. 2020) and *Xanthorrhoea preissii* Endl. (KX822774, Asphodelaceae) as outgroups using the default settings with MAFFT v7.450 (Katoh et al. 2002; Katoh and Standley 2013) in Geneious Prime. Poorly aligned regions of the total alignment were excluded using trimAl v1.2 with default settings and converted to a FASTA file using readAl v1.2 (Capella-Gutiérrez et al. 2009). A maximum likelihood estimation was performed with RAxML v8.2.11 (Stamatakis

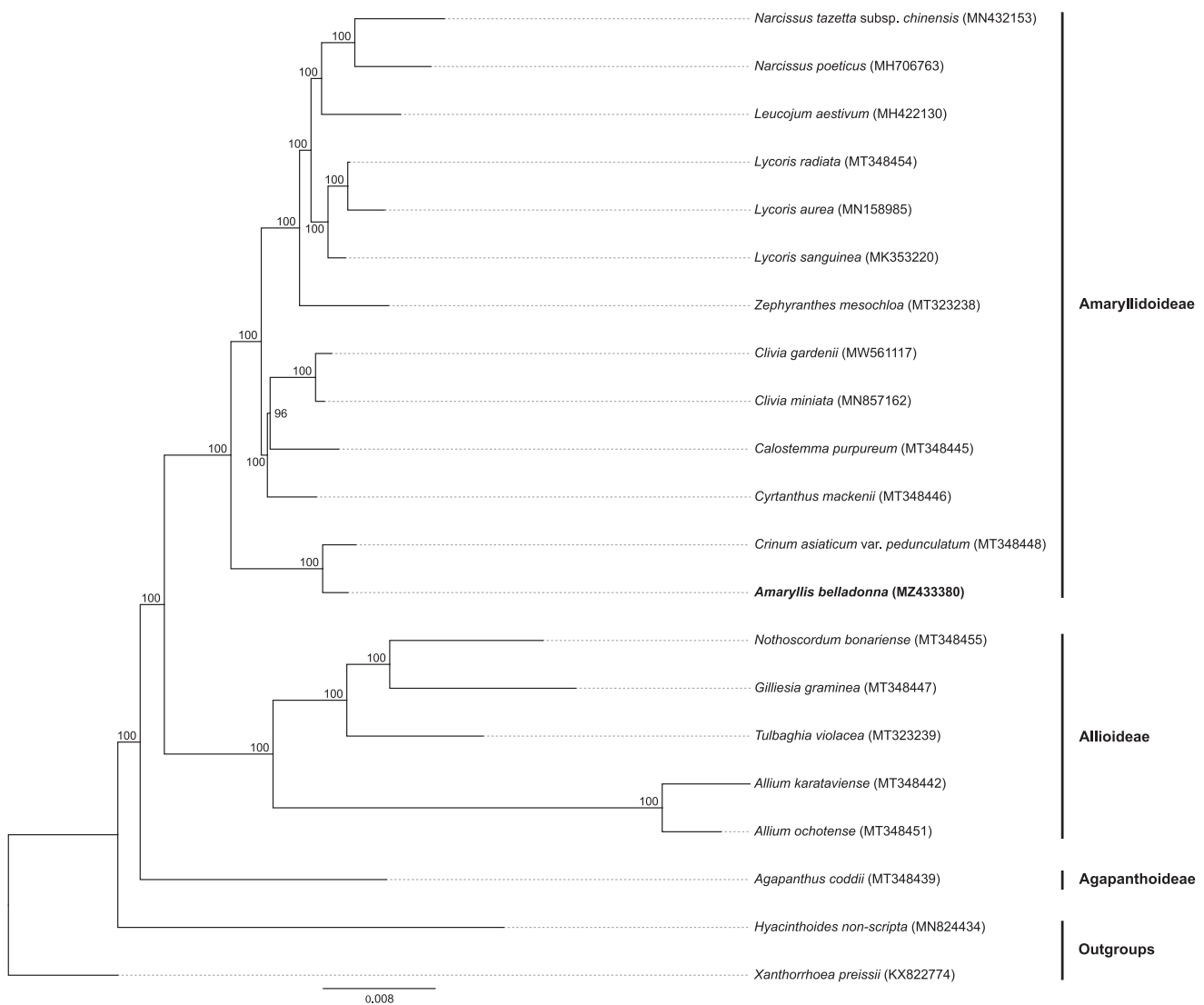


Figure 1. RaxML phylogenetic tree based on 21 asparagalean complete plastome sequences. Bootstrap support values are shown at each branch and GenBank accession numbers are given in brackets, subfamilies of the samples are shown on the right. Text in bold shows the plastome assembled in this study.

2014) in Geneious Prime using the model GTR+G+I and 1000 bootstrap replicates.

The plastome sequence of *A. belladonna* (MZ433380) is 158,145 bp in length, comprising the LSC (85,963 bp), SSC (18,634 bp) and two inverted repeat regions each 26,774 bp. The plastome contains 86 protein-coding, 38 tRNA and eight rRNA genes. Of these eight protein-coding genes, eight tRNA genes and four rRNA genes are duplicated in the inverted repeats. There were high levels of gene synteny between *A. belladonna* and other published Amaryllidoideae plastomes (Könyves et al. 2018; Li et al. 2018; Zhang et al. 2020). The phylogenetic tree produced corresponds to previous topologies based on plastome molecular markers including *matK* (Ito et al. 1999; Rønsted et al. 2012; Chen et al. 2013), *ndhF* and *rbcl* (Chen et al. 2013). Comparison within Amaryllidoideae samples confirms that this subfamily has a lower substitution rate than Allioideae.

The position of *A. belladonna* in the phylogenetic tree is congruent with previous papers (Ito et al. 1999; Rønsted et al. 2012; Chen et al. 2013) and the substitution rate

modeled on the tree is similar to that for other Amaryllidoideae, and lower than that in Allioideae (Figure 1). As the type species of the family Amaryllidaceae and the subfamily Amaryllidoideae, this is a new and important reference in the plastome-based phylogeny of the group.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The annotated plastome sequence is available on GenBank on NCBI (<https://www.ncbi.nlm.nih.gov>) under accession number MZ433380. Raw sequence data were deposited in SRA (BioProject: PRJNA743246); SRA: SRR15031247; BioSample: SAMN20014353).

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