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PLASTOME REPORT

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Complete plastome sequence of *Narcissus pseudonarcissus* L., one of the most iconic European plants

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ABSTRACT

Narcissus pseudonarcissus L. is one of the most iconic plants of the European flora. It is a species of great horticultural interest, but also an endangered and protected plant in the wild as a consequence of loss of natural habitats. Complete plastid genome was assembled from next-generation sequencing data obtaining a circular genome of 160,008 bp long assembly. It comprises a pair of inverted repeat regions, a large single-copy region (108,400 bp), and a small single-copy region (16,434 bp). It encodes 131 genes, including 87 protein coding genes, 37 tRNA genes and seven rRNA genes. Phylogeny showed the strict relationship between *N. pseudonarcissus* and *Narcissus poeticus* L. The complete plastome will provide a useful genetic resource for future conservation programmes, phylogenetic studies and horticultural applications.

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Introduction

The genus *Narcissus* L. first published in Sp. Pl. (1753) in the family Amaryllidaceae J.St.-Hil. (1805), contains 125 species. The genus originated in the Palearctic, spread and differentiated in the Western Mediterranean Region (Blanchard 1990; Mathew 2002). It is currently one of the most traded bulbous plants, with more than 27,000 cultivar names registered (Hanks 2002; Kington 2008). Due to the popularity of the flowers of this genus and facility of propagation, it is now present also outside its native range of distribution, usually as cultivated or naturalized species (Fernandes 1968; Blanchard 1990; Mathew 2002).

Narcissus pseudonarcissus L. (1753) is native to the W-Europe flora. Known worldwide as trumpet daffodils for the shape of the flower corona, the species is of major horticultural interest within the genus (Colling et al. 2010), moreover it has a long history of traditional use in cosmetics and skincare (Boshra et al. 2023).

Despite the great importance, currently there is not a clear phylogenetic framework of the species, for several different factors such as natural hybridization, high phenotypic variability and possible naturalization of some cultivars (Fernandes 1968; Blanchard 1990; Mathew 2002). The current most updated and comprehensive phylogeny of the genus based on nuclear (ITS), plastidial (ndhF and matK) and mitochondrial (cob and atpA) regions has shown that *N. pseudonarcissus* sequences are part of a well-supported and species rich clade including taxa from subsection *Pseudonarcissi*, subsect. *Narcissus* and from section *Ganymedes* (Marques et al. 2017).

Due to habitat loss, *Narcissus* populations are at risk of genetic drift and size reduction; moreover the risk of hybridizations between wild plants and naturalized cultivars is also high. Wild *N. pseudonarcissus* populations, especially at Southern limits of distribution, still consist of several single individuals of reduced height (Figure 1). Reproduction by seeds is essential for the maintenance of the population size and its overall genetic diversity (Colling et al. 2010). A reference genome for the genus is a powerful tool to ascertain the genetic identity of *N. pseudonarcissus* and to assess whether it is native, naturalized or cultivated.

This paper provides basic genetic data to deepen the study of *N. pseudonarcissus* phylogenetic relationships with related species, subspecies and hybrids.

Materials and methods

Fresh leaves of *N. pseudonarcissus* were collected from a single plant in a wild population at Mt. Carmo di Loano, Italy (44.17728, 8.187712 – WGS84). A specimen was deposited at Herbarium of Turin University (https://www.dbios.unito.it/do/home.pl/View?doc=erbario.html, Laura Guglielmone, laura.guglielmone@unito.it) under the voucher number HG – 3574. Leaves sampled for DNA extraction were dehydrated using

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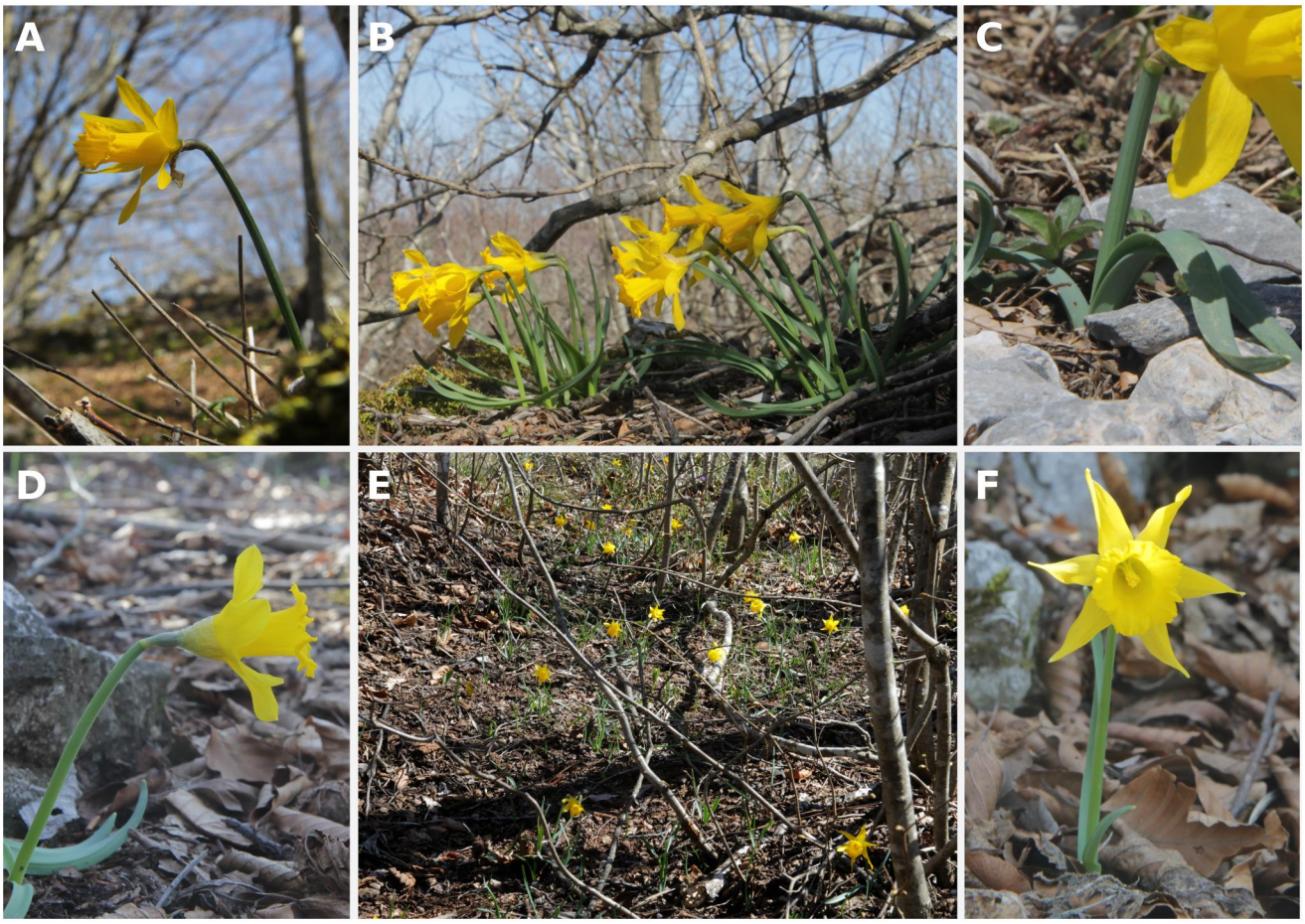

Figure 1. The morphological characteristics of *N. pseudonarcissus*. (A) a single plant, (B) A group of individuals in the wild, (C) Basal leaves, (D) Flower (side view), (E) Habitat, (F) Flower (front view). Photographs were shooted by marco mucciarelli.

silica-gel as described in Chase and Hills (1991). DNA was extracted by using the Macherey-Nagel Plant II kit (Dueren, Germany), following manufacturer's suggestions. Purified DNA was then sequenced by Macrogen (Amsterdam, The Netherlands) using an Illumina TruSeq Nano DNA kit (350 bp insert) on an Illumina HiSeq machine, obtaining 2×150 paired end libraries. Raw libraries were used as input in the organelle assembler NOVOplasty v3.4.3 (Dierckxsens et al. 2016) using the default settings. *Narcissus poeticus* plastome (NC_039825.1) was employed as a reference genome. Sequence of aRblc gene (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) from *Narcissus poeticus* L. (1753) was used as seed sequence. The resultant genome was annotated by Chloe (https://chloe.plastid.org/). The cis-splicing genes, and trans-splicing genes were generated using CPGview (Liu et al. 2023). We reconstructed the phylogenetic position of *N. pseudonarcissus* using available *Narcissus* spp. plastomes and other sequences from the sister genera using the phylogeny from Könyves et al., (2018). Phylogeny was estimated by maximum likelihood (ML) using RAxML (Stamatakis 2008) with 1000 bootstraps. A bayesian approach was also employed using MrBayes (Ronquist et al. 2012) under the GTR + GAMMA + I substitution model calculated with JModelTest2 (Darriba et al. 2012). Comparable positions were selected by means of the gBlock tool (Castresana 2010).

Results

We obtained 10,001,038 raw 150 bp reads (966 Mb) that we did not clean by quality as suggested by the NOVOplasty developers (Dierckxsens et al. 2016) developers; the circular genome of *N. pseudonarcissus* was 160,008 bp including a pair of inverted repeat regions (28,580 bp and 9495 bp) (Figure 2), a large single-copy region (108,400 bp), and a small single-copy region (16,434 bp). The overall G + C content was 49.9% (30.9% A, 19.2% C, 18.6% G, 31.3% T). The plastome encodes 131 genes including 87 protein coding genes, 37 tRNA genes and seven rRNA genes. Fifteen genes (*ndhB*, *petB*, *petD*, *rpl2*, *rps12B*, *atpF*, *ndhB*, *clpP1*, *ndhA*, *pafI*, *rpl16m*, *rpl2*, *rpoC1*, *rps12B* and *rps16*) occur as cys-splicing genes (Supplemental figure 1), while only *sps12* occurs as trans-splicing gene. *N. pseudonarcissus* plastome contains three unique exons, two of which are duplicated in the IR regions.

Most of the genes occurred in a single copy and any of them are multicopy genes with different lengths. Seven protein-coding genes, eight tRNA genes, and three rRNA genes are duplicated. Gene synteny and arrangement analysis revealed that most of *N. pseudonarcissus* genes have the same positions, directions, and nucleotide sequences as in *N. poeticus* (70% consensus identity) (Supplemental figure 2), and this finding suggests that these plastomes are

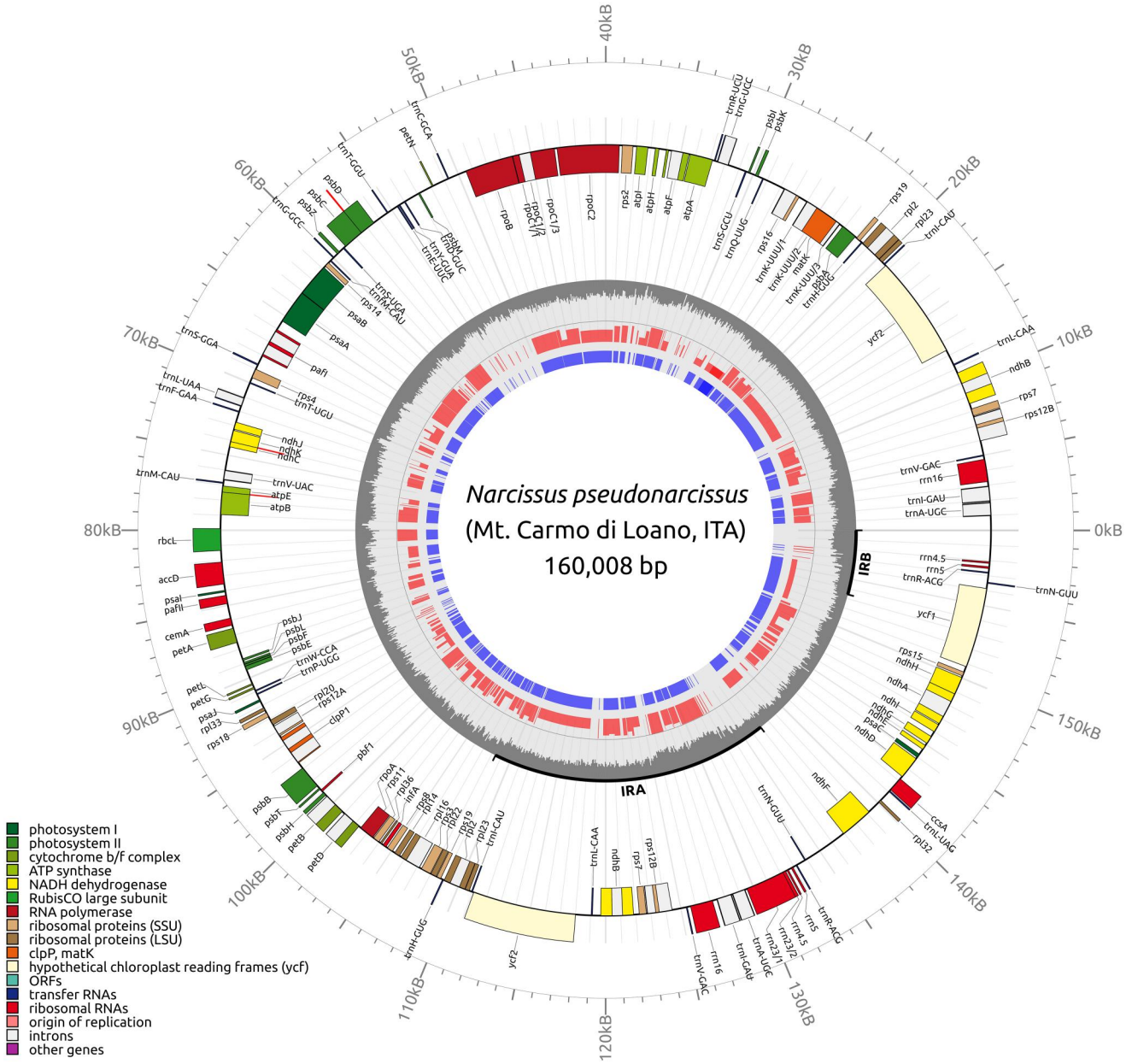

Figure 2. Plastome map of *N. pseudonarcissus*. The map contains five concentric circles. From the inner going outward: i. the long tandem repeats (LTRs) marked with short blue bars; ii. the short tandem repeats (STRs); iii. the size of the LSC, SSC, IRA, and IRB. iv; the GC contents along the plastomes; v. the genes color-coded by their functional classification (see legend on the bottom left). The transcription directions for the inner and outer genes are clockwise and counterclockwise, respectively.

completely syntenic also within *N. tazetta* L. (1753) (Li et al. 2020).

The reads coverage was excellent (Supplemental figure 3) with a mean coverage of ~336 reads per base (range: 150–680×).

The whole plastomes-based phylogeny showed that *N. pseudonarcissus* was closely related to *N. poeticus* (Figure 3), inside the clade Amaryllidoideae, coupled with the well-supported sister clade of Allioideae. The phylogeny tree topologically supports the findings of Marques et al. (2017).

Discussion and conclusions

The complete plastidial genome of *N. pseudonarcissus* L. was sequenced for the first time and found to exhibit a total length of 160,008 bp. There were no significant differences in genome size or gene content when compared to the other available plastidial genomes in *Narcissus* (Li et al. 2020; Könyves et al., 2018). The close genetic relationship observed between *N. pseudonarcissus* and *N. poeticus* L. gives definitive support to the placement of *N. poeticus,* the type species of the genus, in sect. *Pseudonarcissi* and to the new taxonomic circumscription proposed by Marques et al. (2017). Other similarities with *N. poeticus* are the karyotypes (2n = 14, in both species) (Fernandes 1968) and genome sizes which are in the same range of values (26 pg and 23.8 pg 2 C DNA in *N. poeticus* and *N. pseudonarcissus*, respectively) (Zonneveld 2008).

The novel *N. pseudonarcissus* plastome can help to bring new insights into the evolution of the genus. To date, in fact, the very low number of available complete plastomes still

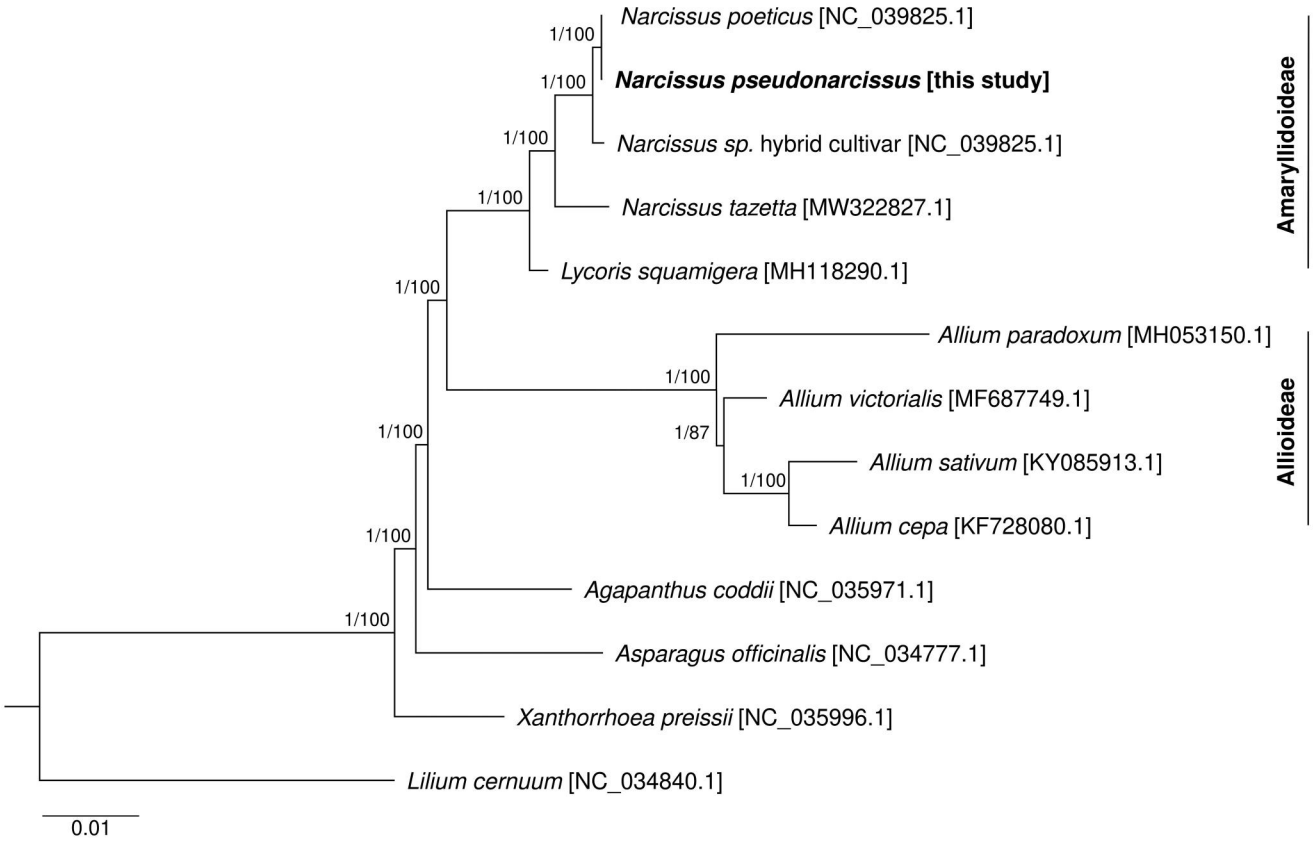

Figure 3. Phylogenetic tree based on the complete chloroplast genome sequence of 13 selected taxa of amaryllidacea. *Lilium cernuum* (GenBank: NC_034840.1; Du et al. 2016) was selected as an outgroup. The posterior probability/bootstrap value is indicated at each branch node. GenBank accession number is listed after the species name. The following sequences were used to populate the tree: NC_039825.1 (Könyves et al. 2018); MW672399 (unpublished); MW322827.1 (Li et al. 2020); KF728080.1 (Huo et al. 2019); KY085913.1 (Huo et al. 2019); MH118290.1 (Jin et al. 2018); MH053150.1 (unpublished); MF687749.1 (Lee et al. 2017); NC_035971.1 (unpublished); NC_034777.1 (Sheng et al. 2017); NC_035996.1 (unpublished).

represents a limit to the purpose. A larger number of sequences is needed, in fact, to gain a realistic understanding of the phylogenetic relationships within this complex group of species, and also to guide studies currently dealing with morphology, biogeography and ecology in wild *Narcissus* spp. In conclusion, the sequenced cpDNA of *N. pseudonarcissus* will provide meaningful information for developing new molecular markers to be applied at the study of the evolutionary history of the genus and within the Amaryllidaceae family. In addition, this is, possibly, a potential tool for horticultural applications.

Author contributions

MA, MM, VF: conception and design; MA, MM: analysis and interpretation of data; MA, MM, VF: drafting of the paper; MM, MA: critical revision of the paper. All authors have approved the final version of the paper and agreed to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. PP313599. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1076687, SRR27971680, and SAMN39949271 respectively.

Permissions

Narcissus pseudonarcissus is not evaluated (NE) by the IUCN at global scale, and in Italy it is not listed as an endangered species by the National Red List (https://www.iucn.it/liste-rosse-italiane.php). Otherwise, in several Italian Regions, *N. pseudonarcissus* is listed in the protected flora and its collection is limited to a few individuals.

For plastome sequencing, DNA was extracted from *N. pseudonarcissus* leaves collected at Monte Carmo di Loano (province of SV, Italy). According to local legislation (LR 30 gennaio 1984 n. 9) *Narcissus* species

fall under Annex B and the collection of five individuals per day per person is permitted (excluding underground organs) (see Art. 3). For this analysis, only two leaves were collected, thus in no way conflicting with the current regulations.

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