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Cytogenomic Differentiation of *Narcissus serotinus* s.l. in Southwestern Turkey: Insights into Polyploidy and Hybridization

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Abstract

The *Narcissus serotinus* complex is one of the most taxonomy-challenging groups within the genus *Narcissus* (Amaryllidaceae). While western Mediterranean populations have been studied thoroughly, eastern Mediterranean populations remain poorly understood. This taxonomic uncertainty has led to confusion about species boundaries, especially regarding the identity and distribution of cytotypes such as *N. serotinus, N. obsoletus*, and *N. miniatus*. This study aimed to clarify the taxonomic status and cytogenetic diversity of *N. serotinus* s.l. populations in southwestern and southern Turkey using an integrative approach. Morphological assessments, karyological analyses, and nuclear DNA content measurements were conducted on eight natural populations in detail.

The analyses showed that all studied populations had a consistent chromosome number of 2n = 30, a cytotype not previously documented in the region. Nuclear DNA content ranged from 48.65 to 54.85 pg, indicating genomic variation despite uniform ploidy. Phenetic clustering based on combined morphological and cytogenetic data confirmed the existence of three main lineages, supporting the distinctiveness of *N. miniatus* and revealing previously unrecognized diversity within Turkish populations.

The discovery of 2n = 30 cytotypes in Turkey expands the known cytogenetic and biogeographic range of the *N. serotinus* complex and supports recognizing *N. miniatus* as a distinct species. Additionally, this study emphasizes the importance of integrative taxonomy—combining morphological, cytogenetic, and genomic methods—in resolving complex plant groups. It also highlights the eastern Mediterranean, especially Turkey, as a key region for studying speciation, hybridization, and plant evolution within the genus *Narcissus*.

Keywords: Amaryllidaceae; chromosome number; DNA content; hybridization; integrative taxonomy; *Narcissus miniatus*; polyploidy

Introduction

The taxonomy of *Narcissus* L. has long been challenging, with roots dating back to early plant records (Fernandes, 1975; Webb, 1980), and many efforts have been made to clarify naming and classification. The genus *Narcissus*, part of the Amaryllidaceae family, comprises about 89 native species and hybrids, mainly found around the Mediterranean, southwestern Europe, and North Africa (Aedo 2010; POWO 2025). It is a well-known example of a plant group that is difficult to classify because of complex evolutionary processes such as polyploidy, hybridization, high phenotypic variability, and a history of human cultivation and selection (Mathew, 2002; Zonneveld 2008; Maryam and Gul, 2025). These factors have fuelled ongoing debates about how to define species, identify synonyms, and classify plants within the genus. The classification of *Narcissus* is still actively being revised, especially among groups that display similar physical traits and complex chromosomal backgrounds (Koopowitz *et al.,* 2017; Mifsud and Lanfranco, 2024).

Among the most problematic groups within the genus is the *Narcissus serotinus sensu lato* (s.l.) complex, which includes autumn-blooming species typically identified by their small flowers, terete or falcate leaves, and narrow coronas that range in colour from pale yellow to orange. These species bloom later in the season than most other *Narcissus* varieties and have adapted to various Mediterranean habitats, from coastal dunes to rocky slopes (Mathew, 2002). Historically, this complex was considered a single, highly variable species with a broad ecological range. However, recent integrative methods combining morphology, chromosome analysis, flow cytometry, and genomic data have challenged this idea, indicating that multiple cryptic species and hybrid derivatives exist within the group (Díaz Lifante and Camacho, 2007; Donnison-Morgan *et al*., 2005).

The cytotaxonomy of *Narcissus serotinus* s.l. is particularly complex. According to Díaz Lifante and Camacho (2007), the group can be divided into three main species based on consistent morphological and chromosomal differences: (1) *N. serotinus* s.str., a diploid (2n = 10) species native to the southwestern Iberian Peninsula and northwestern Morocco; (2) *N. elegans*, a species with 2n = 20 chromosomes found across North Africa and parts of southern Europe; and (3) *N. obsoletus*, a widespread allopolyploid (2n = 30) hybrid that resulted from the crossing of the previous two species (Díaz Lifante et al. 2009). These findings have been confirmed by studies of karyotype morphology, DNA content estimates, and genomic in situ hybridization (GISH) analyses.

Narcissus serotinus s.l. acts as a model system for studying reticulate evolution and allopolyploid speciation in Mediterranean geophytes. The importance of allopolyploidy is especially clear in the diversification of the genus, as it allows the combination of genomes from different species, promoting adaptation to new ecological niches and possibly concealing reproductive barriers (Zonneveld, 2008; Marques *et al*., 2010). The high level of morphological variability in traits such as corona lobing, leaf shape, and

floral structure has often misled early taxonomic efforts, leading to a buildup of synonyms and confusion in naming (Webb, 1980; Fernández Casas, 2008). One of the more debated taxa in this group is *N. miniatus*, first described by Donnison-Morgan *et al.,* (2005) as a distinct triploid species (2n = 30, DNA content ~51 pg) based on specimens from southern Spain. It was suggested that it originated from an ancient hybridization between *N. serotinus* and *N. obsoletus* and was distinguished by its more elongated perianth tube and a darker orange, three-lobed corona. However, other researchers have questioned its status as a separate species, arguing instead that it falls within the phenotypic range of *N. obsoletus* (Díaz Lifante *et al*., 2009). The recent synonymizing of *N. miniatus* with *N. deficiens* by Mifsud and Lanfranco (2024) emphasizes the importance of carefully reevaluating diagnostic morphological traits and their links to cytogenetic profiles.

Despite considerable attention to *N. serotinus* s.l. in populations across the Iberian Peninsula, Morocco, and Malta, a significant knowledge gap remains concerning the eastern range of the complex, especially in Turkey. While Turkey functions as a biogeographically important bridge between the Mediterranean and the Middle East, hosting many endemic and peripheral populations of western taxa, its *Narcissus* flora has not been thoroughly studied in cytotaxonomic detail (Alp *et al*., 2015; Pirhan, 2023). The presence of autumn-flowering populations with morphological traits similar to those of *N. serotinus* s.l. has been documented in southwestern and southern Turkey. However, these populations have yet to undergo systematic cytogenetic or nuclear DNA analyses.

N. serotinus s.l., as observed in Turkey, may include unrecognized diversity or hybrid forms that challenge existing taxonomic classifications. For example, some Turkish populations display morphological features such as three-lobed coronas, terete or falcate leaf cross-sections, and variations in scape height and flower number—key traits for distinguishing species within this complex (Díaz Lifante and Camacho, 2007; Zonneveld, 2008). However, without supporting karyological data—such as chromosome counts, centromere positioning, and asymmetry indices—and measurements of genome size, their taxonomic interpretation remains uncertain.

In this context, *N. serotinus* s.l. populations in Turkey offer a valuable chance to determine whether known cytotypes and morphotypes extend into the eastern Mediterranean or if geographically isolated forms have undergone independent evolutionary divergence. The identification of consistent karyotype structures, flow cytometric DNA values, and cluster differences in morphological traits can help decide whether these populations match existing taxa or represent a new taxonomic group. Additionally, such data may help clarify whether Turkish populations share ancestry with Western Mediterranean groups or have experienced localized genomic evolution.

Díaz Lifante and Camacho (2007) highlighted the importance of combining morphological and cytological data to define taxonomic boundaries within the *N. serotinus* complex. However, their studies did not include Turkish populations. This gap is addressed by the current research, which provides the

first comprehensive dataset on the morphology, karyology, and nuclear DNA content of *N. serotinus* s.l. populations in Turkey. In doing so, this study offers vital information for understanding the diversity and taxonomic structure of the complex in the eastern Mediterranean region.

The current research aims to:

1. Characterize eight geographically diverse Turkish populations of *N. serotinus* s.l. using detailed morphological measurements, including tepal size, corona shape, and leaf anatomy.
2. Determine chromosome numbers and karyotype structure—including total length, chromosome symmetry, satellite presence, and centromeric morphology—using metaphase analysis.
3. Measure nuclear DNA content using flow cytometry and compare the results to established standards for *N. serotinus, N. obsoletus*, and *N. miniatus*.
4. Perform phenetic analyses (e.g., Ward's clustering) to assess whether morphological and cytogenetic traits co-segregate in ways consistent with previous species delimitations.
5. Assess the taxonomic significance of any observed divergence or congruence with western taxa, and discuss the evolutionary role of polyploidy and hybridization in these Turkish populations.

Ultimately, the findings of this study are expected to clarify patterns of cytological variation and morphological differences within *N. serotinus* s.l. and to guide future efforts in taxonomic revision and conservation strategies for *Narcissus* species in the eastern Mediterranean. Additionally, including Turkish populations in the broader story of *Narcissus* evolution enhances the genus's value as a model for understanding polyploid speciation, reticulate evolution, and the impact of geographical isolation on plant diversification.

Materials and Methods

Plant Material

This study involved plant specimens from eight natural populations of *N. serotinus* s.l. collected across southwestern and southern Turkey (Figs. 1 and 2). Fieldwork was conducted between September 2010 and March 2013, during the species' flowering season, focusing on individuals in their natural habitats at various elevations and ecological zones. Geographic coordinates were recorded at each site, and herbarium vouchers were stored at the ISTE Herbarium (Faculty of Pharmacy, Istanbul University) (Table 1). Both live and pressed specimens were used for morphological, cytogenetic, and flow cytometry analyses (Pirhan, 2023).

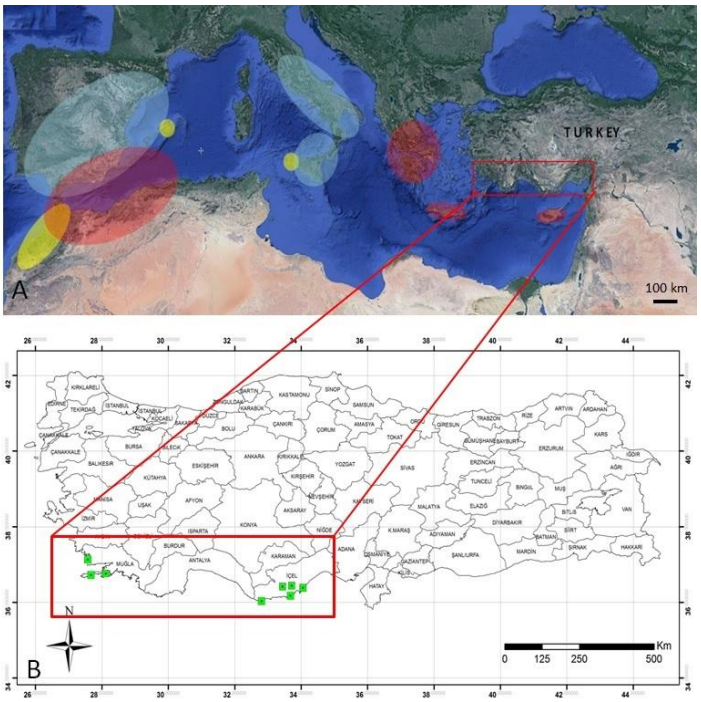

Fig. 1. A) Distribution of *N. serotinus* (blue circle), *N. obsoletus* (red circle), and *N. elegans* (yellow circle) across the Mediterranean basin according to Mill, 1984; D'Amato, 2004; Díaz-Lifante and Camacho, 2007; Zonneveld, 2008; Díaz-Lifante *et al.*, 2009; Mifsud and Caruana, 2010; Troia *et al*., 2013; Pirhan, 2023. B) Distribution of *N. serotinus* s.l. in Turkey and the area examined in this study (red square).

Table 1. Localities of the *Narcissus serotinus* s.l. in Turkey and herbarium vouchers

Plants	ISTE	Localities
A	113700	Muğla: Bodrum, 20.11.2016
B	113701	Mersin: Anamur Ören area, 6.12.2005, 3 m
C	113702	Mersin: Silifke, Ovacık, 15.11.2016, 2 m
D	113706	Mersin: Silifke, Ovacık, coast 15.11.2016, 2 m
E	113707	Mersin: Silifke Taşucu area 15.11.2016, 10 m
F	113703	Mersin: Silifke Taşucu area, 15.11.2016, 2 m
G	113704	Muğla: Marmaris, Taşlıca area, coast, 20.11.2016, 184 m
H	113705	Muğla: Marmaris, Taşlıca area, entrance 20.11.2016, 184 m

Fig 2. Habitat of *Narcissus serotinus* s.l. in Turkey (Muğla, Marmaris).

Morphological Analysis

Sixteen diagnostic morphological characters were chosen based on previous taxonomic research (Díaz Lifante and Camacho, 2007) and measured across all eight populations. Traits included bulb size, scape and leaf length, flower count, tepal size, hypanthial tube length, corona height and shape, and leaf cross-section (Fig. 3). Measurements were taken with digital calipers and a stereomicroscope. Character states were recorded in binary (0/1), and data matrices were created to compare Turkish populations with reference species, including *N. serotinus, N. elegans, N. obsoletus, N. miniatus,* and *N. deficiens* (Donnison-Morgan *et al.,* 2005; Mifsud and Lanfranco, 2024). Morphological similarity patterns were evaluated using hierarchical cluster analysis with Ward's method in the PAST 1.81 software (Hammer *et al*., 2001). Each character and its scoring were clearly specified in the methods section to improve reproducibility and transparency.

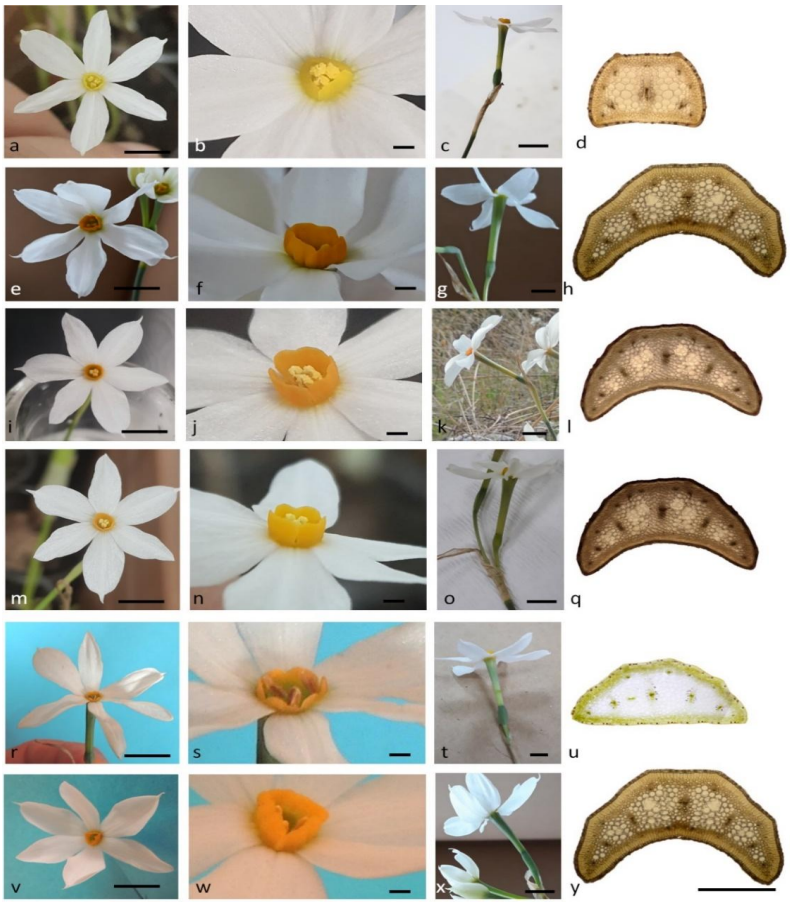

Fig. 3. Flowers, corona, hypanthial tubes and leaves outline shape of *Narcissus serotinus* s.l. specimens in Turkey: A and D (a,b,c,d), B (e, f, g, h), C (I, j, k, l), E and F (m, n, o, q), G (r, s, t, u), H (v, w, x, y). (scale bar 1 cm for flowers and hypanthial tubes; 1 mm for corona and leaves outline shape)

Chromosome Analysis (Karyology)

Cytogenetic analyses were performed on six healthy bulbs from each population. For each individual, 10–15 well-spread metaphase plates were examined. Root tip meristems were prepared using the squash method with Feulgen staining for optimal chromosome visualization. Chromosomes were classified based on centromere position following Levan *et al.*, (1964). Measured parameters included total chromosome length, diploid number (2n), karyotype formula, centromeric asymmetry (M_{CA}), and the coefficient of variation of chromosome length (CV_{CL}), as outlined by Peruzzi and Eroğlu (2013).

Chromosomal data were compared with published information on related taxa: *N. serotinus* (2n = 10), *N. elegans* (2n = 20), *N. obsoletus* (2n = 30), and

N. miniatus (2n = 30) (Zonneveld, 2008; Díaz Lifante *et al*., 2009). Relative chromosome length was also considered to reduce errors caused by variation in chromatin condensation during slide preparation.

Nuclear DNA Content Analysis (Flow Cytometry)

Nuclear DNA content was measured using a Partec CyFlow Space flow cytometer (Partec GmbH, Münster, Germany). For each population, three individuals were analyzed separately, and 5,000 nuclei were counted per sample. The internal standard used was *Triticum durum* Desf. (2C = 25.0 pg), as described by Ulrich and Ulrich, (1991). Nuclei suspensions were prepared with Partec commercial kits following the protocol of Koçyiğit and Tuna (2012). Only results with a coefficient of variation (CV) below 5% were included in the final analysis. Measured DNA values were compared with those reported for relevant *Narcissus* species and used to assess ploidy levels and infer taxonomic relationships (Zonneveld, 2008; Donnison-Morgan *et al*., 2005). In line with reviewer feedback, genome size data were combined with karyological variables in multivariate analyses to improve taxonomic resolution.

Phenetic Analysis

Both morphological and karyological data matrices were analyzed using hierarchical cluster analysis with Ward's method in PAST software. Bootstrap analyses (n = 1000 replicates) were performed to assess the robustness of the dendrogram clusters. The resulting phenograms were used to evaluate the consistency between morphological and cytogenetic datasets. Morphological and chromosomal dendrograms were compared to identify taxonomic groups and evaluate the placement of Turkish populations relative to reference species. This integrative approach offered a framework for determining whether observed variation matched known species boundaries or indicated new evolutionary lineages.

RESULTS

1. Morphological Variation and Phenetic Grouping

The analysis of 16 morphological characters and scores from eight *N. serotinus* s.l. populations in Turkey revealed clear groupings based on floral and foliar traits (Table 2). Ward's hierarchical clustering of the binary-coded data matrix formed three main morphological groups (Fig. 4). The Isparta and Mersin populations showed a distinctly three-lobed corona, narrow leaves, and solitary flowering, aligning morphologically with the Western Mediterranean morphotype *N. miniatus*, which has been suggested as an allopolyploid derived from *N. obsoletus* (Donnison-Morgan *et al.,* 2005).

In contrast, the Burdur and Antalya-Beydağları populations exhibited morphological features such as fully or lightly lobed coronas, a narrower hypanthial tube, and more elongated scapes, which are similar to *N. serotinus* sensu stricto (Díaz Lifante and Camacho, 2007). Leaf cross-section also varied considerably among populations and seemed to be related to cluster

separation. Because of the developmental plasticity of corona lobing observed in live populations, this character was interpreted cautiously. However, the combination of multiple floral and vegetative characters provided enough resolution to distinguish population-level morphological patterns.

Table 2. Morphological Characters, character-states, their coding and scores.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	***serotinus***	***elegans***	***obsoletus***	***miniatus***	***deficiens***
C1	0	0	1	0	0	1	0	0	0	1	0	1	1
C2	0	0	1	0	0	0	0	0	0	1	0	1	1
C3	0	0	0	0	0	0	0	0	0	1	1	0	0
C4	0	0	0	0	1	1	0	0	0	1	1	0	0
C5	1	0	1	1	1	0	0	0	1	1	1	1	1
C6	0	0	1	0	0	0	0	0	1	1	1	1	0
C7	1	1	1	1	1	1	0	0	1	0	0	0	0
C8	0	1	1	0	1	1	0	0	0	1	0	1	1
C9	0	1	1	0	0	0	1	1	0	0	0	0	0
C10	0	1	0	0	0	0	0	0	0	0	0	1	0
C11	0	1	1	0	0	1	1	0	0	0	1	1	0
C12	1	0	0	1	1	1	0	0	1	1	1	0	1
C13	1	0	1	1	1	1	1	0	1	0	0	0	1
C14	0	1	0	0	1	1	1	0	0	0	0	0	1
C15	0	0	0	0	0	0	0	1	1	1	0	0	0
C16	0	0	0	0	1	1	1	0	1	1	0	1	0
1. Bulb width up to 2.5 cm: 0, others 1													
2. Bulb length up to 3 cm: 0, others 1													
3. Leaves length $\leq$ 20 cm: 0, others 1													
4. Leaves wide $\leq$ 2 mm: 0, others 1													
5. Leaf cross section; falcate: 0, others 1													
6. Scape length $\leq$ 20 cm: 0, others 1													
7. Numbers of flowers per scape $\geq$ 3: 0, others 1													
8. Pedicels length $\leq$ 3 cm: 0, others 1													
9. Hypanthial tube length < 2 cm: 0, others 1													
10. Tepals length < 2 cm: 0, others 1													
11. Tepals wide < 1 cm: 0, others 1													
12. Perianth imbricated: 0, not imbricated: 1													
13. Corona colour; orange: 0, others 1													
14. Corona height < 2 mm: 0, others 1													
15. Corona 3-lobed: 0, others: 1													
16. Corona outlines trigonous: 0, others 1													

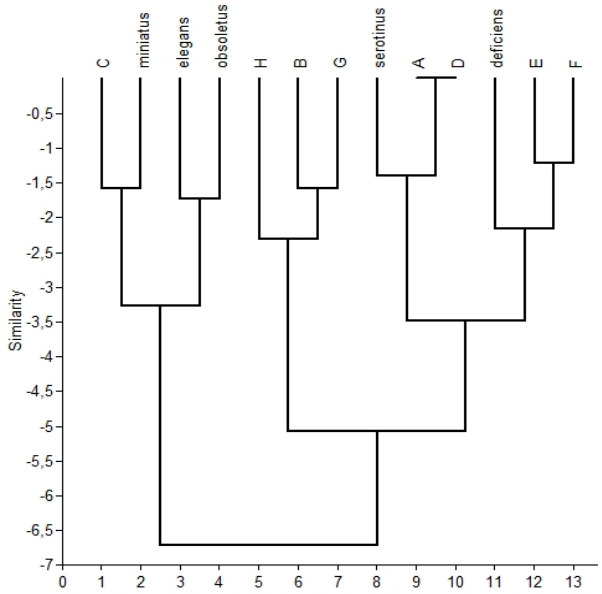

Fig.4. Analysis of *Narcissus serotinus* and related species according to morphological characters using Ward's hierarchical clustering method.

2. Chromosome Number, Karyotype Features and Nuclear DNA Content (2C Values)

Cytogenetic analysis of all eight populations revealed a consistent diploid chromosome number of 2n = 30. This supports the idea that Turkish *N. serotinus* s.l. populations are likely from allopolyploid origins, consistent with previous reports for *N. obsoletus* and *N. miniatus* (Díaz Lifante *et al*., 2009; Zonneveld, 2008).

Karyotype formulas mainly included metacentric (m) and submetacentric (sm) chromosome pairs, indicating symmetrical karyotypes. The total haploid set length ranged from 39.8 µm to 46.2 µm across populations. Notably, satellite chromosomes were found in all populations except the Antalya-Kaş population, suggesting possible regional differences in NOR-associated loci (Table 3).

The coefficient of variation of chromosome length (CV_{CL}) values was low (6.3%–9.8%), indicating moderate chromosomal uniformity. Mean centromeric asymmetry values (M_{CA}) ranged from 17.1% to 22.4%, with no sudden shifts that would suggest derived chromosomal arrangements (Peruzzi and Eroğlu, 2013).

Flow cytometry analysis showed nuclear 2C DNA content values ranging from 50.2 ± 1.1 pg to 53.7 ± 0.9 pg (Table 3). These values match those reported for *N. miniatus* (~51 pg) by Donnison-Morgan *et al*., (2005) and

overlap with *N. obsoletus* as described by Zonneveld (2008). The intra-populational variability observed was minimal (CV < 5%), confirming the reliability of the measurements. Populations from Mersin, Isparta, and Kaş showed slightly higher values (above 53 pg), indicating possible genome expansion caused by transposable elements or residual hybridization effects (Marques *et al.,* 2010). Since all populations have a chromosome count of 2n = 30, these differences in genome size probably reflect variations in genome composition rather than ploidy level itself.

Table 3. Measurements of of *N. serotinus* s.l. in Turkey with related species according to the literature

ISTE No	A1	A2	CV_{cL}	M_{CA}	Total Length [µm]	Karyotype Formula	2n	2C DNA (pg)
A (113700)	0.563	0.306	30.65	40.73	74.61	$2MC^{sat}$ 4MC 12SMC 12STC	30	50.24
B (113701)	0.514	0.362	36.18	35.60	66.24	10MC 12SMC 8STC	30	51.24
C (113702)	0.548	0.329	32.85	38.89	70.24	6MC $4MC^{sat}$ 8SMC $2SMC^{sat}$ 8STC 2TC	30	49.59
D (113706)	0.552	0.375	37.48	37.90	69.97	6MC 16SMC 6STC 2TC	30	52.74
E (113704)	0.594	0.351	35.11	42.55	69.45	6MC 12SMC 12STC	30	48.65
F (113705)	0.562	0.350	35.04	38.82	76.54	$2MC^{sat}$ 4MC 14SMC $2SMC^{sat}$ 6STC 2TC	30	54.85
G (113703)	0.542	0.371	37.07	36.73	70.70	8MC 10SMC 10STC 2TC	30	50.67
H (113707)	0.501	0.352	35.21	35.04	58.66	10MC 12SMC 6STC 2TC	30	50.72
N.serotinus*	0.534	0.293	29.30	36.36	62.54	4MC $2MC^{sat}$ 2SMC 2STC	10	20.80
N. elegans*	0.777	0.389	38.90	48.16	85.79	2MC 2SMC 14STC $2TC^{sat}$	20	30.20
N.obsoletus*	0.751	0.377	37.70	49.50	80.87	8MC 4SMC 16STC $2TC^{sat}$	30	26.10
N.miniatus*	-	-	-	-	-	-	30	51.30

A1 = intrachromosomal asymmetry index; A2 = interchromosomal asymmetry index; CV_{CL} = coefficient of variation of chromosome lengths; M_{CA} = Mean centromeric asymmetry; AI = karyotype asymmetry index. (* According to Diaz-Lifante *et al.,* 2009; Zonneveld 2008).

4. Multivariate Integration of Morphological and Cytogenetic Data

To assess the agreement between morphological and cytogenetic variation, a combined phenetic analysis using both datasets was performed (Tables 2 and 4) (Ward's method, with 1000 bootstrap replicates). The resulting dendrogram (Fig. 5) showed three distinct clusters that mostly matched the previously

identified morphological groups. Group I consisted of individuals with lower 2C values, symmetrical karyotypes, and weakly lobed coronas—traits consistent with *N. serotinus* s.str. Group II included populations with increased DNA content, three-lobed coronas, and broader leaves, morphologically resembling *N. miniatus* or derived forms of *N. obsoletus*. Group III contained intermediate morphotypes, mainly from Kaş and Antalya, which could indicate introgression zones or relic hybrids between the two main lineages (Díaz Lifante *et al*., 2009; Marques *et al.,* 2010).

Table 4. Karyological characters, character-states, their codings and scores.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	***serotinus***	***elegans***	***obsoletus***
C1	1	1	0	0	1	0	0	0	1	0	0
C2	0	1	1	0	0	0	1	1	0	1	1
C3	0	0	1	1	0	1	1	0	1	1	1
C4	1	0	0	1	1	1	1	1	1	1	1
C5	1	0	1	0	0	1	1	0	0	1	1
C6	0	1	0	1	1	0	1	1	0	1	1
C7	1	1	0	1	1	0	1	1	1	1	1
C8	1	1	1	1	1	1	1	1	1	0	0
C9	1	1	0	1	0	1	1	1	0	0	0
C10	0	0	0	0	0	0	0	0	0	1	1
C11	0	1	0	1	1	1	1	1	0	1	1
C12	0	1	0	1	1	1	1	1	0	1	1
C13	1	0	0	0	1	0	0	0	0	1	1
C14	0	0	0	0	0	0	0	0	1	1	0
1. 2 Telocentric chromosomes: 0 yes, 1 no											
2. 6 Metacentric chromosomes: 0 yes, 1 no											
3. 12 Submetacentric chromosomes: 0 yes, 1 no											
4. 8 Subtelocentric chromosomes: 0 yes, 1 no											
5. Total Length (TL)[µm]: 0: 70 < TL, 1: 70 > TL											
6. Satellite on metacentric chromosomes: 0 yes, 1 no											
7. Satellite on submetacentric chromosomes: 0 yes, 1 no											
8. Satellite on telocentric chromosomes: 0 yes, 1 no											
9. DNA content [pg]: 0: 50 > DNA, 1: 50 < DNA											
10. Intrachromosomal asymmetry index (A1): 0: 0.6 > A1, 1: 0.6 < A1											
11. Interchromosomal asymmetry index (A2): 0: 0.35 > A2, 1: 0.35 < A2											
12. Coefficient of variation of chromosome lengths (CVcL): 0: 35 > CVcL, 1: 35 < CVcL											
13. Mean centromeric asymmetry (MCA): 0: 40 > MCA, 1: 40 < MCA											
14. Diploid chromosome numbers: 0: 2n=30, 1: others											

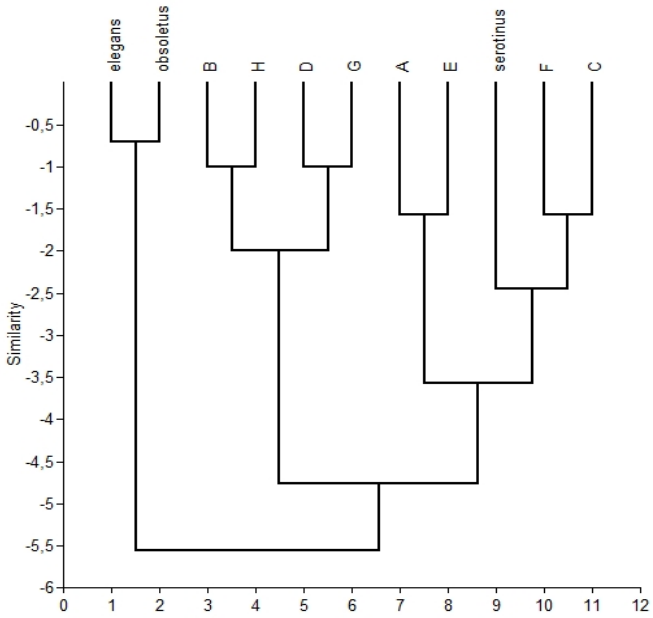

Fig.5. Analysis of *Narcissus serotinus* and related species according to morphological and karyological characters using Ward's hierarchical clustering method.

Diagnostic Key Development and Significance

The construction of a dichotomous key based on morphological traits is essential for resolving taxonomic ambiguities within the *N. serotinus* s.l. complex, especially in regions like Turkey, where hybridization, polyploidy, and phenotypic plasticity make species boundaries unclear. While molecular and cytogenetic analyses offer valuable insights, field identification in floristic and ecological studies still depends heavily on easily observable morphological features (Díaz Lifante and Camacho, 2007; Zonneveld, 2008). Previous research has shown that although corona shape can vary with developmental stage, its diagnostic value improves when combined with floral tube morphology, flower count, and leaf phenology (Díaz Lifante and Camacho, 2007; Zonneveld, 2008). This key directly addresses that issue by including multiple stable traits in a standardized format. It allows for accurate identification of morphotypes in Turkish populations and improves comparability with well-documented Western Mediterranean taxa, ultimately supporting taxonomic clarity and future biogeographic studies.

Dichotomous Key for Turkish *Narcissus serotinus s.l.* Taxa (Based on Morphological Characters)

1. Corona with three distinct lobes; typically produces a single flower; hypanthial tube short to moderately long (10–18 mm); leaves undeveloped or absent during flowering period……2
1. Corona entire or with six shallow lobes; flower solitary or occasionally two; hypanthial tube narrow and long (>18 mm); leaves emerge during or before flowering……..……...……...3
2. Corona lobes dark yellow to orange; scape short (<12 cm); DNA content >52 pg; karyotype symmetric (2n = 30); typically found in Isparta and Mersin populations → ***N. miniatus*** (sensu Donnison-Morgan *et al*., 2005)
2. Corona lobes lighter yellow; scape longer (>12 cm); DNA content ranges between 50–52 pg; observed satellite chromosomes; often transitional morphology→**Intermediate form (*N. miniatus* × *N. obsoletus*?) – "Kaş type"**
3. Hypanthial tube abruptly widened; corona entire with six lobes; stamens adnate less than one-third of filament length; leaf development usually delayed until after flowering; DNA content <51 pg → ***N. serotinus*** (s.str.) (cf. Díaz Lifante & Camacho, 2007)
3. Hypanthial tube gradually widened; corona weakly lobed (3 shallow lobes or slightly emarginate); stamens adnate more than half their length; leaves often present at flowering; DNA content varies (50–53 pg) → ***N. obsoletus*** (wide Mediterranean distribution)

Discussion and Conclusion

Our detailed study of *N. serotinus* s.l. populations in Turkey uncovers previously unknown cytogenetic and morphological variation within the eastern Mediterranean part of this complex. All eight populations examined share a consistent chromosome number of 2n = 30 (Figs. 6, 7), a cytotype not previously reported from this area. This challenges the traditional view that Turkish populations are solely *N. serotinus* (2n = 10) and suggests a more intricate evolutionary history involving allopolyploidy or hidden hybridization events (Díaz Lifante and Camacho, 2007; Zonneveld, 2008). Some *Narcissus* species show polymorphic structures in their karyotypes and banding patterns. B chromosomes have also been noted, often linked to environmental stresses like drought or temperature changes (Pustahija *et al.,* 2024). However, although our study confirmed the presence of polymorphic structures among populations, no B chromosomes were observed.

Morphological variation among populations—especially in floral traits such as corona lobing, hypanthial tube structure, and leaf development—aligns well with karyological and genomic differences. Populations from Mersin and Isparta closely resemble the Western Mediterranean morphotype *N. miniatus*, which was previously identified as an allopolyploid species with distinctive floral features and a larger genome size (Donnison-Morgan et al. 2005). Meanwhile, the Kaş and Antalya populations display intermediate traits, suggesting a possible hybrid origin or an introgression zone between *N.*

miniatus and *N. obsoletus*. These findings are further supported by phenetic cluster analyses that integrate morphological and cytogenetic data, revealing three main groups consistent with different evolutionary paths.

Regarding nuclear DNA content, the Turkish populations showed values ranging from 48.65 to 54.85 pg (2C), which broadly overlap with those of *N. obsoletus* and *N. miniatus*, yet differ from *N. serotinus* s.str., which typically exhibits lower values (Díaz Lifante *et al*., 2009; Zonneveld, 2008). The consistent chromosome number despite genomic variability suggests the presence of stabilized polyploid cytotypes with potentially different parental origins (Zhang *et al*., 2025). This supports the idea that Turkey may serve as both a refuge and a hybridization center for *Narcissus* taxa, located at the biogeographic crossroads of the eastern Mediterranean, the Balkans, and the Middle East. Methods such as multivariate analysis of quantitative morphological features in vegetative and floral parts, descriptive analysis of qualitative traits, and karyotype analysis are used to identify species (Díaz Lifante *et al*., 2024).

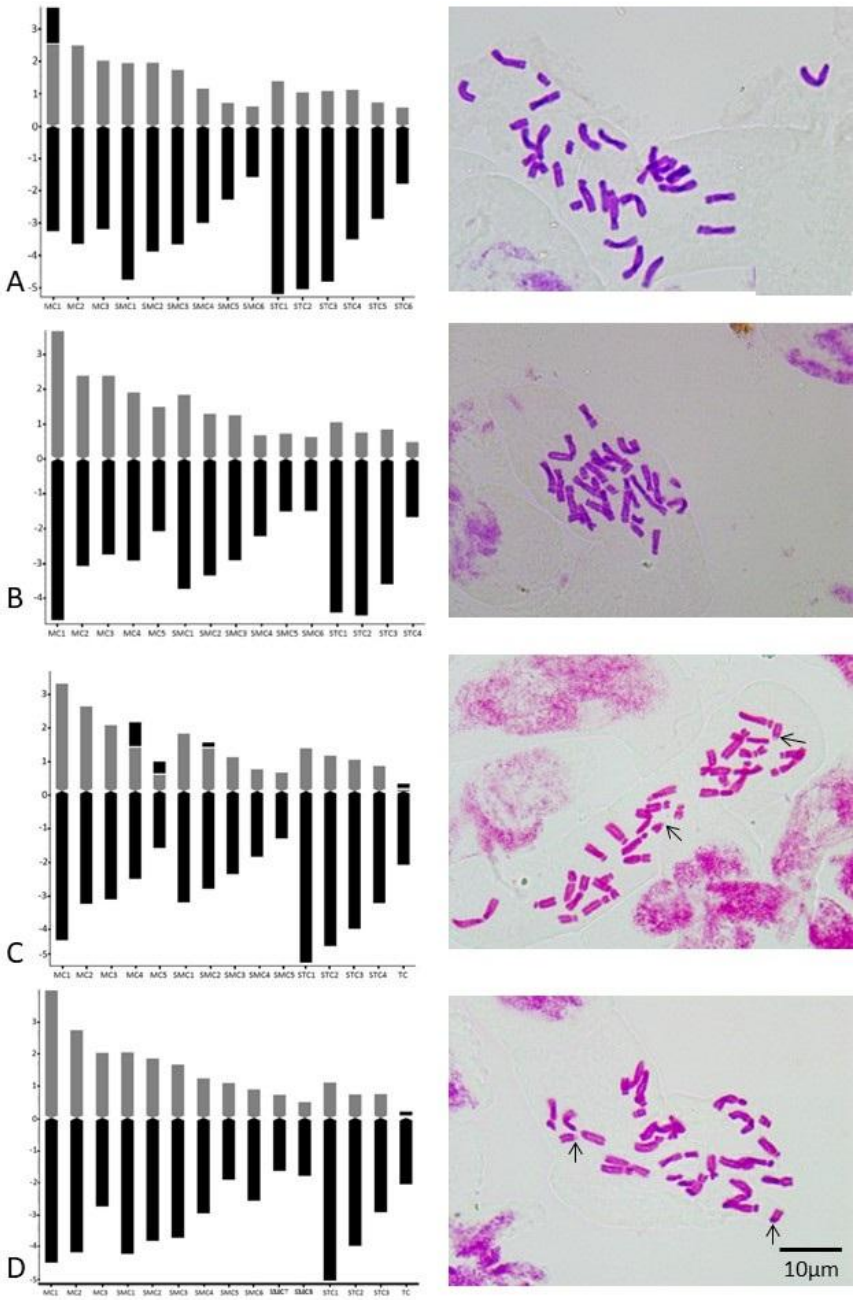

Fig. 6. Mitotic chromosomes and ideograms of *N. serotinus* s.l. with ISTE voucher numbers: A) 113700, B) 113701, C) 113702, D) 113703.

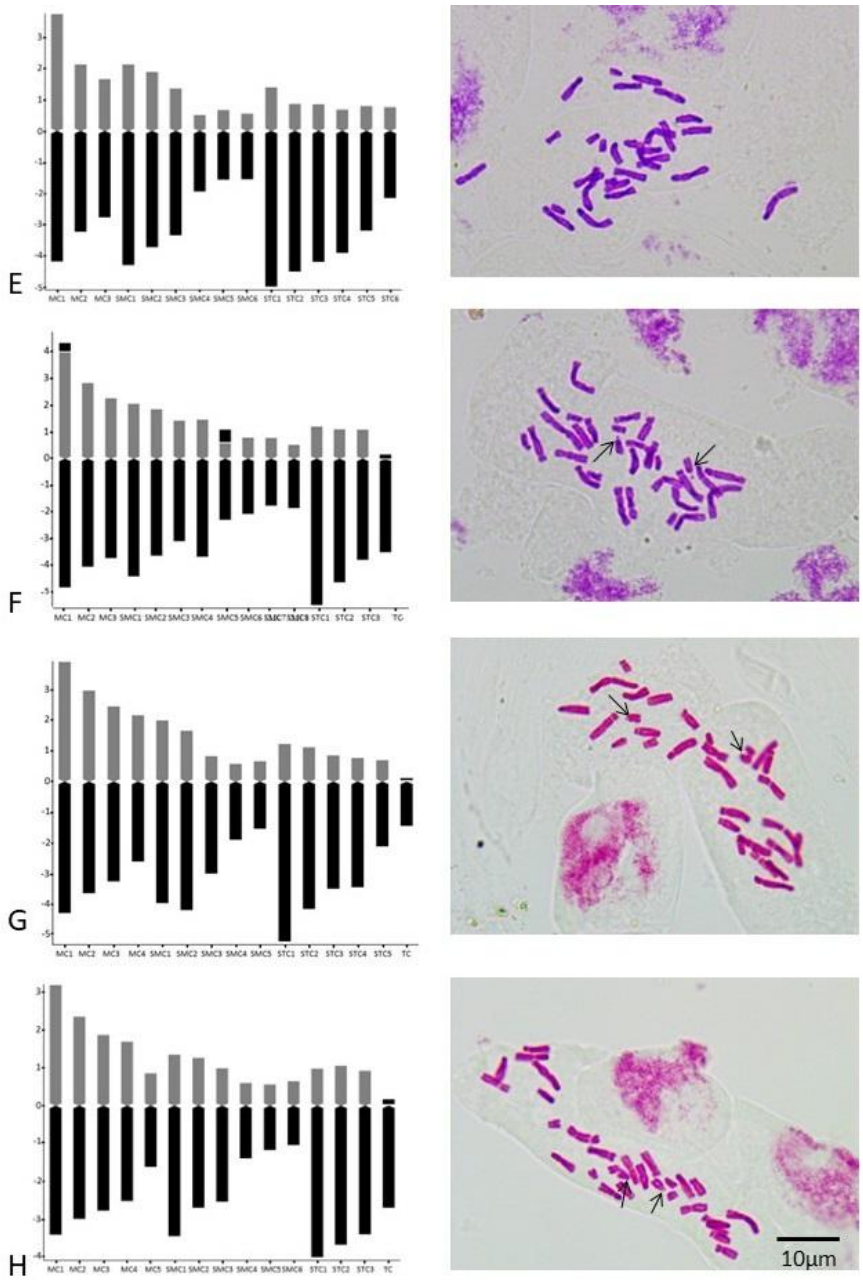

Fig. 7. Mitotic chromosomes and ideograms of *N. serotinus* s.l. with ISTE voucher numbers: E) 113704, F) 113705, G) 113706, H) 113707.

Taken together, these results have significant taxonomic implications. The discovery of stable 2n = 30 cytotypes in Turkey calls for a revaluation of current classifications within the *N. serotinus* complex, which have historically been based on Iberian and North African material. Our findings support recognizing *N. miniatus* as a separate species—based on morphology, cytogenetics, and genomics—and suggest that other intermediate forms might also warrant taxonomic recognition after further molecular research. Conversely, *N. deficiens* may not be a true species but rather an early developmental stage or hybrid derivative, as previously proposed but not formally tested (Mifsud and Lanfranco, 2024).

Conclusion

This study offers important new insights into the biogeographic and taxonomic patterns of *N. serotinus* s.l. in the eastern Mediterranean. It is the first report of 2n = 30 cytotypes in Turkish populations, broadening the known range of cytogenetic diversity within the genus. By integrating morphological, karyological, and flow cytometry data, we reveal the existence of multiple distinct lineages within Turkish *N. serotinus* s.l. populations, some of which closely resemble *N. miniatus* or hybrid intermediates.

These findings are globally relevant, as they expand the taxonomic and geographic range of a model plant group within the Mediterranean biodiversity hotspot. They also show the importance of interdisciplinary approaches—combining field botany, cytogenetics, and genomics—in solving long-standing taxonomic issues. Most importantly, they emphasize the eastern Mediterranean, especially Turkey, as a key region for understanding the evolutionary processes that influence geophytic plant diversity.

Key Contributions to International Literature

1. **First documentation of 2n = 30 *N. serotinus* s.l. cytotypes in Turkey,** revealing a new eastern Mediterranean lineage.
2. **Support the recognition of *N. miniatus* as a separate species,** helping to resolve synonymy disputes.
3. **A practical framework for combining flow cytometry and karyomorphometry** in *Narcissus* taxonomy.
4. **Reinforces Turkey's role as a biogeographic and evolutionary bridge** connecting eastern and western Mediterranean flora.

Further genomic studies, especially involving genome-wide sequencing and comparisons of type specimens, are necessary to fully define species boundaries and trace the evolutionary history of these complex lineages. This work provides a foundation for such efforts and emphasizes the importance of preserving these genetically unique populations, which significantly contribute to the overall story of Mediterranean plant evolution and speciation.

Authors' contributions

E.Z – research concept, supervision, analysis and/or interpretation, critical; M.K. – research concept and design, resources, materials, data collection, literature search, writing manuscript; Ş.A. – research concept, data collection, critical review; M.T. – supervision, critical review; M.E.Ö. – critical review.

Conflicts of interest

Authors declare no conflicts of interest.

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