Chromosome-number diversity and evolution in Nitrariaceae: a critical synthesis of cytological, genome-size and genomic evidence

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Abstract

Chromosome-number data in Nitrariaceae are dispersed across historical cytological reports, regional compilations and recent flow-cytometric and genomic studies, and their biological interpretation is complicated by uneven sampling, taxonomic change and somatic chromosome-number variation. We critically synthesised chromosome-related evidence for the family using ChromoDB as the principal documentary dataset and Plants of the World Online as the contemporary taxonomic framework. The reviewed dataset comprised 218 records, more than 99% of which belonged to *Nitraria* and *Peganum*. A recurrent 12-based chromosome organisation emerged as the best-supported operational framework in both genera, with independently supported diploid and tetraploid states centred on 2n = 24 and 2n = 48. Genuine organismal polyploidy was demonstrated by concordant chromosome counts with flow-cytometric genome-size estimates in *Nitraria* and by regular meiotic behaviour in *Peganum harmala*. In contrast, several *Nitraria* species showed extensive somatic chromosome-number heterogeneity, including documented intraindividual mixoploidy; in other cases, multiple chromosome complements were associated with the same accession or voucher but the exact biological scale remained unresolved. Such additional chromosome classes were therefore not interpreted automatically as independent cytotypes. Genome-size data further showed that chromosome number and nuclear DNA content are only partially coupled. Discordant observations, including n = 6 and historical 2n = 22 in *P. harmala*, 2n = 18 in *Nitraria retusa* and an attributed 2n = 14 in *Tetradiclis tenella*, remain important but do not justify alternative basic numbers or specific dysploid pathways by arithmetic alone. The synthesis also shows that taxonomic reassignment and database structure can substantially alter the apparent chromosome spectrum of a species. We conclude that x = 12 is a robust operational framework for extant *Nitraria* and *Peganum*, but its ancestral status within Nitrariaceae remains unresolved. Future progress requires voucher-based, biologically resolved cytology integrated with meiosis, flow cytometry and chromosome-scale comparative genomics.

Keywords: basic chromosome number; chromosome databases; flow cytometry; genome size; mixoploidy; polyploidy; somatic chromosome variation

1. Introduction

Chromosome number is one of the most fundamental descriptors of genome organisation and has long played a central role in plant cytotaxonomy, systematics and evolutionary biology. Variation in chromosome number can reflect major genomic processes, including whole-genome duplication, chromosome fusion and fission, and other forms of chromosome-number change, and may therefore provide information on both lineage diversification and genome evolution (Guerra, 2008; Rice et al., 2015). At the same time, chromosome numbers require

careful biological interpretation. Numerically similar complements may have different evolutionary origins, whereas apparently different chromosome numbers may represent variation occurring at different levels of organisation, from stable population cytotypes to somatic variation within an individual. Consequently, chromosome-number data are most informative when their taxonomic, bibliographic and biological contexts are preserved (Carta et al., 2020).

Nitrariaceae provides a particularly suitable system in which to examine these issues. The family is currently placed within Sapindales and, under the taxonomic framework adopted by Plants of the World Online, comprises four genera: *Malacocarpus*, *Nitraria*, *Peganum* and *Tetradiclis* (APG IV, 2016; POWO, 2026). These genera have a complex taxonomic history. *Nitraria*, *Peganum*, *Malacocarpus* and *Tetradiclis* were historically associated with Zygophyllaceae under different subfamilial concepts, but morphological and molecular studies progressively supported their separation and their placement within Sapindales (Sheahan & Chase, 1996; Bachelier et al., 2011). More recent phylogenomic analyses have reinforced the distinct position of Nitrariaceae within Sapindales, with the family recovered close to the base of the order in broad nuclear datasets (Joyce et al., 2023; Ma et al., 2023).

Despite its relatively small taxonomic size, Nitrariaceae encompasses considerable ecological, geographical and genomic diversity. *Nitraria* currently includes ten accepted species, whereas *Peganum* comprises four; *Malacocarpus* comprises one accepted species, and *Tetradiclis* comprises two accepted species. Several members of the family are characteristic of arid, saline or otherwise environmentally stressful habitats across Eurasia, North Africa, Australia and the Americas. Recent genomic work has also begun to provide chromosome-scale resources for the family, including a reference genome for *Nitraria sibirica* and a chromosome-scale assembly for *Peganum multisectum* (Ma et al., 2023; Xu et al., 2025). These resources create new opportunities to interpret classical chromosome-number data in the context of genome structure and evolution.

The cytogenetic literature of Nitrariaceae has accumulated unevenly over many decades and is dispersed among original chromosome-count papers, regional studies, historical compilations and recent genomic investigations. This heterogeneity is a general challenge in plant cytogenetics: database aggregation can make dispersed observations accessible, but it does not by itself resolve differences in source provenance, taxonomic usage, sampling scale or biological independence (Rice et al., 2015; Gutiérrez et al., 2023). A value repeated in several secondary sources may ultimately derive from a single historical observation, whereas intensive analysis of one accession may generate several chromosome-number records.

These issues are particularly relevant in Nitrariaceae because the available evidence combines meiotic and somatic counts, B chromosomes, flow-cytometric estimates of nuclear DNA content and chromosome-scale genomic data. Several species of *Nitraria* also show extensive chromosome-number variation among somatic cells (Muratova et al., 2013; Banaev et al., 2024, 2025), so multiple numerical complements may coexist within the same accession or individual. Conversely, meiotic behaviour and genome-size evidence can support genuine differences in organismal ploidy. Distinguishing these biological levels is therefore essential if chromosome-number diversity is to be interpreted evolutionarily rather than merely enumerated.

Taxonomic history adds a second source of ambiguity. Historical chromosome counts may have been published under names now treated as synonyms or under species circumscriptions that differ from current ones. Updating such records to a contemporary taxonomic backbone is necessary for synthesis, but the original identification must remain traceable because taxonomic

reassignment can alter the apparent chromosome spectrum of an accepted species. The distinction between published and currently accepted names is therefore integral to a critical reassessment of the family's cytogenetic record.

A further unresolved question concerns the evolutionary meaning of the chromosome numbers themselves. Previous studies have repeatedly reported complements related to multiples of 12 in *Nitraria* and *Peganum*, but additional chromosome states —including lower and discordant numbers— have also been published. Interpreting this diversity requires distinguishing an operational basic number useful for describing extant variation from an ancestral chromosome number inferred for the family. As emphasised more generally in plant cytotaxonomy, basic chromosome numbers cannot be reconstructed reliably by simple arithmetic division of observed somatic counts (Guerra, 2008). Chromosome-number evolution may involve both polyploid and dysploid processes and identifying their direction and historical sequence increasingly requires integration of cytological data with phylogenetic and genomic evidence (Carta et al., 2020).

Against this background, a family-wide critical synthesis must do more than enumerate chromosome numbers. It should assess the strength and biological scale of the evidence; distinguish stable cytotypes from somatic variation and unresolved historical records, account for changes in taxonomic attribution, and separate uneven documentary coverage from genuine biological variability. These issues are especially important in database-derived analyses, where record abundance is not necessarily proportional to biological sampling or cytotype diversity.

The present study therefore provides a critical synthesis of chromosome-related evidence for Nitrariaceae using ChromoDB as the principal documentary dataset and POWO as the contemporary taxonomic framework. Specifically, we aimed to: (1) assess the taxonomic coverage and distribution of available chromosome evidence across the family; (2) identify the principal recurrent chromosome complements and evaluate the evidence supporting their interpretation as stable ploidy states; (3) distinguish organismal polyploidy from somatic chromosome-number variation and other forms of nuclear variation; (4) critically reassess discordant chromosome numbers and historically proposed basic numbers without relying on arithmetic inference alone; (5) evaluate how taxonomic reassignment and database structure influence the apparent chromosome spectrum of individual taxa; and (6) identify the major cytogenetic gaps and priorities for future study. By integrating documentary provenance, current taxonomy, cytological context, flow-cytometric evidence and chromosome-scale genomic information, the review seeks to provide a more biologically explicit framework for interpreting chromosome-number evolution in Nitrariaceae.

2. Materials and methods

2.1. Study design and taxonomic scope

This study was conducted as a critical review and descriptive synthesis of published chromosome-related evidence for Nitrariaceae. No new experimental data were generated. The analysis was based on an export of ChromoDB and on a taxonomic checklist derived from Plants of the World Online (POWO). The principal aims were to characterise the documented chromosome-number diversity of the family, assess taxonomic coverage, identify discordant or weakly supported records, and distinguish biologically different forms of chromosome variation that may be conflated in flat database summaries.

The taxonomic scope comprised the four genera accepted in the adopted POWO framework: *Malacocarpus*, *Nitraria*, *Peganum* and *Tetradiclis*. Accepted species were used as the principal units for assessing taxonomic coverage. Infraspecific taxa were retained when explicitly represented in the source material but were not counted as additional species. The POWO treatment current during data curation was used as the contemporary taxonomic backbone; historical names were retained separately from the accepted names to preserve the nomenclatural context of the original chromosome observations.

2.2. ChromoDB dataset and analytical unit

The starting dataset consisted of the ChromoDB records assigned to Nitrariaceae, together with their bibliographic provenance, taxonomic assignment, chromosome notation, geographical attribution, verification category and available comments. The working export was preserved as an unchanged reference snapshot, while corrections and additions identified during the review were evaluated separately before incorporation into the curated dataset.

ChromoDB is available at https://chromodb.net/, with the public database interface at https://database.chromodb.net/. The frozen analytical dataset used for this synthesis (data freeze: 6 September 2026) is openly archived in Zenodo (Simon Pallisé & Bosch Daniel, 2026): https://doi.org/10.5281/zenodo.22538050.

A ChromoDB record was treated as a documentary database unit rather than as an independent biological observation. In the current data model, a row represents a chromosome value associated with a taxon, a bibliographic source and a geographical attribution; consequently, several chromosome values reported from the same accession or individual may generate several records, whereas one record may summarise observations from several individuals or populations. Record counts were therefore used to describe documentary coverage and sampling intensity, not to estimate cytotype frequencies or the number of independent biological samples.

Where the source allowed reconstruction of the underlying biological hierarchy, records were interpreted in relation to population, voucher or accession, individual, tissue or cell, and modal versus additional chromosome complements. This distinction was essential in *Nitraria*, where multiple chromosome classes can coexist within a single accession or individual.

2.3. Literature retrieval and source verification

Bibliographic work combined the existing ChromoDB reference corpus with targeted retrieval of original publications and supplementary searches for potentially missing or problematic evidence. Scopus and Web of Science were used as multidisciplinary bibliographic indexes, Google Scholar as a complementary academic search engine, and JSTOR and PubMed Central as additional routes for locating and consulting publications. Academia.edu and ResearchGate were used only as access routes when they facilitated retrieval of otherwise difficult-to-obtain papers; the platform through which a document was accessed was not treated as the bibliographic source of the chromosome observation.

Because the study was built on an existing retrospectively assembled chromosome database, it was not conducted as a de novo systematic review based on a single reproducible search string. Retrieval was iterative and source-oriented. Particular attention was given to discordant chromosome numbers, exceptional values, records based on secondary compilations, and cases whose taxonomic attribution affected biological interpretation. When an original publication could not be consulted, that limitation was retained explicitly rather than treating repetition in later compilations as independent confirmation.

The same bibliographic reference encountered through several platforms was counted once. Likewise, a later publication reproducing or discussing an earlier chromosome count was not regarded automatically as a new independent observation. Publication identity and biological independence were assessed separately whenever the available information allowed this distinction.

Source-level inconsistencies were not silently harmonised. For example, Banaev and Tomoshevich (2021) provide non-identical abbreviated and detailed chromosome-number lists for voucher NSK 3000958; this discrepancy was retained as part of the documentary provenance and the corresponding values were interpreted in their source context.

2.4. Record eligibility and evidence classification

Records were considered eligible when a chromosome-related datum could be associated with a taxon within the study scope and with an identifiable bibliographic source. Missing locality, country or voucher information did not by itself lead to exclusion but restricted the biological or geographical interpretation that could be assigned to the record.

Three broad forms of evidence were distinguished during synthesis: (i) direct cytological observations, including somatic or meiotic chromosome counts and meiotic configurations; (ii) derived or inferred chromosome information reported in a source; and (iii) chromosome-scale genomic evidence. Statements of ploidy without a traceable chromosome observation were not converted into chromosome counts. Chromosome-scale pseudomolecules were retained as genomic evidence but were not treated as direct observations of n or 2n.

ChromoDB verification labels were interpreted as documentary, not experimental, categories. “Original Verified” indicated that the original source had been consulted during database compilation, whereas “Unverified Citation” identified an attribution not yet verified directly against the original publication. These labels were not used as proxies for experimental quality, taxonomic correctness or biological independence. During the present review, records with discordant values, unusual complements or unresolved secondary sourcing were prioritised for renewed source checking.

2.5. Taxonomic and geographical harmonisation

Taxonomic harmonisation was performed against POWO while preserving the distinction between the name used in the original publication and the currently accepted name. A historical synonym could therefore be mapped to an accepted taxon for contemporary synthesis without rewriting the original identification. This procedure was particularly important where taxonomic reassignment altered the apparent chromosome spectrum of an accepted species.

Taxonomic reassignment was based on the adopted POWO synonymy and, where necessary, on the contextual information available in the original source. Geographical coincidence alone was not considered sufficient to transfer a chromosome record between species. When historical identification remained uncertain, the record retained its original published name, and the uncertainty was reflected in its interpretation.

Country and locality information were assigned from the provenance of the biological material whenever this could be established from the source. They were not inferred from author affiliation, the general title of a publication or the known distribution of the species. Unresolved geographical information was left unassigned or treated explicitly as uncertain.

2.6. Treatment of chromosome information

Gametic (n) and somatic (2n) chromosome numbers were retained separately. Approximate counts, ranges, B chromosomes and meiotic configurations were preserved in their reported form. B chromosomes were treated separately from the A-chromosome complement; for example, n = 6 + 0–2B was not converted into n = 6–8. Approximate values were not treated as exact integers.

Ploidy levels were assigned only when a chromosome-number interpretation based on an explicit basic-number framework was corroborated by biologically independent evidence, particularly flow-cytometric genome-size estimates or regular meiotic behaviour. Chromosome-scale genomic information was used as complementary evidence of chromosome organisation but was not considered sufficient, by itself, to assign a cytological n, 2n or ploidy level. Arithmetic divisibility alone was not considered sufficient to establish ploidy. Accordingly, counts compatible with 3x, 5x or other levels were not automatically interpreted as stable organismal cytotypes when they occurred as additional cellular classes within mixoploid material.

The same conservative principle was applied to unusual chromosome numbers. Alternative basic numbers, aneuploidy and dysploidy were not inferred solely from halving a somatic count or from deviation from the predominant 12-based framework. Terms such as “compatible with” were used where arithmetic relationships were suggestive but independent biological support was lacking.

Somatic chromosome-number variation (mixoploidy) was distinguished from organismal polyploidy and from variation in nuclear DNA content detected by flow cytometry. When several chromosome complements were associated with the same accession, voucher or collection, their biological scale was evaluated from the source: they were treated as intraindividual cellular variation only when the publication explicitly established that the counts derived from different cells of the same individual or tissue. Otherwise, they were retained as chromosome-number heterogeneity associated with the same biological material, with the within-individual versus among-individual scale left unresolved. Flow-cytometric 4C, 8C or higher DNA-content classes were treated as evidence of endopolyploid nuclear states where reported by the authors but were not used by themselves to infer the underlying cellular mechanism or a change in whole-individual chromosome complement or ploidy.

2.7. Duplicate handling and biological independence

Potential duplication was evaluated by comparing taxon, source, geographical attribution, chromosome notation and the contextual information available for the underlying material. Import duplicates, repeated citations of an earlier observation and genuinely distinct chromosome observations were treated separately. When a secondary source reproduced an earlier count, the documentary chain was retained, but the repeated citation was not interpreted as independent biological confirmation.

Likewise, separate publications were not assumed to represent independent biological material solely because they had different bibliographic identifiers. Where later studies cited, reused, reanalysed or extended previously characterised accessions, the number of publications was not used as a proxy for the number of independent samples. Conversely, several distinct ChromoDB rows derived from different chromosome classes in one voucher were not interpreted as several independent cytotypes.

2.8. Data synthesis and reproducibility

The synthesis was descriptive. Taxonomic coverage was summarised by genus and accepted species, with coverage expressed as the number of accepted species represented by eligible chromosome-related evidence relative to the number of accepted species in the adopted taxonomic framework. Chromosome-related records were used to characterise documentary representation, recurrent modal complements and the range of reported chromosome evidence. Because record frequency does not equal biological frequency, no estimate of natural cytotype prevalence was derived from the number of database rows.

Interpretation was based on the combined context of chromosome notation, source type, voucher or accession information where available, geographical provenance, modal versus non-modal status, and independent evidence from meiosis, flow cytometry or chromosome-scale genomics. Direct cytological counts inferred values and genomic evidence were kept conceptually distinct throughout the synthesis.

Absence statements were restricted to the reviewed corpus. Thus, taxa for which no evidence was recovered were described as having no chromosome records recovered in the reviewed dataset rather than as globally lacking chromosome information. The analytical snapshot, taxonomic correspondence and bibliographic provenance were retained so that the synthesis can be audited and updated as ChromoDB and the underlying taxonomic framework evolve.

The Zenodo deposit contains the curated chromosome-record and associated bibliographic data underlying the present synthesis and should be treated as the version-specific analytical snapshot. ChromoDB itself is continuously curated and may therefore contain later additions or corrections not represented in the archived dataset.

3. Results

3.1. Dataset and taxonomic coverage

The reviewed dataset comprised 218 chromosome-related records assigned to Nitrariaceae and was linked to 48 bibliographic sources in the frozen analytical dataset. Records were distributed highly unevenly among the four genera recognised in the adopted taxonomic framework: *Nitraria* accounted for 181 records (83.0%), *Peganum* for 35 (16.1%), and *Malacocarpus* and *Tetradiclis* for one record each (0.5% each).

Taxonomic coverage was also heterogeneous. Eight of the ten accepted species of *Nitraria* were represented by chromosome-number evidence, with no records recovered for *N. iliensis* or *N. sphaerocarpa*. All four accepted species of *Peganum* were represented, although *P. multisectum* contributed chromosome-scale genomic evidence rather than a conventional cytological count. *Malacocarpus crithmifolius* and *Tetradiclis tenella* each had a single chromosome record, whereas no record was recovered for *T. corniculata*. Taxonomic coverage by genus is summarised in Table 1.

Table 1. Taxonomic coverage of chromosome-related evidence in Nitrariaceae by genus.

Genus	Accepted species in POWO	Species with chromosome-related evidence	Species with ≥1 direct observation	Species with ≥1 Original Verified observation	Records
Malacocarpus	1	1	1	0	1
Nitraria	10	8	8	7	181
Peganum	4	4	3	3	35
Tetradiclis	2	1	1	0	1
Total	17	14	13	10	218

Note. "Original Verified" is a documentary verification label, not a measure of experimental quality. Within Nitraria*,* N. billardierei *is the only represented accepted species with no Original Verified record in the working export.*

Table 2. Species-level synthesis of chromosome-related evidence in the reviewed Nitrariaceae dataset.

Accepted taxon	Records	Principal chromosome-related evidence	Interpretation / principal caveat
Malacocarpus crithmifolius	1	2n = 24	Single historical attributed count; ploidy unresolved.
Nitraria billardierei	1	2n = 48	Australian material published as N. schoberi and reassigned under current taxonomy; 4x not independently demonstrated.
Nitraria iliensis	0	No record recovered	No chromosome-number evidence recovered in the reviewed corpus.
Nitraria komarovii	4	2n = 48 modal; 60 additional; 2C = 2.23–2.32 pg	Predominantly 4x = 48; 60 treated as additional/non-modal evidence.
Nitraria pamirica	7	2n = 24 and 48; 2C = 1.50 and 3.15 pg	Diploid- and tetraploid-level material strongly supported; additional somatic/nuclear heterogeneity reported.
Nitraria retusa	8	n = 12; 2n = 18, 24, 84, 96	Taxonomically heterogeneous historical evidence; 18 remains discordant; 84/96 derive from material published as N. schoberi var. faurei.
Nitraria roborowskii	1	2n = 36	Compatible with 3x under x = 12, but stable triploidy not demonstrated.
Nitraria schoberi	119	2n = 48 modal; broad additional chromosome range; one FCM-based ~8x accession	Predominantly 4x = 48 with extensive chromosome-number heterogeneity; ~8x condition lacks direct ca. 96 count for that accession.
Nitraria sibirica	30	n = 12; 2n = 24 modal; additional counts; 2C = 1.24–1.34 pg; 12 chromosome-scale units	Predominantly 2x = 24; somatic chromosome heterogeneity and higher DNA-content nuclear states reported.
Nitraria sphaerocarpa	0	No record recovered	No chromosome-number evidence recovered in the reviewed corpus.
Nitraria tangutorum	11	2n = 24 modal; 26, 36, 48, 60, 72 additional; 2C ~ 1.57–1.65 pg	Predominantly 2x = 24; multiple values linked to voucher material, with exact biological scale not always resolved.
Peganum harmala	29*	n = 6, 12, 24; 2n = 22, 24; 12 and 24 bivalents	Widely supported 2x = 24 and demonstrated 4x = 48 cytotype; n = 6 and historical 2n = 22 remain evolutionarily unresolved.
Peganum mexicanum	3	n = 12	Best-documented meiotic state; compatible with 2n = 24, but no direct somatic count in the reviewed corpus.
Peganum multisectum	1	12 chromosome-scale pseudomolecules	Chromosome-scale genomic evidence only; not a direct cytological n or 2n count.
Peganum nigellastrum	2	n = 12; 2n = 24	Meiotic and somatic evidence directly support a 24-chromosome complement.
Tetradiclis corniculata	0	No record recovered	No chromosome-number evidence recovered in the reviewed corpus.
Tetradiclis tenella	1	Attributed 2n = 14	Primary cytological source unresolved; x = 7 or diploidy not inferred.

*Note. * The Peganum harmala total includes 28 species-level records plus one record explicitly assigned to P. harmala var. stenophyllum. Record counts describe documentary representation, not independent individuals, populations or cytotypes.*

Sampling was strongly concentrated in a few taxa. *Nitraria schoberi* contributed 119 records and *N. sibirica* 30, together accounting for 149 of the 181 records of *Nitraria*. Within *Peganum*, most records belonged to *P. harmala*, while the other three species were represented by only one to three records each. This marked species-level imbalance is shown in Figure 1; the principal chromosome evidence and interpretative caveats for all accepted species are summarised in Table 2.

Geographical representation was likewise uneven: Russia, Kazakhstan, China and Tajikistan together accounted for 165 records (75.7% of the dataset), while 10 records lacked a country code (Figure 3).

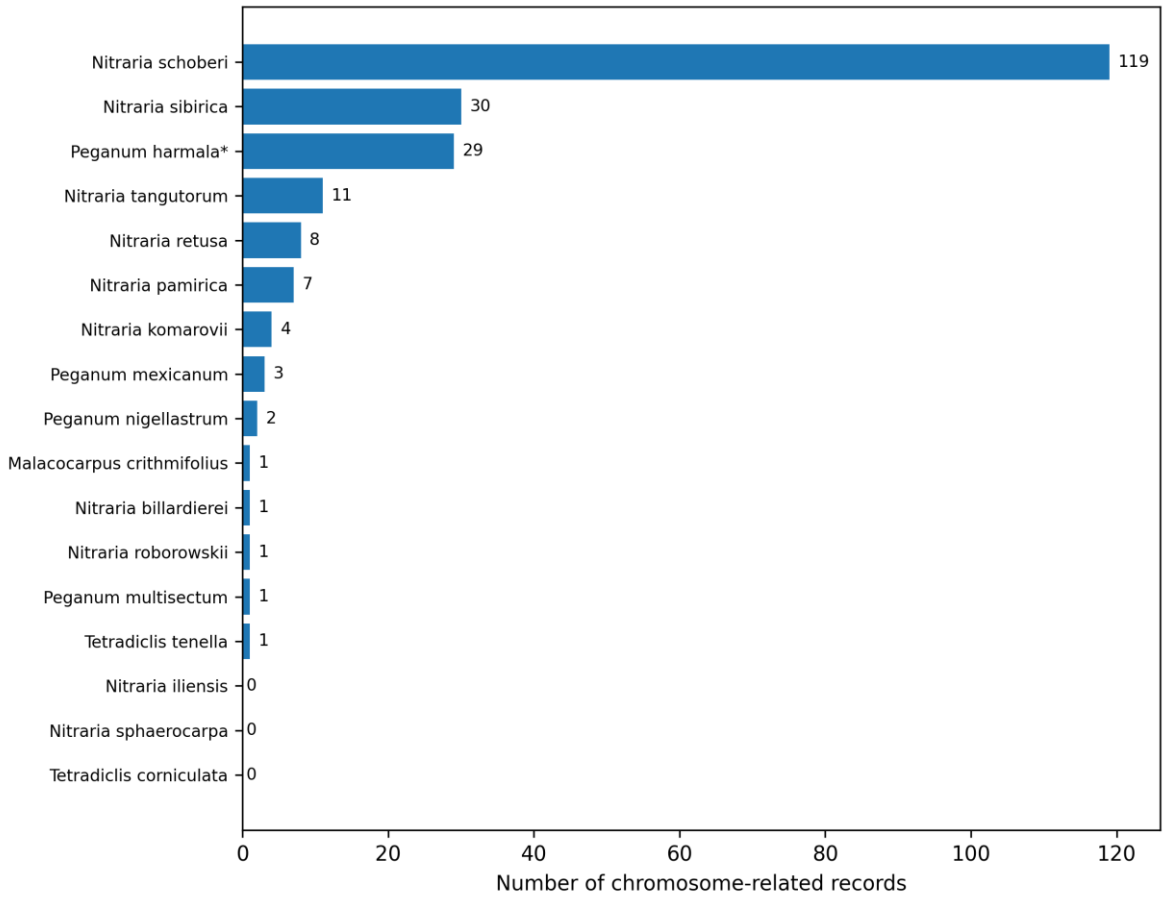

Figure 1. Species-level documentary representation in the reviewed Nitrariaceae dataset (n = 218 records). The P. harmala total (29) includes one record explicitly assigned to P. harmala var. stenophyllum; zero-value taxa had no chromosome-related record recovered in the reviewed corpus.

3.2. Family-wide chromosome-number diversity

Somatic chromosome numbers represented in the reviewed dataset ranged from 2n = 14 to approximately 2n = 96, while meiotic observations included n = 6, n = 12 and n = 24, together with records of B chromosomes. This numerical range comprised biologically different types of observations, including modal complements, distinct cytotypes, non-modal somatic chromosome classes and historical discordant counts.

Direct cytological observations constituted 195 of the 218 records, compared with 21 records classified as inferred chromosome information and two records based on chromosome-scale genomic evidence (Figure 2).

Despite this breadth, chromosome complements related to 12 chromosomes dominated the two best-sampled genera. In *Nitraria*, 2n = 24 and 2n = 48 were the principal recurrent modal complements. In *Peganum*, n = 12/2n = 24 was the most widely documented state, while chromosome-scale genomic evidence from *P. multisectum* was likewise organised into 12 pseudomolecules.

Distinct ploidy levels were documented in both major genera. Within *Nitraria*, independently supported diploid and tetraploid complements occurred among and, in *N. pamirica*, within species. In *N. schoberi*, one Astrakhan accession additionally showed a flow-cytometrically inferred DNA-ploidy level compatible with approximately 8x, but without a direct chromosome

count of ca. 96 for that accession. In *Peganum harmala*, meiotic evidence demonstrated both n = 12/2n = 24 and n = 24/2n = 48 cytotypes.

Several chromosome observations fell outside the dominant 24/48 framework. These included n = 6 and n = 6 + 0–2B and historical 2n = 22 records in *P. harmala*, 2n = 18 in *N. retusa*, and 2n = 14 in *Tetradiclis tenella*. These values were retained as distinct observations without automatically assigning alternative basic chromosome numbers or ploidy levels.

The exceptionally broad numerical spectrum recorded in *Nitraria* was largely generated by additional somatic chromosome classes superimposed on comparatively stable modal complements. Modern studies demonstrated intraindividual chromosome-number heterogeneity in some material, while in other cases multiple values were associated with the same accession or voucher without the exact within-individual scale being fully resolvable from the source. Accordingly, the number of distinct chromosome values represented in the database substantially exceeds the number of independently demonstrated cytotypes.

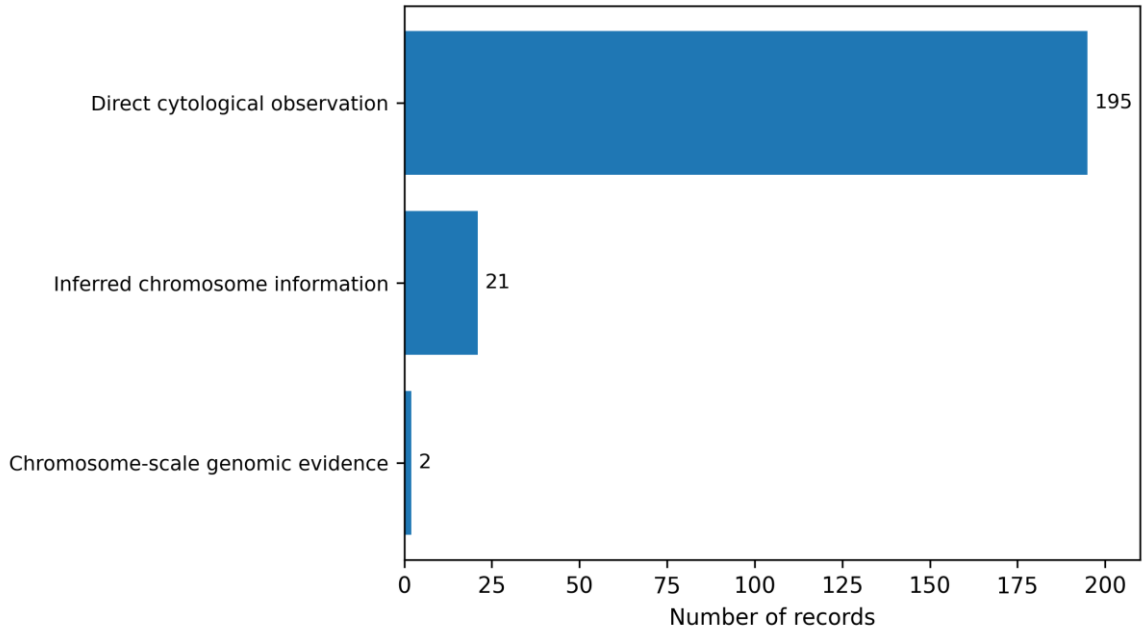

Figure 2. Evidence-class composition of the frozen analytical dataset (n = 218): direct cytological observation (195), inferred chromosome information (21), and chromosome-scale genomic evidence (2).

3.3. *Nitraria*

3.3.1. General cytogenetic pattern

Across *Nitraria*, the best-resolved modern studies identified 2n = 24 and 2n = 48 as the principal recurrent modal chromosome complements. Under the operational basic number x = 12 used in the cytological literature, *N. sibirica* and *N. tangutorum* were predominantly characterised as 2n = 2x = 24, whereas *N. schoberi*, *N. pamirica* and *N. komarovii* were predominantly characterised as 2n = 4x = 48. These assignments were supported in the best-studied material by combinations of chromosome counts and flow cytometry (Muratova et al., 2013; Banaev et al., 2024) and, in N. sibirica, chromosome-scale genomic evidence (Ma et al., 2023).

A characteristic feature of the genus was the coexistence of relatively stable modal complements with extensive somatic chromosome-number heterogeneity. In several species, multiple

chromosome numbers were documented within the same accession, voucher, seedling or individual, depending on the biological resolution provided by the source, including both exact multiples of 12 and counts not corresponding to exact multiples of 12. Where cellular origin within a single individual was explicitly demonstrated, these were treated as somatic mixoploidy; where that scale was unresolved, they were retained more conservatively as chromosome-number heterogeneity associated with the same material rather than as separate population-level cytotypes.

This somatic variation coexisted with independently supported differences in organismal ploidy. In *N. pamirica*, chromosome complements of 24 and 48 were associated with approximately proportional differences in nuclear DNA content in paired accessions from the same locality, supporting distinct diploid- and tetraploid-level material (Banaev et al., 2020). In *N. schoberi*, an Astrakhan accession had 2C = 5.75 pg and was interpreted by flow cytometry as approximately 8x; because the ca. 96 value was inferred rather than obtained as a direct chromosome count for that accession, it was retained as DNA-ploidy evidence compatible with a higher-ploidy condition rather than as independent cytological confirmation (Banaev et al., 2020).

Accordingly, chromosome diversity in *Nitraria* was interpreted at two principal biological levels: stable differences in ploidy among individuals or populations and non-modal chromosome variation among somatic cells. Endopolyploidy, where documented, was treated as an additional and conceptually distinct form of nuclear variation. Chromosome numbers were therefore not assigned to separate cytotypes solely because they represented arithmetic multiples of x = 12.

The intensity of available evidence remained strongly unequal among species. Extensive cellular variation is particularly well documented in the most intensively sampled taxa, whereas several other species are represented by only one or a few chromosome observations. Differences in the known breadth of chromosome variation among species must therefore be interpreted in the context of markedly unequal sampling effort.

3.3.2. *Nitraria schoberi* and *Nitraria sibirica*

Nitraria schoberi and *N. sibirica* were the two best-represented species in the dataset, with 119 and 30 records, respectively. Together they accounted for 149 of the 181 records assigned to *Nitraria*. Modern population-level studies revealed contrasting modal complements: 2n = 48 in *N. schoberi* and 2n = 24 in *N. sibirica*. Under x = 12, these complements were interpreted as predominantly tetraploid and diploid, respectively (Muratova et al., 2013; Banaev et al., 2024).

In *N. schoberi*, 2n = 48 was repeatedly recovered from geographically separated material and remained the modal chromosome number in the most comprehensive recent analyses, although numerous additional non-modal complements were also documented. Flow-cytometric estimates provided independent support for the tetraploid interpretation, with nuclear DNA contents of approximately 2C = 2.93–3.39 pg in material characterised as approximately 4x (Banaev et al., 2024). Thus, 2n = 48 represents the principal chromosome complement of the species rather than simply one value among the numerous counts stored in ChromoDB.

The species nevertheless exhibited exceptionally extensive chromosome-number heterogeneity. Recorded chromosome values in the reviewed corpus extended from 2n = 24 to high values approaching 96, and modern studies associated multiple chromosome classes with individual vouchers or accessions (Banaev & Tomoshevich, 2021; Banaev et al., 2024). Values such as 36, 60 or 72, as well as numerous intermediate counts, were therefore not treated automatically as

independent cytotypes. Where the source explicitly demonstrated that different complements occurred among cells of the same individual, the pattern was interpreted as somatic mixoploidy; otherwise, the within-individual scale was left unresolved. The available data characterise N. schoberi as predominantly 2n = 48 with extensive additional chromosome-number variation.

A distinct higher-DNA-ploidy condition was reported for one accession from the Astrakhan region. Banaev et al. (2020) obtained 2C = 5.75 pg and classified the material by flow cytometry as approximately 8x, corresponding arithmetically to ca. 96 chromosomes under x = 12. The source did not provide a direct chromosome count of ca. 96 for this accession. The record is therefore best treated as DNA-ploidy evidence compatible with an approximately octoploid condition, pending direct cytological confirmation.

Nitraria sibirica showed a complementary pattern. Although ChromoDB contains n = 12 and somatic counts ranging from 2n = 16 to 60, population studies consistently identified 2n = 24 as the modal chromosome complement. Muratova et al. (2013) found 24 chromosomes as the predominant count across southern Siberian populations, despite the occurrence of cells with 36, 48, 60 and other chromosome numbers. Banaev et al. (2024) subsequently confirmed the modal 24 condition and characterised the species as 2n = 2x = 24, with nuclear DNA contents of approximately 2C = 1.24–1.34 pg.

This diploid interpretation is additionally consistent with chromosome-scale genomic evidence. The reference genome of *N. sibirica* was assembled into 12 chromosome-scale units (Ma et al., 2023), while the 2n = 2x = 24 characterisation cited in that study derives from earlier cytological literature rather than from a new chromosome count of the sequenced material. The genomic architecture therefore complements, but does not independently replicate, the cytological evidence supporting 24 as the principal chromosome complement of *N. sibirica*.

As in *N. schoberi*, much of the additional numerical diversity in *N. sibirica* occurred at the cellular level. Recent analyses documented both multiples of 12 and counts not corresponding to exact multiples of 12 within material whose modal complement remained 24. In addition, flow cytometry detected endopolyploid nuclei up to 16C (Banaev et al., 2024). Endopolyploidy and chromosome-number mixoploidy were retained as distinct observations, as increased nuclear DNA content through endoreduplication does not itself establish a change in organismal chromosome complement or ploidy level.

Taken together, the two species illustrate the hierarchical structure of chromosome variation in *Nitraria*. *N. schoberi* is predominantly tetraploid, with 2n = 48, whereas *N. sibirica* is predominantly diploid, with 2n = 24. Both nevertheless contain a broad spectrum of additional somatic chromosome numbers, so that the number of distinct counts recorded substantially exceeds the number of independently supported ploidy states.

3.3.3. *Nitraria tangutorum*, *N. pamirica* and *N. komarovii*

Nitraria tangutorum, *N. pamirica* and *N. komarovii* were represented by substantially fewer records than *N. schoberi* and *N. sibirica*, but modern studies combining chromosome counts with flow cytometry allowed their principal chromosome complements to be resolved with relatively high confidence.

Nitraria tangutorum was represented by 11 records, all from Chinese material. Recorded chromosome numbers were 2n = 24, 26, ca. 26, 36, 48, 60 and 72. Despite this numerical range, the available evidence consistently identified 2n = 24 as the modal complement. Banaev et al. (2020) recorded 24 and 26 chromosomes within the same voucher from Ningxia and obtained a nuclear DNA content of 2C = 1.57 pg, interpreting the material as approximately 2n = 2x = 24.

Subsequent flow-cytometric analyses yielded comparable DNA-content values and retained 24 as the modal chromosome number.

Recent material from Gansu substantially expanded the number of additional chromosome classes documented in the species (Banaev et al., 2025). Individual vouchers were associated with combinations of 24/36/48, 24/36/48/60/72 or 24/48 chromosomes (Banaev et al., 2025). These combinations demonstrate chromosome-number heterogeneity linked to the same voucher material, but the IAPT report alone does not establish whether every value derives from different cells of a single individual, several seedlings, or other biologically connected material. They were therefore not interpreted as separate triploid, tetraploid, pentaploid and hexaploid cytotypes; the precise scale of the heterogeneity was retained as unresolved where the source did not specify it. The best-supported characterisation of *N. tangutorum* remains a predominantly 2n = 24 taxon with substantial additional chromosome-number variation.

Nitraria pamirica was represented by seven records and provided particularly clear evidence for intraspecific ploidy differentiation. Banaev et al. (2020) reported two accessions collected at the same locality in the eastern Pamir with contrasting chromosome-number and nuclear-DNA measurements: one was associated with 2n = 24 and 2C = 1.50 pg, whereas the second was associated with 2n = 48 and 2C = 3.15 pg. The near-doubling of nuclear DNA content in parallel with chromosome number strongly supports diploid- and tetraploid-level material, while avoiding an assumption that every cytological and flow-cytometric measurement necessarily came from the exact same organism unless explicitly documented by the source.

Both accessions originated from the same locality and collection date, showing that the two ploidy levels occur within locally sampled *N. pamirica* material. Subsequent analyses identified 2n = 48 as the modal or typical complement of the species, with nuclear DNA contents of approximately 2C = 3.10–3.30 pg, indicating that the tetraploid condition predominates in the currently sampled material (Banaev et al., 2024). However, the available evidence remains geographically restricted and does not permit estimation of the relative frequency of diploid and tetraploid plants across the species range.

True ploidy differentiation in *N. pamirica* coexisted with additional somatic variability. The species was included among the mixoploid taxa analysed by Banaev et al. (2024), and both chromosome-number heterogeneity and endopolyploidy were reported. *N. pamirica* therefore provides an example in which stable genome-level differences in ploidy and cellular chromosome-number variation occur within the same species.

Nitraria komarovii showed a simpler chromosome pattern. ChromoDB contained four records, all from Kazakhstan, with chromosome numbers of 2n = 48 and 60. A direct count of 2n = 48 was reported by Banaev et al. (2018), and the subsequent comparative study of Banaev et al. (2024) again identified 48 as the modal chromosome number. Nuclear DNA contents of approximately 2C = 2.23–2.32 pg supported its classification as predominantly 2n = 4x = 48 (Banaev et al., 2024).

The only additional chromosome number documented in *N. komarovii* was 2n = 60. Because 48 remained the modal complement and the species was characterised overall as tetraploid and mixoploid, the 60-chromosome observation was retained as a non-modal cellular class rather than interpreted as evidence of a stable pentaploid cytotype. Compared with *N. schoberi* or *N. sibirica*, the currently documented extent of chromosome-number variation is much more limited, although the substantially lower sampling intensity prevents direct comparison of its true frequency or complexity.

The three species therefore show distinct relationships between modal chromosome numbers and additional variation. *N. tangutorum* is predominantly 2x = 24 with multiple higher chromosome classes occurring within individual vouchers; *N. pamirica* includes independently supported 2x and 4x individuals, with 4x = 48 predominating in the available material; and *N. komarovii* is predominantly 4x = 48 with a limited additional cellular class of 60 chromosomes.

3.3.4. Poorly sampled *Nitraria* species

Three accepted species of *Nitraria* were represented by very limited chromosome evidence, and two additional species lacked chromosome records entirely. These taxa were treated separately from the better-sampled species because their available chromosome numbers could not be used to infer modal complements, ploidy frequencies or the extent of somatic variation with comparable confidence.

Nitraria retusa was represented by eight records, including n = 12 and somatic chromosome numbers of 2n = 18, 24, 84 and 96. These values did not derive from a taxonomically uniform set of observations. The counts of 18 and 24 were published directly under *N. retusa*, whereas the high numbers 84 and 96 originated from North African material published historically as *N. schoberi* var. *faurei*, a name treated under the currently accepted concept of *N. retusa* in the present dataset. Consequently, the apparent range 2n = 18–96 was not interpreted as a continuous intraspecific chromosome series.

The directly documented records were themselves heterogeneous. A count of 2n = 18 was reported from Libyan material (Bhattacharya et al., 1971), whereas 2n = 24 and n = 12 were documented from other parts of the species range (Hilu, 1979; Malallah et al., 2001). The n = 12/2n = 24 observations are congruent with the dominant chromosome framework elsewhere in *Nitraria*, but the 2n = 18 record remains numerically discordant. No independent evidence of genome size or meiotic behaviour is available that would justify assigning a separate basic number or stable ploidy level to this complement. Likewise, the historical high counts reported for the taxonomically reassigned faurei material—2n = 96 in Reese (1957) and 2n = 84 and 96 in Reese (1958) were retained as chromosome observations rather than interpreted automatically as stable high-polyploid cytotypes.

Nitraria billardierei was represented by a single record of 2n = 48 from Australian material studied by Reese (1962). The original publication identified the material as *N. schoberi*, whereas the record is assigned to *N. billardierei* under the current taxonomic treatment. Both the original published name and the current accepted name were retained in order to preserve the historical identification while applying the present taxonomic framework.

Although 2n = 48 is numerically compatible with the tetraploid complement established in several Eurasian species of *Nitraria*, no independent genome-size estimate, meiotic evidence or population-level replication is available for *N. billardierei*. The chromosome number was therefore reported as a documented complement without formally assigning the species to a 4x level.

Nitraria roborowskii was also represented by a single chromosome record, 2n = 36, obtained from material collected in Inner Mongolia, China, and linked to voucher NSK 3009806 (Banaev et al., 2025). Under x = 12, the count is numerically compatible with 3x, but no flow-cytometric, meiotic or population-level evidence was available to demonstrate a stable triploid condition. Because 36 chromosomes have also been recorded as a non-modal cellular class in other species of *Nitraria*, the ploidy status of this observation remains unresolved.

No chromosome records were recovered for *N. iliensis* or *N. sphaerocarpa* in the reviewed dataset. Both species were therefore classified as lacking chromosome-number evidence in the present corpus, without inferring chromosome complements from related species. Together with the single-record representation of *N. billardierei* and *N. roborowskii*, these absences show that nominal chromosome coverage of eight of the ten accepted species substantially overstates the evenness of cytogenetic knowledge across the genus.

3.4. *Peganum*

3.4.1. General pattern and *Peganum harmala*

Peganum was represented by 35 records. Of these, 28 were assigned to *P. harmala* and one additional record to *P. harmala* var. *stenophyllum*. All four accepted species possessed some form of chromosome-related evidence, although *P. multisectum* was represented by chromosome-scale genomic evidence rather than by a direct cytological count. Across the genus, n = 12 and 2n = 24 were the most widely distributed chromosome complements.

Most of the chromosome-number diversity of *Peganum* was concentrated in *P. harmala*. The dataset included n = 6, n = 6 + 0–2B, n = 12, n = 24, 2n = 22 and 2n = 24. These values represented different types of evidence rather than a single equivalent series of chromosome races. Repeated somatic and meiotic observations established n = 12/2n = 24 as the most widely documented complement, whereas n = 24 represented a higher-ploidy state, n = 6 a lower meiotic complement, n = 6 + 0–2B the same A-chromosome complement with supernumerary chromosomes, and 2n = 22 remained restricted to historical discordant reports.

The predominant 24-chromosome condition was geographically widespread. Somatic counts of 2n = 24 and meiotic observations of n = 12 were reported from material spanning the Mediterranean region, North Africa and large parts of western, central and southern Asia. Flow-cytometric data from one Iberian and 17 Tunisian populations yielded nuclear DNA contents of approximately 2C = 0.61–0.67 pg, and a direct somatic count of 2n = 24 was obtained from Tunisian material (Hajji et al., 2017).

Clear evidence of a higher ploidy cytotype was obtained from the Kashmir Himalaya and Ladakh Trans-Himalaya. Aslam et al. (2023) documented populations with 12 bivalents, corresponding to 2n = 2x = 24, in Kashmir, and populations with 24 bivalents, corresponding to 2n = 4x = 48, in Ladakh. Meiosis was predominantly regular in both cytotypes, providing direct evidence that the higher chromosome number represents a stable tetraploid complement rather than somatic chromosome-number variation.

An older meiotic record of n = 24 from Mongolia was also present in the dataset (Hanelt, 1973). Thus, the 48-chromosome condition cannot be regarded as restricted to the Himalayan material, although its geographical extent and frequency remain insufficiently resolved.

A second, qualitatively different chromosome condition was documented in Rajasthan, India, where meiotic studies reported n = 6. The same material included observations of n = 6 + 0–2B, documenting between zero and two B chromosomes in addition to the six A chromosomes (Gupta et al., 2016). The B chromosomes were recorded separately and were not incorporated into the basic chromosome count.

Finally, the dataset contained two historical records of 2n = 22, one attributed to Italian material (Negodi, 1937) and one to Xinjiang, China (Ma et al., 1984). These records were retained as discordant chromosome observations. Later literature questioned the interpretation of the

Chinese report (Aslam et al., 2023), but the historical observations were not removed from the synthesis solely on that basis.

At the infraspecific level, *P. harmala* var. *stenophyllum* was represented by a single meiotic record of n = 12 (Singh, 1984), congruent with the predominant complement of the species. No comparable chromosome records were distinguished in the reviewed dataset for var. *harmala* or var. *grandiflorum*.

Overall, the chromosome evidence for *P. harmala* consisted of a widely distributed n = 12/2n = 24 state, a directly demonstrated n = 24/2n = 48 tetraploid cytotype, a locally documented n = 6 complement with B chromosomes, and two unresolved historical 2n = 22 records.

3.4.2. Other *Peganum* species

The remaining three accepted species of *Peganum* were represented by substantially less chromosome evidence than *P. harmala*, but each contributed information consistent with the recurrent 12-chromosome framework observed across the genus.

Peganum mexicanum was represented by three meiotic records, all from Mexican material. Each reported n = 12, including one observation explicitly described as 12 II (Keil & Pinkava, 1979; Ward, 1984; Powell et al., 2017). No alternative chromosome numbers, B chromosomes or higher-ploidy complements were represented in the reviewed dataset. The available evidence therefore supports n = 12 as the best-documented chromosome state of the species. Under the operational x = 12 framework used for comparison within the genus, this is compatible with an expected somatic complement of 2n = 24, although no direct somatic count was represented in the present corpus.

Peganum nigellastrum was represented by two records, both from Mongolian material: one meiotic observation of n = 12 (Hanelt, 1973) and one somatic count of 2n = 24 (Měsíček & Soják, 1972). The agreement between meiotic and somatic chromosome numbers provides direct support for a 24-chromosome complement. No additional chromosome states were represented in the reviewed dataset.

The evidence for *Peganum multisectum* was methodologically different. The species was represented by a single chromosome-scale genomic record rather than by a conventional chromosome count. Xu et al. (2025) produced a genome assembly of approximately 314 Mb, with 98.64% of the assembly assigned to 12 chromosome-scale pseudomolecules. This genomic organisation was retained as independent evidence of a 12-unit haploid chromosome architecture, but it was not treated as a cytological observation of n = 12.

Accordingly, the three poorly sampled species showed no documented chromosome-number diversity comparable with that of *P. harmala*. *P. mexicanum* was consistently represented by n = 12, *P. nigellastrum* by n = 12/2n = 24, and *P. multisectum* by a chromosome-scale genome organised into 12 pseudomolecules. However, the low number of studies does not permit these species to be regarded as cytogenetically invariant.

Taken together, these species broaden the taxonomic distribution of the 12-chromosome condition within *Peganum*. Direct cytological evidence for n = 12 occurs in *P. mexicanum* and *P. nigellastrum*, while *P. multisectum* provides complementary chromosome-scale genomic evidence.

3.5. *Malacocarpus* and *Tetradiclis*

Malacocarpus and *Tetradiclis* were the least represented genera in the reviewed dataset, with one chromosome record each. This limited evidence precluded assessment of intraspecific chromosome variation, cytotype frequencies or confidently established basic chromosome numbers.

Malacocarpus crithmifolius was represented by a single historical record of 2n = 24, attributed to Zakharyeva and Astanova (1968). No meiotic data, flow-cytometric estimates, chromosome-scale genomic evidence or independent population-level replication were available in the reviewed corpus. Accordingly, 2n = 24 was retained as the documented chromosome complement without automatically interpreting it as 2x.

Tetradiclis tenella was represented by a single record of 2n = 14, attributed to Astanova (1993). The record remained among the citations not directly verified from the primary cytological source, and the available compilation did not establish whether Astanova (1993) reported an original observation or reproduced an earlier count. The chromosome number was therefore retained as an attributed record without assigning x = 7 or a diploid ploidy level solely by arithmetic inference.

The second accepted species of the genus, *Tetradiclis corniculata*, had no chromosome records in the reviewed corpus. No inference was therefore made as to whether it shares the 2n = 14 complement attributed to *T. tenella*.

Thus, the chromosome evidence available for these two genera consisted essentially of 2n = 24 in *M. crithmifolius* and 2n = 14 in *T. tenella*, with no data for *T. corniculata*. The extremely limited sampling means that neither the numerical similarity of *Malacocarpus* to the 24-chromosome complements common in *Nitraria* and *Peganum*, nor the divergent 14-chromosome count of *Tetradiclis*, can yet be treated as a genus-level cytogenetic pattern.

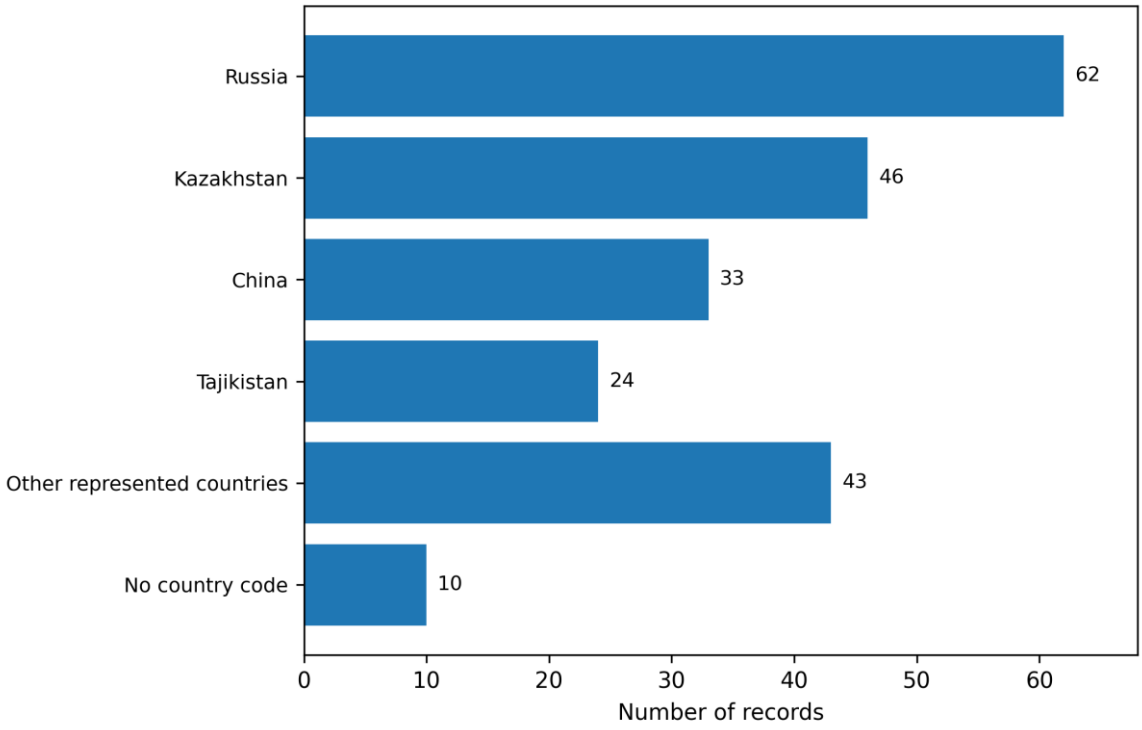

Figure 3. Geographical representation of the 218 chromosome-related records in the frozen analytical dataset. Russia (62), Kazakhstan (46), China (33) and Tajikistan (24) were the four most represented

country codes; 43 records came from all other represented countries combined and 10 lacked a country code.

4. Discussion

4.1. Is x = 12 the central chromosome framework of Nitrariaceae?

The most conspicuous chromosome pattern emerging from the present synthesis is the recurrent importance of 12 chromosomes as a haploid organisation or operational basic number in the two best-sampled genera of Nitrariaceae. In *Nitraria*, the principal modal complements are 2n = 24 and 2n = 48, and modern studies combining cytology with flow cytometry support their interpretation as diploid and tetraploid states, respectively, in several species (Muratova et al., 2013; Banaev et al., 2024). In *Peganum*, n = 12/2n = 24 is likewise the most widely distributed chromosome state, being directly documented in *P. harmala*, *P. mexicanum* and *P. nigellastrum*, while the chromosome-scale genome of *P. multisectum* is organised into 12 pseudomolecules (Xu et al., 2025). Taken together, these independent lines of evidence make x = 12 the best-supported operational chromosome framework for the two principal genera of the family.

The strength of this pattern lies not merely in the numerical recurrence of 24 chromosomes. In *Nitraria*, the 24/48 relationship is independently corroborated by nuclear DNA content in well-characterised material. Particularly compelling is *N. pamirica*, in which paired accessions from the same locality were associated with 2n = 24/2C = 1.50 pg and 2n = 48/2C = 3.15 pg, respectively (Banaev et al., 2020). In *N. sibirica*, a reference genome assembled into 12 chromosome-scale units is consistent with the recurrent cytological 2n = 24 complement, but does not constitute a new chromosome count (Ma et al., 2023). In *P. harmala*, populations with 12 and 24 bivalents support coherent lower- and higher-ploidy chromosome states (Aslam et al., 2023). These lines of evidence make x = 12 a useful operational framework for comparison, but they do not by themselves establish x = 12 as the ancestral basic number.

Nevertheless, the distinction between an operational basic number and an ancestral basic number is critical. The present evidence supports x = 12 as the most useful framework for describing extant chromosome variation in *Nitraria* and *Peganum*, but it does not demonstrate that x = 12 was the ancestral state of Nitrariaceae. Numerical similarity between genera does not by itself establish evolutionary homology, and the strongly uneven taxonomic coverage of the family further limits ancestral reconstruction. More than 99% of the records analysed here belong to *Nitraria* and *Peganum*, whereas *Malacocarpus* and *Tetradiclis* are represented by only one chromosome record each. Any family-wide ancestral hypothesis based primarily on frequency would therefore be heavily biased towards the two best-sampled lineages.

The evidence from *Peganum harmala* illustrates particularly well why the evolutionary meaning of x = 12 remains unresolved. In addition to the widespread n = 12/2n = 24 condition and the demonstrated tetraploid n = 24/2n = 48 cytotype, meiotic material with n = 6 has been reported, including individuals bearing 0–2 B chromosomes (Gupta et al., 2016). Because n = 6 is a directly observed meiotic complement, it cannot simply be dismissed as an arithmetic anomaly. However, its existence does not by itself establish x = 6 as the ancestral basic number of either *Peganum* or Nitrariaceae. The n = 6 state could represent an ancestral condition, a derived chromosome-number reduction, or a lineage-specific reorganisation. Resolving these alternatives requires comparative structural genomic evidence rather than numerical parsimony alone.

The historical reports of 2n = 22 in *P. harmala* introduce an additional complication. These counts were once used to support x = 11 in *Peganum*, but their evidential basis is much weaker than that of x = 12. At least one of the historical 2n = 22 reports has subsequently been questioned, while the widespread occurrence of n = 12/2n = 24 across several species provides substantially broader taxonomic support for a 12-based organisation. Nevertheless, the existence of more than one historical 2n = 22 report argues against simply deleting this state from consideration. At present, x = 11 is best regarded as a historically proposed but insufficiently corroborated hypothesis rather than as an established basic number (Negodi, 1937; Ma et al., 1984; Aslam et al., 2023).

The chromosome evidence from the two poorly sampled genera does not resolve this problem. *Malacocarpus crithmifolius* has a single attributed count of 2n = 24, which is numerically compatible with a 12-based system but lacks meiotic, genomic or flow-cytometric evidence demonstrating x = 12 or a diploid condition. Numerical agreement with *Peganum* or diploid *Nitraria* cannot therefore be treated as proof of chromosome homology. Conversely, the single attributed count of 2n = 14 in *Tetradiclis tenella* is strikingly discordant with the dominant 12-based framework. If confirmed, it may indicate a substantially different chromosome history within the family, but the primary cytological source of this count remains unresolved and *T. corniculata* is entirely unsampled. The current evidence is therefore insufficient to infer x = 7 for *Tetradiclis*, much less to reconstruct a transition between x = 12 and x = 7 at family level. Other discordant numbers reinforce the same methodological caution. The directly checked published report of 2n = 18 in *Nitraria retusa* cannot legitimately be converted into x = 9 merely by halving the somatic number, particularly because n = 12 and 2n = 24 are also documented in the species. Similarly, the existence of extensive somatic mixoploidy in *Nitraria* means that chromosome numbers that are arithmetically compatible with alternative basic numbers or ploidy levels may instead represent non-modal cellular complements. Consequently, ancestral chromosome-number reconstruction in Nitrariaceae cannot be based on the smallest observed divisor or on an unweighted inventory of published counts (Zakharyeva & Astanova, 1968; Astanova, 1993; Bhattacharya et al., 1971; Hilu, 1979; Malallah et al., 2001).

The present evidence therefore supports a hierarchical interpretation. First, x = 12 is the dominant and best-supported operational framework for extant *Nitraria* and *Peganum*. Second, this pattern may reflect a deep feature of chromosome evolution in Nitrariaceae, but its homology across genera remains to be demonstrated. Third, n = 6 in *P. harmala*, the historical 2n = 22 records, 2n = 18 in *N. retusa* and the attributed 2n = 14 of *T. tenella* show that a single unmodified x = 12 series cannot explain all observations currently assigned to the family.

A robust reconstruction of the ancestral chromosome state will therefore require explicit phylogenetic and genomic testing. Particularly informative would be chromosome-scale genomic comparisons between *Nitraria* and *Peganum*, structural analysis of the n = 6 and n = 12 cytotypes of *P. harmala*, and first modern cytogenetic characterisations of *Malacocarpus* and *Tetradiclis*. The genome of *P. multisectum* provides a useful starting framework: its 12 chromosome-scale pseudomolecules strongly reinforce the contemporary importance of a 12-unit organisation, but do not distinguish whether this represents an ancestral x = 12 state or the outcome of an older duplication from a lower basic number (Xu et al., 2025).

For these reasons, the most defensible conclusion is not that x = 12 is the ancestral basic number of Nitrariaceae, but that x = 12 constitutes the central operational chromosome framework presently supported for the family, particularly for *Nitraria* and *Peganum*. Establishing whether

that framework is ancestral, convergent, or itself derived remains one of the major unresolved questions in the chromosome evolution of Nitrariaceae.

4.2. Polyploidy as an evolutionary component of Nitrariaceae

Polyploidy represents a recurrent component of chromosome evolution in Nitrariaceae, but its interpretation requires stricter criteria than the mere occurrence of chromosome numbers that are multiples of a presumed basic number. This is especially important in *Nitraria*, where somatic mixoploidy can generate chromosome classes that superficially resemble conventional polyploid levels. The strongest evidence for true polyploidy therefore comes from cases in which chromosome-number change is supported independently by nuclear DNA content or regular meiotic behaviour.

Within *Nitraria*, the best-resolved species form a broad 2x–4x framework based on x = 12. *N. sibirica* and *N. tangutorum* are predominantly characterised by 2n = 2x = 24, whereas *N. schoberi*, *N. pamirica* and *N. komarovii* are predominantly 2n = 4x = 48. These assignments are not based exclusively on chromosome-number arithmetic; in the modern comparative material, they are supported by flow-cytometric estimates of nuclear DNA content (Banaev et al., 2024). Thus, polyploid differentiation appears to contribute substantially to interspecific chromosome diversity within the genus.

The clearest demonstration of this process occurs in *N. pamirica*. Banaev et al. (2020) documented two individuals collected at the same locality with 2n = 24/2C = 1.50 pg and 2n = 48/2C = 3.15 pg. The approximately twofold increase in both chromosome number and nuclear DNA content provides unusually strong evidence that these plants represent distinct diploid and tetraploid states rather than different cellular classes within a mixoploid individual. Their occurrence at the same locality further demonstrates that ploidy differentiation can occur locally within a single species. Subsequent sampling indicates that the tetraploid condition predominates among the material studied to date, although the geographical distribution and relative frequency of the two cytotypes remain insufficiently known (Banaev et al., 2024).

Nitraria schoberi provides evidence that genome duplication may extend beyond the predominant tetraploid condition, but the strongest higher-ploidy record is flow-cytometric rather than cytological. One Astrakhan accession had 2C = 5.75 pg and was classified as approximately 8x by Banaev et al. (2020), corresponding arithmetically to ca. 96 under x = 12. Because no direct ca. 96 chromosome count was reported for this accession, the observation supports a higher DNA-ploidy condition compatible with octoploidy, pending direct cytological confirmation. It should therefore be distinguished from both verified high chromosome counts in other material and non-modal chromosome classes associated with mixoploid accessions.

The distribution of tetraploidy among several *Nitraria* species raises an important evolutionary question that cannot yet be resolved from chromosome counts alone: did the 4x condition arise once or repeatedly within the genus? *N. schoberi*, *N. pamirica* and *N. komarovii* all possess modal 2n = 48, but equality of chromosome number does not demonstrate a common polyploid origin. Their nuclear DNA contents also differ substantially; for example, N. komarovii has considerably less nuclear DNA than *N. schoberi* or *N. pamirica* despite sharing the same modal chromosome complement (Banaev et al., 2024). These differences demonstrate genome-size divergence among lineages sharing 2n = 48, but do not establish whether that divergence occurred after a shared polyploidisation event, preceded independent polyploid origins, or reflects different progenitor genomes. Phylogenomic and syntenic analyses will be required to distinguish among these alternatives.

Polyploidy is independently demonstrated in *Peganum harmala*, but through a different type of evidence. Aslam et al. (2023) found populations from Kashmir with 12 bivalents and 2n = 2x = 24, whereas populations from Ladakh showed 24 bivalents and 2n = 4x = 48. The predominantly regular meiotic behaviour of both complements establishes them as biologically coherent diploid and tetraploid cytotypes rather than somatic variants. Thus, the 24–48 transition in *P. harmala* represents one of the clearest examples of conventional polyploid differentiation in Nitrariaceae.

The geographical segregation of these cytotypes is potentially important. In the Himalayan material studied by Aslam et al. (2023), diploids occurred in Kashmir and tetraploids in the higher-elevation Ladakh Trans-Himalaya. This pattern is consistent with ecological differentiation between ploidy levels, but the available evidence does not demonstrate that polyploidisation itself caused adaptation to higher, colder or more arid environments. The limited number of sampled populations means that the geographical association should presently be treated as an observed pattern rather than as evidence of adaptive causation.

The comparison between *Nitraria* and *Peganum* therefore indicates that polyploidy has arisen in both major genera but does not establish whether their polyploid events are evolutionarily related. Under the operational x = 12 framework, both contain well-supported 2x = 24 and 4x = 48 states. However, the two genera differ markedly in how those states are expressed and detected. In *P. harmala*, the principal cytotypes occur as discrete populations with regular meiotic configurations. In *Nitraria*, stable ploidy differences are superimposed upon extensive somatic chromosome-number variation, making chromosome counts alone much more difficult to interpret.

This distinction is essential because polyploidy and chromosome-number heterogeneity are not interchangeable phenomena. A 48-chromosome cell within a demonstrably 24-modal individual would not by itself establish a tetraploid cytotype, just as values of 36, 60 or 72 cannot automatically be designated triploid, pentaploid or hexaploid states. In *Nitraria tangutorum*, several such chromosome classes are associated with the same voucher material, but the exact within-individual scale is not fully resolved by the IAPT report. Only when chromosome-number differences are demonstrated at the organismal or population level and are supported by appropriate independent evidence can they be interpreted confidently as stable ploidy states (Banaev et al., 2025).

The poorly sampled species further illustrate the danger of arithmetic assignment. The single 2n = 48 count currently attributed to *N. billardierei* is compatible with tetraploidy under x = 12, but no genome-size, meiotic or population-level data confirm this interpretation. Likewise, the historical 2n = 84 and 96 counts transferred taxonomically to *N. retusa* from material published as *N. schoberi* var. *faurei* cannot presently be treated as demonstrated 7x and 8x cytotypes. These records may ultimately prove to represent high polyploidy, somatic mixoploidy, or a combination of both, but the available evidence does not discriminate among these possibilities (Reese, 1957, 1958, 1962).

Overall, the evidence demonstrates that polyploidy is a genuine and recurrent evolutionary component of Nitrariaceae, rather than an artefact of chromosome-number compilation. Diploid and tetraploid states are independently supported in both *Nitraria* and *Peganum*, and *N. schoberi* provides evidence for an additional higher-ploidy condition. What remains unresolved is the historical structure of these events: how many independent polyploidisations occurred, whether the tetraploid species of *Nitraria* share a common origin, and whether polyploidy contributed directly to ecological or taxonomic diversification.

Resolving these questions will require chromosome-scale genomic comparisons among cytologically characterised diploids and polyploids. Such analyses could distinguish shared whole-genome duplications from recurrent polyploid origins and determine how extensively genome restructuring and DNA loss or gain have modified descendant genomes. Until then, the most robust interpretation is that Nitrariaceae contains a recurrent 2x–4x axis centred on the 24/48 chromosome relationship, locally extended to higher ploidy, but superimposed—particularly in *Nitraria*—on a separate layer of somatic chromosome-number instability.

4.3. Somatic mixoploidy: a defining feature of *Nitraria*?

One of the most distinctive patterns emerging from the present synthesis is recurrent somatic chromosome-number heterogeneity within *Nitraria*. In the best-studied species, modal chromosome complements coexist with additional chromosome classes, but the biological resolution differs among studies: some data demonstrate variation among cells or seedlings, whereas other records associate several values with the same accession or voucher without fully resolving whether they are intraindividual. The strongest evidence for somatic mixoploidy occurs in *N. schoberi* and *N. sibirica*, while *N. tangutorum* and other species also show substantial chromosome-number heterogeneity whose precise scale should be retained as reported. The available evidence therefore suggests that somatic chromosome instability is recurrent in the genus, but its frequency and biological scale remain unevenly documented.

The first strong evidence for this pattern came from population-level studies of *N. schoberi* and *N. sibirica*. Muratova et al. (2013) showed that the modal complements of these species—2n = 48 and 2n = 24, respectively—coexisted with additional chromosome numbers within the same material. In *N. sibirica*, cells with 36, 48 and 60 chromosomes occurred alongside the predominant 24-chromosome complement, whereas *N. schoberi* likewise contained cells with chromosome numbers substantially different from its modal 48. These observations demonstrate that a considerable proportion of chromosome-number diversity in *Nitraria* operates at the somatic and intraindividual level rather than exclusively among genetically distinct population cytotypes.

The later study of Banaev et al. (2024) reinforced this interpretation across five species by explicitly distinguishing modal chromosome numbers from additional cellular complements. *N. schoberi*, *N. pamirica* and *N. komarovii* were centred on modal 2n = 48, whereas *N. sibirica* and *N. tangutorum* were centred on modal 2n = 24. Yet additional chromosome classes were recorded either among plants of the same population or among cells of the same individual. This hierarchical structure is crucial: a species may possess a relatively stable organismal ploidy level while simultaneously displaying substantial cytological heterogeneity within somatic tissues.

Nitraria tangutorum provides an especially instructive example because recent voucher-resolved data reveal a highly ordered but biologically complex pattern. Some accessions contained cells with 24/36/48, others 24/36/48/60/72, and another 24/48. In voucher NSK 3009794, five chromosome classes corresponding arithmetically to 2x, 3x, 4x, 5x and 6x under x = 12 occurred within the same material, although the IAPT report does not fully resolve whether these values derive from different cells of one individual, different seedlings, or other biologically connected material. Treating these as five independent cytotypes would therefore be biologically untenable. The data instead demonstrate that multiple higher chromosome classes can coexist somatically within material whose principal ploidy condition remains diploid (Banaev et al., 2025).

This observation also illustrates why the numerical regularity of some cellular classes should not be overinterpreted mechanistically. In *N. tangutorum*, 24, 36, 48, 60 and 72 form successive multiples of 12, whereas *N. schoberi* and *N. sibirica* additionally contain numerous chromosome numbers that do not correspond to exact multiples of 12. The former pattern might suggest repeated genome multiplication, whereas the latter implies more complex chromosome gain or loss. However, chromosome counts alone cannot determine whether these classes arise through endoreduplication, mitotic nondisjunction, chromosome elimination, irregular cell divisions, or combinations of these processes. The available evidence identifies the phenomenon, but not its cellular mechanism.

The coexistence of chromosome-number heterogeneity and independently supported polyploidy makes *Nitraria* particularly complex. In *N. pamirica*, paired accessions with 24 versus 48 chromosomes and approximately proportional differences in nuclear DNA content support distinct diploid- and tetraploid-level material, while the species has also been reported to show additional somatic chromosome-number and nuclear-DNA heterogeneity. These phenomena must be analysed separately so that additional chromosome classes are not inflated into independent evolutionary cytotypes when their biological scale is unresolved.

Variation in nuclear DNA content represents a further level of complexity. In *N. sibirica*, flow cytometry detected nuclei up to 16C, while the species itself remains predominantly 2n = 2x = 24. These higher DNA-content classes were interpreted by Banaev et al. (2024) as endopolyploid nuclear states, but flow-cytometric DNA profiles alone do not identify the cellular mechanism responsible. They are therefore conceptually distinguished here from directly observed chromosome-number heterogeneity, while recognising that both may coexist in the same biological material and contribute to somatic genomic heterogeneity.

The contrast with *Peganum* is striking. No comparably extensive somatic chromosome mosaicism has yet been documented in that genus. The best-characterised chromosome differences in *P. harmala* occur among individuals or populations and are supported by coherent meiotic configurations, as in the diploid and tetraploid cytotypes documented by Aslam et al. (2023). This suggests that *Nitraria* and *Peganum* may differ fundamentally in the way chromosome variation is expressed: *Nitraria* through pronounced somatic plasticity superimposed on modal ploidy states, and *Peganum* primarily through discrete cytotypes. However, this contrast must remain provisional because the two genera have not been studied using identical cytological approaches. Intensive somatic-cell sampling has been particularly important in revealing the variability of *Nitraria*, whereas many *Peganum* studies have relied mainly on meiosis.

Sampling bias is equally important within *Nitraria*. The strongest evidence for mixoploidy comes from the most intensively investigated species. *N. schoberi* and *N. sibirica* account for the majority of records in the genus, whereas *N. billardierei* and *N. roborowskii* are each represented by a single chromosome observation, and *N. iliensis* and *N. sphaerocarpa* lack records entirely. Absence of documented mixoploidy in these taxa therefore cannot be interpreted as evidence of cytological stability. The apparent concentration of the phenomenon in certain species may partly reflect where cell-by-cell chromosome analyses have actually been performed.

This sampling problem also cautions against describing *Nitraria* simply as a “mixoploid genus”. A stronger conclusion is presently justified at a more restricted level: somatic mixoploidy is a recurrent and prominent feature of several well-studied *Nitraria* species and may represent an important genus-level cytogenetic tendency. Demonstrating that it is truly characteristic of the

genus will require comparable analyses across the poorly sampled species, ideally using standardised protocols that quantify modal chromosome number, frequencies of additional cellular complements, flow-cytometric profiles and tissue-specific variation.

The biological significance of this somatic instability remains largely unresolved. It is not yet clear whether most non-modal chromosome complements are restricted to particular tissues and developmental stages, whether some reach reproductive lineages, or whether they influence phenotype, stress tolerance or fitness. The available chromosome-count data do not establish heredity or adaptive function. Consequently, the evolutionary importance of mixoploidy should not be inferred solely from its numerical extent.

Somatic mixoploidy therefore appears to be one of the most informative features of *Nitraria* cytogenetics, not because every additional chromosome count represents an evolutionary state, but precisely because it reveals how inadequate a flat list of chromosome numbers can be for describing the genus. The emerging picture is of relatively stable modal ploidy states—principally 2x = 24 and 4x = 48—superimposed on an unusually dynamic somatic chromosome environment. Whether this architecture represents a distinctive evolutionary property of *Nitraria* remains to be tested, but it provides a central framework for interpreting both historical and modern chromosome records in the genus.

4.4. Chromosome-number change beyond the dominant 12-based framework

The chromosome-number diversity of Nitrariaceae cannot be reduced to a single 12-based 24/48 series. Several observations fall outside that dominant framework, including the meiotic n = 6 complement and historical 2n = 22 in *Peganum harmala*, 2n = 18 in *Nitraria retusa*, and the attributed 2n = 14 in *Tetradiclis tenella*. These values do not all imply the same evolutionary process. In particular, n = 6 could be related to n = 12 through genome duplication or, alternatively, through chromosome-number reduction in the opposite direction, whereas the 22, 18 and 14 complements raise additional hypotheses involving dysploid or lineage-specific chromosome change. The available evidence is insufficient to reconstruct these mechanisms directly (Negodi, 1937; Bhattacharya et al., 1971; Ma et al., 1984; Astanova, 1993; Gupta et al., 2016).

The strongest evidence for a genuinely lower meiotic chromosome complement occurs *in P. harmala*. Meiotic observations of n = 6 from Rajasthan document six chromosomes at meiosis in at least some material, rather than an inference derived from a somatic count (Gupta et al., 2016). This makes the relationship between n = 6 and the widespread n = 12 state evolutionarily important. Two broad scenarios remain possible: n = 6 may represent an ancestral or previously widespread condition from which the 12-chromosome organisation arose, potentially through genome duplication and subsequent diploidisation; alternatively, n = 6 may represent a derived reduction from an ancestor with n = 12 through chromosome fusion or other structural rearrangement. The chromosome observations themselves do not distinguish between these alternatives, nor do they alone demonstrate gamete functionality.

The genomic architecture of *P. multisectum* provides a potentially powerful framework for resolving this question. Its genome is organised into 12 chromosome-scale pseudomolecules, but this does not establish whether twelve represents the ancestral state or a derived architecture. Comparative synteny between the n = 6 and n = 12 cytotypes of *P. harmala*, ideally using chromosome-scale assemblies from cytologically verified individuals, could discriminate between competing hypotheses. Extensive one-to-two syntenic relationships might support an ancient duplication from six ancestral chromosomes, whereas large-scale fusion relationships

could instead support secondary reduction from a 12-chromosome ancestor. Until such evidence is available, describing n = 6 as equivalent to an ancestral x = 6 would be premature (Xu et al., 2025).

The historical 2n = 22 reports in *P. harmala* pose a different problem. These observations led to the traditional recognition of x = 11 alongside x = 12 in *Peganum*. However, their evidential support is considerably weaker than that of n = 6. The two records derive from Negodi (1937) in Italy and Ma et al. (1984) in Xinjiang, and the latter has subsequently been questioned because 2n = 24 was later reported from the same broad region. Aslam et al. (2023) therefore suggested that the Chinese 2n = 22 may have been erroneous. Nevertheless, the separate historical Italian report prevents the state from being dismissed altogether.

If confirmed, 2n = 22 could represent a dysploid derivative of a 24-chromosome complement, but several alternatives remain possible, including stable aneuploidy, individual somatic variation, technical error or historical misidentification. Consequently, the present data do not justify maintaining x = 11 as an established basic number. More importantly, they illustrate a general principle: an unusual somatic number should not be converted automatically into a new basic number merely by dividing it by two.

A parallel case occurs in *Nitraria retusa*. The directly published 2n = 18 count from Libyan material (Bhattacharya et al., 1971) is difficult to reconcile with the predominant x = 12 framework of *Nitraria*. However, the same accepted species also includes direct records of n = 12 and 2n = 24 (Hilu, 1979; Malallah et al., 2001). The coexistence of these states means that simply interpreting 2n = 18 as a diploid based on x = 9 would be unjustified.

Dysploid change is one possible explanation for the 18-chromosome complement, but it remains only a hypothesis. Confirmation would require repeated observations from independently identified material and, ideally, meiotic analysis, flow cytometry and chromosome-scale genomic information. Such evidence would determine whether 18 represents a stable population-level complement and whether its difference from 24 reflects chromosome fusion, fission or another structural process. Until then, 2n = 18 should be retained as a published discordant observation without assigning either x = 9 or a specific dysploid pathway.

The attributed 2n = 14 in *Tetradiclis tenella* is potentially even more consequential for family-wide chromosome evolution. If independently confirmed as the modal complement of the species, it would demonstrate a chromosome organisation markedly different from the 12-based system dominating *Nitraria* and *Peganum*. However, the record currently derives from Astanova (1993), apparently through a compilation whose relationship to the primary cytological observation remains unresolved. Moreover, no chromosome information is available for *T. corniculata*. It would therefore be premature to infer x = 7 for *Tetradiclis* or to postulate a direct evolutionary transition between x = 12 and x = 7.

These discordant numbers also need to be considered in the context of the extensive somatic instability documented in *Nitraria*. Counts that depart from the predominant multiples of 12 may sometimes reflect stable chromosome evolution, but they may also represent cellular variants within mixoploid individuals. The existence of numerous non-modal chromosome numbers in *N. schoberi* and *N. sibirica* demonstrates that not every deviation from 24 or 48 has equivalent evolutionary significance. Thus, distinguishing heritable dysploid change from somatic numerical instability requires information on the biological scale at which each count occurs.

Taken together, the available data show that chromosome evolution in Nitrariaceae cannot be represented by a single unmodified 24/48 series. The meiotic n = 6 complement in *P. harmala* is securely documented, while 2n = 22, 2n = 18 and 2n = 14 identify additional candidate cases of chromosome-number change outside the dominant framework. What remains unresolved is whether these states reflect genome duplication from a lower complement, dysploid evolution, alternative ancestral chromosome numbers, or more local and heterogeneous origins.

The most productive way forward is therefore not to expand the list of proposed basic numbers—x = 6, 7, 9, 11 and 12—on the basis of arithmetic alone, but to test explicit evolutionary hypotheses using phylogenetically placed and cytologically verified material. Chromosome-scale genomic comparisons could reveal fusion and fission histories, syntenic relationships and ancient duplication events that conventional chromosome counts cannot distinguish. Until such evidence is available, dysploidy should be regarded as a plausible component of chromosome evolution in Nitrariaceae, but not yet as a demonstrated explanation for any particular discordant count.

4.5. Genome size and chromosome evolution

The available flow-cytometric data show that chromosome number and genome size have not evolved in strict parallel within Nitrariaceae. Polyploidisation clearly affects total nuclear DNA content in some cases, but species sharing the same modal chromosome number and inferred ploidy level can differ substantially in 2C values. Genome size therefore provides information that cannot be recovered from chromosome counts alone and is particularly valuable for distinguishing true ploidy differences from superficially similar numerical complements.

The clearest example of proportional change occurs in *Nitraria pamirica*. Paired accessions associated with 2n = 24/2C = 1.50 pg and 2n = 48/2C = 3.15 pg provide strong evidence for diploid- and tetraploid-level material (Banaev et al., 2020). In *N. schoberi*, the Astrakhan accession with 2C = 5.75 pg was interpreted by flow cytometry as approximately 8x, but the corresponding ca. 96 chromosome value was inferred rather than directly counted for that accession. Genome-size evidence therefore strongly supports ploidy differentiation in *Nitraria*, while the degree of independent cytological corroboration differs among cases.

At the interspecific level, however, the relationship is much less simple. Among the predominantly tetraploid species of *Nitraria*, *N. schoberi*, *N. pamirica* and *N. komarovii* all share a modal complement of 2n = 48, yet their nuclear DNA contents differ markedly. Banaev et al. (2024) reported values of approximately 2C = 2.93–3.39 pg in *N. schoberi* and 3.10–3.30 pg in *N. pamirica*, but only 2.23–2.32 pg in *N. komarovii*. Thus, identical chromosome number and nominal ploidy do not imply equivalent genome size.

This disparity demonstrates substantial genome-size divergence among species sharing the same modal chromosome number. The difference is particularly striking in *N. komarovii*, whose nuclear DNA content is markedly lower than that of the other 2n = 48 species. Such variation is compatible with sequence gain or loss, repeat turnover, different ancestral genome sizes, or independent polyploid origins, but the present data do not establish the timing of these changes relative to polyploidisation. Consequently, chromosome number provides only one dimension of genome evolution in *Nitraria*.

The diploid species show a comparable, although less pronounced, pattern. *N. sibirica* and *N. tangutorum* are both predominantly 2n = 2x = 24, yet their nuclear DNA contents differ, with *N. sibirica* showing approximately 2C = 1.24–1.34 pg and *N. tangutorum* approximately 1.57–1.65

pg in the comparative material (Banaev et al., 2024). Thus, even within the same ploidy category and chromosome number, genome size can vary considerably among species.

The comparison between *N. sibirica and N. schoberi* is especially informative because their modal chromosome numbers differ twofold and their genome sizes also separate clearly. N. sibirica combines 2n = 24 with approximately 2C = 1.24–1.34 pg, whereas *N. schoberi* combines 2n = 48 with approximately 2C = 2.93–3.39 pg (Banaev et al., 2024). This relationship broadly supports the diploid–tetraploid distinction, but the values are not simply related by a fixed genome-size multiplier across all species. The additional comparison with *N. komarovii* demonstrates substantial genome-size divergence within the same nominal ploidy class without establishing when that divergence arose.

The chromosome-scale genome of *N. sibirica* adds an evolutionary constraint to this interpretation. Synteny and Ks analyses in Ma et al. (2023) did not identify a lineage-specific whole-genome duplication after the ancestral gamma triplication detected in the broader angiosperm history. This result does not resolve the origin of the 12-based chromosome framework, but it cautions against inferring a recent *N. sibirica*-specific genome duplication from chromosome number alone.

This distinction is important when interpreting the extensive somatic chromosome-number variation of *Nitraria*. A cell with a doubled chromosome number does not necessarily indicate a corresponding stable increase in whole-organism genome size. Conversely, flow-cytometric evidence showing a proportional shift in nuclear DNA content across individuals provides much stronger support for true ploidy differentiation. Genome size therefore functions as an independent line of evidence that helps separate organismal polyploidy from somatic mixoploidy.

The data from *Peganum harmala* provide a useful contrast. Hajji et al. (2017) reported 2C = 0.61–0.67 pg across one Iberian and 17 Tunisian populations, revealing a remarkably narrow genome-size range in geographically separated material representing the predominant 2n = 24 condition. This apparent stability contrasts with the broader interspecific variation documented in *Nitraria*. However, a major limitation remains: comparable flow-cytometric estimates are not yet available for the demonstrated tetraploid 2n = 48 cytotype of *P. harmala* or for the n = 6 material.

These missing data are particularly important. If the tetraploid cytotype of *P. harmala* were found to possess approximately twice the DNA content of the 2n = 24 cytotype, it would provide independent confirmation of genome duplication analogous to that observed in *N. pamirica*. Similarly, genome-size estimates from n = 6 plants could help discriminate between competing evolutionary interpretations. A substantially reduced genome could support one evolutionary trajectory, whereas a similar DNA amount distributed among fewer chromosomes would be more compatible with extensive chromosome fusion without equivalent sequence loss. Chromosome numbers alone cannot resolve this distinction.

The relationship between genome size and phenotype may add a further dimension. Banaev et al. (2024) examined associations between nuclear DNA content, ploidy and pollen morphology in *Nitraria* and generally found increasing pollen dimensions with increasing 2C and 1Cx values. However, *N. komarovii* represented an important exception, combining tetraploidy with comparatively small pollen grains. This suggests that phenotypic correlates of genome size are not strictly determined by chromosome number or ploidy and should therefore be interpreted cautiously.

At present, genome-size evidence remains heavily concentrated in a small subset of Nitrariaceae. No comparable information is available for *Malacocarpus* or *Tetradiclis*, and several poorly sampled *Nitraria* species likewise lack flow-cytometric estimates. This asymmetry limits family-wide comparisons and prevents testing whether the broad genome-size patterns observed in *Nitraria* are representative of Nitrariaceae as a whole.

Nevertheless, the available evidence supports an important general conclusion: chromosome-number evolution and genome-size evolution are only partially coupled in Nitrariaceae. Polyploidisation can produce near-proportional increases in nuclear DNA content, as demonstrated in *N. pamirica*, but subsequent genome-size evolution can generate substantial differences among taxa with identical chromosome numbers and ploidy levels. Accordingly, 2n, ploidy and genome size should be treated as complementary rather than interchangeable cytogenetic variables.

4.6. Taxonomic history changes the apparent chromosome spectrum

Chromosome-number syntheses are unavoidably shaped by the taxonomic framework under which historical records are interpreted. In Nitrariaceae, this is particularly evident in *Nitraria*, where changes in species circumscriptions and synonym alter the apparent chromosome spectrum of individual taxa. A modern database therefore cannot simply replace historical names with currently accepted ones: it must preserve both the name under which the material was originally studied and the accepted name under the contemporary taxonomic treatment.

The clearest example is *Nitraria retusa*. When only records originally published under that name are considered, the documented chromosome evidence is comparatively restricted, including 2n = 18, 2n = 24 and n = 12. However, the present taxonomic treatment also places *N. schoberi* var. *faurei* within the synonym of *N. retusa*. Historical chromosome records published under var. *faurei*, including high complements such as 2n = 84 and 2n = 96, are therefore transferred taxonomically to *N. retusa* in the current synthesis. This single nomenclatural change dramatically expands the apparent chromosome range of the accepted species (Reese, 1957, 1958; Bhattacharya et al., 1971; Hilu, 1979; Malallah et al., 2001).

The biological interpretation of those transferred records is much less straightforward than their taxonomic reassignment. A synonymisation establishes how a historical name is treated under a current taxonomic framework; it does not retrospectively change the identity under which the original investigator examined the material. Thus, the high chromosome numbers reported by Reese should be described as observations made in material identified as *N. schoberi* var. *faurei*, currently treated as *N. retusa*, rather than as if Reese had directly studied material identified according to the modern concept of *N. retusa* (Reese, 1957, 1958).

This distinction is especially important because taxonomic reassignment can alter evolutionary interpretation. If the transferred 84- and 96-chromosome records are presented without their nomenclatural history, *N. retusa* appears to possess an extraordinary range extending from 18 to 96 chromosomes. Yet this range combines observations obtained under different taxonomic concepts, from different geographical material and with different levels of cytological resolution. The resulting numerical spectrum is therefore partly a product of taxonomic history rather than a directly demonstrated biological series within a uniformly circumscribed species.

A second instructive case is *Nitraria billardierei*. The only chromosome count presently attributed to this species, 2n = 48, was published by Reese (1962) from Australian material under the name *N. schoberi*. Under the taxonomic framework adopted here, the record is assigned to *N. billardierei*, while the original published name is retained. This reassignment is

coherent with the current distinction between the Australian species and the Eurasian *N. schoberi*, but it does not justify rewriting the historical observation as though *N. billardierei* had been the identification used in the original study (Reese, 1962).

The correct representation of such a record therefore contains several distinct pieces of information: the original published name, the currently accepted name, the chromosome number, the geographical provenance and the source. This structure preserves both historical evidence and modern taxonomic interpretation without conflating them. In the case of Reese (1962), the scientifically accurate statement is that Australian material published as *N. schoberi* had 2n = 48 and is assigned to *N. billardierei* under the present taxonomic treatment.

Taxonomic revision also affects quantitative summaries. Counts of chromosome records per accepted species, apparent cytotype diversity and chromosome-number ranges can all change when synonyms are reassigned. For *N. retusa*, incorporating var. *faurei* substantially increases both the number of records and the observed numerical range. For *N. billardierei*, taxonomic updating creates essentially the entire currently known chromosome record of the species. Thus, changes in accepted taxonomy can create the appearance of newly recognised chromosome diversity even when no new cytological observation has been made.

This problem extends beyond nomenclature to the interpretation of sampling. A species may appear cytogenetically heterogeneous because records derived from historically distinct taxonomic entities have been merged, while another may appear poorly sampled because older literature remains hidden under obsolete names. Historical synonymy is therefore not merely a taxonomic housekeeping issue; it directly influences how chromosome diversity is reconstructed.

The converse risk is equally important. Modern synonymisation should not be treated as proof that all historical material assigned to the synonym necessarily corresponds unambiguously to the modern accepted species. Historical identifications may themselves have been uncertain, taxonomic concepts may have been broad, and vouchers may be unavailable for reassessment. Where possible, chromosome records should therefore remain linked to voucher material and geographical provenance so that future taxonomic revisions can be propagated without losing the original evidence.

The interaction between taxonomy and cytogenetics is especially consequential when unusual chromosome numbers are involved. High counts reported for the material published as *N. schoberi* var. *faurei*—2n = 96 in Reese (1957), and 2n = 84 and 96 in Reese (1958)—strongly influence the inferred chromosome spectrum of *N. retusa*. Their evolutionary interpretation therefore depends not only on whether the counts were technically correct, but also on whether the historical material is correctly placed within the modern species concept. The taxonomic and cytogenetic uncertainties are inseparable.

Accordingly, chromosome databases should not treat taxonomic updating as a simple overwrite operation. Instead, they should preserve the full chain of interpretation: original identification → taxonomic synonymy or reassignment → current accepted name. For historical cytogenetic records, this provenance is as important as the chromosome number itself.

In Nitrariaceae, the practical consequence is clear: part of the apparent chromosome diversity of individual species is produced by changes in taxonomic circumscription over time. Recognising this does not weaken the historical evidence; rather, it allows that evidence to be used more accurately. The chromosome spectrum of a modern taxon should therefore be understood as a

synthesis of observations made under potentially different historical taxonomic concepts, not as a uniform set of observations generated under the present classification.

4.7. What chromosome databases count—and what they do not

The present review highlights a fundamental limitation of chromosome-number databases: a database record is not necessarily a biological observation at a uniform level of organisation. Depending on the source and the way the information is encoded, one row may represent a chromosome number observed in one cell, one individual, one population, several populations, or a synthesis extracted from a publication. Conversely, a single biological accession may generate multiple records if several chromosome complements are stored separately. Record counts therefore cannot be interpreted directly as numbers of individuals, populations or cytotypes.

This problem is particularly evident in *Nitraria*. Intensive modern studies have documented multiple chromosome classes associated with the same accession, voucher, seedling or individual, and ChromoDB stores many of these values as separate chromosome-number records. A single voucher may therefore contribute several rows corresponding to 24, 36, 48, 60 or other chromosome numbers even when the exact biological relationship among those counts is incompletely resolved. If the hierarchical context is ignored, such records can be mistaken for independent cytotypes.

Nitraria tangutorum provides a particularly clear example. Some recent vouchers contained several chromosome complements simultaneously, including 24/36/48 and, in one accession, 24/36/48/60/72. A flat database representation can legitimately preserve each observed chromosome number, but five rows do not represent five population cytotypes. They represent five cellular chromosome classes within the same material (Banaev et al., 2025).

The same problem occurs on an even larger scale in *N. schoberi*. Its exceptionally broad numerical spectrum partly reflects intensive cell-by-cell investigation rather than an equivalent number of independently evolving chromosome races. Multiple chromosome values have been recorded within single accessions, including both exact multiples of 12 and numerous intermediate values. Consequently, the apparent richness of chromosome numbers in a database query can greatly exceed the number of independently supported organismal ploidy states (Muratova et al., 2013; Banaev et al., 2024).

The converse situation also occurs. In *Peganum*, a publication may document the same cytotype in numerous individuals or populations, yet a chromosome database may summarise this evidence in only one or a few rows. Thus, an intensively sampled, biologically well-supported cytotype can appear numerically underrepresented relative to a mixoploid accession that generated many distinct chromosome values. The number of database records is therefore primarily a property of data structure and reporting granularity, not a direct measure of biological diversity.

A related problem concerns bibliographic independence. Two publications are not automatically two independent biological observations: later studies may reuse, cite, reanalyse or extend previously characterised material. Consequently, publication counts should not be used as a proxy for the number of independent accessions, populations or cytotypes unless the identity of the underlying biological material has been reconstructed.

This distinction suggests that at least four concepts should remain separate in chromosome databases: record, observation, cytotype and biological unit. A record is the database

representation; an observation is the chromosome evidence reported in a source; a cytotype is a biologically coherent chromosome state supported at the individual or population level; and the biological unit may be a cell, tissue, voucher, individual or population. These categories overlap, but they are not interchangeable.

The problem becomes particularly acute in mixoploid organisms. For such taxa, the minimum informative representation cannot be reduced to:

taxon + chromosome number

because this formulation removes the biological scale of the observation. A more informative hierarchy is:

taxon → population → voucher/individual → tissue or cell → modal chromosome complement → additional cellular complements → method → independent evidence of ploidy.

This hierarchy corresponds much more closely to the structure required to interpret *Nitraria*, where the same individual may contain several chromosome classes while still possessing a well-supported modal ploidy level.

The distinction between modal and non-modal complements is especially important. A chromosome number can be completely valid as an observation yet misleading if presented without its status. In *N. komarovii*, for example, 2n = 60 is a genuine recorded value, but the same study identifies 2n = 48 as modal and supports a tetraploid condition through flow cytometry. Presenting “2n = 60” without this context could suggest a stable pentaploid cytotype that is not actually demonstrated (Banaev et al., 2024).

The same principle applies to inferred ploidy. Arithmetic compatibility should not be encoded as biological certainty. A cell with 36 chromosomes is compatible with 3x under x = 12, but this does not establish that the individual is triploid. *N. roborowskii*, represented by a single 2n = 36 record, illustrates the ambiguity: without information on modal chromosome number, cell frequencies, meiosis or genome size, triploidy remains only one possible interpretation (Banaev et al., 2025).

Database design also affects taxonomic interpretation. As discussed above, modern synonymisation can transfer historical records between accepted taxa, changing the apparent chromosome spectrum without generating new cytological evidence. Therefore, the chromosome record must remain linked to its original published name as well as to its current accepted name. A database that stores only the latter loses the historical identity of the observation; one that stores only the former prevents synthesis under current taxonomy.

These issues show why a chromosome database should ideally distinguish raw documentary evidence from biological interpretation. The raw layer should preserve what was reported: taxon name as published, chromosome notation, locality, voucher, source and methodological context. A second interpretative layer may then identify accepted taxonomy, modal complement, probable ploidy, evidence class and biological relationships among records. Mixing these levels risks converting interpretations into apparent primary observations.

The distinction is particularly relevant for chromosome-scale genomic data. The 12 pseudomolecules of *Peganum multisectum* provide strong structural evidence for a 12-unit haploid genome organisation, but they are not a cytological observation of n = 12. A database that treats both evidence types identically would erase a meaningful methodological distinction. Similarly, flow-cytometric evidence can support ploidy assignment without constituting a chromosome count (Xu et al., 2025).

For comparative analyses, the practical consequence is that row frequencies should not be used as cytotype frequencies unless biological independence has been explicitly established. The 119 records assigned to *N. schoberi* do not mean 119 independent populations or cytotypes, just as the much smaller number of *P. harmala* records does not imply weaker biological support for its predominant 2n = 24 complement. Record abundance describes documentary density; biological prevalence requires a different unit of analysis.

This distinction also affects automated synthesis. Queries that simply extract all distinct chromosome numbers for a taxon may produce biologically misleading outputs, especially in organisms with somatic mosaicism. For *Nitraria*, the most useful summary is not the complete list of 2n values but the combination of modal chromosome number, independently supported ploidy level, range and structure of additional cellular variation, and strength of evidence.

Accordingly, chromosome databases would benefit from preserving explicit relationships among records rather than treating them as fully independent rows. Even when legacy database structures cannot accommodate additional fields, the interpretation layer should reconstruct these relationships wherever possible from vouchers, locality, publication and methodological context.

The broader lesson from Nitrariaceae is that chromosome databases do not simply “count chromosomes”; they encode observations made at different biological scales and under different taxonomic and methodological frameworks. Their scientific value therefore depends not only on completeness, but on preserving enough context to reconstruct what each number actually represents. A large inventory of chromosome values is not equivalent to a large diversity of cytotypes, and a database row is not, by itself, an evolutionary unit.

4.8. Sampling gaps and research priorities

The major limitation of current cytogenetic knowledge in Nitrariaceae is not simply the scarcity of chromosome records, but their extreme taxonomic and methodological unevenness. More than 99% of the available records are concentrated in *Nitraria* and *Peganum*, whereas *Malacocarpus* and *Tetradiclis* remain almost completely unresolved. Even within the two better-sampled genera, a small number of species account for most observations. This asymmetry means that absence of documented variation in poorly studied taxa cannot be interpreted as evidence of chromosome stability.

Within *Nitraria*, the highest immediate priority is to obtain the first chromosome data for *N. iliensis* and *N. sphaerocarpa*. Both species currently lack chromosome records in the reviewed corpus, preventing their placement within the principal 2n = 24/48 framework of the genus. Their inclusion is particularly important for reconstructing the distribution of diploid and tetraploid states and for assessing whether somatic mixoploidy is genuinely widespread across *Nitraria* rather than concentrated in the best-studied species. Voucher-based chromosome counts combined with flow cytometry would be sufficient to provide a first robust placement of both taxa.

However, closing these two complete gaps would not by itself solve the sampling problem. *N. billardierei* and *N. roborowskii* are each represented by only one chromosome observation and therefore remain almost as poorly resolved biologically. For *N. billardierei*, modern sampling from several Australian populations should test whether the historical 2n = 48 count is reproducible, whether it represents the modal complement, and whether the species is genuinely tetraploid under the x = 12 framework. Flow cytometry would provide the independent evidence needed to compare its genome size with the tetraploid Asian species (Reese, 1962).

The single 2n = 36 record of *N. roborowskii* likewise requires confirmation. Additional populations should be analysed to determine whether 36 represents a stable organismal complement or a non-modal cellular class in a mixoploid individual. Because 36 is compatible arithmetically with 3x but also occurs as a somatic chromosome class in other *Nitraria* species, chromosome counts should ideally be accompanied by meiotic observations and nuclear DNA-content estimates (Banaev et al., 2025).

Nitraria retusa represents a different priority: the main problem is not absence of numbers, but uncertainty about their biological and taxonomic relationships. New material identified according to the current species concept should be analysed across its range, with particular emphasis on testing the reproducibility of 2n = 18 and 2n = 24. Equally important would be renewed sampling of North African material corresponding as closely as possible to the historical concept of *N. schoberi* var. *faurei*. Combined chromosome counts, flow cytometry and voucher-based identification could establish whether the historical 84- and 96-chromosome records represent stable high-ploidy individuals, somatic mixoploidy or some combination of both (Bhattacharya et al., 1971; Hilu, 1979; Reese, 1957, 1958).

For the intensively sampled *Nitraria* species, the priority shifts from obtaining additional isolated counts to improving biological resolution. Future studies should quantify the frequency of chromosome classes within individuals, compare tissues, determine whether non-modal complements enter reproductive lineages, and distinguish somatic mixoploidy from endopolyploidy. Standardised sampling across *N. schoberi*, *N. sibirica*, *N. tangutorum*, *N. pamirica* and *N. komarovii* would allow the first rigorous comparison of the extent and structure of chromosome mosaicism among species.

Within *Peganum*, the central evolutionary problem remains the relationship among n = 6, n = 12 and n = 24 in *P. harmala*. Cytologically characterised material representing all three states should be analysed using flow cytometry and comparative genomics. In particular, genome-size data from the n = 6 material and the demonstrated 2n = 48 tetraploid are currently missing and would provide an independent test of competing interpretations of chromosome-number change (Gupta et al., 2016; Aslam et al., 2023).

The chromosome-scale genome of *P. multisectum* creates an especially valuable opportunity. The species still lacks a conventional cytological chromosome count in the reviewed corpus, so obtaining a direct somatic count and preferably meiotic evidence should be a straightforward priority. Such data would directly connect the 12 chromosome-scale pseudomolecules with cytological n and 2n values. Comparative synteny between *P. multisectum* and cytologically verified n = 6 and n = 12 material of *P. harmala* could then address whether the present 12-chromosome architecture reflects an ancestral state, genome duplication or chromosome rearrangement (Xu et al., 2025).

P. mexicanum and *P. nigellastrum* also require broader sampling. Their currently uniform n = 12/2n = 24 pattern may reflect genuine chromosome conservatism, but the number of studies is too small to compare their variability meaningfully with that of *P. harmala*. Population-level cytology across their geographical ranges would test whether additional cytotypes or B chromosomes have simply remained undetected.

The most urgent family-level gaps concern *Malacocarpus* and *Tetradiclis*. In *Malacocarpus crithmifolius*, the historical 2n = 24 count should first be confirmed using modern voucher-based material. Meiotic analysis would determine whether n = 12 characterises the species and

whether its numerical similarity to *Peganum* and diploid *Nitraria* reflects genuinely comparable chromosome architecture (Zakharyeva & Astanova, 1968).

In Tetradiclis tenella, the priority is bibliographic as much as experimental: the primary source underlying the attributed 2n = 14 should be identified and critically assessed. Only then can its evidential status be evaluated properly. New chromosome counts from independent populations are nevertheless essential, because a confirmed 14-chromosome complement would have major implications for understanding family-wide chromosome evolution. No chromosome record for *T. corniculata* was recovered in the reviewed corpus; targeted modern cytogenetic sampling of the species is therefore a clear priority. Comparison of the two species would immediately establish whether 2n = 14 is potentially a genus-level pattern (Astanova, 1993).

These priorities point to a broader methodological shift. Future work should avoid adding isolated chromosome numbers without sufficient biological context. The most informative studies would combine voucher-backed identification, population sampling, modal chromosome determination, cell-by-cell assessment of additional complements, meiosis where possible, flow cytometry and chromosome-scale genomics. This integrated design is particularly important in *Nitraria*, where chromosome number alone cannot reliably distinguish organismal ploidy from somatic variation.

A second priority is to improve geographical balance. Several species are represented almost entirely by material from restricted parts of their ranges. Apparent cytogenetic uniformity—or apparent predominance of one ploidy level—may therefore reflect local sampling rather than species-wide structure. Broader geographical coverage is required before cytotype frequencies or biogeographical patterns can be inferred confidently.

Finally, future comparative analyses should explicitly integrate chromosome data with phylogeny. The current dataset identifies strong candidate evolutionary processes—polyploidisation, possible dysploid change, genome-size divergence and somatic chromosome instability—but cannot establish their sequence or number of independent origins. A phylogenomic framework including all four genera would allow chromosome states to be reconstructed explicitly and would test whether the recurrent x = 12 framework reflects common ancestry or convergent evolution.

The present review therefore identifies two complementary research needs. The first is completion of basic cytogenetic coverage, particularly in *N. iliensis*, *N. sphaerocarpa*, *T. corniculata*, *Malacocarpus* and poorly sampled species of *Nitraria* and *Peganum*. The second is increased biological resolution, replacing isolated chromosome numbers with integrated evidence on ploidy, genome size, cellular variation and genomic structure. Addressing both levels will be necessary before the chromosome evolution of Nitrariaceae can be reconstructed with confidence.

5. Conclusions

This family-wide synthesis shows that chromosome evolution in Nitrariaceae cannot be represented adequately by a simple inventory of reported numbers. The strongest recurrent pattern is a 12-based chromosome organisation in the two best-sampled genera, *Nitraria* and *Peganum*. In both, 2n = 24 and 2n = 48 are associated with independently supported diploid and tetraploid states in well-characterised material. Nevertheless, the available evidence supports x

= 12 primarily as an operational framework for interpreting extant variation; it does not yet demonstrate that x = 12 was the ancestral basic number of Nitrariaceae.

Polyploidy is a genuine and recurrent component of chromosome evolution in the family, but chromosome-number multiplication must be distinguished from somatic numerical variation. In *Nitraria*, chromosome counts and flow-cytometric genome-size data support stable diploid- and tetraploid-level differences, while one *N. schoberi* accession provides additional flow-cytometric evidence compatible with approximately 8x but still lacking direct cytological confirmation of ca. 96 chromosomes. At the same time, several species exhibit substantial chromosome-number heterogeneity, with the biological scale ranging from demonstrated cellular variation to voucher-associated variation that remains incompletely resolved. Arithmetic multiples of a presumed basic number therefore cannot be equated automatically with independent cytotypes.

Genome-size data reinforce this distinction while also showing that chromosome number and nuclear DNA content have evolved only partially in parallel. Near-proportional increases in 2C values support genome duplication in some cases, whereas substantial genome-size differences among taxa sharing the same modal chromosome number indicate subsequent genomic divergence. Flow cytometry, meiosis and chromosome-scale genomics therefore provide complementary evidence, but none should be treated as interchangeable with a direct chromosome count.

Several discordant observations remain evolutionarily important precisely because their interpretation is unresolved. The meiotic n = 6 complement in *Peganum harmala*, historical 2n = 22 records in the same species, 2n = 18 in *Nitraria retusa*, and the attributed 2n = 14 in *Tetradiclis tenella* show that the currently documented chromosome diversity cannot be reduced to an unmodified 24/48 polyploid series. However, the present evidence is insufficient to convert these values automatically into alternative basic numbers or specific dysploid pathways. Their evolutionary significance will require repeated, voucher-based cytology integrated with meiosis, genome-size estimation and comparative genomics.

The synthesis also demonstrates that taxonomic history and database structure materially affect the chromosome spectrum reconstructed for a species. Historical observations must remain linked to the names under which they were published while also being mapped, where justified, to current accepted taxa. Likewise, a database row should be interpreted as a documentary unit rather than as an independent individual, population or cytotype. This distinction is particularly consequential in mixoploid taxa, where several valid chromosome-number records may derive from a single accession.

Finally, the family remains extremely unevenly sampled. More than 99% of the reviewed records belong to *Nitraria* and *Peganum*, while *Malacocarpus* and *Tetradiclis* are represented by only isolated historical data. Even within the better-studied genera, several species lack records or are represented by single observations. Future progress will therefore depend less on simply increasing the number of database entries than on obtaining biologically resolved evidence from poorly sampled taxa and populations. Voucher-based cytology, standardised assessment of modal and non-modal chromosome complements, flow cytometry, meiosis and chromosome-scale comparative genomics together offer the most direct route towards reconstructing the origin of the 12-based framework, the history of polyploidy and possible dysploid change, and the evolutionary significance of somatic chromosome instability in Nitrariaceae.

Declarations

Data availability

The frozen analytical dataset underlying this study is openly available in Zenodo (Simon Pallisé & Bosch Daniel, 2026) at https://doi.org/10.5281/zenodo.22538050. The deposit contains the curated chromosome-record and associated bibliographic data used in this synthesis. It should be treated as the version-specific snapshot supporting this manuscript; the continuously curated ChromoDB resource may contain later additions or corrections.

Conflict of interest

The authors declare no competing interests.

Use of generative AI

Generative AI tools were used to assist with literature screening, data organisation, drafting and language editing. AI-generated outputs were critically reviewed by the authors, and factual information was checked against the cited sources wherever the underlying source was accessible. Sources not directly verified from the primary publication are explicitly identified as such in the manuscript. The authors take full responsibility for the content and interpretations presented.

References

Angiosperm Phylogeny Group (APG IV). (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society, 181, 1–20. https://doi.org/10.1111/boj.12385

Aslam, N., Wani, A. A., Ganie, A. H., & Nawchoo, I. A. (2023). Existence of two cytotypes of Peganum harmala L. in Kashmir Himalaya and Ladakh Trans Himalaya of India. Cytologia, 88(2), 111–116. https://doi.org/10.1508/cytologia.88.111

Astanova, S. B. (1993). In A. Takhtajan (Ed.), Numeri Chromosomatum Magnoliophytorum Florae URSS, Moraceae–Zygophyllaceae. Nauka, St Petersburg.

Bachelier, J. B., Endress, P. K., & Ronse De Craene, L. P. (2011). Comparative floral structure and development of Nitrariaceae (Sapindales) and systematic implications. In L. Wanntorp & L. P. Ronse De Craene (Eds.), Flowers on the Tree of Life (pp. 181–217). Cambridge University Press. https://doi.org/10.1017/CBO9781139013321.008

Banaev, E. V., & Tomoshevich, M. A. (2021). In K. Marhold & J. Kučera (Eds.), IAPT Chromosome Data 34/2. Taxon, 70(5), 1149, E2–E3.

Banaev, E. V., Ak-Lama, T. A., Tomoshevich, M. A., Wu, S., Erst, A. A., & Pshenichkina, Y. A. (2025). IAPT chromosome data 47/2: Nitrariaceae. In K. Marhold & J. Kučera (Eds.), IAPT chromosome data 47. Taxon, 74(6), 1628. https://doi.org/10.1002/tax.70073

Banaev, E. V., Tomoshevich, M. A., & Ak-Lama, T. A. (2018). IAPT chromosome data 27: Nitrariaceae. In K. Marhold & J. Kučera (Eds.), IAPT chromosome data 27. Taxon, 67(5), 1042, E6.

Banaev, E. V., Tomoshevich, M. A., & Erst, A. A. (2024). The nuclear DNA content, ploidy, and chromosome numbers in some species of Nitraria and associations with pollen

characteristics. Genetic Resources and Crop Evolution, 71, 2843–2858. https://doi.org/10.1007/s10722-023-01811-5

Banaev, E. V., Tomoshevich, M. A., & Voronkova, M. S. (2020). IAPT chromosome data 32/3. In K. Marhold & J. Kučera (Eds.), IAPT chromosome data 32. Taxon, 69(5), 1127–1128, E6–E8. https://doi.org/10.1002/tax.12322

Bhattacharya, S. S., Khalifa, M. M., & Chaudhri, I. I. (1971). In Á. Löve (Ed.), IOPB chromosome number reports XXXII. Taxon, 20(2/3), 349–350.

Carta, A., Bedini, G., & Peruzzi, L. (2020). A deep dive into the ancestral chromosome number and genome size of flowering plants. New Phytologist, 228(3), 1097–1106. https://doi.org/10.1111/nph.16668

Guerra, M. (2008). Chromosome numbers in plant cytotaxonomy: concepts and implications. Cytogenetic and Genome Research, 120(3–4), 339–350. https://doi.org/10.1159/000121083

Gupta, R. C., Kaur, K., & Singh, V. (2016). Meiotic chromosomal studies in family Zygophyllaceae R. Br. from Rajasthan. Indian Journal of Genetics and Plant Breeding, 76(1), 111–115. https://doi.org/10.5958/0975-6906.2016.00017.1

Gutiérrez, M. L., Rodríguez-González, R., Pascual-Díaz, J. P., Fuentes, I., & Garcia, S. (2023). Online resources useful for plant cytogenetics and cytogenomics research. In T. Heitkam & S. Garcia (Eds.), Plant Cytogenetics and Cytogenomics (Methods in Molecular Biology, Vol. 2672, pp. 549–560). Humana. https://doi.org/10.1007/978-1-0716-3226-0_33

Hajji, A., Vitales, D., Elgazzeh, M., Garnatje, T., & Vallès, J. (2017). First genome size assessment in the genus Peganum and in the family Nitrariaceae: Iberian and North African data on Peganum harmala, including an intensive sampling in Tunisia. Turkish Journal of Botany, 41(3), 324–328. https://doi.org/10.3906/bot-1609-24

Hanelt, P. (1973). In Á. Löve (Ed.), IOPB chromosome number reports XLII. Taxon, 22(5/6), 648–649.

Hilu, K. W. (1979). In Á. Löve (Ed.), IOPB chromosome number reports LXIV. Taxon, 28(4), 395.

Joyce, E. M., Appelhans, M. S., Buerki, S., Cheek, M., de Vos, J. M., Pirani, J. R., Zuntini, A. R., Bachelier, J. B., Bayly, M. J., Callmander, M. W., Devecchi, M. F., Pell, S. K., Groppo, M., Lowry, P. P., Mitchell, J., Siniscalchi, C. M., Munzinger, J., Orel, H. K., Pannell, C. M., Nauheimer, L., Sauquet, H., Weeks, A., Muellner-Riehl, A. N., Leitch, I. J., Maurin, O., Forest, F., Nargar, K., Thiele, K. R., Baker, W. J., & Crayn, D. M. (2023). Phylogenomic analyses of Sapindales support new family relationships, rapid Mid-Cretaceous Hothouse diversification, and heterogeneous histories of gene duplication. Frontiers in Plant Science, 14, 1063174. https://doi.org/10.3389/fpls.2023.1063174

Keil, D. J., & Pinkava, D. J. (1979). In Á. Löve (Ed.), IOPB chromosome number reports LXIII. Taxon, 28(1/3), 271–275.

Ma, X. H., Qin, R. L., & Xing, W. B. (1984). Chromosome observations of some medical plants in Xinjiang. Acta Phytotaxonomica Sinica, 22, 243–249.

Ma, X., Ru, D., Morales-Briones, D. F., Mei, F., Wu, J., Liu, J., & Wu, S. (2023). Genome sequence and salinity adaptation of the desert shrub Nitraria sibirica (Nitrariaceae, Sapindales). DNA Research, 30(3), dsad011. https://doi.org/10.1093/dnares/dsad011

Malallah, G., Al-Dosari, M., & Murín, A. (2001). Determination of chromosome numbers in Kuwaiti flora II. Thaiszia – Journal of Botany, 10, 137–150.

Muratova, E. N., Goryachkina, O. V., & Banaev, E. V. (2013). Karyological studies on Siberian species of Nitraria L. (Nitrariaceae). Turczaninowia, 16(4), 50–54. https://doi.org/10.14258/turczaninowia.16.4.9

Měsíček, J., & Soják, J. (1972). Chromosome studies in Mongolian plants. Preslia, 44, 334–358.

Negodi, G. (1937). Lineamenti sulla cariologia delle Rutaceae e delle Zygophyllaceae. Archivio Botanico (Forlì), 13, 93–102.

Plants of the World Online (POWO). (2026). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. https://powo.science.kew.org/ (accessed 4 September 2026).

Powell, A. M., Powell, S. A., & Jackson, C. (2017). Documented chromosome numbers 2017:1. Miscellaneous counts mostly from western Texas (U.S.A.), one each from New Mexico (U.S.A.) and Mexico. Journal of the Botanical Research Institute of Texas, 11(1), 143–146. https://doi.org/10.17348/jbrit.v11.i1.1145

Reese, G. (1957). Über die Polyploidiespektren in der nordsaharischen Wüstenpflanzen. Flora, 144(4), 598–634.

Reese, G. (1958). Cyto-systematische Notizen zur Gattung Nitraria (Zygophyllaceae). Flora, 146(3), 478–487.

Reese, G. (1962). Zur Chromosomenzahl der australischen Nitraria schoberi L. Portugaliae Acta Biologica, Série A, 6(3–4), 295–297.

Rice, A., Glick, L., Abadi, S., Einhorn, M., Kopelman, N. M., Salman-Minkov, A., Mayzel, J., Chay, O., & Mayrose, I. (2015). The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. New Phytologist, 206(1), 19–26. https://doi.org/10.1111/nph.13191

Sheahan, M. C., & Chase, M. W. (1996). A phylogenetic analysis of Zygophyllaceae R.Br. based on morphological, anatomical and rbcL DNA sequence data. Botanical Journal of the Linnean Society, 122(4), 279–300. https://doi.org/10.1111/j.1095-8339.1996.tb02077.x

Simon Pallisé, J., & Bosch Daniel, M. (2026). Chromosome-number data and taxonomic summary for Nitrariaceae [Data set]. Zenodo. https://doi.org/10.5281/zenodo.22538050

Singh, P. (1984). In Á. Löve (Ed.), IOPB chromosome number reports LXXXV. Taxon, 33(4), 759.

Ward, D. E. (1984). Chromosome counts from New Mexico and Mexico. Phytologia, 56(1), 55–60.

Xu, H., Han, Y., Chi, X., Yu, J., Xia, M., Han, S., Niu, Y., Zhang, F., & Chen, S. (2025). Integration of de novo chromosome-level genome and population resequencing of Peganum (Nitrariaceae): A case study of speciation and evolutionary trajectories in arid Central Asia. Molecular Ecology Resources, 25(4), e14078. https://doi.org/10.1111/1755-0998.14078

Zakharyeva, O. I., & Astanova, S. B. (1968). Chromosome numbers of some wild flowering plant species of Central Asia. Doklady Akademii Nauk Tadzhikskoi SSR, 11(11), 72–75.