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Morphological and genetic variability of critically endangered species from the *Chenopodium neomexicanum* group

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Thesis title

Morphological and genetic variability of critically endangered species from the Chenopodium neomexicanum group

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Objectives of thesis

The Chenopodium neomexicanum species group is widespread in the deserts of the southwestern United States. The center of diversity is located in the Sonoran Desert and adjacent areas, where the following species grow: C. arizonicum, C. neomexicanum s. str., C. palmeri, and C. sonorense. However, these species are very poorly differentiated morphologically, making their identification quite difficult. The current taxonomic concept of this group uses seed characteristics to distinguish between species, but these characteristics are only present at the end of the growing season. In addition, they are relatively small, and the important characteristics are thus minute and relatively unreliable. The aim of this work is (1) to compare seed characteristics using morphometric analysis and (2) to link them to the genetic differentiation of individual species using the RADseq method, which, among other things, will also show whether (3) low interspecific differentiation could be caused by hybridization between individual species.

Methodology

The thesis will be based on (1) morphometric analysis of seeds. This means that the seeds will be measured and photographed in high resolution so that other characteristics of the seeds can be captured. (2) The genetic variability of individual species will be determined using the RADseq method, which will allow the degree of genetic differentiation and possible hybridization between individual species to be determined.

The proposed extent of the thesis

60 pages

Keywords

Chenopodium neomexicanum agg., morphological differentiation, hybridization

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Recommended information sources

Aellen P, Just T. 1943. Key and synopsis of the American species of the genus Chenopodium L. American Midland Naturalist 30: 47–76.

Clemants S.E., Mosyakin S.L. 2003. Chenopodium L. In: Flora of North America Editorial Committee (Ed.), Flora of North America North of Mexico 4. Oxford University Press, New York/Oxford, pp. 275–299.

Fuentes-Bazan S., Mansion G., Borsch T. 2012. Towards a species level tree of the globally diverse genus Chenopodium (Chenopodiaceae). Molecular Phylogenetics and Evolution 62:359–374.

Habibi F., Vit P., Rahiminejad M., Mandák B. 2018. Towards a better understanding of the Chenopodium album aggregate in the Middle East: a karyological, cytometric and morphometric investigation. Journal of Systematics and Evolution 56: 231–242.

Jarvis, D.E., Ho, Y.S., Lightfoot, D.J., Schmöckel, S.M., Li, B., Borm, T.J., Ohyanagi, H., Mineta, K., Michell, C.T., Saber, N., Kharbatia, N.M., Rupper, R.R., Sharp, A.R., Dally, N., Boughton, B.A., Woo, Y.H., Gao, G., Schijlen, E.G., Guo, X., Momin, A.A., Negrão, S., Al-Babili, S., Gehring, C., Roessner, U., Jung, C., Murphy, K., Arold, S.T., Gojobori, T., Linden, C.G., van Loo, E.N., Jellen, E.N., Maughan, P.J., Tester, M., 2017. The genome of Chenopodium quinoa. Nature 542: 307–312.

Mandák B., Krak K., Vít P., Lomonosova M. N., Belyayev A., Habibi F., Wang L., Douda J., Štorchová H. 2018. Hybridization and polyploidization within the Chenopodium album aggregate analysed by means of cytological and molecular markers. Molecular Phylogenetics and Evolution 129: 189–201.

Mandák B., Krak K., Vít P., Pavlíková Z., Lomonosova M. N., Habibi F., Lei W., Jellen E. N., Douda J. 2016. How genome size variation is linked with evolution within Chenopodium sensu lato. Perspectives in Plant Ecology, Evolution and Systematics 23: 18–32.

Vít P., Krak K., Trávníček P., Douda J., Lomonosova M. N. & Mandák B. 2016. Genome size stability across Eurasian Chenopodium species (Amaranthaceae). Botanical Journal of the Linnean Society 182: 637–649.

Wahl H.A. 1954. A preliminary study of the genus Chenopodium in North America. Bartonia 27: 1–46.

Walsh B.M., Adhikary D., Maughan P.J., Emshwiller E., Jellen E.N. 2015. Chenopodium polyploidy inferences from Salt Overly Sensitive 1 (SOS1) data. American Journal of Botany 102: 533–543.

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Author’s Declaration

I, Lady Laura Tuisima Coral de Del Aguila, declare that this master thesis was composed by myself, that the work contained herein is my own, except where I explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification.

Prague, 31th March, 2026

Lady Laura Tuisima Coral de Del Aguila

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Abstract

The taxonomy of the *Chenopodium neomexicanum* aggregate remains poorly resolved due to high morphological variability and potential overlap among closely related taxa. This study integrates seed morphometric analyses with phylogenetic evidence to evaluate species boundaries and evolutionary relationships within this complex. A total of 225 seeds from four taxa (*C. neomexicanum, C. palmeri, C. arizonicum,* and *C. sonorense*) were analyzed using quantitative and qualitative descriptors, complemented by genome-wide SNP data derived from RADseq. Quantitative analyses revealed significant interspecific differences, particularly in seed size, with seed diameter ranging from 0.890 mm in *C. palmeri* to 1.192 mm in *C. neomexicanum*. Principal Component Analysis explained 93.2% of total variation (PC1: 70.3%; PC2: 22.9%), showing a clear gradient separating these taxa, while *C. arizonicum* and *C. sonorense* occupied intermediate and overlapping positions. In contrast, phylogenetic reconstruction revealed well-supported five genetic clusters, although these did not fully correspond to current species delimitation. Morphometric patterns based on genetic clusters further confirmed that most groups overlap extensively, indicating incomplete phenotypic differentiation. These results demonstrate partial congruence between morphological and molecular approaches and indicate that seed morphology alone is insufficient for species delimitation, supporting the need for integrative taxonomic frameworks in *Chenopodium neomexicanum* aggregate.

Keywords: *Chenopodium neomexicanum* aggregate, morphological variation, phylogenetic analysis, seed morphometrics, species delimitation

Abstrakt

Taxonomie komplexu *Chenopodium neomexicanum* zůstává nedostatečně vyřešena v důsledku vysoké morfologické variability a potenciálního překryvu mezi blízce příbuznými taxony. Tato studie integruje morfometrickou analýzu semen s fylogenetickými důkazy za účelem vyhodnocení druhových hranic a evolučních vztahů v rámci tohoto komplexu. Celkem bylo analyzováno 225 semen ze čtyř taxonů (*C. neomexicanum*, *C. palmeri*, *C. arizonicum* a *C. sonorense*) pomocí kvantitativních a kvalitativních deskriptorů, doplněných o genomová data SNP získaná metodou RADseq. Kvantitativní analýzy odhalily významné mezidruhové rozdíly, zejména ve velikosti semen, přičemž průměr semen se pohyboval od 0.890 mm u *C. palmeri* do 1.192 mm u *C. neomexicanum*. Analýza hlavních komponent vysvětlila 93.2 % celkové variability (PC1: 70.3 %; PC2: 22.9 %) a ukázala zřetelný gradient oddělující tyto taxony, zatímco *C. arizonicum* a *C. sonorense* zaujímaly intermediární a překrývající se pozice. Naproti tomu fylogenetická rekonstrukce odhalila pět dobře podpořených genetických klastrů, které však plně neodpovídaly současnému druhovému vymezení. Morfometrické vzorce založené na genetických klastrech dále potvrdily rozsáhlé překrývání mezi většinou skupin, což naznačuje neúplnou fenotypovou diferenciaci. Tyto výsledky ukazují pouze částečnou shodu mezi morfologickými a molekulárními přístupy a naznačují, že samotná morfologie semen není dostatečná pro vymezení druhů, což podporuje potřebu integrativních taxonomických přístupů v rámci komplexu *Chenopodium neomexicanum*.

Klíčová slova:

Chenopodium neomexicanum complex, morfologická variabilita, fylogenetická analýza, morfometrie semen, vymezení druhů

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1. Introduction

The genus *Chenopodium* L. s.l. (*Amaranthaceae*) is a cosmopolitan group that is most varied in temperate regions and includes about 150 species (Kühn, 1993). The majority of the species are annual herbs, though perennial herbs and in a fraction of cases shrubs also exist. They are usually present in arid and semiarid habitats and commonly grow in saline soils. Despite its moderate size, the genus has a long and tangled taxonomic history which has been ambiguous since Linnaeus (1753-1754). Linnaeus defined *Blitum* L. and *Chenopodium* L., with two and 22 species respectively; later authors tended to integrate both into *Chenopodium* (Mandák et al., 2012; Scott, 1978).

The early infrageneric classifications have mainly been based on morphology, the most characteristic being leaf morphology (Linnaeus 1753, 1754; Meyer 1829). Sectional delimitations had been subject to revision (Volkens 1893; Aellen 1960-61; Scott 1978; Clemants & Mosyakin 1996, 2003) and the number and circumscription of infrageneric groups had been the matter of considerable disagreement throughout the nineteenth and twentieth centuries. Even the boundaries of *Chenopodium* and associated genera (for instance *Blitum*) have been treated with varying standards (Clemants & Mosyakin, 1996; Scott, 1978). Although a morphocentric schema was outlined by Clemants and Mosyakin (1996) and adapted later in the Flora of North America (Clemants and Mosyakin 2003), taxonomic instability remains, particularly for species complexes that share overlapping diagnostic characters (Wahl 1954; Uotila 1978).

One particularly well-established and referred to example is the *C. album* aggregate of *Chenopodium* described as a loosely arranged complex of poorly understood races in which many segregate microspecies and infraspecific entities have also been suggested (Mandák et al. 2012). Some of the apparent confusion of this aggregate is likely due to the mixed effect on taxonomy in the aggregate (hybridization and introgression) and multiple reasons that such results (e.g., high level of autogamy, variability in ploidy levels, and strong phenotypic plasticity) is cited (Mandák et al. 2012; Bassett and Crompton 1982). These characteristics exemplify that hybridization and polyploidization have been two factors related to the taxonomic complexity of the genus.

Recent phylogenomic studies have revealed that the *Chenopodium album* aggregate is characterized by a complex genomic composition involving multiple subgenomes (A, B, C and D), which combine through polyploidization to form higher ploidy levels such as tetraploid and hexaploid. In particular, hexaploid taxa such as *C. album* s. str., possess a genomic constitution (BBCCDD) derived from hybridization among distinct diploid ancestors (Mandák et al., 2018; Mandák et al., 2026). This genomic framework highlights that morphologically similar taxa may differ substantially in their subgenomic composition, and that taxonomic units do not necessarily correspond to distinct genomic lineages.

In this evolutionary schema, the North American taxa of *Chenopodium neomexicanum* aggregate belong to the diploid A-genome lineage within the Subclade CH2 (predominantly of American diploid species, 2n = 2x = 18) obtained by phylogenetic analyses of ITS sequences and genome size (Mandák et al., 2016). This subclade, which is also composed of *C. neomexicanum*, also includes species such as *C. fremontii*, *C. pratericola*, *C. incanum*, *C. desiccatum*, *C. leptophyllum*, *C. watsonii*, *C. atrovirens*, and *C. hians*, further demonstrating that they are diploid lineage members of the genus (Mandák et al. 2016). The phylogenetic differences between the CH2 and the Eurasian polyploid family of the *C. album* aggregate further emphasize the evolutionary independence of these American diploid taxa (Mandák et al., 2012, 2018). Phylogeographic analyses with cpDNA also suggests that spread within the diploid-polyploid complex of *Chenopodium* is related to continental expansion of range and differentiation of the lineage (Krak et al., 2019). Although CH2 has been recovered as a monophyletic diploid lineage (Mandák et al. 2016), there is still insufficient internal delimitation of taxa associated with the *C. neomexicanum* aggregate.

This aggregate is not homogeneous and, thus, morphological criteria in this aggregate are not consistent with the identification of distinct evolutionary entities and the correspondence between morphological differentiation and the underlying genetic structure has not been thoroughly tested at population level. The association of species boundaries with ploidy levels has already been demonstrated in *Chenopodium*, and distinct lineages of the genus have been reported to be robustly monophyletic (Mandák et al. 2012; Mandák et al. 2016). In this context, clarifying the taxonomic status of the *C. neomexicanum* aggregate is essential to determine whether the entities currently recognized within this group represent coherent evolutionary lineages rather than continuous phenotypic variation. More precise delimitation of this diploid lineage is thus needed to accurate interpreting patterns of diversification and distribution in North American *Chenopodium*.

By integrating seed morphometric analyses with phylogenetic evidence, this thesis contributes to a more comprehensive understanding of species boundaries, morphological differentiation and potential hybridization in *C. neomexicanum* aggregate.

2. Objective and hypotheses

Objectives

The taxonomy of the *Chenopodium neomexicanum* group remains insufficiently resolved due to high morphological variability and potential hybridization among closely related taxa. Integrating morphological and molecular approaches has proven effective in clarifying species boundaries in complex plant groups, including members of the *Chenopodium album* aggregate.

The main objective of this thesis is to evaluate species boundaries and evolutionary relationships within the *Chenopodium neomexicanum* aggregate by combining seed morphometric analysis with phylogenetic evidence.

To achieve this aim, the thesis pursues the following specific objectives:

1. To characterize the variability of seed morphology within the *Chenopodium neomexicanum* aggregate using quantitative morphometric methods.
2. To identify morphometric patterns and clusters among studied taxa based on seed characteristics.
3. To compare morphological patterns with phylogenetic relationships inferred from molecular data.
4. To evaluate whether morphological intermediacy among populations indicates potential hybridization within the species complex.

Through this integrative approach, the thesis aims to contribute to a better understanding of morphological differentiation, evolutionary relationships, and species delimitation within the *C. neomexicanum* aggregate.

Hypotheses

Based on the current taxonomic uncertainties and previous studies on *Chenopodium* species complexes, the following hypotheses are tested:

H1: Seed morphometric traits differ significantly among taxa within the *Chenopodium neomexicanum* aggregate, reflecting species-level differentiation.

H2: Morphometric groupings derived from seed characteristics correspond to phylogenetic relationships among the studied taxa.

H3: Populations occurring in geographical or ecological contact zones show increased morphological variability or intermediate seed characteristics, suggesting possible hybridization among related taxa.

Together, these hypotheses test whether seed morphology reflects evolutionary relationships and whether it can provide reliable taxonomic signals within this complex group.

3. Literature review

3.1. Variability in *Chenopodium*

3.1.1. Morphological variability

Morphological diversity is a defining characteristic of the genus *Chenopodium* and represents one of the primary reasons for its persistent taxonomic complexity. Species within the genus often display extensive variability in vegetative traits while possessing relatively few stable diagnostic characters. As a result, individual morphological characters are rarely reliable when considered in isolation and must be interpreted cautiously and preferably in combination (Wahl 1954; Uotila 1997). This issue is particularly pronounced within the *Chenopodium album* aggregate, which comprises numerous weakly differentiated and morphologically overlapping taxa with broad geographic distributions (Clemants & Mosyakin 2003; Mandák et al. 2012).

In *C. album* sensu stricto, the remarkable morphological variation observed within the aggregate has been attributed to several factors. These include strong phenotypic plasticity, the presence of multiple autogamous lineages, and variation in ploidy levels (Mandák et al. 2012). However, some commonly proposed explanations require careful evaluation. For example, interploid hybridization has not consistently received support from molecular and cytogenetic studies (Mandák et al. 2012; Vít et al. 2016). Moreover, phylogenetic analyses have suggested that closely related species are often associated with specific ploidy levels rather than consisting of multiple cytotypes within a single species (Mandák et al. 2016).

Consequently, the high morphological variability observed within the *C. album* aggregate should not automatically be interpreted as evidence of ongoing hybridization or cytogenetic instability. Experimental studies conducted under controlled garden and greenhouse conditions have provided important insights into the relative contributions of environmental and genetic factors to morphological variation. Morphometric analyses of representative Iranian species of the *C. album* aggregate grown under standardized greenhouse conditions showed that morphological variation was relatively limited in most species once environmental effects were minimized (Habibi et al. 2018). Field populations that initially appeared strongly differentiated in leaf morphology, leaf arrangement, branching pattern, and inflorescence structure tended to converge morphologically when cultivated under identical conditions, indicating that much of the variation observed in natural populations results from environmentally induced phenotypic plasticity (Habibi et al. 2018). In contrast, *C. album* s. str. maintained a high degree of morphological variability even under controlled conditions, suggesting a stronger genetic component underlying its morphological diversity.

Recent genomic studies have further clarified the evolutionary background of this pattern. Persistent morphological diversity at the species level appears to reflect a complex evolutionary history and a heterogeneous genomic structure rather than solely environmental plasticity. Because vegetative characters in *Chenopodium* are often highly labile and environmentally sensitive, modern systematic treatments increasingly emphasize reproductive characters as more reliable taxonomic markers.

In particular, fruit and seed morphology have proven to be taxonomically informative in several *Chenopodium* groups, including North American taxa of the *C. neomexicanum* complex. Detailed analyses of fruit morphology, pericarp structure, and seed-coat ornamentation have enabled the recognition and separation of closely related species that are otherwise difficult to distinguish based on vegetative traits alone (Benet-Pierce & Simpson 2017, 2019). Large-scale comparative carpological studies within *Chenopodium* and related genera have likewise demonstrated that pericarp anatomy and testa sculpture represent evolutionarily informative characters with diagnostic value at both species and infrageneric levels (Sukhorukov & Zhang 2013).

Hybridization may represent an additional factor influencing morphological variation, although its effects require careful interpretation. Microsatellite analyses of the diploid species *C. ficifolium* and *C. suecicum* have documented the occurrence of both F1 hybrids and later-generation hybrid individuals. While F1 hybrids typically display intermediate morphological traits, later-generation hybrids are often morphologically indistinguishable from their parental taxa (Hodková & Mandák 2018). These findings illustrate both the potential and the limitations of morphological characters in detecting hybridization, particularly in cases involving introgression.

Overall, current evidence suggests that morphological variability in *Chenopodium* results from a combination of phenotypic plasticity, breeding system characteristics, genomic structure, and, in some cases, hybridization. Reliable taxonomic interpretation therefore requires careful evaluation of character combinations across multiple populations and environments, ideally complemented by cytogenetic and molecular evidence.

3.1.2. Karyology

Karyological studies have provided important insights into the structural basis of diversity within the genus *Chenopodium*. Cytological investigations have shown that chromosomes in this genus are generally small and morphologically uniform. Detailed karyotype analyses of *C. quinoa* and *C. berlandieri* subsp. *nuttalliae* from Mexico revealed that their chromosomes are metacentric and extremely small, not exceeding 2.04 µm in length (Palomino et al. 2008). This overall chromosomal uniformity is consistent with earlier cytological studies in the genus and indicates a relatively conserved chromosomal structure despite the considerable taxonomic complexity of *Chenopodium*.

Although chromosome morphology is largely uniform, variation in chromosome number represents an important source of genetic diversity within the genus. Studies focusing on European and Eurasian representatives of the *Chenopodium album* aggregate have shown that individual species often possess similar but distinct chromosome numbers (Mandák et al. 2012, 2016). In some cases, multiple chromosome counts have been reported for what was historically treated as a single species (Mandák et al. 2012). However, such variation is often associated with taxonomic interpretation rather than true cytological instability. In the past, several morphologically distinct taxa within the *C. album* complex were broadly classified under the name *C. album*, which resulted in the merging of cytologically distinct entities into a single taxonomic unit (Mandák et al. 2012).

The genus *Chenopodium* exhibits a relatively stable chromosomal framework characterized by a base chromosome number of **$x = 9$**. This base number gives rise to a clear diploid–polyploid series within the genus. Known cytotypes include diploids ($2n = 2x = 18$), tetraploids ($2n = 4x = 36$), hexaploids ($2n = 6x = 54$), and a single known decaploid species ($2n = 10x = 90$) (Mandák et al. 2012, 2016, 2018). Polyploidy has therefore played a significant role in the evolutionary diversification of the genus.

Importantly, cytogenetic and flow cytometric analyses have demonstrated that individual species are typically associated with specific ploidy levels. As a result, there is often a close correspondence between chromosome number and taxonomic delimitation within the genus (Mandák et al. 2012, 2016). Karyological data thus provide valuable information for understanding species relationships and contribute significantly to the clarification of taxonomic boundaries within the *Chenopodium album* aggregate.

3.1.3. Genome size

The nuclear genomes of species within *Chenopodium*, as well as many members of the family Amaranthaceae, are generally small. Flow cytometric analyses have shown that most taxa fall into the category of "very small genomes" sensu Leitch et al. (1998), with nuclear DNA content (2C) typically not exceeding 2.8 pg. Across *Chenopodium* sensu lato, the mean genome size has been estimated at 1.834 pg, highlighting the overall genomic compactness of the genus (Mandák et al. 2016).

Within the *Chenopodium album* aggregate, genome size variation reflects differences in ploidy levels. The smallest recorded value is 0.945 pg in the diploid species *C. vulvaria*, whereas the largest value, 6.440 pg, has been reported in the decaploid *C. frutescens* (Mandák et al. 2016). Despite this considerable range among cytotypes, the overall genome sizes within the aggregate remain relatively small, with an average value of approximately 1.848 pg (Mandák et al. 2016). These results demonstrate that even highly polyploid taxa in the genus possess relatively compact genomes in absolute terms.

Genome size in *Chenopodium* also appears to be highly stable, similarly to chromosome numbers. A large-scale flow cytometric study conducted by Vít et al. (2016), which analyzed 1,977 individuals representing 17 species of the *C. album* aggregate, revealed remarkable genome size consistency across a Eurasian transect exceeding 7,500 km. The minimal intraspecific variation observed across such a broad geographical range suggests that nuclear DNA content is largely independent of environmental gradients and that species generally represent cytogenetically stable units.

Comprehensive analyses of genome size evolution within *Chenopodium* sensu lato further revealed a pattern that differs from the typical trend observed in many other angiosperms. In most plant groups, polyploidization is followed by substantial DNA loss, resulting in genome downsizing over evolutionary time (Leitch & Bennett 2004; Leitch et al. 2008). In *Chenopodium*, however, Mandák et al. (2016) found little evidence for extensive post-polyploid genome contraction. This suggests that genome size in the genus has remained largely conserved following whole-genome duplication events.

One possible explanation for this pattern is the relatively recent origin of several *Chenopodium* lineages. Mandák et al. (2016) proposed that limited genome downsizing may reflect insufficient evolutionary time for large-scale DNA elimination. This interpretation is consistent with subsequent phylogenetic analyses indicating that most species within the *C. album* aggregate are evolutionarily young, with estimated divergence times not exceeding the Quaternary (Mandák et al. 2018). The combination of recent origin and genomic stability may therefore explain the largely additive genome sizes observed in polyploid taxa.

Indeed, approximate additivity of nuclear DNA content has been demonstrated in several polyploid species. For example, *C. album* sensu stricto, an allohexaploid derived from diploid and tetraploid progenitors, possesses a genome size closely corresponding to the sum of the genome sizes of its presumed parental taxa (Mandák et al. 2018). Similar additive relationships have been observed in other Eurasian polyploid species within the *C. album* aggregate (Mandák et al. 2018).

Taken together, these findings suggest that large-scale genome downsizing has not significantly altered genome size following polyploidization in *Chenopodium*. While genome size provides a reliable cytometric framework for distinguishing ploidy levels and understanding genome stability, it does not reveal the internal genomic composition or evolutionary origin of polyploid taxa. Recent phylogenomic studies have demonstrated that morphologically similar individuals within species may possess different lineage combinations despite having comparable nuclear DNA contents (Mandák et al. 2026). Consequently, genome size data must be integrated with molecular phylogenetic approaches to fully resolve the genomic architecture and evolutionary history of the genus.

3.1.4. Molecular evidence for genus-level relationships in *Chenopodium*

Chloroplast DNA and nuclear ribosomal internal transcribed spacer (nrITS)

The shift from morphology to molecular systematics constituted an essential evolution of relationships among the major taxa of *Chenopodium*. The earliest applications of plastid DNA markers (e.g., *rbcL, atpB–rbcL* spacer) and the nuclear ribosomal internal transcribed spacer (nrITS) provided the initial phylogenetic foundation that was able to validate morphogenic hypotheses (Kadereit et al., 2003, 2005, 2010).

These studies revealed that *Chenopodium* sensu lato is paraphyletic, and has several lineages of different tribes of *Amaranthaceae*.

An in-depth phylogenetic reconstruction of *Chenopodium* s.l. based on cpDNA and nrITS data resulted in the identification of a number of segregate genera such as *Dysphania, Blitum, Oxybasis, Chenopodiastrum, Lipandra,* and *Chenopodium* sensu stricto (Fuentes-Bazán et al., 2012). These analyses greatly clarified relationships between main evolutionary lineages and recast the limits of *Chenopodium* s.str. (which also encompasses the *C. album* aggregate), yet taxon sampling was still incomplete.

Nevertheless, plastid and ribosomal markers also impose intrinsic constraints when tested on polyploid complexes. Chloroplast genomes are usually maternally inherited and nrDNA is subject to concerted evolution. In allopolyploid taxa, these features usually yield the recovery of only one parental signal, hiding reticulate evolutionary histories (Mandák et al. 2016; Mandák et al. 2018). In a community context such as the *C. album* aggregate, abundant allopolyploidy could result in inaccurate topologies in which only cpDNA and nrITS are used.

Analysis of nrITS for the *C. album* aggregate yielded the relationships among diploid taxa with high support but produced unexpected positions of several polyploids (Mandák et al. 2016). Integrating plastid data, it has been shown that the placement to a plastid often reflects uniparental inheritance rather than actual phylogenetic relationship between two pairs. These results underscore the strengths and weakness of early molecular strategies: although there is critical need for them to construct a general diploid framework, this does not suffice to untangle reticulate polyploid origins.

Subgenomic reconstruction and low-copy nuclear markers.

To overcome the limitations of plastid and ribosomal markers in polyploid complexes, low-copy nuclear loci are increasingly used to reconstruct parental relationships and evolutionary histories. Unlike plastid DNA (cpDNA) and ribosomal ITS sequences, which often reflect only maternal inheritance or may undergo concerted evolution, low-copy nuclear genes can retain signals from multiple parental genomes. This makes them particularly suitable for studying reticulate evolution and identifying parental lineages in polyploid species (Sang 2002; Small et al. 2004).

In the *Chenopodium album* aggregate, Mandák et al. (2018) used variation in the third intron of **FLOWERING LOCUS T-like (FTL)** genes as a low-copy nuclear marker to reconstruct subgenomic relationships among diploid and polyploid taxa. Their analysis integrated FTL phylogenies with several complementary datasets, including classical karyological data (Mandák et al. 2012, 2016), genome size estimates obtained by flow cytometry (Mandák et al. 2016; Vít et al. 2016), plastid DNA sequences, and genomic in situ hybridization (GISH). This integrative approach provided a robust framework for identifying parental genomes and reconstructing the evolutionary origins of polyploid species.

The combined analyses revealed **eight major evolutionary lineages (subclades A–H)** within the *C. album* aggregate. Diploid representatives were identified in five of these clades, whereas the remaining three appear to originate from either extinct or currently unsampled diploid progenitors (Mandák et al. 2018). Polyploid species were shown to represent different combinations of these subgenomes, forming a complex evolutionary network involving diploid, tetraploid, and hexaploid taxa.

Tetraploid species generally originated through hybridization between diploid lineages, whereas hexaploid taxa arose from subsequent hybridization events involving tetraploid and diploid ancestors. The decaploid species *C. frutescens* represents a particularly distinctive lineage within this complex, although its precise subgenomic composition has not yet been fully resolved (Mandák et al. 2018).

Overall, the results indicate that polyploid formation in *Chenopodium* is neither random nor the result of a single evolutionary event. Instead, polyploid taxa appear to have originated repeatedly through hybridization among locally available progenitors. This process produced cytogenetically similar polyploid species that nevertheless possess different evolutionary origins and genomic compositions.

More recent genomic analyses based on RADseq data have further contributed to understanding the evolutionary history of polyploid taxa within the *Chenopodium album* aggregate. Mandák et al. (2026) confirmed that *C. album* s. str. has an allohexaploid genomic constitution (‘BBCCDD’, Figure 1) and showed that its subgenomes do not cluster with those of extant diploid or tetraploid species. This pattern suggests that its origin involved ancestral lineages that are no longer represented among currently sampled taxa.

In addition, the study identified multiple subgenomic combinations within *C. album* s. str., indicating that this species originated repeatedly from different genetic lineages. These findings support a complex evolutionary history involving recurrent hybridization and multiple origins of allohexaploid lineages.

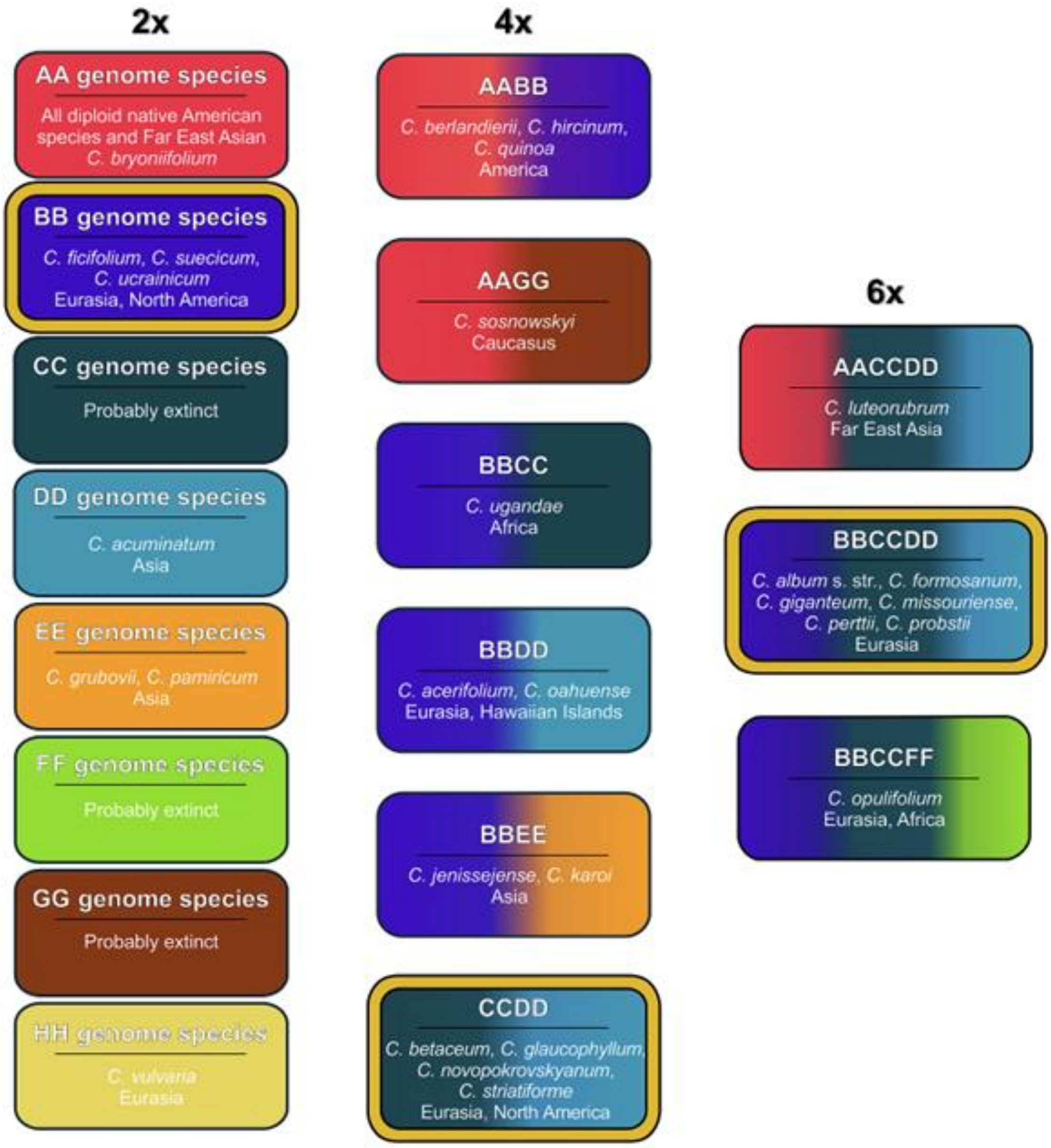


Figure 1. Schematic representation of genomic composition within the Chenopodium album aggregate, showing diploid genomes (A–H) and their combinations in polyploid taxa (e.g., BBCCDD in C. album s. str.). Modified from Mandák et al. (2026).

3.1.5. Importance to the *Chenopodium neomexicanum* aggregate

In contrast to the complex polyploid genomic structure described above, the taxa belonging to the *Chenopodium neomexicanum* aggregate are part of the diploid A-genome lineage (Mandák et al., 2016). These taxa represent a comparatively simpler genomic system, where variation is not driven by multiple subgenomic combinations but occurs within a single diploid lineage.

Such diploid systems provide an important framework for evaluating the relationship between morphological differentiation and genetic structure. Unlike polyploid complexes, where genomic admixture can obscure evolutionary relationships, diploid lineages allow for a clearer interpretation of whether phenotypic variation reflects underlying evolutionary divergence or represents continuous variation within a shared genetic background.

Consequently, the *C. neomexicanum* aggregate represents a relevant model for examining the extent to which morphologically defined taxa correspond to genetically differentiated lineages.

3.2. Taxonomy of *Chenopodium neomexicanum*

Taxonomic treatment of *Chenopodium neomexicanum aggregate*

Within *Chenopodium* sensu stricto, the taxa traditionally associated with *Chenopodium neomexicanum* Standley form a morphologically and ecologically coherent group. This aggregate describes a lineage of annual taxa that are adapted to arid and semi-arid environments of western North America and constitutes, indeed, one unique subgroup in that diploid CH2 clade, which molecular phylogenetic studies indicate (Mandák et al. 2016).

3.2.1. Morphological circumscription.

Chenopodium neomexicanum aggregate are annual herbs that live in lowland desert and semi-desert habitats. They are generally scented intensely and can be resinous-aromatic or skunky when tissues are crushed. This odouriferous nature, while not purely diagnostic, is often featured in the field and serves as a supporting characteristic in species identification. Leaves are variable in shape (lanceolate to rhombic–ovate or flabellate).

Margins are entire to shallowly toothed, and the extent (and quantity) of farinose coating differ significantly among populations and throughout the life cycle. The farinose indument can be thick and well-developed in young leaves, however it is thinned off in more frequent events as one age older.

Like the vegetative characters found in other members of *Chenopodium*, there is also a clear plasticity, particularly in the setting of a variety of environmental conditions (Habibi et al. 2018) and they should be treated carefully. Inflorescences are glomerulate to loosely paniculate, and there are typically five membranous tepals in flowers. The most reliable diagnostic features in the groups are fruiting characters. Seed structures, in general, are horizontal and lenticular. Surfaces range from dull to slightly lustrous and smooth to very faintly rugulose. The pericarp as an important feature, which sticks to the seed coat and is hard to separate. At maturity, tepals are usually somewhat thickened or have a weak keel and encase the fruit. These reproductive traits are common to taxa of the complex and are the primary basis for species-level delimitation (Benet-Pierce & Simpson 2017).

3.2.2. Taxonomic composition

Included species in the aggregate are: *Chenopodium arizonicum* Standley. *Chenopodium flabellifolium* Standley, *Chenopodium neomexicanum* Standley, *Chenopodium palmeri* Standley, *Chenopodium parryi* Standley, *Chenopodium sonorense* Benet-Pierce & M.G. Simpson.

These taxa share a combination of arid-adapted growth form, strongly adherent pericarps, and consistent fruit morphology (Figure 2), which set them apart from other diploid lineages of *Chenopodium.* Although *C. lenticulare* Aellen has formerly been linked to this complex (Benet-Pierce & Simpson 2017), early phylogenetic evidence shows much stronger affinity with other taxa, such as *C. watsonii* and *C. hians* sensu stricto (Mandák et al. 2016). In this respect, *C. lenticulare* is not included in the *C. neomexicanum* aggregate.

Figure 2. Chenopodium neomexicanum completely enclosing the fruit; pericarp adherent; seed horizontal, 1.3-1.5 mm. broad, nearly black, shining, puncticulate, the margin obtuse. Benet-Pierce & Simpson, Chenopodium Taxonomy of North America website (https://plants.sdsu.edu/chenopodium/Groupings/neomexicanum.html).

Geographic distribution and ecological context.

Species of *C. neomexicanum* aggregate are distributed across arid and semi-arid regions, primarily in western North America (Figure 3), including the southwestern United States and northern Mexico. They usually inhabit sandy, rocky, or saline substrates in desert basins, dry washes, and disturbed xeric habitats. The taxa have somewhat overlapping distributions, but each species seems to occupy a somewhat distinct ecological and geographic range from one another, although limits are incompletely documented in some regions of northern Mexico due to historically sparse collecting (Benet-Pierce & Simpson 2017).

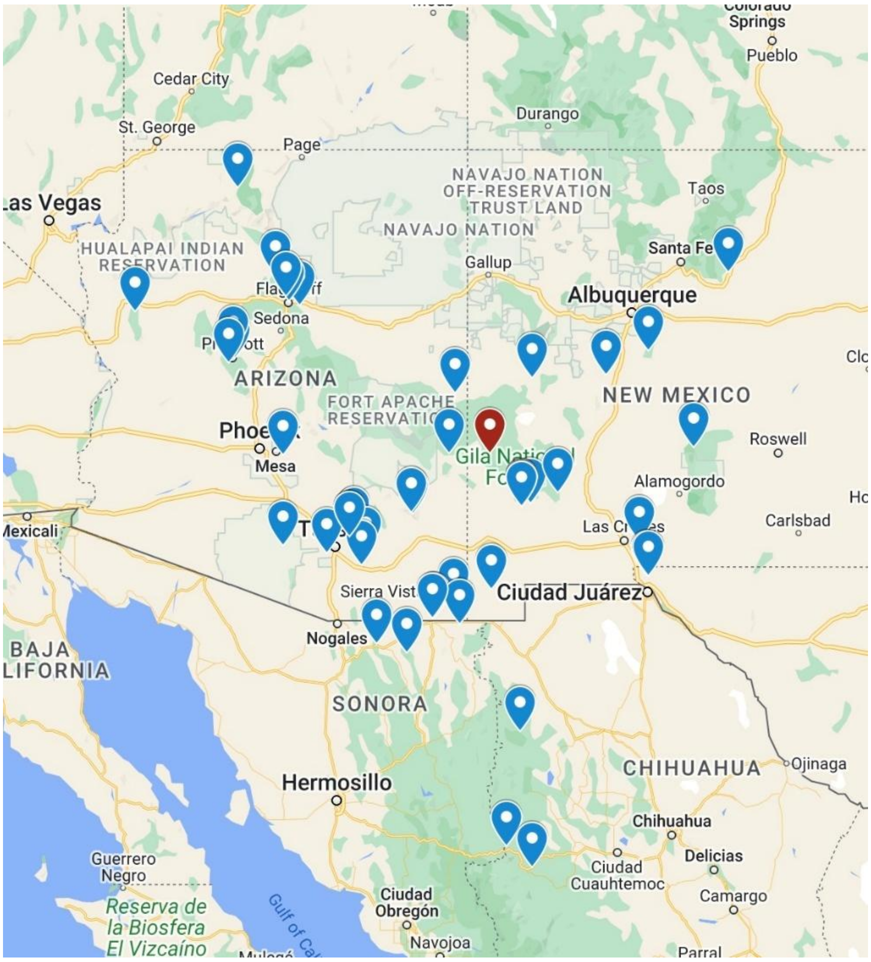


Figure 3. Geographic distribution of Chenopodium neomexicanum across the southwestern United States and northern Mexico, including Arizona, New Mexico, Texas, Sonora, and Chihuahua. Benet-Pierce & Simpson, Chenopodium Taxonomy of North America website (https://plants.sdsu.edu/chenopodium/Groupings/neomexicanum.html).

3.2.3. Taxonomic challenges

The *C. neomexicanum* complex is a group of annual herbs that have many taxonomic problems. The vegetative characteristics (leaf shape and size to farinose coating etc.) also overlap across species and can be highly variable according to the environmental settings. Herbarium specimens do not show mature fruits, or early season leaves, both of which are important for the proper identification. Benet-Pierce and Simpson (2017) showed that a great deal of the historical confusion over these taxa was due to variable vegetative characters and incomplete geographic sampling. Their revised manuscript highlighted the importance of the fruit and seed morphology, especially pericarp adherence, surface sculpture and subtle variations of fruit size and texture, to provide the most reliable and informative characters for species delimitation in the complex.

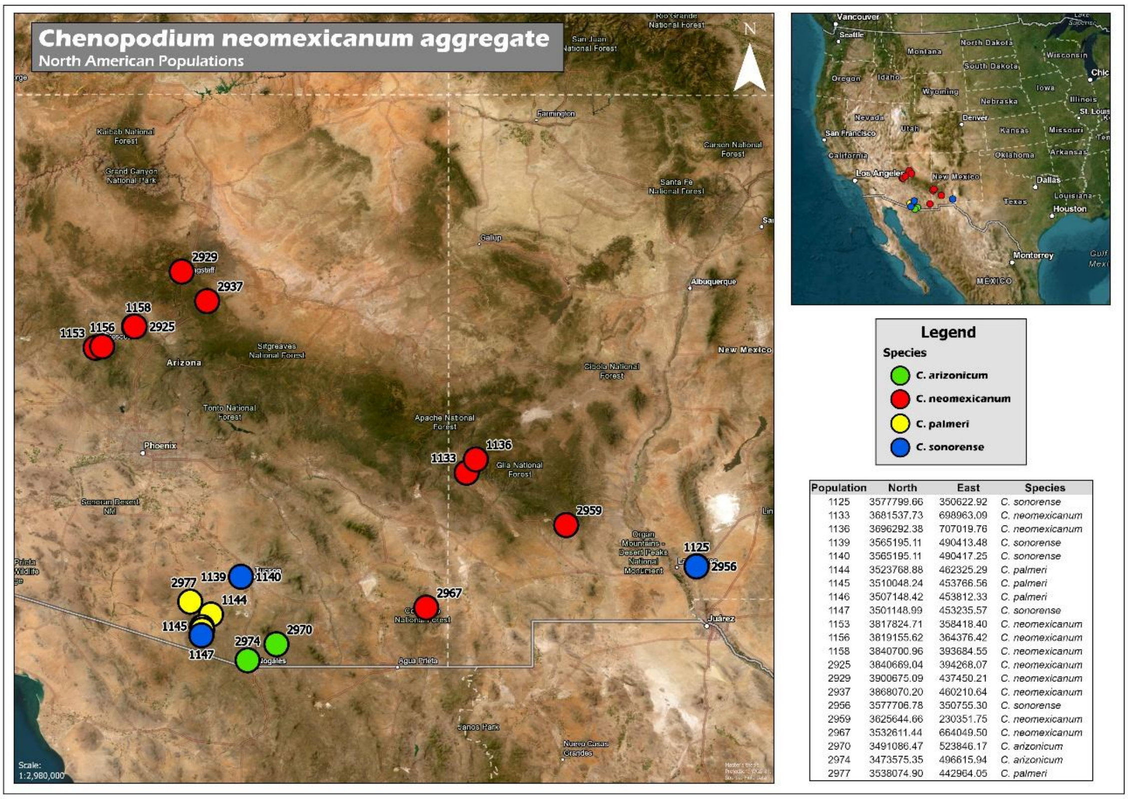
The taxa are morphologically alike but not only environmental variants of one large-scale species; they are separate entities over partially overlapping but ecologically separated ranges in the southwestern US and northern Mexico.

4. Methodology

4.1 Study area and taxonomic scope

This study focused on four species belonging to the *Chenopodium neomexicanum* aggregate: *C. arizonicum*, *C. neomexicanum*, *C. palmeri*, and *C. sonorense*. These taxa occur in arid and semi-arid regions of North America (Figure 4) and are morphologically similar, particularly in seed traits, which has historically complicated their delimitation. The present study aimed to evaluate seed morphological differentiation within this aggregate through an integrative quantitative and qualitative approach.

Specimens were selected to represent the morphological variation across the known distribution range of the aggregate. Species identity was verified using current taxonomic treatments and herbarium determinations prior to morphological analysis.



Population	North	East	Species
1125	3577799.66	350622.92	*C. sonorense*
1133	3681537.73	698963.09	*C. neomexicanum*
1136	3696292.38	707019.76	*C. neomexicanum*
1139	3565195.11	490413.48	*C. sonorense*
1140	3565195.11	490417.25	*C. sonorense*
1144	3523768.88	462325.29	*C. palmeri*
1145	3510048.24	453766.56	*C. palmeri*
1146	3507148.42	453812.33	*C. palmeri*
1147	3501148.99	453235.57	*C. sonorense*
1153	3817824.71	358418.40	*C. neomexicanum*
1156	3819155.62	364376.42	*C. neomexicanum*
1158	3840700.96	393684.55	*C. neomexicanum*
2925	3840669.04	394268.07	*C. neomexicanum*
2929	3900675.09	437450.21	*C. neomexicanum*
2937	3868070.20	460210.64	*C. neomexicanum*
2956	3577706.78	350755.30	*C. sonorense*
2959	3625644.66	230351.75	*C. neomexicanum*
2967	3532611.44	664049.50	*C. neomexicanum*
2970	3491086.47	523846.17	*C. arizonicum*
2974	3473575.35	496615.94	*C. arizonicum*
2977	3538074.90	442964.05	*C. palmeri*

Figure 4. Geographic distribution of Chenopodium neomexicanum aggregate population studied

4.2 Sampling strategy

A total of 225 mature seeds were examined from 4 species within 21 populations (Table 1). Sampling per species was as follows: 22 individuals of *C. arizonicum*, 81 of *C.*

neomexicanum, 53 of *C. palmeri*, and 69 of *C. sonorense*. Only fully developed, undamaged seeds were selected to ensure morphological consistency and to avoid measurement bias caused by immature or deteriorated material.

Each seed was treated as an independent analytical unit. Although multiple seeds may have originated from the same specimen, measurements were conducted individually to capture intra-specific morphological variation.

Seed Imaging and Morphological Evaluation

Seed morphology was analyzed using a KEYENCE VHX digital microscope system (Keyence Corporation, Osaka, Japan), which served as the principal instrument in this study. The system integrates a high-resolution digital imaging sensor, motorized zoom optics, adjustable LED illumination (coaxial and ring lighting), and a calibrated measurement module. The instrument allows real-time image acquisition with extended depth of field and automated scaling, ensuring precise morphometric measurement at magnifications ranging approximately from 100 × to 200×.

Each seed was photographed in two standardized orientations. In the horizontal (equatorial) view, seed diameter, seed width, margin configuration, and beak prominence were evaluated. In the vertical (lateral) view, side view margin thickness, pericarp surface structure, and papilla condition were assessed. Seeds were positioned consistently on a clean glass platform under controlled illumination to minimize angular distortion and shadow artifacts.

All images were digitally archived and coded systematically according to species, individual number, and orientation (e.g., 1125-1-2-V; 1133-1-2-H), ensuring traceability and reproducibility of observations.

Table 1. List of sampled Chenopodium populations in United States region. Latitude and longitude coordinates are in WGS84. Names of collectors are abbreviated: B. Mandák (BM), K. Krak (KK), J. Douda (JD), G. Filippi (GF).

Population Code	**Taxon**	**Latitute (N)**	**Longitude (E)**	**Locality**	**Date of collection**	**Collectors**
1125	*C. sonorense*	32.3271	-106.58703	USA, New Mexico, Las Cruces, Dripping Springs Natural Area	16/09/2019	BM;KK;JD;GF
1133	*C. neomexicanum*	33.25452	-108.86418	USA, New Mexico, Pleasanton, Rd 180	17/09/2019	BM;KK;JD;GF
1136	*C. neomexicanum*	33.38599	-108.77437	USA, New Mexico, Mogollon, Bursum Rd	17/09/2019	BM;KK;JD;GF
1139	*C. sonorense*	32.22333	-111.10174	USA, Arizona, Tucson, Gates Pass Trail	19/09/2019	BM;KK;JD;GF
1140	*C. sonorense*	32.22333	-111.10174	USA, Arizona, Tucson, Gates Pass Trail	19/09/2019	BM;KK;JD;GF
1144	*C. palmeri*	31.84901	-111.39821	USA, Arizona, Tucson, Three Points, Rd 286	20/09/2019	BM;KK;JD;GF
1145	*C. palmeri*	31.72491	-111.48802	USA, Arizona, Tucson, Three Points, Rd 286	20/09/2019	BM;KK;JD;GF
1146	*C. palmeri*	31.69875	-111.4874	USA, Arizona, Tucson, Three Points, Rd 286	20/09/2019	BM;KK;JD;GF
1147	*C. sonorense*	31.6446	-111.4932	USA, Arizona, Tucson, Three Points, Rd 286	20/09/2019	BM;KK;JD;GF
1153	*C. neomexicanum*	34.49232	-112.54202	USA, Arizona, Prescott, Mt Francis Rd	21/09/2019	BM;KK;JD;GF
1156	*C. neomexicanum*	34.50512	-112.47736	USA, Arizona, Prescott, Rd 89, White Spar Campground	21/09/2019	BM;KK;JD;GF
1158	*C. neomexicanum*	34.70282	-112.16087	USA, Arizona, Prescott, Rd 89A	21/09/2019	BM;KK;JD;GF
2925	*C. neomexicanum*	34.7025927	-112.1544956	USA, Arizona, Prescott, Potato Patch Campground	04/09/2025	BM;KK
2929	*C. neomexicanum*	35.2472121	-111.6875334	USA, Arizona, Flagstaff, Forest Service Rd. 164B	05/09/2025	BM;KK
2937	*C. neomexicanum*	34.9543755	-111.4357967	USA, Arizona, Flagstaff, County Rd. 3, Mormon Lake	05/09/2025	BM;KK
2956	*C. sonorense*	32.3262801	-106.5856094	USA, New Mexico, Las Cruces, Dripping Springs Natural Area	10/09/2025	BM;KK
2959	*C. neomexicanum*	32.7356458	-107.877479	USA, New Mexico, Hanover, NM-61	10/09/2025	BM;KK
2967	*C. neomexicanum*	31.91756	-109.2648744	USA, Arizona, San Simon, Rustler Park, Rustler Park Road	12/09/2025	BM;KK
2970	*C. arizonicum*	31.554518	-110.7487478	USA, Arizona, Patagonia, Temporal Rd.	13/09/2025	BM;KK
2974	*C. arizonicum*	31.3967649	-111.0355957	USA, Arizona, Ruby Road	14/09/2025	BM;KK
2977	*C. palmeri*	31.9772657	-111.6036865	USA, Arizona, Kitt Peak National Observatory, along road 386	14/09/2025	BM;KK

4.3. Morphological descriptors

4.3.1. Quantitative descriptors

Three continuous seed traits were measured in millimetres: seed diameter, seed width, and margin width from top. Measurements were obtained directly from calibrated digital images using the integrated software measurement tool of the KEYENCE VHX system.

4.3.2. Qualitative descriptors

Six categorical descriptors were evaluated: margin configuration, beak prominence, side view margin, pericarp surface, pericarp colour, and papilla condition. Character states were defined a priori based on preliminary examination and taxonomic descriptions. Each descriptor was treated as a discrete multi-state categorical variable.

A complete list of descriptors and corresponding character states is provided in Table 2.

Table 2. Qualitative seed descriptors and character states.

Descriptor	**Character states**
Margin configuration	Convex (1); Biconvex (2); Equatorially banded (3); Rounded (4)
Beak prominence	Very weak (1); Weak (2); Prominent (3); Very prominent (4)
Side view margin	Thin (1); Thick (2)
Pericarp surface	Smooth (1); Papillate (2); Broadly pitted (3)
Pericarp colour	Black (1); Greyish (2); Light brown (3); Dark brown (4)
Papilla condition	Collapsed (1); Inflated (2); Absent (3)

4.4. Data recording

Two independent data matrices were constructed. The quantitative matrix included 225 rows (individual seeds) and three continuous variables. The qualitative matrix included 225 rows and six categorical descriptors. Species identity was incorporated as a grouping factor in both matrices.

All data were curated prior to analysis to ensure consistency in coding, absence of missing values, and correct species assignment.

4.5. Statistical analyses

All statistical analyses were performed in R (R Core Team, 2023).

4.5.1. Analysis of quantitative traits

Descriptive statistics (mean, standard deviation, minimum, and maximum) were calculated for each quantitative trait within species. These statistics provided an initial characterization of central tendency and dispersion.

To evaluate overall multivariate differentiation among species based on quantitative traits, a Multivariate Analysis of Variance (MANOVA) was performed using species as a fixed factor and the three morphometric traits as dependent variables. Wilks' lambda (λ) was used as the test statistic. Wilks' lambda represents the proportion of total variance not explained by group differences; lower values indicate stronger multivariate separation.

Each quantitative variable was subsequently analyzed independently. Homogeneity of variances was assessed using Levene's test. When heteroscedasticity was detected, Welch's ANOVA was applied because it is robust to unequal variances and sample sizes. Post-hoc comparisons following Welch's ANOVA were conducted using the Games-Howell procedure. For variables meeting parametric assumptions, one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test was applied.

Principal Component Analysis (PCA) was conducted on standardized quantitative variables. Standardization ensured equal contribution of all variables regardless of scale. Eigenvalues and eigenvectors were extracted to determine the proportion of variance explained by each component (Jolliffe, 2002). Individuals were projected onto the first two principal components to visualize multivariate structure.

Hierarchical clustering was performed using Euclidean distance and Ward's minimum variance method. Euclidean distance preserves geometric relationships in continuous data, while Ward's algorithm minimizes within-cluster variance at each agglomeration step.

4.5.2. Analysis of qualitative traits

State frequencies and percentages were calculated for each descriptor within species. Modal states were identified, and a dominance threshold of $\geq 70\%$ frequency was defined to characterize strongly prevailing character states.

Intra-specific diversity for each qualitative descriptor was quantified using Shannon's diversity index (Shannon, 1948):

$H' = -\Sigma (pi \ln pi)$,

where pi represents the proportion of each character state within species. Higher values indicate greater heterogeneity of character states.

To assess whether qualitative state distributions differed among species, Pearson's chi-square tests were applied. Significant results indicate non-random association between species identity and character states.

4.5.3. Multivariate analyses of qualitative traits

Because qualitative descriptors are multi-state categorical variables, Gower's distance coefficient was used to calculate pairwise dissimilarities among individuals (Gower, 1971). Gower distance accommodates categorical variables and standardizes contributions across descriptors.

Principal Coordinates Analysis (PCoA) was performed on the Gower distance matrix to visualize multivariate relationships among individuals. PCoA extracts orthogonal axes representing major gradients of variation based on eigen decomposition of the centered distance matrix (Legendre & Legendre, 2012).

Species-level differentiation was formally tested using Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001). The model included species as the explanatory factor and the Gower distance matrix as the response. Significance was assessed using 9,999 permutations. The pseudo-F statistic and associated R^2 value were interpreted as indicators of multivariate separation and proportion of explained variation.

Hierarchical clustering of qualitative traits was performed using UPGMA (average linkage) on the Gower distance matrix to assess patterns of aggregation among individuals.

All statistical tests were conducted at a significance threshold of $\alpha = 0.05$.

4.6 Molecular analysis

Molecular data used in this study were provided by the Plant Biodiversity and Evolution Research Group at the Czech University of Life Sciences Prague, Faculty of Environmental Sciences. This research group investigates the phylogeny and phytogeography of *Chenopodium* species. The dataset analysed here was generated as part of ongoing research focused on the evolutionary relationships within the *Chenopodium neomexicanum* aggregate. Below, a brief description of the laboratory and bioinformatic procedures applied by this research group for the phylogenomic analysis of *Chenopodium* species is presented.

4.6.1. DNA analysis and sequencing

Genomic DNA was extracted from plant tissue with the procedure laid out in Štorchová et al., (2000). DNA samples were purified using the NucleoSpin gDNA Clean-up Kit to improve the quality and purity of these genetic products for future use. DNA concentration was quantified using the Qubit High Sensitivity (HS) DNA Kit to ensure sufficient template for sequencing. Genome-wide markers were derived by single-digest RADseq (Restriction-site Associated DNA sequencing) methodology in accordance with the approach of Habibi et al., (2023).

4.6.2. Bioinformatic processing and phylogenetic analysis

Raw sequences were demultiplexed and quality-filtered by means of the process_radtags module found in STACKS v2.68 (Catchen et al., 2013; Rochette et al., 2019). Filtered reads were aligned to the reference genome of *Chenopodium* watsonii in accordance with the BWA bwa-mem algorithm (Li, 2013). This was done according to Young et al., (2023). RAD loci were reconstructed and single nucleotide polymorphisms (SNPs) identified using the ref_map.pl pipeline in STACKS. SNP-filtering was performed using VCFtools (Danecek et al., 2011), retaining loci present in at least 80% of individuals, only biallelic SNPs, a minimum read depth greater than three, and a minor allele frequency above 0.03, while individuals with more than 40% missing data were excluded. A maximum-likelihood phylogenetic tree was reconstructed using RAxML (Stamatakis, 2014) under the K80 substitution model, with node support assessed through 1000 bootstrap replicates. The resulting phylogenetic tree was visualized and edited using FigTree v1.4.4 (Rambaut, 2018).

Genetic clusters were defined based on the phylogenetic tree topology and node support. Well-supported clades (bootstrap ≥ 90) were visually delimited in the tree using vertical boundaries corresponding to each cluster. These delimitations were used to assign individuals to five genetic groups, which were subsequently used for PCA analyses, independently of their species designation.

5. Results

5.1. Quantitative Seed Morphometric Variation

5.1.1. Descriptive Statistics

Quantitative seed traits showed clear variation among the four species of the *Chenopodium neomexicanum* aggregate. The mean seed diameter (1.192 mm) and seed width (0.650 mm) were found to be largest in *C. neomexicanum* and smallest in *C. palmeri* (0.890 mm and 0.457 mm respectively). *Chenopodium arizonicum* and *C. sonorense* showed intermediate mean seed sizes (Table 3).

Variation in seed diameter ranged from 0.686 mm in *C. palmeri* to 1.421 mm in *C. neomexicanum*. In a similar manner, the seed width ranged from 0.239 mm in *C. palmeri* to 0.809 mm in *C. neomexicanum*. Margin width at the top indicated less-substantial differences between different species in the mean values (0.107–0.128 mm), while *C. neomexicanum* presented the maximum value (0.212 mm). The standard deviations for this trait were low across species (0.014–0.022 mm), suggesting little dispersion relative to seed size traits.

Table 3. Mean values, standard deviations, and ranges for each quantitative trait by species; *C.a: C. arizonicum; C.n: C. neomexicanum; C.p: C. palmeri and C.s: C. sonorense*

Quantitative descriptors	***Chenopodium neomexicanum* aggregate**			
	C.a	***C.n***	***C.p***	***C.s***
Number of samples	22	81	53	69
Seed diameter (mm)				
Mean	1.010	1.192	0.890	0.973
Standard deviation	0.084	0.112	0.112	0.075
Minimum	0.854	0.868	0.686	0.737
Maximum	1.160	1.421	1.114	1.132
Seed width (mm)				
Mean	0.517	0.650	0.457	0.532
Standard deviation	0.046	0.083	0.069	0.045
Minimum	0.428	0.453	0.239	0.386
Maximum	0.603	0.809	0.559	0.621
Margin width from top (mm)				
Mean	0.109	0.128	0.107	0.120
Standard deviation	0.014	0.019	0.022	0.017
Minimum	0.084	0.081	0.043	0.090
Maximum	0.133	0.212	0.158	0.155

5.1.2. Multivariate Differentiation (MANOVA)

Multivariate analysis of variance showed that there is an overall highly significant effect of species on quantitative seed traits (Wilks' $\lambda = 0.309$, $F = 36.76$, $p < 0.001$; Table 4). The low Wilks' lambda value reflects pronounced multivariate separation among taxa, indicating that species identity accounts for a considerable share of total morphometric variation.

Table 4. Multivariate analysis of variance (MANOVA) for quantitative seed traits among species of the Chenopodium neomexicanum aggregate

Effect	Df	Wilks' λ	Approx. F	Num Df	Den Df	p-value
Species	3	0.309	36.76	9	533.14	< 0.001
Residuals	221					

5.1.3. Univariate analysis of seeds traits

Univariate analyses confirmed significant interspecific differences for all quantitative traits evaluated (Figures 5-7).

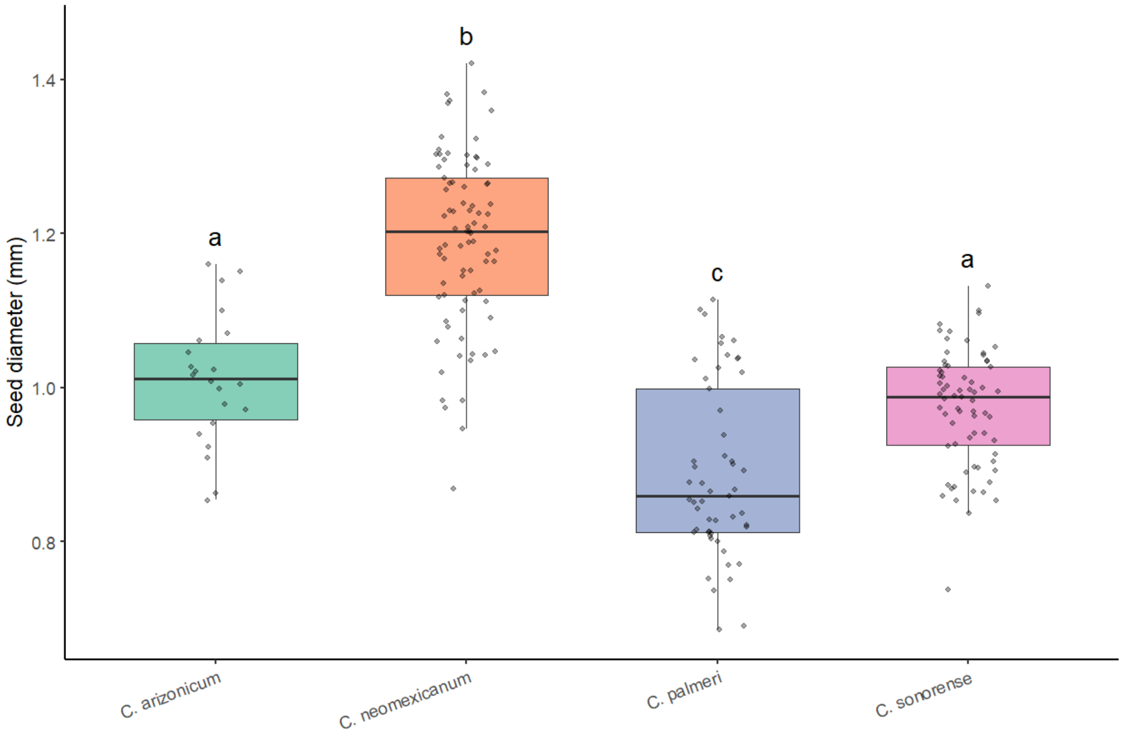


Figure 5. Boxplots of seed diameter (mm) across species of the Chenopodium neomexicanum aggregate. Letters indicate significant differences among species based on Welch's ANOVA followed by Games–Howell post-hoc comparisons (α = 0.05).

Seed diameter differed significantly among species under Welch's ANOVA (F = 95.47, p < 0.001), reflecting heteroscedastic variances. Post-hoc comparisons using the Games–Howell test revealed a clear hierarchical pattern: *C. neomexicanum* exhibited significantly larger seeds than all other taxa, whereas *C. palmeri* showed significantly smaller values. No significant differences were detected between *C. arizonicum* and *C. sonorense*, which formed a homogeneous group (Figure 5).

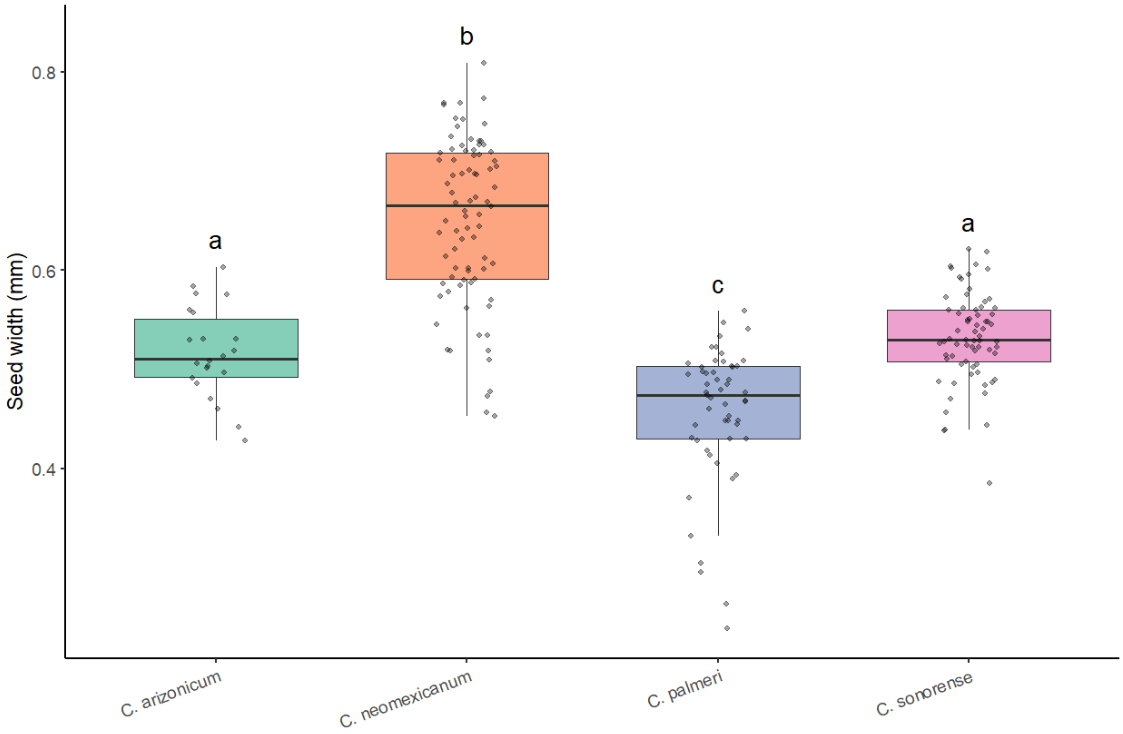


Figure 6. Boxplots of seed width (mm) across species of the Chenopodium neomexicanum aggregate. Letters indicate significant differences among species based on Welch's ANOVA followed by Games–Howell post-hoc comparisons (α = 0.05).

A similar pattern was observed for seed width (Welch's ANOVA: F = 74.76, p < 0.001). Again, *C. neomexicanum* presented the highest values and *C. palmeri* the lowest, while *C. arizonicum* and *C. sonorense* did not differ significantly from each other (Figure 6). The concordance between diameter and width indicates that overall seed size is consistently structured among species.

Margin width from top also showed significant interspecific differentiation (one-way ANOVA: F = 15.18, $p < 0.001$). Tukey's post-hoc test indicated that *C. neomexicanum* had significantly wider margins than the remaining taxa. In contrast to seed size traits, partial overlap was detected among *C. arizonicum*, *C. palmeri*, and *C. sonorense*, which formed intermediate or partially overlapping significance groups (Figure 7).

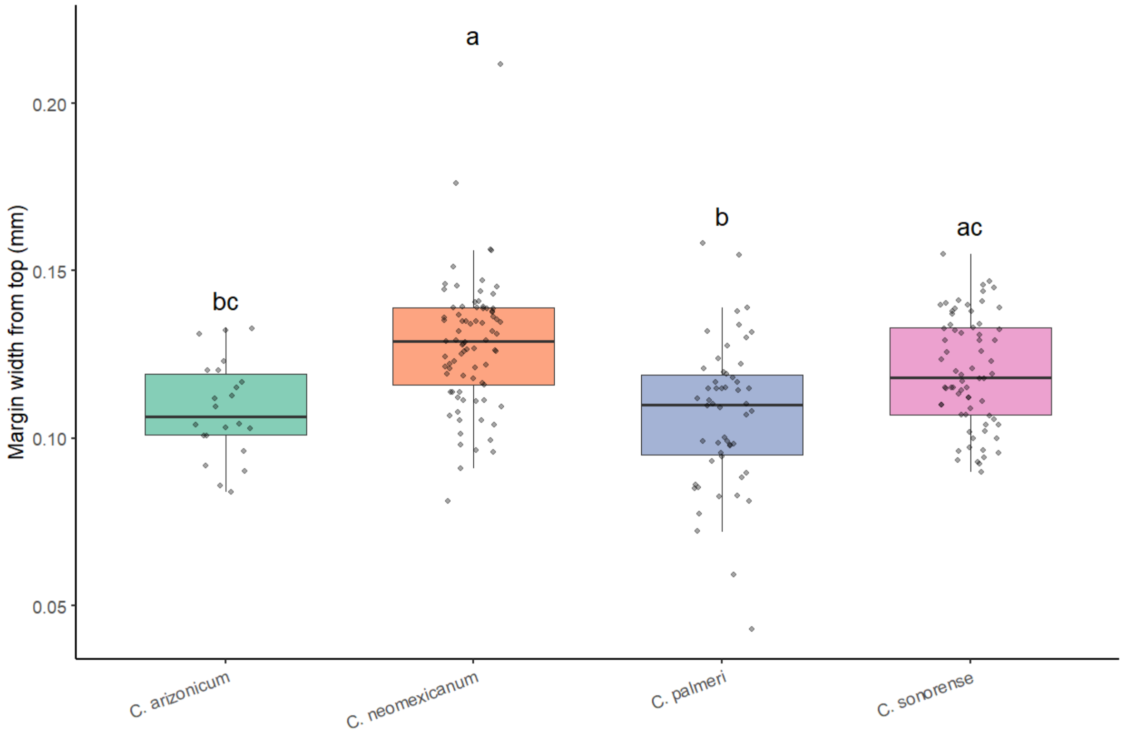


Figure 7. Boxplots of margin width from top (mm) across species of the Chenopodium neomexicanum aggregate. Letters indicate significant differences among species based on one-way ANOVA followed by Tukey's HSD test (α = 0.05).

5.1.4. Principal Component Analysis from quantitative traits

Principal Component Analysis (PCA) was conducted to visualize multivariate structure among individuals; it indicated that the initial two components accounted for 93.2% of the total morphometric variation (Table 5).

Table 5. Eigenvalues and proportion of variance explained by principal components

Component	Standard deviation	Proportion of variance	Cumulative proportion
PC1	1.452	70.3%	70.3%
PC2	0.829	22.9%	93.2%
PC3	0.452	6.8%	100%

The first PC1 explained 70.3% and the second PC2 explained 22.9% of the overall variance. Species were highly structurally grouped along PC1 by visualizing individual scores along the first two principal components (Figure 8). *Chenopodium neomexicanum* and *C. palmeri* were at opposite ends of the first axis indicating high divergence in seed size traits. In contrast, *C. arizonicum* and *C. sonorense* showed only partial overlap and intermediate position. Separation on PC2 was generally lower and caused intra-group dispersion, not strong interspecific segregation. As a whole, the PCA establishes that the quantitative differentiation in the aggregate of *Chenopodium neomexicanum* is determined primarily by variation in seed size and supports findings from MANOVA and univariate analyses.

Figure 8. Principal Components Analysis based on quantitative traits for *Chenopodium neomexicanum* aggregate

5.1.5. Hierarchical clustering based on quantitative traits

Hierarchical clustering based on Euclidean distance and Ward’s method further supported the multivariate differentiation among species (Figure 9).

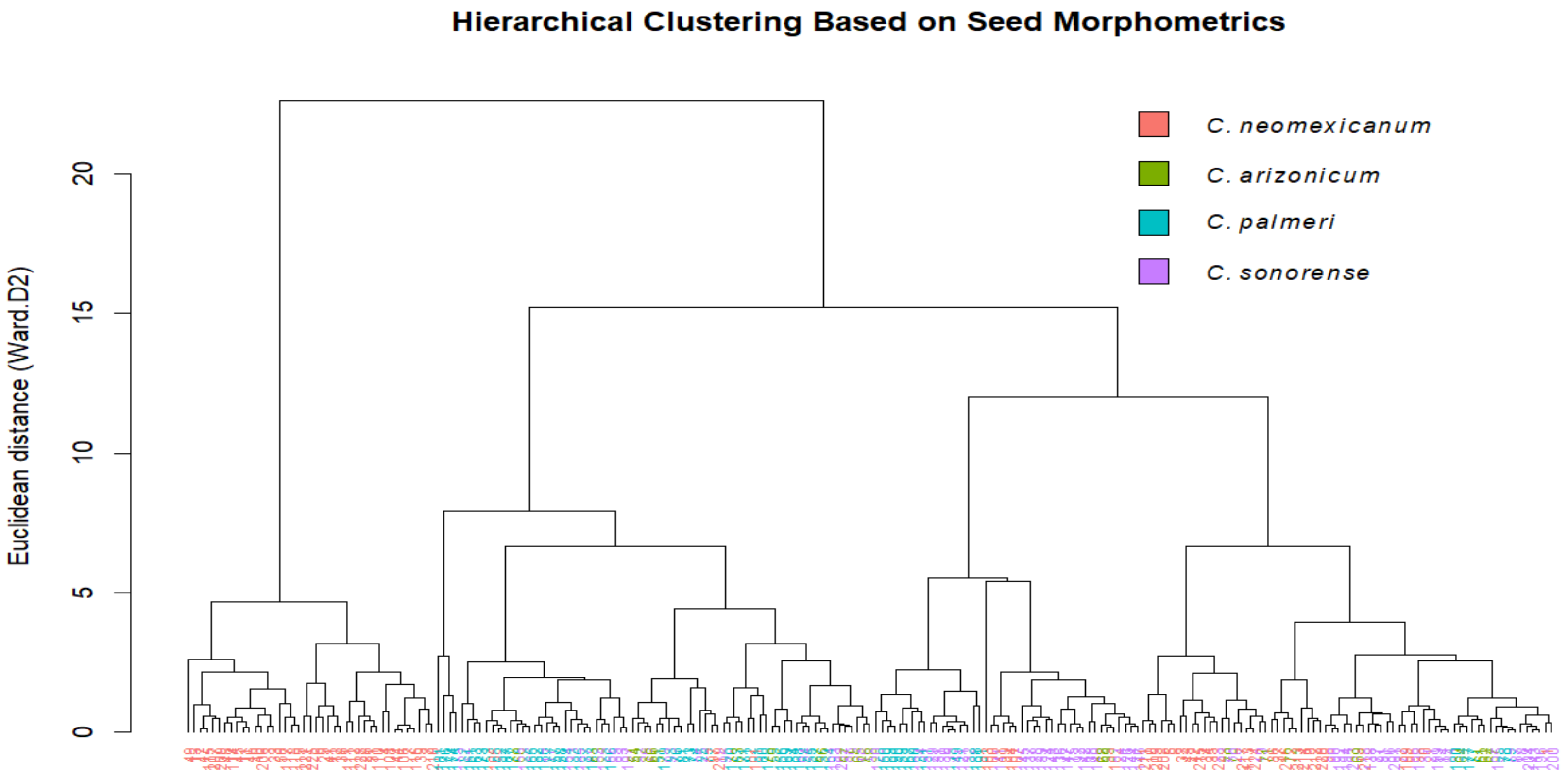


Figure 9. Dendrogram based on seed morphometrics for Chenopodium neomexicanum aggregate

The dendrogram revealed a primary separation consistent with patterns observed in PCA. *Chenopodium neomexicanum* formed a distinct cluster separated from the remaining taxa at higher linkage distances, reflecting its larger seed size. *Chenopodium palmeri* also showed clear clustering tendencies, corresponding to its comparatively smaller seed morphometrics.

In contrast, *C. arizonicum* and *C. sonorense* exhibited closer clustering and partial intermixing at lower linkage distances, consistent with their intermediate morphometric values and the partial overlap observed in the PCA.

Overall, the hierarchical clustering analysis corroborates the strong quantitative structuring within the *Chenopodium neomexicanum* aggregate and reinforces the dominant role of seed size in species differentiation.

5.2 Qualitative Seed morphological Variation

5.2.1. Basic descriptive analysis on qualitative seed morphological variation

Qualitative descriptors identified structured but non-exclusive variation in species in the *Chenopodium neomexicanum* aggregate (Table 6). Even though all character states were represented by taxa, numerous descriptors showed strong differences in proportional representation.

There was dramatic diversity in margin configuration between species. While *C. palmeri* was largely defined by the rounded state (73.6%), *C. arizonicum* was predominantly biconvex (63.6%). *Chenopodium neomexicanum* was also represented by the majority biconvex state (50.6%), although a large proportion of the species exhibited the rounded state (23.5%). *C. sonorense* exhibited relatively intermediate distribution with both rounded (44.9%) and biconvex (34.8%) states highly represented.

Beak prominence showed less interspecific differentiation, with the “very weak” condition dominating across all taxa (65-73%). The “very prominent” state was entirely absent in all species, so that the highly prominent state had relatively limited aggregate discrimination at that species level.

There were different patterns on the side view margin. *Chenopodium arizonicum* was primarily thick (72.7%), while the rest of the species were mainly thin, mainly *C. neomexicanum* (74.1%). Hence, this descriptor adds moderate structural differentiation.

Table 6. Frequency (n) and percentage (%) of qualitative seed character states within species of the Chenopodium neomexicanum aggregate; C.a: C. arizonicum; C.n: C. neomexicanum; C.p: C. palmeri and C.s: C. sonorense

Qualitative descriptors	*Chenopodium neomexicanum* agreggate			
	C.a	***C.n***	***C.p***	***C.s***
Number of samples	22	81	53	69
Margin configuration				
Convex	1 (4.5%)	13 (16.0%)	1 (1.9%)	2 (2.9%)
Biconvex	14 (63.6%)	41 (50.6%)	9 (17.0%)	24 (34.8%)
Equatorially banded	5 (22.7%)	8 (9.9%)	4 (7.5%)	12 (17.4%)
Rounded	2 (9.1%)	19 (23.5%)	39 (73.6%)	31 (44.9%)
Beak prominence				
Very weak	16 (72.7%)	55 (67.9%)	35 (66.0%)	45 (65.2%)
Weak	5 (22.7%)	25 (30.9%)	16 (30.2%)	19 (27.5%)
Prominent	1 (4.5%)	1 (1.2%)	2 (3.8%)	5 (7.2%)
Very prominent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Side view margin				
Thin	6 (27.3%)	60 (74.1%)	36 (67.9%)	42 (60.9%)
Thick	16 (72.7%)	21 (25.9%)	17 (32.1%)	27 (39.1%)
Pericarp color				
Black	21 (95.5%)	58 (71.6%)	35 (66.0%)	56 (81.2%)
Grayinsh	0 (0.0%)	6 (7.4%)	0 (0.0%)	2 (2.9%)
Light Brown	0 (0.0%)	2 (2.5%)	5 (9.4%)	2 (2.9%)
Dark Brown	1 (4.5%)	15 (18.5%)	13 (24.5%)	9 (13.0%)
Papilla condition				
Collapsed	17 (77.3%)	64 (79.0%)	31 (58.5%)	45 (65.2%)
Inflated	0 (0.0%)	15 (18.5%)	21 (39.6%)	22 (31.9%)
Absent	5 (22.7%)	2 (2.5%)	1 (1.9%)	2 (2.9%)
Pericarp Surface				
Smooth	9 (40.9%)	1 (1.2%)	2 (3.8%)	2 (2.9%)
Papillate	2 (9.1%)	11 (13.6%)	32 (60.4%)	47 (68.1%)
Broadly pitted	11 (50.0%)	69 (85.2%)	19 (35.8%)	20 (29.0%)

The color of pericarp in all species strongly favoured the black state, particularly in *C. arizonicum* (95.5%) and *C. sonorense* (81.2%). However, *C. neomexicanum* and *C. palmeri* also indicated greater frequency of dark brown seeds, indicating some chromatic diversity for these taxa.

The collapsed state was dominant for all organisms, especially *C. arizonicum* and *C. neomexicanum* under the papille condition. Indeed, the inflated condition was reported more frequently in *C. palmeri* and *C. sonorense*, indicative of partial seed surface microstructure stratification.

Pericarp surface displayed the starkest interspecific differences. *Chenopodium neomexicanum* presented a broadly pitted state (85.2%), where the papillate, state was the dominant state (60.4% and 68.1% were that of *C. palmeri* and *C. sonorense*, respectively). *C. arizonicum* had greater intra-specific heterogeneity compared to the other species, indicating a more balanced distribution of smooth surfaces and broadly pitted interfaces.

Overall, both qualitative categories show there are differential dominance patterns among species, of which pericarp surface and margin configuration make up the strongest visible separation, whereas beak prominence and pericarp color are linked with broader overlap.

5.2.2 The dominant character states

The most frequent character (modal) state was identified (Table 7) for each qualitative descriptor, with >70% frequency status coded as dominant in the species. Dominance patterns were somewhat variable both for descriptors and taxa.

The dominance of the beak had limited discriminatory influence since all species exhibited a modal “very weak” condition. However, it surpassed the dominance limit once in *C. arizonicum*, inferring generally similar character expressions in the aggregate.

Tables of the side view margin demonstrated contrasting dominant states. *Chenopodium arizonicum* was mostly characterised by a predominant thick margin (72.7%), and *C. neomexicanum* was dominant to thin condition (74.1%). The few other species had intermediate but did not show a majority frequency dominance.

Margin layout exhibited a distinct dominant pattern only in *C. palmeri*, which showed the dominant trend for the rounded state (73.6%). In contrast *C. arizonicum* and *C. neomexicanum* were both largely biconvex, but neither exceeded the dominance threshold. The distribution of *C. sonorense* was much more heterogeneous, with no state being present over 50%.

Pericarp surface displays the strongest species-level structuring. *Chenopodium neomexicanum* had a broadly pitted (85.2%) surface; whereas *C. palmeri* and *C. sonorense* were mainly papillate. In contrast *C. arizonicum* had a widely distributed shape without a dominant state.

Table 7. Frequency (%) of the modal state for each descriptor by species; C.a: C. arizonicum; C.n: C. neomexicanum; C.p: C. palmeri and C.s: C. sonorense, (dominant states ≥70% in bold)

Descriptor	***C.a***	***C.n***	***C.p***	***C.s***
Margin configuration	63.6 (Biconvex)	50.6 (Biconvex)	**73.6 (Rounded)**	44.9 (Rounded)
Beak prominence	**72.7 (Very weak)**	67.9 (Very weak)	66.0 (Very weak)	65.2 (Very weak)
Side view margin	**72.7 (Thick)**	**74.1 (Thin)**	67.9 (Thick)	60.9 (Thick)
Pericarp surface	50.0 (Broadly pitted)	**85.2 (Broadly pitted)**	60.4 (Papillate)	68.1 (Papillate)
Pericarp colour	**95.5 (Black)**	**71.6 (Black)**	66.0 (Black)	**81.2 (Black)**
Papilla condition	**77.3 (Collapsed)**	**79.0 (Collapsed)**	58.5 (Collapse)	65.2 (Collapse)

Pericarp color was highly stable between taxa: black seeds were abundant in *C. arizonicum*, *C. neomexicanum*, and *C. sonorense*. Of note, black too was modal in *C. palmeri*, but the frequency did not approach the dominance threshold.

Papilla state was dominantly dominated by the collapsed state of *C. arizonicum* and *C. neomexicanum*, although *C. palmeri* and *C. sonorense* demonstrated greater heterogeneity and lower overall dominance. Collectively, dominant-state measurements suggest pericarp surface and side view margin are most important for species-level discrimination, while beak prominence and color of the pericarp show more interspecific overlap.

5.2.3. Diversity and interspecific differentiation of qualitative descriptors

The qualitative seed descriptors exhibited contrasting patterns of intra-specific diversity and interspecific differentiation within the *Chenopodium neomexicanum* aggregate (Table 8).

Margin configuration showed moderate to high within-species diversity, with Shannon index values ranging from 0.797 to 1.207 and the highest value observed in *C. neomexicanum* ($H = 1.207$). The chi-square test indicated highly significant among-species differentiation ($\chi^2 = 55.668$, $p < 0.001$), demonstrating that the distribution of margin states differs among species.

Pericarp surface exhibited marked differences in within-species diversity, ranging from low in *C. neomexicanum* ($H = 0.462$) to high in *C. arizonicum* ($H = 0.930$). This descriptor showed the strongest among-species differentiation ($\chi^2 = 111.530$, $p < 0.001$), indicating a pronounced difference in state distribution across taxa.

Table 8. Intraspecific and interspecific variation based on qualitative descriptors C.a: C. arizonicum; C.n: C. neomexicanum; C.p: C. palmeri and C.s: C. sonorense

Descriptor	Shannon Index				χ^2	p-value
	C.a	***C.n***	***C.p***	***C.s***		
Margin configuration	0.983	1.207	0.797	1.134	55.668	<0.001
Beak prominence	0.709	0.680	0.759	0.824	4.020	0.685
Side view margin	0.586	0.572	0.627	0.669	17.096	0.001
Pericarp surface	0.930	0.462	0.796	0.723	111.530	<0.001
Pericarp colour	0.185	0.836	0.841	0.640	18.397	0.028
Papilla condition	0.536	0.590	0.755	0.746	33.015	<0.001

Papilla condition displayed moderate within-species diversity (H = 0.536–0.755) and significant among-species differentiation ($\chi^2 = 33.015$, $p < 0.001$), suggesting that this descriptor contributes to morphological differences among species.

Side view margin showed relatively low within-species diversity (H = 0.572–0.669). Despite this limited diversity, significant among-species differentiation was detected ($\chi^2 = 17.096$, $p = 0.001$), indicating that even subtle variation is structured across taxa.

Pericarp color revealed contrasting levels of within-species diversity, with very low diversity in *C. arizonicum* (H = 0.185) and higher values in *C. neomexicanum* (H = 0.836) and *C. palmeri* (H = 0.841). Although among-species differentiation was statistically significant ($\chi^2 = 18.397$, $p = 0.028$), the magnitude of differentiation was lower compared to margin configuration and pericarp surface.

Beak prominence showed comparable levels of within-species diversity across taxa (H = 0.680–0.824). The chi-square test indicated no significant among-species differentiation ($\chi^2 = 4.020$, $p = 0.685$), suggesting that variation in this descriptor is shared across species rather than taxonomically structured.

5.2.4. Principal Coordinates Analysis (PCoA) based on qualitative traits

Gower distances-based PCoA confirmed the clustering findings. The first two axes described the primary gradients of qualitative variability, but species overlapped significantly (Figure 10). However, while *C. palmeri* showed a higher relationship in the first coordinate axis, *C. arizonicum* was more dominant in the second. *Chenopodium neomexicanum* and *C. sonorense* took intermediate and partially overlapping positions.

Confidence ellipses indicated that species centroids were not completely distinct and thus confirmed the interpretation of incomplete multivariate separation. However, species demonstrated organized patterns of distribution that were similar to differences observed in individual qualitative descriptors.

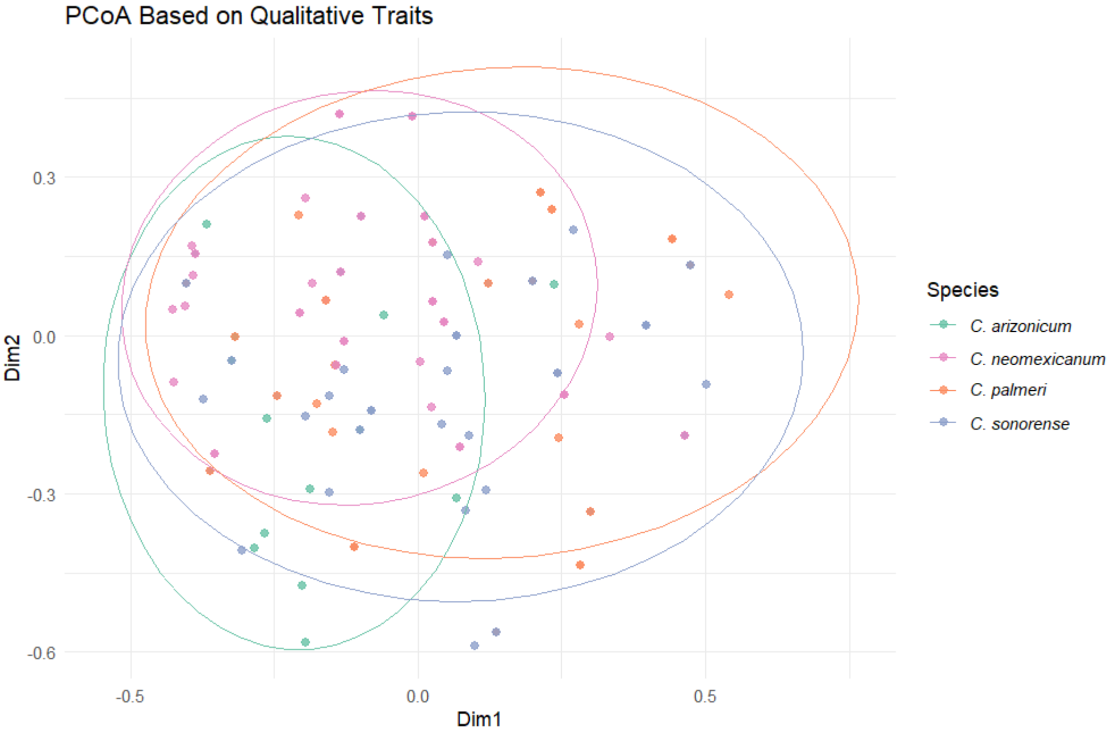


Figure 10. Principal coordinates analysis based on qualitative traits for Chenopodium neomexicanum aggregate

By other hand, PERMANOVA revealed substantial multivariate differentiation among species (Pseudo-F = 13.403, $R^2 = 0.154$, $p < 0.001$), suggesting that species identity accounts for around 15.4% of the variance of qualitative seed traits with respect to each other. Nevertheless, the homogeneity test for multivariate dispersions was also significant ($F = 2.797$, $p = 0.040$), suggesting that species vary in the extent to which they disperse within the greater or lesser distances of within-group variability. This finding demonstrates that the significant PERMANOVA result not only accounts for variation in centroid positions, but also heterogeneity of intra-specific variation.

5.2.6. Hierarchical clustering based on qualitative traits

The UPGMA dendrogram demonstrated partial structuring among species according to qualitative seed characteristics (Figure 11). While species groups exhibited some local aggregation, complete segregation into species-specific clusters was not detected. *Chenopodium neomexicanum* was most closely unified and *C. arizonicum and C. palmeri* spread relatively widely in the branches of the dendrogram. *Chenopodium sonorense* had an intermediate trend with partial clustering, but extensive intermixing. Taken together, the clustering pattern suggests the moderate structuring of multivariable, with qualitative characteristics mediating differentiation but not generating separate species-based clusters.

Combined, the multivariate analyses reveal statistically significant but moderate qualitative differentiation in the aggregate. Species are separate at a multivariate level with considerable overlap and variable internal dispersion indicating structured but incomplete morphological separation.

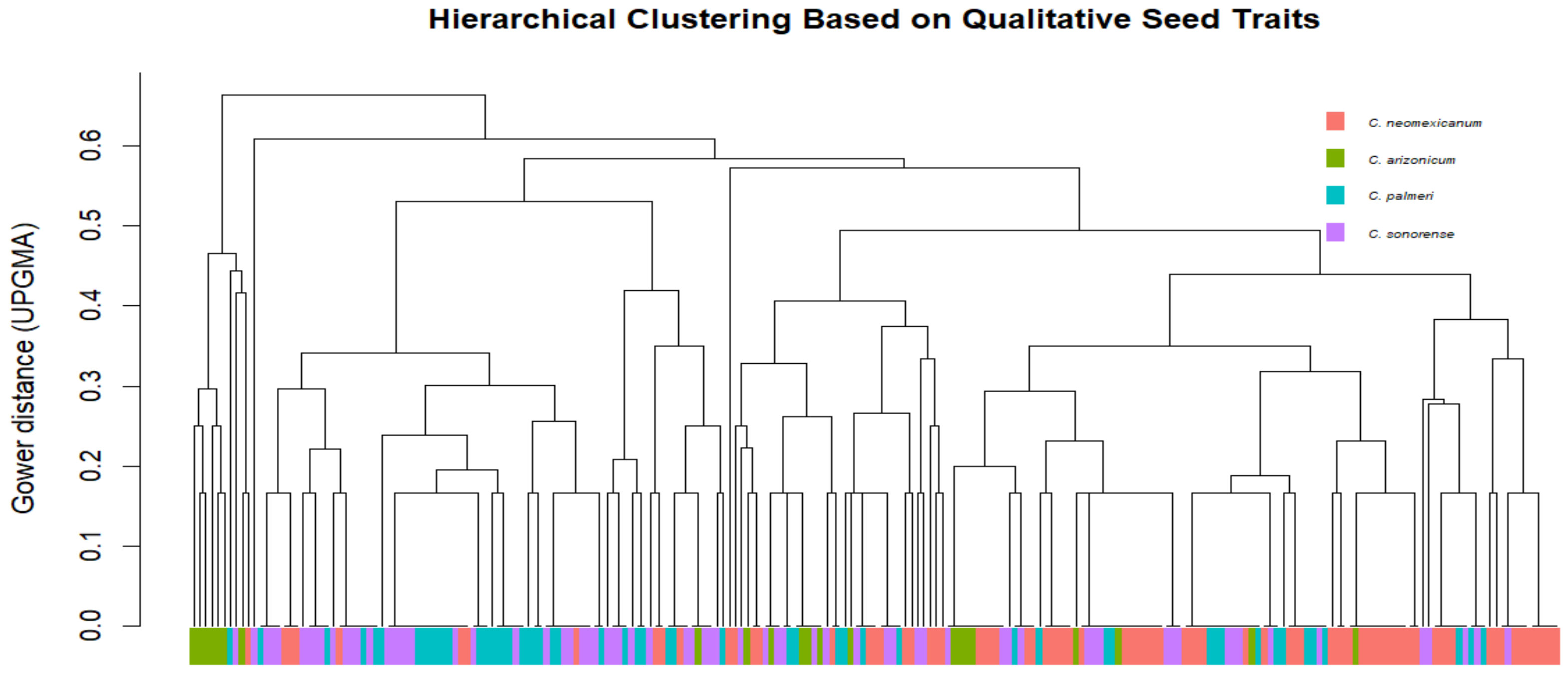


Figure 11. Dendrogram based on qualitative traits for Chenopodium neomexicanum aggregate

5.3. *Chenopodium neomexicanum* aggregate phylogenetic versus morphometric grouping

The phylogenetic tree reveals a well-supported genetic structure within the *Chenopodium neomexicanum* aggregate, with most internal nodes showing high bootstrap values (frequently reaching 100), indicating strong statistical support for the phylogenetic relationships. Based on the phylogenetic topology and considering well-supported nodes (bootstrap ≥ 90), five genetic groups were identified and visually delimited in the tree (Figure 12) using vertical boundaries.

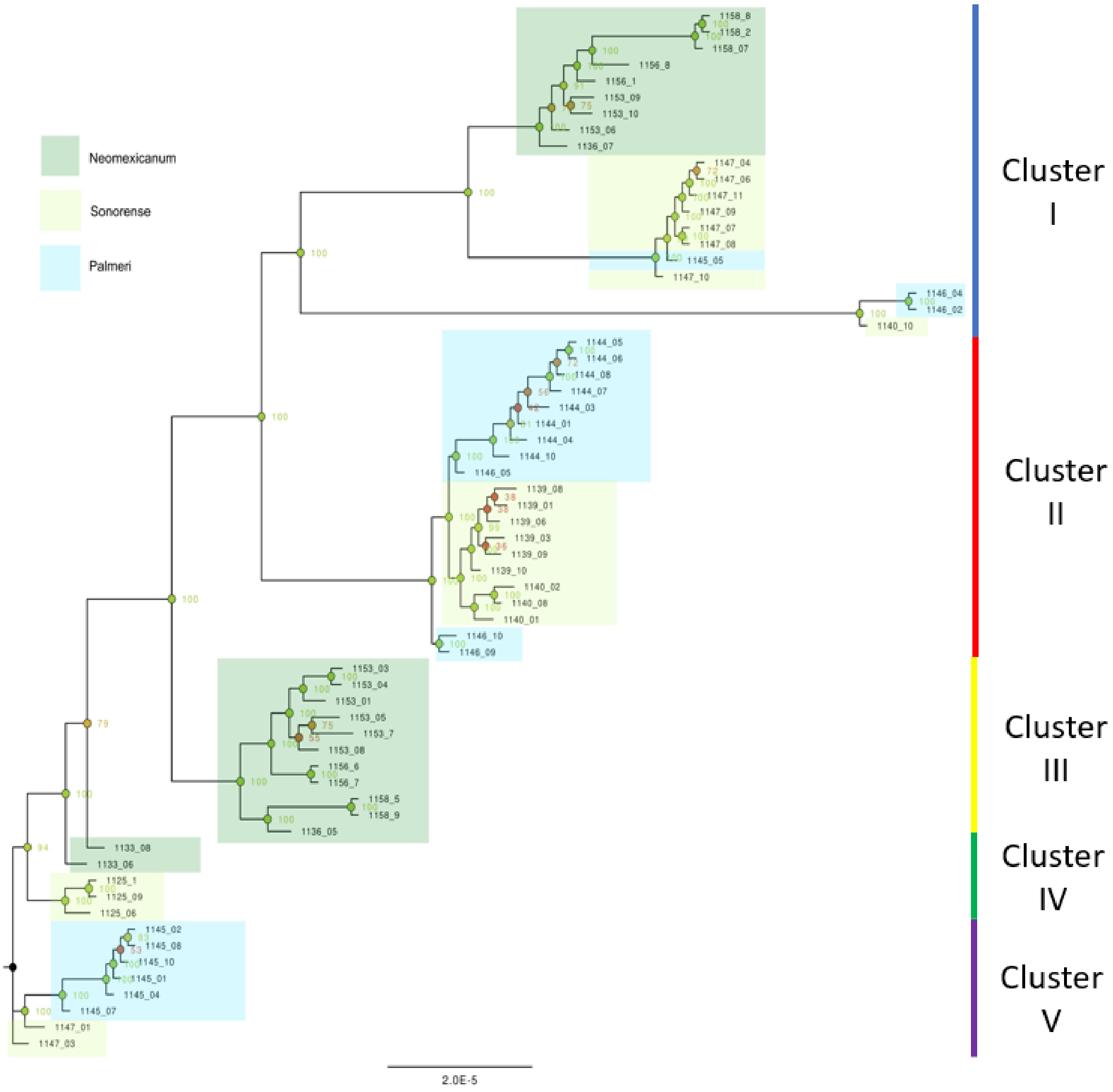


Figure 12. Maximum-likelihood phylogenetic tree of the Chenopodium neomexicanum aggregate based on RADseq-derived SNPs. Five genetic clusters were identified based on tree topology and well-supported clades and are indicated by colored vertical delimitations (blue, red, yellow, green, and purple).

The cluster I (blue delimitation) comprise a well-supported clade including individuals from different taxa, indicating genetic affinity among species. Immediately below, cluster II (red delimitation) groups a distinct lineage that also includes individuals from multiple species.

The third cluster (yellow delimitation) is composed exclusively of individuals assigned to *C. neomexicanum*, forming a cohesive and well-supported group, suggesting the presence of a distinct genetic lineage within this species.

Below this, the fourth cluster (green delimitation) represents another well-supported group. Finally, the cluster V (purple delimitation) comprises a well-supported cluster including individuals from different species, indicating close genetic relationships across taxa.

The principal component analysis (PCA, Figure 13) based on seed morphometric traits, using the five genetic clusters defined from the phylogenetic analysis as grouping units, revealed a structured but partially overlapping pattern of variation among individuals. The first two principal components explained 93.9% of the total variance (PC1: 68.0%, PC2: 25.9%), indicating that most of the morphometric variation is captured within this two-dimensional space.

The main axis of variation (PC1) differentiates genetic clusters along a continuous gradient. Genetic cluster III is clearly separated toward the positive end of PC1, forming a relatively distinct group with limited overlap with other clusters. In contrast, clusters I and V are positioned toward the negative values of PC1 and show considerable overlap between them. Clusters II and IV occupy intermediate positions along this axis and display broad dispersion, overlapping with multiple neighboring clusters.

Along PC2, variation is less associated with clear separation among clusters and primarily reflects within-cluster variability. Most clusters exhibit vertical dispersion, indicating internal morphometric variation, with some individuals showing extreme values along this axis.

The comparison between phylogenetic and morphometric analyses reveals a partial mismatch between genetic structure and phenotypic variation within the *Chenopodium neomexicanum* aggregate. While the phylogenetic reconstruction identifies well-supported genetic clusters, the morphometric analysis shows a more continuous and overlapping pattern of variation among individuals.

This pattern is consistent with the PCA results, where most of the variation is captured along a primary axis that partially separates cluster III, while the remaining clusters occupy intermediate and overlapping positions. In particular, clusters I and V show substantial overlap, and clusters II and IV display broad dispersion, indicating weak morphometric differentiation among these groups.

Moreover, although some genetic clusters exhibit partial phenotypic differentiation, seed morphometric traits do not fully reflect the genetic structure inferred from molecular data. These results suggest that genetic and morphological variation are only partially congruent, highlighting the complex and continuous nature of variation within the *C. neomexicanum* aggregate.

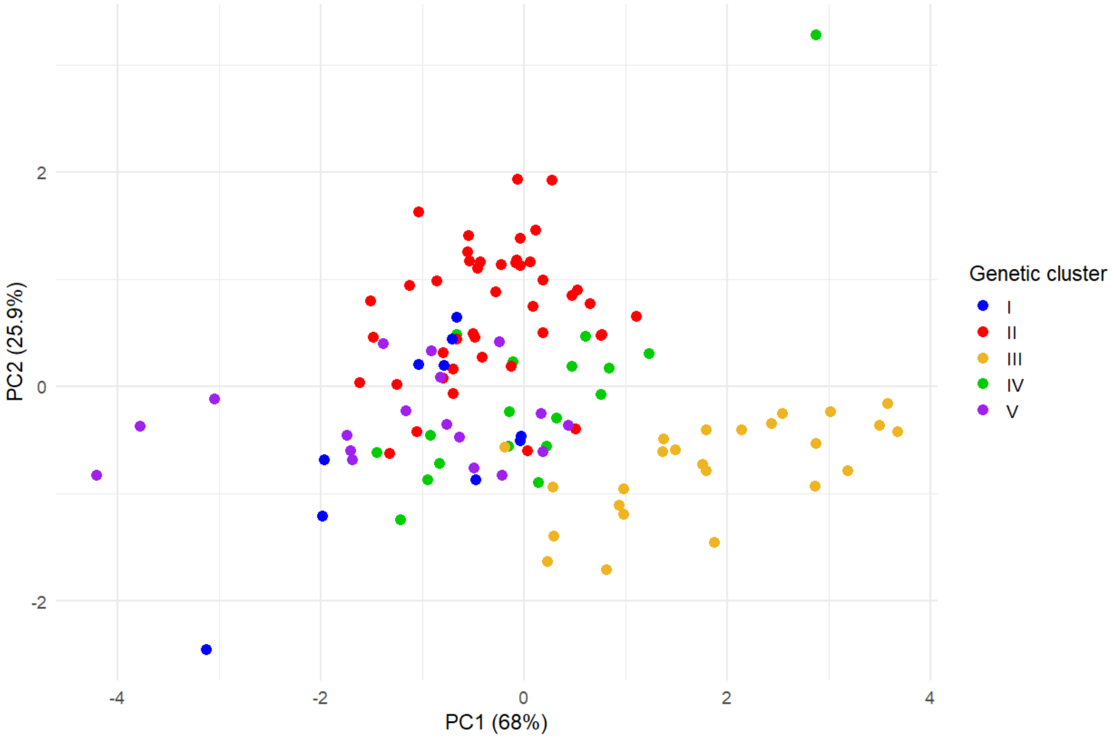


Figure 13. Principal component analysis (PCA) of seed morphometric traits for individuals of the Chenopodium neomexicanum aggregate. Points represent individuals colored according to the five genetic clusters defined from the phylogenetic analysis (see Figure 12).

6. Discussion

The results obtained from quantitative data are in agreement with the diagnostic criteria provided by Benet-Pierce and Simpson, (2017) as seed size is determined by the classification order of the species. Descriptive statistics showed *C. neomexicanum* has the highest mean seed diameter (1.192 mm), *C. palmeri* the smallest value (0.890 mm), and *C. arizonicum* and *C. sonorense* lying intermediate.

Statistical evidence for this gradient can be found in both multivariate (MANOVA, $p < 0.001$) and univariate analyses, in which seed diameter can clearly distinguish taxa together, with *C. neomexicanum* significantly larger versus *C. palmeri* significantly smaller and *C. arizonicum* and *C. sonorense* being a homogeneous intermediate group. Importantly, this variation appears to closely reflect a taxonomic key to the complex (in Appendix 2, Benet-Pierce & Simpson, 2017), with species distinguished mainly for their fruit diameter thresholds (for instance, ~0.8 mm for *C. palmeri*, ≥1 mm for intermediates and 1.2–1.6 mm for *C. neomexicanum*). The agreement between the quantitative and taxonomy thresholds values verifies that seed diameter is a viable, although taxonomically significant, characteristic in the size-related *C. neomexicanum*. The overlap that we noticed between *C. arizonicum* and *C. sonorense* indicates that although diameter does cover well the larger size gradients, the relatively large size gradients may not totally resolve all closely-breeding taxa, reinforcing the requirement for an integrative strategy that combines multiple morphological and molecular markers.

The multivariate analyses based on quantitative seed traits showed a well-organized, but not completely separated, pattern of variation in the *Chenopodium neomexicanum* aggregate. The PCA showed that morphological variation is maintained along continuous gradients, whereas the dendrogram revealed that many clusters include individuals of diverse taxa. Nonetheless, despite this overall overlap, a degree of differentiation could actually be seen between *C. palmeri* and *C. neomexicanum*, indicating that the quantitative features of the seed species capture at least some of the taxonomic structure in the group.

This is consistent with previous taxonomic studies, e.g., Wahl (1954), in which the complex was simplified and was only included with few species recognized, e.g., *C. neomexicanum* and *C. palmeri*. Simultaneously, the representation of multiple populations in the current investigation demonstrates that this stratification is not absolute, with taxa often overlapping morphometric space and indicating that the lineages are likely a bit less fixed than most of the work assumed.

Chenopodium sonorense, however, being not identified in early classifications and its specimens previously assigned to other taxa, is more complicated. The apparent lack of delineation of the taxon in the morphometric studies indicates earlier information on the high morphological similarity across the complex and the reason why it is not recognized in earlier taxonomic systems. Its eventual characterization as a separate species from other *C. neomexicanum* seeds via comprehensive studies of reproductive characteristic traits (Benet-Pierce and Simpson, 2017) suggests that these traits are significant for species delimitation but that morphological differentiation in this group may be marginal and population-dependent. As such, these findings do not entirely support or contradict existing taxonomic explanations, but suggest that species-boundaries of *C. neomexicanum* aggregate are not well delineated under quantitative seed traits. Instead, the patterns detected point to an intricate structure of variation where individual taxa - e.g. *C. palmeri* and *C. neomexicanum* have only partial morphological differentiation, while other taxa, e.g. *C. sonorense*, remain difficult to distinguish. This means that other population data must be considered and morphological and molecular parameters must be pooled together when evaluating species delimitation in this complex unit.

The observed overlap, notably between *C. arizonicum* and *C. sonorense*, is consistent with patterns noted in other morphometric comparisons of quantitative traits. In *Vicia faba*, an examination of agro-morphological traits demonstrated considerable variability among accessions, with the duration of flowering spanning, plant heights, and seed yields. Additionally, Principal Component Analysis (PCA) indicated that the first three components collectively accounted for 84.3% of the overall variation, with the first principal component (PC1) responsible for 44.37% of this variance, primarily linked to factors such as plant height, pod count, seed count, and seed yield (Ammar et al., 2015). Another similar case is in *Lavandula angustifolia* (Pandey et al., 2026), were morphological traits showed broad phenotypic variation, the PCA indicated that the first three components explained 72.78% of the total variation. These results reinforce the idea that PCA space overlap is a frequent phenomenon in biological systems and a continuous pattern of variation, and not a limitation in the methodology.

The qualitative seed traits determined are mostly congruent with the diagnosis guidelines of Benet-Pierce and Simpson (2017) for the *Chenopodium neomexicanum* complex. Of the descriptors discussed, side view margin and pericarp surface most highly agreed with the evidence obtained in the dichotomous key. In the current analysis, *C. arizonicum* had a thick side margin predominant, *C. neomexicanum* had a thin margin predominantly, in line with the dichotomous key, *C. arizonicum* and *C. sonorense* are classified as high-margin in side view, and *C. neomexicanum* as a low margin taxon.

There was also a strong taxonomic correspondence in pericarp surface. *Chenopodium neomexicanum* was largely associated with a broadly pitted surface, agreeing with its description of a broadly pitted, grayish pericarp, while *C. palmeri* and *C. sonorense* were predominantly papillate, that is, as per the smooth papillate pericarp of *C. palmeri* and papillate, banded pericarp of *C. sonorense* described by Benet-Pierce and Simpson (2017).

By comparison, *C. arizonicum* exhibited greater heterogeneity of pericarp surface states, displaying a wider range of variation than expected from the taxonomic key. Individuals exhibited multiple and overlapping surface conditions, suggesting that this trait is more variable within the species than previously described and may reduce its reliability as a diagnostic character when used alone.

Partial but significant agreement with the taxonomic indicator was seen with margin arrangement. The predominance of the round state in *C. palmeri* is in accordance with a lenticular, small-fruited morphology, while the higher frequency of biconvex fruits observed in *C. arizonicum* and *C. neomexicanum* is congruent with the degree of similarity in overall seed outline. Although *C. sonorense* maintained a more moderate state, this reflects the fact that it was the most closely related taxon to *C. arizonicum* in the complex as a whole.

In contrast, beak prominence did not yield much interspecific differentiation and was dominated by the “very weak” state in all taxa. Such low discriminatory significance is consistent with the suggestion that beak prominence is not a strong diagnostic feature emphasized by Benet-Pierce and Simpson (2017), compared to fruit size, side margin thickness and pericarp surface.

Pericarp colour also exhibited broad similarities between species, though the high percentage of black exhibited in *C. arizonicum*, *C. neomexicanum*, and *C. sonorense* is in line with an overall dark colour for each of these taxa. Such differences between dark brown and gray states, especially with *C. neomexicanum* and *C. palmeri*, indicates that colour alone is limited in providing diagnostic information unless analyzed beside the surface pericarp and margin traits.

The patterns seen to be papilla condition can also be interpreted in the context of general carpological evidence. Sukhorukov and Zhang, (2013) revealed that in *Chenopodium,* the pericarp surface tends to become papillate initially but the papillae are in fact collapsed during its maturation which resulted in appearance of craterlike, pitted, or reticulate appearance of the dry fruit. This assists in accounting for why collapsed papillae dominated in *C. arizonicum* and *C. neomexicanum*, and why *C. palmeri* and *C. sonorense* had a greater percentage of inflated papillae or intermediate surface conditions. Thus, differences in papilla status should be interpreted not only as a taxonomic clue, but also a characteristic that may be related to fruit maturity, or the state of preservation.

Furthermore, Uotila's taxonomic research on *Chenopodiastrum* indicates that defining species is more dependent on a mix of qualitative features related to fruit and plant structure than on multivariate morphometrics. For instance, seed diameter varies significantly among different taxa, spanning approximately 1.0 to 1.4 mm across various species (Uotila, 2021). However, meaningful distinctions arise from accompanying characteristics like the adherence of the pericarp, the shape of the seed margins, and the texture of the testa.

The differences between multivariate methods based on quantitative and qualitative seed traits suggest that the quantitative dataset offered superior overall structure to the *Chenopodium neomexicanum* aggregate. The PCA revealed that the first axis accounted for a large fraction of the total variation (70.3%) leading to a distinct morphometric gradient in which *C. neomexicanum* and *C. palmeri* occupied opposite locations, while *C. arizonicum* and *C. sonorense* were intermediate with only a partial overlap. This tendency also appeared in the quantitative dendrogram, where there appeared to be deeper hierarchical segregation and more coherent grouping tendencies between taxa. In contrast, however, PCoA and hierarchical clustering using qualitative seed traits demonstrated wider superposition and lower species resolution.

The ordination space demonstrated strong superposition of taxa, and the qualitative dendrogram showed high intermixing of species across terminal branches. Such findings show that qualitative descriptors, though taxonomically rich in information, are inferior to quantitative traits within their capacity to express the overall multivariate character of a complex.

This characteristic is in accord with the reported morphology studies on other plant families (Ammar et al., 2015; Li et al., 2021; Olawuyi et al., 2025), suggesting that morphological feature is generally associated with continuous variability rather than to the purely fixed clusters. Morphometric studies performed in plants largely depend on quantitative traits for multivariate analyses such as PCA and clustering, while qualitative characters are either excluded, or abstracted away as numerical phenomena.

In comparison, the current study utilized an independent qualitative seed trait set using Gower distance and PCoA that allows an explicit interpretation of categorical variability. This methodology differs from previous work and can be utilized to explain the broader overlap of items found in the qualitative ordination. This also conforms to the taxonomy adopted by Benet-Pierce and Simpson (2017), where qualitative aspects of fruit such as margin configuration, pericarp surface, and papilla condition are combined, rather than used independently.

Moreover, the phylogenetic based on RADseq-derived SNPs identified five well-supported genetic clusters within the *Chenopodium neomexicanum* aggregate. Theses clusters were subsequently used to assigned individuals in the morphometric analysis, allowing a direct comparison between genetic structure and seed traits variation within the same data set.

Morphometric analyses revealed continuous variation and overlap among seed traits across taxa, whereas molecular phylogeny produced a more structured and informative pattern, with several clades supported by strong bootstrap values, indicating clear genetic differentiation among lineages. This contrast suggests that seed morphometric data retain a detectable taxonomic signal but do not fully capture the underlying evolutionary relationships inferred from genome-wide data.

This partial incongruence has been widely documented in *Chenopodium* and other taxonomically complex plant groups. Morphometric analyses within the *Chenopodium album* aggregate have shown that continuous variation and mixed groupings are common, even when genetic data support distinct evolutionary lineages (Mandák et al., 2012). Similarly, phylogenetic studies within *Chenopodioideae* have demonstrated that several morphological characters used in traditional classifications have evolved repeatedly and independently, reducing their phylogenetic reliability (Kadereit & Freitag, 2011). Likewise, broader phylogenetic reconstructions further confirm that traditional taxonomic groupings in *Chenopodium* do not always correspond to monophyletic lineages, emphasizing the importance of molecular data in resolving evolutionary relationships (Fuentes-Bazan et al., 2012). In the same general direction, recent genomic work on *C. album* s. str. reveals a complex evolutionary history with multiple genetic lineages, which may help explain the taxonomic difficulty and morphological variability historically reported in this group (Mandák et al., 2026).

Comparable patterns have also been observed in other plant systems integrating morphological and molecular datasets. In *Psophocarpus tetragonolobus*, morphological analyses based on 17 traits revealed significant variation in vegetative, floral, and phenological characters, while molecular analyses identified major genetic clusters that broadly corresponded to, but did not fully match, morphological differentiation (Olawuyi et al., 2025). In *Vicia faba*, agro-morphological variation was substantial, yet the correlation between morphological and molecular data varied depending on the marker system (Ammar et al., 2015). Similarly, in *Mentha longifolia*, morphological variation was pronounced (e.g., plant height ranging from 28.89 to 89.51 cm), but clustering based on morphology and molecular markers resulted in different group structures (Devi et al., 2022). In *Lavandula angustifolia*, although morphological traits explained a high proportion of total variation (72.78% in the first three principal components), the correspondence with molecular distances remained weak (Mantel r = 0.095, p = 0.007), indicating that phenotypic patterns only partially reflect genetic relationships (Pandey et al., 2026). In *Aspalathus*, some morphological traits corresponded to well-supported molecular clades, yet many characters exhibited homoplasy, limiting their reliability when used alone (Madika et al., 2026).

These consistent patterns across studies indicate that morphological differentiation may be slower, more plastic, or more conservative than genetic divergence, particularly in recently diverged or complex species groups. In this context, the clades identified in the present study, supported by strong bootstrap values, likely represent fundamental evolutionary lineages within the *C. neomexicanum* aggregate. However, the considerable overlap observed in morphometric space indicates that species boundaries remain diffuse when assessed solely through quantitative seed traits.

This pattern becomes even more evident when individuals are grouped according to genetic clusters rather than species identity. Although the molecular phylogeny identifies well-supported genetic clusters, the PCA based on seed morphometric traits shows that most of these clusters overlap substantially in multivariate space. Only one cluster (cluster 3) exhibits partial separation along the main axis of variation, while the remaining clusters are distributed across intermediate positions with considerable overlap. This indicates that morphometric traits do not fully discriminate the genetic structure inferred from molecular data.

In this context, the intermediate position previously observed for *C. sonorense* reflects a general pattern of partial correspondence between morphology and genetic clustering, rather than a species-specific trait. Several clusters lack clear morphometric boundaries, indicating incomplete phenotypic differentiation. This suggests that the evolutionary structure of the *C. neomexicanum* aggregate is not fully captured by seed morphology alone and supports the view of a complex system with only partial congruence among morphological variation, genetic differentiation, and taxonomic classification.

This interpretation is consistent with the taxonomic history of the group, as it was not recognized in earlier classifications (Wahl, 1954) and was later described based on reproductive morphological traits (Benet-Pierce and Simpson, 2017). The mixed morphometric assignment and relatively less distinct phylogenetic position observed here suggest weaker differentiation, supporting the interpretation of this taxon as having blurred boundaries or incomplete divergence within the *C. neomexicanum* complex.

The results provide partial support for the proposed hypotheses. For H1, seed morphometric traits, particularly diameter, showed significant differences among taxa and allowed differentiation between *C. neomexicanum* and *C. palmeri*. However, substantial overlap with other groups indicates incomplete separation. This pattern is consistent when individuals are grouped both by species and by phylogenetically defined genetic clusters, suggesting that morphometric variation captures real differences but does not define discrete boundaries.

Regarding H2, morphometric groupings showed limited correspondence with phylogenetic relationships. Although molecular analyses recovered well-supported clades, grouping individuals according to genetic clusters revealed that the PCA only partially reflects this structure. While one cluster shows a clearer tendency toward separation, the remaining clusters overlap extensively in morphometric space, indicating that seed morphology does not fully represent the genetic structure inferred from molecular data.

For H3, the presence of intermediate positions and increased variability in morphometric space, together with the well-supported genetic structure revealed by phylogenetic analysis, supports a pattern of continuous variation within the complex. Although the molecular data identify clearly supported genetic lineages, these are not reflected as discrete groups in the morphometric analysis, where individuals show substantial overlap.

The lack of clear separation among several groups is therefore consistent with processes such as contact zones, shared ancestral variation, or ongoing gene flow among closely related taxa.

From a conservation perspective, this study is relevant due to the restricted distribution and taxonomic uncertainty surrounding the *Chenopodium neomexicanum* aggregate. By integrating morphometric and molecular approaches, the study improves species delineation within the complex by identifying patterns of morphological variation alongside genetically structured lineages supported by molecular data.

The results reveal the coexistence of well-differentiated lineages, intermediate forms, and poorly defined taxonomic units, highlighting the need to conserve not only clearly delimited species but also the underlying genetic and phenotypic diversity within the complex.

7. Conclusion

According to quantitative morphometric analyses, this study was able to characterize the variability of seed morphology in the *Chenopodium neomexicanum* aggregate. Seed traits, particularly diameter, provide a clear gradient of variation across taxa.

Findings showed that *C. neomexicanum* and *C. palmeri* are the extremes of this gradient; *C. arizonicum* and *C. sonorense* are intermediate, indicating that there is structured but continuous morphological variation within the complex.

Morphometric analyses revealed patterns and some clustering among the taxa; however, these groups were not fully distinct. PCA and dendrogram analyses revealed that the overlap in taxa is substantial even in the presence of taxonomic structuring, providing support that seed morphology as a taxonomic determinant does not guarantee species separations.

Morphological patterns were only partially correlated with phylogenetic relationships inferred from molecular evidence. Whereas molecular analysis confirmed well-supported clades associated with genetic clusters, the results were not well represented in the morphometric analyses, which showed substantial overlap among groups. This partial incongruence was evident in the PCA, where only one genetic cluster exhibited clearer separation, whereas the remaining clusters occupied overlapping and intermediate positions along continuous gradients of variation. The findings suggest that seed morphology, although informative for taxonomic purposes, does not fully capture the evolutionary relationships within the complex.

Furthermore, intermediate morphological patterns observed in the morphometric analysis indicate high variability within the complex. These intermediate positions are not restricted to a single taxon, such as *C. sonorense*, but are distributed across multiple individuals, suggesting incomplete phenotypic differentiation.

Although the phylogenetic analysis identifies well-supported genetic lineages, these are not reflected as discrete groups in morphometric space. This supports a pattern of continuous phenotypic variation within the complex. While this may be consistent with processes such as contact zones or gene flow, there is no direct evidence of hybridisation, and such interpretations should be treated with caution.

8. References

Aellen, P., 1960–61. *Chenopodium*. In: Hegi, G. (Ed.), Illustrierte Flora von Mitteleuropa. Carl Hanser-Verlag, München, pp. 569–659.

Ammar, M. H., Alghamdi, S. S., Migdadi, H. M., Khan, M. A., El-Harty, E. H., & Al-Faifi, S. A. (2015). Assessment of genetic diversity among faba bean genotypes using agro-morphological and molecular markers. *Saudi Journal of Biological Sciences*, *22*(3), 340–350. https://doi.org/10.1016/j.sjbs.2015.02.005

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology,* 26(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Bassett, I. J., & Crompton, C. W. (1982). The genus *Chenopodium* in Canada. *Canadian Journal of Botany*, 60(5), 586–610.

Benet-Pierce, N., & Simpson, M. G. (2017). Taxonomic recovery of the species in the *Chenopodium neomexicanum* (*Chenopodiaceae*) complex and description of *Chenopodium sonorense* sp. nov. *Journal of the Torrey Botanical Society*, *144*(3), 339–356. https://doi.org/10.3159/TORREY-D-16-00013.1

Benet-Pierce, N., & Simpson, M. G. (2019). The Taxonomy of *Chenopodium* Hians, *C. incognitum*, and Ten New Taxa Within the Narrow-Leaved *Chenopodium* Group in Western North America, With Special Attention To California. *Madroño*, *66*(2), 56. https://doi.org/10.3120/0024-9637-66.2.56

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, 22(11), 3124–3140. https://doi.org/10.1111/mec.12354

Clemants, S. E., & Mosyakin, S. L. (1996). *Chenopodium in Flora of North America*. Flora of North America.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Devi, A., Iqbal, T., Ahmad Wani, I., Sharma, G., Verma, S., Noureldeen, A., & Darwish,

H. (2022). Assessment of variability among morphological and molecular characters in wild populations of mint [*Mentha longifolia* (L.) L.] germplasm. *Saudi Journal of Biological Sciences*, *29*(5), 3528–3538. https://doi.org/10.1016/j.sjbs.2022.02.013

Fuentes-Bazan, S., Mansion, G., & Borsch, T. (2012). Towards a species level tree of the globally diverse genus *Chenopodium* (*Chenopodiaceae*). *Molecular Phylogenetics and Evolution*, *62*(1), 359–374. https://doi.org/10.1016/j.ympev.2011.10.006

Gower, J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics*, 27(4), 857–871.

Habibi, F., Mosyakin, S. L., Shynder, O. I., Krak, K., Čortan, D., Filippi, G. A., & Mandak, B. (2023). *Chenopodium ucrainicum* (*Amaranthaceae*), a new "BB" genome diploid species: karyological, cytological, and molecular evidence. *Botanical Journal of the Linnean Society*, *203*(4), 401–410. https://doi.org/10.1093/botlinnean/boad032

Habibi, F., Vít, P., Rahiminejad, M., & Mandák, B. (2018). Towards a better understanding of the *Chenopodium album* aggregate (*Amaranthaceae*) in the Middle East: A karyological, cytometric and morphometric investigation. *Journal of Systematics and Evolution*, *56*(3), 231–242. https://doi.org/10.1111/jse.12306

Hodková, E., & Mandák, B. (2018). An overlooked hybrid between the two diploid *Chenopodium* species in Central Europe determined by microsatellite and morphological analysis. *Plant Systematics and Evolution*, *304*(3), 295–312. https://doi.org/10.1007/s00606-017-1477-9

Jolliffe, I. T. (2002). Principal Component Analysis (2nd ed.). Springer.

Kadereit, G., Borsch, T., Weiging, K., & Freitag, H. (2003). Phylogeny o*f Amaranthaceae* and *Chenopodiaceae* and the Evolution of C4 Photosynthesis. *International Journal of Plant Sciences*, *164*(6), 959–986.

Kadereit, G., & Freitag, H. (2011). Molecular phylogeny of *Camphorosmeae (Camphorosmoideae, Chenopodiaceae*): Implications for biogeography, evolution of C4-photosynthesis and taxonomy. *Taxon*, *60*(1), 51–78. https://doi.org/10.1002/tax.601006

Kadereit, G., Gotzek, D., Jacobs, S., & Freitag, H. (2005). Origin and age of Australian

Chenopodiaceae. Organisms Diversity and Evolution, *5*(1), 59–80. https://doi.org/10.1016/j.ode.2004.07.002

Kadereit, G., Mavrodiev, E. V., Zacharias, E. H., & Sukhorukov, A. P. (2010). Molecular phylogeny of Atripliceae (*Chenopodioideae, Chenopodiaceae*): Implications for systematics, biogeography, flower and fruit evolution, and the origin of C4 photosynthesis. *American Journal of Botany*, *97*(10), 1664–1687. https://doi.org/10.3732/ajb.1000169

Krak, K., Habibi, F., Douda, J., Vít, P., Lomonosova, M. N., Wang, L., & Mandák, B. (2019). Human-mediated dispersal of weed species during the Holocene: A case study of *Chenopodium album* agg. *Journal of Biogeography*, *46*(5), 1007–1019. https://doi.org/10.1111/jbi.13545

Kuhn, U., Bittrich, V., Carolin, C., Freitag, H., Hedge, I.C., Uotila, P., Wilson, P.G., 1993. *Chenopodiaceae*. In: Kubitzki, K. (Ed.), Families and Genera of Vascular Plants, vol. 2. Springer, Berlin, pp. 253–281.

Legendre, P., Legendre, L. (2012). Numerical Ecology (3rd ed.). Elsevier.

Leitch, I. J., Chase, M. W., & Bennett, M. D. (1998). Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. *Annals of Botany*, *82*(SUPPL. A), 85–94. https://doi.org/10.1006/anbo.1998.0783

Leitch I.J., Bennett M.D. (2004). Genome downsizing in polyploid plants. *Biological Journal of the Linnean Society* 82: 651–663.

Leitch I.J., Hanson L., Lim K.Y., Kovarik A., Chase M.W., Clarkson J.J., Leitch A.R. (2008). The ups and downs of genome size evolution in polyploid species of *Nicotiana (Solanaceae).* Annals of Botany 101: 805–814.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.arXiv preprint arXiv:1303.3997.

Li, S., Ji, F., Hou, F., Shi, Q., Xing, G., Chen, H., Weng, Y., & Kang, X. (2021). Morphological, palynological and molecular assessment of *Hemerocallis core* collection. *Scientia Horticulturae*, *285*(April). https://doi.org/10.1016/j.scienta.2021.110181

Linnaeus C (1753) Species *Plantarum*. Holmiae. 1200 p.

Madika, L. K., Sebola, R. J., Zhigila, D. A., Stirton, C. H., & Muasya, A. M. (2026). Phylogenetic relationships in the genus *Aspalathus* L. (Fabaceae; Papilionoideae; Crotalarieae) based on molecular and morphological evidence. *South African Journal of Botany*, *189*(February 2025), 107–119. https://doi.org/10.1016/j.sajb.2025.11.037

Mandák, B., Habibi, F., Čortan, D., Belyayev, A., Jarvis, D. E., Jellen, E. N., Maughan, P. J., Mosyakin, S. L., Uotila, P., & Krak, K. (2026). Multiple origins, singular success: genomic evidence for past recurrent hybridization in *Chenopodium album* s. str. (*Amaranthaceae*). *Molecular Phylogenetics and Evolution*, *215*(October 2025). https://doi.org/10.1016/j.ympev.2025.108500

Mandák, B., Krak, K., Vít, P., Lomonosova, M. N., Belyayev, A., Habibi, F., Wang, L., Douda, J., & Štorchová, H. (2018). Hybridization and polyploidization within the *Chenopodium album* aggregate analysed by means of cytological and molecular markers. *Molecular Phylogenetics and Evolution*, *129*(August), 189–201. https://doi.org/10.1016/j.ympev.2018.08.016

Mandák, B., Krak, K., Vít, P., Pavlíková, Z., Lomonosova, M. N., Habibi, F., Wang, L., Jellen, E. N., & Douda, J. (2016). How genome size variation is linked with evolution within *Chenopodium* sensu lato. *Perspectives in Plant Ecology, Evolution and Systematics*, *23*, 18–32. https://doi.org/10.1016/j.ppees.2016.09.004

Mandák, B., Trávníček, P., Paštová, L., & Kořínková, D. (2012). Is hybridization involved in the evolution of the *Chenopodium album* aggregate? An analysis based on chromosome counts and genome size estimation. *Flora: Morphology, Distribution, Functional Ecology of Plants*, *207*(7), 530–540. https://doi.org/10.1016/j.flora.2012.03.010

Meyer CA (1829) Blitum, *Chenopodium*. In: Ledebour CF, editor. Flora Altaica 1. Berlin. 11–14.

Mosyakin, S. L., & Clemants, S. E. (2002). New nomenclatural combinations in *Dysphania R.* Br. (*Chenopodiaceae*): taxa occurring in North America. *Ukrainian Botanical Journal*, *59*(4), 380–385. https://www.researchgate.net/publication/272417640_New_nomenclatural_combinations_in_Dysphania_RBr_Chenopodiaceae_Taxa_occurring_in_North_America

Olawuyi, O. J., Mutiu, H. A., Akinyoola, O. I., Mudasiru, O. M., Odedeji, J. O., & Azeez, A. A. (2025). Assessment of early morphological traits and molecular characters of *Psophocarpus tetragonolobus* (L.) DC. (winged bean) using ISSR and SCoT markers. *Ecological Genetics and Genomics*, *37*(October), 100416. https://doi.org/10.1016/j.egg.2025.100416

Palomino, G., Hernández, L. T., & De La Cruz Torres, E. (2008). Nuclear genome size and chromosome analysis in *Chenopodium quinoa* and *C. berlandieri* subsp. nuttalliae. *Euphytica*, *164*(1), 221–230. https://doi.org/10.1007/s10681-008-9711-8

Pandey, P., Sharan, H., Verma, S., Kaur, N., Chauhan, R., Singh, S., & Singh, S. (2026). Integrative assessment of morphological and SSR based molecular diversity patterns in lavender (*Lavandula angustifolia* Mill.). *Journal of Applied Research on Medicinal and Aromatic Plants*, *50*(December 2025). https://doi.org/10.1016/j.jarmap.2025.100690

Rambaut, A. (2018). FigTree v1.4.4. http://tree.bio.ed.ac.uk/software/figtree/

Rochette, N.C., Rivera-Colon, A.G., Catchen, J.M., (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Molecular Ecology. 28, 4737–4754.

Sang T. (2002). Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37: 121–147.

Scott, A. (1978). A review of the classification of *Chenopodium* L. and related genera (*Chenopodiaceae*). Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 100: 205–220. *Biota of New Zealand*, 18–23.

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Štorchová, H., Hrdlicková, R., Chrtek, J., Tetera, M., Fitze, D., & Fehrer, J. (2000). An improved method of DNA isolation for plants collected in the field and conserved in saturated NaCl/CTAB solution. *Taxon*, *49*, 79–84.

Sukhorukov, A. P., & Zhang, M. (2013). Fruit and Seed Anatomy of *Chenopodium* and Related Genera (*Chenopodioideae, Chenopodiaceae/Amaranthaceae*): Implications for Evolution and Taxonomy. *PLoS ONE*, *8*(4), 1–18. https://doi.org/10.1371/journal.pone.0061906

Uotila P (1978) Variation, distribution and taxonomy of *Chenopodium suecicum* and *C. album* in N Europe. *Acta Bot Fenn* 108: 1–35.

Uotila, P. (1997). *Chenopodium* L. In: Rechinger, K.H. (Ed.), *Flora Iranica*, No. 172. Akad. Druck-u. Verlagsanstalt, Graz, pp. 24–59.

Uotila, P. (2021). *Chenopodiastrum* (Amaranthaceae s. lato/Chenopodiaceae s. stricto) on Atlantic Islands. *Annales Botanici Fennici*, *58*(1–3), 83–94. https://doi.org/10.5735/085.058.0114

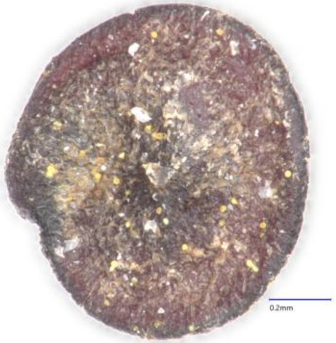
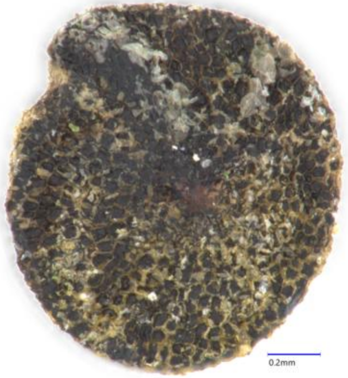
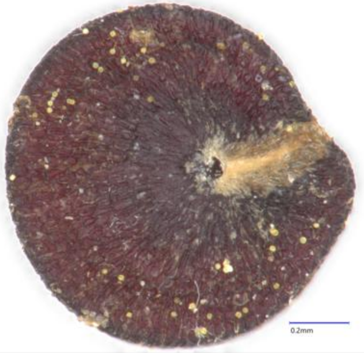
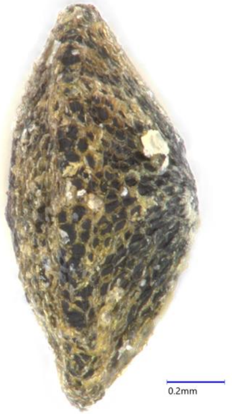
Vít, P., Krak, K., Trávníček, P., Douda, J., Lomonosova, M. N., & Mandák, B. (2016). Genome size stability across Eurasian *Chenopodium* species (Amaranthaceae). *Botanical Journal of the Linnean Society*, *182*(3), 637–649. https://doi.org/10.1111/boj.12474

Volkens , G. (1893) : *Chenopodiaceae*. In . A. Engler, and K. Prantl [eds]. Die nat ü r lichen Pfl anzenfamilien, vol. 3, 1a, 36 – 91. Engelmann, Leipzig, Germany.

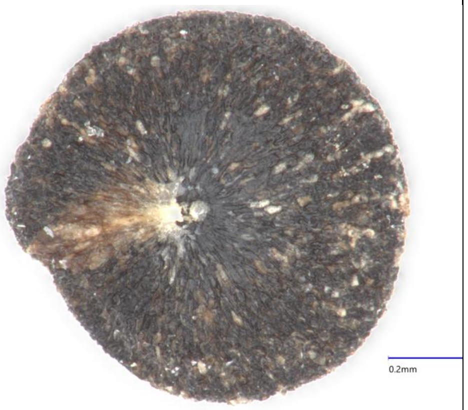
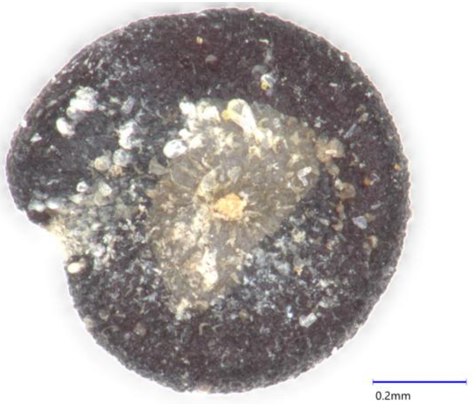
Wahl, H. A. . (1954). A preliminary study of the genus *Chenopodium* in North America. *Bartonia*, *27*(27), 1–46.

Young, L. A., Maughan, P. J., Jarvis, D. E., Hunt, S. P., Warner, H. C., Durrant, K. K., Kohlert, T., Curti, R. N., Bertero, D., Filippi, G. A., Pospíšilíková, T., Krak, K., Mandák, B., & Jellen, E. N. (2023). A chromosome-scale reference of *Chenopodium watsonii* helps elucidate relationships within the North American A-genome *Chenopodium* species and with quinoa. *Plant Genome*, *16*(3), 1–17. https://doi.org/10.1002/tpg2.20349

9. Appendix

1133-6-1	**2929-2-3**	**1156-7-5**	**1153-1-5**
			
			

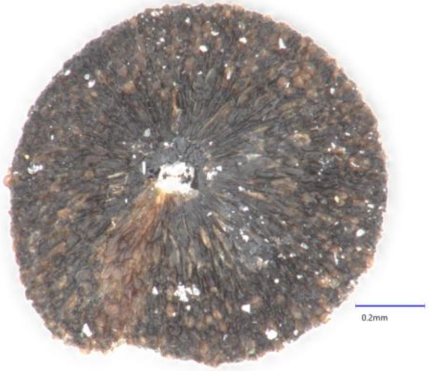
Appendix 1. Photos of Chenopodium neomexicanum

1146-2-1	2977-2-5	2977-3-4	1144-5-4
			
			

Appendix 2. Photos of Chenopodium palmeri

2974-1-1	2970-3-1	2974-3-2	2970-2-1
			
			

Appendix 3. Photos of Chenopodium arizonicum

1140-2-2	2956-4-3	1140-2-1	1147-1-5
			
			

Appendix 4. Photos of Chenopodium sonorense