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Environmental and genetic interactions underlying the morphological and genetic variability of the red juniper (*Juniperus turbinata* Guss.) in northeastern Morocco: Implications for conservation

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Abstract

Plant species often show morphological variations in response to their environments. In this study, we examined the trees, cones, seeds and leaves of three populations of *Juniperus turbinata* in north-eastern Morocco, located in littoral, semi-continental and continental zones, to evaluate how local conditions influence morphological character. Additionally, a genetic study using SSR markers was conducted to assess the genetic diversity and structure within these populations, aiming to determine the genetic contributions to observed morphological differences. The results showed significant morphological variation across zones. Cones from littoral population

were larger and rounder, while those of the semi-continental and continental populations were more elongated and robust. Seeds and leaves characters also varied in patterns linked with altitude and aridity. Genetic analysis revealed moderate differentiation among populations, with higher genetic diversity in the continental site, as confirmed by several calculated indices such as the number of effective and private alleles, Shannon's information index, and observed and expected heterozygosities. Interestingly, the population structure profile identified two distinct genetic pools, with littoral and continental populations clustering closely together. This ~~l~~limited genetic differentiation likely due to gene flow or shared ancestry, suggests the formation of a single genetic group despite geographical separation. The results of this study show that the local environmental factors are the primary drivers of morphological variation, particularly in cones and seeds, while also highlighting a notable genetic component. These findings data are essential for defining conservation strategies adapted to local populations of *J. turbinata* in the context of climate change.

Keywords: *Juniperus turbinata*, Morphological variation, Genetic diversity, Conservation strategies,

I. Introduction

The genus *Juniperus* L. (*Cupressaceae* Gray, nom. cons.) represents a significant component of arid and semi-arid tree and shrub ecosystems in the Northern Hemisphere [1–7]. The geographical distribution of this genus is influenced by a combination of abiotic factors, mainly climate [8–12], biotic interactions [13–15], and its capacity for seed dispersal [16,17].

The genus *Juniperus*, recognized as monophyletic in several studies[3–5,18], is currently classified into three distinct, monophyletic sections [3–5]. The first section, *Caryocedrus* [18] includes a single species native to the Mediterranean region (*J. drupacea* Labill.). The second section, *Juniperus*, includes nine species distributed across East Asia and the Mediterranean circum, among them *J. communis* L. which has a circumboreal range of distribution. The third section, *Sabina* [19] includes 56 species, found in the southwestern United States, Asia, and the Mediterranean region, with additional species present in Africa and the Canary Islands.

Within the *Sabina* section the *J. phoenicea* complex stands out due to its species diversity, which includes *J. phoenicea, J. turbinata*, and *J. canariensis* [20]. This complex spans a vast geographic range, from the Canary Islands and the Atlas Mountains, through North Africa and southern Europe, extending eastward to Jordan and Saudi Arabia. This wide distribution reflects the species' ecological adaptation to various Mediterranean and semi-arid environments.

The first species in the, *J. phoenicea* complex, *J. phoenicea* was initially described by Carl von Linné in 1753, based on specimens collected in Montpellier, southern France. Originally regarded as one of the subspecies within the complex, it was joined in 1844 by a second species, *J. turbinata*, described by Gussone [20] in southern Sicily [21]. Gussone [22] identified two subspecies within this framework: *J. phoenicea subsp. phoenicea* and *J. phoenicea subsp. turbinata* [23]. In 1981, Lebreton and Thivend [24] proposed an additional subspecies, *J. phoenicea subsp. eumediterranea*, originating from Sardinia. However, subsequent research by Farjon [2], and [25,26] concluded that this subspecies was actually synonymous with *J. turbinata*.

The third species, *J. canariensis*, described by Mathou and Guyot [27], is native to the Canary Islands and Madeira. While some authors, including Martínez [28] and [29], suggested reclassifying it as a subspecies of *J. turbinata* under the name *J. turbinata subsp. canariensis*, more recent studies based on volatile oils and DNA analysis[30,31] have refuted this classification.

This study examines *J. turbinata* Guss., a monoecious shrub reaching 8 to 12 meters in height, with rare cases of dioecy. It is distinguished from *J. phoenicea* by its longer leaves, and larger cones [32,33]. The leaves are pointed, and the cones are subglobular to ovoid, measuring 12–14 × 9–11 mm with a rough texture and an ochre or yellowish-brown colour at maturity [34]. *J. turbinata* inhabits coastal habitats on rocks and dunes, It is especially common in France, Greece, Italy, Morocco, Portugal, Spain, and Tunisia [3–5,35–40] . It also grows in the mountains of northwestern Africa, especially in coastal regions.

Although *J. turbinata* was initially described from samples collected in southern Sicily [36], more recent studies have largely excluded the Sicilian population[38–44], with the exception of a DNA-based study by [45]. More recently, the IUCN Conifer Specialist Group proposed revisions proposed by Adams [46–48], recognising *J. phoenicea* subsp. *turbinata* as a distinct species, while limiting *J. phoenicea* s.str. to the typical subspecies [49].

Our study investigates the morphological and genetic diversity of *J. turbinata* Guss. populations in northeastern Morocco [50], providing a pioneer overall analysis of this species in the Oriental region. The first part examines morphological differences among three populations from contrasted environments: a littoral population in Saïdia, a semi-continental population in Oued Elhimer (Jerrada), and a continental population in Abou Lkhel (Figuig).

To assess characteristics such as tree morphology, female cones, seeds, and branchs, a biometric analysis was conducted with the aim of identifying both intra- and interpopulation variations, as well as local adaptations. The second part focuses on the genetic comparison of these populations using leaf DNA and microsatellite markers (SSR) to assess genetic variability, potential gene flow, and population connectivity. This analysis helps to identify populations at risk of genetic isolation, offering insights for their conservation. Moreover, the study explores the relationship between morphological and genetic variations to understand the evolutionary processes and adaptation mechanisms of *J. turbinata* populations in northeastern Morocco. By integrating both approaches, our research provides a multidimensional perspective on intra- and interpopulation variations, supporting more informed management of the species in the face of environmental changes and human impacts.

II. Materials and methods

2.1. Study area

The study was carried out in northeastern Morocco, across three distinct sites representing a continental gradient, ranging from littoral to continental, with an intermediate semi-continental site (Fig. 1). The littoral site located in the province of Berkane, spans 7 km along the littoral dunes from Saidia in the east to the estuary of the Moulouya river in the west, at an average altitude of 57.15 m (± 2.23 m). This ecosystem is dominated by *J.*

turbinata trees of various ages and densities, with a significant fragmentation into unconnected plots. The semi-continental site, located in the province of Jerrada, spans 18,900 hectares in the core the high plateau of eastern Morocco, with an average altitude of 1094.35 m (± 26.855 m). Here *J. oxycedrus*, Tetraclinis articulata, and *J. turbinata* compete, forming a low-density tree distribution over 2 km. The continental site, located in the Saharan Anti-Atlas Mountains at Abou Lkhel in Figuig Province, spans approximately 391,239 hectares, with an average altitude of 1,645.45 m (± 102.46 m). This area is predominantly populated by *J. turbinata* trees at a very low density over a 10 km area. Together, these sites provide significant ecological diversity, allowing for the study of *J. turbinata* ecosystem variation along a continental gradient.

2.2. Sampling method

A spatially stratified random sampling method was employed to investigate the morphological and genetic variations of *J. turbinata* populations, using only mature (adult) trees at each sampling site. This method, inspired by Bitterlich [51] and Keersmaecker [52], aimed to capture both intra- and inter-population variability. Sampling was conducted across all study areas, -littoral, semi-continental, and continental sites- to avoid bias and ensure representative data.

The sampling process began with a randomly selected starting point at the edge of each forested area. Trees were then chosen based on specific criteria: they had to be at least one meter tall, with a minimum distance of 200 meters between two selected trees. This distance was chosen to reduce the effects of competition, and spatial proximity, ensuring that each sampled measured tree was morphologically independent. Furthermore, to better represent population diversity, sampling was done randomly across the area, reflecting the natural distribution of the trees rather than following a single line.

In total, 20 trees were selected in the littoral and semi-continental sites. While 37 trees were sampled from the more scattered continental site to capture a broader distribution. From each tree, 20 mature cones and 20 one-year-old branch sections with mature leaves were collected between November and December 2023, following Bacchetta [53]. Samples were taken from the southern, sun-exposed parts of the tree crowns, at a height between 1 to 2.5 meters. They were placed in labeled paper bags, along with detailed information on the sample's origin, location coordinates, date, and relevant environmental data, as summarised in Table 1.

2.3. Morphological characterisation of *J. turbinata* populations

The biometric measurement protocol used in this study follows the methods outlined by Mazur [54] and Marcysiak [55].The characteristics measured were based on the guidelines set by the International Union for the Protection of New Varieties of Plants [56]. These characteristics include: tree height (TH), number of trunks (NT), trunk diameter (TD), percentage of live crown (PLC), cone length (CL), cone diameter (CD) (calculated as the average of two independent measurements taken at a 90° angle), recta number (RN), number of cone scales (NCS), number of seeds per cone (NSC), seed length (SL), seed diameter (SD), and, the number of leaves on a 5 mm terminal section of a lateral branch (NL).

Measurements were taken using a stereo microscope with an eyepiece scale [39]. In addition to the direct measurements, eight ratios were calculated: the number of trunks to trunk diameter at the base (NT/TD), tree height to trunk diameter (TH/TD), tree height to number of trunks (TH/NT), cone aspect ratio (CL/CD), seed aspect ratio (SL/SD), cone length to seed length (CL/SL), cone diameter to seed diameter (CD/SD), and cone aspect ratio to number of seeds (CS/NSC).

Cone, branch, and seed measurements were made with an accuracy of 0.1 mm. Cone scales were counted after the cones were soaked in water for 24 hours. Seeds were extracted from the cones, air-dried, and then measured. The length of the terminal lateral with leaves was measured along its side, rather than diagonally, as described by Marcysiak [55]. In total, twelve measured characters and eight calculated ratios were included in the study. Full list of character provided in Table 2.

2.4 Genetic diversity of *Juniperus* populations

2.4.1 DNA extraction and PCR amplification

Each DNA preparation was obtained from a single plant. DNA was extracted from 200 mg of crushed leaves using CTAB buffer, following the methods of Doyle and Rajčević [57,58] with some modifications. After extraction and washing, the DNA was re-suspended in 1 mL Tris-EDTA buffer and treated with RNase to remove any RNA contamination [43–49,51,52,59]. DNA concentration was measured with a NanoDrop™ UV-Visible spectrophotometer (Thermo Fisher Scientific) and its quality was assessed using 0.8% agarose gel electrophoresis.

DNA was then diluted to a concentration of 10 ng/µL and stored at -20°C for subsequent use in polymerase chain reactions (PCR) [60]. Six genomic SSR (gSSR) markers developed from the genome sequence (Table 3) were selected for their polymorphism and amplification efficiency [61]. These SSR markers included di- and

trinucleotide motifs, and their forward primers were labeled with a fluorophore, either 6-FAM or HEX. PCR reactions were performed in a thermal cycler (Multigene, Labnet International, Inc.) in a total volume of 15 µL, containing 20 ng of genomic DNA, 0.23 µM of each primer, and 7.5 µL of 2× Hot-Goldstar Master Mix (Eurogentec), which provides a reaction medium containing 1 unit of Hot Gold Star DNA polymerase, 200 µM dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 15 mM Tris-HCl and 0.01% (v/v) stabiliser.

The PCR amplification program for markers J005458 and J064797 included an initial step at 94°C for 4 minutes, followed by 35 cycles of 94°C for 35 seconds, 56°C for 35 seconds, 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. For the other four markers, the amplification program included an initial step at 94°C for 4 minutes, followed by 35 cycles of 94°C for 35 seconds, 58°C for 35 seconds, 72°C for 45 seconds, and a final extension at 72°C for 7 minutes.

2.4.2 Fragment analysis

PCR products were diluted 1:10 in ultrapure water for fragment analysis. Each FAM-tag was multiplexed with a HEX-tag. Samples were denatured for 5 minutes at 95°C and then placed on ice for 2 minutes. The different alleles in each sample were separated by capillary electrophoresis on a 3500 genetic analyser (Applied Biosystems). GeneMapper6 software was then used to assign a base pair size to each SSR allele, using the GeneScan™-600LIZ™ internal size standard (Applied Biosystems) . A genetic profile was thus generated for each sample analysed.

2.4.3 Molecular data analysis

GenAlEx 6.5 software [62,63] was used to calculate several genetic parameters as the number of alleles per locus (Na) which indicates the allelic richness at each locus, the effective number of alleles per locus (($Ne = \frac{1}{\Sigma pi^2}$ ²) where pi is the frequency of the ith allele in the population) adjusts this measure by accounting for allele frequencies, providing a more accurate estimate of genetic diversity, the number of private alleles (Np) which corresponds to alleles specific to a given population and absent in other populations studied, the observed heterozygosity (Ho), expected heterozygosity (He = 1 - Σpi²) provide information on the actual proportion of heterozygous individuals and expected genetic diversity, respectively. Wright's fixation index ($F = 1 - \frac{Ho}{He}$) according to Wright [64] assesses the degree of inbreeding within populations, Shannon index (I = 1*Σ(pi * Ln pi)) is used to assess the overall genetic diversity of populations, fixation index (FST) is a measure of the genetic

differentiation between populations, giving insight into the degree of their genetic separation, and gene flow (Nm = [(1/FST) - 1]/4) indicates the flow of genes between populations [64].

Polymorphism information content (PIC) values were computed using Polygene software, taking into account the number of alleles per locus and their relative frequencies in the population, following the methods of Botstein [65] and Roldan-Ruiz [66]. Additionally, molecular analyses were performed with GenAlEx 6.5, including analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA). The PCoA, based on a dissimilarity matrix, was used to assess genetic relationships among the genotypes of *J. turbinata*. Finally, an unrooted phylogenetic tree was constructed using the Neighbor-Joining (NJ) method [67] with Darwin 6.0.021 software [68].

2.4.5 Genetic structure

The genetic structure was assessed to determine the most likely number of genetic clusters by applying the Bayesian clustering approach implemented in STRUCTURE v2.3.4 software [69]. To detect genetic groups, default parameters were applied with 100,000 repetitions of the Markov chain Monte Carlo (MCMC), with a burn-in period starting at 10,000 and ten repetitions per run for each number of groups chosen (i.e. K = 1-10). To determine the most likely K modal distribution, the delta K [70] was defined using STRUCTURE HARVESTER [71]. To check the most likely group membership coefficient in ten runs of STRUCTURE and STRUCTURE HARVESTER, we used CLUMPP v.1.1.2 [72]. The corresponding graphs were produced using DISTRUCT [69,73,74].

2.5 Data analysis

In order to compare the *J. turbinata* populations and assess their relationships based on the studied characters, several statistical analyses were employed. Data normality was tested using the Shapiro-Wilk test, and variance homogeneity was examined with Levene's test. A one-way ANOVA was conducted to compare the means of the three groups for each variable. When significant differences were detected, Tukey's test was applied to identify specific pairwise differences. All analyses were performed using IBM SPSS Statistics 25 software, with a significance level set at 0.05. Principal component analysis (PCA), a dimensionality reduction technique, was used to simplify a set of variables into fewer components. The Kaiser-Meyer-Olkin (KMO) index was calculated to assess the correlation between variables and determine the appropriateness of the data for PCA (only variables with a KMO value greater than 0.5 were included).

III. Results

3.1. Morphological characterisation of *J. turbinata*

3.1.1. Variability of morphological characters

A total of 20 traits were evaluated to compare three *J. turbinata* populations in north-eastern Morocco, including twelve measurable traits and eight calculated traits. These measurable traits included four tree characteristics (TH, NT, TD, and PLC), four cone characteristics (CL, CD, RN and NCS) three seed characteristics (SL, SD and NSC), and one leaf characteristic (NL). The calculated ratios comprised three tree shape indices (NT/TD, TH/TD and TH/NT), and five cone and seed ratios (CL/CD, SL/SD, CL/SL, CD/SD and CS/NSC). The ANOVA results show significant differences in most of morphological characters between the three studied populations, except for four variables (Table 2). Specifically, the number of trunks per tree (NT), the calculated ratio of tree height to number of trunks (TH/NT), and the number of recta and cone scales (RN and NCS) did not differ significantly among the *J. turbinata* trees from the three regions studied.

The tallest specimens of *J. turbinata* are found at the semi-continental site with an average height of 4.76m, followed by those at the littoral site (3.86m), while the trees at the continental site are the shortest (3.12 m). Regarding the trunk base diameter, the largest diameters are observed in trees from the littoral region (139 cm), followed by those from the semi-continental site (120 cm), while the trees from the continental site show the smallest diameters (63 cm). In terms of the percentage of living parts, the populations at the semi-continental and littoral sites have the highest percentages (86 and 85%, respectively), whereas the trees at the continental site show the lowest percentage (52%), reflecting local differences in environmental conditions.

In terms of pomological characters, the longest cones are found in the littoral population (9.94 mm), followed by the continental population (7.89 mm), while the semi-continental population has the shortest cones (7.56 mm). Regarding cone diameter, measured from two perpendicular angles, the largest cones are observed in the littoral population (8.87 mm), while the semi-continental and continental populations display similar values (6.30 mm and 6.37 mm, respectively). There is no significant variation in the number of recta and scales between populations, with values around 4 and 6, respectively.

The number of seeds per cone is highest in the semi-continental population (7.56), followed by the continental (5.77) and littoral (4.67) populations. Seed length is greatest in the littoral population (5.08 mm), followed by the semi-continental (4.52 mm) and continental (3.47 mm) populations. Concerning seed diameter, the continental population has the highest value (4.77 mm), followed by the littoral population (4.63 mm), while the semi-

continental population shows the smallest diameter (1.86 mm). The number of leaves in the 5 mm terminal branch is higher in the continental and semi-continental sites, with averages of 25 and 24.62, respectively, compared to the littoral site, which averaged 19.44 leaves.

The analysis of morphological ratios reveals other noteworthy characteristics. The ratio between the number of trunks and the trunk base diameter indicates that the trees in the littoral population, with a higher ratio (0.04), show greater competition among trunks, suggesting a robust morphology. Additionally, the ratio between tree height and trunk diameter shows that trees in the littoral population have a better proportion (0.08), suggesting more favorable vertical growth compared to other populations. Moreover, the ratio between tree height and number of trunks indicates that the trees in the littoral population (3.08) have a more elongated architecture, possibly in response to light requirements.

For cones, the length-to-diameter ratio in continental populations (1.24) indicates a more oval shape, while cones from semi-continental populations are rounder (1.19). Regarding seeds, the length-to-diameter ratio is highest in the semi-continental population (2.83), indicating an elongated shape favorable for dispersal. The relationship between cone length and seed length shows that cones from continental populations have a better proportion (2.49), suggesting increased reproductive efficiency. Similarly, the ratio between cone diameter and seed diameter reveals that cones in semi-continental populations have a higher ratio (3.93), indicating resinous structures that better protect the seeds. Finally, the ratio between the overall cone aspect and the number of seeds is highest in the littoral population (0.24), suggesting a cone shape optimized for seed production.

These observations highlight the influence of environmental conditions and local adaptations on the morphology of *J. turbinata* populations and underline the importance of incorporating these variations into conservation strategies for the species.

3.1.2. Principal component analysis

To assess the characteristics of *J. turbinata* trees according to their morphological characters, a principal component analysis (PCA) was carried out, integrating measurements on the trees, cones, seeds and leaves across 20 quantitative characters. The results of the PCA (Fig.2) showed that the first two principal components explained 50.18% and 35.85% of the observed variation respectively, totalling 86.03% of the overall variation. As shown in Figure 2, the populations of *J. turbinata* were organised into three groups in the space defined by the first two axis. According to the first axis (PC1), the semi-continental and continental populations both have low coordinates and were clearly distinguished from the littoral population with high coordinates. The second

axis (PC2) further differentiates the semi-continental (high coordinates) from the continental population (low coordinates).

3.2. Genetic analysis of *Juniperus* populations

3.2.1. Genetic diversity across loci

The results of the genetic analysis of *J. turbinata*, based on six SSR loci, provide crucial information about the genetic diversity and structure of the populations studied. Several genetic indices were calculated. The Table 4 shows variation in genetic diversity of *Juniperus* across different loci. The markers A3 (J064797) and A2 (J006365) accounted for the highest number of alleles (17.33 and 14.67, respectively), while A5 (J015521) registered the smallest value (10). Ne values range from 5.955 A1 (J005458) to 10.777 A2. High Ne values (like at A2 and A3) show that alleles are more evenly distributed, increasing the diversity captured by these loci. The Shannon Information Index (I) values further support this diversity, with high scores ranging from 2.511 for A3 to 1.997 for A5 with an average of 2.252 reflecting both the richness and evenness of alleles across loci. The results of the observed heterozygosity (Ho), which reflects the actual genetic diversity within populations show that for littoral samples, Ho values range from 0.250 to 0.700, with an average of 0.466. For semi-continental samples, values range from 0.300 to 0.550, with an average of 0.458. In contrast, the observed diversity is higher for continental region samples, with Ho values ranging from 0.270 to 0.811 and an average of 0.504. As for the expected heterozygosity (He), the values vary depending on the sites studied. For littoral samples, He ranges from 0.804 to 0.923 for each locus, with an average of 0.865 ± 0.019, indicating relatively high genetic diversity. Samples from the semi-continental zone show values between 0.775 and 0.866, averaging 0.821 ± 0.016, revealing slightly lower genetic diversity than littoral populations. Concerning the samples from the continental region, He values range from 0.790 to 0.932, with an average of 0.892 ± 0.022, highlighting even greater genetic diversity.

The observed heterozygosity (Ho) consistently falls below the expected heterozygosity (He), leading to positive fixation index (F) values across all loci ranging from 0.218 for the marker A5 to 0.696 for the marker A2 with an average of 0.444. Additionally, all loci have high PIC values close to or above 0.90, with the marker A2 (J006365) showing higher PIC value (0.95) whereas A1 (J005458) has the lowest PIC value (0.89). The high PIC values obtained in this study, with an average of 0.92, indicate that the loci used are highly informative for distinguishing between individuals or groups in the population. Regarding the Hardy-Weinberg equilibrium

(HWE) test, the results show that the observed genetic variation in all loci deviates from what is expected under the assumptions of the HWE model.

3.2.2. Allelic Pattern and Genetic Diversity Indices of *J. turbinata* populations across growing sites

Analysis of the results obtained for *J. turbinata* populations using six SSR markers revealed notable differences in genetic diversity between littoral, semi-continental and continental sites (Table 5). The continental population shows the greatest allelic diversity, with an average number of alleles (Na) of 16.83, an effective number of alleles (Ne) of 10.76, and a Shannon information index (I) of 2.51. In comparison, the littoral population has less genetic diversity (with an Ne value of 8.30 and an I index of 2.26), while the semi-continental population exhibit even lower values, with an Ne of 5.84 and an I index of 1.98. This comparative analysis indicates that the continental population is genetically more diverse, suggesting a potentially greater capacity to adapt to varied environmental conditions. In contrast, the littoral and semi-continental populations exhibit lower genetic variability, which may make them more susceptible to environmental pressures.

Regarding heterozygosity, the continental population displays an expected heterozygosity (He) of 0.892 ± 0.022, slightly higher than that of the littoral (He = 0.865 ± 0.019) and semi-continental (He = 0.821 ± 0.016) populations. The observed heterozygosity (Ho) is also higher in the continental population (Ho = 0.505 ± 0.094) compared to the littoral (Ho = 0.467 ± 0.074) and semi-continental (Ho = 0.458 ± 0.040) populations. Regarding private alleles, the continental population registered the highest number (Np = 6.167±0.7) followed by the littoral population (Np = 5.33±0.84) while the lowest value was seen for semi-continental population (Np = 3±1.12).

As for the inbreeding coefficient (F), it remained relatively steady across the three populations with high values (0.456, 0.439 and 0.438) pointing to a substantial degree of inbreeding.

In sum, these results highlight that the continental population possesses a richer and potentially more resilient genetic diversity, while the littoral and semi-continental populations display comparatively lower levels of genetic diversity.

3.2.3. Analysis of Molecular Variation reveals significant genetic differentiation among individuals.

The analysis of molecular variance (AMOVA) is a statistical method used to partition genetic variation among different hierarchical levels. In our study, Table 6 presents the main results of AMOVA analysis, indicating that only 7% of the total genetic variation is attributed to differences between populations (sites).

In contrast, 43% of the genetic variation arises from differences between individuals within populations. This high intra-population component highlights considerable genetic diversity among individuals within each site. Furthermore, 50% of the genetic variation is explained by differences within the individuals themselves.

The F_{ST} value found in our study is 0.07, indicating a moderate level of genetic differentiation between the three populations. In our study, the Nm value of 3.302 (Table 6). Nm values above 1.0 generally prevent strong population differentiation, which is supported here by an F_{ST} value of 0.07, reflecting a moderate level of genetic differentiation.

Analysis of Molecular Variation (AMOVA), along with Nei's genetic distance and genetic diversity indices, was performed on the hierarchical population to assess the genetic structure between *J. turbinata* populations based on growing sites. The AMOVA results showed that the highest genetic variation (50%) occurred within individuals, while genetic differentiation among populations was minimal, accounting for only 7% of the total variance, indicating no significant structure. Additionally, Nei's genetic distance analysis revealed a high gene flow (Nm = 3.302) and a moderate F_{ST} value (0.07).

The paired F_{ST} values calculated between the littoral, semi-continental, and continental populations of *J. turbinata* reveal low to moderate differentiation, with values ranging from 0.046 to 0.068 (Supplemental Table 1). The F_{ST} value of 0.068 between the littoral and semi-continental populations suggests some isolation, although gene flow is still present. The lower value of 0.046 between the littoral and continental populations indicates less differentiation, likely due to greater genetic exchange or more recent divergence. The F_{ST} value of 0.049 between the semi-continental and continental populations reflects also moderate differentiation. Although the three populations exhibit some genetic differentiation, the data suggest the presence of gene flow, particularly between littoral and continental populations. The littoral population showed more genetic proximity to the continental population than to the semi-continental population. These results are critical for understanding population connectivity and guiding conservation efforts.

3.2.4. Genotypes' relationships and population structure

Principal coordinate analysis (PCoA) provides in-depth insights into the genetic structuring of *J. turbinata* populations in two-dimensional space, highlighting genetic relationships among individuals (Fig. 3). The PCoA axes explain 11.5% of the total molecular variation. It is interesting to note that the three populations are divided into two distinct groups along the first principal coordinate (Group 1: Semi-continental; Group 2: Littoral and Continental), except a few individuals resulting likely from crosses between these groups. In addition, all the littoral individuals have high coordinates on both axes. In contrast, semi-continental individuals show average coordinates on axis 2, while being more dispersed along axis 1. Finally, continental individuals are distinguished by low coordinates on axis 2 and high coordinates on axis 1.

The unrooted neighbour-joining tree generated by the Darwin software represents the studied *J. turbinata* individuals (Fig. 4) and illustrates the relationships between them based on their relative genetic distance. All the individuals in the littoral population (in green) group together on one side, except individuals S8 and S15. Similarly, all the individuals in the semi-continental population are on the opposite side, except for O17. In the continental population, ten individuals (F33, F11, F22, F1, F26, F36, F5, F3, F16 and F8) are outside the main group. These thirteen individuals (S8, S15, O17, F33, F11, F22, F1, F26, F36, F5, F3, F16 and F8) appear to be hybrids resulting from crossing events between populations.

Additionally, a structure analysis was carried out on the dataset to describe the genetic structure of *J. turbinata* populations in north-eastern Morocco. The Delta K method (ΔK) was used to determine the optimal number of genetic groups in the populations studied. This method is based on the second-order rate of change of the log-likelihood (Ln P(D)) of the data as a function of the number of clusters (K). The maximum value of Delta K was observed at K = 2 (Fig. 5 A), suggesting two main genetic groups. In other words, *J. turbinata* individuals from the three concerned sites can be grouped primarily into two distinct genetic sets, indicating that individuals share common genetic variation within each cluster, while exhibiting notable differences with the other cluster.

Analysis of the genetic structure, sorted by regions (Fig. 5 B), reveals a clear separation into two main groups: one group comprising the littoral and continental populations, and another composed exclusively of semi-continental individuals, except some few genotypes. This differentiation indicates that littoral and continental individuals show greater genetic similarity than the semi-continental ones. Whereas this later population appears to be genetically isolated. Although being clustered in the same group, it is noteworthy that samples from the littoral region seems to be genetically pure than those from the continental region. Indeed, certain continental individuals displayed shared genetic material with the semi-continental site. This observation is confirmed also

by PCoA and the phylogenetic tree, where some genotypes from the continental and semi-continental regions are juxtaposed. These distinctions are essential for formulating conservation strategies, as each genetic group may represent a distinct management unit with specific needs in terms of preserving genetic diversity.

3.2.5. Genetic analysis based on genetic clusters sorted by Q

In the first phase of the genetic analysis, each sampling site was treated as representing a distinct population of *J. turbinata*. This initial approach made it possible to collect data on allelic and genetic diversity, revealing notable differences between the sites. The genetic structure showed that individuals from littoral and continental sites share similar genetic characteristics, thus forming a single genetic population (called Pop1), distinct from the semi-continental one (called Pop2) (Fig. 5 C).

An in-depth re-evaluation of the genetic diversity based on the genetic clusters was undertaken to better understand these distinctions. The second phase of the study focuses on a detailed analysis of this revised structure, considering the new population classifications and exploring the implications of this genetic distribution on the diversity and resilience of *J. turbinata* populations.

The results provide a detailed comparison of genetic parameters across the six loci in two populations of *J. turbinata*, (Supplemental Tables 2 and 3). Population 1 had a significantly higher number of alleles (Na) (24.167) than population 2 (8.00), indicating greater allelic diversity. However, when we look at frequent alleles (N_a Freq $\geq$ 5%), this difference reduces to 7.5 for population 1 and 6.833 for population 2, suggesting that most of the alleles in population 1 are rare. The effective number of alleles (Ne) is almost three times higher in population 1 (14,311) than in population 2 (5,023), reinforcing the idea of greater genetic diversity in the former. The Shannon information index (I) was also higher in population 1 (2.845 compared with 1.792), indicating a more homogeneous distribution of alleles. In addition, population 1 had a significantly higher number of private loci (Np), with 17,833 compared with only 1,667 for population 2, indicating greater genetic specialisation.

Neither population shares common alleles locally at frequencies of 25% or 50%, indicating a low occurrence of shared alleles. Observed (Ho) and expected (He) heterozygosity rates were slightly higher in population 1 (Ho: 0.497, He: 0.924) compared with population 2 (Ho: 0.435, He: 0.791), highlighting greater expected genetic variability in the former. The expected unbiased heterozygosity (uHe) corroborates these trends, reaching 0.932 for population 1 compared with 0.813 for population 2. Finally, the fixation indices (F), which are relatively similar between the two populations (0.462 for population 1 and 0.446 for population 2), indicate a comparable level of inbreeding, although population 1 has a more complex and diverse genetic pattern.

The AMOVA analysis of *J. turbinata* populations according to the genetic clusters sorted by Q was quite similar to that of populations sorted by regions (Supplemental Table 4). This consistency in AMOVA results implies that the genetic differentiation among the three populations in the initial grouping was effectively between the semi-continental individuals and the others. The first grouping may have masked genetic similarities between the littoral and continental populations, which was highlighted by the second grouping.

Additionally, the results of the PCoA analysis were identical to those of the first PCoA, grouping clearly individuals from the littoral region with those from the continental region into a single group, while the second group consists of individuals from the semi-continental region (Supplemental Fig. 1). This indicates a clear distinction between both populations based on genetic data and further confirms the result of the genetic structure.

IV. Discussion

4.1. Morphological study

The results of this study revealed that morphological variations in *J. turbinata* Guss. in northeastern Morocco are influenced by specific environmental factors and local genetic adaptations. These differences highlight the importance of considering these aspects in conservation strategies to preserve this species' genetic and ecological diversity [35] .

4.1.1. Impact of environmental conditions on tree morphology

The studied populations -continental, semi-continental, and littoral showed differences in morphology and structure, which can largely be attributed to the environmental conditions of each region.

The continental site (region of Figuig), characterised by high temperatures, severe drought and poor soils, imposes significant constraints on tree growth [75]. Continental trees are smaller (3.12 ± 0.31 m) with a sparse canopy (52.26% cover), reflecting an adaptation to a hostile environment [76]. The limitation of their vertical development and the reduction in trunks underline a survival strategy in response to extreme environmental conditions[77–79].

The semi-continental site (located on the high plateau of eastern Morocco) offers more favourable conditions, such as greater availability of water resources and a more moderate climate. The trees are taller (4.76 ± 0.32 m), and their canopy is denser (86.25%), indicating conditions conducive to optimised growth both vertically and

laterally [78]. The larger diameter of the trunks reflects better adaptation to this milder environment [80] and does not reflect the approximate age of the tree [81,82].

The littoral site, while facing environmental challenges such as soil salinity [83] and constant winds [84], has a relatively moderate climate in terms of temperature and humidity. In response to the persistent winds, the trees adopt a more compact, bushy form with a relatively dense canopy (85.5%). This morphology contributes to their stability and resistance [85]. The *J. turbinata* trees studied are classified as large trees, according to Lepointe [86].

The cones and leaves of *J. turbinata* populations show significant variations [87] linked to local conditions.

Samples from the littoral site are distinguished by longer (9.94 ± 0.06 mm) and wider cones, while semi-continental cones are narrower. The smallest cones are from the continental, consistent with the region's harsh climatic conditions [39]. This variability highlights the importance of the environment for the species' reproduction.

Samples from the littoral site show the longest leaves (19.44 leaves per 5 mm branch), followed by those from the semi-continental (24.62 leaves per 5 mm branch). The shortest leaves were observed in the continental (25.06 leaves per 5 mm branch), probably due to the light requirements of the genus *Juniperus* [5]. These differences illustrate the gradual adaptation of populations to local constraints [88].

Populations of *J. turbinata* in northeastern Morocco show a remarkable ability to adapt to various environments, from severe continental conditions to more favourable semi-continental and littoral environments. Considering these adaptations in conservation strategies is crucial to ensuring the long-term survival of this species. The differences observed in the morphology and structure of the trees demonstrate the significant influence of local conditions on their development and, consequently, on the conservation measures to be adopted.

Principal Component Analysis (PCA) of *J. turbinata* populations reveals distinct morphological patterns between continental, semi-continental, and littoral populations, indicating significant morphological similarities and divergences.

The PCA extracted two major components. The first component is strongly correlated with several morphological characters, such as CL (0.885), CD (0.909) and NL (-0.863), indicating that these characteristics significantly influence the total variation. The second component is associated with other characters like SD (-0.803) SL/SD (0.862), and CD/SD (0.811).

Populations from different regions show specific correlations with these components. Littoral populations are positively correlated with the first component, indicating that their morphology is influenced by greater cone length and width. However, in the second component, these populations are spread between positive and negative values, suggesting variability in growth characteristics, particularly SD and other character related to adaptation to littoral environmental conditions.

In contrast, semi-continental populations are negatively correlated with the first component and positively correlated with the second. This means these populations tend to have smaller cones (negative correlation with CD) but show more developed growth characteristics, such as CD/SD, suggesting adaptation to varied, though less extreme, conditions than those found on the coasts.

Continental populations, on the other hand, are negatively correlated with both components. This reflects smaller cones CD and narrower seeds SD, as well as less pronounced growth characteristics, likely due to harsher environmental conditions that reduce growth vigor.

In summary, the PCA reveals that larger cones distinguish littoral populations, while semi-continental and continental populations display different morphological adaptations to their respective environments, with growth and development characteristics varying according to specific environmental pressures.

4.2. Genetic analysis

The genetic study of *J. turbinata* populations in northeastern Morocco aims to better understand the genetic structure and diversity of this species in a context of habitat fragmentation and increasing environmental pressures. Indeed, although their vast geographical distribution, *Juniperus* populations are fragmented and discontinuous [89–91]. Population genetics allows us to assess genetic variation within and between populations, providing insights into the species' capacity to adapt to local conditions and withstand environmental changes. For *J. turbinata*, genetic analysis is essential to identify the most vulnerable populations and those with genetic characters that promote resilience. By integrating these data with morphological observations, this study contributes to a comprehensive understanding of the species' diversity and evolutionary dynamics, which is crucial for developing effective conservation strategies tailored to local specificities.

4.2.1. Allelic and Genetic diversity between populations

In this investigation we examined 77 trees at three separate sites in northeastern Morocco. The results emphasize the considerable genetic diversity of *J. turbinata* populations in these regions demonstrating the effectiveness of

SSR markers in revealing this diversity through allele numbers and genetic diversity (He). Using six genomic SSR markers we identified 80.01 alleles, with an average of 13.335 alleles per locus. For comparison, [61] analysed 83 individuals of *J. phoenicea ssp. turbinata* from the stabilised dunes of the Reserva Biológica de Doñana (province of Huelva, Spain) using 19 SSR markers, including six in common with in this study, and identified 93 alleles, with an average of 4.89 alleles per locus. Similarly, Meloni [92] detected 90 alleles in 109 individuals of *J. phoenicea* using three separate SSR markers. Boratyński [42] found 76 alleles in their study, with an average of 2.11 alleles per locus. Despite significant geographical differences between the 14 populations of *J. phoenicea* spread across north-west Africa (88 individuals), southern Europe (279 individuals) and south-west Asia (24 individuals), a total of 391 individuals showed little genetic variation between these populations Boratyński [42]. However, all these studies report lower values than those we obtained, even though the number of samples was smaller (77 individuals spread over three populations).

Genetic diversity plays a central role in plant improvement efforts, as it provides a better understanding of population variability. One of the key parameters for quantifying this diversity is the expected heterozygosity index (He), which reflects the theoretical level of genetic diversity. The higher the value of He, the greater the diversity is, meaning that the population has a larger genetic reservoir, which is essential for plant selection and adaptation.

In this study, the values of expected genetic diversity (He) averages 0.859 and vary depending on the sites studied. The semi-continental zone show He value averaging 0.821, for littoral samples, He averages 0.865 and for the samples from the continental region, He value averages 0.892, highlighting even greater genetic diversity than the other sites. These results emphasize that genetic diversity fluctuates depending on the region, likely due to environmental and historical factors specific to each site. Despite these variations, it is important to note that genetic diversity is considered high across all regions studied. These finding allows us to highlight the importance of genetic diversity for the resilience of plant populations in the face of environmental changes and selection pressures.

The mean He value in our study (0.85) was significantly higher than those reported in other studies. For instance, Boratyński [42] found an overall mean He of 0.195 across 244 individuals of *J. turbinata* from nine regions in six different countries. The same study reported average He values of 0.210, 0.209, and 0.239 for Moroccan populations located, respectively, along the Atlantic coast south of Tangier, in the High Atlas at Tizi-n-Tagalm, and between Agouti and Azilal. This finding highlights notable geographic variation in genetic diversity across

Moroccan populations. For *J. phoenicea*, Boratyński [42] found a low mean He value of 0.172, based on 147 individuals from five regions across three countries. Similarly, Sánchez-Gómez [93] reported He values lower than our mean for *J. phoenicea* populations in Spain (0.153 - 0.202) and for *J. turbinata* populations across 16 Mediterranean countries, from Jordan to Portugal (0.147 - 0.229). Even lower values (0.101 - 0.146) were reported by Meloni [92] for *J. phoenicea* populations in Cyprus and Italy. These results contrast sharply with our data, which indicate significantly higher genetic variability.

For related species, most studies found in the literature reported He values lower than our results for populations of *J. coreana* (0.148 - 0.190), *J. rigida* (0.149 - 0.210), *J. squamata* (0.543) in southwest China, also *J. sabina* in China (0.456), and *J. excelsa* in Turkey (0.524), as reported in the studies of Huh [94], Ju [95], Evren [96], and Lu [97]. Only *J. communis* populations in Saxony (Germany) stand out with He averaging 0.84 [98], very close to our mean of 0.859, suggesting a similarly high level of genetic variability. These comparisons demonstrate that our study on *J. turbinata* reveals some of the highest levels of genetic diversity, comparable only to the findings for *J. communis*, while far exceeding those reported for Moroccan or Mediterranean populations of *J. turbinata* and related species.

Regarding the observed heterozygosity (Ho), the value averages 0.476, with variations depending on the sampling sites. For semi-continental samples, the average Ho is 0.458, while littoral samples exhibit an Ho of 0.467. In contrast, samples from the continental region show a higher observed heterozygosity, averaging 0.505. Similarly, García et al. [61] reported a range of Ho values from 0.11 to 0.68 for *J. turbinata* in stabilized coastal dunes within the Doñana Biosphere Reserve, Spain

The F coefficient provide a direct measure reflecting and quantifying how well Ho aligns with He. Values exceeding 0.15 generally raise concerns about inbreeding depression. Practically, this coefficient may guide management decisions, with values over 0.25 indicating a need for immediate intervention to prevent further declines in genetic health, which is the case in our study. Indeed, the F coefficient in this investigation registered high values averaging 0.444. In comparison with other studies on *J. turbinata*, Sánchez-Gómez [93] reported average F values of 0.065 for populations from the Canary Islands, 0.169 for those in the central and eastern Mediterranean basin, and 0.113 for those from the Iberian Peninsula and Morocco. Boratyński [42] observed even lower values, ranging between -0.110 and 0.013, across Europe and North Africa, while García [61] reported an extremely lower F value of 0.0035 in a regional study of the Parque Natural del Alto Tajo (Guadalajara province, Spain). For *J. phoenicea*, the fixation index also showed lower values than our findings.

Sánchez-Gómez [93], reported an average value of 0.158 for populations in Spain, Boratyński [42] documented values ranging from -0.076 to 0.084 for populations in Spain, France, and Andorra. All these finding reflects a generally lower inbreeding compared to *J. turbinata* in this study.

Among other *Juniperus* species, the inbreeding coefficient displayed considerable variability. For instance, Reim [98] reported F values between 0.19 and 0.41 for *J. communis* populations in Germany, Slovakia, Norway, and Italy. Similarly, Huh [94] found an average F value of 0.481 (with a maximum of 0.674) for *J. coreana*. High F values were also observed for *J. squamata* in China, ranging from 0.523 to 0.559, with a mean of 0.543 [95]. The high fixation index found in this study suggests a reduction in heterozygosity within these populations. Therefore, organization of in situ conservation measures is required to maintain at least the ongoing genetic situation

The polymorphism information content (PIC) reflects the ability of a marker to identify genetic differences within a population. In general, a PIC above 0.5 indicates that the marker is highly informative. All microsatellite markers exhibited high informativeness, as reflected by their PIC values with an average of 0.92, indicating that the loci used are highly polymorphic and informative for *J. turbinata* populations. In contrast, the markers we used and developed by García [61] were tested on a juniper species, revealing low PIC values ranging from 0.3 to 0.39. PIC values obtained in this study suggest strong allelic diversity, allowing for good genetic differentiation between individuals.

4.2.2. Genetic differentiation between populations

The fixation index (F_{ST}), ranging from 0 to 1, helps assess the genetic differentiation between populations. Theoretically, a value of 0 indicates the absence of genetic differentiation, while a value of 1 means that the populations are completely fixed and differentiated [98–101]. Values below 0.05 indicate low differentiation, suggesting that population share a similar gene pool. Values between 0.05 and 0.15 reflect moderate differentiation, indicating some genetic distinction among populations. Values from 0.15 to 0.25 signify high differentiation, often due to limited genetic exchange or adaptation to different environments. Values above 0.25 represent very high differentiation, indicating completely isolated populations [6,7,102]. According to these criteria, the three populations of *J. turbinata* analysed in this study display moderate genetic differentiation of 0.07.

572 Gene flow (Nm) is another important parameter that measures the degree of genetic material exchange between
573 populations. It is inversely correlated with F_{ST}, as high gene flow reduces genetic differentiation. When Nm is
574 less than 1, this reflects limited gene exchange between populations, while a value greater than 1 indicates
575 significant genetic exchange [103]. The obtained F_{ST} value of 0.07 and Nm value of 3.302 offer valuable insights
576 into the genetic differentiation and gene flow between the studied *J. turbinata* populations in eastern Morocco.

577 This moderate differentiation suggests that the populations are not completely genetically isolated and that some
578 level of gene flow occurs between them. Moreover, this genetic differentiation could be influenced by factors
579 such as geological variations or ecological conditions that facilitate the mechanisms of seed dispersal or pollen
580 transport. promoting gene flow between populations. Since the value of Nm is high, this indicates that there is
581 significant genetic exchange between populations, which limits their divergence over time. The combination of a
582 moderate F_{ST} value and a high Nm may also indicate the presence of asymmetric genetic exchanges, where a
583 specific population could be a predominant source of genes for others, or experience sporadic and irregular
584 exchanges over time. Populations of *J. phoenicea* are often pioneers, occupying specific habitats such as littoral
585 dunes and rocky cliffs, where some individuals can be over 1.000 years old [81]. This species is mainly wind-
586 pollinated [102]. The long-distance transport of pollen by wind to ensure pollination has been reported and
587 discussed in several studies [99,104–110]. *Juniperus* pollen is no exception, as a recent study tracing the reverse
588 trajectory of *J. ashei* pollen showed that it had been transported from central Quebec to Repulse Bay,
589 approximately 3000 km [111]. Additionally, animals often transport seeds over long distances [112,113].
590 Particularly, *Juniperus* relies on bird-mediated seed dispersal, also called ornithochory. While most conifers have
591 dry, wind-dispersed seeds contained in hard cones, *Juniperus* have seeds in fleshy, berry-like cones that attract
592 birds. This seed dispersal allows seeds to travel long distances, thereby promoting the colonization of new
593 habitats and the diversification of populations [113].

594 Furthermore, gene flow can disseminate beneficial alleles, boosting genetic variation and adaptive evolution in
595 stressful environments. Private alleles, prevalent in all populations, especially continental samples, are unique
596 genetic variations essential for distinguishing genotypes and identifying key genomic regions in *Juniperus*
597 species. They enhance allele diversity and are valuable for developing new *Juniperus* cultivars with desirable
598 economic traits through registration and breeding programs.

599 The studied populations of *J. turbinata* exhibit some significant genetic divergences, as confirmed by the
600 analysis of molecular variance (AMOVA), with a high significance (p-value < 0.001). This moderate genetic

differentiation between populations represents only 7% of the total molecular variance, suggesting that most genetic variation lies elsewhere. Indeed, 50% of the genetic variability was observed within the individuals, highlighting a high degree of intra-individual diversity. This indicates that, although the populations are relatively genetically distant, much of the genetic diversity is found at the individual level rather than between populations. Previous studies corroborate these findings. Boratyński [42] reported that about 57% of the genetic variation is found within populations of *J. turbinata* from Greece and Turkey, when analysing the genetic distances between individuals. Furthermore, Meloni [92] reported that only 16% of the total genetic variance was found between populations within the regions studied. These results align with ours, where a relatively small portion of the variance is attributed to population differentiation.

Other species within the genus *Juniperus* show similar trends. For example, in *J. oxycedrus*, Juan [114] found that 47.42% of the genetic variation was within populations. In the same context, another study investigating the genetic diversity of *J. phoenicea* using isozymes also demonstrated the presence of a level of intra-population genetic variation, comparable to that in other European conifers [115]. These studies confirm that although populations may be differentiated within the *Juniperus* genus, a large part of the genetic diversity remains within individuals or within populations.

These observations are important for understanding the conservation dynamics and management of *J. turbinata*. The low percentage of genetic differentiation between populations, combined with the high intra-individual diversity, suggests that conservation strategies should focus on preserving genetic diversity within populations, while considering genetic isolation between different groups.

4.2.3. Population structure and genetic relationships

This study combined three complementary methods – Principal Coordinates Analysis (PCoA), Neighbour-Joining (NJ) tree, and population structure analysis – to examine the genetic structure and relationships among *J. turbinata* individuals, following the recommendations of Wang [115]. The diversity panel, comprising 77 individuals, was separated into three groups corresponding to the sampled populations. However, the structure profiles revealed that these three populations belong to two distinct genetic pools. The littoral and semi-continental populations do not share common ancestors, while the continental individuals exhibit a genetic mix between these two populations.

The presence of this genetic mixture is supported by the Nm value (3.302), indicating high gene flow between both genetic clusters. Furthermore, the results from the PCoA, NJ tree, and structure profile show that

geographical distance does not significantly influence the observed genetic differentiation. Although the three studied regions are distant (with distances ranging from 80 to 400 km) and separated by natural and artificial barriers such as mountains, cities, and roads, the seeds of *J. turbinata* have likely been exchanged over time, particularly towards the continental region, located in the south. This study highlights the importance of combining various approaches – morphological and genetic– to refine the taxonomic delimitation of the *J. phoenicea* complex. Morphologically, two distinct groups were identified: one comprising individuals from littoral areas and the other gathering populations from semi-continental and continental regions, likely due to climatic similarities between these two sites compared to the littoral site (Saïdia). However, genetic analyses reveal a different structure, the semi-continental population from Oued Elhimer form a distinct genetic group, while littoral (Saïdia) and continental (Figuig) populations, despite their geographical distance, show strong genetic similarity. This suggests that edaphoclimatic conditions may have a greater influence on the morphological characteristics of *Juniperus* than genetic factors do. A similar phenomenon was observed in the study conducted by Jiménez [116], where the Gran Canaria population was found to be genetically closer to that of El Hierro, despite the geographic distance between these islands. These genetic similarities between geographically distant populations, both in our study and that of Jiménez [116], highlight the complexity of genetic flow in *Juniperus*. Indeed, pollen can travel great distances, and the fruits are dispersed by various frugivores, such as the blackbird (*Turdus merula*) and thrushes [113,117], which transport seeds along their migratory routes. Thus, *Juniperus* genetic flow does not follow the north-south stepwise differentiation model. This model, used to explain the genetic structure of other species, such as *Gallotia* spp. [118] or *Olea europaea* L. [119], seems inadequate for *J. phoenicea*. Instead, the study reveals that factors such as pollen and seed dispersal by frugivores, as well as local environmental conditions, play a predominant role in shaping the genetic structure of this species, making genetic flow more complex than expected.

Thus, the taxonomic delimitation of *J. phoenicea* requires a more nuanced consideration of the interactions between environmental factors and genetic flow, which do not always conform to traditional geographical differentiation models.

4.2.4. Implications for the conservation of *J. turbinata* populations

Conservation strategies for *J. turbinata* populations can be implemented both in-situ and ex-situ. In-situ conservation focuses on preserving genetic diversity by protecting populations in their natural habitats and by reintroduction of young *J. turbinata* plants to rejuvenate existing populations along with reforestation efforts in

suitable areas as well as maintaining ecological interactions and supporting local adaptations. For the long-term preservation of this species, ex-situ conservation is essential. This approach involves establishing seed banks and living collections (e.g., in botanical gardens) to serve as a genetic reservoir for *Juniperus* species. These resources can be utilized in future restoration efforts if natural populations experience significant decline.

This investigation has direct implications on the conservation of *J. turbinata* in northeastern Morocco. Primarily, the high level of genetic diversity found within populations rather than between them indicates that conservation strategies should prioritize maintaining genetic diversity within each population. Furthermore, the high inbreeding found in all *J. turbinata* populations may reduce genetic diversity, leading to inbreeding depression, where reduced heterozygosity compromises individual fitness, reproductive success, and resilience to environmental stresses. Addressing this issue through increasing cross-population breeding is required.

In addition, the genetic clustering based on the structure profile reveals the presence of two distinct genetic pools. Each pool may represent a distinct management unit with specific needs in terms of preserving genetic diversity. Our results show genetic proximity between the continental (Figuig) and littoral (Saidia) populations. However, this latter (littoral population) has undergone significant degradation, impacted by the AZUR development project in Saidia. According to [40,50], this expansion led to a 63% loss of *J. turbinata* maquis between 2004 and 2021 in this area. Furthermore, recent research [41] on the germination of seeds from this species reveals that seeds from the littoral population (Saidia) have low germination potential, unlike those from the continental population (Figuig), whose germination potential is considerably higher. This finding paves the way for ecological restoration actions using seeds from the continental population to restore degraded littoral areas, thereby contributing to the preservation of the fragile ecosystems in this region [50].

V. Conclusion

This comprehensive study on *J. turbinata* in northeastern Morocco integrates both morphological and genetic analyses. The morphological results revealed distinct adaptations among regions, particularly between the littoral and continental populations, highlighting specific adaptive responses to varying environmental conditions. The analysis of morphological characters, such as tree height and the dimensions of cones and seeds, underscores significant differences despite the populations' genetic proximity. From a genetic perspective, the study demonstrates high genetic diversity and moderate population differentiation, indicating unrestricted gene flow among the sampled locations, illustrated by low F_{ST} values and sufficient genetic connectivity, which allows for

genetic exchanges despite geographical distances. These findings suggest that most of the genetic diversity resides within the populations, reinforcing the importance of protecting them to maintain the species resilience.

This study emphasizes the need for an integrated approach to the conservation of *J. turbinata*, which considers both morphological specificities and genetic dynamics. Conservation efforts should focus on the preservation of individual populations and the promotion of genetic diversity through continuous monitoring of gene flows. This is essential to ensure the longevity and diversity of this species in the face of increasing environmental challenges.

Author contributions

Mehdi BOUMEDIENE: Writing- Original draft, Investigation, Data curation; Chaymae BENKIRANE: Investigation, Data curation; Saida LHOUSNI: Investigation, Data curation; Christina M. MÜLLER Validation, Data curation; Larbi RHAZI: Writing - Review & Editing ; Ahmed MARHRI: Conceptualization, Validation ; Alicia AYERDI GOTOR: Writing - Review & Editing; Majida CHARIF: Investigation, Ressources ; Mohammed BELLAOUI: Methodology, Validation ; Malika ABID: Conceptualization, Supervision, Project administration; Nargis SAHIB: Conceptualization, Supervision

Data availability

Data available on request from the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare no competing interests.

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Table 1. Geographical coordinates and average climatic conditions of the three sampling regions between 2019-2022.

Site	Latitude	Longitude	Altitude (m)	Area in hectares	Average annual temperature (°C)		Average annual precipitation (mm)
					Min	**Max**	
Littoral site **(Saidia)**	35.09363763 35.11514601	-2.25636434 -2.32276416	57.15 ±2.23	30	7.67	32.22	262.625
Semi-continental site **(Jerrada)**	34.42172189 34.41928104	-1.88995441 -1.88019053	1094.35±72.9	63	0.9	34.12	273.25
Continental site **(Figuig)**	32.47337315 32.22803617	-1.08276935 -1.65916036	1645.45±39.4	391 239	0.7	40.82	99.65

Character	Variable	Littoral site	Semi-	Continental	Average	Df	F-value	p-value

Table 2. One-way ANOVA of quantitative morphological parameters carried out on trees, cones, seeds and leaves of *Juniperus turbinata*.

	code		continental site	site	value			
Tree height (m)	TH	3.86 ± 0.28 [ab]	4.76 ± 0.32 [a]	3.12±0.31 [b]	3.91	2	6.414	0.0026**
Number of trunks Per tree	NT	1.9 ± 0.36 [a]	2.1 ± 0.28 [a]	1.45±0.11 [a]	1.81	2	2.208	0.1170NS
Trunk diameter (cm)	TD	139 ± 17.05 [a]	120.21±9.81 [a]	63.15±9.23 [b]	107.45	2	8.805	0.0003***
Percentage of living part	PLC	85.5 ± 3.38 [a]	86.25 ± 2.9 [a]	52.56±7.04 [b]	74.77	2	10.698	0.0000***
Cone length (mm)	CL	9.94 ± 0.06 [a]	7.56 ± 0.02 [c]	7.89±0.09 [b]	8.46	2	199.45	0.0000***
Cone diameter (mm)	CD	8.87±0.04 [a]	6.30±0.02 [b]	6.37±0.07 [b]	7.18	2	447.124	0.0000***
Recta number Per cone	RN	4.25 ± 0.06 [a]	4.10 ± 0.04 [a]	4.21±0.05 [a]	4.19	2	1.384	0.2570 NS
Number of scales Per cone	NCS	6.17 ± 0.03 [a]	6.20 ± 0.06 [a]	6.11±0.02 [a]	6.16	2	1.525	0.2242 NS
Number of seeds per cone	NSC	4.67 ± 0.03 [c]	7.56 ± 0.03 [a]	5.77±0.04 [b]	6.00	2	793.029	0.0000***
Seed length (mm)	SL	5.08 ± 0.23 [a]	4.52 ± 0.23 [a]	3.47±0.17 [b]	4.36	2	16.633	0.0000***

Seed diameter (mm)	SD	4.63 ± 0.44 [a]	1.86 ± 0.14 [b]	4.77±0.20 [a]	3.75	2	32.076	0.0000 [***]
Number of leaves in the 5mm terminal branch	NL	19.44 ± 0.1 [b]	24.62 ± 0.07 [a]	25.06±0.44 [a]	23.04	2	61.51	0.0000 [***]
Number of trunks /Diameter at the base of the trunk	NT/TD	0.04±0.01 [a]	0.3±0.012 [ab]	0.01±0.001 [b]	0.11	2	4.443	0.015 [*]
Tree height /Diameter at the base of the trunk	TH/TD	0.08±0.01 [a]	0.04±0.006 [b]	0.026±0.002 [b]	0.04	2	23.19	0.0000 [***]
Tree height / Number of trunks	TH/NT	3.08±0.37 [a]	3.35±0.5 [a]	2.41±0.29 [a]	2.94	2	1.82	0.1681 [NS]
Aspect ratio of the cone (Length / diameter)	CL/CD	1.12±0.005 [c]	1.19±0.005 [b]	1.24±0.013 [a]	1.24	2	25.356	0.0000 [***]
Aspect ratio of the seed (Length / diameter)	SL/SD	1.83±0.42 [b]	2.83±0.33 [a]	0.83±0.09 [c]	1.83	2	15.813	0.0000 [***]
Length of the	CL/SL	2.04±0.1 [ab]	1.78±0.11 [b]	2.49±0.12 [a]	2.1	2	8.416	0.0005 [***]

cone/seed length								
Cone diameter /seed diameter	CD/SD	3.26±0.75 [a]	3.93±0.39 [a]	1.54±0.16 [b]	2.91	2	10.19	0.0001***
Cone aspect ratio/seed number	AS/NSC	0.24±0.002 [a]	0.21±0.0007 [b]	0.15±0.003 [c]	0.2		178.04	0.0000***

*Results were expressed as mean ± standard error. Within a row for the same measured parameters, means followed by the same letters are not significantly different ($p > 0.05$), according to the Tukey post-hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, not significant (NS) $p > 0.05$.*

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1010 **Table 3.** Microsatellites (SSR) and amplification parameters for molecular characterization of

1011 *Juniperus turbinata*

Locus name	Primer sequence 5'- 3'	Motif	Size (bp)	Tm (°C)	Fluorescent labeling
J005458 **(A1)**	F : GACCCTATGTAGCCATGCCC R : GCTTGCGGTTATGGAGAAGG	$(TATG)^{10}$	169-256	56	FAM
J006365 **(A2)**	F : TTAGATGCGCGTACAAATGC R : GGTTGCGTATGACTTTTGTTTGG	$(CA)^{13}$	182-216	58	HEX
J064797 **(A3)**	F : CGGTCATCTTAATTGTTTTAGTGCG R : TCATTCATGTAGTAACTTGGAAAACAC	$(TC)^{22}$	192-252	56	FAM
J127300 **(A4)**	F : TGCTAGTGTACCATTCTCCATC R : GAGCCATATTTGGTTGTTACTTGG	$(AC)^{17}$	192-242	58	HEX
J015521 **(A5)**	F : AGCCTCATTCACGAGGTCTG R : CACCTATGCAGAAAATCGAAAGC	$(CT)^{18}$	160-187	58	HEX
J084596 **(A6)**	F : GGGAGCTCTAAGCCAACATC R : AGGCTGACTTGTGGTCATAC	$(CT)^{21}$	228-277	58	FAM

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Table 4. Genetic diversity indices across 6 SSR loci for *Juniperus turbinata* populations.

Markers names	Na	Ne	I	Ho	He	F	PIC	HWE (Sig)
J005458 (A1)	11.67	5.955	2.035	0.366	0.827	0.561	0.89	***
J006365 (A2)	14.67	10.777	2.482	0.273	0.901	0.696	0.95	***
J064797 (A3)	17.33	10.733	2.511	0.503	0.877	0.423	0.94	***
J127300 (A4)	12.67	6.725	2.186	0.461	0.849	0.457	0.93	***
J015521 (A5)	10.00	6.773	1.997	0.654	0.829	0.218	0.90	***
J084596 (A6)	13.67	8.819	2.303	0.602	0.874	0.312	0.92	***
Means	13.335	8.297	2.252	0.476	0.859	0.444	0.921	

Na = Number of Alleles, Ne = Number of Effective Alleles, I = Shannon's Information Index, Ho = Observed Heterozygosity, He = Expected Heterozygosity, F= Fixation index, PIC: polymorphism information index, HWE= Hardy-Weinberg equilibrium test.

Table 5. Allelic patterns between *Juniperus turbinata* populations based on growing sites.

	Littoral site	Semi-continental site	Continental site
Na	13.000±1.291	10.167±0.792	16.833±1.851
Na Freq >=5%	8.500±1.057	7.500±0.224	7.500±0.719
Ne	8.296±1.259	5.837±0.528	10.757±1.567
I	2.262±0.135	1.982±0.075	2.513±0.150
Np	5.33±0.84	3±1.12	6.167±0.7
Number of locally common alleles (≤25%) and (≤50%)	0	0	0
Ho	0.467±0.074	0.458±0.040	0.505±0.094
He	0.865±0.019	0.821±0.016	0.892±0.022
uHe	0.887±0.020	0.843±0.017	0.904±0.022
F	0.456±0.092	0.439±0.051	0.438±0.099

Na = Number of Alleles, Ne = Number of Effective Alleles, I = Shannon's Information Index, Np= number of private alleles, Ho = Observed Heterozygosity, He = Expected Heterozygosity, uHe = Unbiased Expected Heterozygosity, F = Fixation Index. All values are presented as mean ± standard error.

Table 6. Analysis of molecular variance (AMOVA), fixation index and gene flow between *Juniperus turbinata* populations based on growing sites.

	Degree of freedom (df)	Sum squares (SS)	Mean square (MS)	Estimated variance (Est.Var.)	% of variation
Among Pops	2	27.595	13.798	0.203	7%
Among Indiv	74	288.859	3.904	1.228	43%
Within Indiv	77	111.5	1.4480	1.448	50%
Total	153	427.955		2.878	100%
Fixation index (FST)			0.07		
Gene flow (Nm)			3.302		