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Genetic diversity hotspot at the edge of the species range: integrating plastome phylogeography with ecological niche modelling for the conservation of *Ulmus glabra* Huds. in the Italian peninsula

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Abstract

Background. The Italian peninsula is a widely recognised genetic diversity hotspot and one of the main glacial refugia for European temperate trees, which are now increasingly threatened by climate change, anthropogenic pressure, and pathogens. This study, focusing on the wych elm (*Ulmus glabra*) in its Italian range, integrated plastome phylogeography with lineage-based ecological niche modelling (ENM) and protected area gap analysis, aiming at: (1) reconstructing the evolutionary history of the species and identifying main phylogenetic lineages; (2) assessing habitat suitability and evaluating the impacts of climate change on each lineage; (3) performing a spatially explicit conservation assessment, incorporating genetic and ecological information.

Results. Phylogeographic analyses of 75 trees revealed 42 haplotypes and a deep separation between Alpine (ALP) and Apennine (APE) lineages, with the latter showing higher nucleotide diversity and substructuring into two haplogroups, corresponding to north-central and south-central Apennines. The deep separation between ALP and APE was confirmed by significant N_{ST} and G_{ST} statistics ($N_{ST} > G_{ST}$, $p \leq 0.01$). Our findings suggest a multiple refugia scenario for the species in the Italian peninsula, with the Apennines supporting a ‘refugia-within-refugia’ model. Niche analysis highlighted significant ecological differentiation between ALP and APE (niche overlap $D = 0.18$). ENMs for the two lineages predicted a future decrease in habitat suitability, mainly in the arid regions of south-central Italy; however, while APE lineage was found to be well represented within protected areas, often coinciding with potential climatic refugia, most ALP populations are not protected.

Conclusions. The strong genetic and ecological divergence between *U. glabra* lineages underscores the need for lineage-specific conservation. Priority actions should include expanding *in situ* conservation in the Alps, establishing Genetic Conservation Units in the identified climate-refugia and enhancing connectivity in south-central Italy. As a complementary measure, *ex situ* conservation should maximize genetic diversity, preserving all identified haplogroups.

Keywords: chloroplast genome resequencing, ecological niche modelling, phylogeography, temperate trees, *Ulmus glabra*; conservation strategy

Background

Patterns of population genetic diversity provide insights into species migration driven by climate oscillations over time, particularly cycles of range reduction and expansion related to the survival in glacial refugia, followed by post-glacial recolonization [1-4]. Glacial refugia typically show greater genetic diversity due to long-term demographic stability, while post-glacial recolonization areas often exhibit reduced diversity due to founder effects [5-7]. However, contact zones of different recolonization routes can form admixture ‘melting pots’ [3]. For European temperate trees, three main glacial refugia were identified south to 45°N: the Iberian, Italian and Balkan peninsulas [8]. Nonetheless, recent evidence suggests a more complex scenario, with multiple micro-refugia within larger ‘safe areas’ (the so-called ‘refugia-within-refugia’ model), supported by patterns of endemism, genetic diversity and regional complexity [9-11]. As reservoirs of genetic and species diversity, past and future climate refugia are considered key conservation priorities [12-15]. Marginal and relict populations serve as sources of genetic variability and local adaptations, essential for maintaining evolutionary potential and long-term survival of species [*e.g.* 16-19]. Genetic diversity is indeed one of the three biodiversity levels prioritized by the IUCN [20]. Identification and management of Gene Conservation Units (GCUs) has been proposed as an effective *in-situ* strategy to protect genetic diversity and evolutionary potential [21]. Conservation biology also extensively relies on Ecological Niche Models (ENMs) to assess environmental suitability under current and future climates. Based on species’ ecological requirements derived from occurrence data and current environmental conditions (realised niche), ENMs allow to predict habitat suitability and potential distribution under future climate scenarios [22, 23]. Integrating phylogeographical information into ENMs enables investigation of intraspecific differential response to climate change, improving model accuracy and guiding targeted conservation [24].

In addition to the direct effects of climate change, temperate forest ecosystems are increasingly threatened by multiple additional stressors [25, 26]. One of the most severe and pervasive is anthropogenic pressure, which leads to the degradation, reduction and fragmentation of forest habitats [27, 28]. In turn, fragmented and isolated populations are more prone to genetic erosion, potentially resulting in a reduction of fitness and evolutionary potential, and to local extinction due to stochastic and demographic events [29-31]. Moreover, additional important threats include the spread of newly emerging pathogens and diseases [32], as well as the introduction of non-native invasive species (e.g., ornamental trees) and cultivated varieties [33, 34]. These organisms can compromise the survival of native species either by disturbing the ecological balance of the colonised environment through competition or regime shifts [35, 36], or by hybridizing with closely related native species, leading to genetic pollution [37-39]. Notably, the aforementioned threats often act synergistically, with complex feedback loops and non-linear interactions that can amplify their impacts and accelerate the decline of forest ecosystems [40].

Wych elm (*Ulmus glabra* Huds., Ulmaceae) is a temperate broadleaved tree species with the northernmost and widest natural range among European elms [41], spanning from Southern Italy (including Sicily island) to above the Arctic Circle (Beiarn, Norway), and extending westwards to Portugal and eastwards to the Urals [42-44]. In its southern range it is found only in mountainous areas: for this reason, it is also known as ‘mountain elm’ [44, 45]. In the last decades, the distribution of wych elm showed signals of an upward shift, probably due to increasing temperatures [46, 47]. Indeed, this species is adapted to cool and humid summers, and requires rich, moist and non-acidic soils [44, 48, 49]. It is typical of hemiboreal and temperate mixed deciduous forests, where it occurs with sporadic individuals especially along streams, often associated with other noble trees such as *Fraxinus* spp., *Acer* spp. and *Tilia* spp. [50]. It is a diagnostic species of the Alliance *Tilio-Acerion* Klika 1955 [51] and a typical species associated to a priority habitat of EU concern (Annex I Directive 92/43/EEC and Bern Convention), the priority habitat 9180* "*Tilio-Acerion* forests of slopes, screes and ravines". In Italy, wych elm grows in small and fragmented stands along the Alps and Apennines and, with relict populations, in Sicily [52].

Wych elm trees play an important economic and cultural role in rural landscapes, where they have been exploited for a variety of uses; in the past, the species was also indirectly affected by land-use changes and human management of the mixed forest stands in where it naturally grows [44]. Human pressure from logging and habitat disruption has therefore severely impacted wych elm populations for a long time. As a result, the species survives in small, fragmented populations, often exhibiting a high degree of differentiation and an elevated risk of genetic erosion, particularly in its southern range [53]. In addition, since the beginning of the last century an even more concerning threat emerged for European elm species: the spread of Dutch Elm Disease (DED). DED is a destructive tracheomycosis that decimated elm populations throughout Europe, North America, part of Asia and New Zealand. DED is caused by the invasive pathogenic fungi *Ophiostoma ulmi* Nannf. and the more aggressive *Ophiostoma novo-ulmi* Brasier, introduced in Europe in 1910s and 1970s, respectively, and responsible for two successive epidemic waves [54-56]. Despite being highly susceptible to DED, to date *U. glabra* has been preserved at the northernmost latitudes and highest elevations, where insect vectors (bark beetles of genus *Scolytus*; fam. Curculionidae) cannot easily survive [57, 58]; however, rising temperatures may soon alter this balance. As an effort to control the spread of DED, Europe and North America have begun introducing resistant elms, including non-native species and artificially produced hybrids. However, this has given rise to a more subtle threat: hybridization and introgression between exotic and native elm species, potentially negatively affecting the fitness of the latter due to genetic pollution. The phenomenon has already been documented in natural stands for *U. pumila*, native from Siberia and northern China, with *U. rubra* in North America and with *U. minor* in Europe; so far it is not reported in nature for *U. glabra*, but the species can hybridize under controlled conditions [35, 59]. For all the above mentioned reasons, *U. glabra* is currently listed as "Vulnerable" in Europe on the IUCN Red List of Threatened Species [59], with past population declines of 10–40% and projected future declines exceeding 30% across its European range this century.

Despite the potential contribution of population genetics and phylogeography to wych elm conservation, little is still known about the genetic diversity within and among European populations of the species. Goodall-Copestake et al. [60] found no clear evidence for regional geographic structure in *U. glabra* from different European provenances, but their results were based on a limited set of genetic markers (5 RAPD and 3 ISSR), as the primary goal of their study was the taxonomic assignment of *Ulmus* spp. trees held in *ex-situ* collections, and not the investigation of within-species genetic diversity. Conversely, a detailed study of relict wych elm populations on the Spanish Central System showed low intra-population genetic diversity (probably due to small population size), signals of past and recent bottleneck events and high inter-population genetic differentiation [53]. Finally, the Balkan peninsula has been identified as the main glacial refugium for the congeneric *U. laevis* Pall., a species with similar ecological requirements, but more tolerant to aridity and growing exclusively in lowlands [61]. However, the relict populations of *U. laevis* found in Iberia and Italy, characterised by unique haplotypes, indicate that these areas may also have played an important role in the survival of this species [61, 62], and possibly for other elms.

Within this context, investigating the genetic diversity and differentiation of wych elm populations at the southern edge of its range, along with assessing current and future habitat suitability, becomes urgent both to illuminate the species' evolutionary dynamics and to inform conservation strategies. In this study, focusing on *U. glabra* in the Italian Peninsula – a putative glacial refugium for temperate tree species – we aimed to: (1) investigate plastome diversity to reconstruct the evolutionary history of the species and identify the main phylogenetic lineages; (2) assess habitat suitability and evaluate the impacts of climate change on each lineage, by projecting lineage-specific Ecological Niche Models (ENMs) under future climate scenarios; and (3) perform a spatially explicit conservation assessment, by integrating species occurrence data, genetic information, and ENM results in the context of the national network of protected areas. Based on the integration of the different findings, we developed long-term conservation strategies for *U. glabra* within its Italian range, aimed at preserving the species' evolutionary potential and ensuring the long-term viability of its populations in the face of multiple current and future threats.

Methods

1. Sampling, sequencing and chloroplast genome assembly

A total of 78 leaf specimens of *U. glabra* were included in the analyses, of which 75 were collected along the Italian peninsula and 3 were included as external comparisons (2 from France and 1 from Bosnia-Herzegovina; sampling localities are shown in Fig. 1 and details are reported in Table S1, Additional file 1). Leaves were dried in silica gel and stored at -20°C until DNA extraction. Tissues were pulverised using liquid N_2 and genomic DNA was extracted from ~25 mg dry weight using the Invisorb Spin Plant Mini Kit (Invitek Molecular, Berlin, DE). DNA concentration was measured by using Qubit 2.0 Fluorometer (Thermo

Fisher, Waltham, US-MA), while integrity was evaluated by electrophoresis in 1% agarose gel. Genomic DNA was sequenced with 2×150 cycle on the Illumina Novaseq platform at Novogene (Cambridge, United Kingdom) and Genewiz (Leipzig, Germany) in different sequencing rounds. Sequencing reads were deposited in GenBank (BioProject ID: PRJNA1067409). All voucher specimens were identified by Dr. Alberto Santini and deposited at the herbarium of the Institute for Sustainable Plant Protection of the National Research Council of Italy (IPSP, CNR). The voucher ID numbers are: IPSP-UG01 to IPSP-UG78.

De novo assembly of cp genome was performed for each sample by using the NOVOPlasty and GetOrganelle toolkits [64, 65], with default parameters and using the sequence of the plastidial gene *mat*K as seed and the NCBI complete plastidial sequence of *U. glabra* MT165931 as reference [66]. When necessary, the genome assembly was completed by manually aligning the contigs to the reference. Assembled sequences were aligned using the MAFFT online service v.7 (available at https://mafft.cbrc.jp/alignment/server/[67, 68]) and trimmed with ClipKIT, setting the mode argument *kpic-gappy* to retain parsimony-informative and constant sites, while removing sites exceeding the default gappyness threshold of 0.9 [69]. All the subsequent analyses were performed on this ClipKIT output.

2. Phylogeographic and genetic diversity analysis

A maximum-likelihood phylogenetic tree (substitution model K80) of *U. glabra* specimens was built using the function *pml_bb* implemented in the R package *phangorn* v.2.11.1 [70], including an *U. glabra* sequence available in GenBank (accession MT165931) for comparison and *U. pumila* (GenBank: MW279236.1) as an outgroup. The K80 model was chosen as it accounts for differing rates of transitions and transversions, a relevant factor in chloroplast genomes, while maintaining model simplicity appropriate for intra-specific comparisons. Chloroplast haplotypes were inferred following the workflow by Toparslan et al. [71], implemented in the R package *pegas* [72-74]. Haplotypes distribution was mapped with the R package *leafletR* [75], grouping samples according to administrative regions (see Table S1, Additional file 1). The phylogenetic relationships among haplotypes were estimated and visualised through a parsimony network (*haploNet* function in *pegas*), and a neighbor-joining tree was built according to Saitou and Nei [76]. A Bayesian hierarchical clustering of plastidial sequences was implemented with *fastbaps* [77], with partition inferred under the algorithm by Heller and Ghahramani [78]. Finally, a principal component analysis (PCA) was performed using the *adegenet* R package (v2.1.8) [79, 80].

Standard genetic diversity estimates were performed on the entire dataset, on samples from each administrative region and on samples from each main phylogenetic group (indicated as ‘regions’ and ‘groups’, respectively, as reported in Table S1, Additional file 1). The main three groups (*i.e.*, S_AP, N_AP and ALPS) were defined based on the results of the previous phylogenetic analyses and PCA; sample UL1 from Bosnia-Herzegovina was excluded from diversity estimates for being the only Balkan representative.

Nucleotide diversity (π), haplotype diversity (H_d), average gene diversity (within regions and groups) corrected for sample size (H_s) [81] and total gene diversity (H_t) were calculated with the R packages *pegas* and *adegenet* [72-74, 79, 80]. Distribution of pairwise genetic distances among *U. glabra* accessions in the main three sampling area (*i.e.* the groups ALPS, N_AP and S_AP) was visualised as violin plot by using the R function *pairDistPlot* implemented by the package *adegenet* [79, 80].

To test for phylogeographic structure, G_{ST} and N_{ST} statistics were calculated according to Pons and Petit [82], as implemented in PermutCpSSR v2, with 1000 permutations to test statistical significance and considering the three above-mentioned haplogroups. Finally, analysis of molecular variance (AMOVA) was performed to test for population differentiation, with the R package *poppr* [83], again setting regions and groups as reported in

Table S1, Additional file 1. To test for significance, a randomisation test was performed using the function *randtest* from the *ade4* package [84].

Possible demographic changes, both considering the whole dataset and at haplogroup level were investigated through the calculation of Tajima's D and Fu's Fs (neutrality tests) and sum of square deviations (SSD) and Harpending's raggedness index (H_{Rag}) (mismatch parameters) using WinArl35 v.3.5.2.2 [85]. Moreover, plots of mismatch distribution were produced using DNAsp v.6.12 [86]. Typically, significantly negative results in neutrality tests, as well as non-significant mismatch parameters, indicate potential historical demographic expansions. Conversely, non-significant Tajima's D and Fu's Fs, together with significant SSD and H_{Rag} and a multimodal mismatch distribution indicate that populations evolved following a neutral model [87 and references therein].

3. Ecological niche models

We based our ENMs for *U. glabra* on the Grinnellian niche concept [88], assuming that the broad-scale distribution of a species over space and time is primarily determined by environmental variables [89, 90]. Based on the outcome of the phylogeographic analysis (Results, section 2), separate ENMs were performed for the two main *U. glabra* intra-specific branches (hereafter, ALP and APE) which, based on sampling distribution, correspond to the Alpine and Apennine ranges, respectively. In order to define the spatial extent of the environmental layers used for each lineage-based models (ALP and APE) and, consequently, to delimit the corresponding subsets of occurrence records, we adopted a combined approach integrating genetic, geographic, and bioclimatic information. First, we relied on the results of the phylogeographic analysis (see Results, section 2), assigning each occurrence record to the geographically closest population with available genetic data. Due to the clear geographic correspondence of the observed phylogeographic structure between the two main lineages, this procedure did not result in any ambiguity, except for an intermediate zone located between the southern Alps and the northern Apennines, where no genetic data were available. In this case, we adopted Colle di Cadibona (Liguria region) as the boundary between the ALP and APE ranges. This boundary not only represents the conventional geographic limit between the two mountain chains, but also has ecological and bioclimatic significance. Indeed, in the ecoregion maps of Italy [91], which were developed by integrating climatic, physiographic, and biogeographic data and specifically designed as a framework for ecological modelling and biodiversity conservation, Colle di Cadibona is considered the southern limit of the Alpine eco-province (see Fig. 1b therein). Regarding the presence points used in the ENMs, only one observational record (point 4 in Table S2), not associated with genetic data, was located in a potentially intermediate area. This record corresponds to Monte Moria (Piacenza Apennines), which lies approximately 120 km distant from the adopted Alps/Apennine boundary and is geographically part of the northern Apennine chain. Therefore, this point was included in the APE subset.

Occurrence points of *U. glabra* were used to model the ecological niche of the species with regard to bioclimatic and pedological conditions, and predict changes in habitat suitability in the next decades based on climate projections. The occurrence datasets for ALP and APE (Table S2, Additional file 1) were build combining: (i) the sampling sites of the 75 trees included in our genetic analysis; (ii) additional A. Santini personal observations deriving from field monitoring; and (iii) the available Italian records for the species, retrieved from Mauri et al. [92](EU-Forest dataset) and Caudullo et al. [63] chorological maps. From the latter dataset, four isolated points in urban parks and rural areas within the Po Plain (thus, outside the native range of the species), most likely corresponding to ornamental trees, were discarded. Occurrence records were filtered at the resolution of current bioclimatic layers (30 arc-sec, see below) using the ‘Extract by locations’ tool in QGIS v.3.34.7 [93], and calculating the new coordinates as centroids of the selected cells. A total of 121 points were used as input for the models (70 for ALP and 51 for APE).

A set of bioclimatic and edaphic variables were selected as environmental predictors of the species distribution, according to specific literature and previous ecological studies on the species [49, 94]: bio1 (annual mean temperature), bio4 (temperature seasonality), bio5 (maximum temperature of the warmest month), bio6 (minimum temperature of the coldest month), bio7 (temperature annual range), bio10 (mean temperature of the warmest quarter), bio11 (mean temperature of the coldest quarter), bio12 (annual precipitation), bio14 (precipitation of the driest month), bio15 (precipitation seasonality), bio18 (precipitation of the warmest quarter), bio19 (precipitation of the coldest quarter), continentality index, moisture index, growing degree days above 5°C (gdd5), and soil pH and organic carbon content in the first 15 cm of thickness. All the bioclimatic layers from the historical period 1970-2000 (hereafter ‘current’) at a resolution of 30 arc-sec were downloaded from the WorldClim v.2.1 online repository (https://www.worldclim.org/data/index.html, accessed in May 2024) [95]. Continentality index, moisture index and gdd5 were calculated using the *envirem* R package [96]. Raster layers of soil organic carbon content (g/kg) and pH at a resolution of 250 m were obtained from the SoilGrids database (available at https://soilgrids.org/ [97]). All the raster layers were clipped at the extension of the two Italian subranges (ALP and APE), by means of the ‘Extract by mask’ tool in QGIS and rescaled at 30 arc-sec resolution. To avoid dependency among predictors and prevent overfitting, all the selected variables were tested for collinearity using Pearson’s correlation tests, and for multicollinearity based on variance inflation factors (VIFs), using the *vifstep* function of the R package *usdm* [98, 99]. Pearson’s coefficient $|r| = 0.85$ and VIF = 10 were considered as critical thresholds [99, 100]; when two variables were highly correlated, only one of them was retained, based on its assumed ecological relevance for the species. Eleven variables were excluded from further analyses as a result of correlation (≥ 0.85) and multicollinearity (VIF ≥ 10), and final models were built with the following list of variables: bio1, bio4, bio15, bio18, bio19 and soil pH.

Under future climate change, populations from the Apennines could potentially shift their range northward, tracking favourable climatic conditions into the southern Alps, provided that suitable habitats are available. To test this hypothesis, we also ran ENMs for the APE lineage under both current and future climatic scenarios, using presence points for the APE lineage but bioclimatic variables covering the entire Italian Peninsula (i.e., without clipping the rasters to the extent of APE lineage's assumed distribution; for more details, see Supplementary Information 3 in Additional file 2).

In order to compare the niches of the two *U. glabra* intraspecific lineages and test for ecological differentiation, we assessed the niche overlap, niche similarity, and niche equivalence using the environmental principal component analysis method (PCA-env; [101]; see Supplementary Information 1, Additional file 2 for details), as implemented in the R package *ecospat* v. 4.1.0 [102].

ENMs for current and future scenarios, for both ALP and APE, were built using MaxEnt v.3.4.4 (available at: http://biodiversityinformatics.amnh.org/open_source/maxent/; [103, 104]. Models were built by setting product, threshold and hinge equal to false in order to avoid overparametrisation [105] and performing 10 replicates run for cross-validation. Habitat suitability maps were generated using a logistic link function, to yield a suitability value between 0 and 1 [103]. Model performance was evaluated using the Area Under the Curve (AUC) [103]; when AUC > 0.8, the model's prediction is considered to be good, while models with AUC > 0.9 are considered excellent predictors [106, 107]. The relative contribution of each environmental variable in current ENMs was evaluated by their permutation importance in the model and jackknife test [104].

4. Future habitat suitability variation

For future climate projections, two emission scenarios (i.e., Shared Socioeconomic Pathways, SSP2-4.5 and SSP5-8.5) were considered to predict habitat suitability for wych elm for two timeframes (2041-2060 and 2061-2080). SSPs describe both socioeconomic development pathways and associated greenhouse gas emissions, and are used in the CMIP6 framework to project climate change. SSP2-4.5 represents an intermediate scenario with greenhouse gas concentrations stabilizing around 4.5 W/m² by 2100, leading to a projected global mean surface air temperature increase of approximately 1.8 °C. SSP5-8.5 represents a high-emission scenario with continuously increasing greenhouse gas concentrations, resulting in a projected global mean surface air temperature increase of approximately 3.7 °C. For each timeframe and each SSP, three CMIP6 global circulation models (GCMs) were considered for MaxEnt modelling: CMCC-ESM2 [108], MIROC6 [109], and MPI-ESM1-2-HR [101]. Ensemble models were calculated as the weighted average of the three GCM output models, based on the AUC of each. To evaluate differences among future scenarios, we performed pairwise correlation tests between the habitat suitability maps generated for the ALP and APE lineages, for the different timeframes and climate scenarios. Significance of correlations was tested using the modified t test developed by Dutilleul et al. [111] to control for potential effects of spatial autocorrelation (R package: SpatialPack; [112]).

Based on the rasters resulting from current and future ENMs, spatial maps for the future habitat suitability variation (Δ = future - current suitability) for *U. glabra* in ALP and APE were generated by using R (*raster* and *dplyr* packages; [113, 114] and reclassifying each pixel into three classes: decrease ($\Delta < -0.05$), stable ($-0.05 \leq \Delta \leq +0.05$), increase ($\Delta > +0.05$). In addition, to gain a more ecological meaningful interpretation of predicted future variations, the elevation of pixels showing increase or decrease in habitat suitability was extracted from Copernicus digital elevation model (DEM) for Europe at 30 arc-sec [115]. Moreover, to gain a more ecologically meaningful interpretation of suitability variations, we calculated future changes in the overall extent of suitable areas for five suitability classes (very low, low, medium, high and very high) separately, defined by dividing the predicted habitat suitability range for current models into five equal intervals. The area falling in each class (in pixels) was calculated for each raster, as well as the % variation between each future scenario and the current one, according to the formula: variation (%) = (Δ/(current area)) x 100.

Lastly, current habitat suitability score and the variation in each future scenario (*Δ*) were calculated at the local scale for each observation and sampling site, in order to highlight possible habitat loss for specific *U. glabra* haplogroups.

5. Spatially explicit conservation assessment

To assess the representation of *U. glabra* populations within Italy's conservation network, we integrated georeferenced genetic and observational data with protected areas (Natura 2000 and Natural Parks). We evaluated spatial overlap by identifying species occurrences and haplotypes falling inside or outside protected areas. Haplotypes were classified as "protected" if found at least once within a protected area; observational records were assigned to lineages and haplogroups based on geography, following the results of our phylogeographic analysis. We also conducted a two-tier proximity analysis; Level 1: for all records outside protected areas, we calculated the minimum distance to the nearest protected site; Level 2: for observational records only, we computed the distance to the nearest genetic sampling site. These analyses helped identify conservation-relevant sites near protected areas and highlight spatial gaps in the genetic dataset.

Lastly, we assessed how habitat suitability for the species aligns with protection status across all georeferenced *U. glabra* sites (genetic and observational), using current suitability values and future variations predicted by lineage-based ENMs.

By combining the three current suitability categories (Low, < 0.3; Medium, 0.3–0.6, and High, > 0.6) with the three future change categories (decreasing, stable, increasing), we established nine composite categorical variables, each corresponding to a distinct dynamic suitability profile (e.g., 'High and Decreasing', 'Medium and Stable', etc.). These were compared across protection status groups (protected vs unprotected).

All spatial analyses were performed in QGIS v.3.34; statistical analysis of the derived data were performed in R [74]; further methodological details and rationale are reported in Supplementary Information (Additional file 6).

Results

1. Sequencing and cp genome assembly

Specimens sequencing generated ~150 Gb of raw data with a total of ~1.4 billion reads. The number of reads per sample ranged from ~8 million to ~51 million (mean number of reads ~18 million). Complete cp genome assembly was obtained for all 78 *U. glabra* samples, with genome size ranging from 159,040 to 159,578 nucleotides. Mean cpDNA sequencing depth was 681×, with at least 91.3% of bases with quality score ≥ 30 in all the samples (Table S1, Additional file 1).

2. Phylogeography and genetic diversity of wych elm in the Italian peninsula

After clipKIT trimming, a total of 221 polymorphic sites were detected, which led to the identification of 44 different haplotypes. Most haplotypes were found only in one or two samples; haplotype H3, found in nine individuals (eight in Piedmont and one in Valle d'Aosta regions), was the most represented, probably due to the extensive sampling effort in this area (Table S1, Additional file 1; Fig. 2). The maximum likelihood phylogenetic tree showed two distant lineages: the first one, quite homogeneous, comprising all the samples from Northern Italy (sampled across the Alps chain) and France, while the second one was more branched. In fact, three additional groups were clustered: one included samples from the North-central Apennines (*i.e.*, Tuscan-Emilian Apennines); another, more heterogeneous, comprised all the samples from the South-central Apennines (from Marche to Basilicata regions); finally, sample UL1 from Bosnia-Herzegovina formed a separate branch of the tree (Fig. 3). The haplotype network highlighted the presence of three main Italian haplogroups defined on a geographic basis, in accordance with the phylogenetic tree (Fig. 4A). In particular, samples from the Alps formed a distinct cluster together with samples from France (for a total of 20 haplotypes), which were closely related to samples from Trentino-Alto Adige (Central-eastern Alps). Samples from the Apennines clustered in two main groups: one corresponding to West-central Apennines (Tuscany and Emilia-Romagna, comprising 8 haplotypes) and the other to East-central and South-central Apennines (15 haplotypes), where samples from Abruzzo and Campania (South-central Apennines) showed a high intra-regional variability (Table S1, Additional file 1; Fig 4A). Sample UL1 from Bosnia-Herzegovina formed a distinct separate edge of the haplotype network. Overall, a higher genetic variability was highlighted for *U. glabra* across the Apennines (in particular the South-central Apennines), compared to the Alpine chain, at least in terms of haplogroups, although the spatial coverage of the sampling was not homogeneous. The analysis of the phylogenetic relationships among haplotypes revealed again the presence of four phylogroups, in accordance with previous analyses (Fig. S1, Additional file 3). Results of the fastbaps clustering were in accordance with previous analyses, although samples from the South-central Apennines were split and assigned to three different clusters (Fig. S2, Additional file 3), revealing hierarchical substructure in this geographic region. Finally, the PCA confirmed the presence of four main genetic groups defined on a geographic basis, with PC1 and PC2 explaining the 62.2% and the 22.4 % of the total variance, respectively (Fig. 4B).

Nucleotide diversity (π) at haplogroup level ranged from 0.22×10^{-4} for the Alps to 0.91×10^{-4} for the Southern Apennines (Table 1), while overall $\pi = 2.71 \times 10^{-4}$. Within groups the highest level of nucleotide diversity was observed in the samples from the Abruzzo region ($\pi = 1.27 \times 10^{-4}$). Nucleotide diversity was lower across the Alps and higher in the Southern Apennines. Haplotype diversity was lower in the Abruzzo region (ABR, $H_d = 0.6$) while the highest value was found in the Valle d'Aosta and Emilia-Romagna regions (VDA and EMR, respectively; $H_d = 1$). At haplogroups level, haplotype diversity was lower in the Northern Apennines and higher in the Southern Apennines ($H_d = 0.88$ and 0.95, respectively), while overall $H_d = 0.97$ (Table 1). Gene diversity at regional level was comprised between 0.032 (France, FR) and 0.146 (Emilia-Romagna, EMR); at haplogroup level was lower across the Alps (Hs = 0.04) and higher in the Southern Apennines (Hs = 0.13); total Hs = 0.33 (Table 1). Mean pairwise genetic distances among individuals were considerably higher in the Southern Apennines (0.098), followed by the Northern Apennines (0.023) and the Alps (0.018), as highlighted in Fig. S3, Additional file 3.

The difference between N_{ST} and G_{ST}, with N_{ST} significantly higher than G_{ST}, revealed the existence of a strong phylogeographic pattern along the Italian peninsula ($G_{ST} = 0.124$, $N_{ST} = 0.367$; $p \leq 0.01$). The AMOVA partitioning revealed a moderate but significant genetic structuring, with 15% of the variation occurring among groups, 14% among populations within groups, and 71% within populations (Table 2). Variance within populations was significantly higher than expected. The resulting F-statistics for the overall sample were: $F_{ST} = 0.293$, $F_{SC} = 0.163$ and $F_{CT} = 0.151$.

Both neutrality tests (Tajima's D and Fu's Fs) were not significant, both when all the samples were considered together and when they were divided by haplogroup (Table 3). Consistently, both mismatch tests (SSD and H_{Rag}) were significant (with the only exception of

H_{Rag} for N_AP, Table 3) and mismatch distribution plots showed a multimodal and ragged profile (Fig. S4, Additional file 3), indicating no demographic expansions.

3. Ecological niche models

Niche overlap between the two *U. glabra* lineages (ALP and APE) was found to be low (Schoener's D = 0.1867; Fig. 5A). Niche equivalency test showed that niche overlap between the two lineages is significantly smaller than the null distribution (p = 1), indicating that ALP and APE lineages do not occupy equivalent niches. Consistently, niche similarity tests resulted in non-significant p-values ($p > 0.05$), confirming that the two niches are not more similar than expected by chance (see Supplementary information, Additional file 2).

MaxEnt models of current habitat suitability showed generally good predictive performance, for both the ALP and APE lineages (AUC = 0.837 and 0.821, respectively, Fig. 5; see also Supplementary Information 2, Additional file 2). Habitat suitability maps were generally consistent with the current geographic distribution of the species (as deduced by the species occurrence data; Fig. 5A). For the ALP lineage, the areas resulting in medium to high suitability values corresponded to the southern range of the Alpine chain, from which they branch off towards medium and low elevation areas of the internal valleys. For APE, they matched the relief of the Apennine chain and surrounding heights, with the largest highly suitable areas that can be identified in the northern part of the Apennines, in the central area of the peninsula in the correspondence to the Gran Sasso and Maiella massifs, and in the South-west (mountain regions of Calabria). However, several presence points resulted to be located in pixels with medium or low predicted suitability (*i.e.* suboptimal conditions). Indeed, current habitat suitability values for pixels corresponding to the observational records varied largely: for the ALP subset, suitability scores ranged from 0.186 to 0.790 (mean: 0.549 ± 0.15), with 54/79 sites (68%) falling within the range 0.40-0.70 (medium suitability); for the APE subset suitability scores showed a higher variability, ranging from 0.072 to 0.868 (mean

0.533 ± 0.22). Only 11/80 presence points (13.75%) fell within the “high” suitability class in the ALP subset, while 18/73 in the APE subset (24.66%) (Table S4, Additional file 4).

4. Future habitat suitability variation

Pearson’s correlation coefficients between the habitat suitability maps generated for the different timeframes (2041-2060 and 2061-2080) and future climate scenarios were all higher than 0.999 for the ALP models and between 0.991 and 0.999 for APE; all the correlations were highly significant after Dutilleul's correction for spatial autocorrelation ($p < 1\times10^{-5}$). Therefore, we focused on models SSP5 8.5 2061-2080 (further in time and worst-case emission scenario) for further analyses.

Analysis of pixels with significant future decrease ($\Delta < -0.05$) and increase ($\Delta > +0.05$) of habitat suitability for the ALP lineage showed a generally stable or slightly increasing ($\Delta < 0.1$) predicted suitability, with the increments being mainly concentrated in the Central-eastern Alps (Fig. 5B; Table S4, Additional file 4). In this area, an apparent northern expansion of the species was predicted, which also corresponded to an altitudinal shift: indeed, the majority of pixels characterised by a predicted increase in suitability were in the range 1,000-2,000 m above sea level (a.s.l.) (with a peak between 1,600 and 1,800 m a.s.l.), most of them lying between 45°N and 47°N (Fig. S7A, Additional file 3). The only areas with an increase in habitat suitability higher than 0.1 were concentrated in the coastal region of South-western Alps: in this area *U. glabra* is predicted to benefit the most from future climate change (Fig. 5B). On the other hand, no area is expected to experience a significant reduction ($\Delta < -0.05$) in future habitat suitability as inferred by our model, except for the internal portion of South-western Alps, but the predicted variation was always < -0.1.

For APE lineage, the most relevant and continuous areas showing significant increments of habitat suitability in the future were: (a) a narrow range spanning from North-west to south-east, corresponding to the internal part of North-central Apennines, extending towards south approximately until 42°N; (b) in the southern part, the Tyrrhenian coastal strip from the Cilento mountains (Campania) to the coastal mountain range of Calabria (Serre and Aspromonte mountains; Fig. 5B). The remaining areas below 42°N were entirely characterised by stable or decreasing habitat suitability, with the most extensive decreases in the eastern part of Southern Apennines and in the Gargano promontory (Puglia), in Monti Simbruini and Monti Lepini (Lazio) and, to a minor extent, in the Apuan Alps (Northern Tuscany) and Subapennines relieves of southern Tuscany. Considering altitudinal trends, no general pattern emerged for the Apennines, but a high fraction of pixels showing a future increase in suitability were located below 1,000 m a.s.l. (Fig. S7B, Additional file 3). It should also be noticed that the majority of pixels with a predicted suitability increase is located above 1,600 m a.s.l. and corresponds to south-central latitudes (below 42°N); in this part of the Italian peninsula, however, also lower elevation sites showed an increasing suitability, on the southern Tyrrhenian side. When models were built using presence points for the APE lineage but bioclimatic variables covering the entire Italian Peninsula, very similar results were obtained (for details, see Supplementary Information 3 in Additional file 2). These models identified the south-western Alps as a potentially suitable area for the APE lineage in Northern Italy under current climatic conditions (habitat suitability > 0.4; Fig. S6a in Additional file 2). On the other hand, future projections indicate in this area extensive clusters of pixels with decreasing habitat suitability for the APE lineage, almost perfectly matching areas that currently show medium to high suitability (Fig. S6b). Consequently, the only areas in the Alps that are moderately suitable for the APE lineage are predicted to become unsuitable in the future. However, this result should be treated with caution and taken as a general indication due to the methodological limitations described in Supplementary Information 3.

When analysing future variation in the different habitat suitability classes separately, for the ALP lineage, all future scenarios showed a decrease in the percentage of area falling into both the ‘very low’ and ‘high’ suitability classes, and a corresponding slight increase in the intermediate suitability classes (Table S5, Additional file 1). In particular, in the worst-case scenario (SSP5 8.5 2061-2080) highly suitable areas showed an overall variation of -26% compared to the current scenario. Conversely, for the APE lineage, the overall habitat suitability was predicted to be relatively stable in all classes, with less pronounced fluctuations, between +0.1% for the “high suitability” class and -3.7% for the “low suitability class” in the SSP5 8.5 2061-2080 scenario.

Lastly, considering wych elm presence points, the predicted habitat suitability was overall stable in future scenarios for the ALP subset (average variation: -0.002, for SSP5 8.5 2061-2080), but all the 11 observations falling into the "high suitability" class (all located in Piedmont) resulted in slightly decreasing future suitability (maximum variation: -0.060), except for two with stable value (+0.001); all future increases > 0.05 corresponded to current suitability values < 0.3 (low suitability), having therefore low ecological meaning. For the presence points in the APE subset, a similar situation emerged, with an overall average future variation of +0.001, but with 12/18 points in the "high suitability" class which resulted in a future slight decrease in habitat suitability (maximum variation: -0.022).

5. Spatially explicit conservation assessment

Among sites with genetic data, 60% (45/75) fall within protected areas, but protection levels differ sharply between lineages: only 20% of ALP sites (7/35) are protected, compared to 95% of APE sites (38/40). At the haplotype level, 69% (29/42) of Italian haplotypes are represented within protected areas, including 68.4% of ALP and 100% of APE haplotypes. For observational records without genetic data, the proportion of protected sites is lower (33.3%) but follows the same pattern: higher for APE (54.8%) than for ALP (15.6%).

Level 1 of proximity analysis revealed that nearly all unprotected sites lie close to protected areas: 100% of genetic-data sites and 96.1% of observational records are within 10 km, with only two ALP sites located 10–20 km away. Level 2 of proximity analysis showed that most observational records (83.3%) are located over 10 km from the nearest genetic sampling site, with 52.6% over 50 km away – a pattern consistent across both lineages. Presence sites within protected areas showed higher average current habitat suitability (0.60 ± 0.18) than those outside (0.51 ± 0.18). However, lineage-level analyses revealed contrasting patterns. In ALP, suitability values were similar between protected and unprotected sites. In APE, protected sites had significantly higher suitability (0.63 vs. 0.34; Wilcoxon test, $p < 0.001$).

Analysis of combined trends in current suitability and future variation also highlighted contrasting patterns among lineages in relation to conservation status. Considering potential climatic refugia, defined as sites with medium to high current suitability and a favourable trajectory (*i.e.*, stable or increasing) under climate change, they represent a comparable fraction of the total presence sites in the two lineages (ALP = 81%, APE = 83%), but their distribution with respect to protection status differs markedly. Indeed, while in ALP most of them are located in unprotected areas (accounting for 71% of total sites), in APE, the majority are found within protected areas (73% of total sites). A more detailed description of all the results of spatially explicit conservation assessment is reported in Supplementary Information (Additional_file_6); raw results of the overlap and proximity analyses between *U. glabra* occurrences and protected area network are reported in Table S5. For a synoptic table with the conservation priorities and corresponding foundational analyses for informing *U. glabra* conservation strategy, see Table 4.

Discussion

The Italian Peninsula has long been recognised as a glacial refugium for temperate tree species [116]. More recently, attention has turned to the evolutionary value of peripheral refugia, where populations persist under suboptimal or variable conditions and may harbour unique genetic variants [117]. Understanding the spatial patterns of genetic diversity, ecological differentiation, and habitat suitability dynamics across southern Europe is thus essential to guide conservation, especially for long-lived forest species such as the wych elm (*U. glabra*).

1. *Plastome-based phylogeography of* Ulmus glabra *in the Italian peninsula*

The high cp haplotype diversity observed in Italian *U. glabra* populations indicates long-term demographic stability that enabled the accumulation and maintenance of genetic variation, likely reflecting the role of the Italian Peninsula as a glacial refugium [3, 118, 119]. Phylogenetic analyses revealed two major lineages – Alpine (ALP) and Apennine (APE) – with further sub-structuring in the Apennines into North-central and South-central haplogroups. Although population differentiation was moderate overall, the AMOVA and F-statistics supported a clear phylogeographic split between the Alpine and Apennine regions. This significant genetic differentiation between Alps and Apennines mirrors patterns observed in other temperate broadleaved trees, such as *Fagus sylvatica* [120], and the deep divergence between the two may even predate the Pleistocene, as proposed for other species in Italy [121], though molecular dating would be needed to confirm this for *U. glabra*.

Overall haplotype diversity (Hd = 0.945) was comparable to that of *U. laevis* [61], despite the latter analysis spanning a broader European area, but nucleotide diversity was considerably higher (overall $\pi = 2.71\times10^{-4}$, haplogroup-based $\pi = 0.22–0.91\text{x}10^{-4}$; *U. laevis*: $\pi \approx 1\times10^{-5}$), indicating deeper divergence among haplogroups. In contrast, overall gene diversity was lower in *U. glabra* (0.326 vs. 0.960). Similar patterns were observed in France and the Czech Republic, where *U. glabra* showed greater diversity than *U. minor* despite both suffering DED-related bottlenecks [49, 122, 123]. These differences may be linked to reproductive strategies: *U. glabra* primarily reproduces sexually and rarely produces root suckers, while other elms such as *U. minor* and *U. laevis*, are more prone to vegetative reproduction [124, 125].

While haplotype diversity was similar in the three main haplogroups (ALPS = 0.92; N_AP = 0.88; S_AP = 0.94), nucleotide and gene diversity showed variation across regions, with particularly high values in the Southern Apennines. Indeed, the southernmost haplogroup was the most diverse, as confirmed by FastBAPS clustering and pairwise genetic distances, with evidence for geographic sub-structure. The observed genetic patterns support a ‘refugia within refugia’ model for the species in the Italian peninsula, particularly in the South-central Apennines, shaped by Pliocene-Pleistocene climatic oscillations and sea level changes [126]. In contrast, the lower diversity in the Alps may reflect survival during cold stages in less fragmented peripheral refugia.

Two French samples sharing haplotypes with the Italian Alps suggest possible post-glacial recolonization of northeastern France from southern Alpine refugia – though more extensive data are needed to confirm this. Finally, the sample from Bosnia-Herzegovina showed deep divergence from Italian haplogroups, suggesting a marked differentiation of Balkan populations, though this interpretation remains tentative, being based on a single sample.

2. *Niche differentiation, lineage-based ENMs and future scenarios*

Niche overlap and similarity analyses revealed significant ecological differentiation between ALP and APE lineages, supporting separate ENMs and suggesting potential eco-evolutionary divergence. The observed deep phylogenetic split suggests a long time span for local adaptation, and future genomic analyses targeting the nuclear genome may help clarify whether adaptive evolution has occurred.

Despite good AUC values (ALP = 0.837, APE = 0.821) and general agreement with the species’ known distributional range, MaxEnt models classified several sites of presence as low-to-medium suitability. Similar results were reported for the species by Zimmermann et al.

[127] in Switzerland (and with a similar model AUC, 0.88). This outcome may suggest that model performance may vary across environmental contexts. However, species occurrence in areas predicted as poorly suitable does not necessarily indicate low model accuracy: populations may persist under suboptimal conditions due to historical legacies or ongoing processes [128]. These findings may also reflect, at least in part, the known limitations of ENMs relying on presence-only and bioclimatic data, which may miss key factors such as substrate availability and biotic interactions, while the coarse resolution of environmental data can obscure local microclimatic variation – issues common in plant distribution modelling [129, 130]. Among the biotic pressures affecting elms, DED deserves particular mention, as it has caused severe population declines across Europe, resulting in range contractions and, locally, niche replacement [131]. Lastly, anthropogenic pressure and land-use changes, long shaping the Italian landscape, have fragmented broadleaved mixed forests, where wych elm persists often in relict areas [132, 133]. Mismatches between predicted suitability and current presence could also reflect climate-driven decline already underway, assuming a temporal lag between environmental change and range shift, which is not rare in long-lived trees [134]. Alternatively, persistence in marginal conditions may reflect phenotypic plasticity or ongoing adaptation allowing the species to persist under environmental stress – mechanisms that, while enabling survival, may increase vulnerability to pathogens and forest dieback [135, 136].

ENM predictions under future climate change scenarios showed evidence for a slight northward and upward shift of the species' suitable habitat in the central-eastern Alps (Fig. 5B). Upward altitudinal shift for *U. glabra* in future climatic scenario was also predicted by Zimmermann et al. [127] in Swiss Alps. Moreover, when evaluating future changes in the different suitability class separately, our models indicated a slight but widespread decline in highly suitable habitats – representing the species' ecological optimum – across the Alps, especially in known areas of species presence (occurrence points). A notable exception was the coastal region of the southwestern Alps (Ligurian Alps), where habitat suitability is predicted to benefit the most from future climate change, most likely due to its geographic position between the sea and the mountains, which promotes abundant precipitation.

In the Apennines, future projections showed that much of the southern range is predicted to experience stable or declining habitat suitability, with the most pronounced decreases in the Lazio mountains, in the central-eastern part of the Southern Apennines and in the Gargano promontory (Apulia region), roughly corresponding to the most arid regions of continental Italy [137], which are predicted to face strong negative effects from climate change. Regional case studies and modelling efforts consistently support the link between increasing aridity and declines in tree productivity and habitat suitability for several species across central and southern Italy. For example, dendrochronological evidence and ENMs document drought-driven reductions in growth and future habitat suitability in the central and southern Apennines for another temperate broadleaved species, *Fagus sylvatica* [138, 139]. Similarly, in southern and central Italy, several oak species (including *Quercus cerris*, *Q. pubescens*, and *Q. robur*) as well as the narrow-leaved ash (*Fraxinus angustifolia*) have recently exhibited increased decline and mortality linked to recurrent drought and rising aridity, with growth reductions and dieback episodes closely associated with warm, dry seasons [140, 141]. However, localized areas of increasing future suitability were also identified in southern Italy by our models, particularly in mountain regions along the southern part of the Tyrrhenian coast – from the Cilento to the Aspromonte mountains: these areas fall within the so-called "Mediterranean mountainous climate" subregion [137], where relatively high rainfall may buffer the negative effects of climate warming.

3. *Conservation implications*

Europe's Pan-European Forest Genetic Resources Strategy emphasizes the need to conserve the genetic diversity of native trees, highlighting the key role of Genetic Conservation Units (GCUs) – designated forest areas (*in situ* or *ex situ*) aimed at dynamically preserving evolutionary processes. Conservation planning should account for both past evolutionary dynamics and future pressures such as species range shifts under climate change [142]. Notably, no *in situ* GCUs have yet been established for *U. glabra* in the Italian Peninsula [143], highlighting the urgent need for robust, science-based criteria to guide their selection.

A primary outcome of this study is the identification of deep phylogeographic structure in *U. glabra* populations in Italy, aligned with the Alpine-Apennine separation and associated with divergent ecological niches, suggesting distinct evolutionary trajectories. These findings underscore the need to preserve both lineages to safeguard the species' evolutionary potential and long-term resilience. Their marked genetic differentiation, emerged at cpDNA, and ecological divergence, together with niche differentiation and lack of co-occurrence (based on our data), supports their recognition as separate Evolutionarily Significant Units (ESUs). *In situ* conservation should focus on identifying suitable habitats within each lineage's range while avoiding translocations that could disrupt local adaptation. Ideally, finer-scale genetic substructure, particularly within the APE lineage, should also inform GCU designation. Complementary analyses of nuclear DNA would be essential to integrate the cpDNA-based information in the definition of ESUs, allowing a more comprehensive characterization of the species' genetic diversity and enabling the detection of possible recent admixture events between the Alpine and Apennine lineages in potential contact zones.

ENM future projections call for targeted conservation in the driest regions of southern Italy, where suitability is projected to decline most sharply, threatening local haplotypes. Conversely, suitable areas for GCUs could be located in the inner and eastern parts of Northern and Central Apennines (for the North-central Apennine haplogroup and, partly, South-central Apennine haplogroup), as well as in the Southern Tyrrhenian mountains up to Aspromonte (for the South-central Apennine haplogroup), which show more stable or even improving environmental suitability. Our findings showed that the potential for a natural migration corridor of the Apennine lineage into the southern Alps appears unlikely (or at least not a winning strategy). For the same reasons, potential assisted migration initiatives or, more generally, translocations of plant material from Apennine populations hosting at-risk haplotypes into Alpine areas also appear inadvisable.

A spatial analysis combining current and future habitat suitability, haplotype distributions, and protected areas revealed contrasting conservation prospects for the two lineages. While the APE lineage is relatively well represented within protected areas – often coinciding with both favourable current and future habitat suitability (i.e., potential climatic refugia) – the ALP lineage appears more vulnerable, with most ALP populations lying outside them. However, the proximity of many unprotected populations to existing protected areas, offers opportunities to enhance conservation without creating entirely new reserves. Assisted migration or reforestation with locally adapted material from nearby sources may offer a convenient way to expand in situ conservation in the Alps, provided that suitable habitats within protected areas can be identified. Nevertheless, uneven genetic sampling – especially in central and southern Italy – underscores the need for expanded data collection to better inform conservation planning, particularly for the highly diverse and structured APE lineage.

Conservation efforts must also aim to maintain connectivity among populations to mitigate genetic erosion. Promoting gene flow through ecological corridors and habitat restoration may be particularly crucial in south-central Italy, where populations appear more fragmented and genetically differentiated. Moreover, since *U. glabra* often occurs at low densities, silvicultural practices supporting natural regeneration may be needed, especially in areas most affected by projected suitability losses.

Pests and pathogens, especially DED, pose an additional threat to European elms. Although *U. glabra* has so far been less affected than other elms, future climate shifts could reshape the insect–fungus–climate dynamics influencing DED spread [56]. The crucial role of standing genetic variation in maintaining the ability of natural populations to respond to emerging diseases is widely recognized [144]. In elms, susceptibility to DED has been found to be variable among species and among genotypes within species, often on a geographic basis [145, 146], but to our knowledge, no published studies have so far demonstrated a direct correlation between pre-infestation genetic diversity levels and DED severity, across natural elm populations. However, overwhelming evidence from other tree-pathogen systems (*e.g.*, ash dieback, [147]; chestnut blight, [148]; sudden oak death, [149]) strongly supports the critical role of genetic diversity in disease resistance and recovery. Under this perspective, *in situ* conservation of genetically unique and diverse populations becomes even more crucial. *Ex situ* conservation, typically implemented for the species through clonal archives or field plantations, can complement *in situ* efforts, acting as a buffer against potential future local pathogen outbreaks. Moreover, it could provide an additional means to preserve the genetic diversity of the most threatened populations, as well as to produce high-quality, genetically diverse seed for reforestation [150]. In Italy, an *ex situ* collection for *Ulmus* spp. was established in Florence in the late 1990s by the National Research Council (C.N.R.), for native germplasm characterisation, conservation and eventual future utilization [150, 151], and could serve as a basis for an expanded conservation program. Future research should include screening for variation at candidate DED-resistance genes [152], which may allow more direct assessment of susceptibility across *U. glabra* populations.

Finally, competition with invasive species and potential hybridization with non-native elms (e.g., *U. pumila*) are considered additional threats to the species, with the latter leading to genetic pollution in native elm species [38]. Natural hybridization and introgression between

U. glabra and *U. minor* have also been reported in several areas of Europe where the species coexist [153, 154]. Although spontaneous hybridization is generally not considered a threat to species, as it is a natural evolutionary process that can even enhance genetic diversity and potentially increase fitness, the case of elms is more complex. The distribution of certain species and their cultivated clones has been strongly influenced by human activity; moreover, anthropogenic environmental changes may have facilitated gene flow between species in some areas. The extent to which this may have negative consequences for *U. glabra* remains a complex and unresolved issue. However, direct evidence of natural hybridization between *U. glabra* and *U. minor* is lacking in Italy, where the two species are largely allopatric and gene flow is limited by ecological and altitudinal segregation between them, though it cannot be entirely ruled out in some areas at the edge of wych elm’s range. Our chloroplast data showed no evidence of inter-specific hybridization in the studied populations; however, nuclear genomic data would be necessary to fully rule out this possibility or to better investigate its genetic consequences.

Conclusions

In this study, by integrating phylogeographic analysis with ecological niche models and future projections, we shed light on the past evolutionary history of an ecologically important temperate tree, *Ulmus glabra*, in the Italian peninsula and assessed potential future dynamics, providing a basis for effective long-term conservation strategies, grounded in evolutionary and ecological evidence. Altogether, our findings call for lineage-specific and geographically targeted conservation planning that combines *in situ* and *ex situ* approaches, with the ultimate goal of sustaining the species populations and preserving its evolutionary potential in the face of climate change, habitat fragmentation and increasing pathogen threats.

Declarations:

Ethics approval: not applicable

Consent for publication: all the material used to build maps is copyright free or reference is indicated in caption.

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Authors' contributions:

A.Santini, F.S. conceived and planned the study. A.Santini, A.Selvaggi, L.G., F.P., A.P. and S.V. participated in specimens sampling. S.V. and F.P. carried out laboratory work. S.V., A.M., S.T., and F.S. were responsible for bioinformatics analysis. S.V. and A.M. wrote the initial draft of the manuscript and all authors provided critical feedback on both the analyses and the manuscript.

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Ethics approval and consent to participate: Leaf material was collected non-destructively from wild *Ulmus glabra* individuals in accordance with relevant national legislation in Italy, France and Bosnia-Herzegovina. Sampling was conducted in public forests or natural areas, as no specific permit was required for *U. glabra*, which is not a protected species, and only small, non-destructive leaf fragments were collected. Permission from the landowner was obtained in cases of access to private land.

Competing interests: The authors declare that they have no competing interests.

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Figures

Fig. 1 A) Distribution range of *U. glabra* from Caudullo et al. [63] and B) sampling sites for the present study (see Table S1, Additional file 1, for points coordinates). C) Wych elm specimen, photo by A.Santini.

Fig. 2 Map of wych elm haplotypes distribution. Samples were grouped according to administrative regions as reported in Table S1, Additional file 1, with pie-charts coordinates calculated as averages of specimen geographic coordinates. Pie-charts size is proportional to the number of samples. Different shades of the same colour represent different haplotypes within the same haplogroup (*i.e.* green/yellow: Northern Italy and France; blue: North-central

Apennines; red/purple: South-central Apennines; pink: Balkans). The map was built using the R package *leafletR* [75].

Fig. 3 Maximum likelihood phylogenetic tree of *U. glabra* samples based on chloroplast sequences. The tree is rooted with *U. pumila* (GenBank: MW279236.1) as outgroup; an *U. glabra* sequence available in GenBank (accession MT165931) was added for comparison. In addition to the sample from the Balkans (UL1), three main haplogroups are identified and indicated in the figure: Alps, North Apennines (N_AP), and South Apennines (S_AP).

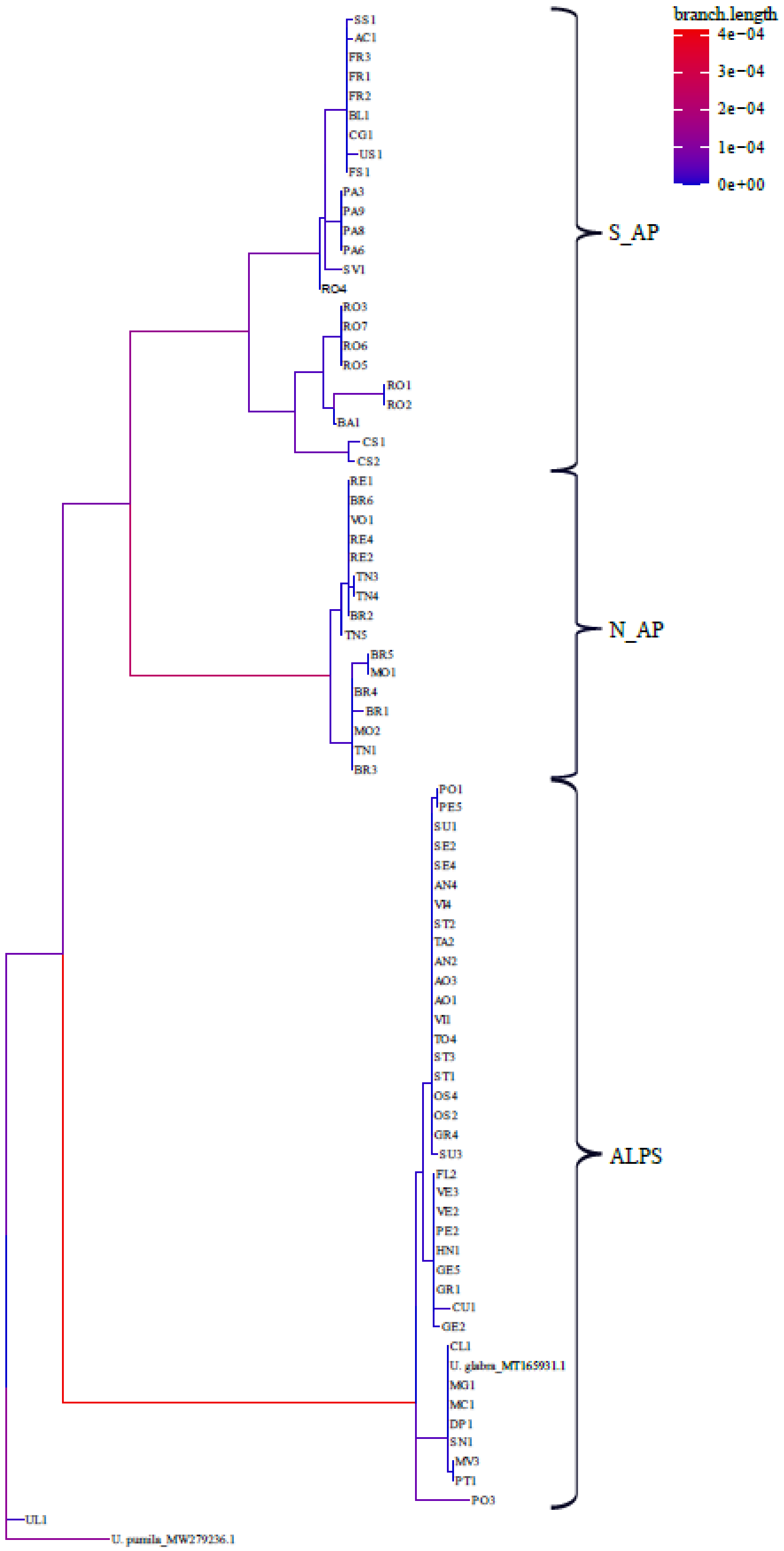

branch.length
4e−04
3e−04
2e−04
1e−04
0e+00
S_AP
N_AP
ALPS
SS1
AC1
FR3
FR1
FR2
BL1
CG1
US1
FS1
PA3
PA9
PA8
PA6
SV1
RO4
RO3
RO7
RO6
RO5
RO1
RO2
BA1
CS1
CS2
RE1
BR6
VO1
RE4
RE2
TN3
TN4
BR2
TN5
BR5
MO1
BR4
BR1
MO2
TN1
BR3
PO1
PE5
SU1
SE2
SE4
AN4
VI4
ST2
TA2
AN2
AO3
AO1
VI1
TO4
ST3
ST1
OS4
OS2
GR4
SU3
FL2
VE3
VE2
PE2
HN1
GE5
GR1
CU1
GE2
CL1
U. glabra_MT165931.1
MG1
MC1
DP1
SN1
MV3
PT1
PO3
UL1
U. pumila_MW279236.1

Fig. 4 A) Haplotypes network. Colours indicate administrative regions as listed in Table S1, Additional file 1, and circle size is proportional to the number of individuals. Black bars on the branches indicate the number of SNPs between haplotypes. B) Principal Component Analysis (PCA) performed on plastome sequences of *U. glabra* specimens. Dots represent individuals, while inertia ellipses and colours represent different regions as reported in Table S1. The *x* and *y* axes represent the first and the second Principal Components, respectively, with the relative proportion of explained variance in brackets.

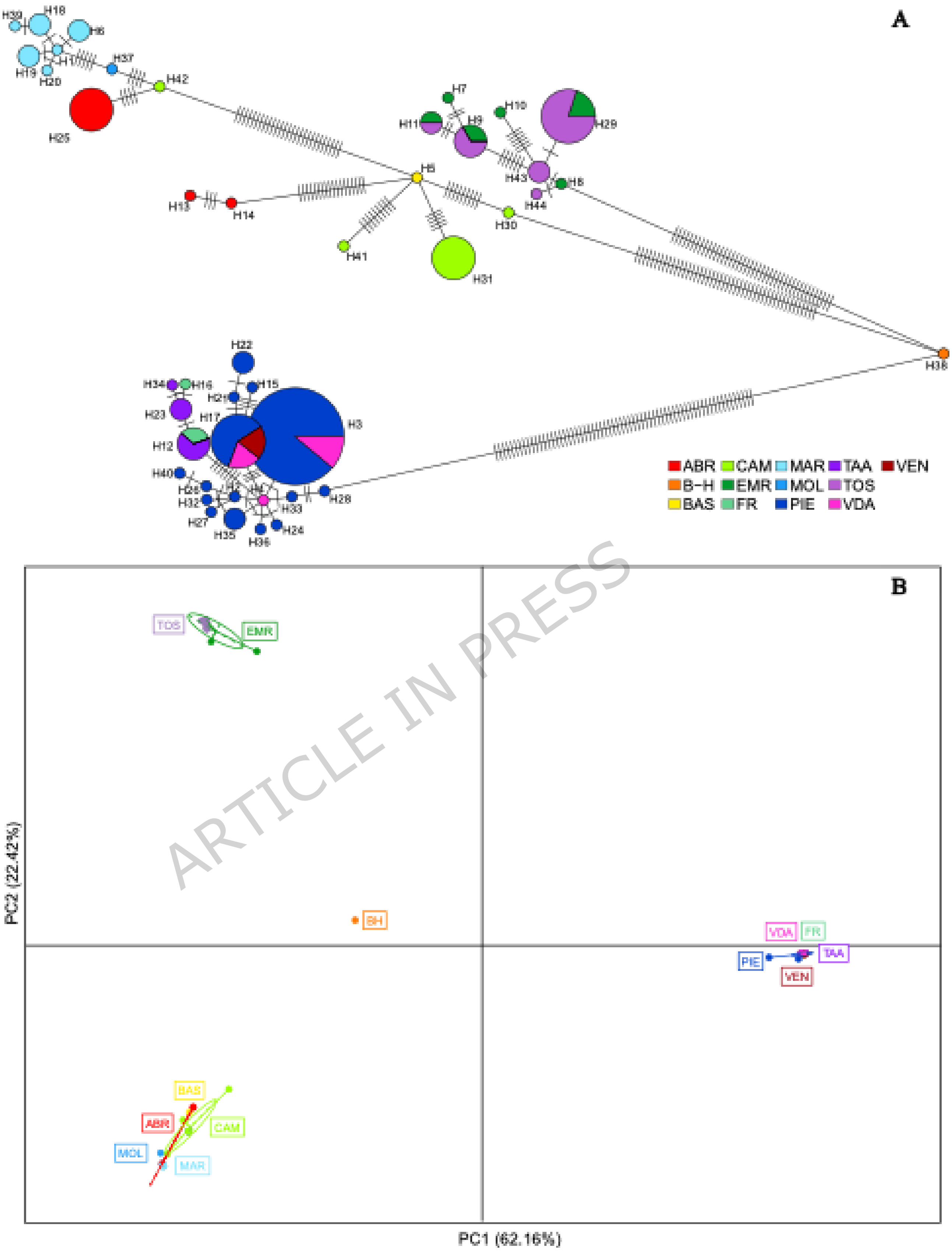
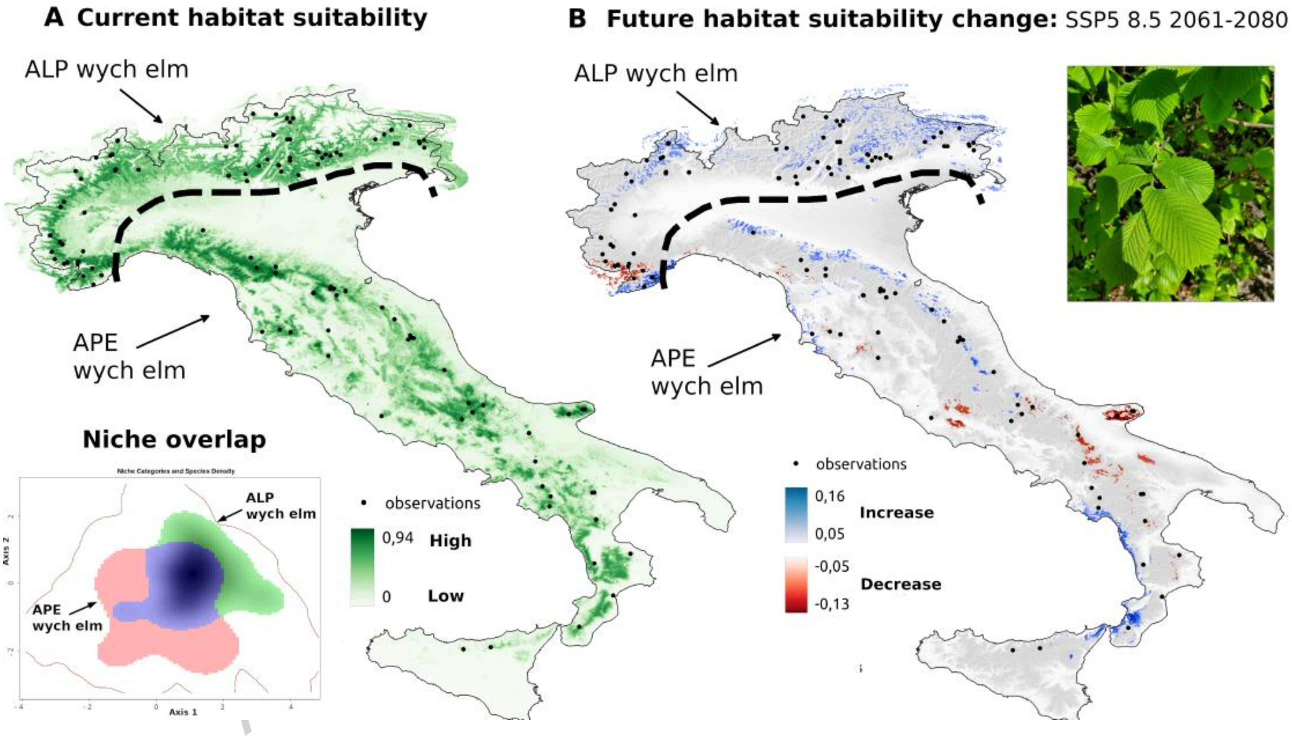

A
H39
H18
H5
H19
H1
H20
H37
H42
H25
H7
H10
H11
H9
H29
H6
H43
H8
H44
H13
H14
H30
H41
H31
H38
H22
H34
H16
H15
H21
H23
H17
H3
H12
H40
H26
H2
H4
H32
H33
H28
H27
H24
H35
H36
🟥 ABR
🟧 B-H
🟨 BAS
🟩 CAM
🟩 EMR
🟩 FR
🟦 MAR
🟦 MOL
🟦 PIE
🟪 TAA
🟪 TOS
🟪 VDA
🟥 VEN
B
TOS
EMR
BH
VDA
FR
TAA
PIE
VEN
BAS
ABR
CAM
MOL
MAR
PC2 (22.42%)
PC1 (62.16%)

Fig. 5 Current (A) and future (SSP5 8.5 2061-2080; B) habitat suitability maps for the Alpine (ALP) and Apennine (APE) *U. glabra* lineages. Dots represent current presence points used for ecological niche modelling. Current habitat suitability is indicated in shades of green, while future suitability variation is reported in blue (suitability increase) or red shades (suitability decrease). Niche overlap between ALP and APE lineage is based on current bioclimatic conditions and calculated by using the R package *ecospat*.



Tables

Table 1 Basic statistical information of genetic diversity calculated for the entire dataset and for regions and haplogroups as defined in Table S1, Additional file 1. Nucleotide diversity (π), haplotype diversity (H_d), their respective variance (σ^2), the average gene diversity within regions and haplogroups (gene diversity, Hs) and the total gene diversity (Hs calculated on the entire dataset, *i.e*. Ht) were calculated with the R packages *pegas* and *adegenet*. Populations: ABR = Abruzzo, BAS = Basilicata, CAM = Campania, EMR = Emilia-Romagna, FR = France, MAR = Marche, MOL = Molise, PIE = Piedmont, TAA = Trentino-Alto-Adige, TOS = Tuscany, VDA = Valle d'Aosta, VEN = Veneto. Groups: ALPS = Alps, N_AP = Northern Apennines, S_AP = Southern Apennines.

Pop	**number of samples**	**number of haplotypes**	**π (×10^{-4})**	**σ^2_π 10^{-9})**	**H_d**	**σ^2_{Hd} (×10^{-3})**	**Hs**
ABR	6	3	1.272	5.765	0.600	45.370	0.126
BAS	1	1	NA	NA	NA	NA	NA
CAM	7	4	0.690	1.677	0.714	31.356	0.110
EMR	6	6	0.215	0.217	1	4.630	0.146
FR	2	2	NA	NA	NA	NA	0.032
MAR	9	6	0.014	0.004	0.917	3.315	0.067
MOL	1	1	NA	NA	NA	NA	NA
PIE	26	15	0.102	0.048	0.898	2.286	0.041
TAA	5	3	0.038	0.017	0.800	16.640	0.034
TOS	10	5	0.257	0.251	0.822	7.898	0.112
VDA	3	3	0.168	0.226	1	37.037	0.044
VEN	1	1	NA	NA	NA	NA	NA
Group							
ALPS	37	20	0.217	0.159	0.922	0.884	0.040
N_AP	16	8	0.228	0.186	0.875	3.040	0.115
S_AP	24	15	0.908	2.210	0.946	0.642	0.132
Total	78	44	2.711	0.173	0.973	0.061	0.326

Table 2 Results of the analysis of molecular variance (AMOVA). Populations and groups correspond to regions and haplogroups as reported in Table S1, Additional file 1. ***P≤0.001.

Source of variation	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**Variance (σ)**	**Variance on total (%)**
Among groups	2	1313.6	656.8	12.6	15 ***
Among populations within groups	9	515.0	57.2	11.8	14 ***
Within populations	65	2959.6	45.5	59.6	71 ***
Total	76	4788.2	63	84	

Table 3 Neutrality test statistics (Tajima’s D and Fu’s Fs) and mismatch parameters (SSD and H_{Rag}) and the respective p-values calculated for the three Italian groups (as defined in Table S1, Additional file 1) and for all Italian samples. Asterisks indicate significant p-values.

Group	**Tajima's D**	**p**	**Fu's Fs**	**p**	**SSD**	**p**	**H_{Rag}**	**p**
ALPS	0.95825	0.846	5.76096	0.972	0.12781	0.01**	0.18761	0.02*
N_AP	2.34174	0.999	4.1892	0.962	0.15094	0.01**	0.19965	0.08
S_AP	0.54832	0.771	6.75923	0.99	0.0534	0.02*	0.09745	0***
Total	1.91189	0.94	10^{39}	1	0.03473	0***	0.03722	0***

Table 4 Integrated conservation framework for *U. glabra*: linking genetic data, ecological models, and management priorities. Notes: [1] threats may act synergistically; [2] future perspectives; not treated in the present study; [3] Genetic erosion may result from threats 1, 2, and 4, and can exacerbate the impacts of threats 2 and 4; however, it warrants distinct consideration due to its role in increasing extinction risk; [4] based on the assumption that maintaining genetic diversity enhances evolutionary potential and disease resilience; cp = chloroplast; nuc = nuclear.

Threats[1]	**Potential conservation measures**	**Scientific questions**	**Foundational analyses**
(1) Habitat loss, degradation, and fragmentation	**Static *in situ*:** • Protected areas • Sustainable forest management; restoration	• Is the current network of protected areas adequate to preserve populations and evolutionary lineages?	• Spatial overlap and proximity analyses between populations and protected areas
(2) Climate change	**Dynamic *in situ***: • Genetically and ecologically informed selection of **GCUs**; prioritize GCUs within predicted climate refugia; **assisted migration** ***Ex situ*:** • Living collections maximizing genetic diversity	• How will climate change affect habitat suitability for distinct genetic lineages? • Do protected areas match with environmentally stable habitats under future climate scenarios? • Are there suitable source and target areas for assisted migration?	• Phylogeographic (cp data) and population genetics (nuc data) analyses • Niche differentiation analysis • Lineage-based ENMs • Projections of future habitat suitability (and analysis of elevational trends) • Adaptation genomics[2]
(3) Genetic erosion[3]	• All above-listed measures • **Temporal monitoring of genetic diversity**	• What are current levels of genetic diversity within and among populations? Which populations are most at risk of genetic erosion?	• Population genetics analyses (nuc data)
(4) Diseases and pathogens (e.g. DED)	• **Conservation of resistant lineages/genotypes** • **Conservation of adaptive potential** through genetic diversity[4] • ***Ex situ* conservation** as a safeguard against outbreaks-driven population collapse	• Do phylogenetic lineages differ in pathogen susceptibility? • Does within-population genetic variation influence disease resilience?	• All of the above-mentioned analyses[1] • Assessment of differential susceptibility among lineages/populations[2]
(5) Competition with invasive alien species and genetic pollution	• Appropriate **silvicultural practices** (e.g., protecting seedlings) • **Controlling** and managing **the spread of non-native elms**	• Are natural forest stands declining due to competition with non-native trees? • Are there evidence of hybridization with non-native elms?	• assessment of population trends[2] • genetic analysis

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