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Disentangling subspecies delimitation in *Heldreichia bupleurifolia* Boiss. (Brassicaceae) through genomic and quantitative morphological approaches

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ABSTRACT

Heldreichia bupleurifolia Boiss., the only species in its genus, is distributed across the Taurus and Anatolian Diagonal Mountains in Türkiye and presents considerable taxonomic challenges. The species is currently divided into five subspecies and one variety and has long posed challenges for infraspecific classification due to overlapping morphological traits and unresolved phylogenetic relationships. To address these issues and clarify the boundaries of the described subspecies and their evolutionary history, this study integrates comprehensive morphometric analyses of 502 individuals from 36 populations, as well as SNP- and locus-based genomic analyses from RAD-seq data of 136 individuals from 20 populations. While morphometric assessments showed significant differentiation in foliar and fruit traits, especially between western and eastern populations, genomic analyses identified distinct genetic clusters corresponding to geographic regions. High levels of genetic admixture and morphological plasticity were seen in the Central Taurus populations, indicating either historical hybridization or the preservation of ancestral polymorphisms. In accordance with morphological and admixture analyses, coalescent-based subspecies delimitation supported a three-subspecies model, resulting in revised taxonomic classifications that included new combinations. To further contextualize these results within an evolutionary perspective, we adopted the speciation continuum framework, interpreting morphological, genetic, and reproductive differentiation as components of a continuous process of divergence. These results emphasize the drawbacks of using morphology alone for taxonomy and the value of integrative methods for determining subspecies boundaries in areas with complex topography.

1. Introduction

Delimiting species and/or subspecies is challenging because of the complex nature of evolutionary processes and biodiversity. Speciation is characterized as a gradual process where populations gradually diverge over time due to accumulating genetic, morphological, and ecological variations (Naciri & Linder, 2015; Padilla-García et al., 2018; Stankowski & Ravinet, 2021; Vences et al., 2024). In the early stages of speciation, such differences are often unclear, making the distinction between species and subspecies difficult. The occurrence of hybridisation and introgression, especially between closely related species, leads to the formation of intermediate forms, further complicating taxonomic classification (De Queiroz, 2007, 2020; Twyford and Ennos, 2012; Harrison & Larson, 2014).

In order to overcome species delimitation problems, integrative methods are increasingly preferred (Karbstein et al., 2024). Traditional methods, based on morphological and single-marker DNA data, may overlook minor genetic and ecological variations, particularly in closely

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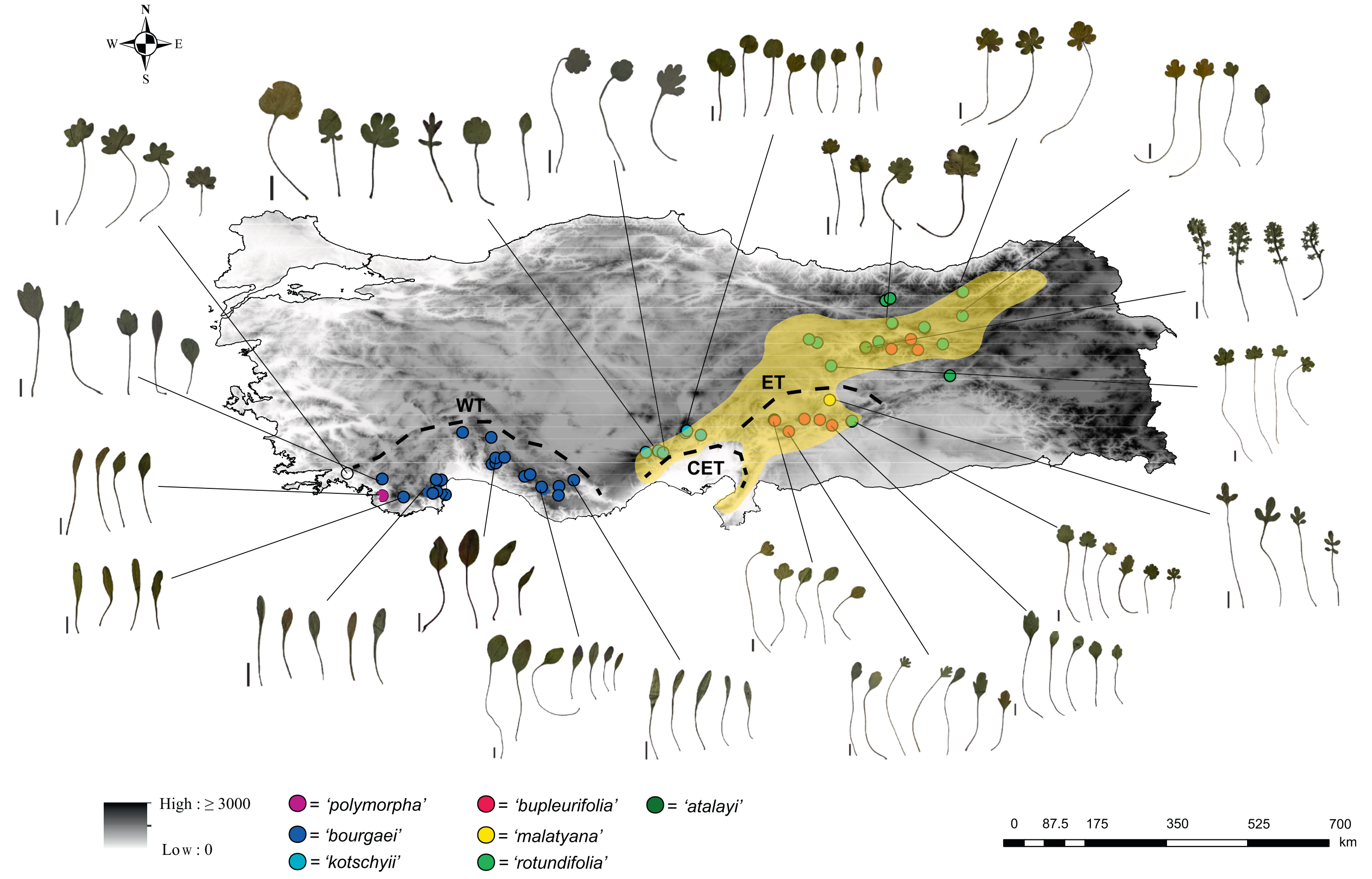


Fig. 1. Distribution and basal leaf morphology of *Heldreichia bupleurifolia*. Infraspecific taxa are shown by colors on the map. WT = Western Taurus, CT= Central Taurus, ET = Eastern Taurus Mountains. Anatolian Diagonal highlighted by yellow area on map. The uncoloured circle in the westernmost part of the WT region indicates the new variety "*sandrasica*". For details, please refer to the discussion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

related species or those undergoing speciation. Therefore, integrative approaches that combine morphological, genetic, ecological, and biogeographic evidence provide a more comprehensive view of speciation and yield more reliable species delimitation (De Queiroz, 1998; Edwards & Knowles, 2014; Mera-Rodríguez et al., 2024; Padial et al., 2010; Vitecek et al., 2017).

Located between temperate and subtropical regions, Anatolia exhibits extraordinary biodiversity, shaped by a combination of complex tectonic history, diverse topography, and varied climate (Huang et al., 2019; Şekercioğlu et al., 2011; Özüdoğru & Mummenhoff, 2020). The region's highly dynamic environment promotes speciation, as steep elevational gradients, interconnected yet dissected mountain chains, localized microclimates, and fine-grained edaphic heterogeneity jointly create barriers and corridors that reinforce genetic and ecological differentiation (Atalay, 2006; Şekercioğlu et al., 2011; Gür, 2013, 2016). Although the region is biologically diverse, research on species differentiation, speciation, and delimitation is rather limited, particularly in mountain ecosystems, and has been carried out mainly on animals (Sağlam et al., 2014; Kaya & Çıplak, 2017). Similarly, plant studies have been limited and have primarily depended on one or two genes (Parolly et al., 2010; Ansell et al., 2011; Özüdoğru et al., 2022).

Heldreichia bupleurifolia Boiss., as the only member of its monotypic genus, exhibits a predominantly Anatolian distribution, occurring mainly in the alpine zones of the Taurus Mountains and the Anatolian Diagonal –two principal biogeographic axes acting dually, serving as corridors and as barriers- with an isolated disjunct record reported from Lebanon (Hedge, 1965; Parolly et al., 2010) (Fig. 1). Recent studies further indicate that *H. bupleurifolia* is an autotetraploid and belongs to the tribe Biscutelleae within the latest Brassicaceae phylogeny (Özüdoğru et al., 2017; Guo et al., 2021; Hendriks et al., 2023). Adapted to mobile scree habitats, this species grows clonally via its perennial underground rhizomes. It occurs at elevations between 1100 and 3100 m in Anatolia, with the majority of populations occurring above 2000 m in alpine zones (Hedge, 1965; Parolly et al., 2010; Özüdoğru & Yıldırım, 2019). The first comprehensive study on the genus *Heldreichia* was carried out in Flora Orientalis (Boissier, 1867), which included six species (*H. bourgaei* Boiss., *H. bupleurifolia*, *H. rotundifolia* Boiss., *H. kotschyi* Boiss, *H. silaifolia* Hook.f. & Thomson and *H. longifolia* Boiss.) from various mountainous regions across Türkiye, Afghanistan, and Persia (Iran). Excluding the last two taxa, which are now classified under different genera and occur outside Türkiye, *H. kotschyii* originally described from the central Taurus region, was regarded as a synonym of *H. rotundifolia* by Hedge (1965). Consequently, *Heldreichia* was recorded in the Flora of Turkey with three species, to which *H. atalayi* was subsequently added as a fourth (Hedge, 1965; Tan & Sorger, 1986). Parolly et al. (2010) conducted an extensive taxonomic reassessment, reclassifying all previously recognized species within the genus as infraspecific ranks of *H. bupleurifolia.* They also described a new subspecies, *H. bupleurifolia* subsp. *polymorpha* Parolly, Nordt, Eren & Mumm., thus unifying the genus into a single species comprising multiple subspecies and varieties. This updated classification was formally adopted in the Turkish vascular plant checklist (Mutlu, 2012). In 2019, the discovery of *H. bupleurifolia* subsp. *malatyana* Özüdoğru & Yıldırım in Levent Valley, Malatya, Türkiye increased the total number of recognized taxa within *H. bupleurifolia* to six (Özüdoğru & Yıldırım, 2019). All these taxa will be referred to by the following names for simplicity in the text: '*atalayi*', ‘*bourgaei*’, ‘*bupleurifolia*’, ‘*malatyana*’, ‘*kotschyii*’, ‘*polymorpha*’ and ‘*rotundifolia*’.

Parolly et al. (2010) demonstrated that the taxa encompassed by *H. bupleurifolia* exhibit substantial morphological and geographical overlap, and ITS-based phylogenetic analysis was unable to delineate them. The key diagnostic character for the genus *Heldreichia* in the literature is the morphology of basal leaves. Unlike *‘bourgaei’* (with linear leaves) and *‘bupleurifolia’* (with oblong-spatulate leaves), which have entire leaves, *‘rotundifolia‘, ‘atalayi’,* and *‘malatyana’* are distinguished by its characteristic 3–5 lobed or dissected leaves. Geographically, *‘bourgei’* is restricted to the Western Taurus Mountains, whereas *‘bupleurifolia’* occupies the eastern part of the Western Taurus, the Central Taurus Mountains, and the southern part of the Anatolian Diagonal. In contrast, *‘rotundifolia’* is distributed throughout the northern part of the Anatolian Diagonal and also in the Central Taurus Mountains. Although the geographic ranges broadly match the diagnostic characters reported in the Flora of Turkey, Parolly et al. (2010) showed that range information alone is inadequate for resolving intraspecific variation, particularly in the Central Taurus Mountains (e.g., Aladağlar), and that finer morphological or genetic data are required. Many populations in this region show remarkable morphological transitions, particularly in lobe formation on leaves, with both lobed and entire leaves frequently observed within the same populations. This pattern corresponds closely with the morphological variation described for *‘polymorpha’* by Parolly et al. (2010). However, populations in this area bearing mixed or *rotundifolia*-like leaves (with 3–7 lobes) have historically been classified as *H. kotschyii*. Furthermore, phylogenetic analysis based on the ITS region of nuclear DNA confirmed that these populations do not form distinct genetic clusters neither western ‘*polymorpha’* nor eastern ‘*rotundifolia/bupleurifolia’* (Hedge, 1965; Parolly et al., 2010).

To date, studies on *Heldreichia* populations have been limited and have exclusively utilized ITS markers, which have failed to provide sufficient phylogenetic resolution for subspecies delimitations. Thus, the evolutionary history of the genus and the boundaries among its subspecies remain ambiguous. Although Parolly et al. (2010) conducted extensive sampling in the Taurus Mountains, their analysis of Anatolian Diagonal populations was based solely on herbarium specimens, which prevented the detection of notable leaf morphological variation in populations from Kahramanmaraş and Malatya (Eastern Taurus), the latter being the type locality of *H. bupleurifolia*.

Although this study primarily addresses the infraspecific taxonomic problems within *H. bupleurifolia*, it should be considered that speciation is now understood as a continuous process rather than a discrete event. Taxa typically diverge gradually across multiple axes such as genetic, morphological, ecological, and reproductive dimensions (Stankowski & Ravinet, 2021; Bolnick et al., 2023). The “speciation continuum” framework recognizes that divergence may unfold along independent axes such as genetic differentiation, ecological adaptation, and reproductive isolation, which may not always evolve synchronously. The speciation hypercube model proposed by Bolnick et al. (2023) and subsequently used in empirical studies such as Gorospe et al. (2025), allows researchers to locate taxa along a continuum of divergence by combining data from these multiple dimensions. Given the availability of genomic, morphological, and geographic data in this study, we integrate this conceptual framework to further elucidate the evolutionary trajectories within *H. bupleurifolia* and contextualize our taxonomic conclusions within a broader speciation landscape.

The present study integrates comprehensive morphological and genetic analyses of *H. bupleurifolia* across its entire distribution range in Anatolia and aims to: 1) evaluate morphological and genomic variation across the distribution range of *H. bupleurifolia* in Anatolia; 2) uncover phylogenetic relationships among subspecific lineages using RAD-Seq data and phylogenomic approaches; 3) propose revised classifications for morphologically ambiguous or transitional populations; 4) interpret the evolutionary and biogeographic history of the species within the context of Anatolia’s complex mountainous landscapes and the consept of speciation continuum.

2. Material and methods

2.1. Sampling and fieldwork

Field studies were conducted across the potential distribution areas of the genus in Türkiye, based on the localities recorded in the Flora of Turkey, local floras, and herbarium records (ANK, B, E, EGE, G, HUB, ISTE, K, KONF). The research was primarily conducted in the Taurus Mountains and the Anatolian Diagonal, which runs from the northeast of Türkiye to the southwest, where it splits into two branches toward the Mediterranean, one through the Amanos Mountains and the other through the Central Taurus Mountains. Fieldwork carried out in 2019, 2021, 2022 and 2023 resulted in the collection of specimens from 46 different populations. Leaf samples were collected from ten different individuals per population for use in molecular studies and were dried in silica gel. Herbarium specimens were prepared from five to thirty individuals per population for morphological analyses and were deposited in the Hacettepe University Herbarium (HUB). Detailed information of the studied populations is presented in Table S1.

2.2. Morphological studies

A total of 502 individuals from 36 populations were included in the morphological analyses (Table S2). Guided by previous findings (Parolly et al., 2010) and initial evaluations, the morphological study focused on the characteristics of the basal leaf and fruit. Populations were grouped for morphological analyses based on geography and the classification proposed by Parolly. Only the entire-leaved populations located west of the Central Taurus Mountains, which were accepted as *bupleurifolia* by Parolly, were grouped together with other entire-leaved populations and those classified as ‘*bourgaei’*.

Traits such as floral structures (sepals, petals, filaments, and anthers), seed morphology, plant height and root architecture and were excluded because they did not provide reliable characters for morphological differentiation. Qualitative and quantitative morphological characters of basal leaves and fruits are presented in Table S3. Basal leaf measurements were taken using a standardised ruler, whereas fruit measurements, requiring higher precision, were conducted digitally using ImageJ (Schneider et al., 2012).

To integrate both qualitative and quantitative traits within a unified multivariate framework, the qualitative traits were assigned numerical codes based on defined morphological categories. These values were then converted into factor types and treated as categorical variables before analysis. This process allowed for the simultaneous assessment of categorical and continuous variables via Factorial Analysis of Mixed Data (FAMD).

Morphological variation among populations was evaluated through a multivariate approach FAMD and Canonical Discriminant Analysis (CDA). The FAMD was performed using the FAMD () function from the FactoMineR package in R, and individual coordinates on the first five dimensions were extracted (Lê et al., 2008, R Core Team, 2025). To identify the variables contributing most to the first dimension (Dim.1) and the second dimension (Dim.2), weighted contributions were calculated by multiplying the original contribution values by the percentage of variance explained by each dimension. The top 15 variables were then visualised as bar plots using ggplot2 (Wickham, 2016). Subsequently, the first three FAMD dimensions were used as input variables for CDA to assess separation between populations. The model was constructed using the lm () and candisc () functions, and the resulting canonical biplot displayed group centroids along with the directional influence of the FAMD dimensions as vectors projected onto the canonical axes.

To account for potential bias arising due to unequal sample sizes among populations, a secondary analysis was conducted using a balanced subset of the data. For this purpose, a maximum of 20 individuals per taxon were randomly selected to construct a balanced dataset. The same multivariate framework –comprising FAMD and CDA- was applied to assess whether clustering patterns and variable contributions differed under conditions of equal sampling size.

2.3. Genomic analysis

2.3.1. DNA isolation, RadSeq libraries and sequencing

DNA isolation was carried out as described in Yılmaz et al. (2025). Both library construction and sequencing were performed by Floragenex (Eugene, OR, USA). The collection information of the specimens used in the study is given in Table S1.

2.3.2. Data preparation (filtering & mapping) SNP and locus selection for phylogenetic analyses

Individual sequences were aligned to the reference genomes of *Arabidopsis thaliana* (L.) Heynh. and *Megadenia pygmaea* Maxim. –the latter being more closely related to *Heldreichia*- using the BWA-MEM algorithm (Li, 2013) as described by Lamesch et al. (2012) (Yang et al., 2021). Since alignment with *M. pygmaea* resulted in only 9 % more alignment success than with *A. thaliana*, we decided to proceed with alignment to the more refined and well-annotated *Arabidopsis* genome. The alignment files, initially generated in SAM (Sequence Alignment Map) format, were subsequently converted to BAM (Binary Alignment Map) format, followed by sorting and indexing using samtools v1.21 (Li et al., 2009). Duplicate reads were detected and marked with the MarkDuplicates tool in Picard v2.27.4 (Broad Institute, 2024). SNPs were identified using a probabilistic framework implemented in ANGSD, which is specifically tailored for next-generation sequencing data. Polymorphic loci were identified separately for each cluster. Genotype likelihoods were estimated using the samtools model, and results were output accordingly. Major and minor alleles were determined, allele frequencies were calculated, and read counts were computed at each site. Low-quality bases and reads were removed by setting a minimum base quality threshold of 20 and a minimum mapping quality of 30. Sites were retained only if they exhibited an average read depth of at least 6X per individual and were present in at least 50 percent of individuals within each population. To assess the impact of allele frequency on downstream analyses, two separate datasets were generated using Minor Allele Frequency (MAF) threshold of 0.05 and 0.33, respectively. From these results, common polymorphic loci were extracted. Genotypes were called using the maximum a posteriori method implemented in ANGSD, with genotype calling enabled through the doGeno option set to 5. A flat prior was applied by setting the doPost option to 2, and only genotypes with a posterior probability of ≥95 % were retained, as defined by the postCutoff threshold. Genotype data were then exported in Variant Call Format by enabling the doBcf option.

2.3.3. Genomic structure of populations

To investigate the genetic structure of the populations, we carried out a principal component analysis (PCA) using the snpR package (v1.2.79; Hemstrom & Jones, 2023), which implements the smart PCA algorithm (Price et al., 2006; Patterson et al., 2006). Genetic variation was visualized by plotting the first two principal components (PC1 and PC2) in R with the ggplot2 package (Wickham, 2016).

In addition, individual ancestry proportions were estimated using the program NGSadmix (Skotte et al., 2013), which relies on genotype likelihoods. We evaluated K values from 1 to 6, running six replicates for each K to account for stochastic effects and ensure consistent convergence. The most likely number of genetic clusters was determined using the ΔK method (Evanno et al., 2005), which assesses changes in log-likelihood values between consecutive K values. Admixture patterns for each K were subsequently visualized in R.

2.3.4. Phylogenomic analyses

Phylogenomic analyses were conducted using two distinct data sets. The first dataset comprises SNP data from two individuals per population, with the exception of the ENGIZEK and GOKBEL_2 populations, which are represented by a single individual each. To evaluate the impact of SNP filtering on phylogenetic inference, we generated two SNP subsets based on the MAF threshold of 0.05 and 0.33, as previously described in Section 2.3.2. The resulting SNP matrices were subsequently converted into PHYLIP format using the vcf2phylip script (Ortiz, 2019), which facilitates the generation of input matrices for phylogenetic inference across multiple formats. SNP-based phylogenetic analyses were then conducted for each subset independently. Maximum likelihood trees were inferred with RAxML v.8.0.17 (Stamatakis, 2014) using the GTR + G model of molecular evolution and 1000 bootstrap replicates. Since our study focused on examining differentiation within *H. bupleurifolia* rather than testing the monophyly of *Heldreichia*, we selected *Noccaea oppositifolia* (Pers.) Al-Shehbaz & Menke –a relatively distant relative of *Heldreichia* for which RADSeq data was available- as the outgroup.

The second dataset, which included all individuals, was derived from a filtered set of 3,477 RAD loci (including invariant sites) selected using snakeRAD—a custom Snakemake-based pipeline developed to identify loci suitable for phylogenomic analyses from raw RADseq alignments (bam files). The pipeline integrates multiple bioinformatic tools to automate and standardize key processing steps, including alignment indexing, locus detection, quality filtering, depth calculation, sequence extraction, and multi-sample alignment. It then identifies shared RAD loci across individuals, retaining only those loci that are present in at least one individual per population to ensure broad representation. This filtering resulted in a final set of 1,085 loci. From this subset, the 500 loci with the highest parsimony information content were selected using AMAS (Borowiec, 2016), concatenated, and then subjected to phylogenetic analyses using RAxML v.8.0.17 (Stamatakis, 2014) and MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003).

The second dataset was also employed to construct individual gene trees using the RAxML in a non-concatenated approach. These gene trees were then used for species tree estimation via ASTRAL-III (Zhang et al., 2018) using a multi-species coalescent framework.

2.3.5. Subspecies delimitation

Subspecies delimitation analysis was performed using the Bayes Factor Delimitation (BFD*) framework implemented in SNAPPER v1.1.5 (Bryant et al., 2012; Stoltz et al., 2021) within BEAST v2.7.7 (Bouckaert et al., 2014). Input SNP data were generated by filtering the VCF file to retain only bi-allelic sites with a MAF threshold of 0.33 and by including two individuals per population. This filtered VCF file was converted into a binary NEXUS format using vcf2phylip with the nexus-binary option. The resulting NEXUS file was then imported into BEAUti to generate XML configuration files. Model parameters and priors were set following the BFD SNAPPER tutorial (Leaché & Bouckaert, 2018), and MCMC analyses were run for 10,000,000 generations, sampling every 1,000 steps. In the *Taxon Sets* panel, we defined six alternative species delimitation scenarios, representing competing hypotheses of species boundaries based on geographic and genetic structure.

Each XML file was independently executed in BEAST, after which path sampling and stepping-stone sampling analyses were carried out using the PathSampler utility. These analyses were conducted with the following settings: the shape parameter alpha was set to 0.3, the number of path steps was defined as 48, and the MCMC length for each step was 500,000 iterations, with a burn-in of 10 % and a preliminary burn-in phase consisting of 100,000 iterations. Marginal likelihood estimates (MLEs) for each subspecies delimitation model were calculated using both path sampling and stepping-stone sampling methods. Bayes factors (BFs) were subsequently derived by comparing the MLE of each alternative model to that of the reference model, following the procedure described by Kass and Raftery (1995), whereby the difference in MLEs is

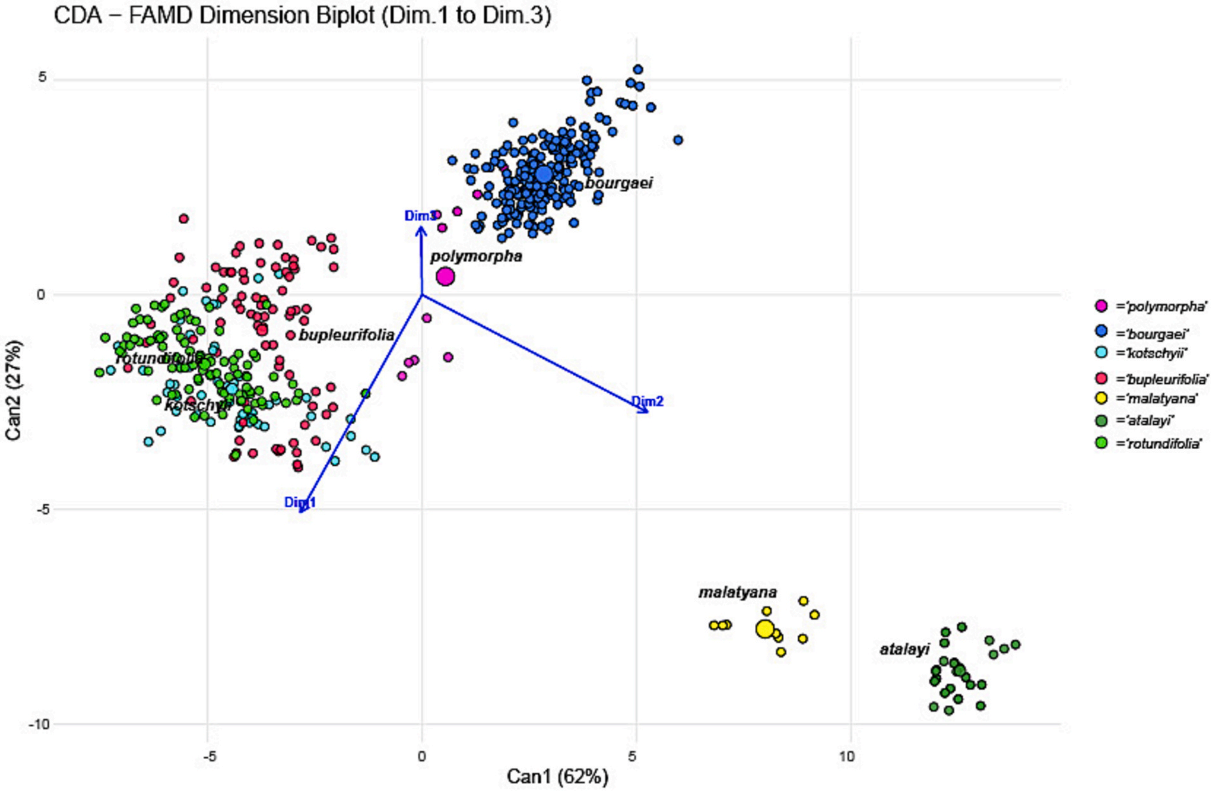


Fig. 2. Canonical discriminant analysis (CDA) based on 19 morphological characters and seven predefined groups corresponding to seven taxa including”*kotschyii*” treated as synonym of “*rotundifolia*”. Color codes follow Fig. 1.

multiplied by two. The model yielding the highest Bayes factor was considered to have the strongest support and was selected as the most likely subspecies delimitation scenario.

2.3.6. Multidimensional differantiation

Following Bolnick et al. (2023) and Gorospe et al. (2025), we quantified multidimensional differentiation among 16 populations spanning the geographic, morphological and genetic breadth of the *H. bupleurifolia* complex. Genetic differentiation was measured as pairwise FST estimated from genotype-likelihood data: for each population we inferred site allele frequency (SAF) with ANGSD (−GL 1 −doSaf 1) against the TAIR10 reference, computed a folded joint two-dimensional SFS for each population pair with realSFS (−fold 1), obtained locus-wise FST with *realSFS fst index*, and summarised genome-wide (global) FST with *realSFS fst stats*; downstream analyses used the pairwise global FST values. Phenotypic differentiation was calculated as Euclidean distances between population centroids in a morphometric PCA space built from leaf and fruit traits. To estimate climatic differentiation, WorldClim v2.1 (19 BIO variables) was used. After PCA on population means, Euclidean distances between centroids were taken as pairwise climatic divergence. Geographic distances were computed as great-circle distances between population coordinates with the R package geodist v0.1.1 (Padgham and Sumner, 2024). The filtered SNPs were matched to population assignments and converted to BA3-SNPs (Mussmann et al., 2019) input with **bcf2ba3** (Rannala, 2022). Directional migration rates were estimated with BA3-SNPs using default mixing step sizes (deltaM, deltaA, deltaF) and default burn-in settings. For each population pair, directional rates were summarized as posterior means and standard deviations. As a proxy for reproductive isolation between two populations, one minus the average of their opposing directional migration rates was used.

Contemporary directional migration rates were estimated with BA3-SNPs and used to derive a reproductive-isolation proxy (RI proxy) such that larger values denote lower contemporary gene flow. All distance matrices (RI proxy, FST, phenotypic, climatic, geographic (Table S5-9)) were then normalized and integrated via PCA to summarise the principal axes of differentiation; three largely independent criteria—RI proxy, genetic differentiation and phenotypic differentiation—were retained as orthogonal axes to construct a “speciation cube” as defined by Bolnick et al. (2023), allowing visualisation of the speciation continuum within the complex.

3. Results

3.1. Morphological studies

Canonical Discriminant Analysis (CDA), applied to these FAMD dimensions (the first three dimensions), showed clear population-level structuring: ‘atalayi’ and ‘malatyana’ are well separated, ‘*bourgaei*’ forms a compact cluster, ‘*bupleurifolia*’, “*kotschyii*” and ‘*rotundifolia*’ substantially overlap, and ‘*polymorpha*’ lies between the bupleurifolia–rotundifolia scatter and the ‘*bourgaei*’ cluster, with limited overlap with ‘*bourgaei*’. The first two canonical axes accounted for nearly 90 % of the total variation (Can1: 62 %, Can2: 27 %), capturing the major axes of morphological divergence across the range (Fig. 2, S1).

On FAMD dimensions 1–2, the strongest contributions came from categorical leaf characters related to leaf shape and dissection, whereas fruit characters contributed less (Fig. S2). In Fig. 2, the arrows represent the projection of the first three FAMD dimensions into the CDA space rather than raw variable loadings, and their orientation indicates that the observed separation among taxa aligns primarily with foliar-driven gradients.

The results of a PERMANOVA based on the first three FAMD dimensions ($R^2 = 0.89$, $F = 830.44$, $p < 0.001$) confirmed that the observed morphological differences among taxa are statistically significant and not due to random variation. This highlights the taxonomic relevance of the morphological structuring, even in the presence of

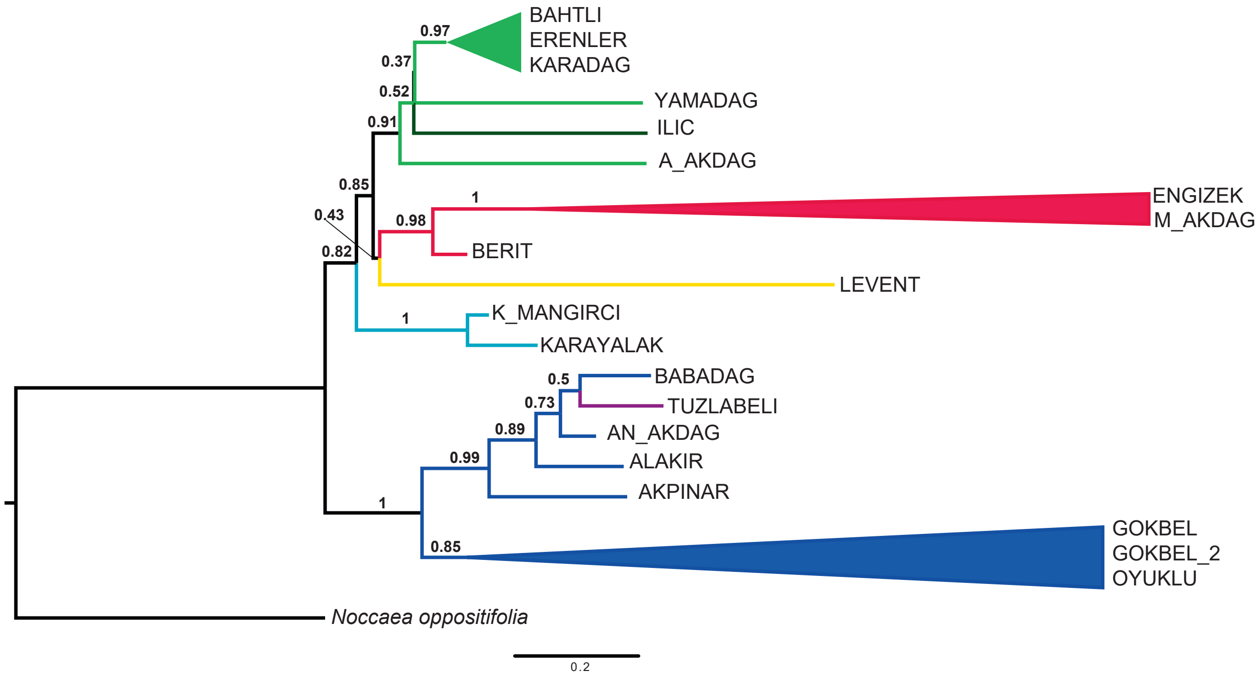


Fig. 3. ASTRAL species-tree generated from 500 RAXML gene-trees. Each locus contains > 120 individuals with a few exceptions. Numbers at each node represent the local posterior probability (local PP). Color codes follow Fig. 1. The population names appearing on the tree are explained in Table S1.

character continuity and overlap.

In the analysis conducted using a balanced sample (Results are not presented), in which a maximum of 20 individuals were randomly selected from each population, the overall clustering structure was largely preserved, although a slight reduction in the clarity of group separation was observed.

The same categorical leaf characters contributed most strongly to FAMD dimensions 1–2. In addition, the population from İliç, Erzincan −which exhibits a pinnate-pinnatifid leaf morphology characterized by leaves divided down to the midrib and further subdivided segments, identical to the type described as '*atalayi*' based on a single specimen from Akdağ, Adıyaman- clustered separately in the analyses.

3.2. *Genomic structures of populations*

The first two principal components (PC1 and PC2) together explained 38.03 % of the genetic variation among the *H. bupleurifolia* populations (Fig. S3A). The PCA diagram showed clear separation among most taxa. '*malatyana*' formed a distinct and well-separated cluster along the PC2 axis. '*bupleurifolia*', and '*rotundifolia*' formed closely related but individually distinct clusters along the positive axis of PC1, indicating genetic proximity and minor differentiation. '*kotschyi*' was positioned intermediately between these groups, while '*polymorpha*' and '*bourgaei*' formed overlapping clusters along the PC1 axis, grouping closely together.

Admixture analysis (Figs. S3B, S4) determined the optimal number of genetic clusters to be K = 3. Individuals from the western Taurus Mountains, including '*polymorpha*' and '*bourgaei*', predominantly grouped within Cluster 1, exhibiting little to no genetic admixture with Cluster 2 and 3. Cluster 2 comprised individuals from populations distributed across the Anatolian Diagonal, which also exhibited a high degree of genetic homogeneity. In contrast, populations such as ENGIZEK, BERIT, and KARAYALAK displayed signatures of admixture between Clusters 1 and 2, indicating potential zones of genetic contact or introgression. Notably, individuals from the Levent population ('*malatyana*') formed a distinct group corresponding to Cluster 3, suggesting a unique genetic lineage or divergent evolutionary history.

3.3. *Molecular phylogenetics based on SNP and locus data sets*

The species tree inferred using ASTRAL from 500 independent RAD loci reveals a well-supported and geographically structured phylogeny among populations sampled across the Taurus Mountains and the Anatolian Diagonal (Fig. 3). Most internal branches are supported by high local posterior probabilities, indicating strong phylogenetic resolution.

The Western Taurus populations − encompassing '*bourgaei*', '*polymorpha*', and spathulate-leaved forms previously classified as '*bupleurifolia*' − form a well-supported clade (local PP = 1.0), representing the most basal split in the dataset. This indicates a long-term evolutionary distinctiveness of the Western Taurus relative to all other regions.

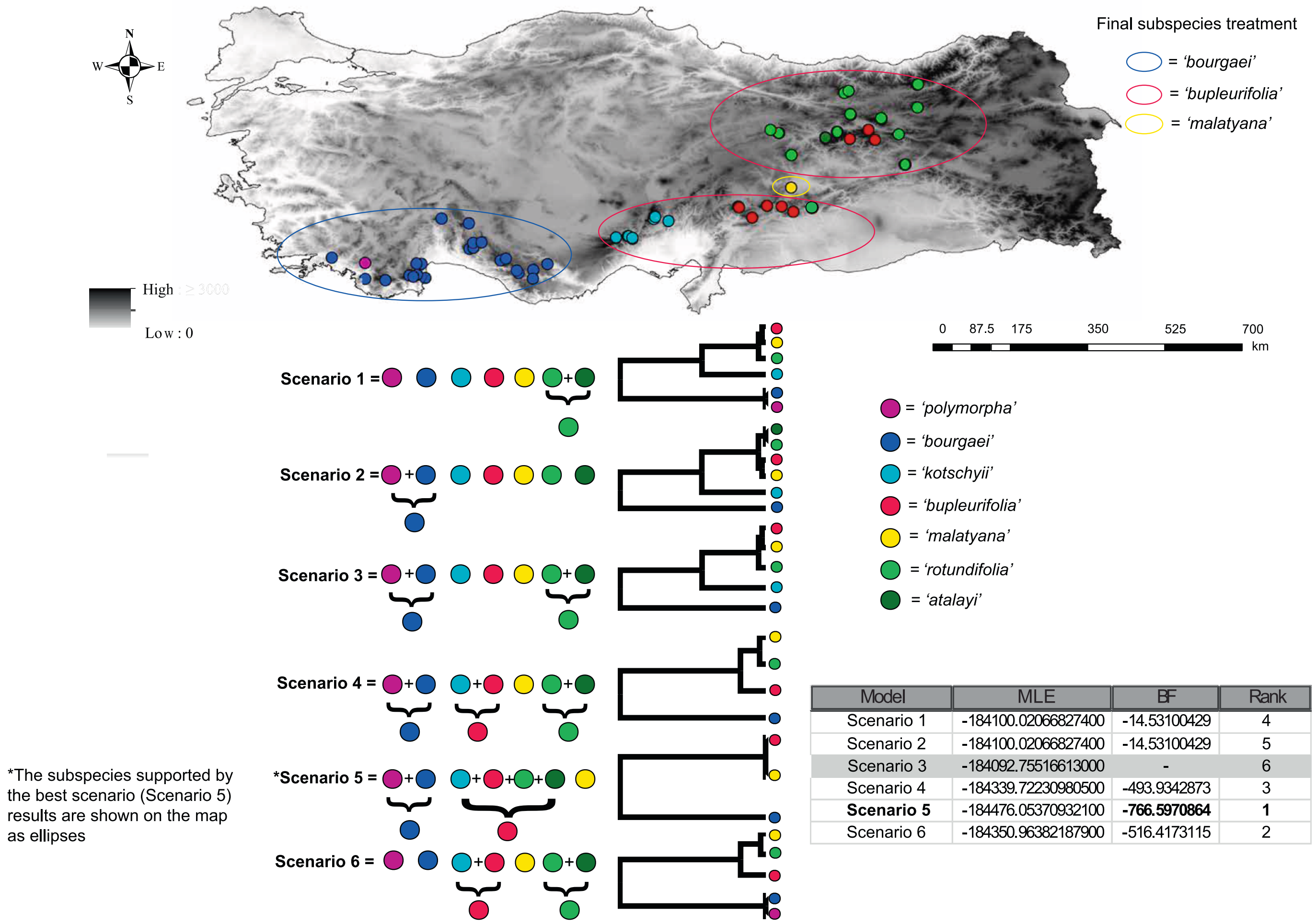
The sister clade to the Western Taurus includes all remaining populations (local PP = 0.82) and comprises three major geographic groups: the Central Taurus, Anatolian Diagonal, and Eastern Taurus.

The Central Taurus populations, historically assigned to '*kotschyii*', form a well supported clade (local PP = 1) and are the first to branch off within this broader non-Western group (local PP = 0.82). This basal position suggests a relatively early divergence of Central Taurus lineages.

The Anatolian Diagonal group, comprising *rotundifolia* populations including *atalayi*, forms a strongly supported clade (local PP = 0.91), indicating a coherent and distinct evolutionary lineage within the highlands.

The Eastern Taurus clade (local PP = 0.98) includes populations from both the eastern Taurus range and the northeastern portion of the Anatolian Diagonal, including the type locality of *bupleurifolia*. However, position of the Eastern Taurus populations within the tree unresolved. In particular, the branch uniting *malatyana* (LEVENT)—restricted to the Levent Valley in Malatya—with the rest of the Eastern Taurus has low support (local PP = 0.43), indicating uncertainty in its placement. While LEVENT may represent an early-diverging lineage within the Eastern Taurus, the current data are insufficient to determine its exact phylogenetic position.

The five major clades identified in the coalescent-based species tree—Western Taurus, Central Taurus, Eastern Taurus, Anatolian Diagonal, and Levent Valley—were also recovered in SNP-based



Model	MLE	BF	Rank
Scenario 1	-184100.02066827400	-14.53100429	4
Scenario 2	-184100.02066827400	-14.53100429	5
Scenario 3	-184092.75516613000	-	6
Scenario 4	-184339.72230980500	-493.9342873	3
Scenario 5	-184476.05370932100	**-766.5970864**	**1**
Scenario 6	-184350.96382187900	-516.4173115	2

Fig. 4. SNAPPER subspecies delimitation scenarios. A Assignment of accessions studied to recent subspecies classification of *H. bupleurifolia* with"*kotschyii*" treated as synonym of "*rotundifolia*". The colour code follows Fig. 1. B Various potential subspecies delimitation scenarios. Based on the current study, scenario 5 is the most probable one against scenario 3 (highlighted by grey, the five-subspecies framework derived from SNP-based phylogenetic analyses) and highlighted in bold in the inset table.

phylogenetic reconstructions (Figs. S5-6). However, their relative topological positions differed, underscoring the influence of data type and analytical approach on phylogenetic inference.

3.4. Subspecies delimitation

To evaluate alternative subspecies delimitation hypotheses, we used SNAPPER to compare six demographic scenarios (Fig. 4) using the MAF threshold of 0.33 filtered SNP dataset. We chose a high MAF threshold to reduce SNP counts–thereby increasing computational efficiency in coalescent-based species delimitation with SNAPPER–and to prioritize widely shared over rare alleles. The resulting dataset is enriched for information-dense, parsimony-informative sites, which typically provide more reliable phylogenetic signal for species/subspecies delimitation when per-population sample sizes are modest (n ≈ 2 per population). The highest-ranked model (Scenario 5; Bayes factor = –766.597 relative to Scenario 3, which reflects the five monophyletic lineages obtained from SNP based analayses) supports a three-subspecies scenario. This model aligns with the ASTRAL-based phylogeny, resolving three subspecies-level groups: (i) Western Taurus populations—including '*bourgaei*' and '*polymorpha*'—as one subspecies; (ii) '*malatyana*' as a distinct subspecies; and (iii) populations from the Central Taurus, Eastern Taurus, and the Anatolian Diagonal—including '*atalayi*', '*bupleurifolia*', '*rotundifolia*', and '*kotschyii*'—as a third subspecies.

3.5. Multidimentional differentation

The three-dimensional speciation cube (Fig. 5A; Table S4-9) visualizes the extend and heterogeneity of divergence among *H. bupleurifolia* population pairs. Our multidimensional analyses indicated that *H. bupleurifolia* populations diverged along distinct trajectories within the speciation continuum. An aggregated trajectory across all pairwise comparisons demonstrates a general increase in differentiation along the continuum. Principal component analysis (PCA) of divergence metrics (Fig. 5B) showed that the first two axes explained 67 % of total variance (PC1 = 47.5 %, PC2 = 19.5 %). PC1 captured genetic and morphological differentiation together, while PC2 was more strongly associated with the RI proxy.

4. Discussion

This study integrates extensive genomic and morphological data to resolve taxonomic complexity in *H. bupleurifolia*. A total of 502 individuals from 36 populations were analysed, sampling across the species' morphological diversity and geographic distribution. In parallel, genome-wide RAD-seq data obtained from 136 individuals provided a high-resolution phylogenetic framework based on 61,286 SNPs and 500 loci. Together, these data provide a robust basis to evaluate the species' infraspecific boundaries, which have proven difficult to define using morphology or molecular evidence alone.

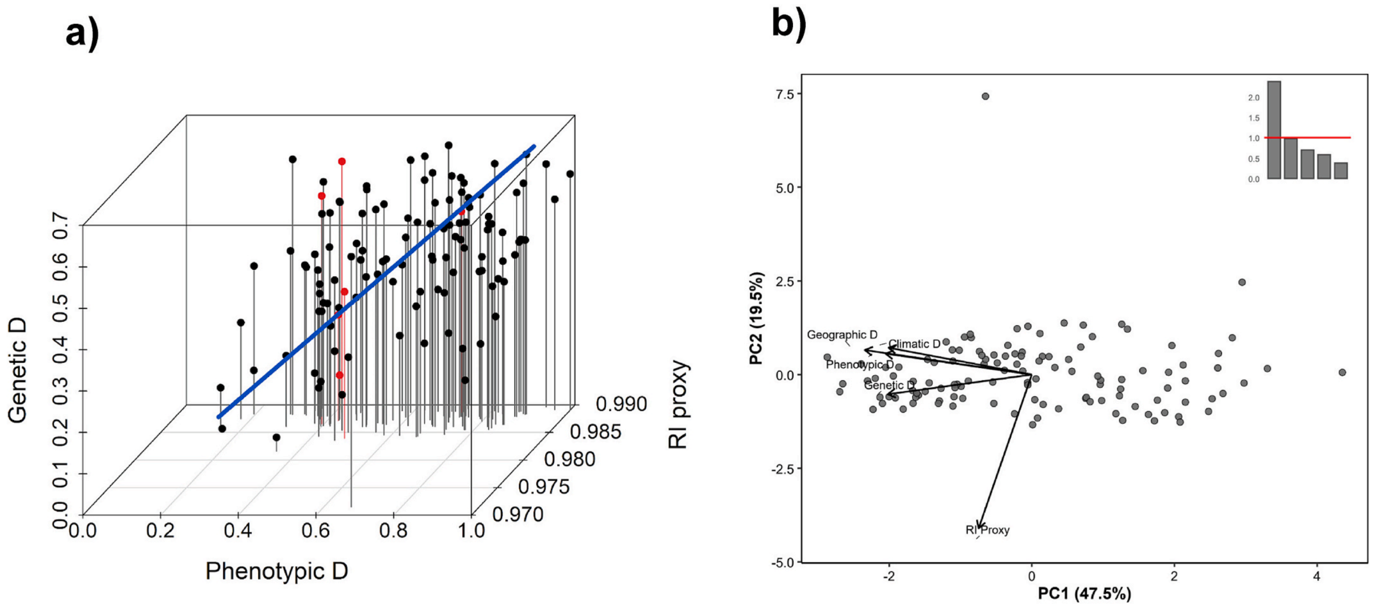


Fig. 5. Multidimensional analysis of *H. bupleurifolia*. A Genetic, phenotypic, and reproductive differentiation (RI proxy, inferred from migration estimates) among population pairs. The blue line represents the consensus trajectory of divergence, while red points highlight population pairs showing progressive differentiation along the speciation continuum. B Principal component analysis (PCA) summarizing all divergence metrics. Each dot denotes a population pair, and vectors show the loadings of individual measures on the first two axes. The inset graph displays eigenvalues for the five PCA components, with the red line marking the broken-stick threshold. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.1. Morphological variation fails to resolve infraspecific boundaries

Our results reveal that while traditional morphological traits, particularly categorical leaf characters related to leaf shape and dissection, are partially informative for distinguishing geographically isolated populations of *H. bupleurifolia*, they fall short of resolving infraspecific boundaries across much of the species' range. Morphometric analyses show that entire-leaved forms in the Western Taurus (traditionally identified as '*bourgaei*' and '*bupleurifolia*', but treated as 'bourgaei' in this study) are relatively well-differentiated from the more dissected-leaved forms found in the Eastern Taurus and Anatolian Diagonal (e.g., '*rotundifolia*'). However, in the Central Taurus—particularly in the Aladağlar and Bolkar Mountains – leaf morphology varies dramatically even within individuals, ranging from entire to 3–7 lobed, making taxonomic assignment ambiguous. This complexity is compounded by overlapping fruit traits, such as style length and fruit width, which provide little resolution among populations.

These findings corroborate earlier concerns (Parolly et al. 2010) about the limitations of morphology-based taxonomy in *Heldreichia* and highlight the need for integrative approaches. Notably, '*malatyana*' and '*atalayi*' emerge as the two morphologically coherent units across all analyses. Of these, '*atalayi*' is characterized only by its bipinnatifid leaves, while '*malatyana*' has a large number of consistently expressed traits—long pedicels, deeply pinnatifid leaves, and elongate fruits—supporting its recognition as a distinct taxon, a conclusion further reinforced by its genetic distinctiveness.

While morphological data provide some resolution for certain taxa, they fail to capture the full scope of phenotypic and evolutionary variation in central populations. A key reason for this shortfall may lie in the historical reliance on herbarium specimens. Because such collections often represent limited snapshots—both spatially and temporally—they tend to obscure intra-individual variability and environmentally induced plasticity, particularly in traits like leaf dissection. Our extensive field-based sampling, by contrast, reveals continuous morphological transitions that are not visible in preserved material alone. This limitation is especially evident in the eastern Taurus region, including areas south of the Anatolian Diagonal (e.g. Mt. Engizek, Mt. Berit), where specimens exhibit both entire and lobed leaf forms even within the same population. Remarkably, individuals collected from the type locality of *H. bupleurifolia* (Malatya, Akdağ), situated east of both the Bolkar and Aladağlar ranges, also show this duality in leaf morphology. This underscores a critical limitation of morphology-based delimitation in topographically and climatically complex regions like the Taurus Mountains.

Taken together, these patterns suggest that the morphological complexity in *Heldreichia* may reflect convergent evolution, retention of ancestral polymorphism, or phenotypic plasticity-processes that typically cannot be resolved without genomic data.

Lastly, integrating genome-wide divergence metrics within a speciation-continuum framework suggests that the morphological differentiation observed in *H. bupleurifolia*, which has a polygenic basis linked to ion transport and homeostasis (Yılmaz et al., 2025), indicates an advanced phase of divergence. In this phase, ecological adaptation and reproductive isolation are more closely aligned. Elevated RI proxy values, alongside significant phenotypic and geographic differentiation, suggest that multiple population pairs reside at the upper end of the speciation continuum. This is indicative of incipient speciation rather than mere intraspecific variation. The presence of populations with different morphotypes in the intermediate zones of the cube suggests that leaf dissection, genomic divergence and reproductive isolation, while correlated, may progress independently across lineages.

4.2. Genetic patterns and phylogenetic discordance

The morphological complexity observed in populations in the Central Taurus region ('*kotschyii*') is corroborated by molecular evidence. These populations, including those with mixed leaf types in the eastern Taurus, exhibit significant genetic admixture (Fig. S3B). This may explain why the Central Taurus populations cluster with western groups in SNP-based phylogenies but not in gene-tree/species-tree approach. In any case, it is clear that the populations in this region are complex both morphologically and phylogenetically.

Our subspecies delimitation analyses using SNAPPER favor a scenario grouping the central Taurus populations ('*kotschyii*') with the mixed-leaved eastern Taurus populations (‘*bupleurifolia*’) and ‘*rotundifolia*’ + ‘*atalayi*’ populations found across Anatolian Diagonal. In contrast, for the western mixed-leaved ‘*polymorpha*’, the situation is much clearer and this population (TUZLABELI) is found with ‘*bourgaei*’ in all molecular-based analyses without genetic admixture. Similarly, populations from the eastern Western Taurus that were previously identified as ‘*bupleurifolia*’ due to their spatulate leaves −classified as ‘*bourgaei*’ in our study based on their entire leaf margins- consistently clustered with‘*bourgaei*’ in all phylogenetic analyses, which supports our interpretation (Fig. 3, S5-6).

Defining species and subspecies boundaries has long presented a significant challenge in plant taxonomy, especially for taxa occurring topographically and ecologically complex regions with continuous morphological variation (Cheng et al., 2021; Larsson et al., 2022; Padin & Calviño, 2023; Zhang & Chen, 2024). These challenges are particularly related to the mountainous systems/regions known for their high biodiversity and endemic richness (Janiczek et al., 2025; Ioan et al., 2025). The Taurus Mountains span southern Anatolia and function not only as a major physical barrier but also as a critical biodiversity refuge and dispersal corridor, while hosting a wide array of microclimates (Atalay, 2006; Médail & Diadema, 2009; Özüdoğru et al., 2020; Ioan et al., 2025).

These mountain regions offer highly dynamic environments, where climatic, edaphic, and habitat conditions can shift dramatically over very short distances. This promotes and sometimes restricts genetic and morphological differentiation among populations, making them important regions for speciation processes (Özüdoğru and Mummenhoff, 2020; Larsson et al., 2022; Janiczek et al., 2025). While habitat discontinuities in mountain systems can drive strong genetic divergence in some taxa, others exhibit more complex patterns shaped by historical connectivity rather than discrete geographic barriers (Janiczek et al., 2025).

Throughout the Quaternary period, glacial and interglacial cycles have profoundly influenced the genetics, morphology, and distribution dynamics of plant populations, as in other organisms (Bennett and Provan, 2008; Stewart et al., 2010; Gür, 2016). In northern Europe, advancing glaciers forced many species to retreat to southern refugia; during interglacial periods, these species recolonised their previous ranges (Hewitt, 2004; Schönswetter et al., 2005; Gür, 2017). However, in less glaciated regions like Anatolia, these dynamics operated differently. Especially in Anatolia’s high mountain systems, it has been proposed that species shifted from higher to lower elevations during glacial periods and retreated upward again during interglacials (Çıplak, 2008; Médail & Diadema, 2009; Özüdoğru et al., 2020). This elevational movement may have led to population isolation in narrow valleys or intermittent contact and genetic exchange (Stehlik, 2003; Bettin et al., 2007; Westergaard et al., 2011).

These interpretations and related taxonomic complexity in the Central Taurus (‘*kotschyi*’), especially populations around Aladağlar and Bolkar Mountains, can be contextualised in light of the glacial-interglacial dynamics of the Quaternary. Anatolian mountain systems served as both refugia and dispersal corridors. Unlike northern latitudes, where species were forced to retreat to southern refugia, Anatolian taxa could track suitable climates through short altitudinal shifts within deeply dissected topography (Médail & Diadema, 2009; Gür, 2016, Özüdoğru et al., 2020; Özüdoğru & Mummenhoff, 2020). This model explains the frequent morphological transitions observed in Central Taurus populations: during glacial periods, populations may have been isolated in separate valleys, while interglacial periods likely facilitated contact and admixture, giving rise to morphologically intermediate or hybrid populations. The unique geomorphology of the Aladağlar, characterised by steep vertical zoning and narrow canyons, would have further contributed to genetic isolation and microrefugial persistence. Similar instances of phylogeographic discontinuity have been recorded in other Anatolian taxa. For example, Doğru-Koca (2024) describes a significant genetic distinction between *Antalia* Doğru-Koca and *Kundmannia* Scop. This shows how historical allopatry and climate changes from the Pliocene to the Quaternary periods have affected plant diversity in Anatolia. This pattern is analogous to that seen in *H. bupleurifolia*.

Additionally, in the central region of the Bolkar Mountains, populations display remarkable uniformity in phenetic structure, corresponding to subsp. *rotundifolia*. This taxon, long referred to as *H. kotschyi* previously (e.g., Boissier, 1867) is characterised by consistently lobed leaves, small fruits, and the shortest styles among all *Heldreichia* populations. The ‘*rotundifolia*’ populations in the high alpine zones of the Aladağlar may have historically contacted more eastern lineages such as ‘*bupleurifolia*’, potentially giving rise to intermediate or clonal lineages. As Parolly et al. (2010) note, the unique geological structure of the Aladağlar, with steep vertical zoning and isolated canyon systems, likely promotes genetic isolation, further complicating morphological interpretations.

There are two main approaches for inferring phylogeny from multilocus sequence data. The traditional concatenation method is rooted in the total evidence paradigm and it combines gene sequences into a single dataset, assuming that increasing the number of variable sites enhances phylogenetic accuracy. However, this approach assumes that gene trees have the same topology across loci. When topological discordance exists, it is generally regarded as a phylogenetic error rather than the results of biological processes such as incomplete lineage sorting (Edwards, 2009; McVay & Carstens, 2013). As an alternative, certain methods have been designed to infer phylogeny while accounting for topological discordance among loci arising from coalescent processes (Carstens & Knowles, 2007; Degnan & Rosenberg 2009).

In light of the evidence of admixture in certain *Heldreichia* populations and the inconsistencies among the gene trees, we consider the ASTRAL topologies -despite their ambiguity in resolving relationships among the five main lineages- as the most plausible across our analyses. Consequently, the taxonomic framework adopted here is based on the three-subspecies model supported by SNAPPER subspecies delimitation, as this approach aligns with both phylogenetic uncertainty and with geographic and morphological patterns. From a taxonomic standpoint, this approach appears to be the most coherent, as alternative classifications would make it impossible to resolve infraspecific relationships. Following the reclassification of ‘*rotundifolia*’ to varietal level under *H. bupleurifolia* subsp. *bupleurifolia*, the genetically coherent populations in the central Taurus region, which resemble either ‘*rotundifolia*’ or ‘*bupleurifolia*’ in morphology, are now assigned to var. *rotundifolia* and var. *bupleurifolia* under subsp. *bupleurifolia*.

4.3. *Taxonomically cryptic cases*

Finally, two cryptic cases deserve particular attention. The first concerns *H. atalayi*, a taxon known only from its type specimen collected on Akdağ in Adıyaman, Eastern Taurus and not collected again since its initial description. This taxon was treated by Parolly et al. (2010) as a variety of *H. bupleurifolia* subsp. *rotundifolia*. However, during our fieldwork, specimens attributable to this taxon were collected from İliç (Erzincan), a locality that is both geologically distinct from the original site in Akdağ, Adıyaman (serpentine *vs.* limestone), and geographically distant from it. Comprehensive surveys conducted by our team on Akdağ, along with a floristic study of this mountain, identified only *H. bupleurifolia* subsp. *rotundifolia* in that area (Mutlu and Avcı, 2023). Importantly, Prof. Dr. Şinasi Yıldırımlı, who collaborated with Kit Tan at the Edinburgh Herbarium during the period when *H. atalayi* was described, indicated that the specimen likely originated from Erzincan but was mislabelled as being from Adıyaman −a claim that is fully consistent with our findings.

The second case involves herbarium specimens from Sandras Mountain (Muğla) that were provided by Prof. Dr. Hasan Yıldırım after the project. These specimens currently represent the westernmost known distribution of *H. bupleurifolia.* While their multiple lobed basal leaves resemble those of *H. bupleurifolia* subsp. *rotundifolia*, the notably large fruits are characteristic of *H. bupleurifolia* subsp. *bourgaei*. In our revised diagnostic key, subsp. *bourgaei* is primarily differentiated from other subspecies by fruit size. Taking into account both the fruit morphology and the pronounced geographic structuring observed within *Heldreichia* in terms of neutral genetic variations (PCA and Admixture), we tentatively classify this population as a variety of subsp. *bourgaei*, despite the lack of supporting genetic data, pending further investigation.

4.4. Taxonomical treatment

Heldreichia bupleurifolia Boiss. in Ann. Sci. Nat., Bot., ser. 2, 17: 186. 1842.

Type: Türkiye, Malatya: in monte Akdagh Cappadociae, Aucher 292 (Holotype: "Mak Dag", s.d., Aucher-Eloy 292 (G-BOIS [G00150392!]; Isotype: BM [BM000582585, BM000582586 https://data.nhm.ac.uk/object/e44feb95-d261-45f2-9eef-a3faa26391ba/1748251284062], G [G00096399!], K [K000484315!], P P01817519 [https://science.mnhn.fr/institution/mnhn/collection/p/item/p01817519?listIndex=31&listCount=516], P01817520 [https://science.mnhn.fr/institution/mnhn/collection/p/item/p01817520?listIndex=32&listCount=516], P01817521, [https://science.mnhn.fr/institution/mnhn/collection/p/item/p01817521?listIndex=33&listCount=516]).

Glabrous, glaucous rhizomatous geophyte with a slender, long-creeping rootstock up to 1 cm in diameter. **Stems** erect, 5–40(–50) cm tall, variously branched in the region of the inflorescence. **Leaves** coriaceous to subsucculent. **Basal leaves** long-petiolate; lamina linear, oblong-spathulate or obovate; cuneate, truncate or cordate at base, (0.7–)1–3(–5) × 0.5–3 cm; trilobed, palmately (up to –7 lobes) or 1–3 pinnately lobed with a length-to-width ratio of 0.6–1.5 mm; petiole 2–15 mm. **Stem leaves** decrease quickly in size upwards, often absent in fruiting material. **Flowers** white, tinged lilac; petals obovate, shortly clawed, 4 × 3 mm; **sepals** membranous at margins, spreading. **Filaments** ascending, broad at base, with a small lateral tooth; **anthers** ovate. Lateral nectaries semi-annular, intrastaminal, confluent with median. Fruiting pedicels are filiform, (2.5–)5–9(–15) mm, suberect, sometimes reflexed. **Fruit** (silicula) obovate sometimes obcordate to oval, cuneate or truncate at base, truncate or obcordate at apex (3.5–)4–7(–8.5) × (3.5–)5–8.5(–11) mm, angustiseptate; septum < 1 mm wide, with a midvein. Valves strongly compressed, keeled, non-winged. **Style** (0.3-)0.5–1.5 mm; stigma entire or weakly bilobed. **Ovules** 2 per fruit. **Seeds** non-mucilaginous, brown, 1.8–2.5 x 1.8–2 mm, weakly winged, with a small umbo at the apex. Cotyledons are flat, radicle accumbent, thin and longer than the cotyledons.

*The description was revised primarily based on Parolly et al. (2010), incorporating the findings of the present study.

1.Fruit large, 6–8.5 × 7.5–9.5(−10.5) mm; if smaller, basal leaves entire…… subsp. **bourgaei.**

1.Fruit smaller, (4.5-) 5 (−6) × 3–6(6.5) mm; if larger, basal leaves both entire and lobed on the same individual……………………………………………………………………………**.2.**

2. Stems branching from the base (up to 20 branches), branching dichotomously towards the apex; leaves deeply pinnatifid; pedicels distinctly long, (9.5-) 11–15 mm….subsp. **malatyana.**

3. Stems branches generally fewer; leaves entire, trilobed to palmately lobed (up to 7 lobes) or 1–3 pinnately lobes; pedicels short, (3-)4–6.5(−8) mm……………………subsp. **bupleurifolia**

−***Heldreichia bupleurifolia*** Boiss. subsp. ***bupleurifolia***

1. Basal leaves entire or both entire and lobed on the same individual; petiole generally long, up to 13 cm……………………………………………………………………………………… var. **bupleurifolia.**

1. Basal leaves deeply lobed; petioles mostly shorter than 9 cm….……………………………………… **2.**

2. Basal leaves always 2(3) pinnatifid; petioles shorter, up to 6 cm; leaf length always greater than width, leaf length/width ratio > 1.5 ………………………………………………..var. **atalayi.**

2. Leaves mostly 3–5 lobed, rarely 7-lobed or pinnatifid; petioles longer, up to 9 cm; leaf length not always greater than width, leaf length/width ratio < 1.5 ………………var. **rotundifolia.**

Heldreichia bupleurifolia Boiss. subsp. ***bupleurifolia*** var. ***rotundifolia*** (Boiss.) I.D.Can & Özüdoğru, **comb. nov.** ≡ *H. rotundifolia* Boiss. *Boiss. in Ann. Sci. Nat., Bot. ser. 2, 17: 185. 1842.* = *Heldreichia kotschyi* Boiss. in Ann. Sci. Nat., Bot. ser. 2, 17: 186. 1842. =*H. bupleurifolia* subsp. *rotundifolia* (Boiss.) Parolly et al. in Taxon 59: 198. 2010.

Type: Monte Olympo Armeniae Turcicas, Aucher-Eloy 293 (Auch. exs. 293!) Lectotype: (designated in Al-Shehbaz & Barriera, 2019): Türkiye "in Olympo Armenia", s.d., *Aucher-Eloy 293* (G-BOIS [G00150313!]; Isolectotype: BM [BM000582603], G [G00096292!], K [K000484143!], MO [MO1617689], P [P01817524, P01817525, P01817526]).

Heldreichia bupleurifolia Boiss. subsp. ***bupleurifolia*** var. ***atalayi*** (Kit Tan) I.D.Can & Özüdoğru, **comb. nov.** ≡ *H. atalayi* Kit Tan in PI. Syst. Evol. 154: 113. 1986 = *H. bupleurifolia* subsp. *rotundifolia* var. *atalayi* (Kit Tan) Parolly, Nordt & Mumm in Parolly in Taxon 59: 187. 2010.

Type: Türkiye, Adıyaman: east flanks (lower slopes) of AkDag, 33 km from Adıyaman, open scree 1300 m, 17.6.1983 (E [E00373127!]).

Heldreichia bupleurifolia Boiss. subsp. ***bourgaei*** (Boiss.) Parolly, Nordt & Mumm var. ***bourgaei*** ≡ *H. bourgaei* Boiss., Flora Orientalis 1: 320, 1867.

Type: Türkiye, Antalya: In alpine rocky areas of Akdağ, 3 July 1860, Bourgeau 19. Holotype: In glareosis regionis alpinis montis Akdagh Lyciae, 3.VII.1860, *Bourgeau 19* (G-BOIS [G00150391!]; Isotype: B [B100673498, B100673499], E [E0373125, E00373126], G [G00096398, G00096400, G00446238], GOET [GOET002606], K [K000484139], LE [LE00012865], P [P06648028, P06648034, P06648037, P06648039], W [W0075594, W18890072809, W18890152678].

1.Basal leaves entire, linear or lanceolate………………………………………… var. **bourgaei.**

1 Basal leaves (3-)5-lobed lobed or mixture of simple and 3-lobed……………………….…**2**

2.Basal leaves a mixture of simple and 3-lobed………………………………..var. **polymorpha.**

2.Basal leaves (3-)5-lobed lobed…………………………………………………var. **sandrasica**

Heldreichia bupleurifolia Boiss. subsp. ***bourgaei*** (Boiss.) Parolly, Nordt & Mumm var. ***polymorpha*** (Parolly, Nordt, Eren & Mumm.), I.D. Can & Özüdoğru **comb. & stat nov.** ≡ *Heldreichia bupleurifolia* subsp. *polymorpha* Parolly, Nordt, Eren & Mumm. in Parolly in Taxon 59: 187. 2010.

Type: Türkiye, Muğla, Fethiye, Çameli, southern Tuzla Pass, 1390–1440 m, steep limestone scree slope, north-facing, 20.6.2005, Ulrich 5/6a (holotype: B; isotypes: AYDN, E, herb. Parolly)

Heldreichia bupleurifolia Boiss. subsp. ***bourgaei*** (Boiss.) Parolly, Nordt & Mumm. var. ***sandrasica*** I.D.Can & Özüdoğru **var. nov.** Fig. S7

Type: Muğla: Köyceğiz, Sandras Mountain, from Gökçeova lake to Kartal Lake, serpentine, stony area, N: 37′ 04″ 26, E: 028′ 49″ 31, 1804 m, 18.10. 2008, H.Yıldırım 1481 (Holotype EGE! isotypes: ANK, HUB, HBH, NGBB.

Diagnosis: A new variety clearly distinguished from the other two varieties by its consistently (3-)5-lobed leaves (vs. exclusively entire leaves in var. *bourgaei*, and highly variable leaves — from entire to 1–3-lobed, often mixed within individuals — in var. *polymorpha*).

−***Heldreichia bupleurifolia*** Boiss. subsp. ***malatyana*** Özüdoğru & Yıldırım

Type: Türkiye, Malatya: Akçadağ, Levent Canyon, the path down to the canyon, small slopes with loose rocks, 1275 m, 30 vi 2010, H. Yıldırım 1745 (Holotype EGE [EGE-43206!]) Isotype (EGE [EGE-43207!], HUB!)

Declaration of Generative AI and AI-assisted technologies in the writing process

ChatGPT was used to identify grammatical issues in parts of the manuscript. All content was subsequently reviewed and edited by the authors.

CRediT authorship contribution statement

Ilgın Deniz Can: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Emrullah Yılmaz:** Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Kurtuluş Özgişi:** Resources, Project administration, Conceptualization. **Hakan Gür:** Resources, Project administration, Conceptualization. **İsmail Kudret Sağlam:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Project administration, Methodology, Investigation, Conceptualization. **Barış Özüdoğru:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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.

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Data accessibility statement.

The raw sequencing reads have been submitted to the NCBI Sequence Read Archive (SRA) under accession number PRJNA1272478 and the other relevant files (scripts, trees, VCF files etc.) can be accessed at https://osf.io/qxrct.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2025.108518.

Data availability

I have shared the link in the manuscript

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