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# Distribution and morphological differentiation of native alder taxa (*Alnus* Mill.) in the Iberian Peninsula

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ABSTRACT

The riparian environments of Europe host a remarkable richness of plant communities, often dominated by a single tree species. Understanding the identity of this species, including its morphology and distribution, is essential for the effective management and conservation of biodiversity. In Iberian alder forests, *Alnus lusitanica* Vít, Douda & Madák appears to be more common than *Alnus glutinosa* (L.) Gaertn., which dominates most of western Europe. Previous studies based on limited sampling suggested *A. lusitanica* was widespread in the western and southern Iberian Peninsula, while *A. glutinosa* was scattered in the Pyrenees and across the Cantabrian Range. Although some morphological differences have been observed, the two species are mainly differentiated by their ploidy levels: *A. lusitanica* is tetraploid, and *A. glutinosa* is diploid.

This study aims to document the detailed distribution and morphological differentiation between *A. lusitanica* and *A. glutinosa* in the Iberian Peninsula, and determine whether putative hybrids (triploids) exist. Fresh and herbaria samples covering the entire Iberian range of *Alnus*, plus others from Europe, were collected. Ploidy levels were determined by flow cytometry. A morphometric study was also carried out with 26 variables and ratios. The results indicate that *A. glutinosa* is more widespread than previously reported and no triploid hybrids were detected. The distinction from *A. lusitanica* can be made using a set of characters. These are described in a new identification key that successfully identifies 87.5% of specimens.

## 1. Introduction

Effective management of forest landscapes requires a comprehensive understanding of the species that shape their ecological diversity. In Europe, the two most relevant classifications of forest communities, EUNIS (Chytrý et al., 2020) and European Forest Types (European Environment Agency, 2006), together with the Habitats Directive focused on forest conservation (Council Directive 92/43/ECC), are primarily based on the dominant woody species. Consequently, it is crucial to recognize the diagnostic tree species of upland but also of riparian forests (Leblanc et al., 2024). Riparian forests, in particular, are gaining increasing attention since they hold remarkable biological richness (Naiman et al., 1993; Biurrun et al, 2016; Leo et al., 2019) and play critical ecological functions, providing a range of ecosystem services (de la Fuente 2018; Riis et al., 2020). Despite their relevance, river ecosystems and associated forests remain poorly studied and conserved (Tockner & Stanford, 2002; Richardson et al., 2007; Hoppenreijs et al., 2022) and hence greater efforts are needed to fully understand their diversity.

Forests, particularly those in riparian biotopes, support a diversity that remains largely unexplored, with new species, including trees, still being discovered (Hopkins, 2007; Cheek et al., 2020; Du et al., 2020; Manzitto-Tripp et al., 2022). To comprehend biodiversity, it is necessary to have detailed knowledge of its components. Ideally, this knowledge

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should be defined using intergrated criteria which combine genetics, ploidy levels, morphology, distribution, and ecology (Dayrat 2005; Queiroz 2007). Morphology remains the fundamental tool for species recognition (Dayrat, 2005), but subtle or absent morphological differences make species discrimination challenging, leading to pseudo-cryptic and cryptic species being described (Mann & Evans, 2008). Objective morphological analyses using numerous characters are critical. In addition, ploidy level data can be relevant for detecting cryptic taxa (Kobrlova et al., 2016; Zhang et al., 2023), and detailed distribution maps are equally essential for effective biodiversity recognition and conservation planning. Integrating distinct approaches provides a comprehensive view of biodiversity, yet it can complicate taxonomic ranking when different methods yield inconsistent results (Kobrlova et al., 2016; Wang et al., 2022).

*Alnus* Mill. is a monophyletic genus of the Betulaceae family, consisting of riparian tree species that form alder forests. Its 41 accepted taxa are distributed across the northern hemisphere and the Andes (POWO, 2024). In Europe, thanks to the remarkable work of Vít et al. (2017), six native species are recognized (POWO, 2024): *Alnus cordata* (Loisel.) Duby, *A. alnobetula* (Ehrh.) K.Korch, *A. incana* (L.) Moench, *A. glutinosa* (L.) Gaertn., and the recently described *A. rohlenae* Vít, Douda & Mandák and *A. lusitanica* Vít, Douda & Mandák. Two species have been identified in the riparian forests of the Iberian Peninsula, the diploid *Alnus glutinosa* (L.) Gaertn (*2n = 28*), and the tetraploid *A. lusitanica* Vít, Douda & Mandák (*2n = 4x = 56*), and they show distinct distribution ranges (Mandák et al., 2016; Vít et al., 2017). *Alnus glutinosa* extends across Europe, from the Scandinavian Peninsula and western Russia to the Mediterranean basin, reaching some northern localities of the Iberian Peninsula. This broad distribution appears to have arisen due to its post-glacial expansion from southern marginal regions of Europe (Mandák et al., 2016). In contrast, *A. lusitanica* is present in the western half of the Iberian Peninsula and in Morocco (Harvdová et al., 2015; Mandák et al., 2016; Vít et al., 2017). Formerly considered part of *A. glutinosa, A. lusitanica* is now recognized as a separate species (Vít et al., 2017) which likely arose from autopolyploidy (Mandák et al., 2016).

In the past decade, considerable progress has been made in understanding the distribution of the two *Alnus* species in the Iberian Peninsula (Vít et al., 2017; Sanna et al., 2023; Martín et al., 2024), yet the precise geographical limits of both species remain unclear due to a lack of studies with extensive sampling. Likewise, the diagnostic morphological traits that have been provided to date (Vít et al., 2017) do not appear to fully resolve the identification of alder specimens across the entire Iberian distribution range (pers. obs.). *Alnus glutinosa* exhibits substantial morphological variability (Colagar et al., 2016; Gholamiterojeni et al., 2019; Jurkšienė et al., 2021) that might have obscured differences with *A. lusitanica* (Gomes Marques et al., 2022, 2024; Vít et al., 2017). Moreover, it is important to note that identifying both species may also be challenging due to potential hybridisation events between them. Recent evidence from eastern Europe shows tetraploid *A. rohlenae* hybridising with diploid *A. glutinosa* to produce viable triploids (Šmíd et al., 2020). This fact, along with genetic admixture evidence (Mandák et al., 2016; Šmíd et al., 2020), suggests potential gene flow despite ploidy differences. In this context, the possibility of hybridisation events between *A. lusitanica* and *A. glutinosa* in contact zones should be considered. Despite these observations, comprehensive morphological and ploidy studies of *A. glutinosa* and *A. lusitanica* across their entire Iberian range are lacking.

Alder forests are key components of the riparian vegetation across the western and northern Iberian Peninsula, hosting a remarkable assemblage of plants with Atlantic, Eurosiberian, sub-Mediterranean, Macaronesian, and even Paleotropical affinities (Lara et al., 2007; Garilleti et al., 2012; Loidi, 2017). In addition, these forests play different ecological roles, such as stabilisation of watercourses and nitrogen fixation (Sabater et al., 2000; Bjelke et al., 2016). Due to its biogeographical relevance in Europe, alder forests are classified as a priority habitat (91E0*) under Annex I of Directive 92/43/EEC. However, in recent decades, European alder forests have been increasingly affected by the oomycete *Phytophthora alni* Brasier & S.A.Kirk. This pathogen reduces the tree's canopy by affecting the roots and vascular system (Bjelke et al., 2016) and has already damaged alder forests of northern and western territories of Spain and Portugal (Solla et al., 2010; Martín et al., 2024; Kanoun-Boulé et al., 2016; Bregant et al., 2023). In this context, a comprehensive understanding of the characteristics and distribution of *A. glutinosa* and *A. lusitanica* would be helpful for implementing conservation actions.

The lack of research across the entire distribution of the two alder species in the Iberian Peninsula hinders the acquisition of detailed knowledge about their full distribution ranges, their potential for hybridisation, and the key diagnostic morphological traits needed to identify them. To address these aspects, we carried out an extensive sampling and analysed ploidy levels and morphological characters to: 1) accurately determine the distribution of *Alnus glutinosa* and *A. lusitanica*, 2) identify putative hybrids, and 3) identify key morphological characters to enable robust species discrimination. Such enhanced data are vital for supporting the conservation of riparian ecosystems structured by Iberian alder species.

## 2. Material and methods

### 2.1. Study species

In the Iberian Peninsula, the diploid species *Alnus glutinosa* is found in isolated sites along the eastern and western Pyrenees and the western Cantabrian Range. In contrast, the tetraploid *A. lusitanica* has been reported more widely, with dispersed records across all of Portugal, in multiple locations in northern Spain, and in scattered sites in central and southern Spain (Vít et al., 2017; Sanna et al., 2023). Both species are wind-pollinated and self-incompatible riparian trees that can reach up to 35 m in height, although they rarely exceed 20 m (Lara et al., 2007) (Fig. 1). The trees's root system establishes actinorhizal nodules with *Frankia alni* (Woronin 1866) Von Tubeuf 1895, thereby enabling the tree to fix nitrogen (McVean, 1953). These alders mostly grow on acidic substrates, along riverbanks with perennial water flow due to their high water requirements (Lara et al., 2007).

### 2.2. Field sampling

This study includes 181 samples from an equal number of *Alnus* individuals (Fig. 2, **Annex I**): 160 samples collected by us in Spain from

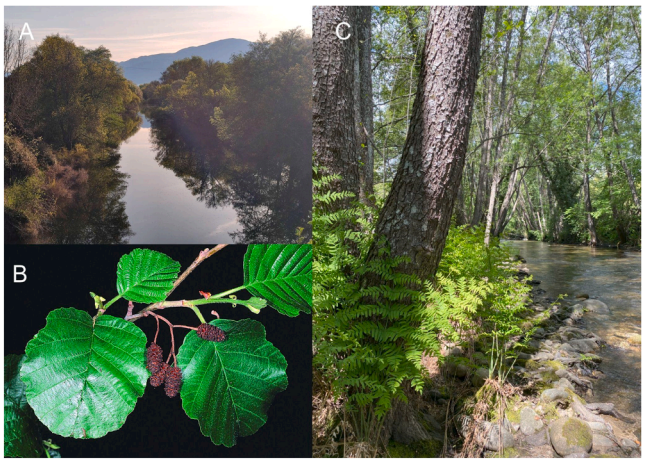


**Fig. 1.** A: Alder forest, Caparra River, Cáceres, Spain. B: Leaves and female catkins of an *A. lusitanica* individual. C: Alder trees with *Osmunda regalis* growing at the base of the trunks in Pedro Chate River, Cáceres, Spain.

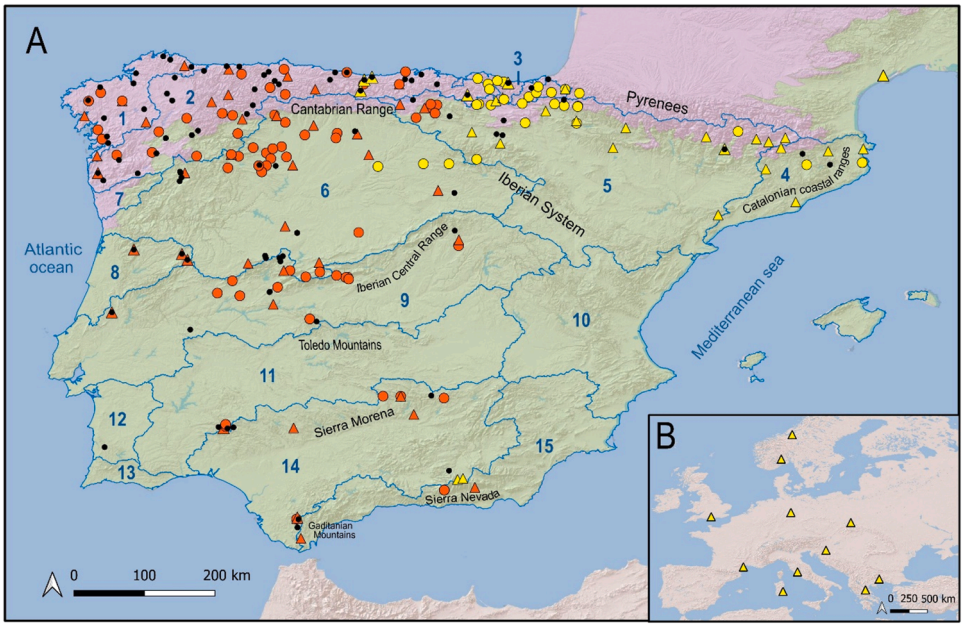


**Fig. 2.** Geographical location of *Alnus* samples used in this study. Orange symbols correspond to *A. lusitanica* and yellow symbols to *A. glutinosa*. **A)** Iberian samples. Triangles represent individuals used in morphometric analysis. Black points correspond to localities analysed in previous studies (Havrdová et al., 2015; Vít et al., 2017; Gomes Marques et al., 2022; Sanna et al., 2023; Martín et al., 2024). The biogeographic Eurosiberian and Mediterranean regions are indicated with pink and green colour respectively. Blue lines and numbers refer to the main hydrographic districts: 1-Galician coastal, 2-Miño, 3-Basque Country internal basins, 4-Internal basins of Catalonia, 5-Ebro, 6-Douro, 7-Cavado, Ave and Leça, 8-Vouga, Mondego and Lis, 9-Tagus, 10- Júcar, 11-Guadiana, 12-Sado and Mira, 13-Algarve basins, 14-Guadalquivir, 15-Andalusian Mediterranean basins. **B)** Samples from outside the Iberian Peninsula used in the study. Triangles indicate the location of individuals used in the morphometric analysis.

142 localities, and 21 herbarium specimens from other parts of Europe (16 specimens) and specific Spanish locations (5 specimens). Spanish samples were collected between April 2023 and December 2023 throughout most of the *Alnus* distribution range. Branches with mature leaves and, when possible, mature female catkins of the 160 *Alnus* sampled individuals were collected. Samples were chosen from analogous trees of similar heights and solar exposure, excluding basal branches and shoots. The samples were carefully dried (**Table S1**), following standard collecting and curatorial practices. In particular cases, fresh material was also collected from the same individual. For herbarium specimens, the five samples from northern Spain were loaned by FCO, all of them previously used in Sanna et al. (2023), whereas the 16 European samples from outside Spain, including Portugal, were obtained from PRA (nine samples, all cited by Vít et al., 2017), and MA (seven specimens). Herbaria acronyms follow Index Herbariorum (Thiers, 2024) (**Table S2**).

### 2.3. Estimation of DNA ploidy level

To determine the distribution of *Alnus glutinosa* and *A. lusitanica* and search for discriminant morphological characters, the taxonomic identity of each individual was assessed by its ploidy level (Mandák et al., 2016; Vít et al., 2017; Martín et al., 2024). The ploidy level was obtained using the flow cytometry protocol of Márquez-Corro et al. (2023). Most analyses were conducted on dried leaf samples, except for three individuals, for which both fresh and dry samples were analysed to verify the accuracy of the method using only dried samples. In two additional cases, fresh material was also analysed to enhance resolution and confirm putative hybrids. In all cases *Petroselinum crispum* (Mill.) Fuss (2C = 4.50 pg; Obermayer et al., 2002) was used for the internal calibration. General Purpose Buffer (GPB; Loureiro et al., 2007) was used and supplemented with 3 (w/v) % PVP-40 (Pellicer et al., 2020) for sample processing. One millilitre of buffer was added to the target sample and internal standard, then the leaves were co-chopped with a razor blade. An additional 1 ml of buffer was added, and the sample was filtered through a 30 μm pore size CellTrics filter (Sysmex). Afterward, 100 μl of propidium iodide (PI, 1 mg/ml; Sigma) was added, and the sample was kept on ice for ca. 20 minutes. Finally, the samples were analysed using a CyFlow Space cytometer (Sysmex) fitted with a Cobolt Samba laser (532 nm). We stopped the flow cytometer after the target sample and the standard had reached at least 800 nuclei per fluorescence peak.

### 2.4. Multivariate morphometric analysis

A morphometric approach was employed to evaluate the possibility of discriminating *Alnus lusitanica* from *A. glutinosa*. A total of 19 characters (semi-quantitative, quantitative and binary) and seven ratios were considered as explanatory variables for the 80 samples measured: 40 from each species (Table 1). The characters used by Vít et al. (2017) were initially selected. However, those that were not very informative were discarded as they focused not only on the differentiation of *A. glutinosa* and *A. lusitanica* but also of *A. incana* and *A. rohlenae*. The characters employed were: *Distance from lamina base to maximum lamina width along the central vein* (BC); *distance from lamina base to maximum lamina width* (BM); *female catkin length* (CL); *female catkin stalk length* (CS); *female catkin width* (CW); *dorsal lamina hairiness* (DH); *presence of hairs on annual shoots* (HA); *presence of hairs on buds* (HB); *presence of hair floccules in lateral vein axils* (HF); *lamina length* (LL); *lamina margin indentation* (LM), *leaf lobation* (LO); *lamina apex* (LS); *lamina width* (LW); *petiole length* (PL); *number of pairs of lateral veins* (PV); *shape of annual shoot lenticels* (SA); and *ventral lamina hairiness* (VH). The characters DH, HF, LM, LO, LS, SA and VH needed to be re-scaled to reflect the variability observed in these *Alnus* species and the character lamina base (LB) was included (**Figure S1**). Three leaves and one catkin per individual were measured. The program *ImageJ* (Schneider et al., 2012) was employed to estimate quantitative characters (**Figure S2**). Semi-quantitative characters were scored using numerical scales as is reflected in Table 1. To obtain a unique value for each individual, the mean of the leaf characters was calculated. Missing values were replaced

**Table 1**
List of analysed morphological characters.

| | Abbrev. | Character description | Unit |
|---|---|---|---|
| 1 | LL | Lamina length | cm |
| 2 | LW | Lamina width | cm |
| 3 | BM | Distance from lamina base to maximum lamina width | cm |
| 4 | BC | Distance from lamina base to maximum lamina width along the central vein | cm |
| 5 | PL | Petiole length | cm |
| 6 | PV | Number of pairs of lateral veins | Number |
| 7 | VH | Ventral lamina hairiness | Scale (1: glabrous, 2: individual hairs, 3: lightly hairy) |
| 8 | DH | Dorsal lamina hairiness | Scale (1: glabrous, 2: individual hairs, 3: lightly hairy) |
| 9 | HF | Presence of hair floccules in lateral vein axils | Scale (1: glabrous or individual hairs, 2: hairy, 3: densely hairy) |
| 10 | LS | Lamina apex | Scale (1: most prominent points of margin above the central vein, 2: most prominent points aligned with the central vein, 3: most prominent points of margin below the central vein) |
| 11 | LM | Leaf margin dentation | Scale (1: denticulate; 2: denticulate-dentate, 3: dentate; 4: dentate-obtusely dentate, 5: obtusely dentate, 6: obtusely dentate-crenate) |
| 12 | LO | Presence of leave lobation | Scale (1: unlobed or lightly lobed, 2: lobed, 3: highly lobed) |
| 13 | LB | Lamina base | Scale (Taking margins up to the height of the first pair of veins, 1: wedge, angle $> 90°$; 2: lightly wedge, $< 90°$; 3: straight, angle $\approx 180°$) |
| 14 | HA | Presence of hairs on annual shoots | Binary (0: absence; 1: presence) |
| 15 | HB | Presence of hairs on buds | Binary (0: absence; 1: presence) |
| 16 | SA | Shape of annual shoot lenticels | Scale (1: circular, 2: oval and circular, 3: oval) |
| 17 | CS | Catkin stalk length | cm |
| 18 | CL | Catkin length | cm |
| 19 | CW | Catkin width | cm |
| 20 | LW/LL | Ratio of distance from lamina width and length | Ratio |
| 21 | BC/LL | Ratio of distance from lamina base to maximum lamina width along the central vein and lamina length | Ratio |
| 22 | PL/LL | Ratio petiole length and lamina length | Ratio |
| 23 | CS/PL | Ratio of catkin stalk length and petiole length | Ratio |
| 24 | PV/LL | Number of veins per 1 cm of lamina length | Ratio |
| 25 | CL/CS | Ratio of catkin length and catkin stalk length | Ratio |
| 26 | CL/CW | Ratio of catkin length and with | Ratio |

with the taxon mean obtained from at least 90% of its individuals [following Vít et al. (2017)]; for example, if a leaf value was missing, the individual's mean was calculated by including the substituted value along with the other recorded measurements.

Morphometric analyses were performed using the *MorphoTools2* package (Šlenker et al., 2022) in *RStudio v.4.2.2* (Posit team, 2024). Basic statistics (minimum, 5th percentile, mean, 95th percentile and maximum) were calculated. Twelve variables fitted a normal distribution (**Table S3**). The non-parametric Spearman's correlation coefficient was employed to verify the potential correlation between characters. In cases where pairs of characters showed a Spearman's coefficient $\geq 0.70$, one of them was discarded in the following analyses (Overholser & Sowinski, 2008). Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA) were used to explore the relationship between both species. Then, we employed a Classificatory Discriminant Analysis (ClDA), using the non-parametric k-nearest neighbour discriminant function, to determine the percentage of correctly classified individuals. Three different approaches were used to select the variables included in the ClDA: variables identified by stepwise selection, those most highly correlated with the first canonical axis (from the CDA analysis), or those most correlated with the first principal component (from the PCA analysis), all aimed at maximizing the percentage of correctly classified individuals. The cross-validation method was employed to determine the discriminant power, and the selected k value provided the highest success rate. The characters included in the most successful ClDA, along with those correlated with them, were compared between the two species using different methods: the ANOVA test for continuous normally distributed characters, the Mann-Whitney-Wilcoxon test for non-normally distributed continuous variables, and contingency tables and Fisher's exact test (with Holm's correction for multiple comparison) for semi-quantitative characters. The odds ratios (OR) from Fisher's exact test indicate the relative probability of observing each level of each trait in *Alnus lusitanica* compared to that of *A. glutinosa*: $OR < 1$ indicated a higher probability of observing a particular level of the trait in *A. lusitanica* than in *A. glutinosa*, $OR > 1$ indicated a lower probability of observing a particular level of the trait in *A. lusitanica* than in *A. glutinosa*, and $OR = 1$ indicated no difference in the probability of a particular level of the trait between the two taxa.

Finally, based on Vít et al. (2017), two more ClDAs were done to evaluate their identification success in our data: 1) an analysis based on the most tightly correlated characters with their canonical axis, and 2) an analysis based on the characters used in their determination key for *A. lusitanica* and *A. glutinosa*.

## 3. Results

### 3.1. Flow Cytometry

The approximate mean 2C-values estimated from the dried samples were 2.8 pg (CV% ranging from 5.49% to 22.85%) for *Alnus lusitanica*, and 1.34 pg (CV% ranged from 6.28% to 17.99%) for *A. glutinosa*. Despite the wide range of coefficients of variations, it was possible to distinguish the peaks (**Figure S3**) and hence be confident of the ploidy level assigned to each sample. Fresh material was analysed for some individuals (051, 052, and 053) to verify that dried leaves were suitable for ploidy level determination. These provided the same results in terms of species identification based on ploidy level. Thus, both types of material identified *A. lusitanica* for the individuals analysed i.e. (i) fresh material of individual *051* $2C = 2.35 \pm 0.055$ pg (CV = 2.53%) vs. dried material of individual *051* $2C = 2.53 \pm 0.005$ pg (CV = 6.67%), (ii) fresh *052* $2C = 2.30 \pm 0.004$ pg (CV = 3.28%) vs. dried *052* $2C = 2.33 \pm 0.011$ pg (CV = 7.82%), and (iii) fresh *053* $2C = 2.29 \pm 0.001$ pg (CV = 2.55%) vs. dried *053* $2C = 2.70 \pm 0.010$ pg (CV = 8.92%).

### 3.2. Distribution

Based on the ploidy levels determined by flow cytometry, 53 diploid individuals corresponding to *Alnus glutinosa* and 106 tetraploid individuals corresponding to *A. lusitanica* were detected. *Alnus glutinosa* was shown to be widely distributed in the north and northeast of the Iberian Peninsula, mainly thriving throughout the entire range of the Pyrenees but also in the Ebro basin. It was also the prevalent taxon in the Basque Country internal basins (located between the Pyrenees and the Cantabrian Range, Fig. 2). Likewise, *A. glutinosa* showed an intrusion within the Cantabrian Range, surrounded by *A. lusitanica*. It was also seen to penetrate into the eastern region of the Douro basin and Iberian System, and into the Catalonian Coastal Ranges within the Mediterranean region with a disjunct locality in the southeast corner of the Iberian Peninsula, specifically on the northern slope of the Sierra Nevada

(Fig. 2). In contrast, *A. lusitanica* was distributed mainly throughout the western side of the Iberian Peninsula, from the Cantabrian Range to the Gaditanian Mountains. Its range also encompassed the entire Iberian Central Range, Toledo Mountains, Sierra Morena, and the Sierra Nevada. Thus, it was found both in coastal and inner territories within the Eurosiberian and Mediterranean regions, across most of the main hydrographic districts: Galicia coast, Miño and Sil, Cantabrian, Douro, Ebro, Tagus, Guadiana, Guadalquivir and in the Andalusian Mediterranean basins (Fig. 2).

### 3.3. Potential hybridisation

The flow cytometry analysis of dried material from three individuals suggested that their ploidy level might be triploid (**Table S1**, symbol "*"), raising the possibility that they were hybrids formed between the two Iberian species. The use of fresh material of two of them (*098* and *103*) however, showed that they were diploid, corresponding to *Alnus glutinosa*, thus ruling out their hybrid origin (fresh *098* 2C = 1.22 ± 0.005 pg, CV = 6.46%; fresh *103* 2C = 1.26 ± 0.023, CV = 6.37%). A third putative hybrid (dry *089* 2C = 1.87 pg, CV = 10.95%) remained unresolved due to the inability to obtain a suitable fresh sample.

### 3.4. Multivariate morphometric analysis

Out of the 19 characters and seven ratios measured (Table 1), four characters (LW, BM, BC and CW) and three ratios (PV/LL, PL/LL and CL/CS), were correlated with another variable and were therefore discarded (Table 2). The remaining 19 variables were included in the Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA). The PCA showed a large morphological overlap between the two species (Fig. 3). However, axis PC1 distinctly separated some Iberian *Alnus glutinosa* specimens with low values of *petiole length* (PL) and *lamina length* (LL) from a group of *A. lusitanica* samples with greater values of the *catkin stalk length/petiole length* ratio (CS/PL) and *ventral lamina hairiness* (VH). Individuals of *A. glutinosa* from the Cantabrian intrusion (Fig. 2) appeared to be mixed in with most of the *A. lusitanica* individuals, whereas the two *A. glutinosa* samples from the Sierra Nevada grew close to each other and separate from the *A. lusitanica* samples. The contributions of each character to PC1 are shown in Table 2.

**Table 2**
Contribution of the characters shown in Table 1 to the first component axis in PCA and CDA. Discarded characters because they correlated with others are marked with "-".

| | PCA | CDA |
|---|---|---|
| HA | 0.052 | 0.128 |
| HB | -0.084 | -0.097 |
| SA | -0.156 | -0.051 |
| CS | 0.222 | 0.586 |
| CL | 0.049 | 0.326 |
| CW | - | - |
| LL | -0.292 | -0.151 |
| LW | - | - |
| BC | - | - |
| BM | - | - |
| PL | -0.375 | -0.030 |
| PV | -0.253 | -0.255 |
| VH | 0.332 | 0.227 |
| DH | 0.266 | -0.068 |
| LS | 0.176 | 0.528 |
| LM | 0.206 | 0.153 |
| LO | 0.179 | 0.326 |
| LB | 0.280 | 0.392 |
| HF | -0.049 | -0.029 |
| LW/LL | 0.203 | 0.575 |
| BC/LL | -0.178 | -0.352 |
| CS/PL | 0.399 | 0.367 |
| CL/CW | 0.158 | 0.449 |
| PV/LL | - | - |
| PL/LL | - | - |
| CL/CS | - | - |

The CDA also exhibited overlap between *Alnus glutinosa* and *A. lusitanica* (Fig. 4). The three most highly correlated variables with the first canonical axis (Can1) were the same variables provided by stepwise approximation: *catkin stalk length* (CS), *lamina width/lamina length* ratio (LW/LL) and *lamina apex* (LS). Their contributions to the Can1 are included in Table 2. The ClDA based on the CDA reached the maximum percentage of individuals correctly assigned to the predefined groups (i.e. 87.50 %) by including CS, LW/LL and LS variables (Table 3). The rate of correctly classified *A. lusitanica* individuals was 85 %, while for *A. glutinosa* it reached 90 %. These variables, and the ratio *catkin length/catkin stalk length* (CL/CS) which correlated with CS, showed differences between *A. lusitanica* and *A. glutinosa: A. lusitanica* had greater values of CS and LW/LL, smaller values of CL/CS and a higher frequency of straight lamina apex (LS) than *A. glutinosa* (Fig. 5**, Tables S4, S5**). However, the overlap between the two species is remarkable, especially in LS (Fig. 5). The ClDA based on PCA, showed a lower maximum rate of success classifying the individuals and a higher number of variables were needed (13 variables, Table 3).

## 4. Discussion

### 4.1. Distribution of Alnus in the Iberian Peninsula

The number of Iberian localities considered in this study for both *Alnus lusitanica* and *A. glutinosa* collected by us (142 localities), is greater than the total number of localities considered in previous studies (94 sites) (Havrdová et al., 2015; Vít et al., 2017; Gomes Marques et al., 2022; Sanna et al., 2023; Martín et al., 2024). In those previous studies the Mediterranean and Pyrenean regions were highly underrepresented, with the central area of the Pyrenees being particularly overlooked. In contrast, our study offers comprehensive information on the distribution of both taxa in the Iberian Peninsula with extensive representation of Pyrenees and Mediterranean interior and eastern regions. In any case, it is noted that Iberian alders are acidophilic, making alder forests more common in the siliceous western half of the Iberian Peninsula, and since they thrive in humid climates, they are mainly restricted to mountainous areas in the Mediterranean region (Lara et al., 2007; Rodríguez Fernández et al., 2014).

*Alnus glutinosa* had been previously reported in scattered locations in the eastern and western Pyrenees and the eastern part of the Cantabrian Range (Vít et al., 2017; Sanna et al., 2023; Martín et al., 2024), with a single locality reported in central-western Spain (Sánchez Anta et al., 1987). However, our analyses find that *A. glutinosa* extends beyond the localised eastern part of the Cantabrian Range, encompassing the entire range of the Pyrenees, the Iberian System and the Douro basin (near some populations of *A. lusitanica*). Furthermore, *A. glutinosa* was also recorded across the Catalonian Coastal Ranges up to the Tarragona province, where it has its natural southern limit. Additional populations were also found further south, on the northern slopes of Sierra Nevada, although their natural origin is doubtful. Overall, this study reveals that populations of *A. glutinosa* are more common and widespread than previously reported and highlights that this species can occur in areas with distinct climatic characteristics. It can grow in areas with an oceanic Atlantic climate, such as in the eastern Cantabrian Range, and also in temperate, submediterranean and alpine climates in the Pyrenees, although it is scarce in the Pyrenean central zones (Lara et al., 2007). It has also been recorded in continental and oceanic Mediterranean areas, such as the Ebro Valley and the Catalonian Coastal Ranges, respectively, but at a lower frequency and less extensively.

Prior to this study, the Iberian distribution of *A. lusitanica* was reported to cover practically all mountain systems in the western half of Spain and all of Portugal, occurring in both the Eurosiberian and Mediterranean regions (Vít et al., 2017; Sanna et al., 2023; Martín et al., 2024). However, these previous studies concentrated their sampling in

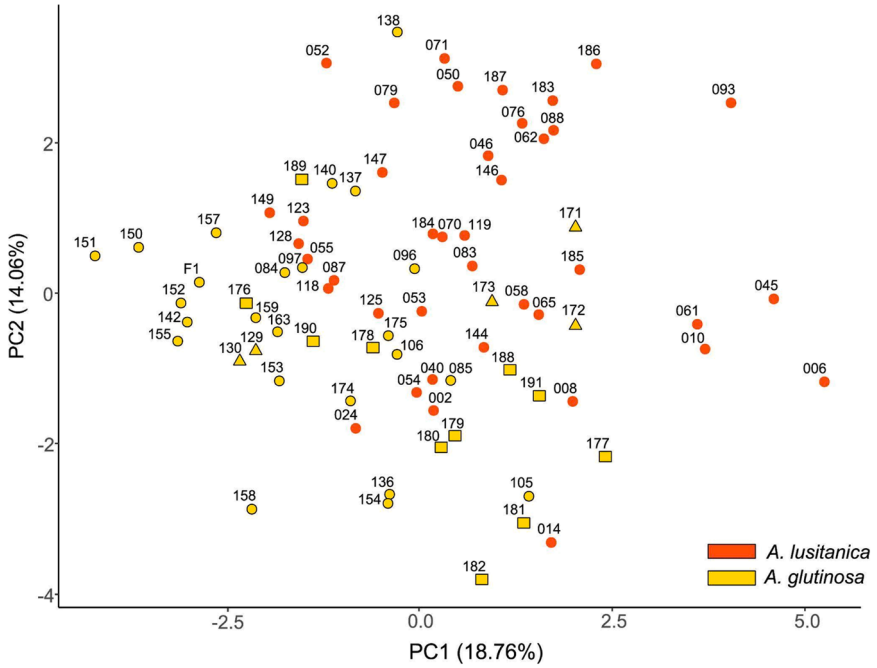


**Fig. 3.** Principal component analysis (PCA) plot of 80 *Alnus* samples (40 *A. glutinosa* and 40 *A. lusitanica*) using 19 morphometric characters. Squares represent samples from outside the Iberian Peninsula, triangles indicate individuals from the contact zones in the Sierra Nevada and the Cantabrian Range (Deva and Nansa rivers), and circles represent the remaining samples. Numbers correspond to the identification code of each sample (Table S1).

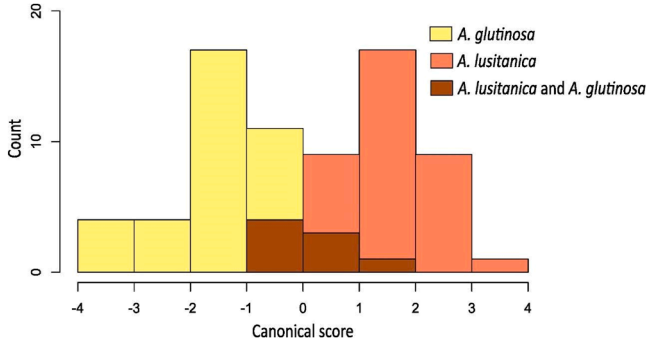


**Fig. 4.** Canonical discriminant analysis (CDA) based on 80 *Alnus* samples (40 *A. glutinosa* and 40 *A. lusitanica*) using 19 morphometric characters. The x-axis represents the canonical score, which corresponds to the discriminant function that maximizes the separation between the two species based on the analysed traits. The y-axis shows the number of individuals.

the northern part of the Peninsula. Here, we have found tetraploids within the same distribution range but also in lowlands of inner areas, within most of the major hydrological basins of the Iberian Peninsula. Moreover, our data based on flow cytometry identification have also shown a greater presence and continuity in the Central System Range and Sierra Morena compared with previous studies (Vít et al., 2017; Sanna et al., 2023; Martín et al., 2024). These new insights into the distribution of *A. lusitanica* suggest it may be more successful than *A. glutinosa* in Mediterranean areas which can experience intense droughts and high summer temperatures. In part, this could be due to the increased potential adaptability of tetraploid *A. lusitanica* to extreme conditions as reported in other polyploid species (e.g. reviewed in Van de Peer et al., 2021). Such advantages, arising from increased genetic and epigenetic diversity upon which selection can act (e.g. Lepais et al., 2013; Mortier et al., 2024) may contribute to overcoming the challenges of establishing new polyploids which initially can experience reproductive difficulties (Hagen et al., 2023; Mortier et al., 2024).

A similar distribution pattern to that observed for *Alnus glutinosa* and *A. lusitanica* is known from other genera on the Iberian Peninsula. For example, diploid *Hedera helix* L. is predominantly found in the eastern half of the Iberian Peninsula whereas the tetraploid *H. hibernica* Poit. and hexaploid *H. iberica* (McAll.) Ackerf. & J.Wen are common in the western and southern regions (González-Toral et al., 2021). Similarly, *Betula pendula* Roth dominates in the north and northeast whereas *B. pubescens* Ehrh. dominates in the northwest (Moreno & Peinado, 1990). At the intraspecific level, the case of *Quercus ilex* L. is also illustrative. It exhibits one lineage in the western part of the Iberian Peninsula and another in the eastern part, both originating from North African populations (Petit et al., 2005). A further example, which is very similar, is that of *Frangula alnus* Mill. This species has one lineage occurring in

**Table 3**
Classificatory Discriminant Analysis (using the k-nearest neighbour method) of 40 *Alnus glutinosa* and 40 *A. lusitanica* individuals based on: i) CDA (or stepwise); ii) PCA; iii) CDA from Vít et al. (2017); iv) Vít et al. (2017) determination key. "k" refers to the optimal *k* value. Characters and ratios used in each Classificatory Discriminant Analysis: i) CS, LW/LL, LS; ii) CS/PL, PL, VH, LL, LB, DH, PV, CS, LM, LW/LL, LO, BC/LL, LS; iii) LS, SA, CS, CL/CW; iv) LS, SA, CS, CL/CW, LB, PL. (See Table 1 for key to abbreviations).

| | k | Taxon | Classified taxon | | Correctly classified (%) |
|---|---|---|---|---|---|
| | | | *A. glutinosa* | *A. lusitanica* | |
| i) CDA (and stepwise) | 30 | *A. glutinosa* | 36 | 4 | 90 |
| | | *A. lusitanica* | 6 | 34 | 85 |
| | | Total | 42 | 38 | 87.5 |
| ii) PCA | 9 | *A. glutinosa* | 35 | 5 | 87.50 |
| | | *A. lusitanica* | 10 | 30 | 75.00 |
| | | Total | 45 | 35 | 81.25 |
| iii) CDA Vít et al. (2017) | 19 | *A. glutinosa* | 28 | 12 | 70.00 |
| | | *A. lusitanica* | 10 | 30 | 75.00 |
| | | Total | 38 | 42 | 72.50 |
| iv) Vít et al. (2017) determination key | 27 | *A. glutinosa* | 32 | 8 | 80.00 |
| | | *A. lusitanica* | 10 | 30 | 75.00 |
| | | Total | 42 | 38 | 77.50 |

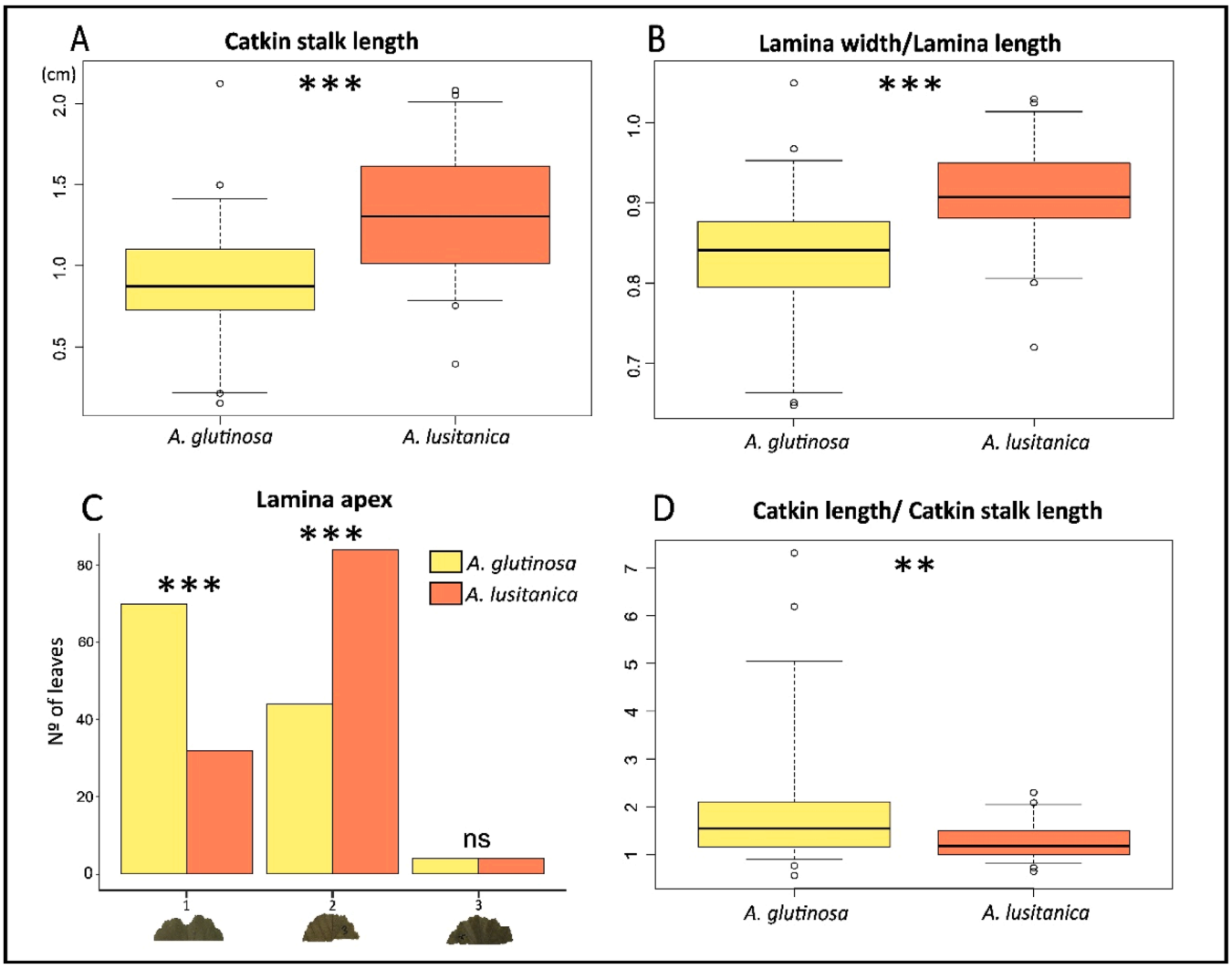


**Fig. 5.** Distribution of the variables included in the Classificatory Discriminant Analysis (ClDA) based on the Canonical Discriminant Analysis (CDA). Significance levels from ANOVA (A, B), Fisher's exact test (C) and Mann-Whitney-Wilcoxon test (D) are represented by: "***" p value < 0.001, "**" 0.001 < p value < 0.05, "ns" p value > 0.05.

the Pyrenees and the majority of the European continent, and the other lineage localised in the northwest, west, and south of the Iberian Peninsula (Petit et al., 2005). As observed in *Alnus glutinosa* (Mandák et al., 2016), the Pyrenean populations of *F. alnus* contributed to the expansion across Europe, as they belong to the same haplogroup (Petit et al., 2005). Although the Iberian Peninsula served as an important refuge during the Last Glacial Maximum (LGM), in some cases, only certain regions contributed to post-glacial expansion (Petit et al., 2005; Rodríguez-Sánchez et al., 2010; Nieto Feliner, 2014). Populations that did not contribute to the post-glacial colonisation of northern European regions would be considered relict populations, as reported for *Fagus sylvatica* L. (Magri et al., 2006).

Despite the predicted autopolyploid origin of *Alnus lusitanica* from *A. glutinosa* (Havrdová et al., 2015; Mandák et al., 2016; Sanna et al., 2023), areas where the two species grow adjacent to each other or coexist remain scarce. Although alders are anemophilous and their pollen can disperse over long distances (McVean, 1953), geographical proximity is often key to understanding genetic relationships between species as well as understanding whether there are potential opportunities for introgression or hybridization to occur (Šmíd et al., 2020). Sanna et al. (2023) reported one area along the Deva and Nansa rivers in the Cantabrian Range, and Martín et al. (2024) found mixed populations in the Ebro basin. Here, we show new contact zones in the Douro basin, in the Iberian System and in the northern slopes of Sierra Nevada. However, populations of *A. glutinosa* from Sierra Nevada may be a legacy of old plantations in the mid-20th Century (Arias Abellán, 1981). In contrast, both the previous northern records of *A. lusitanica* (Sanna et al., 2023) and our southern records occur in areas of Sierra Nevada where plantations have never been recorded.

### 4.2. Potential hybridisation

Cryptic polyploid taxa are relatively common (e.g. Kobrlova et al., 2016; Serrano & Ortiz, 2023). Integrative taxonomy increasingly recognizes ploidy levels as a key tool for identifying cryptic taxa, particularly those arising through autopolyploidy in sympatric scenarios (Oberprieler, 2023). However, different ploidy levels are sometimes regarded as cytotypes of the same taxon, typically when morphology, genetics or other data and criteria fail to identify clear differences [e.g. *Adansonia digitata* L. (Cron et al., 2016); *Centaurea stoebe* L. (Španiel et al., 2008); *Vicia cracca* L. (Eliášová et al., 2014)]. Taxonomic delimitation can also be complex due to the ease of hybridisation between sympatric polyploids and diploids (Pinheiro et al., 2010; Robertson et al., 2010; De Hert et al., 2012). *Alnus* hybrids usually arise from diploid species and show $2n = 28$ (Furlow, 1979; King, 2000; Banaev & Bažant, 2007; Jurkšienė et al., 2021; Villani et al., 2021). However,

*Alnus glutinosa* (diploid) and the tetraploid *A. rohlenae (2n = 4x = 56)* are morphologically similar and generate sympatric triploid hybrids (Šmíd et al., 2022). The hybridisation between *A. glutinosa* and *A. rohlenae* indicates potential gene flow (Šmíd et al., 2020). Previous studies focused on *A. lusitanica* and *A. glutinosa* detected mixed populations or contact zones, but no hybrids were found (Sanna et al., 2023; Martín et al., 2024). Similarly, only diploid and tetraploid individuals of *A. lusitanica* and *A. glutinosa* were observed in our study. The absence of hybrids may be due to differences in phenology between *A. lusitanica* and *A. glutinosa*, as has been proposed for other *Alnus* species (Banaev & Bažant, 2007), but accurate data for this are needed to confirm or refute this explanation.

### 4.3. Morphological delimitation

Pseudocryptic and cryptic taxa have historically remained unnoticed due to just subtle or no morphological differences with their sister taxa [e.g. *Navarretia linearifolia* (Howell) L.A.Johnson (Johnson & Cairns-Heath, 2010); *Linaria incarnata* complex (Vigalondo et al., 2015); *Symphytum tuberosum* complex (Kobrlova et al., 2016); *Lewinskya affinis* complex (Vigalondo et al., 2019); *Jasione* gr. *crispa* complex and *J. sessiliflora* s.l. (Serrano & Ortiz, 2023)]. Nevertheless, the use of molecular tools has significantly contributed to revealing (pseudo)cryptic taxa (Mann & Evans, 2008) and simultaneously or consequently, detailed morphological studies have been able to identify previously unnoticed characters that can be used diagnostically (Vigalondo et al., 2015; Kobrlova et al., 2016). In this context, morphological studies that incorporate a large number of characters, specimens from the entire distribution range, and statistical methods are needed to ensure objectivity and robust results (Vanderhoeven et al., 2002; Vigalondo et al., 2015; Kobrlova et al., 2016).

*Alnus lusitanica* and *A. glutinosa* have been considered cryptic autopolyploid species (in a broad sense) due to their morphological similarities, though some differences have been reported. Gomes Marques et al. (2022; 2024) uncovered morphological and biochemical differences in seeds and seedlings, and Vít et al. (2017) provided a key based on adult morphological characters. Some of those characters were also shown to be key distinguishing features in our study: i.e. *catkin stalk* (CS) appears longer in *A. lusitanica* than *A. glutinosa*, while the *lamina apex* (LS) is frequently emarginate in *A. glutinosa* whereas it is often straight in *A. lusitanica*. In the present study, two more diagnostic characters have been identified: *Alnus lusitanica* tends to have higher values of *lamina width/lamina length* ratio (LW/LL) and lower values of *catkin length/-catkin stalk* ratio (CL/CS), while *A. glutinosa* usually has longer but narrower leaves and higher CL/CS ratio. In contrast, some key characters proposed by Vít et al. (2017) lack significant taxonomic importance in our analysis: *leaf lamina length/petiole length* ratio (LL/PL), *female catkin length/female catkin width* ratio (CL/CW), *leaf base* (LB) and *shape of the annual shoot lenticels* (SA). Indeed, the discriminant capacity of the variables proposed by Vít et al. (2017) in their identification key and in their CDA are lower than the discriminant capacity of the variables here proposed (Table 3). Nevertheless, due to the overlap of these variables (Fig. 5), it is necessary to consider them all simultaneously to correctly identify large numbers of individuals (but not all of them). The differences between our results and those obtained by Vít et al. (2017) could be attributed to the greater sample size and distribution range of *A. lusitanica* individuals analysed in our study. This may also account for the initial morphological variation they recorded for each character, which differed from that we found, and which consequently necessitated the rescaling of many traits. These findings emphasise the importance of exhaustive sampling.

Both taxa exhibit considerable morphological variation across their respective geographic ranges (Fig. 3) as already noted in previous studies of *Alnus glutinosa* (Colagar et al., 2016; Gholamiterojeni et al., 2019; Jurkšienė et al., 2021). Vít et al. (2017) also found greater differences between individuals of *A. lusitanica* and *A. glutinosa* from the Iberian Peninsula than between individuals of *A. lusitanica* from the Iberian Peninsula and non-Iberian individuals of *A. glutinosa*. This pattern is also partially reflected in our results (Fig. 3), where, according to PC1, the individuals of *A. glutinosa* that show the greatest divergence from *A. lusitanica* are from the Iberian Peninsula. In the case of *A. lusitanica*, Gomes Marques et al. (2024) showed that seedlings from individuals of the southern Mediterranean region exhibited specific characteristics associated with drought. This could be related to the distinct haplotypes of *A. lusitanica* from southern Spain (Havrdová et al., 2015; Sanna et al., 2023). However, our PCA results also reflect the lack of a geographical pattern linking certain morphological characters to southern individuals of *A. lusitanica*. Overall, the morphological variability of both species found in our study does not follow a specific geographic pattern within the Iberian Peninsula.

We have shown that simultaneously considering a set of morphological characters allows us to discriminate a high percentage of individuals of *Alnus lusitanica* and *A. glutinosa*. However, the similarity between the two species is very high as even the ranges of the discriminant variables overlap to a greater or lesser extent. Morphological similarity between diploids and autopolyploids is common (Vanderhoeven et al., 2002; Cires et al., 2009; Ramsey & Ramsey, 2014). In the case of these two alder species, their observed morphological similarites does not necessarily reflect an ongoing or a recent speciation process (Bickford et al., 2007). Instead, the genetic data support the relictual nature of the tetraploid individuals (Havrdová et al., 2015), thus our results may reflect morphological stasis (McDaniel & Shaw, 2003) or that the time needed to evolve morphological differences is longer than has so far elapsed since autopolyploid event or events (Vít et al., 2017).

### 4.4. Conservation and management considerations

Alder forests are currently designated as a priority habitat under Annex I of the Council Directive 92/43/EEC, classified within habitat 91E0*. These forests are recognized for their significant ecological and biogeographical value and therefore must be conserved and protected to ensure their preservation and, where needed, recovery. The official description (European Comisión DG Environment 2013) currently includes only two species of alder: *Alnus glutinosa* and *A. incana*. In Spain, however, the alder forests are now characterized by the presence of both *A. lusitanica* and *A. glutinosa*. In Portugal, these forests consist solely of *A. lusitanica*. Nevertheless, updating the description of this European habitat is felt to be unnecessary, as the new species, *A. lusitanica*, is implicitly associated with the species (*A. glutinosa*) originally used to define alder forests, including those in Spain and Portugal.

The new species *Alnus lusitanica* is an Iberian-North African endemic that, *a priori*, would not be considered threatened according to the IUCN criteria (IUCN 2012). However, alder populations across much of Europe, including northern and western Iberia, are under threat from the spread of the pathogen *Phytophthora alni* (Bjelke et al., 2016). It was registered in Europe for the first time in the United Kingdom in 1993 (Gibbs, 1995) and in Spain (Miño river) in 2010 (Solla et al., 2010). The alder dieback caused by this pathogen significantly threatens the stability of alders, leading to a decline in the structural integrity of their roots and branches. The consequent reduction in canopy cover, as well as the quantity and quality of leaf litter, results in alterations to the ecosystem both above and below the waterline (Bjelke et al., 2016). The oomycete *P. alni* survives poorly in cold winter temperatures, helping alder forests recover, while extreme temperatures and drought limit its incidence (Aguayo et al., 2014; Bjelke et al., 2016). Nevertheless, the effect of low temperatures has been shown to be more effective in slowing down tree decline than drought (Aguayo et al., 2014), so particular attention should be paid to the southern populations of *A. lusitanica* and those of *A. glutinosa* located in the oceanic warm areas of northeastern Spain.

The high genetic diversity levels recently detected in *Alnus lusitanica*

could be crucial for identifying resistant individuals (Martín et al., 2024). Management strategies such as forestation should strictly control the geographical origin of the individuals used and consider the genetic diversity in the location being targeted for management. This approach appears to have been overlooked in the past, as evidenced by the analysis of samples of *A. glutinosa* from Sierra Nevada, which might have come from old plantations (Arias Abellán, 1981), although their actual geographic origin would benefit from a phylogeographic study. The presence of these *A. glutinosa* samples within the distribution range of *A. lusitanica* could have put such alder forests from the south of the Iberian Peninsula at risk from genetic contamination. This finding highlights the importance of considering natural genetic diversity in management strategies, as highlighted previously (Beatty et al., 2015; Mingeot et al., 2016).

### 4.5. Taxonomic treatment

The taxonomic treatment should be in accordance with species delimitation, which is supported by various lines of evidence, among which the divergence of lineages based on genetic differences is particularly prominent (Queiroz, 2007, 2020). In the case of *Alnus glutinosa* and *A. lusitanica,* some genetic variability has been reported yet no robust phylogeny supports both species as independent lineages. Harvdová et al. (2015) detected five unique haplotypes that distinguished *A. lusitanica* from *A. glutinosa,* and Sanna et al. (2023) confirmed two of them. Recently, Martín et al. (2024) found a distinct genetic structure based on ten microsatellite markers yet both species exhibited an important inter-population genetic variability. *Alnus lusitanica* has unique haplotypes in the southern half of the Iberian Peninsula and northern Africa (Sanna et al., 2023), along with three distinct gene pools (Martín et al., 2024). Similarly, *A. glutinosa* presents several haplotypes, although one predominates across Europe (Havrdová et al., 2015). Nevertheless, despite all these pieces of evidence, the most complete phylogeny currently available (Havrdová et al., 2015) indicates that if one recognises *A. lusitanica* as a species then *A. glutinosa* is paraphyletic. Thus, all the genetic variability recorded so far in *A. glutinosa* and *A. lusitanica* could be leading towards overestimating the number of *Alnus* species, as has already been recorded in other cases (Derkarabetian et al., 2022; Karbstein et al., 2024).

Species delimitation and the subsequent taxonomic treatment should also consider the extent of morphological and ecological divergences between individuals (Queiroz 2007). In this regard, *Alnus lusitanica* and *A. glutinosa* are very similar. We have previously highlighted that both species show notable overlaps in most of the morphological variables analysed. Both species also usually grow along riverbanks on acidic or decarbonated substrates (Lara et al., 2007; Loidi, 2017). Although *A. lusitanica* seems to have adaptations to the Mediterranean region (Gomes Marques et al., 2024), the two species occupy both Mediterranean and Eurosiberian areas. These similarities favour the existence of several contact zones and even mixed populations (Sanna et al., 2023; Martín et al., 2024).

The ploidy level of the individual currently remains the most conclusive diagnostic feature for distinguishing the two alder species, *A. glutinosa* and *A. lusitanica*. Ploidy levels are crucial for identifying cryptic sympatric species emerging from autopolyploidy (Oberprieler, 2023), especially if it is also confirmed that there is an absence of gene flow or hybrid individuals. Nonetheless, there are taxonomic proposals which include divergent ploidy levels as cytotypes within the same species, particularly in instances where there is an absence of compelling genetic, morphological, or ecological evidence (e.g. Eliášová et al., 2014; Cron et al. 2016). Thus, the recognition of *A. lusitanica* as a species-level entity could be debated. It is therefore recommended that a comprehensive genetic or genomic study should be conducted to build a robust phylogeny for the *A. glutinosa* complex and thus, provide the necessary support for recognising *A. lusitanica* at species level. Otherwise, *A. glutinosa* remains as a paraphyletic species which contradicts the species concept (Queiroz, 2007).

## 5. Identification key for native *Alnus* species in the riparian foresta of the Iberian Peninsula

It is recommended that several leaves are analysed from an individual and that the full set of characters are considered to ensure the correct identification of the species.

1a. Leaf width/length ratio 0.7–1, lamina apex generally straight, often emarginate and rarely obtuse. Catkin stalk length 0.4–2.1 cm and catkin length/catkin stalk ratio 0.6–2.3
***A. lusitanica***

1b. Leaf width/length ratio 0.6–1, lamina apex generally emarginate, often straight and rarely obtuse. Catkin stalk length 0.1–2.1 cm and catkin length/catkin stalk ratio 0.6–7.3
***A. glutinosa***

## 6. Conclusion

The two tree riparian species of alder currently described for the Iberian Peninsula show a notable morphological and ecological similarity, and there are currently no genetic or genomic studies that support them being two completely independent lineages, such that they primarily differ at the ploidy level.

Our results highlight the importance of exhaustive studies analysing many individuals sampled from a large number of localities in order to ensure a detailed understanding of the geographical distribution and the boundaries of the taxa. This has revealed that the two species of Iberian *Alnus*, the widespread *A. lusitanica* and the less widespread *A. glutinosa*, occur in climatically and biogeographically contrasting regions.

New contact zones between the two species of Iberian alders have been detected. However, triploid hybrids could not be found and seem to be rare or non-existent. Further population studies in the contact and mixed zones using genomic approaches are necessary to definitively resolve the evolutionary processes operating in these regions.

The morphometric approach has demonstrated that both species exhibit extensive morphological variability. The significant overlap between *Alnus glutinosa* and *A. lusitanica* means that it is necessary to consider a combination of diagnostic characters for the accurate identification of a significant percentage of individuals, although correctly identifying all individuals is not currently possible.

Distribution and genetic knowledge of both species and their populations is key for their conservation, particularly in the context of their current decline due to *Phytophthora alni*.

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## CRediT authorship contribution statement

**Macarena Cuerdo:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **José Ignacio Márquez-Corro:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Francisco Lara:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ricardo Garilleti:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Ilia J. Leitch:** Writing – review & editing, Resources, Funding acquisition. **Eduardo Cires:** Writing – review & editing, Resources, Data curation.

**David G. del Olmo:** Writing – review & editing, Data curation. **Alba Romero:** Writing – review & editing, Data curation. **Carmen Andrés:** Writing – review & editing, Data curation. **Eduardo Ballesteros:** Writing – review & editing, Data curation. **Álvaro Prado:** Writing – review & editing, Data curation. **Juan A. Calleja:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, 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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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2025.152723.

## Data availability

Data will be made available on request.

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