

Original Article

Can we rescue *Centranthus* (Caprifoliaceae: Valerianoideae) from the *Valeriana* sea?

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ABSTRACT

Centranthus is a small Mediterranean taxon that was recognized as a distinct genus throughout the 19th and 20th centuries, but initially and more recently was included in *Valeriana*. The authors propose a molecular phylogenetic study involving all the species and subspecies in *Centranthus* and confirming its monophyly. Two datasets were used for the phylogeny based on nuclear and plastid markers with Sanger sequencing. The first dataset (DS-I) was based on the nuclear rRNA cistron and 10 plastid markers (five genes, three introns, and two intergenic spacers) and was integrated with literature data for a wide sample of Valerianoideae; the second dataset (DS-II) involved an additional analysis exclusively focused on *Centranthus* taxa, incorporating five additional plastid markers (four intergenic spacers and one gene) to achieve better resolution within this group. According to the results, the Spanish endemic *V. longiflora* is identified as the closest living relative of *Centranthus*. Despite previous hypotheses, *Centranthus* reliably originated in western continental Europe not in the Sardo-Corse system; in addition, two different lineages precociously originated, one including all and only the annual species, and the other including all and only the perennial ones. All our analyses suggest that the *Centranthus* clade is nested within a larger *Valeriana* with extremely strong support. Therefore, and given the relationship with *V. longiflora*, with the present state of knowledge, the separation of *Centranthus* from *Valeriana* appears at least to be not well justified.

Keywords: endemic flora; ITS; Mediterranean; molecular systematics; phylogeny; plastid markers; Valerianeae

INTRODUCTION

The phylogenetic history of Valerianoideae (Caprifoliaceae) is problematic, as testified by disagreement regarding the taxonomic recognition of many taxa above the species rank, especially in the Americas (e.g. [Weberling 1961](#), [Larsen 1986](#), [Kutschker 2011](#)). Surprisingly, these difficulties intensified after the application of molecular methods ([Backlund and Bremer 1997](#), [Raymúndez et al. 2002](#), [Bell 2004, 2007](#), [Hidalgo et al. 2004, 2010](#), [Bell and Donoghue 2005](#), [Bell et al. 2012, 2015](#), [Bell and Gonzalez 2018](#)). Basing on molecular results, widely accepted genera characterized by evident synapomorphies or autapomorphies have been discussed and merged within *Valeriana* L. ([Christenhusz et al. 2018](#)). This is the case of *Centranthus* DC. (Caprifoliaceae, Valerianoideae), a Mediterranean group of nine species ([Raab-Straube 2017](#); [Table 1](#); [Fig. 1](#)) represented by annual or

rhizomatous perennial herbs ([Richardson 1976](#)). This treatment, however, is not yet universally accepted, nor is the inclusion itself of Valerianaceae in Caprifoliaceae (e.g. [Bartolucci et al. 2024](#)).

Necker (1802, sub *Kentranthus*, nom. inval.) first proposed to separate monostaminate species with spurred flowers from *Valeriana*. This proposal was initially controversial, because some scholars refused to segregate *Centranthus* at the generic level, noting that its general resemblance as well as flower shape in some species are very similar to those of some *Valeriana*, and regarding the number of stamens as being inconstant and misleading (e.g. [Tenore 1824–1829](#), [Viviani 1830](#), [Bertoloni 1833](#)). Nevertheless, *Centranthus* was later widely accepted until recently (e.g. [Candolle 1805](#), [Bentham and Hooker 1873](#), [Hock 1882](#), [Graebner 1906](#), [Weberling 1961](#), [Richardson 1976](#), [López](#)

Table 1. Synopsis of *Centranthus* taxa and their native distribution (see also Fig. 1 and Supporting Information File S1).

Section	Taxon	Distribution
<i>Calcitrapa</i> Lange	<i>C. calcitrapae</i> subsp. <i>calcitrapae</i>	Macaronesia and Mediterranean
	<i>C. calcitrapae</i> subsp. <i>orbiculatus</i> (Sm.) Meikle	Cyprus
	<i>C. calcitrapae</i> subsp. <i>trichocarpus</i> I.Richardson	S & SE Spain and Balearic Islands
	<i>C. macrosiphon</i> Boiss.	S & SE Spain, NW Morocco, and N Algeria
<i>Centranthus</i> DC.	<i>C. angustifolius</i> (Mill.) DC.	SE France to NW Switzerland and N Central Italy
	<i>C. lecoqii</i> Jord.	S France, Andorra, and NE Spain
	<i>C. longiflorus</i> Steven subsp. <i>longiflorus</i>	E Mediterranean to Transcaucasus and N Iraq
	<i>C. longiflorus</i> subsp. <i>junceus</i> (Boiss. & Heldr.) I.Richardson	Albania and NW Greece
	<i>C. longiflorus</i> subsp. <i>kellereri</i> (Stoj., Stef. & T.Georgiev) I.Richardson	S Bulgaria
	<i>C. nevadensis</i> subsp. <i>atlanticus</i> (I.Richardson) Dobignard	N Algeria and N Morocco
	<i>C. nevadensis</i> subsp. <i>battandieri</i> (Maire) Maire	N Algeria and N Morocco
	<i>C. nevadensis</i> Boiss. subsp. <i>nevadensis</i>	N Morocco and Spain
	<i>C. nevadensis</i> subsp. <i>sieberi</i> (Heldr.) I.Richardson	Crete
	<i>C. ruber</i> (L.) DC. subsp. <i>ruber</i>	SE Europe, NW Africa, W Turkey
<i>Nervosae</i> Rouy	<i>C. ruber</i> subsp. <i>sibthorpii</i> (Boiss.) Hayek	N Macedonia (Crkvice), S Albania, and Greece
	<i>C. amazonum</i> Fridl. & A.Raynal	E Sardinia
	<i>C. pontecorvi</i> Bacch. & Brullo	SW Sardinia
	<i>C. trinervis</i> Viv.	S Corse

Taxonomy according to Richardson (1975) and Raab-Straube (2017), with the exception of *C. calcitrapae* subsp. *orbiculatus* and *C. pontecorvi* (see main text).

Martínez and Devesa 2007). Finally, after molecular studies revealed the *Centranthus* clade as intimately nested in *Valeriana* s.l. (Bell and Donoghue 2005, Hidalgo et al. 2010), *Centranthus* was sometimes again treated as a synonym of *Valeriana* (Christenhusz et al. 2018, Fedoronchuka 2023, POWO 2024, WFO 2024).

Morphologically, the group of interest to the present study is quite homogeneous (Eriksen 1989), being characterized by glabrous and usually glaucous leaves, cymose inflorescences with dense partial inflorescences, flowers usually zygomorphic with an internal longitudinal membrane, gibbous or spurred, with calyx-teeth inrolled in the flower but developed later into a plumose pappus, achene usually glabrous, and with reduced sterile loculi (Richardson 1976, López Martínez and Devesa 2007); finally, despite some previous counts (e.g. Pouques 1949), it is consistently tetraploid ($2n = 32$, $x = 8$) (Tucci and Ricciardi 1978, Hidalgo et al. 2010). Three sections are generally recognized (Richardson 1975) (Table 1; Fig. 1): *Centranthus* sect. *Calcitrapa* Lange includes two circum-Mediterranean annual species of open dry habitats, also characterized by divided upper leaves, gibbous or shortly spurred corolla, three-fid stigma, and fruit sometimes hairy. *Centranthus* sect. *Nervosae* Rouy once included only *C. trinervis* Viv. (= *C. nervosus* Moris), a rupicolous perennial plant endemic to Sardinia and Corse and characterized by undivided leaves, corolla gibbous near the middle, and three-fid stigma; later, however, the Sardinian populations were segregated into a new species, i.e. *C. amazonum* Fridlender & A.Raynal (Fridlender and Raynal-Roques 1998). Finally, a third species from Sardinia, *C. pontecorvi* Bacch. & Brullo, was recently described (Bacchetta et al. 2024). *Centranthus trinervis* (and consequently its allied species *C. amazonum* and *C. pontecorvi*) is believed to be relict and ancestral in *Centranthus* (Contandriopoulos 1962). The third and richest section (*Centranthus* sect. *Centranthus*)

comprises perennial rhizomatous herbs with undivided leaves, corolla spurred near the base, and stigma entire.

The present study is based on the most comprehensive sampling of *Centranthus*, along with representatives of *Valeriana* and related genera (e.g. *Fedia* Adans., *Valerianella* Mill., *Plectritis* DC., and *Nardostachys* DC.). The primary aims are to test the monophyly of *Centranthus* and its eventual distinctness from *Valeriana*, and to investigate its relationship with *V. longiflora* Willk., which previously resulted in an intriguing sister position (Hidalgo et al. 2004, 2010). To this end, we carried on Sanger sequencing of the nuclear rRNA cistron and 10 plastid molecular markers, already well used in *Valeriana* phylogeny (Bell et al. 2012, 2015). An additional deep phylogenetic reconstruction was carried out exclusively in *Centranthus* taxa using five other variable plastid markers (De Castro et al. 2025).

MATERIAL AND METHODS

Nomenclature

For simplicity, we here preliminarily adopt a traditional circumscription of *Centranthus* (Richardson 1975), as outlined in the Euro + Med Checklist (Raab-Straube 2017; see Table 1 and Supporting Information File S1, sheet 1). However, we treat *C. calcitrapae* subsp. *orbiculatus* (Sm.) Meikle (= *Valeriana orbiculata* Sm.) as a separate taxon and report it preliminarily as a subspecific taxon. In fact, this taxon has been widely debated, with many regarding it as a mere synonym of *C. calcitrapae* subsp. *calcitrapae* (e.g. Richardson 1975), and others recognizing it as a separate species (POWO 2024).

Plant sampling

A taxonomically exhaustive *Centranthus* dataset was obtained both by field sampling and access to herbaria (APP, CAG, MA,

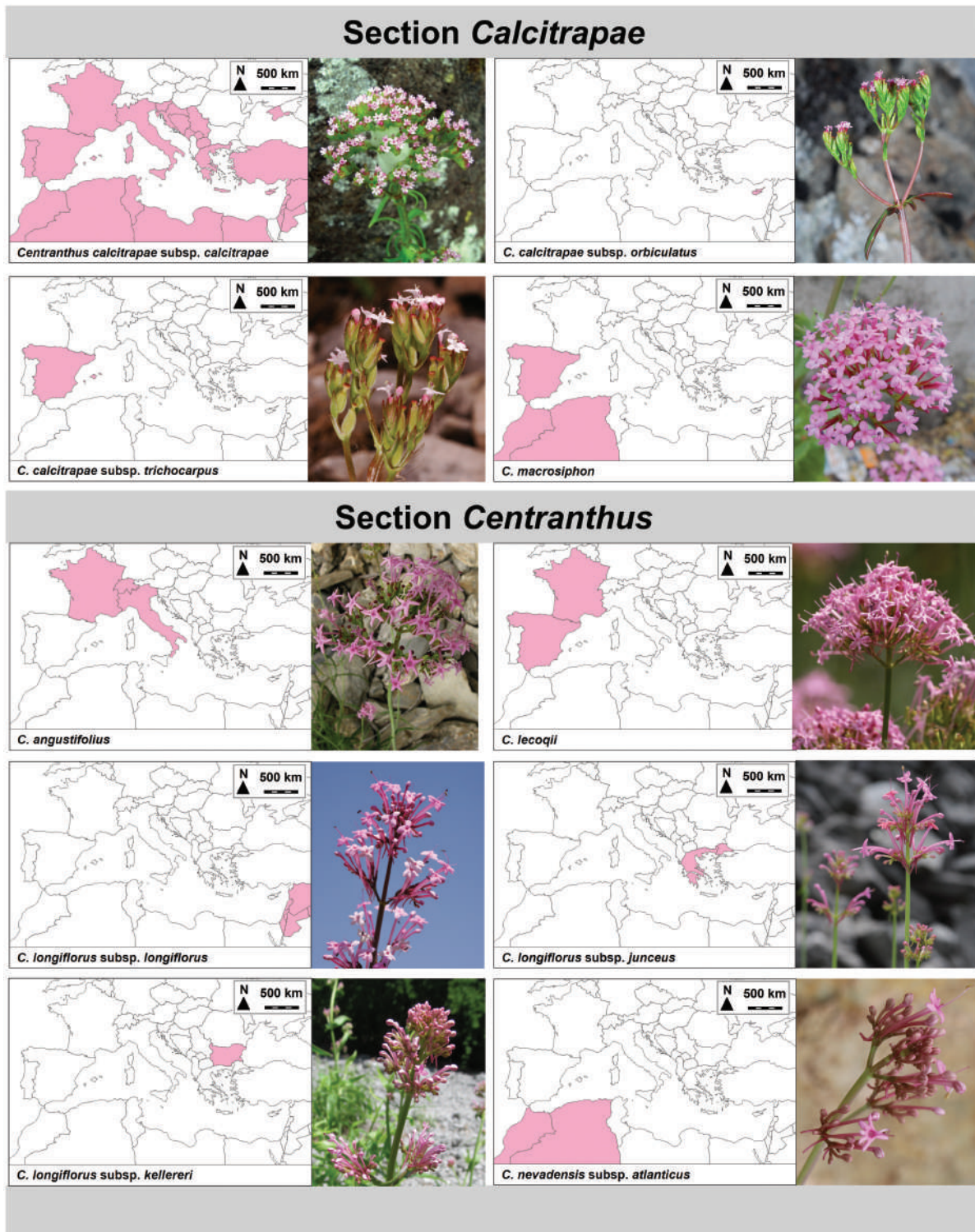


Figure 1. Native distribution and inflorescence of each investigated *Centranthus* species. Distribution according to POWO (2024) except for *C. calcitrapae* subsp. *trichocarpus* and *C. pontecorvi* (Richardson 1975, Bacchetta *et al.* 2024). Photo copyright holder (author, and additional information); taxon order matches the figure: *C. calcitrapae* subsp. *calcitrapae* (B. Pierini, *Acta Plantarum Forum*); *C. calcitrapae* subsp. *orbiculatus* [C. S. Christodoulou, Flora of Cyprus—a dynamic checklist website (R. Hand, G.N. Hadjikyriakou, C.S. Christodoulou, <http://www.flora-of-cyprus.eu/>)]; *C. calcitrapae* subsp. *trichocarpus* (L. Serra Laliga); *C. macrosiphon* (G. Nicoletta, *Acta Plantarum Forum*); *C. angustifolius* (D. Longo, *Acta Plantarum Forum*); *C. lecoqii* (F. Turmo Gort, Flickr personal account); *C. longiflorus* subsp. *longiflorus* (O. Fragman-Sapir, POWO site); *C. longiflorus* subsp. *junceus* (K. Goula, Flora of Greece Web); *C. longiflorus* subsp. *kellereri* (M. Valachovic); *C. nevadensis* subsp. *atlanticus* (A. Homrani Bakali); *C. nevadensis* subsp. *battandieri* (A. Homrani Bakali, Plant Biodiversity of South-Western Morocco—Teline website); *C. nevadensis* subsp. *nevadensis* (A. Homrani Bakali, Plant Biodiversity of South-Western Morocco—Teline website); *C. nevadensis* subsp. *sieberi* (N. Turland, Flickr personal account); *C. ruber* subsp. *ruber* (O. De Castro); *C. ruber* subsp. *sibthorpii* (M. Sonnleitner, Flora Ionica Working Group); *C. amazonum* (G. Bacchetta); *C. trinervis* (C. Piazza); *C. pontecorvi* (G. Bacchetta).

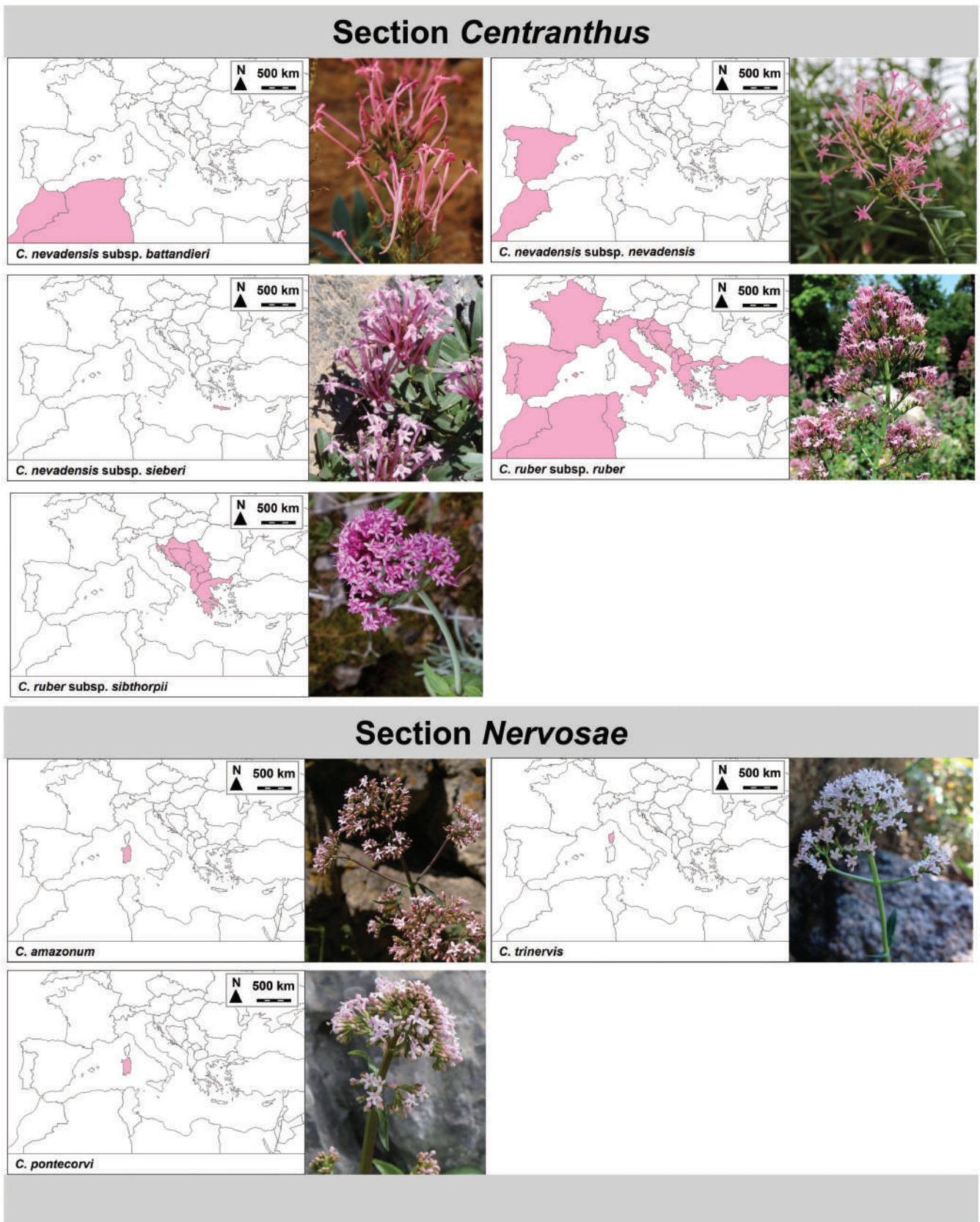


Figure 1. Continued

MAIC, NAP, and SAV; herbarium codes according to Thiers 2024) and from the 'Office de l'Environnement de la Corse' (OEC) (Supporting Information File S1, sheet 1; Fig. 1). Due to a recent genetic study on Sardinian populations of *C. amazonum* (De Castro *et al.* 2025), we included four samples from different localities in the analyses, including the morphologically divergent population recently described as *C. pontecorvi* (Bacchetta *et al.* 2024). Additionally, accessions of *Valeriana longiflora* Willk. [subsp. *longiflora* and subsp. *pau* (Cámara) P.Monts.] were employed. This taxon was chosen because it resulted in a sister position to *Centranthus* in the phylogeny of Hidalgo *et al.* (2004, 2010), although those authors utilized only two plastid markers separately [i.e. *matK* gene and *trnL*^(UAA)-*trnF*^(GAA) intergenic spacer (IGS)].

Taxa dataset for phylogeny

Two taxa datasets were utilized for the phylogeny of *Centranthus*. The first dataset (DS-I) also incorporates data from relevant literature on Valerianaceae/Valerianoideae phylogenies (Bell and Donoghue 2003, 2005, Bell 2004, 2007, Bell *et al.* 2012, 2015) to ensure the inclusion of representative taxa of *Valeriana* from both the Old and New World, as well as the traditionally recognized genera *Fedia*, *Nardostachys*, *Plectritis*, *Valerianella* (hereafter *V-la*), and *Patrinia* Juss. (this last, belonging to the tribe *Patrineae*, was employed as an outgroup) (Supporting Information File S1, sheet 1). In total, 79 taxa were analysed, with 54 sourced from the literature, for this first phylogenetic analysis, utilizing 11 molecular markers [one ribosomal cistron = nrDNA (ITS1 + 5.8S RNA gene + ITS2); 10 = cpDNA].

A second dataset (DS-II) comprised 28 taxa sampled for a focused phylogenetic analysis of *Centranthus*. This analysis used five additional plastid markers in addition to the 10 previously utilized in the first dataset (DS-I; Supporting Information File S1, sheet 1). In this new dataset, we added further specimens of *Centranthus*, and a single representative for each of the remaining Mediterranean genera, i.e. *V. montana* L., *V-la locusta* (L.) Laterr., and *F. cornucopiae* (L.) Gaertn. (these additional specimens, along with their vouchers and locality information, are listed in Supporting Information File S1, sheet 1 with the symbol '●'). This supplementary analysis aimed to assess potential improvements in discriminating between taxa within *Centranthus* that were not observed in the first phylogeny (DS-I). In total, 16 molecular markers were utilized, comprising one nuclear ribosomal cistron and 15 plastid DNA markers. *Fedia cornucopiae* and *V-la locusta* were employed as outgroups.

A list of specimens utilized for both phylogenetic analyses (including taxon, origin, GenBank accession, and reference) is provided in Supporting Information File S1, sheet 1.

Molecular markers dataset

As nuclear markers, the ribosomal cistron (ITS1 + 5.8S gene + ITS2) was amplified and sequenced using literature data (De Castro *et al.* 2021). Additional nuclear markers, such as low-copy genes (LCNGs: geranylgeranyl reductase, *ChlP*; serine glyoxylate aminotransferase, *Agt1*; and hydroxymethylglutaryl-CoA synthase, *HmgS*) used by Bell *et al.* (2015), were tested in *Centranthus* taxa. Unfortunately, these three markers were excluded from our analyses due to ambiguous results and

difficulties in interpretation within *Centranthus*. Specifically, multiple bands caused by heterozygosity patterns for the marker *Agt1*, due to the presence of a variable intron within the gene, were observed. Additionally, the presence of a large number of ambiguous bases and low efficiency in amplification were noted for the remaining two gene markers, *ChlP* and *HmgS*.

Furthermore, 10 plastid regions, already examined by Bell *et al.* (2012, 2015), were amplified and sequenced (first phylogeny dataset, DS-I). These regions include five genes (*accD*, *rpoC1*, *ndhJ*, *ycf5*, and the first 5' part of *matK*), three introns [*trnG*^(UCC), *trnK*^(UUU), and *trnL*^(UAA)], and two intergenic spacers [*psbM-trnD*^(GUC) and *trnL*^(UAA)-*trnF*^(GAA)]. In addition to these plastid markers, a subsequent screening using various primer libraries was performed to identify more variable plastid markers for exclusive use in *Centranthus* accessions (second phylogeny dataset, DS-II). The markers employed included four intergenic spacers [*trnW*^(CCA)-*trnP*^(UGG)-*psaI*], *trnS*^(GCU)-*trnG*^(UCC), *accD-psaI*, and *rpS15-ycf1*] and the 3' portion of the *matK* gene (De Castro *et al.* 2025). Details of the primers are provided in Supporting Information File S1, sheet 2.

DNA extraction, amplification, and sequencing

DNA extraction was conducted using the E.Z.N.A. Plant DNA DS Mini Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. DNA visualization was carried out by agarose gel electrophoresis, and quantification was performed using a Qubit version 3 fluorometer (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA).

Genomic DNA amplifications were executed in a volume of 20 µL, comprising 5–10 ng of DNA template, 0.25 µM of each primer, and Phire Plant Direct PCR Master Mix (Thermo Fisher Scientific). PCR cycling parameters were according to the manufacturer's instructions for the PCR Master Mix, and the annealing temperature for each primer pair is provided in Supporting Information File S1, sheet 2. Amplicons were purified using both PEG8000 precipitation (15% or 20% PEG, 2.5 M NaCl) and the innuPREP PCRpure Kit (Analytik Jena). The purified templates were sequenced in a final volume of 5 µL following instructions for the BrightDye Terminator Cycle Sequencing Kit (ICloning, Beijing, China). Subsequently, the reactions were purified using the BigDye XTerminator Purification Kit (Applied Biosystems, Thermo Fisher Scientific) and sequenced using an automated sequencer (3130 Genetic Analyzer, Life Technologies, Thermo Fisher Scientific).

The obtained sequences were assembled and examined using Chromas Pro v.2.1.10 software (Technelysium Pty Ltd, South Brisbane, Australia), aligned, and edited using BioEdit v.7.2.5 software (Hall 1999) or MEGA v.10.1.13 (Kumar *et al.* 2018). The resulting sequences have been deposited in GenBank and detailed in Supporting Information File S1, sheet 1. The aligned matrices are available as Files S1 and S2.

Phylogenetic analyses

Sequences were analysed excluding primer-PCR artefacts present in several GenBank accessions. Furthermore, due to incomplete plastid sequences in the 5' or 3' regions (except for *rpoC1* and *accD* genes), the corresponding matrices were shortened where possible to avoid significant ambiguity in the phylogenetic

analysis of the samples (Supporting Information Files S2 and S3).

The phylogenetic relationships were evaluated using two analytical approaches: Bayesian inference (BI, Yang and Rannala 1997) and maximum likelihood (ML, Felsenstein 1981). The model of molecular evolution was selected using the Akaike information criterion (AIC) within JModeltest v.2.1.10 software (Darriba *et al.* 2012). To assess potential incongruence between the nuclear and plastid matrices, the incongruence length difference (ILD) test of Farris *et al.* (1994) was conducted using Winclada v.1.61 software (Nixon 2002).

For BI, MrBayes v.3.2.6 software (Ronquist *et al.* 2012) was employed. Two runs of four Markov chains (three hot, one cold) were executed for 15 000 000 generations, with sampling every 1500 generations. The first 15% and 26% were discarded as burn-in for the first dataset and 7% and 2% for the second (ITS and combined plastid matrices, respectively). Convergence diagnostics were assessed using Tracer v.1.7.1 software (Rambaut *et al.* 2018).

ML inference was performed on the same datasets using IQ-TREE via its web server portal (<http://iqtree.cibiv.univie.ac.at/>; Trifinopoulos *et al.* 2016; accessed June 2024). Bootstrap analyses were conducted with 1000 replicates. Phylograms were visualized using FigTree v.1.4.4 software (<http://tree.bio.ed.ac.uk/software/figtree/>, accessed June 2024).

RESULTS

Table 2 displays the most likely substitution model and character information for each marker in both datasets. The ILD test indicated significant incongruence ($P = .001$) between the nuclear and plastid matrices for both datasets, necessitating separate analyses. Additionally, information regarding the plastid concatenated matrices is provided in Table 2.

The phylogenetic analyses of both datasets using the ML approach yielded topologies highly similar to those obtained with BI for both plastid and nuclear matrices (except for three *Valeriana* taxa, see # symbol in the cp-tree). As depicted in Figures 2–4, both datasets utilized the ML phylogram as a reference tree.

According to the analysis of the first dataset (DS-I) using plastid markers (Fig. 2), *Nardostachys* formed a basal grade to the other species. Successively, a clade including *V. celtica* L., *V. saxatilis* L., and *V. elongata* Jacq. and, in turn, a *Fedia/Valerianella* clade, was basal to the remaining sampled species of *Valeriana*, *Plectritis*, and *Centranthus* (hereafter ‘Wide-*Valeriana* Clade’).

Within the ‘Wide-*Valeriana* Clade’, a well-supported dichotomy is evident which separated a group of *Valeriana* species from the northern hemisphere (from both the Old and New World), and another group in turn subdivided into *Plectritis* + *Valeriana* species of Central and South America and a highly supported clade including *V. longiflora* sister to *Centranthus*. Within this latter clade, the basal grade of *V. longiflora* was also strongly supported. Furthermore, the samples belonging to the two subspecies of *V. longiflora* segregated independently. Regarding *Centranthus*, specimens belonging to sect. *Calcitrapae* collapsed in a single clade (hereafter ‘Annual Clade’), but which was robustly sister to the other accessions (hereafter ‘Perennial Clade’). The ‘Perennial Clade’ comprised a *C. lecoqii*

Jord. + *C. nevadensis* Boiss. subsp. *nevadensis* group dichotomously sister to the remaining *Centranthus* accessions. Among them, some clades were well supported: (i) the two subspecies of *C. ruber* (L.) DC.; and (ii) a group with *C. nevadensis* subsp. *sieberi* (Heldr.) I.Richardson sister to (iii) the Sardinian–Corse specimens (hereafter ‘*Nervosae* Clade’). In this last clade, no reliable discrimination in taxonomic groups was detected.

In the nuclear phylogeny (Fig. 3), several differences were observed, particularly in the southern/central American accessions, but the positions of *Nardostachys*, of the ‘alpine *Valeriana*’ taxa, and of the *Fedia-Valerianella* clade were confirmed. Regarding the clade comprising *V. longifolia* and *Centranthus*, this phylogeny matches with the plastid one, albeit with lower clade resolution. Better discrimination is observed in the ‘Annual Clade’, where *C. macrosiphon* is positioned basal to *C. calcitrapae* taxa. Additionally, the ‘*Nervosae* Clade’ is well supported. A notable difference from the plastid tree is the emergence of a new grouping formed by *C. longiflorus* subsp. *kellereri* (Stoj., Stef. & T.Georgiev) I.Richardson and *C. longiflorus* subsp. *longiflorus*. For further details, compare Figure 3 with Figure 2.

According to the second dataset (DS-II), the plastid phylogeny (illustrated in Fig. 4) corroborates the previous topology observed in DS-I (Fig. 2), with improved discrimination among the taxa of the ‘Annual Clade’ and the ‘Perennial Clade’. Notably, in the former, *C. macrosiphon* appears basal to *C. calcitrapae* taxa, consistent with observations from the nuclear dataset of DS-I (Fig. 3). Within the ‘*Nervosae* Clade’, the plastid phylogeny exhibits clear division into two well-supported clades (Fig. 4): one consisting of *C. trinervis* and *C. amazonum* from Gossolè and Mt. Corraisi of central Sardinia (coded P1 and V1, Supporting Information File S1, sheet 1), and the other composed of the sister position of *C. amazonum* from Codula di Luna of central Sardinia (coded R1, File S1, sheet 1) and *C. pontecorvi* from southwestern Sardinia (coded U1, File S1, sheet 1). However, it is of note that the corresponding nuclear tree does not align with this plastid phylogram (data not shown), due primarily to lower resolution of some taxa within the ‘*Nervosae* Clade’, as evident from previous analyses with DS-I (Fig. 2).

DISCUSSION

Phylogenetic incongruence between nuclear and plastid markers

In this study, the authors who previously conducted phylogenetic studies on the Valerianaceae (Hidalgo *et al.* 2004, Bell *et al.* 2012, 2015) observed phylogenetic incongruence (nr vs. cp data), particularly among South American *Valeriana* taxa, a pattern also corroborated by our data. A possible explanation that generates the greatest noise in the congruence between nuclear and plastid phylogenetic topologies for the *Valeriana* group is incomplete lineage sorting (ILS), which is one of the primary causes of incongruences in nuclear markers (Wendel and Doyle 1998), as highlighted by Bell *et al.* (2012, 2015) in their dataset analysis. Hybridization and introgression may also contribute to significant discrepancies, given the distinct inheritance pattern of the nuclear and plastid genome (Wendel and Doyle 1998, Freeland *et al.* 2012). Unlike the plastid genome, the nuclear genome undergoes recombination, which can alter phylogenetic signals and complicate the interpretation of evolutionary

Table 2. Characteristics of the molecular marker matrices used for both datasets (DS-I = 79 taxa; DS-II = 28 taxa).

Molecular marker	Aligned length (bp)	Missing taxa (%)	Variable sites (%)	Parsimony-informative sites (%)	Model of sequence evolution (BI inference ^a)
First dataset (DS-I): 79 taxa					
nrDNA					
ITS (ITS1 + 5.8S + ITS2)	699	0	406 (58.1)	279 (40)	GTR+G
cpDNA					
<i>trnL</i> ^(UAA) intron	548	0	215 (39.2)	130 (23.7)	TVM+G (GTR+G+I)
<i>trnL</i> ^(UAA) - <i>trnF</i> ^(GAA) IGS	391	1 (1.3)	155 (40)	100 (25.6)	TVM+G (GTR+G)
<i>matK</i> (-5') gene	319	3 (3.8)	165 (51.7)	74 (23.2)	TVM+G (GTR+G)
<i>trnK</i> ^(UUU) intron	815	5 (6.3)	412 (50.6)	214 (26.2)	TVM+G (GTR+G)
<i>trnG</i> ^(UCC) intron	617	2 (2.5)	186 (30.1)	126 (20.4)	GTR+G
<i>psbM-trnD</i> ^(GUU) IGS	916	0	217 (23.4)	153 (16.7)	TVM+G (GTR+G)
<i>accD</i> gene	262	0	70 (26.7)	48 (18.3)	TVM+G (GTR+I)
<i>ndhI</i> gene	353	0	53 (18.7)	36 (10.2)	TVM+G (GTR+G)
<i>rpoC1</i> gene	580	4 (5)	101 (17.4)	61 (10.5)	TVM+I+G (GTR+G)
<i>ycf5</i> gene	222	3 (3.8)	20 (9)	16 (7.2)	GTR
Plastidial concatenated matrix	5023		1594 (31.7)	985 (19.6)	Partitioned
Second dataset (DS-II): 28 taxa					
nrDNA					
ITS (ITS1 + 5.8S + ITS2)	678	0	167 (24.6)	103 (15.2)	GTR+G
cpDNA					
<i>trnL</i> ^(UAA) intron	513	0	31 (6)	18 (3.5)	HKY
<i>trnL</i> ^(UAA) - <i>trnF</i> ^(GAA) IGS	355	0	40 (11.3)	21 (5.9)	TVM (GTR)
<i>matK</i> (-5') gene	317	0	40 (12.6)	18 (5.7)	TVM (GTR)
<i>trnK</i> ^(UUU) intron	767	0	121 (15.8)	51 (6.6)	TVM+G (GTR+G)
<i>trnG</i> ^(UCC) intron	589	0	64 (10.9)	34 (5.8)	GTR+I+G
<i>psbM-trnD</i> ^(GUU) IGS	561	0	78 (13.9)	33 (5.9)	TIM2 (GTR)
<i>accD</i> gene	259	0	21 (8.1)	12 (4.6)	HKY
<i>ndhI</i> gene	353	0	21 (5.9)	6 (1.2)	HKY+I
<i>rpoC1</i> gene	574	0	43 (7.5)	22 (3.8)	TVM (GTR)
<i>ycf5</i> gene	222	0	4 (1.8)	1 (0.4)	HKY+I
<i>trnW</i> ^(CCA) - <i>trnP</i> ^(UGG) - <i>psaJ</i> IGS	932	0	119 (12.8)	51 (5.5)	TVM (GTR)
<i>trnS</i> ^(GCU) - <i>trnG</i> ^(UCC) IGS	739	0	122 (16.5)	51 (6.9)	TVM+G (GTR+I)
<i>accD-psaI</i> IGS	723	0	82 (11.3)	34 (4.7)	GTR+I
<i>rps15-ndhF</i> ^b IGS	542	0	79 (14.6)	31 (5.7)	TVM (GTR)
<i>matK</i> (-3') gene	775	0	112 (14.4)	61 (7.9)	TVM (GTR)
Plastidial concatenated matrix	8221		977 (11.9)	444 (5.4)	Partitioned

^aClosest model by the absence in MrBayes software settings.^bThe *ndhF* gene is present instead of the *ycf1* gene in the cpDNA of *Valeriana jatamansi* (NC_067975), used as a reference.

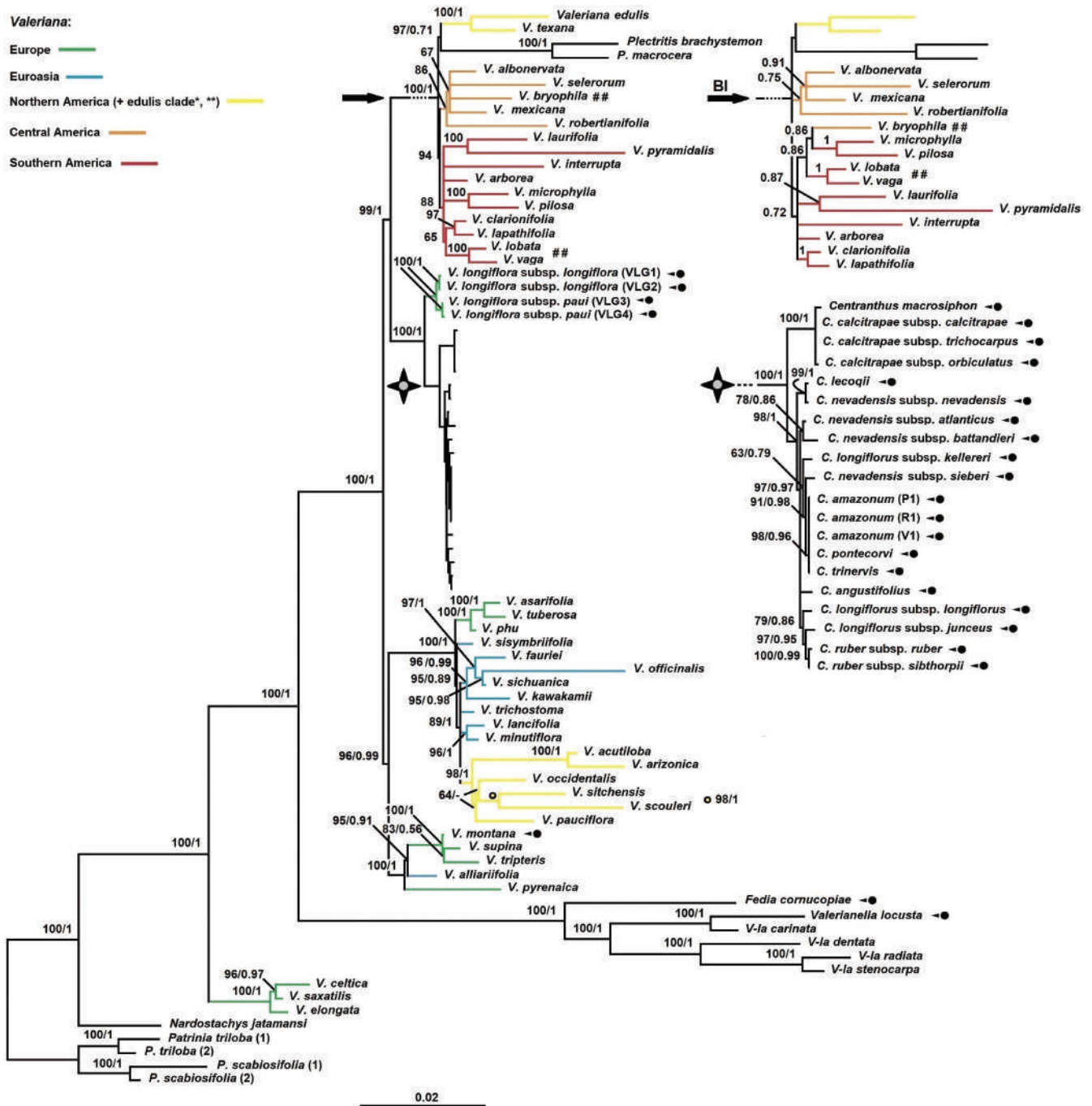


Figure 2. Maximum-likelihood phylogram of the 79-taxon dataset (DS-I) consisting of newly generated and previously published sequences from 10 plastid markers [*trnL*^(UAA) intron, *trnL*^(UAA)-*trnF*^(GAA) IGS, *matK*(-5') gene, *trnK*^(UUU) intron, *trnG*^(UUC) intron, *psbM*-*trnD*^(GLU) IGS, *accD* gene, *ndh* gene, *rpoC1* gene, and *ycf5* gene]. The newly generated sequences are indicated by the symbol '●'. Maximum-likelihood bootstrap values followed by Bayesian posterior probabilities are shown above the branches (BS/PP values >50%). On the right: arrow symbol, detail of the Bayesian tree (BI) where three taxa (marked with #) are incongruent in their position in the maximum-likelihood tree; star symbol, enlarged detail of the *Centranthus* group. For sample information, see [Supporting Information File S1, sheet 1](#), and for the plastid matrices, refer to [Supporting Information File S2](#).

relationships. Differences in mutation rates between nuclear and plastid markers further provide insights across varying temporal scales (Rousseau-Gueutin et al. 2018). Generally, plastid DNA evolves more slowly than nuclear DNA, which is more prone to polymorphisms, gene duplications, rapid diversification, and locus homogenization (Wendel and Doyle 1998).

In the specific case of nuclear sequences obtained from *Centranthus* taxa, they exhibit lower resolution compared to plastid markers, particularly within sect. *Centranthus*, where the ITS marker demonstrates homogenization and minimal variability. This phenomenon probably results from a combination of the aforementioned factors, although it remains challenging

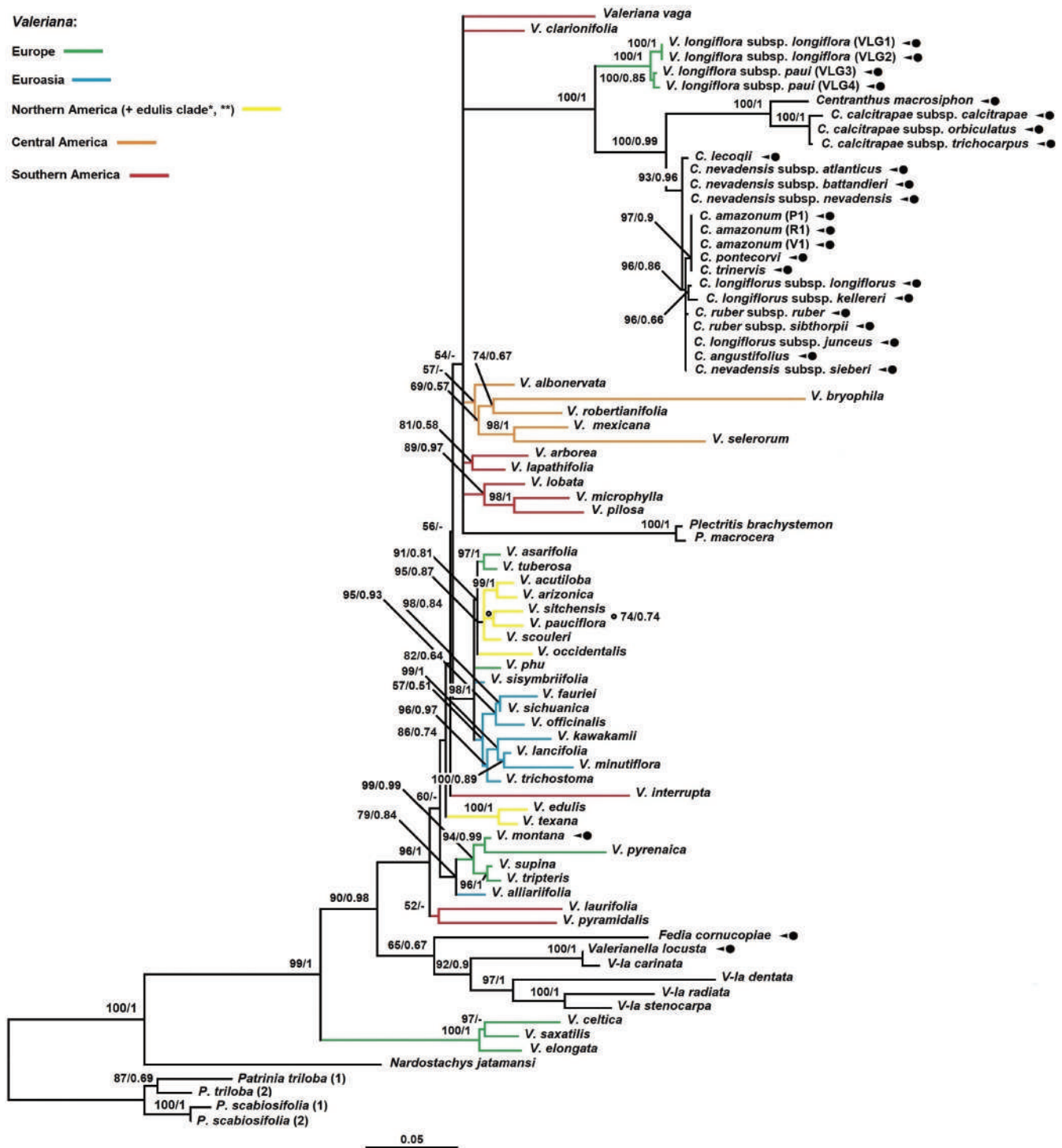


Figure 3. Maximum-likelihood phylogram of the 79-taxon dataset (DS-I) consisting of newly generated and previously published sequences from internal transcribed spacers (ITS1 + 5.8S gene + ITS2). The newly generated sequences are indicated by the symbol '●'. Maximum-likelihood bootstrap values followed by Bayesian posterior probabilities are shown above the branches (BS/PP values >50%). For sample information, see [Supporting Information S1, sheet 1](#), and for the ITS matrix, refer to [Supporting Information File S2](#).

to attribute it precisely to any single cause or combination of causes due to the complexity of the biological processes involved (Wendel and Doyle 1998). A genome-wide analysis using whole genome sequencing (WGS) of *Centranthus* taxa could provide greater clarity on the phylogenetic and genomic evolution of the group (O. De Castro, unpubl. work).

Phylogeny of Valerianoideae and monophyly of the *Centranthus* clade

Our phylogenetic reconstruction of Valerianoideae largely concurs with previous reconstructions (e.g. [Hidalgo et al. 2004, 2010](#), [Bell et al. 2012, 2015](#)). None of these reconstructions adequately meets the generic circumscriptions proposed over

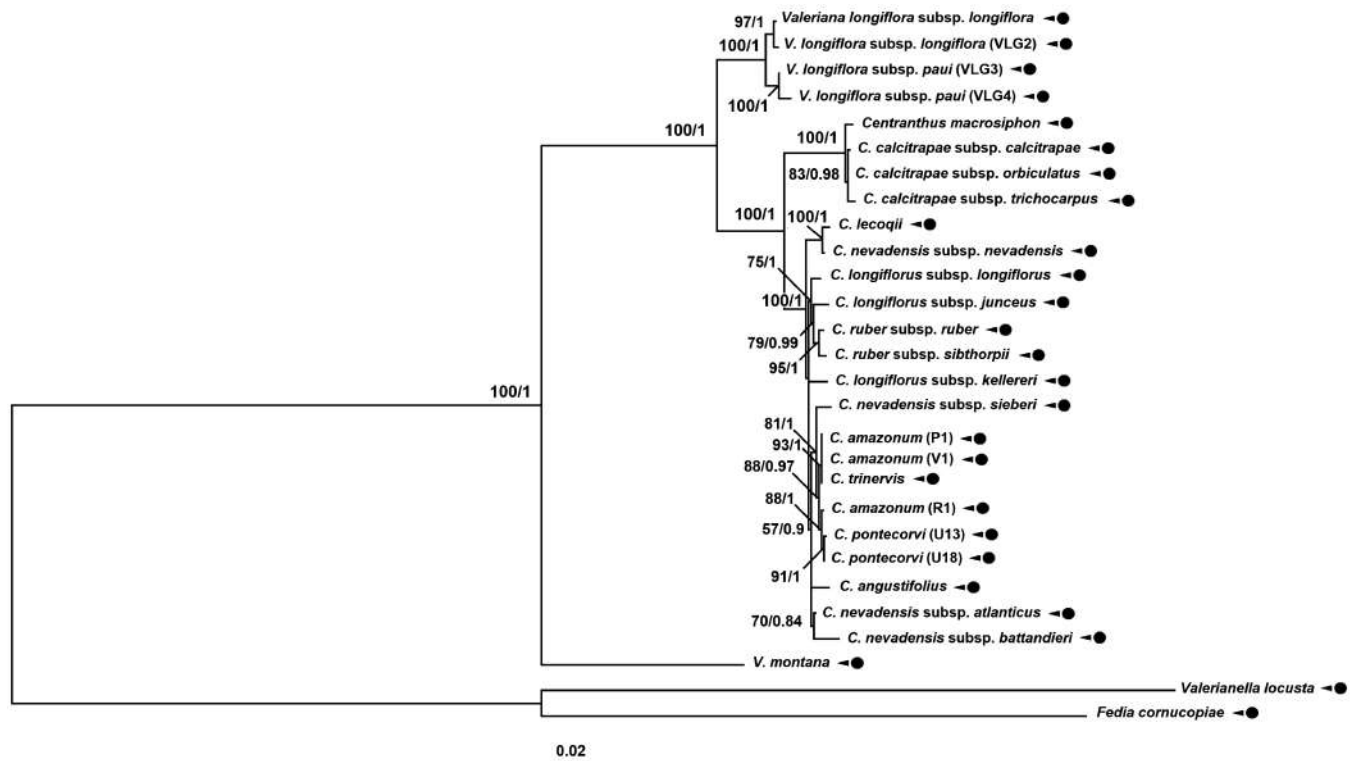


Figure 4. Maximum-likelihood phylogram of the 28-taxon dataset (DS-II) consisting of newly generated sequences (symbol ‘●’) from 15 plastid markers [*trnL*^(UAA) intron, *trnL*^(UAA)-*trnF*^(GAA) IGS, *matK*(-5′) gene, *trnK*^(UUU) intron, *trnG*^(UUC) intron, *psbM*-*trnD*^(GUC) IGS, *accD* gene, *ndhJ* gene, *rpoC1* gene, *ycf5* gene, *trnW*^(CCA)-*trnP*^(UGG)-*psaJ* IGS, *trnS*^(GCU)-*trnG*^(UCC) IGS, *accD*-*psaI* IGS, *rps15*-*ndhF* IGS, and *matK*(-3′) gene]. Maximum-likelihood bootstrap values followed by Bayesian posterior probabilities are shown above the branches (BS/PP values >50%). For sample information, see [Supporting Information S1, sheet 1](#), and for the plastid matrices, refer to [Supporting Information File S3](#).

time. In fact, except *Patrinia* and, in turn, *Nardostachys*, which are confirmed to be basal to all the remaining taxa, any conciliation between the molecular results and the traditional classification based on morphology remains highly problematic.

For example, a small group of Alpine *Valeriana* (the so-called ‘Celtica Clade’, [Bell *et al.* 2015](#)) is confirmed to be sister to the ‘Wide-*Valeriana* Clade’, including all the remaining taxa. Analogously, even if *Plectritis* was found to be monophyletic in both the plastid and the nuclear tree ([Figs 2, 3](#)), its position varies internally to South American *Valeriana* groups.

In addition, the phylogenetic trees do not satisfactorily reflect the geography, which sometimes is ‘a better predictor than taxonomy’ ([Hilpold *et al.* 2014](#)). For example, in the plastid tree ([Fig. 2](#)), we find a robustly supported group of northeastern American species included in a clade with Eurasian species, while other North American taxa such as *Plectritis* are included with South American *Valeriana* species in a different clade. The situation is even more obscure in the nuclear tree ([Fig. 3](#)). These results, however, might support the hypothesis of [Eriksen \(1989\)](#), according to whom the Valerianoideae colonized the New World at different times and across different routes (at least four, according to [Bell and Donoghue 2005](#)).

Thus, a fully convincing phylogeny of Valerianoideae seems still far away, presumably due to the rapid and parallel diversifications of some clades which probably colonized new areas in waves ([Bell 2004, 2007, Bell and Donoghue 2005](#)). This phenomenon took place especially in the Mediterranean Basin and in the Americas, where Valerianoideae are especially diverse.

Nevertheless, at the present state of knowledge, the choice to merge all Valerianoideae into a very wide *Valeriana* ‘super-genus’, except *Patrinia* and *Nardostachys* ([Christenhusz *et al.* 2018](#)), seems premature. In fact, given the numerous species hitherto not sequenced, this choice may not be the most definitive and therefore risks promoting nomenclatural inflation. In this regard, for example, the *Fedia*-*Valerianella* clade was well supported in our tree and sister to all the remaining *Valeriana*-*Centranthus* taxa, except the ‘Celtica Clade’. If this clade were kept distinct, maintaining at least *Valerianella* would be justified. *Fedia* and *Valerianella* species are dichotomously branched annuals with rich partial inflorescences, calyx toothed in pairs (one or two pairs in *Fedia*, mostly three in *Valerianella*) ([Richardson 1975](#)) and fruit polymorphism ([Martin and Mathez 1990, Xena and Mathez 1998](#)). In addition, *Fedia* is readily distinguished from all the other Valerianoideae based on the number of stamens (two) and from *Valerianella* (*V-la*) also by its flower colour. However, [Arnelas *et al.* \(2022\)](#) showed that the Spanish endemic *V-la. fusiformis* Pau, with peculiarly stipitate and fusiform fruits, forms a paraphyletic group with the major *Valerianella* clade. Regardless, their results also strongly suggest that a genus *Valerianella*, including *Fedia*, could stand separately from *Valeriana*.

The joining link between *Valeriana* and *Centranthus*

In all the reconstructions ([Figs 2, 3](#)), the ‘*Centranthus* Clade’ is undoubtedly monophyletic, but robustly nested in the ‘Wide-*Valeriana* Clade’, confirming the results of previous authors (e.g.

Hidalgo *et al.* 2010, Bell *et al.* 2015, Arnelas *et al.* 2022). On the one hand, this agrees with the synapomorphies shared by *Centranthus* taxa, including their most relevant autapomorphy, i.e. reduction in stamen number to one. On the other hand, it reflects the numerous features shared with *Valeriana* (Del Guacchio 2002), including the thyrsoid inflorescence (Weberling 1961), and fruit with extremely reduced sterile locules (Jacobs *et al.* 2010) and surmounted by a pappus, among various others. A pappus-like structure also occurs in *V-la hirsutissima* Link, but clearly by convergence (Richardson 1975). Indeed, according to this latter author, the only reliable character to distinguish *Centranthus* from *Valeriana* is the number of stamens.

By the contrary, any closer relationship with the 'Fedia-Valerianella Clade' (Graebner 1906, cf. Richardson 1975) can be excluded and is clearly due to convergence. More importantly, our results point to the crucial position of *V. longiflora*. This taxon in fact was sister to all *Centranthus* species in all phylogenetic reconstructions (Figs 2–4), in agreement with previous molecular results (Hidalgo *et al.* 2004, 2010). *Valeriana longiflora* is a threatened chasmophytic species, endemic to NE Spain (Vázquez Pardo *et al.* 2007). The species is often regarded as constituted by two different subspecies (e.g. Raab-Straube 2017), i.e. *V. longiflora* subsp. *longiflora* and *V. longiflora* subsp. *pau* (Cámara) P.Monts., while other authors (e.g. POWO 2024) place them in synonymy. This latter treatment is rejected by our results, because the representatives of the two subspecies segregate in two different groups in both nuclear and plastid trees.

From a morphological point of view, *V. longiflora* has interesting features, such as the autapomorphic features of its achene (costae parallel and appressed, calyx persisting as a cupuliform structure). Its pinkish and long corolla suggests specialized pollination, which is regarded as a driving trend in *Centranthus* diversification (Paglia 1900).

Thus, our results appear to discourage the segregation of *Centranthus* as a distinct genus, owing to the presence of a living intermediate between the Mediterranean *Valeriana* taxa (i.e. *V. longiflora*) and *Centranthus* itself.

The diversification of *Centranthus* throughout the Mediterranean

It is generally acknowledged that the annual lineages derived from perennial ancestors (Hjertaas *et al.* 2023), a well-known trend in the Mediterranean Basin, for example in the sister subfamily Dipsacaceae (Caputo *et al.* 2004). This is only partially verified in *Centranthus*, because it split precociously into an annual and a perennial clade, due to a rapid adaptive radiation. However, both lineages presumably derived from a perennial ancestor, similar to *V. longiflora*, which indirectly supports the general phylogenetic trend from perennial to annual. The origin of the *Centranthus* radiation is therefore located in western Europe, where the sister taxon still occurs and where both *Centranthus* lineages are represented. The same area has been indicated as the probable diversification centre for the 'Valerianella-Fedia Clade' (Arnelas *et al.* 2022). At the origin of the radiation of *Centranthus*, as well as with the success of some of its taxa, there is the trend to flower zygomorphy which deals with the dorsal position of the single stamen and a better adaptation to Lepidoptera, as testified by the purple-pinkish corolla

and saccate/spurred tube (Fig. 1). Paglia (1900) suggested that the nectariferous spur is internally covered by dense hairs to prevent access by small insects. Paglia also observed a peculiar mechanism in the flowers of the proterandrous *C. ruber*, in which the stamen retroflects after pollination and at the same time the style moves up to receive the successive pollinator. According to Paglia, this mechanism on the one hand supports cross-pollination, while on the other its efficacy justifies the stamen reduction. However, the spread of *Centranthus* has also been enhanced by its efficient dispersal by means of the pappus (Del Guacchio 2002), a feature not occurring in *V. longiflora*.

In the 'Perennial Clade', according to the plastid trees (Figs 2, 4), *C. nevadensis* subsp. *battandieri* and *C. nevadensis* subsp. *atlanticus* group together, but surprisingly *C. nevadensis* subsp. *nevadensis* is completely external and is found in a well-supported clade with *C. lecoqii*. Nevertheless, the topology of the plastid tree might partly reflect a relevant geographical influence, to which the plastid signal is notoriously prone, because the former two subspecies are endemic to NW Africa, while *C. nevadensis* subsp. *nevadensis* occurs also in Spain (where the SW European *C. lecoqii* occurs too). Regardless, the basal position of these two latter taxa in the 'Perennial Clade' indirectly supports a SW European origin of *Centranthus*. Unfortunately, the nuclear tree does not provide further information in this regard (Fig. 3). Moreover, the Cretan *C. nevadensis* subsp. *sieberi* is not directly related to any of the remaining subspecies in any tree. Besides, its position as a sister to the 'Nervosae Clade' in the plastid tree is remarkable, and this may be due to ancient hybridization or to ILS. Regardless, and due to its distantly disjunct distribution, *C. sieberi* seems to deserve specific status. Within the 'Perennial Clade', only a 'Nervosae Clade' is well supported in both trees (Figs 2, 3) and might justify a corresponding *C. sect. Nervosae*; however, the recognition of a monophyletic group corresponding to *C. sect. Centranthus* cannot be confirmed, making a comprehensive subdivision of *Centranthus* still premature. In addition, our data cannot support or reject the recent split of *C. trinervis* into different taxa, as ITS did not provide any clustering and the plastid signal is only obscurely linked to geography (Fig. 4). As our data do not support any hypothesis of dramatic genetic impoverishment of the clade, we infer that the 'Nervosae Clade' may have originated in Oligocene (from 37 to 24 Mya), when the Sardo-Corse system separated from Continental Europe (Caković and Frajman 2023) (age of the Valerianoideae crown-group mostly estimated as 58–50 Mya, Stevens 2017). By contrast, Arenales *et al.* (2022) proposed a much less old dating, i.e. 20.34 ± 3.7 Mya for the divergence of the core Valerianoideae and about 20.34 ± 3.7 Mya for *Centranthus*. Regardless, despite previous statements (Contandriopoulos 1962) based mainly on the morphological similarity of these species with some *Valeriana* taxa and probably suggested by their relictual state, the 'Nervosae Clade' is not basal in *Centranthus* (Figs 2–4). Indeed, little can be added, because the obtained trees are substantially unresolved. However, without doubt, the current distribution of perennial *Centranthus* (with the partial exception the ubiquitous *C. ruber* subsp. *ruber*) appears as the clear consequence of mountain fragmentation by vicariance after glacial events (Fig. 4), a phenomenon well known in Mediterranean flora (De Luca *et al.* 2022).

From a taxonomic view, our results do not integrate with any available treatment (e.g. Richardson 1975, Raab-Straube 2017, Christenhusz *et al.* 2018). For example, they do not support the current taxonomic treatment within *C. sect. Nervosae*, which indeed seems to be contradicted by the placement of *C. amazonum* specimens in two different clades (two specimens grouped with *C. trinervis*, the other with *C. pontecorvi*) (Fig. 4). Besides, they do not justify the inclusion at subspecific rank of *C. sieberi* under *C. nevadensis* and do not support the division into different taxa of *C. calcitrapae*. Finer analyses and wider samplings are required to reliably assess the taxonomic value of several taxa, such as *C. amazonum* and *C. pontecorvi*, and *C. calcitrapa* subsp. *trichocarpus* and subsp. *orbiculatus*. The hypothesis of Hidalgo *et al.* (2010) of a hybridogenous origin of *C. lecoqii* from *C. ruber* and *C. angustifolius* cannot be ruled out, due to the incongruent positions in the plastid and nuclear reconstructions here (Figs 2–4); nevertheless, our results point to the involvement of *C. nevadensis* or a closer taxon. A similar incongruence also involves *C. longiflorus* subsp. *kellereri*, which seems, also on this basis, probably better retained as a distinct species (Christenhusz *et al.* 2018). By contrast, and as stated above, our results strongly suggest keeping as distinct, at least at subspecific rank (López Martínez and Devesa 2007), *V. longiflora* subsp. *longiflora* and subsp. *pau* (cf. POWO 2024).

CONCLUSIONS

Centranthus is confirmed to be a monophyletic group, precociously split into two lineages, one including all and only the annual taxa, and the other including only and all the perennial species (Figs 2, 3). Within the latter, only a Sardo-Corse clade, corresponding to *C. sect. Nervosae*, can be reliably defined (Figs 2–4). However, finer techniques are required not only for testing the taxonomic value of the taxa recently described within this clade (Di Iorio *et al.* 2024, De Castro *et al.* 2025; O. De Castro, unpubl. work), but also to clarify the relationships among the perennial species. Presumably, *Centranthus* originated in SW Europe, where the nearest relative still occurs. In fact, we were able to confirm that the Spanish *V. longiflora* is not only morphologically, but also genetically intermediate between *Valeriana s.s.* and *Centranthus*; this suggests, with the present state of knowledge, that *Centranthus* is better kept as an infrageneric taxon under *Valeriana*.

SUPPLEMENTARY DATA

Supplementary data are available at *Botanical Journal of the Linnean Society* online (Supporting information S1, S2 and S3).

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CONFLICT OF INTEREST

No potential conflict of interest was confirmed by the authors.

DATA AVAILABILITY

The data supporting this article are provided as Supporting Information File S1, S2 and S3.

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