TITLE: MOLECULAR IDENTIFICATION AND DIVERSITY ASSESSMENT OF TYRRHENIAN *ROMULEA* SPECIES (IRIDACEAE)

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Abstract

Taxonomic assignments based only on morphology are often insufficient for delimiting species, particularly in polyploid complexes with extensive morphological overlap. This limitation hinders biogeographic and conservation issues. The genus *Romulea* (Iridaceae), distributed across Africa and the Mediterranean Basin, exemplifies this challenge. Despite its remarkable diversity, Mediterranean *Romulea* has remained largely understudied at the molecular level. Here, we present the first multilocus genotype analysis of several taxa of the Mediterranean region, with a sampling focused on the Tyrrhenian biogeographic province. Using the hybseq method with the universal Angiosperms353 target capture kit, we sequence nuclear (on target) and plastid loci (off target) for 272 individuals representing 18 putative taxa. Nuclear SNP genotyping yield 8,168 high-confidence loci, revealing 19 genetic groups. $H\mathrm{ind}/H_{\mathrm{E}}$ analyses indicate a predominance of allopolyploid inheritance. Analyses of genetic diversity reveal incomplete differentiation, introgression, or plastid capture, which complicate species delimitation. Several taxa, including *R. x melitensis*, *R. corsica*, and *R. bulbocodium*, show genomic signatures consistent with hybrid origins. Four taxa (*R. assumptionis*, *R. revelieri*, *R. ligustica*, *R. rollii*) are consistently well differentiated across nuclear and plastid datasets, supporting their recognition as distinct species. The widespread species *R. ramiflora* and *R. columnae* contain well-differentiated groups that are either isolated or close to other taxa, calling for further analysis to differentiate cryptic speciation from historical introgression. Our findings question the current taxonomic status of several narrow endemic species. Based on robust genetic boundaries, we propose various solutions for further taxonomic studies. The plastid phylogeny reveals a star-like diversification pattern, consistent with rapid radiation in the Tyrrhenian region. These results provide a primary genomic framework for integrative taxonomy in *Romulea*, highlighting the central role of polyploidy and reticulate evolution in shaping Mediterranean diversity and offering key insights for biogeography, systematics and conservation.

Key words: species, diversity, genomic, variation, hybseq, Angiosperms353, biogeography, systematic

Introduction

Taxonomic assignments constitute a critical foundation of biodiversity science, underpinning species description, classification, and biogeographical inference (Diniz Filho *et al.*, 2023). When based exclusively on morphology, they may introduce systematic biases that misinform evolutionary, ecological, and conservation studies. This limitation becomes particularly acute in plant species complexes distributed over wide geographical areas (Pinheiro *et al.*, 2018; Gargano *et al.*, 2023). In such circumstances, robust species delimitation requires a quantitative assessment of genetic diversity and its spatial structure. This approach is addressed through phylogeographic frameworks integrating population genetics and phylogenetics (Leaché *et al.*, 2018; Criado-Ruiz *et al.*, 2025; Davis *et al.*, 2025). Despite their importance, species delimitation and the delineation of conservation units are rarely considered in Mediterranean plant species conservation literature (Heywood, 2014; Médail & Baumel, 2018; Salmerón-Sánchez *et al.*, 2021; Bobo-Pinilla *et al.*, 2022).

In the Mediterranean Basin, several plant species complexes remain in urgent need of molecular systematics to disentangle taxonomic uncertainties and elucidate evolutionary relationships. The genus *Romulea* Maratti (Iridaceae) represents a paradigmatic case. This clade of geophytic monocots, distributed across Africa and the Mediterranean Basin, exhibits pronounced taxonomic richness and considerable morphological variation, and consequently a great taxonomic complexity. However, *Romulea* taxa remains severely underrepresented in molecular phylogenetic datasets. In the South African Cape Floristic Region, which constitutes the principal centre of diversity with approximately 76 recognized taxa, *Romulea* has been intensively characterized from a morphological, karyological, and floral biological standpoint (Manning & Goldblatt, 2001). Nonetheless, the only available molecular investigation incorporated a single representative in a broader *Crocoideae* phylogeny (Goldblatt *et al.,* 2008), thereby precluding any meaningful inference of intrageneric relationships. The Mediterranean *Romulea* species have received only partial treatment, often limited to early 20th-century monographs (Béguinot, 1907; 1908; 1909) or regional floristic accounts (Frigani & Iiriti, 2011; Mifsud, 2015). There remains a significant discrepancy between the detailed analytical approaches of the 20th century and the more synthetic perspectives presented in major syntheses such as Flora Europaea (Marais 1980), reflected in current international taxonomic checklists (POWO, 2025). Cytogenetic investigations (Peruzzi *et al.*, 2011) have revealed a predominance of tetraploid cytotypes (4x=36 chromosomes), as well as pentaploid and hexaploid cytotypes for eleven Mediterranean taxa. This pattern contrasts sharply with the South African taxa, which are predominantly diploids although doubts subsist regarding the chromosome basic number (Maning & Golatt, 2001). Despite these insights, molecular studies targeting Mediterranean *Romulea* are rare, with a handful of nucleotidic sequence accessions deposited in public repositories for studies in the Iridaceae or the Asparagales (Souza-Chies *et al.,* 1997; Chen *et al.,* 2013; Harpke *et al.*, 2013). Recent detailed morphological assessment focused exclusively on three taxa of Malta archipelago (Mifsud, 2021), partly due to inherent difficulties to design taxon sampling.

The present study forms part of a broader integrative taxonomic initiative aimed at resolving species boundaries and reconstructing the evolutionary history of *Romulea* of the Mediterranean Basin. We concentrate here on the taxa occurring in an area (Figure 1) centred around the Corsica-Sardinian microplate which separated from the continent 29 Myrs ago (Alvarez, 1976; Speranza *et al.*, 2002). This region, called here Tyrrhenian area, exhibits high floristic diversity, an exceptional rate of endemism, and strong the floristic links between the islands, highlighting a shared biogeographical history (Médail, 2002). This is also the area where Mediterranean *Romulea* were mostly studied (Frigani & Iirti, 2011; Peruzzi *et al.*, 2011). Using the hybseq method (Weitemier et al., 2014) with the Angiosperms353 target capture kit (Baker *et al.*, 2022), our objectives are to (i) generate multilocus genetic data for a representative set of Mediterranean *Romulea* taxa, ii) delimit genetic groups corresponding to putative species-level lineages, iii) assess their evolutionary distinctiveness through population genetic and phylogenetic inference, and iv) reconcile molecular groupings with independent morphological identifications conducted by expert botanists.

Material & Methods

Sampling design

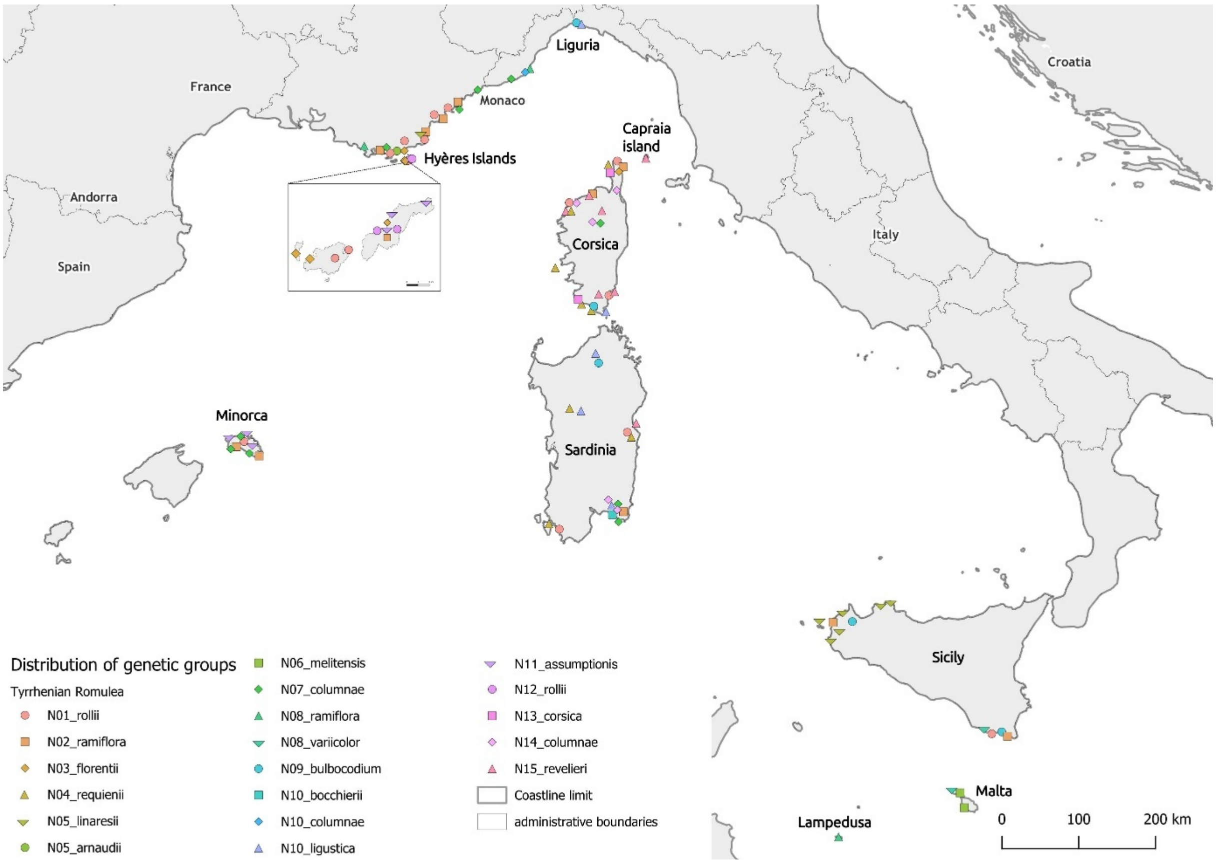

Figure 1: Sampling of *Romulea* populations. Symbols and colours according to the 19 genetic groups revealed by this study.

Field collections were performed across four Mediterranean countries within the Tyrrhenian biogeographic province (Figure 1), targeting all taxa of *Romulea* occurring in this area (Table 1). Whenever possible, multiple populations and individuals were sampled per taxon to capture intra-population and inter-population genetic variation. Field identification relied on current taxonomic keys, with provisional assignments recorded *in situ*. Putative hybrids were noted based on intermediate floral morphology and recorded as such in Table 1. *Romulea rosea*, an alien species introduced from South Africa and naturalized in France, was also included. Additionally, DNA of *R. saldanhensis*, *R. dichotoma*, and *R. pratensis*, all native to South Africa, was obtained from the Royal Botanic Gardens, Kew DNA Bank, while sequence data for *R. monadelpha*, also from South Africa were obtained from PAFTOL data base. All metadata, including precise geographic coordinates and ENA accession numbers (ENA project PRJEB85456) are provided in supplementary material (Supplement 1). The genotypes and their metadata are also archived in the ZENODO digital repository under accession #####.

Chromosome counts obtained from Peruzzi *et al.* (2011) and from Manning & Goldblatt (2001), for Mediterranean and South African taxa respectively, were reported on table 1. Preliminary analyses of genome size estimation by flow cytometry, conducted for this study, reported a small range of variation around 3 pg/2c which was not consistent with chromosome counts variation. Technical issues due to the choice of internal standard or the conservation of the tissues were suspected, as well as the possibility of a negative correlation between chromosome size and chromosome number (Peruzzi *et al.,* 2011) and/or disploidy. As a consequence, flow cytometry analyses did not allow to inform ploidy levels and were not used here.

Table 1: *Romulea* sampling (full metadata in Supplement 1). Powo = current taxonomy according to powo.science.kew.org (consulted 05/11/2025); N = number of samples genotyped for this study. Chromosomes numbers (2n) are from Peruzzi *et al.,* (2011) excepted to South African taxa which are from Manning & Goldblatt (2001).

Romulea taxon	**Powo**	**N**	**Areas**	**2n**
R. arnaudii Moret	accepted	10	Provence	-
R. arnaudii x ***columnae***		3	Provence	-
R. arnaudii x ***rollii***		1	Provence	-
R. assumptionis Font Quer	Romulea columnae subsp. assumptionis	8	Minorca, Hyères Islands	56
R. bocchierii Frignani & Iiriti	accepted	3	Sardinia	45
R. bulbocodium (L.) Sebast. & Mauri	accepted	13	Liguria, Sardinia, Sicily	36
R. columnae Sebast. & Mauri	accepted	37	Corsica, Liguria, Malta, Minorca, Provence, Sardinia	27, 45
R. columnae x ***ramiflora***		3	Provence	-
R. corsica Jord. & Fourr.	accepted	6	Corsica	-
R. florentii Moret	accepted	17	Hyères Islands, Provence	-
R. insularis Sommier	accepted	2	Capraia Island (Tuscan Archipelago)	45
R. *x* ***jordanii*** Beg.	accepted	1	Corsica	-
R. ligustica Parl.	accepted	14	Corsica, Liguria,	36

			Sardinia	
R. linaresii Parl.	accepted	14	Sicily	54
R. x ***melitensis*** Bég.	accepted	6	Malta	-
R. aff. ramiflora		4	Minorca	36
R. ramiflora Ten.	accepted	34	Corsica, Liguria, Minorca, Provence, Sicily	36
R. requienii Parl.	accepted	23	Corsica, Sardinia	36
R. revelieri Jord. & Fourr.	accepted	19	Corsica, Sardinia	36
R. rollii Parl.	Romulea columnae subsp. rollii	36	Hyères Islands, Minorca, Provence, Sardinia	36
R. variicolor Mifsud	accepted	18	Malta, Sicily	-
R. dichotoma (Thunb.) Baker	accepted	1	South Africa	30
R. pratensis M.P.de Vos	accepted	1	South Africa	44
R. rosea (L.) Eckl.	accepted	2	Provence (exotic)	18
R. saldanhensis M.P.de Vos	accepted	1	South Africa	24

DNA extraction and quality control

Leaf tissue samples were silicagel dried for several weeks prior to DNA extraction, which was conducted at at the Molecular and Cell Biology facility of IMBE (Marseille, France). Approximately 20-30 mg of dried tissue were fragmented into pieces < 5 mm and homogenized using a FastPrep-24 tissue grinder (MP biomedicals) with three glass beads per tube at the lowest speed (45m/s) for 45 seconds, repeated once. Following centrifugation, genomic DNA was extracted using the Nucleospin II Kit (Macherey-Nagel) with the following modification: lysis was performed with 600 µl of PL1 buffer for 1 h at 65°C. To minimize carryover of debris, 250 250µl of lysate were carefully pipetted from the supernatant, leaving ~200µL at the bottom of the tube. thereby improving 230/260 purity ratios. DNA was eluted in two sequential steps (60 µl and 30 µl) of PE buffer. DNA concentration and purity were assessed first using a Nanodrop One spectrophotometer (Thermo scientific) and subsequently validated with a Qubit HS dsDNA fluorometric assay (HS kit, Thermo Fisher Scientific). Samples yielding < 10 ng/µl or suboptimal purity ratios (<1.0 at A260/A230) were re-extracted when tissue material was available. High-quality DNA extracts were normalized to a concentration between 8 and 11 ng/µl and organized into 24-sample pools.

Genomic library preparation, target capture and sequencing

Normalized DNA samples were shipped to the IGENseq service (ICM, CHU Pitié-Salpêtrière, Paris, France) for library preparation, target enrichment, and sequencing. A total of 150 ng of genomic DNA per sample were subjected to enzymatic fragmentation for 15 min to generate insert sizes ranging 200-450 bp. Libraries were prepared with the NEBNext Ultra II FS DNA Library Prep Kit (New Englands Bio lab), quantified with Qubit HS and SPARK fluorometry, and equimolarly pooled in groups of 24 for hybrid capture with the Angiosperms353 probe kit (Baker *et al.*, 2022). Hybridization-based target enrichment was performed with the MyBaits v5 kit following the manufacturer's standard protocol, using 500 ng of pooled libraries as input. Post-capture amplification consisted of 15 PCR cycles using KAPA HiFi HotStart polymerase (Roche sequencing solutions), followed by purification with Ampure XP magnetic beads. Sequencing was carried out on an Illumina NextSeq 2000 platform with paired-end 150 bp reads, generating approximately 400 million reads for 192 samples.

Bioinformatics pipeline and genotyping

Given the absence of a reference genome for *Romulea* and the unknown ploidy levels of several taxa, we adopted a reference-based genotyping-by-sequencing approach designed to maximize locus recovery while minimizing paralogous interference. Raw reads were demultiplexed and quality-filtered using fastp (Chen *et al.*, 2018), retaining reads with Phred scores ≥30. A representative set of 42 *Romulea* samples was selected to assemble target loci using HybPiper v2.16 (Johnson *et al.*, 2016) with the Mega353 target reference (McLay *et al.*, 2021). Supercontig (exons and introns) were extracted, and loci with <20 sample coverage or >2 paralog warnings were excluded. Multiple sequence alignments were performed using MAFFT with default parameters (Katoh *et al.*, 2002), and trimmed with TrimAl (Capella-Gutiérrez *et al.*, 2009) in automatic mode and manual curation in AliView (Larsson, 2014) to remove spurious regions. Consensus sequences for each locus were generated using the EMBOSS (Madeira *et al.*, 2024) cons tool and concatenated into a custom reference used for read mapping.

Variant discovery was conducted using BWA-MEM (Li & Durbin, 2009) for alignment, duplicate removal with SAMBAMBA (Tarasov *et al.*, 2015), and SNP calling with FreeBayes (Garrison & Marth 2012) under the following parameters: ploidy = 2, use-best-n-alleles = 4, min-mapping-quality = 30, min-base-quality = 20, min-coverage = 6, min-alternate-count = 3, no-population-priors, hwe-priors-off. Resulting VCF files were filtered using BCFtools (Danecek *et al.*, 2021) to retain only bi-allelic SNPs with minor allele frequency ≥0.01.

Genotype calling was performed with polyRAD (Clark *et al.*, 2019), following best practices for polyploid genotyping (Cohen & Turgman-Cohen, 2023; Gorospe *et al.*, 2025; Phillips *et al.*, 2025). Potential ploidy levels were parameterized as tetraploid. The vcf was imported in polyRAD with the function VCF2RADdata with default parameters. The $H\mathrm{ind}/H_E$ index (Clarck *et al.*, 2022) was used to estimate if the genotypes analysed here follow a diploid or a polysomic polyploid inheritance. It is independent of genotype calling. $H\text{ind}/H_E$ = (k-1)/k (1-F) where k is the ploidy level and F the inbreeding rate. After excluding loci having $H\text{ind}/H_E < 0.10$ or >1.0, the expected $H\text{ind}/H_E$ distribution was simulated under diploid, autotetraploid or autohexaploid inheritance and two inbreeding coefficients (0 and 0.5). The overdispersion parameter was recalculated post-filtering, and continuous genotype probabilities were estimated with IteratePopStruct and GetWeightedMeanGenotypes functions. To estimate the most likely value for the inbreeding parameter for IteratePopStruct function, individuals exhibiting mean $H\text{ind}/H_E$ values > 0.8 were removed to avoid allopolyploid outliers, simulations $H\text{ind}/H_E$ were conducted for several inbreeding values. The observed distribution of $H\text{ind}/H_E$ was similar to the simulated distribution for an inbreeding of 0.2 and this value was used to perform the continuous genotype calling for all individuals.

Genetic structure analyses

To characterize genetic structure accounting for uncertain ploidy levels, we employed a non-model-based clustering strategy. Euclidean genetic distances were calculated from continuous genotype data, followed by hierarchical clustering analysis (HCA) using Ward’s minimum variance algorithm. Cluster membership was defined by dendrogram truncation at levels maximizing congruence with prior taxonomic expectations. When groups with several taxa were obtained, each group was split into the corresponding taxa in relation and according to the clustering of the genotypes. Genotypic similarity matrices were constructed as 1 – normalized Euclidean distances and visualized as heatmaps, enabling direct inspection of group distinctiveness and potential admixture. To further evaluate groups separation, we performed Discriminant Analysis of Principal Components (DAPC) using the adegenet R package (Jombart & Ahmed, 2011), with the groups previously defined as a priori discriminant factor. Cross-validation (xvalDapc) and optimization of the α-score (optim.a.score) were used to avoid overfitting. Where admixture signals were detected, Neighbor-net analyses (Huson & Bryant, 2024) were performed on reduced data subsets to refine group boundaries.

In details, HCA was conducted with the ward algorithm, minimising within cluster variance on Euclidean distance between genotype (R dist and hclust functions). Cutree function was used to truncate and design clusters. Then the clusters were displayed on the dendrogram with the rec.hclust function. If a grouping according to taxa was observed in one cluster new groups were designed. The heat map of genotypic similarity was computed on a matrix of genotypic simalrities obtained after the following transformations: (i) division of Euclidean distances by their maximum distance to obtain values between 0 and 1 (normalized Euclidean distances) and (ii) the similarities were obtained by subtracting this distance from 1. A zero value was assigned to the diagonal of the matrix and then the heatmap was performed with the heat map function and the genotype ordered in row and columns according the dendrogram obtained above, allowing the observation of genetic groups on the heatmap. Colours were chosen to reflect genotypic similarities from grey (low values) to intense magenta (great similarities). With this method, isolated genetic groups, not connected among them by gene flow, will form square of genotypes aligned along the diagonal in a background of low similarities (grey), their colours will change according to the level of their divergence (magenta). However, in case of admixture between groups, patches or magenta, more or less intense (genetic similarity) will appear at the intersection of row and columns corresponding to the groups. The DAPC analysis was conducted with these group as a priori factor (dapc function, adegenet R package) after having selected with xvalDapc and the optim.a.score functions the the optimal number of principal components to avoid overfitting. For subset analyses, Heat map of genetic similarities were done as described above and Euclidean genetic distances between genotypes were converted to nexus format with the write.nexus.dist function (phangorn R package; Schliep, 2011) to draw network with the Neighbor-net method implemented in the SplitsTree App (Huson and Bryant, 2024). Principal component analysis were done with the dudi.pca function (ade4 packages). DAPC and PCA results were drawn with the ggplot2 R package (Villanueva & Chen, 2019).

Plastid genome variant calling and phylogenetic inference

Given heterogeneous off-target plastid recovery among individuals, a variant-calling based phylogenetic approach was adopted. A reference (Figure S1, Supplement 2) was first assembled from one *R. florentii* specimen using GetOrganelle (Jin *et al.,* 2020) with default parameters and annotated with CPGAVAS2 (Shi *et al.,* 2019). Reads were mapped to this plastome reference, and SNP calling was conducted with Freebayes in haploid mode (ploidy = 1). The resulting VCF was converted to FASTA format using vcf2phylip (Ortiz, 2019) and parsimony- informative sites were retained with ClipKit (Steenwyk *et al,.* 2020). Sequences with > 30% missing data were discarded. Maximum likelihood phylogenetic trees were reconstructed in IQTREE (Minh *et al.,* 2020) under a GTR+ASC substitution model, which is adapted to SNP data, with 100 parsimony starting trees and 1,000 unsuccessful iteration stop criterion. Node support was evaluated using ultrafast bootstrap (1,000 replicates; with -bnni option; Minh *et al.,* 2013) and SH-aLRT likelihood ratio tests (Guindon *et al.,* 2010).

Results

Nuclear SNP dataset assembly

Following quality filtering and the exclusion of three low-coverage samples, an average of 12 million paired-end reads per sample was retained, with a mean on-target capture efficiency of 47%. Based on HybPiper recovery statistics, 331 loci were retained after discarding genes absent in >50% of samples and those with >2 paralog warnings. After alignment, trimming, and manual curation, the concatenated supermatrix comprised approximately 316 kbp of nuclear sequence data. Consensus reference sequences derived from these loci were subsequently used for SNP discovery and genotype calling.

After stringent filtering (MAF ≥ 0.01, $H\text{ind}/H_E$ between 0.10 and 1.0), the final dataset contained 272 individuals genotyped at 8,168 high-confidence bi-allelic SNPs, with an overall missing data rate of 9%. Completeness heatmap (Figure S2 in Supplement 2) generated with vcfR revealed no systematic bias across individuals, confirming the dataset was suitable for downstream population-genetic analyses.

Simulations of expected $H\text{ind}/H_E$ under three modes of inheritance yielded a range between 0.10 and 0.77 (Figure 2A). The distribution of observed $H\text{ind}/H_E$ across loci was bimodal, with a primary mode at approximately 0.55 and a secondary mode near 1.0 (Figure 2B). The observed peak at 0.56 is consistent with an autotetraploid inheritance model with a low inbreeding rate, whereas the peak near 1.0 is indicative of fixed heterozygosity, suggesting allopolyploid inheritance for a large subset of loci. At the individual level, $H\text{ind}/H_E$ values ranged from 0.5 to 0.95 with a pronounced mode near 0.75 (Figure 2C), indicating that the majority of individuals exhibit inheritance patterns compatible with allopolyploidy than with strict autopolyploidy.

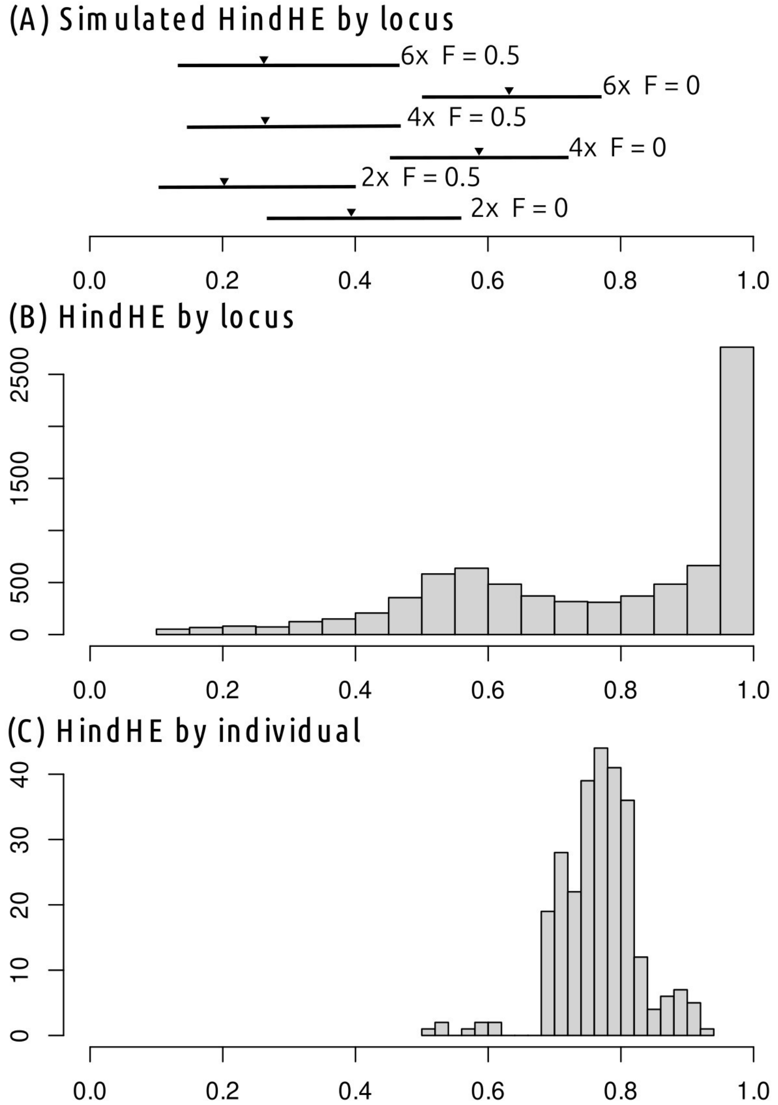

Figure 2: *H*ind/H_E analyses. (A) 95% CI and mean of values by locus after simulations acording to diploid, autotetraploid or autohexaploid inheritance and two inbreeding values (F). (B) and (C) Histograms of observed values by locus or by individual.

Genetic similarities and grouping

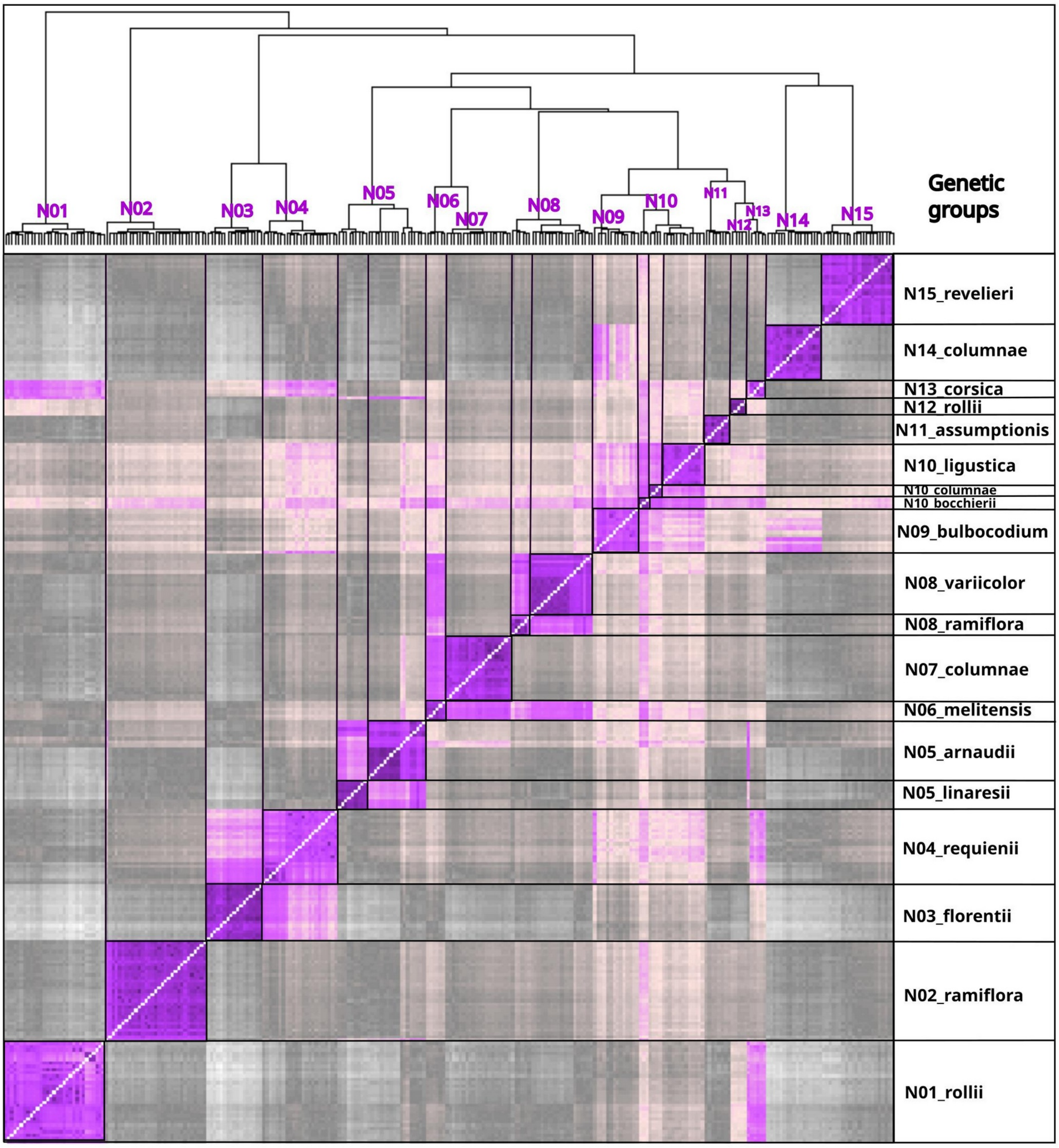

Figure 3: Heatmap of similarities between 272 genotypes of 8168 biallelic SNPs for the *Romulea* taxa studied. Magenta intensity is proportional to genotypic similarities. Genotypes were organized according to a hierarchical clustering (Ward algorithm) of their dissimilarities, and 15 clusters were designed by truncation of the dendrogram, then some clusters were subdivided in respect to putative taxa, for finally designing 19 genetics groups.

Genetic structure and clustering analyses

Hierarchical clustering of Euclidean genetic distances delineated 15 primary clusters (Figure 3). Incorporation of taxonomic priors and examination of substructure led to the recognition of four additional subclusters, resulting in 19 genetically coherent groups. Heatmaps of genotypic similarity confirmed that most groups were strongly differentiated, forming distinct high-similarity blocks along the diagonal. Nevertheless, off-diagonal similarity signals were detected, indicative of gene flow or shared ancestry among several groups. Examples include pronounced similarity between N01 (*R. rollii*), N04 (*R. requienii*) and N13 (*R. corsica*), as well as between N06 (*R. x melitensis*) and its putative parental clusters N07 (*R. columnae*) and N08 (*R. variicolor*). Putative hybrids identified during fieldwork between *R. arnaudii* × *R. columnae* and between *R. arnaudii* × *R. rolli* were supported by their intermediate placement in the heatmap.

Following cross-validation to prevent overfitting, 13 principal components explaining 52% of the total genetic variance were retained for DAPC (Figure 4). The resulting discriminant functions separated the 19 genetic groups with variable degree of resolution. N01 (*R. rolii*), N02 (*R. ramiflora*), N07 (*R. columnae*), and N15 (*R. revelierei*) were among the most genetically isolated clusters. N08 (*R. variicolor* and *R. ramiflora*), N11 (*R. assumptionsis*), and N14 (*R. columnae*) were also well-separated but closer, consistent with a more recent divergence or incomplete lineage sorting. The groups N03 (*R. florentii*), N04 (*R. requienii*), and N05 (*R. arnaudii* and *R. linaresii*) exhibited a partial overlap despite their geographical separation. Within N08 (*R. variicolor* and *R. ramiflora*) and within N10 (*R. ligustica*, *R. columnae* and *R. bocchierii*), genetic separation was minimal, consistent with either historical or ongoing admixture. N09 (*R. bulbocodium*) was isolated but always close and at intermediate distance between N10 and N14, suggesting a hybrid origin.

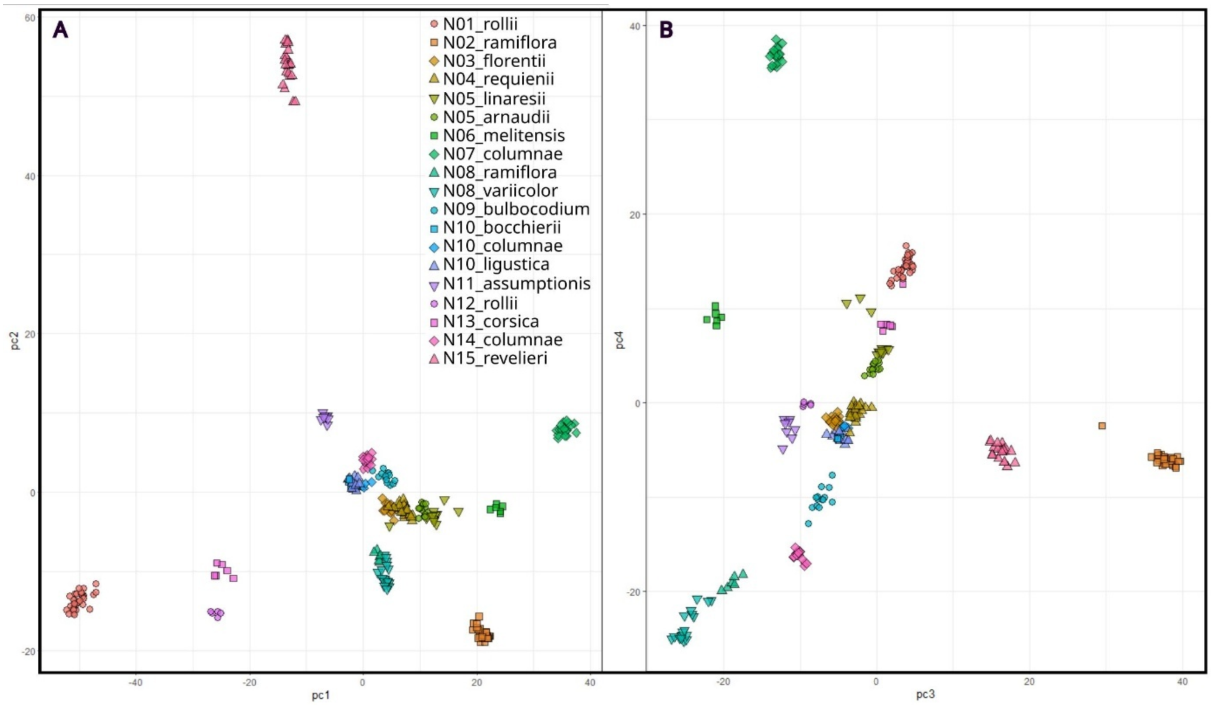

Figure 4 Discriminant analysis of principal components (DAPC) of 272 genotypes of 8168 biallelic SNPs for the *Romulea* taxa studied. The genetic groups were used as *a priori* factors and the first 13 principal components (52% of the variance) were chosen to conduct the DAPC. The first four DAPC axes are shown (A: axes 1 and 2; B: axes 3 and 4).

To further refine genetic groups and investigate admixture signatures, Neighbor-net analyses were conducted on selected groups (Figure 5). In the N01-N04-N13 triad, N13 (*R. corsica*) occupied an intermediate position between N01 (*R. rollii*) and N04 (*R. requienii*), confirming its hybrid origin. Similarly, N06 (*R.* x *melitensis*) exhibited an intermediate position between N07 (*R. columnae*) and N08 (*R. variicolor*). N09 (*R. bulbocodium*) displayed an intermediate position between N10 (*R. ligustica*) and N14 (*R. columnae*) but some genotypes were closer to N10. The genotypes of *R. bocchieri* were in intermediate position between *R. ligustica* and *R. bulbocodium*. Although, N03 (*R. florentii*), N04 (*R. requienii*), and N05 (*R. arnaudii* and *R. linaresii*) display an important proximity according to DAPC (Figure 4), Neighbor-net analyses (Figure 5) support that they are forming two isolated genetic groups. Despite their current geographical isolation, the Nnet network suggests that *R. arnaudii* and *R. florentii* derive from *R. linaresii* and *R. requienii*, respectively.

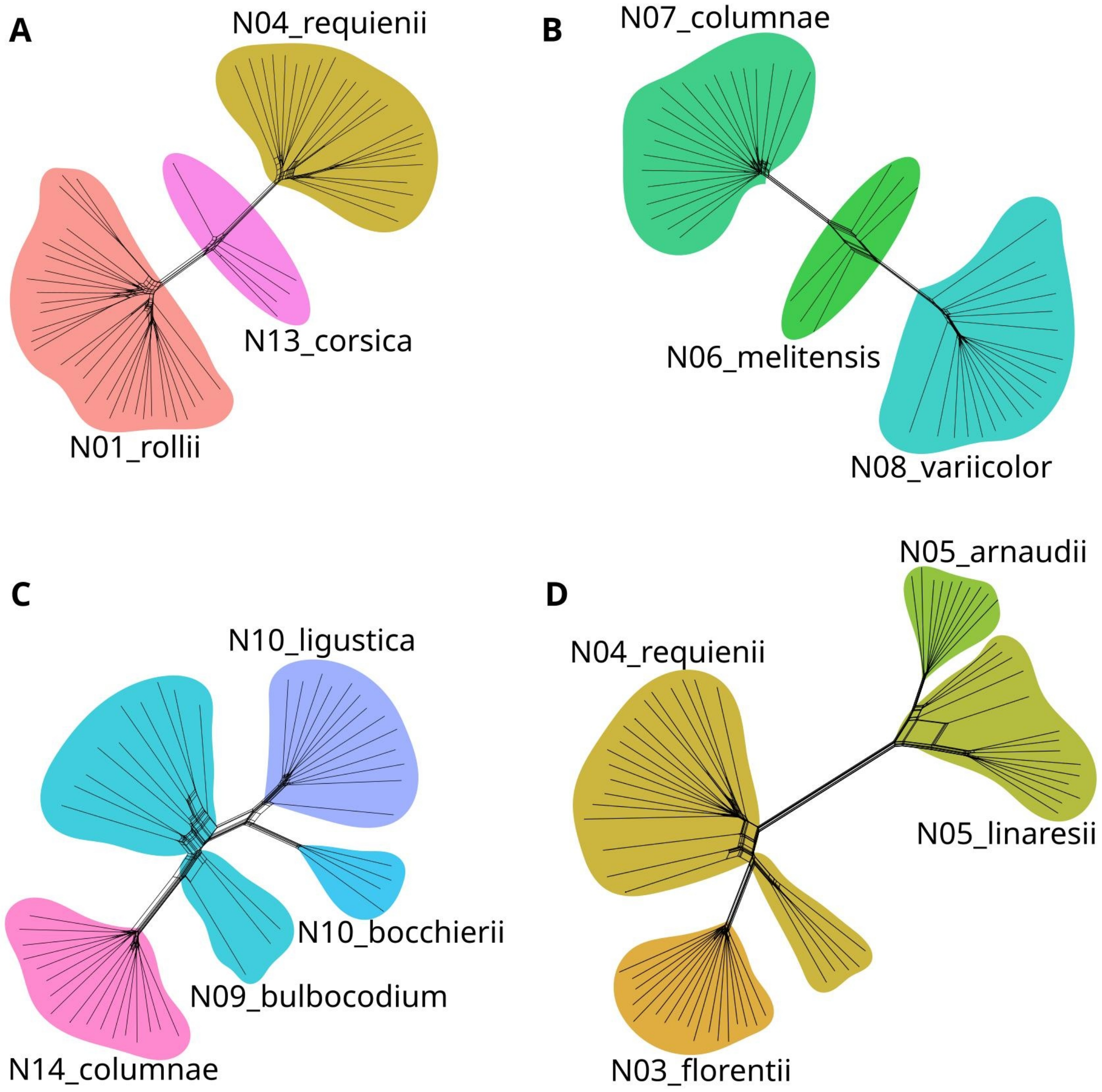

Figure 5: Neighbor-net networks of genetic relationships between selected groups of the *Romulea* genotypes, drawn at the same scale.

Plastid phylogenomics

The plastome assembled for *R. florentii* having a length of 151 kbp was used for variant calling. Variant-based plastid phylogeny was reconstructed from 262 haplotypes across 965 parsimony-informative sites, with an overall missing data rate of 4% (Figure S3, Supplement 2). After excluding South African haplotypes, the resulting Mediterranean clade was strongly supported as monophyletic and contained 798 parsimony informative sites. Maximum likelihood analyses resolved 23 well-supported clades which were numerically indexed for cross-comparison with nuclear genetic groups.

Although terminal clades exhibited high bootstrap and SH-aLRT support, several deeper nodes lacked robustness, resulting in a star-like diversification pattern consistent with rapid radiation. The Tyrrhenian haplotypes were partitioned into six major lineages, one of which encompassed clades 6 to 23, though the relationships among these internal clades remained poorly resolved.

Review of *Romulea* genetic groups

The nineteen genetic groups, based on nuclear genotypic distances, were matched to plastid haplotype clades, taxonomic assignments, and geographic areas (Table 2). We review these groups individually, focusing on their distinctiveness and correspondence with morphological identifications.

N01. This group is distributed across six areas and corresponds exclusively to *R. rollii*. It is associated predominantly with plastid clade P14, which is also present in *R. corsica* and represents the maternal contribution in that taxon.

N02. Found in six areas, this group includes *R. ramiflora*, *R. aff ramiflora* from Minorca, and hybrids involving *R. columnae*. It is characterized by exclusive association with plastid clade P3. Most individuals identified as *R. ramiflora* fall within this group.

N03 and N04. These two groups are closely related. N03 corresponds to *R. florentii*, endemic to Provence and the Hyères Islands, while N04 corresponds exclusively to *R. requienii*, endemic to Corsica and Sardinia. Nnet analysis shows that *R. florentii* derives from *R. requienii*. Both share plastid lineages, although N04 exhibits greater nuclear genetic diversity. *Romulea requienii* functions as the paternal parent of *R. corsica*.

N05. This group encompasses *R. arnaudii* and *R. linaresii*, narrow endemics of Provence and northern Sicily, respectively. Their differentiation remains incomplete in all analyses (Figure 4 and Figue 5). Two closely related plastid clades are specific to N05: P20, which is unique to *R. linaresii*, and P21, which is shared between *R. linaresii* and *R. arnaudii*. Within P21, the haplotypes of *R. arnaudii* form a monophyletic group, supported by bootstrap and SH-aLRT values of 88/88.

N06. This group corresponds to *R. x melitensis*, a narrow endemic hybrid of Malta. Nuclear clustering places it as intermediate between indicates N07 (*R. columnae*) and N08 (*R. variicolor*), consistent with a hybrid origin involving these two parental lineages.

N07. Genotypes within this group were identified primarily as *R. columnae*, with a minority identified as *R.* aff. *ramiflora* in Minorca. N07 is found in six areas, with main sampling in Provence and Minorca. It contains three plastid clades, among which P18 is the most common and specific to N07 and N06. N07 represents the maternal lineage of *R. x melitensis*.

N08. This group includes all *R. variicolor* genotypes from Sicily and Malta, a subset of *R. ramiflora* individuals from multiple localities, and some *R. columnae* individuals. Within N08, all *R. ramiflora* and a majority of *R. variicolor* are associated with plastid clade P23, whereas clade P22 is restricted to *R. variicolor*. *R. ramiflora* genotypes in this group are genetically closer to *R. variicolor* than to the *R. ramiflora* individuals of N02.

N09. This group corresponds to *R. bulbocodium* and includes a single genotype of *R. corsica*. Analyses of genetic similarity (Figure ###), particularly the heatmap and PCA, indicate that *R. bulbocodium* shares affinities with both N10 (*R. ligustica*) and N14 (*R. columnae*), suggesting these lineages as potential parental taxa. Plastid diversity is high in N09, with fourteen sequenced samples revealing a polyphyletic pattern.

N10. This group contains *R. ligustica* genotypes, which form a distinct groups and possess a private plastid lineage. *Romulea ligustica* occurs in three regions: Liguria, Corsica and Sardinia. Additionally, three genotypes clustering near *R. ligustica* were morphologically identified as *R. columnae* (two individuals from Liguria) or as a hybrid between *R. ramiflora* and *R. columnae* (one individual from Provence). The genotypes of *R. bocchierii* form one group within N10. As shown above, this endemic of Sardinia is close to *R. ligustica* and *R. bulbocodium*, but it has its own plastid lineage (P01) which is also the most basal in plastid phylogeny.

N11. This group includes *R. assumptionis* genotypes collected in Minorca and the Hyères islands. They are also identified by the plastid clade P06. Plastid relationship show that French haplotypes derived from Minorcan populations.

N12. Although these genotypes of Hyères islands, *identified as R. rollii*, share strong nuclear similarity with N01 (*R. rolii*) and belong to the same plastid lineage, they consistently form a separate genetic group in all analyses.

N13. This group is composed mainly of *R. corsica*. Analyses indicate a hybrid origin with *R. requienii* and *R. rolli* as parental taxa. Four out of six plastid haplotypes, belong to the *R. rollii* lineage, while two form a distinct lineage near the base of the plastid phylogenetic tree. N13 also contains a hybrid between *R. arnaudii* and *R. rollii*.

N14. This group corresponds to a second genetic group of *R. columnae*. Genotypes are clearly discriminated but remain genetically close to N09 (*R. bulbocodium*) and N10 (*R. ligustica*), with which hybridization has occurred. N14 includes populations from Corsica and Sardinia, and most individuals carry plastid clade P15, which is also shared with *R. bulbocodium* (N09) and *R. revelieri* (N15).

N15. This group corresponds primarily to *R. revelieri*, endemic of Corsica and Sardinia, as well as *R.*x *jordanii* (a putative hybrid between *R. revelieri* and *R. ramiflora*) and *R. insularis* (endemic to Capraia island). The latter two fall entirely within the genetic diversity of *R. revelieri*. N15 is associated with four plastid clades, two of which are exclusive to *R. revelieri* and two that are shared with other taxa.

Table 2: Review of plastid data, taxonomy and distribution for 19 nuclear genetic groups of *Romulea*

Genetic_groups	**n**	**Plastid_clades ***	**Taxa**	**Capr.**	**Cors.**	**Hyer.**	**Ligu.**	**Malt.**	**Mino.**	**Prov.**	**Sard.**	**Sici.**
N01_rollii	31	**P14 (28**), P23	R. rollii (31)		6	6			3	10	3	3
N02_ramiflora	31	**P03**	R. ramiflora (29), R. columnae x ramiflora, R. aff ramiflora		9	1			4	11	3	3
N03_florentii	17	**P08**	R. florentii (17)			12				5		
N04_requienii	23	**P11 (10)**, P10, P09, P08	R. requienii (23)		14						9	
N05_arnaudii	13	**P21 (10)**, P19	R. arnaudii (10), R. arnaudii x columnae							13		
N05_linaresii	14	**P20 (12)**, P21,	R. linaresii (14)									14
N06_melitensis	6	P18	R. x melitensis (6)					6				
N07_columnae	20	**P18 (14)**, P19, P05	R. columnae (16), R. columnae x ramiflora, R. aff ramiflora		2		1	2	7	7	1	
N08_ramiflora	6	**P23**	R. ramiflora				3			2		1
N08_variicolor	19	**P23 (14)**, P22, P18, P04	R. variicolor (18), R. columnae					6				13
N09_bulbocodium	14	**P13 (4),** P15, P12, P04	R. bulbocodium (13), R. corsica		1		4				3	6
N10_bocchierii	3	**P01**	R. bocchierii (3)								3	
N10_columnae	3	P05, P19	R. columnae (2), R. columnae x ramiflora				2			1		
N10_ligustica	14	**P07 (13)**, P01	R. ligustica (14)		3		4				7	
N11_assumptionis	8	**P06**	R. assumptionis (8)			3			5			
N12_rollii	5	**P14**	R. rollii (5)			5						
N13_corsica	6	**P14 (4),** P02	R. corsica (5), R. arnaudii_x_rollii		5					1		
N14_columnae	17	**P15 (14)**, P18	R. columnae (17)		9						8	
N15_revelieri	22	**P17 (13)**, P16, P15, P14, P20	R. revelieri (18), R. insularis, R. x jordanii	2	17						3	

*** in bold the main plastid clade with its number of samples, in underlined when they are private**

Discussion

Species constitute the fundamental operational units of biodiversity research, and their accurate delimitation is essential for linking observed diversity patterns to underlying evolutionary processes (e.g. Queiroz, 2007; Galtier, 2019). Our multilocus nuclear and plastid datasets together provide the first comprehensive molecular framework for *Romulea* of the Mediterranean region, enabling an evaluation of species boundaries, hybridization, and reticulate evolution in this genus. While our results reveal a generally strong concordance between genetic groups and current taxonomic concepts, they also highlight instances of incomplete differentiation, introgression, and plastid capture, indication that species boundaries in *Romulea* are both permeable and shaped by historical reticulation.

Evidences for reticulate evolution

The distribution of Hind/H_E across loci and individuals was incompatible with strictly diploid or autopolyploid inheritance models. The high number of loci having Hind/H_E value near one is indicative of a fixed heterozygosity which is the likely imprint of an allopolyploid origin, consistent with the existence of hybridization events in the evolutionary history of the Mediterranean *Romulea*. Our findings thus corroborate the hypothesis that polyploidization and hybridization have been major drivers of diversification in this genus. Moreover, several taxa (e.g., *R. x melitensis, R. corsica, R. bocchierii* and *R. bulbocodium*) exhibit genetic clustering consistent with hybrid origins, further supporting the role of reticulate evolution. In *R. x melitensis*, the nuclear data and plastid identity converge on a biparental origin involving N07 (*R. columnae*) and N08 (*R. variicolor*). By contrast, *R. corsica* and especially *R. bulbocodium* appear to have more complex hybrid origins, possibly involving multiple parental lineages and repeated hybridization events, as suggested by their association with several plastid clades. The hybrid origin of the narrow Sardinian endemic *R. bocchierii* (Peruzi *et al.*, 2011), involving *R. bulbocodium* and *R. ligustica,* is also supported.

Several cases of hybridization, confirmed or discovered here, involved *R. columnae* as a parent in these hybrids. Interestingly, *R. columnae* has a very different morphology (small white flowers) from most other species of *Romulea*. Our assessment of reticulate evolution is exacerbated by at least five cases of plastid capture, where the plastid haplotype of a taxon did not match its nuclear genetic background. Examples include Sicilian populations of *R. rollii* sharing the P23 plastid clade with *R. variicolor,* few individuals of *R. revelieri* having plastid haplotypes shared with other taxa (P14, P15, and P19), or certain *R. arnaudii* individuals carrying plastid haplotypes characteristic of *R. columnae* (P19). It is remarkable that these plastid captures were observed between populations belonging to well differentiated genetic groups. These findings support that introgression has been active during the evolutionary history of Mediterranean *Romulea* taxa.

Species boundaries and taxonomic implications

Our results confirm the distinctiveness of four taxa (*R. assumptionis* (N11), *R. revelieri* (N15), *R. ligustica* (N10), and *R. rollii* (N01)) which are supported as independently evolving lineages by both nuclear and plastid data. These taxa can thus be considered “robust species” under a genotypic cluster concept of species delimitation (Mallet, 1995). According to Peruzzi *et al.*, (2010), *Romulea revelieri*, *R. ligustica* and *R. rolli* are tetraploid whereas *R. assumptionis* is an hexaploid (Contandriopoulos, 1992). N02 (*R. ramiflora*) and N07 (*R. columnae*) are clearly isolated genetic groups, which represent a substantial part of the genetic variance analysed here. However, other groups were identified as *R. ramiflora* or *R. columnae*. *Romulea ramiflora* forms two nuclear groups, only one of which (N02) exhibits clear plastid and nuclear distinctiveness. This latter could be considered as a robust species. The second group (N08) was rarely observed in 3 areas (Provence, Liguria and Sicily). It is closely related to *R. variicolor*, abundant in Malta and very rare in Sicily, with genetic overlap and shared plastid identity. More data are necessary to decipher its role in the origin of *R. variicolor*. *Romulea columnae* is represented by three separate genetic groups (N07, N10, N14), suggesting that what is currently treated as a single species may instead constitute a complex of partially isolated lineages, which frequently hybridise with other genetic groups. Given its wide distribution across the Mediterranean, the study of *R. columnae* should be expanded to include additional populations. The correlation of these different lineages with the karyological variability reported by Peruzzi et al. (2011) merits further investigation.

In addition, our study questions the status of several narrow endemics. *Romulea* x *jordanii* (endemic of Corsica) and *R. insularis* (endemic of Capraia Island, Tuscan Archipelago) are genetically indistinguishable from *R. revelieri* (common in Corsica but rare in Sardinia), supporting the hypothesis that they may represent intraspecific polymorphism or karyotype variation (*R. insularis* being pentaploid, Peruzzi *et al.*, 2011) rather than distinct species. *Romulea* x *jordanii* is usually considered as an hybrid between *R. revelierei* and *R. ramiflora*. It appears in mixed populations. It is not always easy to distinguish, and the only specimen we were able to find was not typical (pale yellow corolla throat). It is possible that it was a somewhat unusual *R. revelierei*. Although close in our analyses, the two pairs of taxa (*R. arnaudii* / *R. linaresii*; *R. florentii* / *R. requienii*) form two independent genetic groups that are well distinct from the other genetic groups. They should be considered as two robust species hypotheses but, currently, both contain two taxa. *Romulea arnaudii* (endemic from Saint-Tropez, Provence) and *R. linaresii sensu stricto* (endemic of N Sicily) are more differentiated than the previous case, but, despite being separated by a large geographic distance, they are still very close since they share a single genetic group (N05) and plastid haplotypes. Their plastid differentiation is limited to private haplotypes, suggesting that they divergence recently, possibly during the late Pleistocene. The differentiation between *R. requienii* (Corso-Sardinian endemic) and *R. florentii* (endemic from Hyères Islands and Cap Bénat, Provence) is more evident. However, the latter is part of the genetic diversity *of R. requienii*, suggesting incomplete lineage sorting. For both cases, investigating the causes of their geographical isolation with the support of divergence time analyses, will be necessary to definitively assess their taxonomic status.*Romulea bocchierii*, a very rare endemic to Sardinia with a pentaploid karyotype, is confirmed to be an hybrid but holds a unique plastid lineage that branches at the root of the plastid phylogenetic tree. This underlines its distinctiveness and originality. The sole population of *R. bocchierii* grows on a plateau on substrate originating from Paleozoic metamorphic rocks. Currently, the only other species found in the same habitat is *R. ligustica*. The currently known populations of *R. bulbocodium* in Sardinia are more than 100 km away from the population of *R. bocchierii*. *Romulea variicolor* is also supported by molecular data, but its close relationship with one group of *R. ramiflora* need further analyses to decipher the distinctiveness of this taxon. Finally, two rare and endemic taxa, *R. corsica* and *R. x melitensis*, are also supported as well as their clear hybrid origin.

In summary, our study shows that genetic boundaries are fairly robust despite evidence of reticulate evolution, which undoubtedly had a significant contribution to the diversification of the *Romulea* genus in the Mediterranean. After this first assessment, it remains an important issue concerning *R. bulbocodium*. This aggregate exhibits great variability, but it is still poorly understood in terms of taxonomy. Despite its limited sampling in this study, it is one of the most common taxa in the Mediterranean. Our first results suggest that *R. bulbocodium* establishes a genetic link with several genetic groups and its intermediate position between *R. ligustica* (N10) and *R. columnae* (N14), revealed by genotypic similarities, is clearly challenged by its wide plastid diversity. Further studies encompassing more western Mediterranean and Atlantic populations are needed to better understand the evolutionary patterns and the links between the diverse taxonomic entities described within *R. bulbocodium* aggregate.

Biogeographic and evolutionary insights

Because of their occurrence throughout the Mediterranean basin and on all the major islands, *Romulea* taxa constitute a fascinating model for documenting the complex historical biogeography of this area. In this first molecular study, the genetic data reveal striking patterns of evolutionary links among populations separated by large geographic distances and insular barriers. This pattern of relatedness despite isolation is a recognised feature of the Tyrrhenian biogeography, with numerous endemic taxa shared between the various islands for plants (Médail, 2022) and animals (Bidegaray-Batista & Arnedo, 2011). To interpret these similarities in terms of connectivity or common ancestry predating the separation of the islands from the mainland, it is necessary to compare the likely times of divergence of the populations with the geological history of their areas of occurrence. Certain studies have revealed divergence times compatible with palaeogeographic phenomena linked to the separation of the Proto-Ligurian massif from the continent and the rotation and isolation of Corsica and Sardinia (Salvo *et al.*, 2010). However, other studies have revealed more recent divergences, suggesting long-distance dispersal (Molins *et al.,* 2011; Carnicero *et al.,* 2017; 2018) or the maintenance of a physical connection, still hypothetical, between the islands and the continent until the end of the Miocene (Ketmaier *et al.,* 2006). Some pairs of lineages, which are still very close, such as *R. florentii* versus *R. requienii*, or *R. arnaudii* versus *R. linaresii*, or even the two current areas of presence (Balearic Islands and Hyères Islands in Provence) for *R. assumptionis*, clearly raise doubts about isolation events more ancient than the Pliocene. The observed star-like topology of the plastid phylogeny further supports a scenario of Pleistocene diversification. Thus, the present study opens an important issue for performing divergence time analyses of the several genetic groups revealed here in relation to the palaeogeography of the Mediterranean Basin.

Conclusions and future directions

Our study provides the first genome-scale assessment of *Romulea* taxa in the Mediterranean Basin, uncovering a complex interplay of polyploidy, hybridization, plastid capture, and incomplete lineage sorting. Our findings underscore the need for more extensive genomic studies. These investigations should initially verify the allopolyploid nature of Mediterranean *Romulea*, followed by the identification of the homeologs genomes and their ancestry. This would allow to refine properly the relationships between the different taxa. The aim of molecular identification of taxa has offered opportunities for exchange and discussion between partners from several countries on the validity of taxa in Mediterranean *Romulea*. It appears that, in general, the morphological and molecular identifications correspond fairly well, but not completely. A comprehensive integrative taxonomic revision of the genus will have important implications for conservation and biogeography within the Mediterranean basin hotspot.

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Supplementary material

Supplement 1: Metadata of all genotypes (Excel file)

Supplementary material 2: Figure S1, S2 and S3

Supplementary material 1

Id_raw_data	id_analyses	taxon	Country	Area	nuclear_group	Plastid_group	Site	Longitude	Latitude
23AB_A01	AssFrLev12	R_assumptionis	France	Hyeres_islands	N11_assumptionis	P06	Hyères île du Levant	6,47032	43,0226
23AB_A02	ColFrHye23	R_columnae_subsp_columnae	France	Provence	N07_columnae	P19	Hyères Notre-Dame de Consolation	6,12781	43,09792
23AB_A03	RolFrHye15	R_rollii	France	Provence	N01_rollii	P14	Hyères Plage du Ceinturon	6,15886	43,0887
23AB_A04	RamFrSar14	R_ramiflora	France	Provence	N02_ramiflora	P03	Sainte-Maxime Pointe des Sardinaux	6,67158	43,31329
23AB_A05	ColFrHye13	R_columnae_subsp_columnae	France	Provence	N07_columnae	P19	Hyères Le Fenouillet	6,09669	43,1347
23AB_A07	RamFrVil33	R_ramiflora	France	Provence	N02_ramiflora	P03	Villeneuve-Loubet parc de Vaugrenier	7,12838	43,6211
23AB_A09	RamFrVil31	R_ramiflora	France	Provence	N02_ramiflora	P03	Villeneuve-Loubet parc de Vaugrenier	7,12838	43,6211
23AB_A11	ColFrTur32	R_columnae_subsp_columnae	France	Provence	N07_columnae	P19	La Turbie Mont Justicier	7,4105	43,74751
23AB_A12	RamFrGar22	R_ramiflora	France	Provence	N02_ramiflora	P03	La Garde Le plan	6,01542	43,1184
23AB_A14	RolFrCan65	R_rollii	France	Provence	N01_rollii	P14	Cannes La Croix des Gardes	6,98522	43,56083
23AB_A15	RolFrHye13	R_rollii	France	Provence	N01_rollii	P14	Hyères Plage du Ceinturon	6,15886	43,0887
23AB_A16	ColRamFrAnt32	R_columnae_x_ramiflora	France	Provence	N07_columnae	P19	Antibes Bois de Garoupe	7,1295	43,56392
23AB_A17	ColRamFrAnt41	R_columnae_x_ramiflora	France	Provence	N10_columnae	P05	Antibes Cap d'Antibes	7,14269	43,55331
23AB_A18	RamFrSar15	R_ramiflora	France	Provence	N02_ramiflora	P03	Sainte-Maxime Pointe des Sardinaux	6,67158	43,31329
23AB_A19	ArnFrSt73	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,69568	43,2618
23AB_A20	FloFrBor12	R_florentii	France	Provence	N03_florentii	P08	Bormes-les-Mimosas	6,36219	43,1125
23AB_A21	ArnFrSt61	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,69149	43,2701
23AB_A25	ArnFrSt32	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,67847	43,2775
23AB_A27	RolFrHye14	R_rollii	France	Provence	N01_rollii	P14	Hyères Plage du Ceinturon	6,15886	43,0887
23AB_A29	LinSiGal42	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Monte Gallo	13,28943	38,21136
23AB_A30	RolFrCol41	R_rollii	France	Provence	N01_rollii	P14	Collobrières montagne de Marescau	6,3686	43,21918
23AB_A33	RolFrLev34	R_rollii	France	Hyeres_islands	N12_rollii	P14	Hyères Ile du Levant	6,46875	43,03157
23AB_A35	RolFrLev21	R_rollii	France	Hyeres_islands	N12_rollii	P14	Hyères Ile du Levant	6,46881	43,03157
23AB_A36	RamFrSar11	R_ramiflora	France	Provence	N02_ramiflora	P03	Sainte-Maxime Pointe des Sardinaux	6,67158	43,31329
23AB_A37	ArnColFrSt11	R_arnaudii_x_columnae	France	Provence	N05_arnaudii	P20	Saint Tropez	6,69624	43,2631
23AB_A38	ColRamFrHyr12	R_columnae_x	France	Provence	N02_ramiflora	P03	Hyères Les	6,19296	43,11751

		_ramiflora					Vieux Salins		
23AB_A39	ArnFrSt13	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,67445	43,27764
23AB_A40	ArnFrSt52	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,68195	43,2747
23AB_A41	ReqCoMon23	R_requienii	France	Corsica	N04_requienii	P11	Monacia-d'Aullène Mucchiu Biancu	8,96721	41,48683
23AB_A42	RolFrLev24	R_rollii	France	Hyeres_islands	N12_rollii	P14	Hyères Ile du Levant	6,46881	43,03157
23AB_A43	AssFrLev13	R_assumptionis	France	Hyeres_islands	N11_assumptionis	P06	Hyères île du Levant	6,47032	43,0226
23AB_A44	ArnRolFrSt11	R_arnaudii_x_rollii	France	Provence	N13_corsica	P14	Saint Tropez	6,69284	43,2689
23AB_A46	FloFrLev32	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île du Levant	6,45759	43,02759
23AB_A47	ReqCoCen41	R_requienii	France	Corsica	N04_requienii	P08	Centuri Funtana Vecchja	9,35047	42,96875
23AB_A48	BulSiBai22	R_bulbocodium_agg	Italy	Sicily	N09_bulbocodium	NA	Sicile Bala del Corello	13,26769	38,20199
23AB_A49	ArnFrSt74	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,69568	43,2618
23AB_A51	RamFrGar23	R_ramiflora	France	Provence	N02_ramiflora	P03	La Garde Le plan	6,01542	43,1184
23AB_A52	ArnColFrSt22	R_arnaudii_x_columnae	France	Provence	N05_arnaudii	P20	Saint Tropez	6,69068	43,2533
23AB_A54	RolFrLev32	R_rollii	France	Hyeres_islands	N12_rollii	P14	Hyères Ile du Levant	6,46875	43,03157
23AB_A55	RolFrCan63	R_rollii	France	Provence	N01_rollii	P14	Cannes La Croix des Gardes	6,98522	43,56083
23AB_A56	ReqCoRev31	R_requienii	France	Corsica	N04_requienii	P11	Calvi Revellata	8,72166	42,56266
23AB_A58	ArnFrSt112	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,67811	43,2465
23AB_A61	LinSiCof51	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Monte Cofano	12,66129	38,09818
23AB_A62	ReqCoCen43	R_requienii	France	Corsica	N04_requienii	P08	Centuri Funtana Vecchja	9,35047	42,96875
23AB_A63	ReqCoRev33	R_requienii	France	Corsica	N04_requienii	P11	Calvi Revellata	8,72166	42,56266
23AB_A65	AssFrLev14	R_assumptionis	France	Hyeres_islands	N11_assumptionis	P06	Hyères île du Levant	6,47032	43,0226
23AB_A67	FloFrBor14	R_florentii	France	Provence	N03_florentii	P08	Bormes-les-Mimosas	6,36219	43,1125
23AB_A68	ColFrTur33	R_columnae_subsp_columnae	France	Provence	N07_columnae	P05	La Turbie Mont Justicier	7,4105	43,74751
23AB_A69	RevCoBit13	R_revelieri	France	Corsica	N15_revelieri	P17	Sotta Bitalza	9,14292	41,5936
23AB_A70	BulSiMar43	R_bulbocodium_agg	Italy	Sicily	N09_bulbocodium	P13	Marslo cds Son Silvestro	12,49201	37,78211
23AB_A71	LinSiCof52	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Monte Cofano	12,66129	38,09818
23AB_A72	RolFrRam53	R_rollii	France	Provence	N01_rollii	P14	Ramatuelle Salagrue	6,65137	43,22993
23AB_A73	ColFrHye21	R_columnae_subsp_columnae	France	Provence	N07_columnae	P19	Hyères Notre-Dame de Consolation	6,12781	43,09792
23AB_A74	LinSiGal41	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Monte Gallo	13,28943	38,21136
23AB_A75	ArnColFrSt31	R_arnaudii_x_c	France	Provence	N05_arnaudii	P20	Saint Tropez	6,69076	43,2533

		olumnae							
23AB_A76	LinSiMar41	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P22	Marslo cds Son Silvestro	12,49201	37,78211
23AB_A77	RevCoBit11	R_revelieri	France	Corsica	N15_revelieri	P15	Sotta Bitalza	9,14292	41,5936
23AB_A78	FloFrPor23	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île de Port-Cros	6,41853	43,01235
23AB_A79	ArnFrSt92	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,6904	43,2549
23AB_A83	FloFrPor24	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île de Port-Cros	6,41853	43,01235
23AB_A85	RamFrVil32	R_ramiflora	France	Provence	N02_ramiflora	P03	Villeneuve-Loubet parc de Vaugrenier	7,12838	43,6211
23AB_A88	ArnFrSt102	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,68279	43,25014
23AB_A89	ArnFrSt22	R_arnaudii	France	Provence	N05_arnaudii	P22	Saint Tropez	6,67631	43,2783
23AB_A90	LinSiBai24	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Bala del Corello	13,26769	38,20199
23AB_A92	FloFrPor21	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île de Port-Cros	6,41853	43,01235
23AB_A93	FloFrLev31	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île du Levant	6,45759	43,02759
23AB_A95	FloFrPor25	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île de Port-Cros	6,41853	43,01235
23AB_A96	BulSiBos53	R_bulbocodium_agg	Italy	Sicily	N09_bulbocodium	P16	Bosco Scorace	12,77301	37,98992
23AB_B01	BulSaLimA7	R_bulbocodium_agg	Italy	Sardinia	N09_bulbocodium	P15	Mt Limbara	9,141944	40,852222
23AB_B02	VarSiMo6a	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Cava d'Aliga	14,68599	36,72359
23AB_B03	MelMaXew12b	R_melitensis	Malta	Malta	N06_melitensis	P19	Gozo Xewkija	14,256	36,02865
23AB_B04	VarSiMo8c	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Spiaggetta Sampieri	14,73183	36,71949
23AB_B06	RamSaCreA1	R_ramiflora	Italy	Sardinia	N02_ramiflora	P03	Codoleddu Monte Cresia	9,39361	39,25333
23AB_B07	VarMaGh9a	R_variicolor	Malta	Malta	N08_variicolor	P23	Gozo Gharb	14,20406	36,07233
23AB_B08	VarSiMo7a	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Punta Corvo di Sampieri	14,70652	36,71742
23AB_B09	ColSaArrB2	R_columnae_subsp_columnae	Italy	Sardinia	N14_columnae	P15	Bau Arrexini	9,399444	39,2722
23AB_B10	ReqSaBolB2	R_requienii	Italy	Sardinia	N04_requienii	P10	Padru Mannu Bolotama	8,79722	40,3555
23AB_B11	RevSaTeA4	R_revelieri	Italy	Sardinia	N15_revelieri	P18	Teletotes Dorgali Baunei	9,552500	40,09722
23AB_B13	RolSaSoA5	R_rollii	Italy	Sardinia	N01_rollii	P14	Solina Masainas	8,5788	39,02638
23AB_B14	MelMaXew12a	R_melitensis	Malta	Malta	N06_melitensis	P19	Gozo Xewkija	14,256	36,02865
23AB_B15	BulSaLimA5	R_bulbocodium_agg	Italy	Sardinia	N09_bulbocodium	P15	Mt Limbara	9,141944	40,852222
23AB_B17	ColCoSca2	R_columnae_subsp_columnae	France	Corsica	N14_columnae	P15	Corscia Scala	9,05262	42,3623
23AB_B18	VarSiMo7b	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Punta	14,70652	36,71742

							Corvo di Sampieri		
23AB_B19	ReqCoMez2	R_requienii	France	Corsica	N04_requienii	P11	Ajaccio Mezzumare	8,591727	41,877173
23AB_B20	LigCoBon1	R_ligustica	France	Corsica	N10_ligustica	P07	Bonifacio Cala Longa	9,24744	41,40446
23AB_B21	RevSaTeA7	R_revelieri	Italy	Sardinia	N15_revelieri	P18	Teletotes Dorgali Baunei	9,552500	40,09722
23AB_B22	VarSiMo5a	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Fornace Pisciotto	14,75743	36,71178
23AB_B23	BocSaCodA7	R_bocchierii	Italy	Sardinia	N10_bocchierii	P01	Attopiano di Codoleddu	9,393056	39,252778
23AB_B24	BocSaCodA8	R_bocchierii	Italy	Sardinia	N10_bocchierii	P01	Attopiano di Codoleddu	9,393056	39,252778
23AB_B25	ReqSaCatA7	R_requienii	Italy	Sardinia	N04_requienii	P09	Stagno S. Catarina	8,501389	39,085
23AB_B27	VarSiMo8b	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Spiaggetta Sampieri	14,73183	36,71949
23AB_B28	BulSiMod4b	R_bulbocodium_agg	Italy	Sicily	N09_bulbocodium	P13	Raguza Between Mar. di Modica and Sampieri	14,76939	36,71079
23AB_B29	ColSaLurA3	R_columnae_subsp_columnae	Italy	Sardinia	N14_columnae	P15	Luras Ghiesa Campestre	9,399444	39,2722
23AB_B31	RolFrFre71	R_rollii	France	Provence	N01_rollii	P14	Fréjus Maison forestière des Cantonniers	6,7881	43,48928
23AB_B33	ReqSaBolB3	R_requienii	Italy	Sardinia	N04_requienii	P10	Padru Mannu Bolotama	8,79722	40,3555
23AB_B35	VarSiMo5c	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Fornace Pisciotto	14,75743	36,71178
23AB_B36	RamSaCreA2	R_ramiflora	Italy	Sardinia	N02_ramiflora	P03	Codoleddu Monte Cresia	9,39361	39,25333
23AB_B39	ColSaArrB3	R_columnae_subsp_columnae	Italy	Sardinia	N14_columnae	P15	Bau Arrexini	9,399444	39,2722
23AB_B40	MelMaMel10b	R_melitensis	Malta	Malta	N06_melitensis	P19	Mellieha	14,34812	35,93766
23AB_B41	AssMiPre03	R_assumptionis	Spain	Minorca	N11_assumptionis	P06	Cala Pregonda	4,04683	40,05262
23AB_B43	MelMaMel10a	R_melitensis	Malta	Malta	N06_melitensis	P19	Mellieha	14,34812	35,93766
23AB_B44	BulLiGen2	R_bulbocodium_agg	Italy	Liguria	N09_bulbocodium	P04	Via Razzara superiore Genova Sestri Ponente	8,82349	44,43771
23AB_B45	VarSiMo7c	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Punta Corvo di Sampieri	14,70652	36,71742
23AB_B46	RevCoPig2	R_revelieri	France	Corsica	N15_revelieri	P18	Bastia Pignu	9,40136	42,69688
23AB_B48	CrsCoBar1b	R_corsica	France	Corsica	N13_corsica	P14	Rogliano Barcaghju	9,409781	43,005573
23AB_B49	ReqSaBolB1	R_requienii	Italy	Sardinia	N04_requienii	P10	Padru Mannu Bolotama	8,79722	40,3555
23AB_B50	BocSaCodA2	R_bocchierii	Italy	Sardinia	N10_bocchierii	P01	Attopiano di Codoleddu	9,393056	39,252778

23AB_B51	RamCoSal3	R_ramiflora	France	Corsica	N02_ramiflora	P03	Porto-Vecchio Salines	9,28625	41,58388
23AB_B52	ReqCoRev1	R_requienii	France	Corsica	N04_requienii	P11	Calvi Revellata	8,72202	42,56334
23AB_B53	LigLiRio2	R_ligustica	Italy	Liguria	N10_ligustica	P07	Rio Molinassi	8,83514	44,43758
23AB_B54	BulLiGen4	R_bulbocodium_agg	Italy	Liguria	N09_bulbocodium	P12	Via Razzara superiore Genova Sestri Ponente	8,82349	44,43771
23AB_B55	VarSiMo5b	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Fornace Pisciotto	14,75743	36,71178
23AB_B59	FloFrBag1	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyeres île de Bagaud	6,36117	43,0093
23AB_B61	BulSiMod4c	R_bulbocodium_agg	Italy	Sicily	N09_bulbocodium	P13	Raguza Between Mar. di Modica and Sampieri	14,76939	36,71079
23AB_B63	ReqCoBar1	R_requienii	France	Corsica	N04_requienii	P10	Rogliano Barcaghju	9,417346	43,009815
23AB_B64	RamCoSal2	R_ramiflora	France	Corsica	N02_ramiflora	P03	Porto-Vecchio Salines	9,28625	41,58388
23AB_B65	RolCoBar3b	R_rollii	France	Corsica	N01_rollii	P14	Rogliano Barcaghju	9,409989	43,00573
23AB_B66	LigSaBolC1	R_ligustica	Italy	Sardinia	N10_ligustica	P07	Padru Mannu Bolotama	8,79722	40,3561
23AB_B67	VarSiMo6b	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Cava d'Aliga	14,68599	36,72359
23AB_B68	RolSaSoA8	R_rollii	Italy	Sardinia	N01_rollii	P14	Solina Masainas	8,5788	39,02638
23AB_B70	RamSiMod3b	R_ramiflora	Italy	Sicily	N02_ramiflora	P03	Raguza Between Mar. di Modica and Sampieri	14,76939	36,71079
23AB_B71	ReqCoMez3	R_requienii	France	Corsica	N04_requienii	P11	Ajaccio Mezzumare	8,591727	41,877173
23AB_B72	ReqCoSta1	R_requienii	France	Corsica	N04_requienii	P11	Bonifacio Stagnolu	9,10829	41,41874
23AB_B73	LigSaSinA2	R_ligustica	Italy	Sardinia	N10_ligustica	P07	Sinnai Barranca	9,327222	39,286944
23AB_B74	RevCoBit2	R_revelieri	France	Corsica	N15_revelieri	P15	Sotta Bitalza	9,14271	41,5934
23AB_B75	LigLiRio1	R_ligustica	Italy	Liguria	N10_ligustica	P07	Rio Molinassi	8,83514	44,43758
23AB_B77	RamCoOst1	R_ramiflora	France	Corsica	N02_ramiflora	P03	Palasca Ostriconi	9,05689	42,65911
23AB_B78	RamCoBar2	R_ramiflora	France	Corsica	N02_ramiflora	P03	Rogliano Barcaghju	9,4081	43,00506
23AB_B79	RamSiMod3a	R_ramiflora	Italy	Sicily	N02_ramiflora	P03	Raguza Between Mar. di Modica and Sampieri	14,76939	36,71079
23AB_B80	RolCoSal1	R_rollii	France	Corsica	N01_rollii	P14	Porto-Vecchio Salines	9,28625	41,58388
23AB_B81	ColCoPig1	R_columnae_subsp_columnae	France	Corsica	N14_columnae	P15	Bastia Pignu	9,39804	42,69647
23AB_B84	ColCoRev1	R_columnae_subsp_columnae	France	Corsica	N14_columnae	P15	Calvi Revellata	8,72123	42,56871
23AB_B85	RamCoOst2	R_ramiflora	France	Corsica	N02_ramiflora	P03	Palasca Ostriconi	9,05689	42,65911

23AB_B86	RolSaSoA6	R_rollii	Italy	Sardinia	N01_rollii	P14	Solina Masainas	8,5788	39,02638
23AB_B87	RevCoLos2	R_revelieri	France	Corsica	N15_revelieri	P18	Belgodere Losari	9,00624	42,64233
23AB_B88	CrsCoSop1	R_corsica	France	Corsica	N13_corsica	P02	Figari Saline Soprane	9,07228	41,46574
23AB_B89	RevCoCro3	R_revelieri	France	Corsica	N15_revelieri	P14	Calenzana Crovani	8,67221	42,47695
23AB_B90	ColMaMel11a	R_columnae_subsp_columnae	Malta	Malta	N07_columnae	P19	Mellieha	14,34812	35,93766
23AB_B92	VarMaDi15a	R_variicolor	Malta	Malta	N08_variicolor	P23	Dingli	14,37517	35,85643
23AB_B93	VarMaMe1b	R_variicolor	Malta	Malta	N08_variicolor	P19	Mellieha	14,334742	35,979575
23AB_B94	LigCoBon3	R_ligustica	France	Corsica	N10_ligustica	P07	Bonifacio Cala Longa	9,24744	41,40446
23AB_B95	ColCoSqu2	R_columnae_subsp_columnae	France	Corsica	N07_columnae	P20	Soveria San Quilicu	9,16826	42,349
23AB_C01	VarSiMo8a	R_variicolor	Italy	Sicily	N08_variicolor	P24	Raguza Modica Spiaggetta Sampieri	14,73183	36,71949
23AB_C04	LigLiRio3	R_ligustica	Italy	Liguria	N10_ligustica	P07	Rio Molinassi	8,83514	44,43758
23AB_C06	ColLiCer1	R_columnae_subsp_columnae	Italy	Liguria	N10_columnae	P19	Cervo path to Cape Mimosa	8,12204	43,92872
23AB_C08	RevCoBag2	R_revelieri	France	Corsica	N15_revelieri	P18	Moltifao Bagliettu	9,18921	42,48271
23AB_C10	ReqSaTelC3	R_requienii	Italy	Sardinia	N04_requienii	P10	Teletotes Dorgali Baunei	9,5525	40,096389
23AB_C11	RamSaCreA7	R_ramiflora	Italy	Sardinia	N02_ramiflora	P03	Codoleddu Monte Cresia	9,39361	39,25333
23AB_C12	ReqSaCatA6	R_requienii	Italy	Sardinia	N04_requienii	P09	Stagno S. Catarina	8,501389	39,085
23AB_C13	ReqCoRev2	R_requienii	France	Corsica	N04_requienii	P11	Calvi Revellata	8,72202	42,56334
23AB_C15	FloFrBag2	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyeres île de Bagaud	6,36117	43,0093
23AB_C16	RolSiMo2b	R_rollii	Italy	Sicily	N01_rollii	P24	Raguza Marina di Modica	14,769508	36,710877
23AB_C17	LigCoBon2	R_ligustica	France	Corsica	N10_ligustica	P07	Bonifacio Cala Longa	9,24744	41,40446
23AB_C18	ColCoPig2	R_columnae_subsp_columnae	France	Corsica	N14_columnae	P15	Bastia Pignu	9,39804	42,69647
23AB_C19	ColSaLurA2	R_columnae_subsp_columnae	Italy	Sardinia	N07_columnae	P19	Luras Ghiesa Campestre	9,399444	39,2722
23AB_C22	RevCoBen3	R_revelieri	France	Corsica	N15_revelieri	P17	Lecci Benedettu	9,33033	41,61969
23AB_C23	BulLiGen1	R_bulbocodium_agg	Italy	Liguria	N09_bulbocodium	P12	Via Razzara superiore Genova Sestri Ponente	8,82349	44,43771
23AB_C26	LigSaTemB7	R_ligustica	Italy	Sardinia	N10_ligustica	P07	Tempio Pausania	9,098889	40,95722
23AB_C27	RamFrRap63	R_ramiflora	France	Provence	N02_ramiflora	P03	Saint Raphaël Pointe de l'Observtoire	6,9188	43,449
23AB_C29	RamCoBar3	R_ramiflora	France	Corsica	N02_ramiflora	P03	Rogliano Barcaghju	9,4081	43,00506

23AB_C31	BulSiMod4a	R_bulbocodium_agg	Italy	Sicily	N09_bulbocodium	P13		Raguza Between Mar. di Modica and Sampieri	14,76939	36,71079
23AB_C32	ReqCoRev3	R_requienii	France	Corsica	N04_requienii	P11		Calvi Revellata	8,72202	42,56334
23AB_C33	RevCoBit3	R_revelieri	France	Corsica	N15_revelieri	P17		Sotta Bitalza	9,14271	41,5934
23AB_C34	LigLiRio5	R_ligustica	Italy	Liguria	N10_ligustica	P07		Rio Molinassi	8,83514	44,43758
23AB_C35	ColCoSca1	R_columnae_subsp_columnae	France	Corsica	N14_columnae	P15		Corscia Scala	9,05262	42,3623
23AB_C36	MelMaDin14c	R_melitensis	Malta	Malta	N06_melitensis	P19		Dingli	14,37517	35,85643
23AB_C37	ReqCoBar2	R_requienii	France	Corsica	N04_requienii	P08		Rogliano Barcaghju	9,417346	43,009815
23AB_C39	RolCoRev1	R_rollii	France	Corsica	N01_rollii	P14		Calvi Revellata	8,72075	42,5625
23AB_C41	ColSaSerC3	R_columnae_subsp_columnae	Italy	Sardinia	N14_columnae	P19		Monte Serpeddi	9,281009	39,3525
23AB_C42	ColCoRev2	R_columnae_subsp_columnae	France	Corsica	N14_columnae	P15		Calvi Revellata	8,72123	42,56871
23AB_C44	JorCoPor1	R_jordanii	France	Corsica	N15_revelieri	P20		Porto-Vecchio salines	9,28625	41,58388
23AB_C46	RamLiAnd1	R_ramiflora	Italy	Liguria	N08_ramiflora	P24		Colla Micheri Andora	8,15876	43,96632
23AB_C47	ColLiPom1	R_columnae_subsp_columnae	Italy	Liguria	N07_columnae	P05		Pompeiana trail to Mount Faudo at the Chapel of St. Bernard	7,89253	43,86108
23AB_C48	RamLiAnd2	R_ramiflora	Italy	Liguria	N08_ramiflora	P24		Colla Micheri Andora	8,15876	43,96632
23AB_C49	RevCoPig1	R_revelieri	France	Corsica	N15_revelieri	P18		Bastia Pignu	9,40136	42,69688
23AB_C50	ColSaSerC4	R_columnae_subsp_columnae	Italy	Sardinia	N14_columnae	P19		Monte Serpeddi	9,281009	39,3525
23AB_C51	RolSiMo2a	R_rollii	Italy	Sicily	N01_rollii	P24		Raguza Marina di Modica	14,769508	36,710877
23AB_C52	ReqSaBolB4	R_requienii	Italy	Sardinia	N04_requienii	P10		Padru Mannu Bolotama	8,79722	40,3555
23AB_C53	CrsCoSop2	R_corsica	France	Corsica	N09_bulbocodium	P15		Figari Saline Soprane	9,07228	41,46574
23AB_C54	LigSaTemB4	R_ligustica	Italy	Sardinia	N10_ligustica	P07		Tempio Pausania	9,098889	40,95722
23AB_C55	VarSiMo5d	R_variicolor	Italy	Sicily	N08_variicolor	P24		Raguza Modica Fornace Pisciotto	14,75743	36,71178
23AB_C57	ColLiPom2	R_columnae_subsp_columnae	Italy	Liguria	N10_columnae	NA		Pompeiana trail to Mount Faudo at the Chapel of St. Bernard	7,89253	43,86108
23AB_C58	RevCoLos3	R_revelieri	France	Corsica	N15_revelieri	P18		Belgodere Losari	9,00624	42,64233
23AB_C59	ColMaMel11b	R_columnae_subsp_columnae	Malta	Malta	N07_columnae	P19		Mellieha	14,34812	35,93766
23AB_C60	ColCoPig3	R_columnae_subsp_columnae	France	Corsica	N14_columnae	P15		Bastia Pignu	9,39804	42,69647

23AB_C61	FloFrBor51	R_florentii	France	Provence	N03_florentii	P08	Bormes-les-Mimosas Port du Pradet	6,36737	43,09237
23AB_C62	RevCoCro1	R_revelieri	France	Corsica	N15_revelieri	P14	Calenzana Crovani	8,67221	42,47695
23AB_C64	ReqCoSta3	R_requienii	France	Corsica	N04_requienii	P11	Bonifacio Stagnolu	9,10829	41,41874
23AB_C65	RolSiMo2c	R_rollii	Italy	Sicily	N01_rollii	P24	Raguza Marina di Modica	14,769508	36,710877
23AB_C66	VarMaMe1a	R_variicolor	Malta	Malta	N08_variicolor	P24	Mellieha	14,334742	35,979575
23AB_C67	RevCoSal2	R_revelieri	France	Corsica	N15_revelieri	P18	Porto-Vecchio Salines	9,28761	41,58036
23AB_C68	FloFrBor42	R_florentii	France	Provence	N03_florentii	P08	Bormes-les-Mimosas Pointe de la Galère	6,33177	43,08996
23AB_C69	LigSaSinA6	R_ligustica	Italy	Sardinia	N10_ligustica	P07	Sinnai Barranca	9,327222	39,286944
23AB_C73	VarMaDi13c	R_variicolor	Malta	Malta	N08_variicolor	P23	Dingli	14,37517	35,85643
23AB_C74	RevSaTeA2	R_revelieri	Italy	Sardinia	N15_revelieri	P18	Teletotes Dorgali Baunei	9,552500	40,09722
23AB_C76	VarMaDi13a	R_variicolor	Malta	Malta	N08_variicolor	P04	Dingli	14,37517	35,85643
23AB_C78	RamLiAnd4	R_ramiflora	Italy	Liguria	N08_ramiflora	P24	Colla Micheri Andora	8,15876	43,96632
23AB_C81	RevCoBag1	R_revelieri	France	Corsica	N15_revelieri	P18	Moltifao Bagliettu	9,18921	42,48271
23AB_C82	RolCoRev2	R_rollii	France	Corsica	N01_rollii	P14	Calvi Revellata	8,72075	42,5625
23AB_C85	RevCoPig3	R_revelieri	France	Corsica	N15_revelieri	P18	Bastia Pignu	9,40136	42,69688
23AB_C86	ReqSaTelC2	R_requienii	Italy	Sardinia	N04_requienii	P10	Teletotes Dorgali Baunei	9,5525	40,096389
23AB_C87	CrsCoSop3	R_corsica	France	Corsica	N13_corsica	P02	Figari Saline Soprane	9,07228	41,46574
23AB_C88	ColCoSqu3	R_columnae_subsp_columnae	France	Corsica	N07_columnae	P19	Soveria San Quilicu	9,16826	42,349
23AB_C89	RevCoSal3	R_revelieri	France	Corsica	N15_revelieri	P17	Porto-Vecchio Salines	9,28761	41,58036
23AB_C91	MelMaDin14a	R_melitensis	Malta	Malta	N06_melitensis	P19	Dingli	14,37517	35,85643
23AB_C93	RolCoBar1	R_rollii	France	Corsica	N01_rollii	P14	Rogliano Barcaghju	9,409989	43,00573
23AB_C94	CrsCoBar2	R_corsica	France	Corsica	N13_corsica	P14	Rogliano Barcaghju	9,409781	43,005573
23AB_C95	CrsCoBar1	R_corsica	France	Corsica	N13_corsica	P14	Rogliano Barcaghju	9,409781	43,005573
23AB_C96	AssMiPre01	R_assumptionis	Spain	Minorca	N11_assumptionis	P06	Cala Pregonda	4,04683	40,05262
24AB_A03	ColCoRev3	R_columnae_subsp_columnae	France	Corsica	N14_columnae	P15	Calvi Revellata	8,72123	42,56871
24AB_A04	BulLiGen3	R_bulbocodium_agg	Italy	Liguria	N09_bulbocodium	P12	Via Razzara superiore Genova Sestri Ponente	8,82349	44,43771
24AB_A07	LigSaBolC6	R_ligustica	Italy	Sardinia	N10_ligustica	P01	Padru Mannu Bolotama	8,79722	40,3561
24AB_A09	ColCoSqu1	R_columnae_subsp_columna	France	Corsica	N14_columnae	P15	Soveria San	9,16826	42,349

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24AB_A12	ColSaArrB1	R_columnae_subsp_columnae	Italy	Sardinia	N14_columnae	P15	Bau Arrexini	9,399444	39,2722
24AB_A13	FloFrBor41	R_florentii	France	Provence	N03_florentii	P08	Bormes-les-Mimosas Pointe de la Galère	6,33177	43,08996
24AB_A20	RamCoSal1	R_ramiflora	France	Corsica	N02_ramiflora	P03	Porto-Vecchio Salines	9,28625	41,58388
24AB_A35	LigSaSinA1	R_ligustica	Italy	Sardinia	N10_ligustica	P07	Sinnai Barranca	9,327222	39,286944
24AB_A51	RamCoOst3	R_ramiflora	France	Corsica	N02_ramiflora	P03	Palasca Ostriconi	9,05689	42,65911
24AB_A52	RamCoBar1	R_ramiflora	France	Corsica	N02_ramiflora	P03	Rogliano Barcaghju	9,4081	43,00506
24AB_A62	RolCoRev3	R_rollii	France	Corsica	N01_rollii	P14	Calvi Revellata	8,72075	42,5625
24AB_A75	ReqSaTelC1	R_requienii	Italy	Sardinia	N04_requienii	P10	Teletotes Dorgali Baunei	9,5525	40,096389
24AB_A78	BulSaLimA1	R_bulbocodium_agg	Italy	Sardinia	N09_bulbocodium	P15	Mt Limbara	9,141944	40,852222
24AB_A93	ColSaSerC1	R_columnae_subsp_columnae	Italy	Sardinia	N14_columnae	P19	Monte Serpeddi	9,281009	39,3525
24AB_B25	RamFrRap62	R_ramiflora	France	Provence	N02_ramiflora	P03	Saint Raphaël Pointe de l'Observtoire	6,9188	43,449
24AB_B45	cfVarMiAlc1401	R_cf_variicolor	Spain	Minorca	N02_ramiflora	P03	Alcalfar Vell	4,2832880	39,8335050
24AB_B64	ColSaLurA1	R_columnae_subsp_columnae	Italy	Sardinia	N14_columnae	P15	Luras Ghiesa Campestre	9,399444	39,2722
24AB_C03	LinSiGal3	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Monte Gallo	13,28943	38,21136
24AB_C04	RolFrPor1	R_rollii	France	Hyeres_islands	N01_rollii	P14	Hyères île de Porquerolles	6,189875	43,003934
24AB_C15	LinSiGal1	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Monte Gallo	13,28943	38,21136
24AB_C20	RolFrPor2	R_rollii	France	Hyeres_islands	N01_rollii	P14	Hyères île de Porquerolles	6,189875	43,003934
24AB_C29	RolFrPor3	R_rollii	France	Hyeres_islands	N01_rollii	P14	Hyères île de Porquerolles	6,189875	43,003934
24AB_C39	LinSiBai1	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Bala del Corello	13,26769	38,20199
24AB_C41	RolFrTre1	R_rollii	France	Provence	N01_rollii	P14	Collobrières Treps	6,360852	43,254172
24AB_C44	LinSiCor1	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P22	Monte Cofano	12,6616	38,09793
24AB_C51	RamMiMarc	R_ramiflora	Spain	Minorca	N02_ramiflora	P03	La Marcona	4,0088050	39,9939750
24AB_C52	RamFrCer3	R_ramiflora	France	Provence	N08_ramiflora	P24	Sanary-sur-mer Pointe du Cerveau	5,78732	43,16204
24AB_C54	ColSiLam1	R_ramiflora	Italy	Sicily	N08_ramiflora	P24	Lampedusa	12,595846	35,511637
24AB_C56	LinSiGal2	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Monte Gallo	13,28943	38,21136
24AB_C61	RamFrLev1	R_ramiflora	France	Hyeres_islands	N02_ramiflora	P03	Hyères île du Levant	6,45749	43,02762
24AB_C63	ColSiLam2	R_columnae_subsp_columna	Italy	Sicily	N08_variicolor	P24	Lampedusa	12,595846	35,511637

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24AB_C64	RamMiGal2	R_ramiflora	Spain	Minorca	N02_ramiflora	P03	Cala Galdana	3,9567130	39,9367740
24AB_C67	InsItCap1	R_insularis	Italy	Capraia_island	N15_revelieri	P18	Capraia	9,81817	43,032853
24AB_C69	RollFrCol1	R_rollii	France	Provence	N01_rollii	P14	Collobrières Boussicaut	6,36336	43,21616
24AB_C77	RolFrLev2	R_rollii	France	Hyeres_islands	N12_rollii	P14	Hyères île du Levant	6,45749	43,02762
24AB_C78	LinSiLev1	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Trapani Ile Levanzo	12,3426	37,98734
24AB_C85	LinSiBai3	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Bala del Corello	13,26769	38,20199
24AB_C90	LinSiBai2	R_linaresii_subsp_linaresii	Italy	Sicily	N05_linaresii	P21	Bala del Corello	13,26769	38,20199
24AB_C91	RamMiGal1	R_ramiflora	Spain	Minorca	N02_ramiflora	P03	Cala Galdana	3,9567130	39,9367740
24AB_D08	RollFrCro3	R_rollii	France	Hyeres_islands	N01_rollii	P14	Hyères île de Port-Cros	6,38645	43,01093
24AB_D09	RollFrCro4	R_rollii	France	Hyeres_islands	N01_rollii	P14	Hyères île de Port-Cros	6,38645	43,01093
24AB_D14	AssMiBas1	R_assumptionis	Spain	Minorca	N11_assumptionis	P06	Bassa Son Morell	3,8784130	40,0501020
24AB_D22	FloFrFau1	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île de Port-Cros	6,37648	43,0051
24AB_D23	InsItCap2	R_insularis	Italy	Capraia_island	N15_revelieri	P18	Capraia	9,81817	43,032853
24AB_D25	RamFrCer2	R_ramiflora	France	Provence	N08_ramiflora	P24	Sanary-sur-mer Pointe du Cerveau	5,78732	43,16204
24AB_D39	FloFrLev3	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île du Levant	6,45753	43,02739
24AB_D44	ColFrHye1	R_columnae_subsp_columnae	France	Provence	N07_columnae	P19	Hyères Notre-Dame de Consolation	6,12773	43,098
24AB_D49	RolMiRod2	R_rollii	Spain	Minorca	N01_rollii	P14	Muntanya de ses Fonts Rodones	4,0599190	39,9603280
24AB_D59	ColMiCas2	R_columnae_subsp_columnae	Spain	Minorca	N07_columnae	P19	Castell de Santa Àgueda	4,0056540	40,0235170
24AB_D60	FloFrLev1	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île du Levant	6,45753	43,02739
24AB_D63	RolMiRod1	R_rollii	Spain	Minorca	N01_rollii	P14	Muntanya de ses Fonts Rodones	4,0599190	39,9603280
24AB_D64	RolFrCro2	R_rollii	France	Hyeres_islands	N01_rollii	P14	Port-Cros Marma	6,401913	43,016846
24AB_D78	cfVarMiBai1	R_cf_variicolor	Spain	Minorca	N07_columnae	P20	Binicodrell de Baix	4,0504990	39,9178520
24AB_D79	cfVarMiCuq2	R_cf_variicolor	Spain	Minorca	N07_columnae	P19	Ses Cuques	4,0505670	39,9176720
24AB_D80	ColMiBer1	R_columnae_subsp_columnae	Spain	Minorca	N07_columnae	P19	Es Berrecks de Santa Anna	3,9480520	39,9350970
24AB_D82	RolMiLli1	R_rollii	Spain	Minorca	N01_rollii	P14	Llinàritx Nou	4,0714730	39,9917370
24AB_D83	cfVarMiBai4	R_cf_variicolor	Spain	Minorca	N07_columnae	P20	Binicodrell de Baix	4,0504990	39,9178520
24AB_D85	FloFrFau2	R_florentii	France	Hyeres_islands	N03_florentii	P08	Hyères île de Port-Cros	6,37648	43,0051
24AB_D90	RamSiTra1	R_ramiflora	Italy	Sicily	N02_ramiflora	P03	Trapani	12,49435	37,97813
24AB_D94	AssMiMon1	R_assumptionis	Spain	Minorca	N11_assumptionis	P06	Morell	4,2114700	39,9347510
24AB_D95	AssMiEsc3	R_assumptioni	Spain	Minorca	N11_assumpti	P06	Escudero	3,8524530	40,0507020

		s			onis				
24AB_E03	ColMiCan3	R_columnae_subsp_columnae	Spain	Minorca	N07_columnae	P20	Platja de ses Canassies	4,0574980	39,905797
24AB_E06	ColMiAl1	R_columnae_subsp_columnae	Spain	Minorca	N07_columnae	P19	Alcalfar Vell	4,2832880	39,8335050

Supplementary material 2

TITLE: MOLECULAR IDENTIFICATION AND DIVERSITY ASSESSMENT OF TYRRHENIAN *ROMULEA* SPECIES

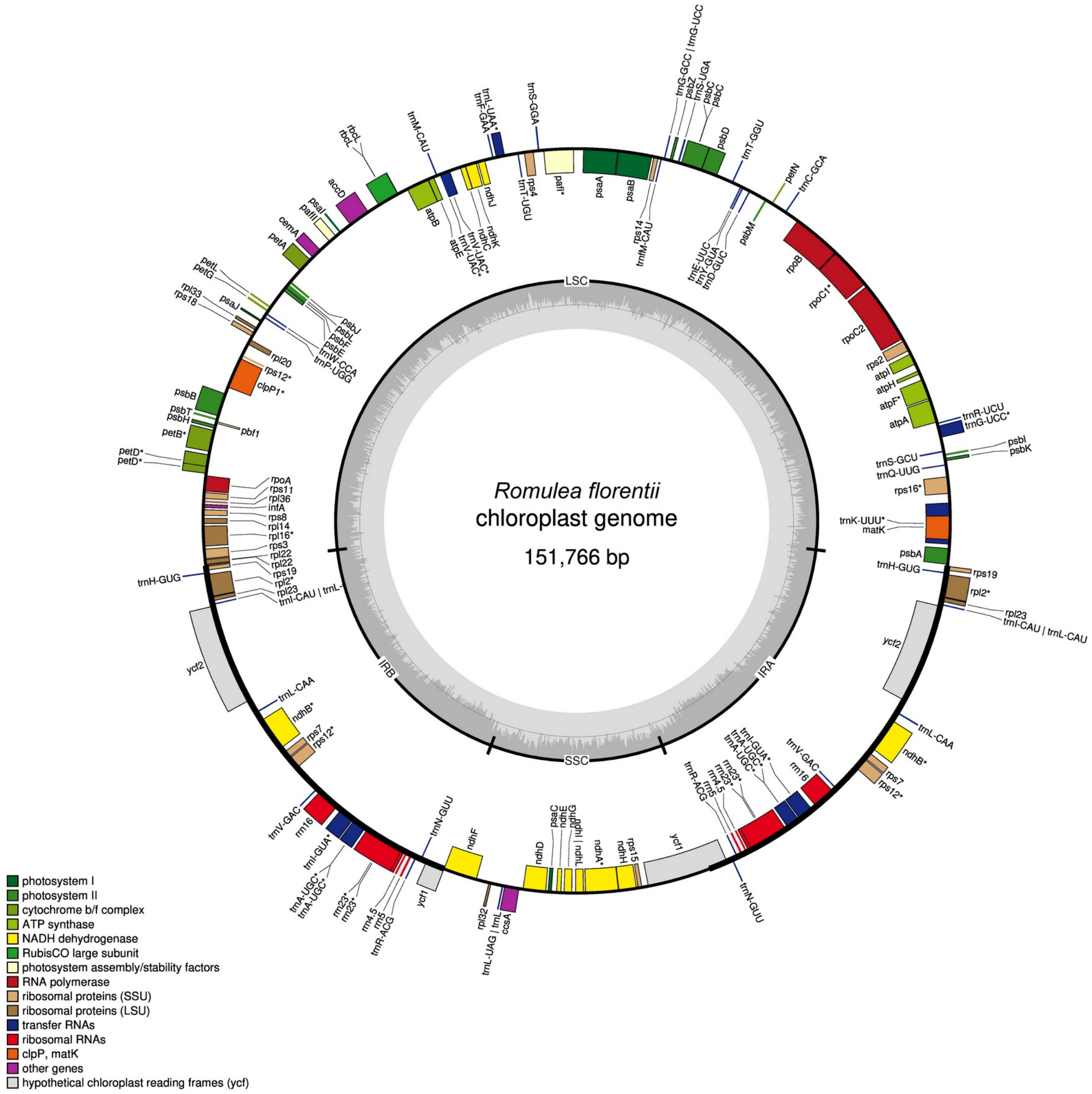


Figure S1 : Plastome map of Romulea florentii, 151 kbp.

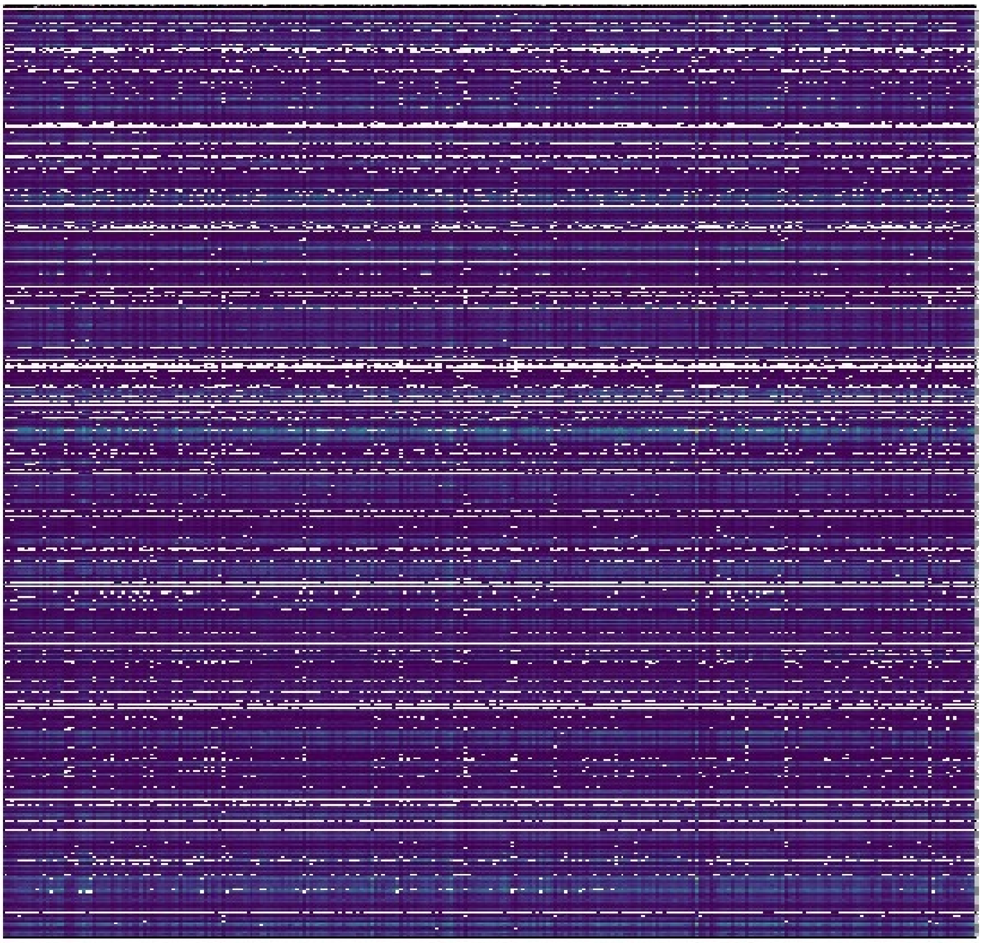


Fig. S2 Data completeness of 272 *Romulea* genotypes for 8168 loci biallelic SNPs and an overall missing rate of 9%. Loci in rows and individual in columns.

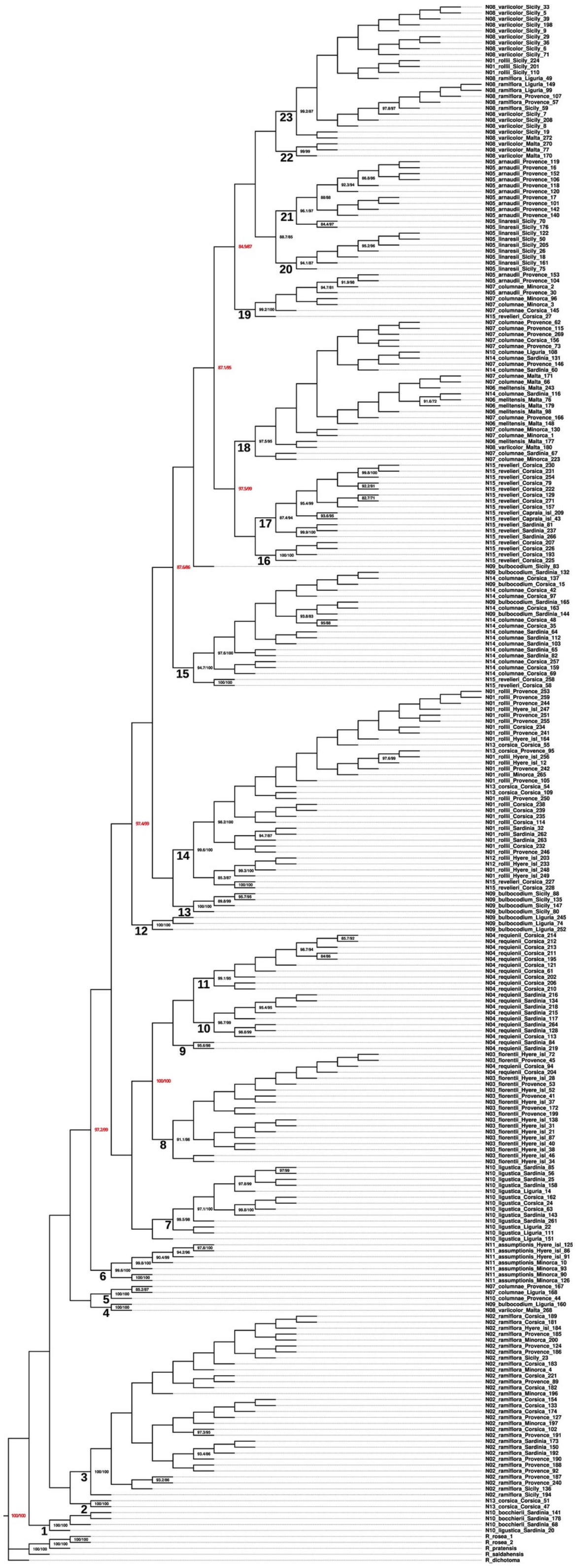


Figure S3 : Maximum likelihood tree of 262 plastid haplotypes based on 965 parsimony informative sites from the whole plastome. 23 clades are designed for comparison with nuclear genetic groups

Figure 1

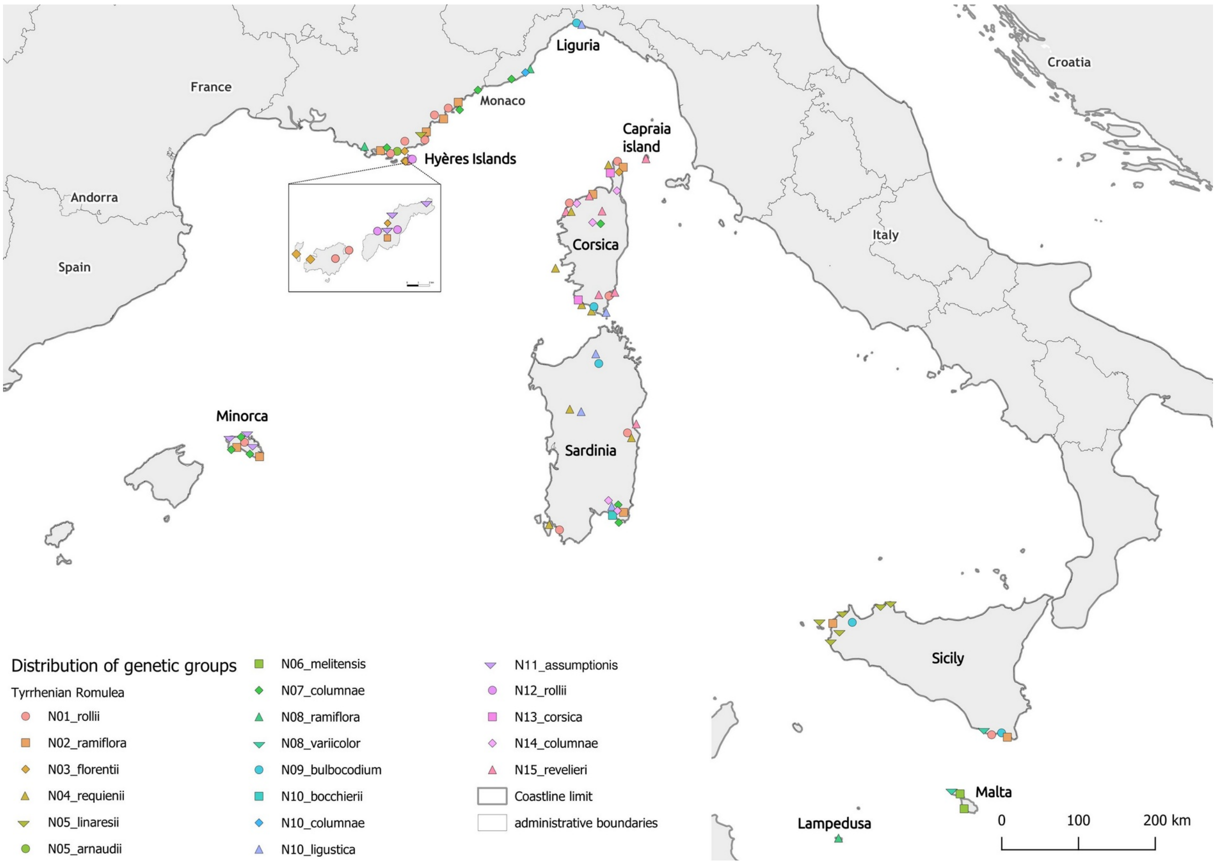

France
Liguria
Monaco
Croatia
Capraia island
Hyères Islands
Andorra
Spain
Corsica
Italy
Minorca
Sardinia
Sicily
Malta
Lampedusa
0
100
200 km
Distribution of genetic groups
Tyrrhenian Romulea
N01_rollii
N02_ramiflora
N03_florentii
N04_requienii
N05_linaresii
N05_arnaudii
N06_melitensis
N07_columnae
N08_ramiflora
N08_variicolor
N09_bulbocodium
N10_bocchierii
N10_columnae
N10_ligustica
N11_assumptionis
N12_rollii
N13_corsica
N14_columnae
N15_revelieri
Coastline limit
administrative boundaries

Figure 2

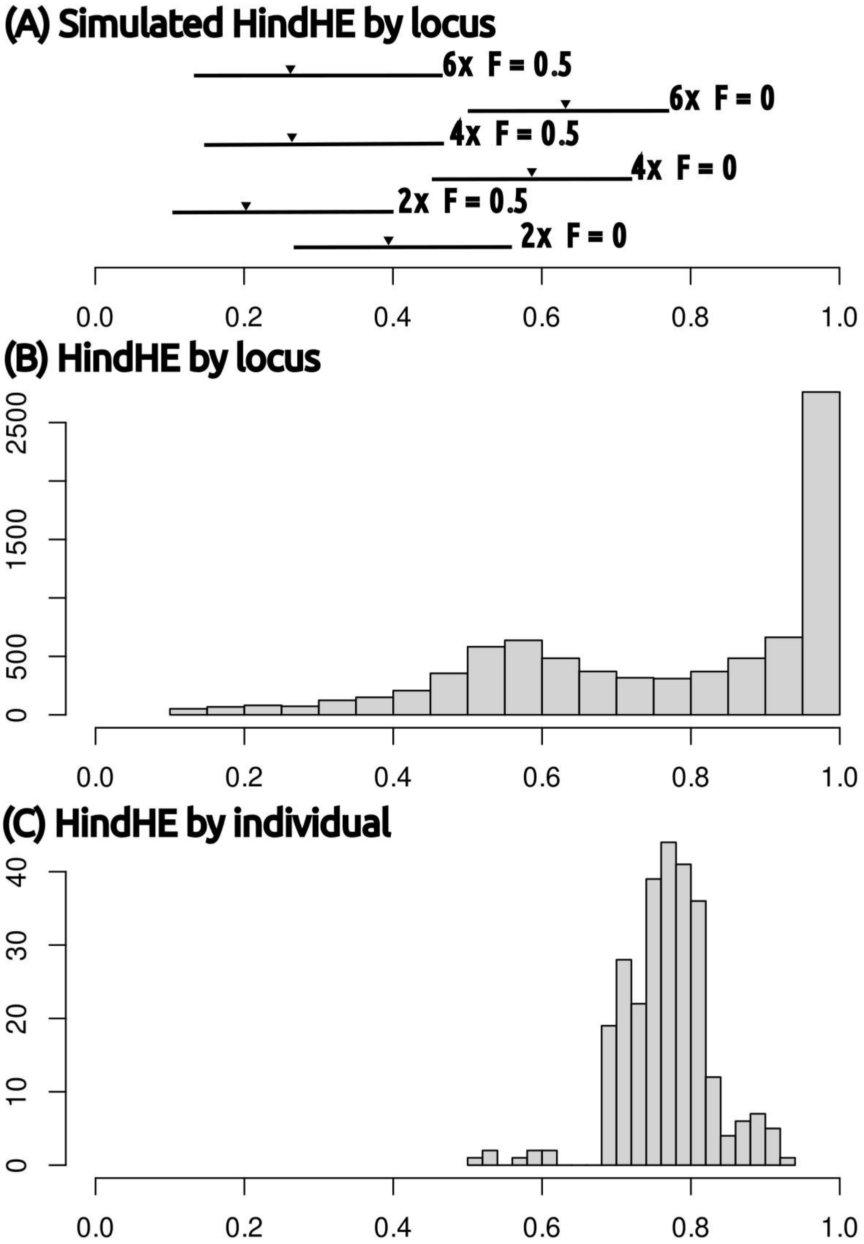

(A) Simulated HindHE by locus
6x F = 0.5
6x F = 0
4x F = 0.5
4x F = 0
2x F = 0.5
2x F = 0
0.0
0.2
0.4
0.6
0.8
1.0
(B) HindHE by locus
0
500
1500
2500
0.0
0.2
0.4
0.6
0.8
1.0
(C) HindHE by individual
0
10
20
30
40
0.0
0.2
0.4
0.6
0.8
1.0

Figure 3

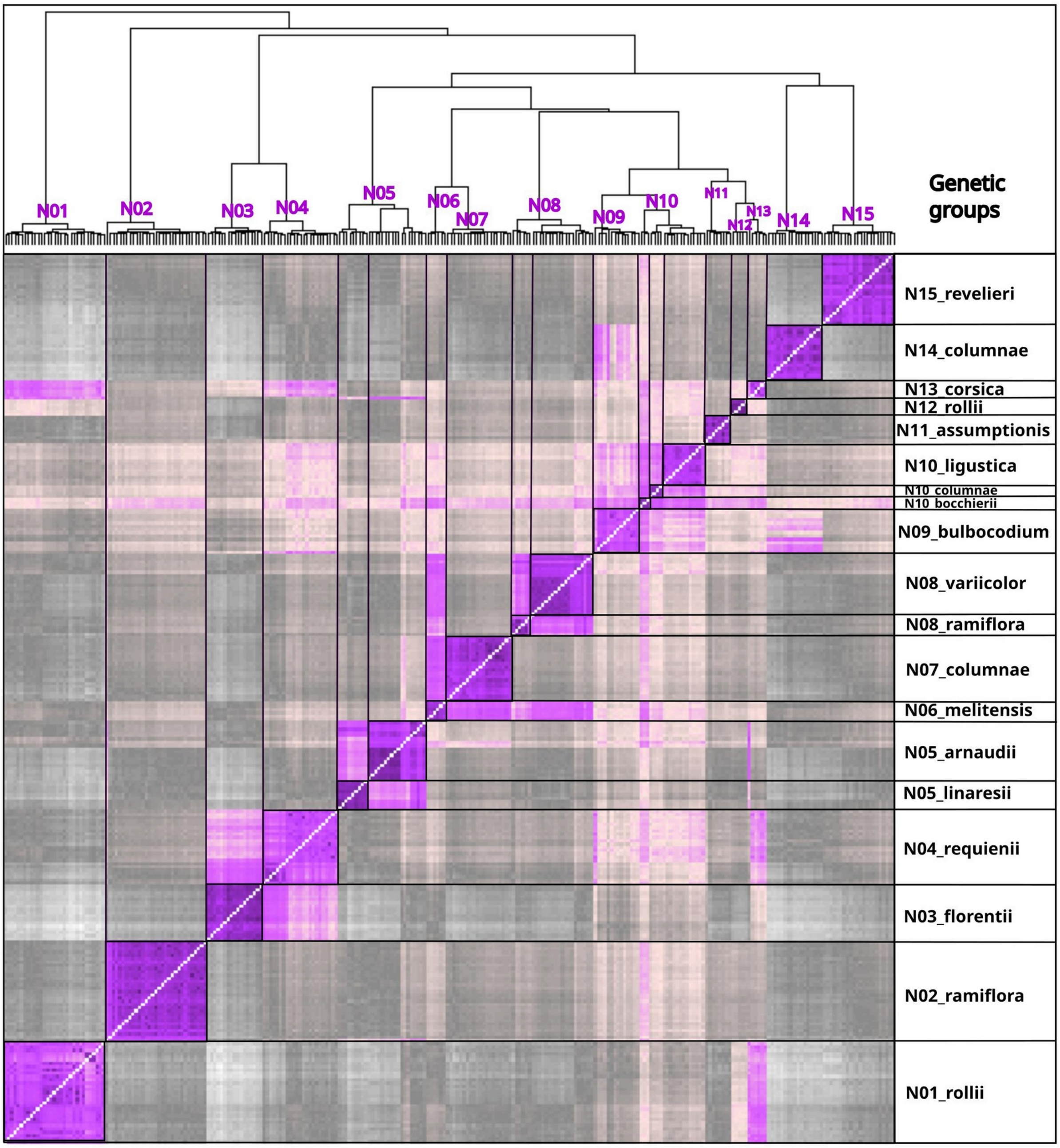

N01
N02
N03
N04
N05
N06
N07
N08
N09
N10
N11
N12
N13
N14
N15
Genetic groups
N15_revelieri
N14_columnae
N13_corsica
N12_rollii
N11_assumptionis
N10_ligustica
N10_columnae
N10_bocchierii
N09_bulbocodium
N08_variicolor
N08_ramiflora
N07_columnae
N06_melitensis
N05_arnaudii
N05_linaresii
N04_requienii
N03_florentii
N02_ramiflora
N01_rollii

Figure 4

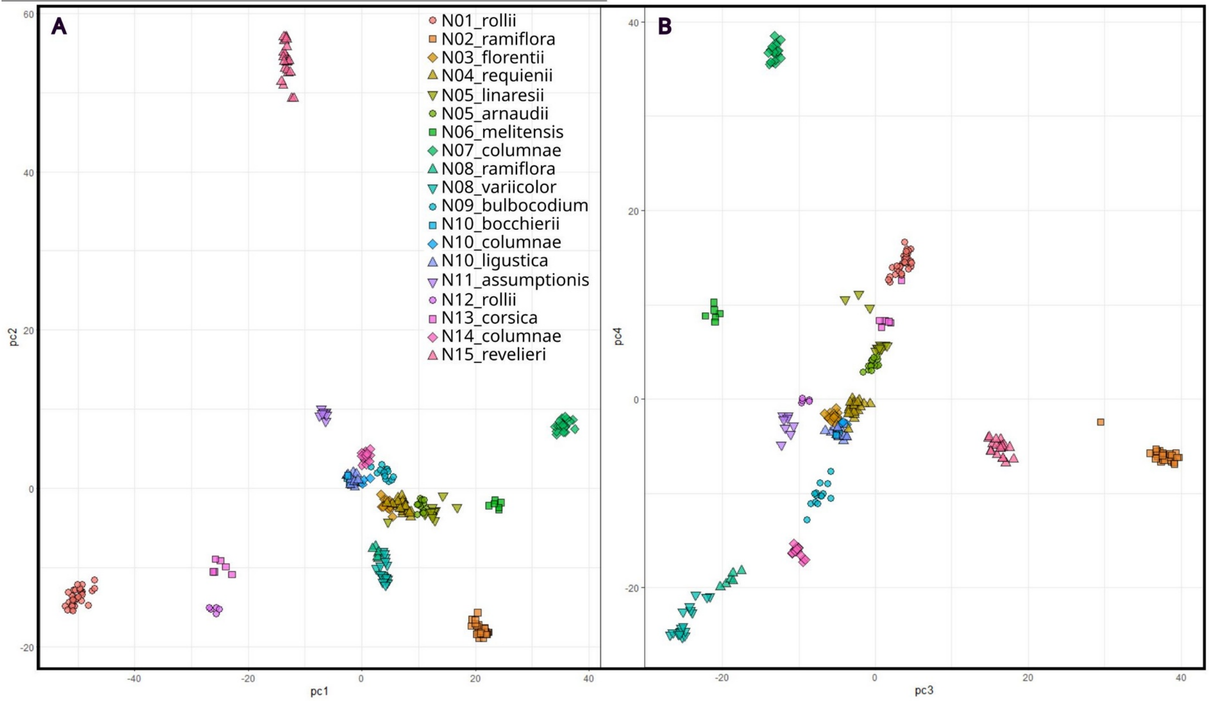
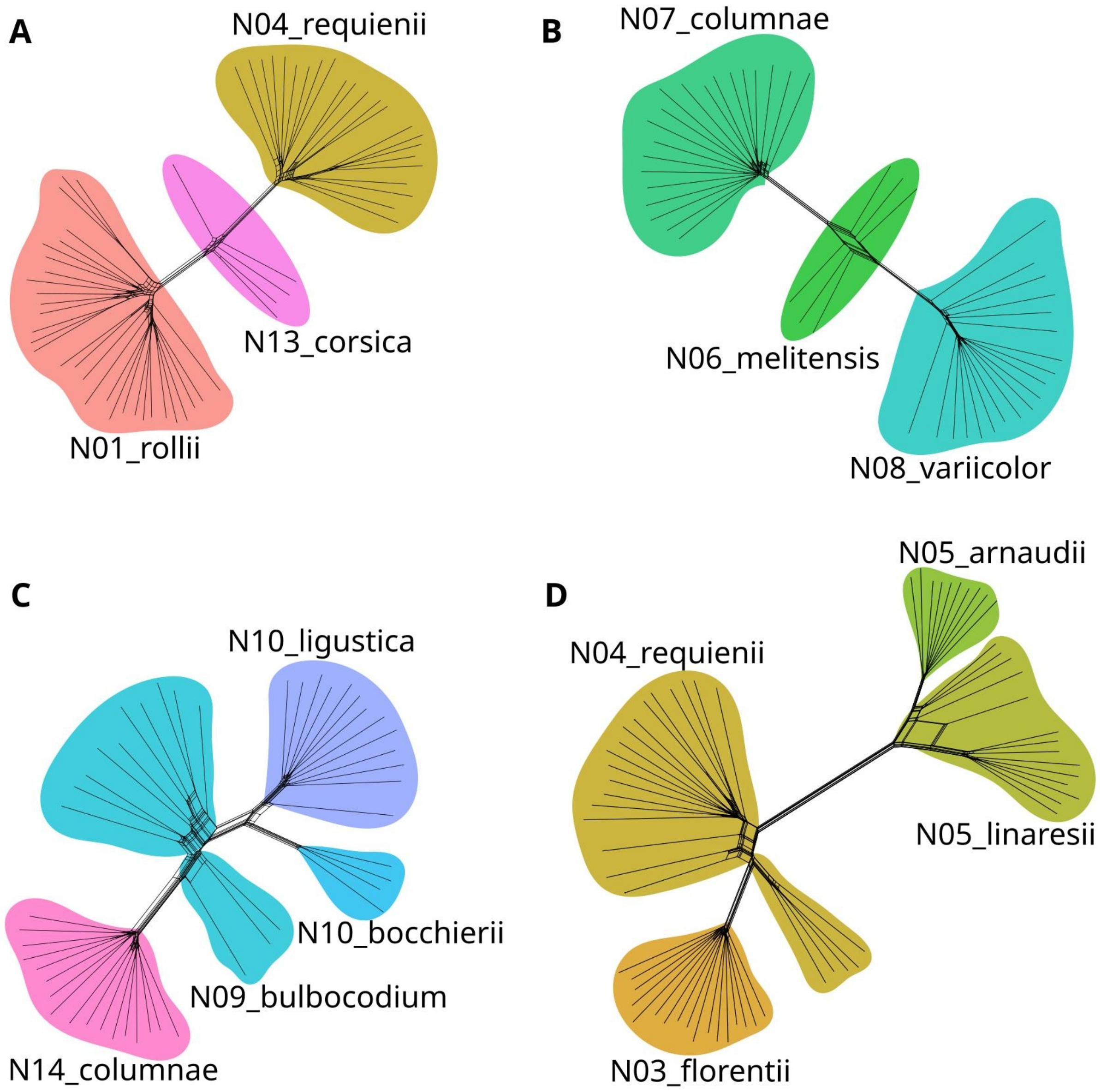

A
B
● N01_rollii
■ N02_ramiflora
◆ N03_florentii
▲ N04_requienii
▼ N05_linaresii
● N05_arnaudii
■ N06_melitensis
◆ N07_columnae
▲ N08_ramiflora
▼ N08_variicolor
● N09_bulbocodium
■ N10_bocchierii
◆ N10_columnae
▲ N10_ligustica
▼ N11_assumptionis
● N12_rollii
■ N13_corsica
◆ N14_columnae
▲ N15_revelieri
pc2
60
40
20
0
-20
-40
-20
0
20
40
pc1
pc4
40
20
0
-20
-20
0
20
40
pc3

Figure 5


A
N04_requienii
N13_corsica
N01_rollii
B
N07_columnae
N06_melitensis
N08_variicolor
C
N10_ligustica
N10_bocchierii
N09_bulbocodium
N14_columnae
D
N05_arnaudii
N04_requienii
N05_linaresii
N03_florentii