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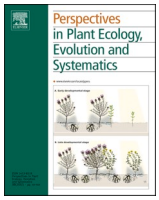

Genetic differentiation of the *Capparis spinosa* group in the Mediterranean area

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ABSTRACT

The *Capparis spinosa* group is represented in the Mediterranean by a complex of taxa widespread in North Africa, the Middle East, and southern Europe. The taxonomy of this group used to be based on morphological characters with little work on the genetics of the group, and there is still much to be learned about its evolutionary history and diversification. We sampled 431 individuals of two subspecies and five varieties of *C. spinosa* and analysed them using highly informative EST-SSR markers to evaluate the population genetic diversity, structure and differentiation of the species in the Mediterranean. In addition, comparisons with the genetic profiles of *C. spinosa* subsp. *cartilaginea,* the putative ancestral taxon were made to investigate the phylogeographic history and possible gene flow across taxa. Integrated Bayesian approaches showed: *i)* a high divergence among *C. spinosa* subsp. *spinosa* var. *canescens*, *C. spinosa* subsp. *spinosa* var. *aegyptia* and the three varieties belonging to *C. spinosa* subsp. *rupestris* (var. *rupestris*, var. *ovata* and var. *myrtifolia*), with a clear separation between var. *aegyptia* and var. *canescens* which allows to consider var. *aegyptia* as a subspecies of *C. spinosa*; *ii)* a significant correlation between genetic divergence and geographic distance between the five varieties studied; *iii)* that the different varieties in the Mediterranean may have been derived from *C. spinosa* subsp. *cartilaginea*. Further genomic investigations are required to confirm our results. However, the findings presented allows us to suggest the genus *Capparis* can be considered a model for the study of the gene flow and differentiation in species occurring in a wide range of habitats.

1. Introduction

The genus *Capparis* L. includes about 150 species of shrubs, small trees and climbers (POWO, 2022), occurring over a wide range of habitats in the Old World tropical and subtropical regions, mainly in the Indo-Pacific area, with outliers in the Mediterranean and Central Asia (Souvannakhoummane et al., 2018, 2020; Fici, 2017). The *Capparis spinosa* group comprises several taxa - constituting the sect. *Capparis* - distributed in Southern Europe, Northern and Eastern Africa, Madagascar, Western, Central and Southern-Eastern Asia, Australia and Oceania (Jacobs, 1965; Fici, 2012; Maurya et al., 2023), characterized by plesiomorphic features for the whole genus, like simple hairs and a single flower in the leaf axils. The presence of several variants of this group in different Gondwanian landmasses constitutes a complex biogeographic problem (Fici, 2004). The *C. spinosa* group consists of shrubs showing marked phenotypic polymorphism, mainly in the Mediterranean area, with intermediate forms across taxa (Heywood, 1964). This high polymorphism can be explained by different features, such as plasticity, hybridization processes, selection of cultivated forms and eco-geographic differentiation (Fici, 2014). The hybridization process is facilitated by the mixed reproductive system of *C. spinosa*. Indeed the plants are andromonoecious and produce both hermaphroditic and

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male flowers, which increase the abundance of pollen and the outcrossing rate (Pegiou et al., 2023). The ecological adaptations of the different members of the group have not been carefully investigated. Zohary (1960) pointed out the difficulty of developing a satisfactory taxonomic classification of this "chaotic group". He regarded the representatives of the genus *Capparis* in the Mediterranean and neighboring areas as relics of a tertiary xero–tropical flora, which gave origin to descendants partially retaining tropical traits (Zohary, 1973). Fici (2001) hypothesized that the climatic and geopedological conditions can be considered distinctive speciation factors for *C. spinosa* in this area, at the North-Western border of its whole distributional range.

Several taxa of different ranks have been described within the *C. spinosa* group in the Mediterranean and Middle East, while the narrower or wider species concept adopted in many regional floristic studies resulted in discordant taxonomic treatments. Boissier (1867) recognized a single species, *C. spinosa* L., in a wide area from the Mediterranean to India split into six varieties. Zohary (1960) reported five species, i.e. *C. spinosa*, *C. ovata* Desf., *C. leucophylla* DC., *C. cartilaginea* Decne., *C. mucronifolia* Boiss., in the Mediterranean and middle Eastern countries, of which the former three are able to frequently intercross and thus were split into several varieties. Across the whole paleotropical and subtropical distribution range of the group, St. John (1965) recorded six geographically differentiated species, whereas Jacobs (1965) recognized a single polymorphic species *C. spinosa*, split into five varieties. A subdivision of *C. spinosa* into subspecies was proposed by Maire (1965) for the taxa occurring in Northern Africa, and more recently by Higton and Akeroyd (1991) and Tan (2002) for those of Southern Europe. Inocencio et al. (2006), adopting a narrow species concept, recognized ten species and several intraspecific taxa from the Mediterranean area to central Asia. More recently, taking into account the whole distribution range of the group, *C. spinosa* was considered as a unique species, split into six subspecies including several varieties, of which subsp. *spinosa* and subsp. *rupestris* (Sm.) Nyman are present in the Mediterranean (Fici, 2014, 2015). In these classifications the subspecies mostly show geographical vicariance, except in the Mediterranean area where subsp. *spinosa* and subsp. *rupestris* have parapatric distributions (Fici, 2001), whereas the varieties are referred to forms clearly differentiated within the subspecies, which in some cases show intermediates in the contact areas (Hedge and Lamond, 1970; Blakelock and Townsend, 1980).

Currently, data on the genetic differentiation of varieties of *C. spinosa* are available for some countries or provinces and provide an incomplete overview about the geneflow in this interesting and critical group. Moreover, studies have used dominant molecular markers, such as AFLP, RAPD and ISSR, which are not very informative (Inocencio et al., 2005; Moubasher et al., 2011; Özbek and Kara, 2013; Al-Safadi et al., 2014; Gristina et al., 2014; Aichi-Yousfi et al., 2016; 2022; Mahmodi et al., 2022), or plastidial markers (Maurya et al., 2022, 2023; Pegiou et al., 2023). The advent of Next Generation Sequencing (NGS) technologies enabled the development of a first panel of Expressed Sequence Tag (EST)-SSRs through *de novo* transcriptome assembly of *C. spinosa* subsp. *rupestris* (Mercati et al., 2019). EST-SSR have the potential to facilitate evolutionary analyses in a wide variety of taxa and may be the best way forward for studies with limited resources (Ellis and Burke, 2007). Moreover, they are ideal molecular markers to isolate useful gene functions and identify correlations between related species. Indeed, being located in the coding regions, EST-SSRs results are highly transferable to related taxa; thus, they can be considered an efficient tool to assess the genetic diversity and to describe the evolution of a species (Zhou et al., 2019; Zhang et al., 2019), including *C. spinosa*.

The present paper investigates the genetic differentiation and the evolutionary trends of the most representative varieties of *C. spinosa* group in the Mediterranean area, by using efficient EST-SSRs previously isolated (Mercati et al., 2019). A large number of samples, collected along a south-north transect, from the Tamanrasset massifs (Algeria), near the Tropic of Cancer, to the central Mediterranean (Italy), covering a representative part of the genetic diversity of the group in the Mediterranean, was investigated for the first time through an integrated Bayesian approach. Our results provide new insights into the genetic structure of relict populations of the rocky outcrops of the Saharan massifs, which are refugia for ancient floristic elements providing relatively wet conditions in desert areas (Davis, 1951; Danin, 1978). We also develop a hypothesis of the phylogeographic history of the group inferred from samples of *C. spinosa* subsp. *cartilaginea* (Decne.) Maire & Weiller, collected in Egypt, Kenya, Oman, Somalia, and Yemen. This taxon is widespread in tropical and subtropical areas of Eastern Africa and Middle East, and is a putative xero-tropical ancestor of the Mediterranean forms (Fici, 2001). The analysis of genetic diversity and population structure, in combination with modelling gene flow allowed us to clarify the relationships among the evolutive lineages of *C. spinosa*, to shed light on the correct taxonomic classification of some critical varieties of the species and its putative evolution.

2. Material and methods

2.1. Sample collection

Four hundred thirty-one (431) samples from 82 natural populations belonging to the *C. spinosa* group were collected from Italy (Rome 41° 54' 10.0152'' N 12° 29' 46.9176'') to Southern Algeria (Tamanrasset, 22°47'6"N 5°31'22.001"E). Based on morphological traits and following the nomenclature adopted by Fici (2014), the samples were identified as two subspecies and five varieties widespread in the Mediterranean and Saharan massifs: *C. spinosa* subsp. *spinosa* var. *aegyptia* (AEGY), *C. spinosa* subsp. *spinosa* var. *canescens* (CAN), *C. spinosa* subsp. *rupestris* var. *rupestris* (RUP), *C. spinosa* subsp. *rupestris* var. *myrtifolia* (MYR), *C. spinosa* subsp. *rupestris* var. *ovata* (OVA). Twenty-three samples belonging to five populations that could not clearly be identified by means of morphological characters (Unidentified taxon 1 - UNI1 - and

Table 1
Samples of *C. spinosa* collected across the Mediterranean area. The samples belonged to five varieties (AEGY, CAN, OVA, MYR, RUP), to two unidentified groups (UNI1, UNI2), and to the ancestral taxon *C. spinosa* subsp. *cartilaginea* (CART). CAN-A samples were from Algeria; CAN-I from Italy. Classification based on Fici (2014), (2015). See Table S1 for a detailed description of the morphology of the taxa. All samples were collected in their natural habitat.

Species	Sub-species	Variety	Group ID	Number of populations	Number of samples
Capparis spinosa	*C. spinosa* subsp. *spinosa* L.	var. *aegyptia* (Lam.) Boissier	AEGY	23	142
		var. *canescens* Cosson	CAN-A	3	16
			CAN-I	9	25
	C. spinosa subsp. *rupestris* (Sm.) Nyman	var. *ovata* (Desf.) Batt.	OVA	7	52
		var. *myrtifolia* (Inocencio, D. Rivera, Obón & Alcalaraz) Fici	MYR	10	56
		var. *rupestris* (Sm.) Viviani	RUP-A	9	28
			RUP-I	17	89
	Unidentified	-	UNI1	1	6
		-	UNI2	4	17
	C. spinosa subsp. *cartilaginea* (Decne.) Maire & Weiller	-	CART	5	9
		TOTAL		*88*	*440*

Unidentified taxon 2 - UNI2) were also included in the genetic analysis (Table 1; Table S1; Table S2; Fig. 1; Fig. S1). Finally, to investigate the phylogeographic history of the *C. spinosa* group in the Mediterranean area, the genetic profiles of 9 samples belonging to *C. spinosa* subsp. *cartilaginea* (CART) were included in the present study (Table 1; Table S2).

2.2. DNA extraction and genotyping

Genomic DNA was extracted from 100 mg of powdered, frozen, young leaf tissue of each sample collected using the Macherey-Nagel Plant Kit (Düren, Germany). The purity and quantity of the DNAs were assessed by a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). All the genotypes were characterized by using 12 Expressed Simple Sequence Repeat microsatellites (EST-SSR) previously isolated (Mercati et al., 2019). PCR reactions were performed following the procedures reported in Mercati et al. (2019), using primers fluorescently labelled with FAM, VIC, and NED. The fragments were separated by capillary electrophoresis using an ABI PRISM 3130 Genetic Analyser (Applied Biosystems) to detect polymorphisms. Fragments were sized and binned into alleles using Gene Mapper v. 4.1 software (Applied Biosystems).

2.3. Genetic diversity and population structure

Phylogenetic analysis and Principal Coordinates Analysis (PCoA) on the whole dataset were performed to estimate the overall relationships among the five varieties belonging to the *C. spinosa* group widespread in the Mediterranean (Table 1). A distance-based dendrogram, using Nei's genetic distance (Nei, 1972) and NJ (Neighbor joining) algorithm, with bootstraps based on 1000 re-sampling to assess the robustness of the inferred evolutionary relationships in the dendrogram, was obtained with R/poppr (Kamvar et al., 2015) and visualized through R/ggtree (Yu et al., 2017), while PCoA was carried out with R/adegenet (Jombart, 2008).

The number of genetic pools (*K*) was computed using STRUCTURE (Pritchard et al., 2000). The fitting number of model complexity explaining the structure of the datasets was calculated following Evanno et al. (2005). Twenty independent runs (100,000 burn-in, 500,000 Marchov Chain Monte Carlo) for each *K* (1−10) were carried out using the admixture model with correlated marker frequency and default parameters. The runs were averaged using CLUMPP (CLUster Matching and Permutation Program; Jakobsson and Rosenberg, 2007) and the histograms were obtained with the DISTRUCT program (Rosenberg, 2004). Individuals with ancestry probabilities < 0.70 were considered an admixture group, while those with higher values were assigned to the corresponding cluster. Bayesian analysis was performed also by Discriminant Analysis of Principal Components (DAPC) implemented in R/adegenet. The number of principal components (PCs) retained was evaluated using the cross-validation procedure. The *K*-means algorithm 'find.clusters' was adopted to verify the assignment of individuals to clusters. The analyses were performed independent of geographic origin.

2.4. Population genetic statistics, Wright's fixation index (Fst) and isolation by distance (IBD)

Population genetic statistics including observed heterozygosity (*Ho*), expected heterozygosity (*He*), and inbreeding coefficient (*F*), were computed using R (https://www.bioconductor.org). Pairwise genetic

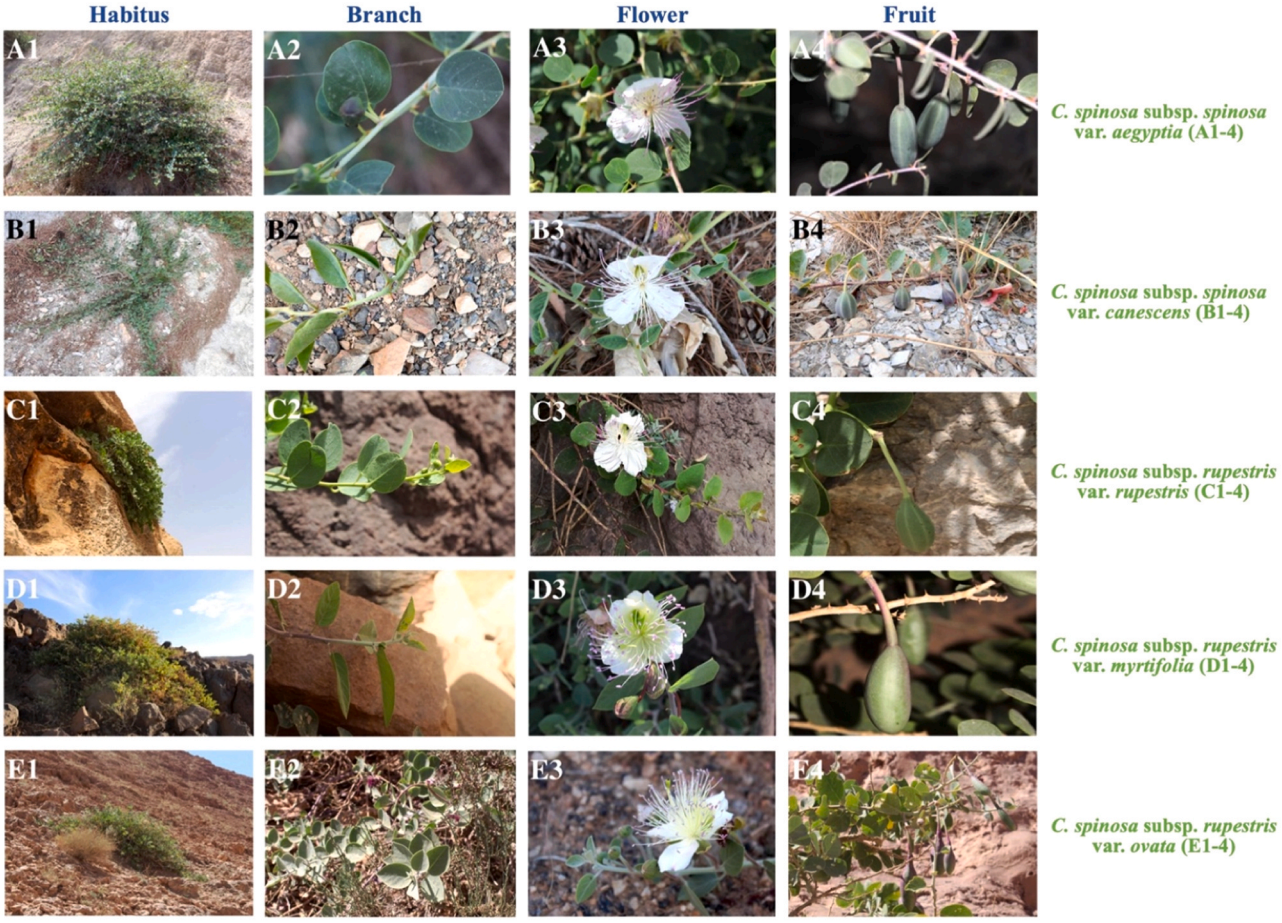

Fig. 1. Morphological characteristics of the different taxa of the *C. spinosa* group.

differentiation index (*Fst*) (Wright, 1965) among varieties was computed using R/HierFstat (Goudet, 2005). A NJ-tree based on *Fst* values was developed with R/adegenet. In addition, we tested for IBD using a Mantel test between the genetic (Nei, 1972) and geographic distances between the groups investigated (Table 1) with R/adegenet.

2.5. Inference of spatial population structure through ancestry coefficient

To assess the genetic differentiation and spread of varieties belonging to the *C. spinosa* group, a spatial ancestry estimation through R/TESS3 was performed (Caye et al., 2016). The collection was split into seven groups, according to the genetic results, the taxonomic classification (Fici, 2014, 2015), and the geographic origin: AEGY, OVA, MYR, RUP-I, RUP-A, CAN-A, and CAN-I. A and I indicated the country of origin, Algeria and Italy, respectively. The ancestry coefficient was assessed using the whole dataset (440) by including 9 genotypes from eastern Africa and south-western Asia belonging to *C. spinosa* subsp. *cartilaginea* (CART), considered the xero-tropical ancestor (Fici, 2001) (Table S2). CART genotypes were useful to shed light on the origin of the varieties of the *C. spinosa* group in the Mediterranean area included in the present study (Table 1), which were not included in previous analyses due to their small number of genotypes. After a cross-entropy criterion evaluation for each value, the best *K* was selected (Frichot et al., 2014; Caye et al., 2016). The Q-matrix values for the best *K* were interpolated on a geographic map.

2.6. Gene flow evaluation within C. spinosa varieties

A ML-tree of data collected and a gene flow model among the six varieties were developed by TreeMix (Pickrell and Pritchard, 2012). The mean length, variance in length, and number of haplotypes for each group and microsatellite were calculated. Fifty independent ML searches following the procedure described by Mercati et al. (2021) were carried out, including the -micro flag. To filter the results based on the likelihood values, the R/cfTrees module was used (Zecca et al., 2020). Duplicates were deleted and the best-scoring ML tree was selected. Then, to obtain the optimal number of migration events (*m*) between groups, each number of migrations (from 0 to 4) was tested 10 times using bootstrap replicates and different random seeds each time. The choice of the best *m* was automated through R/OptM (Fitak, 2019). The residuals from the fitted models were evaluated and visualized using the R script *plot_resid*.

2.7. Approximate Bayesian Computation (ABC) for modelling the differentiation among varieties

To establish the most likely gene flow scenarios during the differentiation of the *C. spinosa* group in the Mediterranean area an ABC analysis was carried out (Cornuet et al., 2014). Six varieties (AEGY, OVA, MYR, RUP, CAN, and CART; Table 1; Table S1; Table S2) were included in the modelling. A core collection was selected through R/corehunter (Thachuk et al., 2009) by using the allele coverage allocation strategy to maximize the allele proportion. Three main scenarios for the possible origin of the Mediterranean varieties were evaluated: *i*) Scenario 1 assumed that RUP, AEGY and CAN groups originated at different times from CART - a tropical and subtropical subspecies -, and assumed a secondary flow from RUP to MYR and then to OVA; *ii*) Scenario 2 assumed firstly gene flow from CART to MYR and RUP, then to OVA. Independently, AEGY and CAN derived from CART; *iii*) Scenario 3 considered that AEGY, CAN, and RUP evolved independently from CART, while gene flow from CART generated MYR and then OVA. For all the scenarios, 3×10^6 simulations were carried out by DIY-ABC. The posterior probabilities (with 95% confidence intervals - CI) of each scenario were assessed following both a logistic regression and a direct approach on the 1% of simulated datasets closest to the observed dataset, evaluating the ability of ABC approach to discriminate between scenarios.

3. Results

3.1. Phylogenetic and principal coordinates analysis

The phylogenetic analysis of the large germplasm collection was able to separate the different varieties of caper here studied. Indeed, the *C. spinosa* group in the Mediterranean resulted in four main clusters that separated the genotypes belonging to CAN, AEGY, RUP, and a cluster consisting of the two closely related taxa MYR and OVA (Fig. 2A; Table 1; Table S2), that included also 12% of RUP genotypes (Fig. 2A; Table 1). The two unidentified taxa, UNI1 and UNI2, were grouped with CAN and MYR/OVA varieties, respectively (Fig. 2A). PCoA confirmed the genetic relationships revealed by cluster analysis (Fig. 2B), and separated the genotypes belonging to *C. spinosa* subsp. *spinosa* from those of *C. spinosa* subsp. *rupestris*, with nearly 60% of variance explained by the first two PCoA-axes; furthermore, AEGY was clearly distinguished from CAN samples within the subs. *spinosa*.

3.2. Discriminant Analysis of Principal Components (DAPC) and STRUCTURE analysis

The DAPC analysis with $K = 3$ (Fig. 3A), the best cluster number, separated all AEGY genotypes (light brown) from a second cluster comprising CAN genotypes (brown) that also included the UNI1 population, and from a cluster of the *C. spinosa* subsp. *rupestris* (MYR, OVA, RUP; green), that included also UNI2 genotypes.

The optimum *K* was also evaluated by STRUCTURE, providing an optimal value at 4 (Fig. S2). In agreement with phylogenetic and PCoA analyses, the varieties were very distinct in the STRUCTURE analysis (Fig. 3B). Each group showed a prominent ancestry membership (> 0.7) to an owner pool, with only 4 % of admixture profiles in the full dataset (18 out of 431 samples). AEGY genotypes were entirely ascribable to pool 1 (light brown), 97 % of CAN and 50 % of UNI1 genotypes were attributable to pool 2 (brown), while the other 50 % of UNI1 showed admixture. One-hundred three (103; 88 %) out of 117 RUP samples belonged to pool 3 (light blue), while pool 4 (green) was formed by UNI2, MYR, and OVA genotypes (Fig. 3B).

3.3. Genetic diversity, fixation index, and isolation by distance (IBD)

Several genetic statistics, such as the observed (*Ho*) and the expected heterozygosity (*He*), the inbreeding coefficient (*F*) and the Shannon's index (*I*) were evaluated for each variety (Table S3). The mean value of *I* was 0.541, with similar values for four (MYR, OVA, CAN, and UNI2) out of seven groups investigated, while the lowest values were recorded for AEGY-populations and the highest for RUP -populations. AEGY showed also the lowest values of both *Ho* and *He*, while comparable values within the dataset, ranging from 0.173 (MYR) to 0.284 (UNI2) and from 0.241 (MYR) to 0.320 (OVA) were observed, except in RUP and UNI1 (Table S3). *F* values were positive for all the groups and the mean value was 0.160, ranging from 0.075 (RUP) to 0.472 (AEGY). Only the two unidentified taxa showed negative values (-0.164 for UNI1 and −0.021 for UNI2).

MYR and AEGY groups showed the highest genetic distance according to pairwise *Fst* values (Table S4). Large genetic divergences were observed between AEGY and OVA, as well as AEGY *vs* UNI2, RUP and CAN. The lowest *Fst* pairwise value was recorded between CAN and UNI1, followed by MYR and UNI2 (Table S4). The NJ (Neighbor-Joining) tree based on *Fst* values (Fig. S3) split up all the varieties, including UNI1 and UNI2, showing a structure consistent with previous results. MYR, OVA, and UNI2 groups formed one main branch and differed greatly from CAN and AEGY groups, included in two other main branches, while RUP was close to the other varieties of the *C. spinosa* subsp. *rupestris* (MYR and OVA). AEGY and CAN groups were separated,

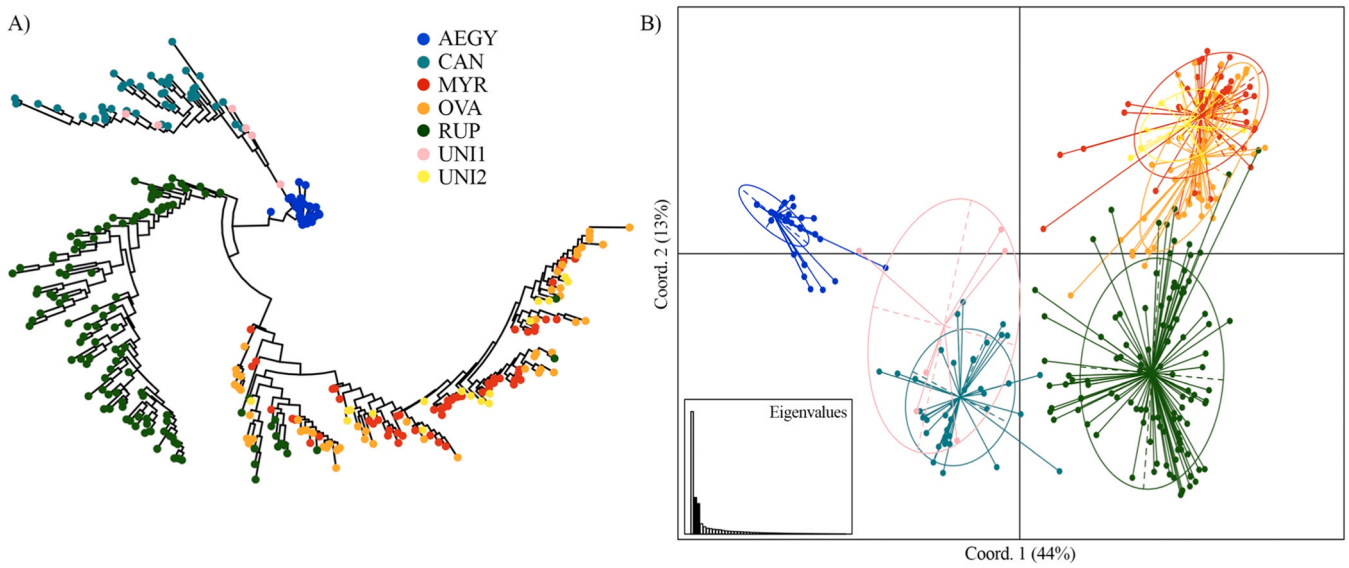

Fig. 2. Genetic relationship among 431 genotypes of *C. spinosa* collected in the Mediterranean (Table 1; Table S1; Table S2). **A)** NJ dendrogram. **B)** PCoA based on the first (44 % of variance explained) and second (13 % of variance explained) axes. The colour of the samples indicates their taxonomic classification according to Fici (2014); (2015). AEGY: *C. spinosa* subsp. *spinosa* var. *aegyptia*; CAN: *C. spinosa* subsp. *spinosa* var. *canescens*; MYR: *C. spinosa* subsp. *rupestris* var. *myrtifolia*; OVA: *C. spinosa* subsp. *rupestris* var. *ovata*; RUP: *C. spinosa* subsp. *rupestris* var. *rupestris*; UNI1: Unidentified 1; UNI2: Unidentified 2.

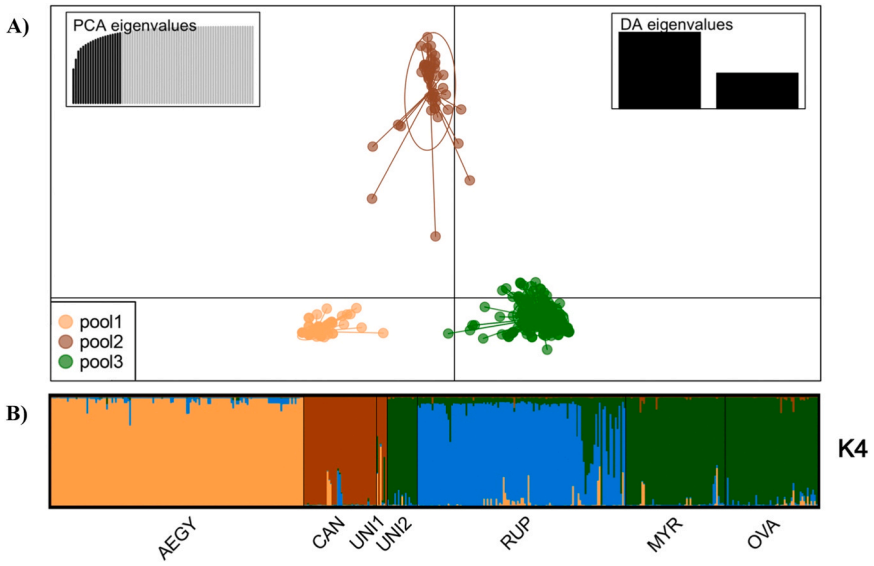

Fig. 3. Population structure of the samples of *C. spinosa* collected in the Mediterranean (Table S1; Table S2). **A)** DAPC scatter plot based on the first and second Linear Discriminants (LD) at the best K (3). **B)** Barplot of the posterior probability evaluated by STRUCTURE at the optimal $K = 4$ (Fig. S2).

with CAN closely related to UNI1 samples, in agreement with the PCoA and STRUCTURE results (Fig. 2; Fig. 3). The *C. spinosa* varieties showed an IBD pattern ($p < 0.001$) between genetic differentiation and geographic distances. The pattern of Fst-values between varieties was in agreement with the results from the STRUCTURE, PCoA, and NJ analyses. In addition, according to these finding, genotypes belonging to UNI1 were similar to those of CAN group, while the genotypes of UNI2 were near to those of MYR group. Therefore, based on *Fst* values and genetic diversity, in agreement with IBD results and the geographic origin, the samples were divided into seven groups, AEGY, OVA, MYR, RUP-I (I = Italy), RUP-A (A = Algeria), CAN-I (I = Italy), and CAN-A (A = Algeria).

3.4. Evaluation of the differentiation of C. spinosa varieties through ancestry coefficient analysis

For the analysis of ancestry the genotypes of *C. spinosa* subsp. *cartilaginea* (CART) (Table 1; Table S2) were added to the dataset to better reflect the evolution of the *C. spinosa* group. The ancestry analysis by the cross-entropy criterion estimated a single fixed $K = 6$ (Fig. S4), confirming the distinctness of the varieties of *C. spinosa* (Fig. 4; Table S5). Interpolating the ancestry coefficients on a geographical map highlighted that CART might harbour ancestral traits compared to the taxa widespread in the Mediterranean, as hypothesized by Fici (2001) (Fig. 4A). Furthermore, four out of the eight groups investigated (AEGY, CART, MYR, and OVA) belonged to a specific pool (membership >

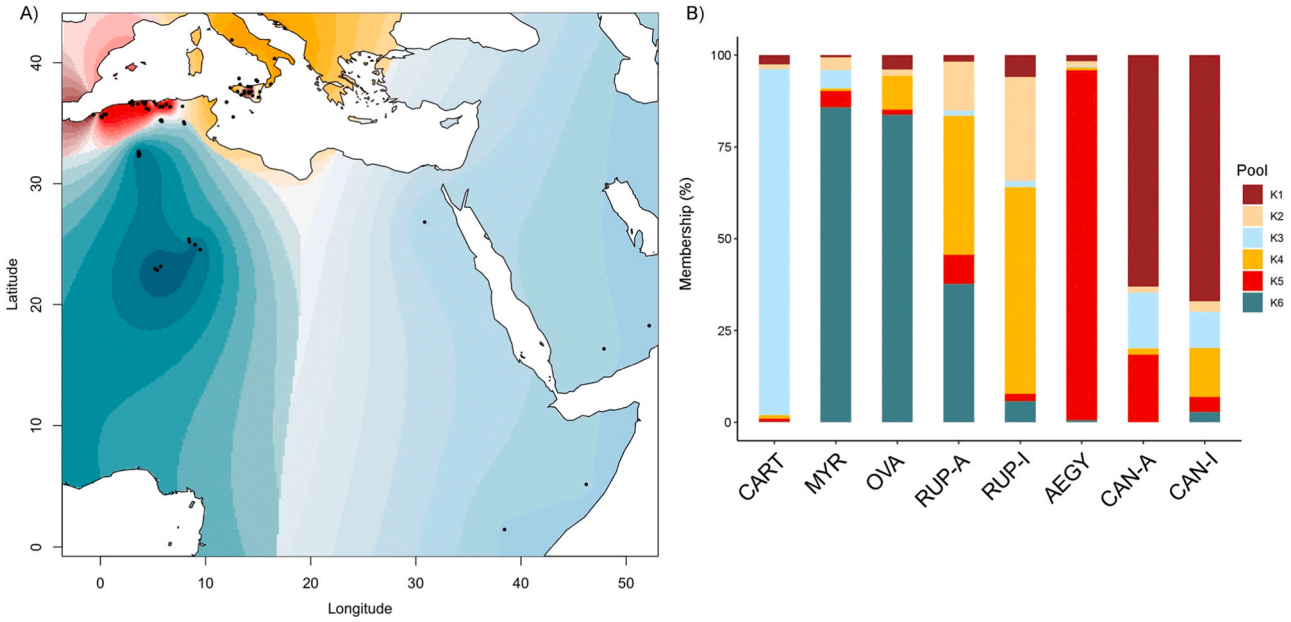

Fig. 4. Ancestry coefficient evaluation of *C. spinosa* populations in the Mediterranean, including the samples from *C. spinosa* subsp. *cartilaginea* (CART) (Table S2). The number of ancestral populations ($K = 6$) was chosen after the evaluation of a cross-entropy criterion for each K (Fig. S4). **A)** The Q-matrix values ($K = 6$) were interpolated on a geographic map, using the coordinates (Latitude; Longitude) of each population (Table S2). **B)** Bar plot showing the proportion of ancestry due to each of the six pools (*K1 -K6*) in the eight groups investigated (AEGY, CAN-A, CAN-I, CART, OVA, MYR, RUP-A, and RUP-I; Table S5).

80 %); the others, RUP-A, RUP-I, CAN-A, and CAN-I, showed an admixed structure (Fig. 4B; Table S5).

3.5. Evaluation of gene flow by estimation of putative migration events

To well describe the complex evolution of the *C. spinosa* group in the Mediterranean, a putative migration chart of ancestral populations based on gene flow was developed. Fifty preliminary ML (Maximum Likelihood) searches displayed the same value of ln (likelihood), with the same topology and branch lengths, therefore one out of fifty ML was selected at random. The ML-tree showed the relatedness across taxa, supporting the diversification process of varieties of *C. spinosa*, with horizontal branch lengths proportional to the amount of genetic drift that occurred within each group. Increasing the number of migration events (*m*) from 0 to 4 did not result in saturation of the log-likelihood models and the models including migrations showed higher residual values and SEs than the ML-tree without migrations (data not shown). Therefore, the model without *m*, showing the lowest SE (± 0.9), was chosen as the best fit (Fig. S5).

3.6. The best scenarios for the differentiation of C. spinosa varieties in the Mediterranean

Three scenarios for the possible differentiation of *C. spinosa* in the Mediterranean were tested using ABC approach (Fig. 5). To reduce the computational power required, the ABC analysis was performed using a core collection for each group (125 samples analysed) in which the whole genetic diversity was preserved. The posterior probability calculated by both the direct and the logistic approach was highest for Scenario 2 (Fig. S6). This scenario assumed a gene flow directly from CART to MYR and then to the other two varieties of subsp. *rupestris* (OVA and RUP). The groups AEGY and CAN are assumed to both have branched from CART (Fig. 5).

4. Discussion

The genus *Capparis* is considered a relic of the tertiary xero-tropical flora in the Mediterranean and Middle East, which gave rise to extratropical derivatives (Zohary, 1973) within the *C. spinosa* group. The taxa belonging to this group often show a high phenotypic plasticity as well as intermediate forms (Heywood, 1964; Fici, 2001). This remarkable variation resulted in discordant taxonomic classifications and in the description of several taxa. Here, for the first time, a large number of samples of the main varieties of *C. spinosa* subsp. *spinosa* and subsp. *rupestris* in the Mediterranean was used to assess their phylogenetic relationship, using high informative EST-SSRs as an efficient tool to analyse the evolutionary process across the varieties under study.

Our results provide novel insights into the divergence history of this group in the Mediterranean. In particular, we showed for the first time that the variety *aegyptia* (AEGY) is clearly distinguished from var. *canescens* (CAN), within the subspecies *spinosa*. AEGY is also well differentiated from all the varieties belonging to the subsp. *rupestris* (RUP, MYR, and OVA), and therefore it could be considered a distinct subspecies of *C. spinosa*, i.e. subsp. *aegyptia* (Lam.) Kit Tan & Runemark, as proposed by Tan (2000). This taxon, widespread in the Mediterranean, from northern Africa to Greece, and in the Middle East, is characterized by small, subfleshy, orbicular to obovate, glabrous leaves, often glaucous or blue-glaucous (Zohary, 1960; Maire, 1965; Tan, 2002; Fici, 2014). The other variety belonging to subsp. *spinosa*, i.e. var. *canescens* (CAN), is widespread in a large area from the Mediterranean eastwards to India and Nepal, and if compared to AEGY shows larger, oblong to elliptic-obovate, more or less pubescent leaves (Blakelock and Townsend,1980; Fici, 2014). The subsp. *rupestris* is represented by three varieties, of which MYR occurs in the central Sahara mountains (Tibesti, Ennedi, Hoggar, Tassili) and is characterized by ovate or ovate-lanceolate, more or less coriaceous, glabrous or puberulous leaves, with stiff apical mucro. RUP is widespread along the Mediterranean coasts and shows peculiarly coriaceous to succulent, orbicular to obovate, glabrous leaves, while OVA, recorded from northern Africa to southern Italy, has thinner, ovate or ovate-elliptical, pubescent leaves (Zohary, 1960; Maire, 1965; Inocencio et al., 2006; Fici, 2014). Interestingly, in the phylogenetic, PCoA, and STRUCTURE analyses the populations of RUP were clearly differentiated from the ones of the other two varieties of subsp. *rupestris* (MYR and OVA), which are in some cases merged. These conclusions are also confirmed by the *Fst* index among all the pairwise comparisons. However, the treatment of MYR and OVA as

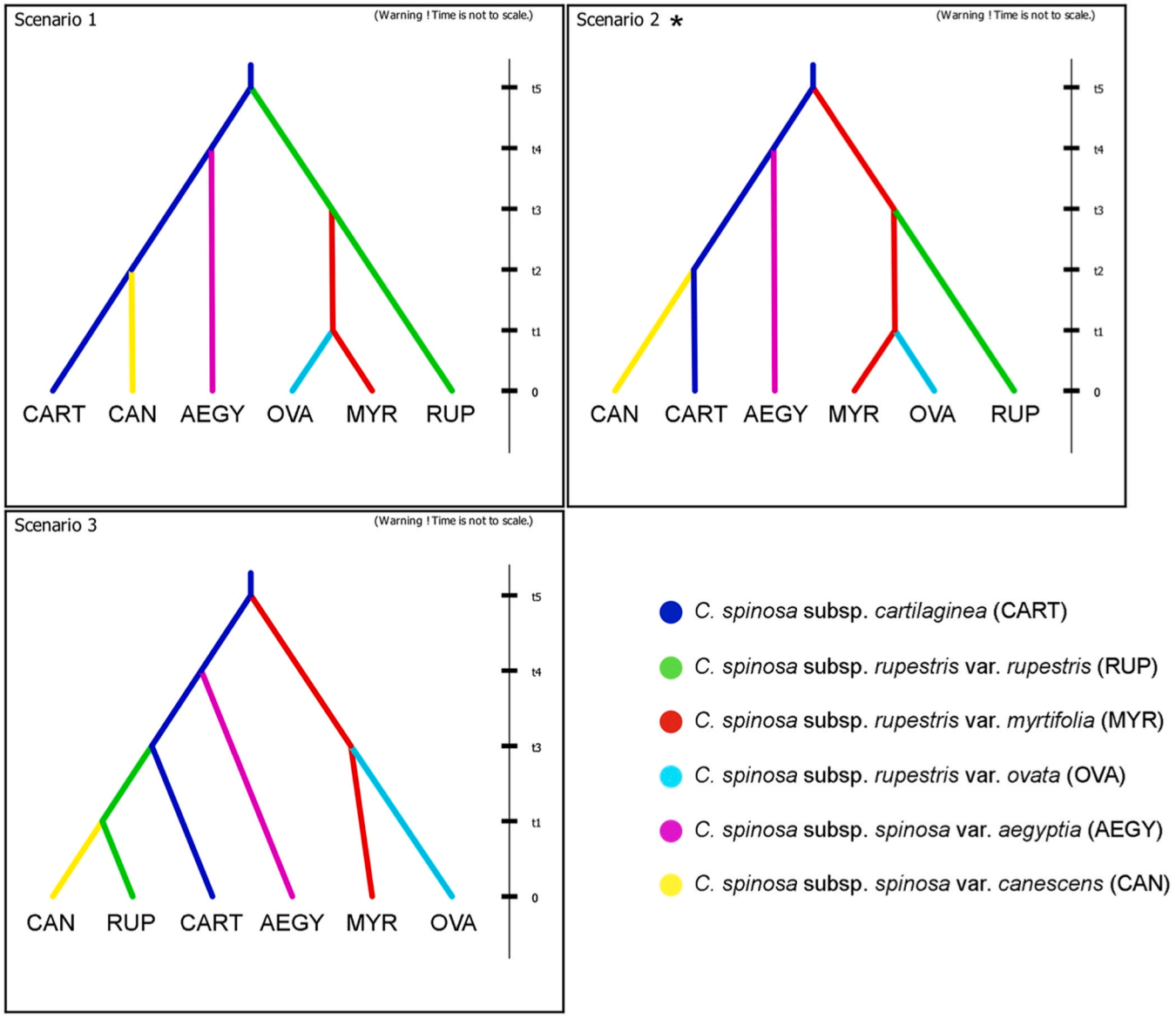

Fig. 5. Schematic representation of three scenarios for the gene flow across varieties of *C. spinosa* in the Mediterranean, based on ABC analyses. The vertical bar in each box indicates the time axis (t0 = present). Among the scenarios tested, Scenario 2 (*) is the most likely one based on both the direct and the logistic regression approach (Fig. S6).

different taxa is well supported by their morphological characters and distinct areas of distribution. As a results of the genetic analyses we were also able to assign the two morphologically unidentified taxa, UNI1 to CAN, and UNI2 to MYR, although UNI2 was genetically also very close to OVA.

The differentiation between two subspecies of *C. spinosa*, subsp. *spinosa* and subsp. *rupestris*, was expected, given the well-known phenotypic differentiation previously reported (Zohary, 1960; Coode, 1965; Maire, 1965; Hedge and Lamond, 1970; Higton and Akeroyd, 1991; Marcos Samaniego and Paiva, 1993; Saadaoui et al., 2009; Fici, 2014; Gristina et al., 2014; Mercati et al., 2019). In contrast, the genetic differentiation of AEGY from the other variety belonging to *C. spinosa* subsp. *spinosa* (CAN) was not reported before. The taxonomic classification of AEGY has historically been controversial, since this taxon was firstly described by Lamarck (1785) as a separate species, and then reduced to a variety of *C. spinosa* by Boissier (1867), an interpretation accepted in many taxonomic revisions and floristic reports (Zohary, 1960; Maire, 1965; Pottier-Alapetite, 1979; Coode, 1965; Elamin, 1983; Boulos, 1995; Fici, 2014). Furthermore, in the last century AEGY has been considered a synonym of CAN by some authors (Hedge and Lamond, 1970; Nyberg, 1996; Boulos, 1999). The genetic statistics and STRUCTURE profiles here reported indicate that AEGY is distinct from both the subspecies *spinosa* and *rupestris*, in agreement with the classification proposed by Tan (2002), who considered AEGY a distinct subspecies of *C. spinosa*. Interestingly, the genetic divergence between the varieties of *C. spinosa* increased significantly with their pairwise geographic distance. This result could be explained by the isolation (mainly due to deserts and mountains) and subsequent lack of gene flow and local adaptation of the groups.

Our results suggest a central role for the tropical and subtropical subsp. *cartilaginea* (CART) in the evolutionary history and diversification of *C. spinosa* in the Mediterranean. Furthermore, we also inferred the existence of at least three gene flow events that gave rise to the different varieties in this area. CART is widespread in rocky habitats in several areas of eastern Africa, Madagascar and Middle East, with its distribution extending northwards to Egypt, including Sinai, and Israel (Fici, 2015). In the past, this taxon has been considered a separate species by Zohary (1960) and Elffers et al. (1964), but they have stressed its close

relationship to *C. spinosa* subsp. *spinosa*. In agreement with this classification, Maire (1965) reduced it to a subspecies of *C. spinosa*. Fici (2001; 2015) pointed out that this subspecies is characterized by an erect habit and fleshy leaves and hypothesized that it is closely related to a xero-tropical ancestor of the widespread Mediterranean taxa.

The *K5* pool in the ancestry analysis encompassed the samples belonging to AEGY, suggesting its diversification from CAN, which showed the highest ancestry value for the *K1* pool. This result is in agreement with the analyses of the genetic relationships and the population structure, and the models of gene flow, that showed a distinction between AEGY and CAN, supporting the taxonomic treatment by Tan (2002), who considered var. *canescens* a distinct subspecies (subsp. *aegyptia* (Lam.) Kit Tan & Runemark). *K6* contributed most to MYR and OVA. This pool was also present to an appreciable degree in RUP (higher in Algerian samples than Italian as expected, due to their geographic distribution), underlining a possible genetic exchange between MYR or OVA and RUP, a hypothesis confirmed by the genetic relationships between the two varieties and the pattern of drift identified by the gene flow analysis. Notably, the populations belonging to RUP (both RUP-A and RUP-I) showed multiple memberships, but with a highest ancestry value for *K4* pool. Finally, the *K2* pool contributed to all varieties, but in particular to RUP-I and RUP-A. Our conclusions are also supported by the most likely evolutionary scenario indicated by ABC analysis.

Our findings suggest a key role for CART in the evolution of *C. spinosa* in the Mediterranean, because of gene flow from CART to MYR and then to OVA and RUP. MYR, with its relict populations in the Saharan massifs, is the oldest among the varieties of *C. spinosa* subsp. *rupestris*. From these RUP, widely spread in the Mediterranean, and the mainly North African OVA should have been originated. Our results are in agreement with Maurya et al. (2023), who considered CART, RUP and OVA a sister clade. Gene flow from CART could have generated both AEGY and CAN, confirming the pivotal role of CART in the evolution of the *C. spinosa* group in the Mediterranean, as shown by ancestry evaluation, and gene flow, that supported CART as ancestral variety.

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CRediT authorship contribution statement

Sakina Bechkri: Writing – review & editing. **Silvio Fici:** Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Francesco Mercati:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization. **Guglielmo Puccio:** Writing – review & editing, Formal analysis. **Francesco Carimi:** Writing – review & editing, Conceptualization. **Francesca La Bella:** Writing – review & editing, Investigation. **Alessandro Silvestre Gristina:** Writing – review & editing, Resources. **Youmna Melzi:** Writing – review & editing, Writing – original draft, Resources, Investigation, Conceptualization. **Marcello Zerbo:** Writing – review & editing, Validation, Investigation, Formal analysis. **Douadi Khelifi:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data presented in this study are available in the text or Supplementary material here

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ppees.2024.125794.

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