Molecular markers for botanic identification among *Brassica* species

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Collecting wild relatives of *Brassica* (n=9) species is sometimes puzzling as the morphology within a species is variable and often descriptors do not completely discriminate between the different taxa. This has lead to dubious taxonomical determinations and some have been discussed for many years without any firm conclusions. We have identified four complicated cases deserving investigation:

“Wild” kale on Helgoland, Germany
“Wild” kale from Rødvig, Denmark
“Wild” kale at Monte Conero, Italy
“Wild” kale on Isola di Ponza, Italy

Here we present a preliminary study of the kale found at Monte Conero.

On Monte Conero near Ancona on Italy’s Adriatic coast, a population of *Brassica montana* (formerly named *B. oleracea* subsp. *robertiana*) is reported by Onno (1933) and by Biondi *et al*. (2002). This species is indicated by Pignatti (1982) as having a fragmented relictual distribution, but the population at this locality, south of Ancona, has been considered an escaped population of cultivated *B. oleracea* by Snogerup *et al.* (1990).

To bring genetic considerations into the discussion we decided to apply genetic markers in the study. Our approach was to compare the sampled population with well known populations of agreed taxonomical status from classic locations.

We selected as references 2 accessions of *B. montana* (▲) and 3 accessions of *B. incana* (■) as the locality under study is close to the distribution area of these species (Snogerup *et al.* 1990), Figure 1. Furthermore, cultivated references of *B. oleracea* (●) were selected, including two traditional landraces of leafy kale from Sicily and Calabria and two locally cultivated cauliflower varieties (●) from the Marche region. Finally, we tested a sample of kale from Monte Conero (◆) – accession UNICT4198, Table 1.

Table 1. Material analyzed in this study.

Holding Genebank	Accession number	Collection number	Plants	Genus	species	Variety	ORIGCTY	Sampstat	Collecting site
ITA 227	UNICT4198	LM05-13	11	Brassica	?		ITA	W	Monte Conero
ITA 227	UNICT3841	S536	9	Brassica	oleracea	acephala	ITA	LR	Caltavuturo, Sicily
Collected		S303	6	Brassica	oleracea	acephala	ITA	LR	Stilo, Calabria
Donated by N. Acciarri			21	Brassica	oleracea	botrytis	ITA	CV	Marche
Donated by N. Acciarri			19	Brassica	oleracea	botrytis	ITA	CV	Marche
ITA 227	UNICT4202	LM05-1	12	Brassica	incana		ITA	W	Lago Albano, Lazio
ESP003	6800-85	It-100	18	Brassica	incana		ITA	W	Isola del Giglio, Tuscany
ITA 227	3494		12	Brassica	incana		ITA	W	Agnone Bagni, Sicily
ESP003	6802-85	It-103	16	Brassica	montana		ITA	W	Stazzema, Tuscany
ESP003	6817-85	Fr-117	20	Brassica	Montana		FRA	W	Le Croupatier

Materials and methods
All accessions were either requested from indicated genebanks, donated or collected by authors.

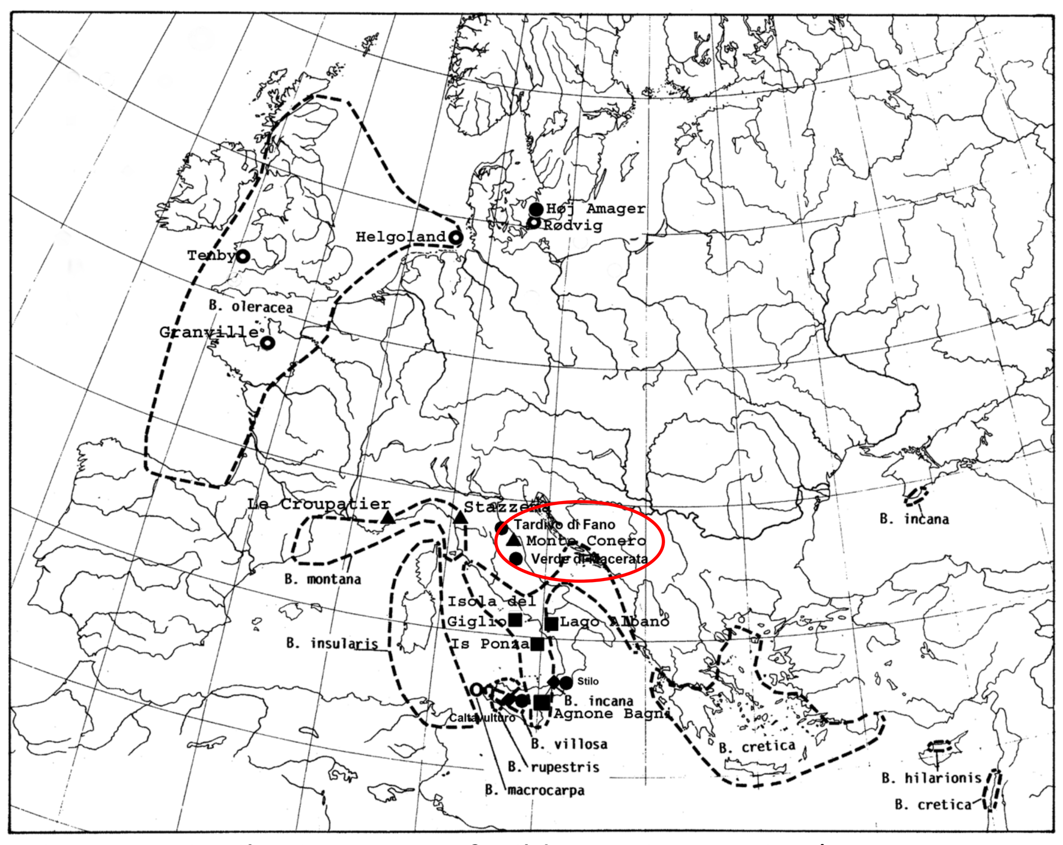

Figure 1. Distribution areas of wild *Brassica* species (Snogerup et al. 1990) and collection sites of invesigated accession

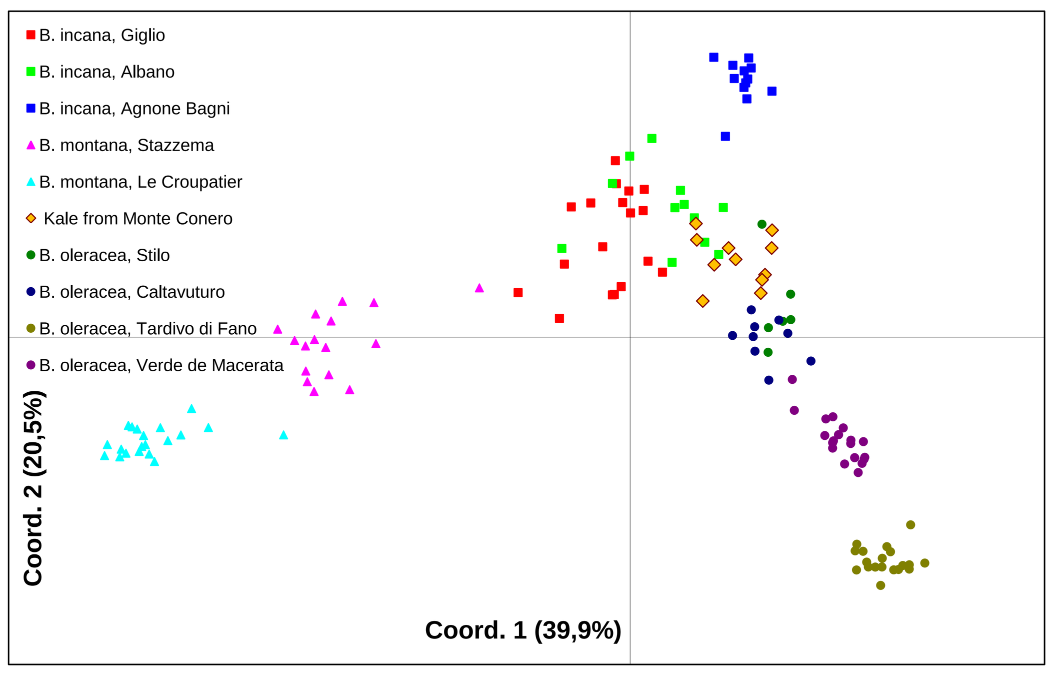

Figure 2. Principal coordinate analysis shows the genetic similarity of the tested Brassica populations based on ISSR data.

Table 2. Assignment analysis of the Monte Conero population to reference populations. FGH: First Generation Hybrids.

Reference populations	Assigned to Monte Conero				
log likelihood ->	0	0-FGH	1	1-FGH	2
Verde di Macerata	0	0	0	0	0
Agnone Bagni	0	0	0	0	0
Le Croupatier	0	0	0	0	0
Isola del Giglio	1	0	0	0	0
Lago Albano	1	0	0	0	0
South Italian kale	9	1	7	0	2
Stazzema	0	0	0	0	0
Tardivo di Fano	0	0	0	0	0
Giglio x SouthItKale	-	8	-	3	-
Lago Albano x SouthItKale	-	2	-	0	-
Allocation to none	0	0	4	8	9

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DNA was isolated, PCR amplified using inter simple sequence repeat (ISSR) markers according to Andersen *et al.* 2008.
DNA fragments were separated on gels which were stored as electronic files and converted to binary matrices using the Cross Checker software (Buntjer and Otsen 1999).
Data were analyzed using GenAlEx (Peakall and Smouse 2006) for genetic diversity and structure. AFLPop (Duchesne and Bernatchez 2002) was applied for assignment analysis.

Results and discussion
The structures of populations were analyzed using principal coordinate analysis in GenAlEx. *Brassica montana* populations are grouping together and are separate from the other species. *Brassica incana* populations are also nicely grouped and distinct from both *B. montana* and *B. oleracea*. All *Brassica oleracea* samples are separate from the other two species, and the cauliflowers and the leafy kales are grouping according to varieties. Finally, the population of kale from Monte Conero is plotting between *B. incana* from Lago Albano and the leafy kales from South Italy.

Assignment analysis of the Monte Conero Population.
The use of log-likelihood values of 1 and 2 resulted in many samples assigned to no populations. We thus used a log-likelihood value of 0, which resulted in the assignment of 9 individuals to the cultivated kales from South Italy, while 2 individuals were assigned to *Brassica incana* from Isola del Giglio and Lago Albano, respectively. When the first generation hybrid option was applied, one individual was assigned to the cultivated kales form South Italy, while the remaining ten individuals were assigned to “hybrids” with *Brassica incana*, specifically eight to Isola del Giglio x South Italian Kale and two to Lago Albano x South Italian Kale. We realize that these specific hybrids are not possible in nature due to long distances between the populations. However, the interspecific hybrid nature between cultivated kale and wild *Brassica incana* suggested by the genetic analysis of the Monte Conero population is strongly signaled here.

Conclusions
This controversial Monte Conero population has been treated at least three times in the scientific literature, twice as *B. montana*, once as *B. oleracea* and now our data add new perspectives as a possible hybrid between *B. oleracea* and *B. incana*. There certainly is a strong need for verification of the taxonomy, the descriptors and the methods used to distinguish between (n=9) *Brassica* taxa.

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