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 led 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 Radvig, 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. oleracea (■) 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.

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

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 Olsen 1999). Data were analyzed using GenAlEx (Peakall and Smouse 2006) for genetic diversity and structure. AFLPpop (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.

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

![Figure 2. Principal coordinate analysis shows the genetic similarity of the tested Brassica populations based on ISSR data.](image)

![Figure 1. Distribution areas of wild Brassica species (Snogerup et al. 1990) and collection sites of investigated accession](image)

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