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RESEARCH ARTICLE

# On the origin of artichoke and cardoon from the *Cynara* gene pool as revealed by rDNA sequence variation

**Gabriella Sonnante · Anna Vittoria Carluccio · Roser Vilatersana · Domenico Pignone**

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**Abstract** The evolutionary history of artichoke and cultivated cardoon and their relationships to wild allies of the genus *Cynara* are not fully understood yet. To try resolve the evolutionary patterns leading to the domestication of these two crops, a study of molecular evolution was undertaken. The species *C. cardunculus*, including artichoke, cultivated and wild cardoon, together with four wild *Cynara* species were taken into consideration. Internal (ITS) and external (ETS) rDNA transcribed spacers were used as markers of nuclear genome, the *psb*A-*trn*H spacer as a marker of chloroplast genome. Sequences were analysed using phylogenetic analysis packages. Molecular data indicate that the whole genus is quite recent and that the domestication of artichoke and cultivated cardoon, crops diverging for reproduction system and use, are independent events which diverge in time and space. As for wild *Cynara* species, an evolutionary pattern consistent with their present geographical distribution was hypothesized in relation to the climatic changes occurring in the Mediterranean during the last 20 millennia: *C. humilis* and *C. cornigera* appeared to have differentiated first, *C. syriaca* and *C. baetica* were differentiated in a second period, while *C. cardunculus* showed to be the most recent and plastic species. The high plasticity of *C. cardunculus* has not only allowed its nowadays wide distribution, but has also given the potential for domestication.

**Keywords** Artichoke · Cardoon · Domestication · Evolution · Genus *Cynara* L. · Origin

Dedicated to the memory of Richard Neville Lester (1937–2006) who greatly contributed to the understanding of the evolution, domestication, and genetic resources of eggplants as well as to the biosystematics and taxonomy of allied species.

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G. Sonnante (✉) · A. V. Carluccio · D. Pignone
Institute of Plant Genetics, CNR,
Via Amendola 165/A, 70126 Bari, Italy
e-mail: gabriella.sonnante@igv.cnr.it

R. Vilatersana
Botanic Institute of Barcelona, CSIC-ICUB, Passeig del Migdia S/N, 08038 Barcelona, Spain

## Introduction

The genus *Cynara* L. (Asteraceae) includes wild Mediterranean species, and two crops: the artichoke, cultivated for its edible heads and pharmaceutical properties (Bianco 1990), and the cultivated or leafy cardoon, grown for its fleshy stems and leaf stalks (Dellacecca 1990). According to Wiklund (1992), eight species constitute the genus: *C. cardunculus* L., *C. syriaca* Boiss., *C. auranitica* Post, *C. cornigera* Lindley, *C. algarbiensis*

Cosson, *C. baetica* (Spreng.) Pau, *C. cyrenaica* Maire et Weiller, and *C. humilis* L., positioning *C. tournefortii* Boiss. et Reuter. in a new genus, later reincluded in *Cynara* by Robba et al. (2005). All wild species are perennial and the genus is characterized by large spiny leaves and heads. *Cynara algarbiensis*, *C. baetica*, *C. humilis* and *C. tournefortii* are principally of Western Mediterranean distribution, while *C. cornigera*, *C. cyrenaica* and *C. syriaca* are distributed in the Eastern part of the Mediterranean. *Cynara cardunculus*, present in almost all the Mediterranean, is reported to contain two wild subspecies, namely subsp. *cardunculus* and subsp. *flavescens* Wiklund, differing for bracts characters and geographical distribution: the former is distributed from Cyprus to Greece, Central and Southern Italy, Sicily and Sardinia, the latter is diffused in the Iberian peninsula and Macaronesian region (Wiklund 1992). Morphological differences refer especially to bract colour, shape and spines, subsp. *cardunculus* showing longer and more acute spines, and subsp. *flavescens* possessing a yellowish margin in the middle involucral bracts.

Previous classifications considered the cultivated artichoke as a separate species: *C. scolymus* L. However, Wiklund (1992) included cultivated artichoke, leafy cardoon and wild cardoon, in a single species: *C. cardunculus* L. The same conclusion was reached by Basnizki and Zohary (1994), Rottenberg and Zohary (1996) and Rottenberg et al. (1996) on the basis of the results of a crossing programme and isozyme analyses. Therefore, the species *C. cardunculus* was divided into three varieties: the wild var. *sylvestris* Lam., and two cultivated ones, var. *scolymus* (L.) Fiori, the artichoke, vegetatively propagated, and var. *altilis* DC., the leafy cardoon, generally propagated by seed. This nomenclature will be used hereafter when considering the cultigroup, and 'wild cardoon' will indicate *C. cardunculus* var. *sylvestris*; the other wild species will be indicated after their Latin name.

Hybridization experiments have shown that wild cardoon is fully interfertile with cultivated material and therefore it belongs to the primary genepool of artichoke and leafy cardoon (Basnizki and Zohary 1994; Rottenberg and Zohary 1996); this evidence together with other ones, such as isozymes, RAPDs, and AFLPs (Rottenberg et al. 1996; Sonnante et al. 2002, 2004), have suggested that the two crops have evolved from the wild cardoon genepool, which can therefore be considered their progenitor.

Nevertheless these evidences are not exhaustive enough also in the light of the suggested division of the wild cardoon into two subspecies (Wiklund 1992). As a crop, artichoke is supposed to be relatively recent (about 1st century AD), and the beginning of its cultivation is not easily derived from historical literature due to linguistic uncertainty (Foury 1989). Similar doubts regard the origin of cultivated cardoons.

Among the other wild species recognised, *C. syriaca* was initially considered as a possible donor of genes to the cultivated artichoke (Zohary and Bazniski 1975), however some other evidences (Rottenberg and Zohary 1996; Rottenberg et al. 1996), including a recent analysis based on AFLP markers (Sonnante et al. 2004) do not support this hypothesis.

Recently, the phylogeny of the genus *Cynara* was analysed by Robba et al. (2005) using sequences of the internal transcribed spacers of the 18-25S ribosomal DNA. Nevertheless, this study concentrated mostly on the relationships among wild species of the genus, deserving no attention to the evolution of the crops.

Ribosomal DNA (rDNA) is a multigene family with thousands nuclear copies in eukaryotes, arranged in tandem arrays. Artichoke and its wild allies possess ribosomal loci of the same length (Tucci and Maggini 1986; Maggini et al. 1988). The region of 18S-5.8S-25S rDNA contains the mature rRNA coding sequences, which are quite conserved in the evolution of even differentiated species, and two internal transcribed spacers (ITS1 and ITS2), which, on the contrary, diverge more rapidly; these regions evolve cohesively within a single species and exhibit only limited sequence divergence within single individuals (Arnheim et al. 1980). For this reason, these regions have often been used for assessing relationships and evolution among closely related taxa (Baldwin 1992; Sonnante et al. 2003). In some cases, ITS sequence variation is appreciable also within the same species and allows studies at the population level (Schaal and Learn 1988; Yuan and Küpfer 1995; Kollipara et al. 1997; Aïnouche and Bayer 1999).

Similarly to ITS regions, the external transcribed spacer (ETS), a region immediately upstream of the 18S rDNA, is under less selective pressure and thus evolves quite rapidly, often at different rates than ITS (Baldwin and Markos 1998; Markos and Baldwin 2002), providing a further means to improve the resolution of phylogenetic analyses among closely related plant species.

A third DNA sequence of phylogenetic utility is represented by the non-coding region of chloroplast DNA (cpDNA) acting as spacer between *psb*A and *trn*H genes (Kim et al. 1999; Holderegger and Abbott 2003).

In our study, the variation of ITS, ETS and chloroplast *psb*A-*trn*H regions was used to try clarify phylogenetic relationships between cultivated artichoke, cultivated cardoon and their wild progenitors and allies. A number of different samples belonging to the three varieties of *C. cardunculus* were used in the analysis in order to better disclose possible phyletic trends. Other wild species, namely *C. syriaca*, *C. cornigera*, *C. humilis*, and *C. baetica*, were also used to unravel the relationships among wild taxa and those between wild species and *C. cardunculus*.

## Materials and methods

### Plant material

Plant material was obtained from the artichoke and cardoon living collection held at the Institute of Plant Genetics (IGV), Bari, Italy; from field collections carried out by IGV and by the Botanic Institute of Barcelona, Spain; from Dr. Macua, Spain (Spanish cultivated cardoons), and from Dr. Lev-Yadun, Israel (*C. syriaca*). Five artichoke samples were chosen in order to be representative of the different morpho-productive varietal groups ('Spinosi', 'Violetti', 'Catanesi' and 'Romaneschi'; Bianco 1990); four samples of cultivated cardoons were representative of major productive areas of this crop (Italy and Spain); six samples of wild cardoons were representative of its geographical range.

**Table 1** List of the material analysed

| Sample | Cultivated variety[a] | Abbreviation | Origin |
|---|---|---|---|
| *C. cardunculus* var. *scolymus* | CAT Catanese | Scol Catanese | Italy |
| *C. cardunculus* var. *scolymus* | CAT Locale di Mola | Scol Locale Mola | Italy |
| *C. cardunculus* var. *scolymus* | ROM Romanesco | Scol Romanesco | Italy |
| *C. cardunculus* var. *scolymus* | VIO Violetto di Toscana | Scol Violetto Toscana | Italy |
| *C. cardunculus* var. *scolymus* | SPI Spinoso Sardo | Scol Spinoso Sardo | Italy |
| *C. cardunculus* var. *altilis* | Bianco Avorio | Alt Bianco Avorio | Italy |
| *C. cardunculus* var. *altilis* | Gigante di Romagna | Alt Gigante Romagna | Italy |
| *C. cardunculus* var. *altilis* | Verde de Peralta | Alt Verde Peralta | Spain |
| *C. cardunculus* var. *altilis* | Blanco de Peralta | Alt Blanco Peralta | Spain |
| *C. cardunculus* var. *sylvestris* | | Sylv Italy | Italy |
| *C. cardunculus* var. *sylvestris* | | Sylv Sicily | Sicily, Italy |
| *C. cardunculus* var. *sylvestris* | | Sylv Spain1 | Spain |
| *C. cardunculus* var. *sylvestris* | | Sylv Spain2 | Spain |
| *C. cardunculus* var. *sylvestris* | | Sylv Spain3 | Spain |
| *C. cardunculus* var. *sylvestris* | | Sylv Greece | Corfu, Greece |
| *C. syriaca* | | *C. syriaca*1 | Israel |
| *C. syriaca* | | *C. syriaca*2 | Israel |
| *C. baetica* | | *C. baetica*1 | Spain |
| *C. baetica* | | *C. baetica*2 | Spain |
| *C. cornigera* | | *C. cornigera*1 | Egypt |
| *C. cornigera* | | *C. cornigera*2 | Egypt |
| *C. humilis* | | *C. humilis*1 | Spain |
| *C. humilis* | | *C. humilis*2 | Portugal |
| *Silybum marianum* | | *Silybum marianum* | Italy |

Abbreviation column refers to the names used in the trees

[a] CAT: Catanesi type; ROM: Romaneschi type; VIO: Violetti type; SPI: Spinosi type

Two samples per each wild relative available from different geographical regions, and one sample of *Silybum marianum* L. were also used (Table 1).

## DNA isolation, PCR amplification, and sequencing

Genomic DNA, from the material listed in Table 1, was extracted from young leaves according to Sonnante et al. (2002).

As for the ITS spacers, amplifications were performed using the primer forward 18Sdir: CGTAACAAGGTTTCCGTAGG and the reverse 25Scom: AGCGGGTAGTCCCGCCTGA which allow to amplify the region comprised between the 3′ terminus of the 18S rDNA and the 5′ terminus of 25S rDNA, thus including the ITS1 and ITS2 spacers (Sonnante et al. 2003). A total volume of 50 µl for each reaction contained 50 ng DNA, 50 mM KCl, 10 mM Tris–HCl pH 9.0, 1.5 mM $MgCl_2$, 0.1 mM each nucleotide (dATP, dCTP, dGTP, dTTP), 2.5 units of *Taq* DNA polymerase and 0.2 µM each primer. The amplification program included a cycle at 94°C for 3 min, 35 cycles at: 94°C for 30 s, 52°C for 30 s, 72°C for 45 s and a final elongation at 72°C for 7 min.

For ETS amplification, the primer forward ETS-Car-1: TTCGTATGCTTCGGT (Kelch and Baldwin 2003) was initially used, together with the primer reverse 18S-ETS: ACTTACACATGCATGGCTTAA (Baldwin and Markos 1998). Primer ETS-Car-1 had been designed by sequencing upstream from the 18S gene into the IGS of *Carduus nutans* and *Cirsium andrewsii* and locating a region of conserved sequence in the two taxa. Direct sequencing from this amplicon was not reliable enough, therefore, the PCR product was cloned and sequenced, allowing the design of a more internal and specific primer for the *Cynara* taxa analysed in the present study. This primer, named CynETSFor (GCTGCATGCCTCTTCAGTT), was subsequently used for all analyses. ETS amplification protocol was similar to that described for ITS region, except for the annealing temperature which was 57°C.

The chloroplast intergenic spacer *psb*A-*trn*H was amplified using the primers *psb*A-F: CGAAGCTCCATCTACAAATGG and *trn*H-R: GCCAAGTGGATCAAGGCAAGT designed on the basis of corresponding sequences of other species (Hamilton 1999). The amplification protocol used was the same as for ITS.

PCR products from all reactions were run on an agarose gel; all taxa showed a single band per amplified region. Amplificates were purified using 'Qiaquick PCR purification kit' (Qiagen, Milan, Italy) and directly sequenced using a Beckman CEQ 8800 sequencer (Beckman Coulter, Milan, Italy). Three different amplificates per sample were sequenced in order to prove the reliability of the method. Sequences were deposited in the EMBL database (accession numbers AJ404744, AJ831529 through AJ831538, AM269938 through AM269946, AM267301 through AM267320).

## Data analysis

Sequences of the same domain were aligned using the program CLUSTAL W (http://www.ebi.ac.uk/clustalw/).

For phylogenetic reconstruction, the aligned sequence data matrix was analysed using PAUP* (4.0b10, Swofford 2000). Characters were equally weighted and their states were unordered. Parsimony analyses were carried out with gaps treated as fifth state, and heuristic tree search options included random sequence addition, TBR branch swapping, MULPARS on, steepest descent off and 1,000 bootstrap replicates. Maximum likelihood analyses were conducted using the HKY'85 model, with rate heterogeneity (G50.5) and a transition/transversion ratio of 2, heuristic tree search, as-is sequence addition, TBR tree swapping, MULPARS on, steepest descent off and 1000 bootstrap replicates. Sequences were also analysed with MEGA 3.1 package (Kumar et al. 2004) using CNI level = 1 with initial tree by random addition and bootstrap test with 100 repetitions.

The Incongruence Length Difference (ILD) test was carried out to determine whether the ETS and ITS data sets were incongruent (Farris et al. 1994), and was implemented using the partition homogeneity option in PAUP*. Search options were as described in bootstrap analyses.

All analyses were carried out on ITS and ETS regions separately and on the combined ITS +

ETS sequences. The combined sequences were also used to perform minimum evolution analysis with MEGA using search options set at close-neighbour-interchange level = 2 and maxtree option set to 1, enforcing the molecular clock divergence time option (Nei and Kumar 2000).

The chloroplast sequences were not subjected to phylogenetic analysis (see results section).

## Results

### ITS

The analyses were conducted on 15 samples of *C. cardunculus* representative of the three varieties *scolymus*, *altilis* and *sylvestris*, plus eight samples representative of the four wild species used in the present study: *C. syriaca*, *C. baetica*, *C. cornigera* and *C. humilis*. *Silybum marianum* was chosen as an outgroup.

Usually very clear and unambiguous sequences were obtained, but, in some cases, double peaks were observed in some positions. The positions including more than one nucleotide were given as degenerated using the appropriate degeneration code.

The amplified region included 719 bp in most samples analysed, of which 41 bp belonged to 18S gene, 258 bp to ITS1 spacer, 165 bp to 5.8S gene, 217 bp to ITS2 spacer, and 38 bp to 25S gene. However, length variations in the two spacers were observed: in particular, ITS1 was 257 bp in all the cultivated artichokes, in *C. syriaca*, and in *C. cornigera* for a single base deletion at a different position for each taxon, and 259 bp in the outgroup which contained an additional nucleotide also in ITS2. All *Cynara* species showed a higher G+C content in both ITS1 and ITS2 (average 63.0% and 63.4% respectively) as compared to 5.8S region (53.8%) which was taken as a reference, since it was the only complete ribosomal gene sequence analysed.

In *Cynara* the ITS1 region contained 20 variable sites, all of them parsimony informative; ITS2 showed 21 variable, all parsimony informative, sites. If the outgroup sequence was considered, variable sites were 45 and 40 for ITS1 and ITS2 respectively.

As regards *C. cardunculus*, no variation within artichoke varieties was observed. When comparing artichoke sequences to those of wild and cultivated cardoon, four and two base substitutions were recorded in ITS1 and ITS2 respectively, even though, in some samples a nucleotide polymorphism was observed, thus reported as degenerated in the analysis. As expected, a higher number of differences was observed between *C. cardunculus* and the other wild *Cynara* species.

It is worth noticing that in *C. humilis*, differences were observed in the 5.8S gene, which displayed three autapomorphic base substitutions, while all the remaining *Cynara* species had a conserved sequence.

### ETS

A similar procedure as for ITS analysis was used for the ETS region to assign degenerate nucleotides at positions where polymorphisms were observed. The partial ETS region sequenced included 64 bp of the 18S gene and in total was 426 bp in most species, while it was 428 bp in *C. cornigera* for a two base insertion, and 425 bp in *C. humilis* for a single base deletion; the outgroup sequence was 427 bp. Differently from what observed for the internal spacers, the partial ETS sequence had a lower G+C content, equal to 50.7%. In *Cynara* species, the partial ETS spacer revealed 38 variable sites, 36 of which parsimony informative, and 2 singletons. If the outgroup sequence was considered, variable sites were 66.

### Chloroplast *psb*A-*trn*H spacer

The sequence of the amplified product was 444 bp, of which 25 bp belonged to *psb*A gene, 388 bp to the internal spacer, and 31 bp to *trn*H gene. The spacer had an average G+C content of 26%. This sequence was highly conserved and did not show any variation among all the species analysed.

### Phylogenetic analyses

Phylogenetic analyses were initially carried out for ITS and ETS separately. The ITS parsimony consensus tree (Fig. 1) grouped *C. cardunculus*

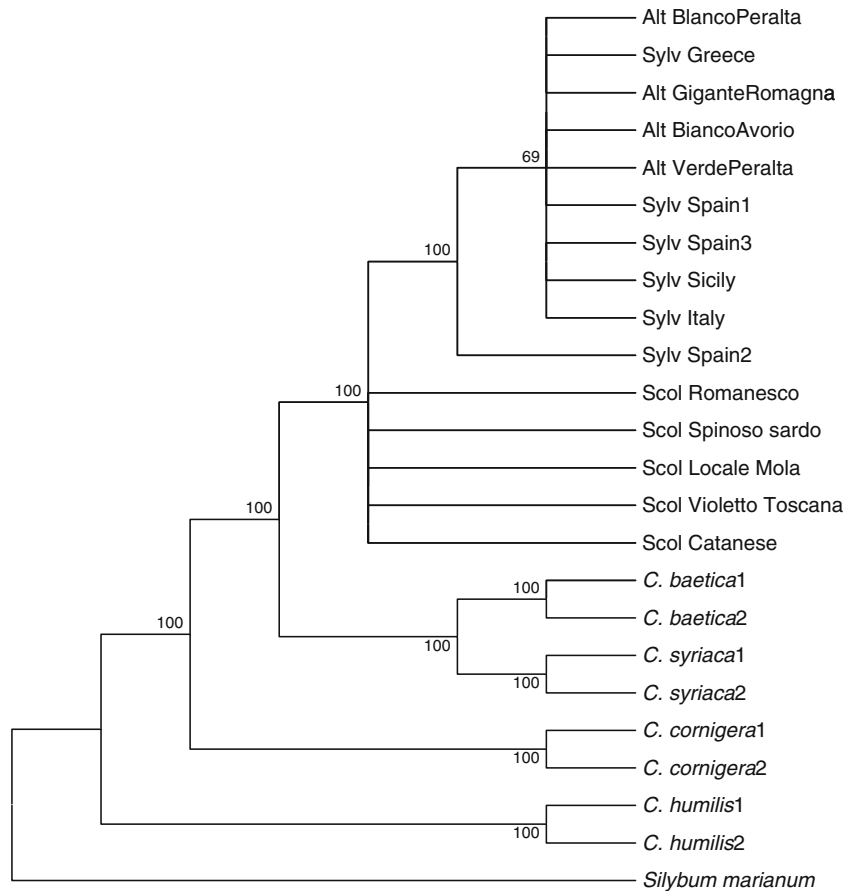


**Fig. 1** ITS parsimony consensus tree

separated from the other species, and all the wild cardoons together with the cultivated cardoons in a nested branch. The artichoke varieties were grouped together. The branch closer to the *C. cardunculus* species complex was that including *C. syriaca* and *C. baetica*; *C. cornigera* and *C. humilis* occupied separate branches. Parsimony analysis identified 100 unfiltered best trees with a tree length of 102 steps; consistency index was CI = 0.9314 and retention index was RI = 0.9255.

A slightly different picture appeared when analysing the parsimony consensus tree derived from ETS data (Fig. 2a). In this case, the wild cardoons from Italy, Sicily, and Greece, formed a nested cluster in the *C. cardunculus* cluster. The relationships with the other wild species are not well resolved. Parsimony analysis identified 250 unfiltered best trees with a tree length of 66 steps, CI = 0.9848 and RI = 0.9733. A better resolution is obtained after Maximum likelihood analysis which delivered a tree, with a sum of branch lengths of SBL = 0.1666, in which *C. baetica* and *C. syriaca* form a cluster basal to the *C. cardunculus* one and *C. humilis* and *C. cornigera* are separated (Fig 2b).

The ILD test resulted in a probability of 0.75, indicating that the ITS and ETS data sets were not significantly incongruent, and could be merged in the analysis. When the combined ITS and ETS sequences were used for the analysis, a better picture of the phylogenetic relationships was disclosed. Parsimony analysis produced a consensus tree (Fig. 3a) 156 steps long, with CI = 0.9423 and RI = 0.9497; unfiltered best trees were 218. In this tree the *C. cardunculus* species complex is composed of two groups, one including the samples belonging to var. *scolymus*, the other including the vars. *sylvestris* and *altilis*; in this latter group the *sylvestris* samples from Italy, Sicily, and Greece form a distinct nested cluster, while the wild cardoon samples from Spain were included within the group of cultivated cardoons (var. *altilis*).

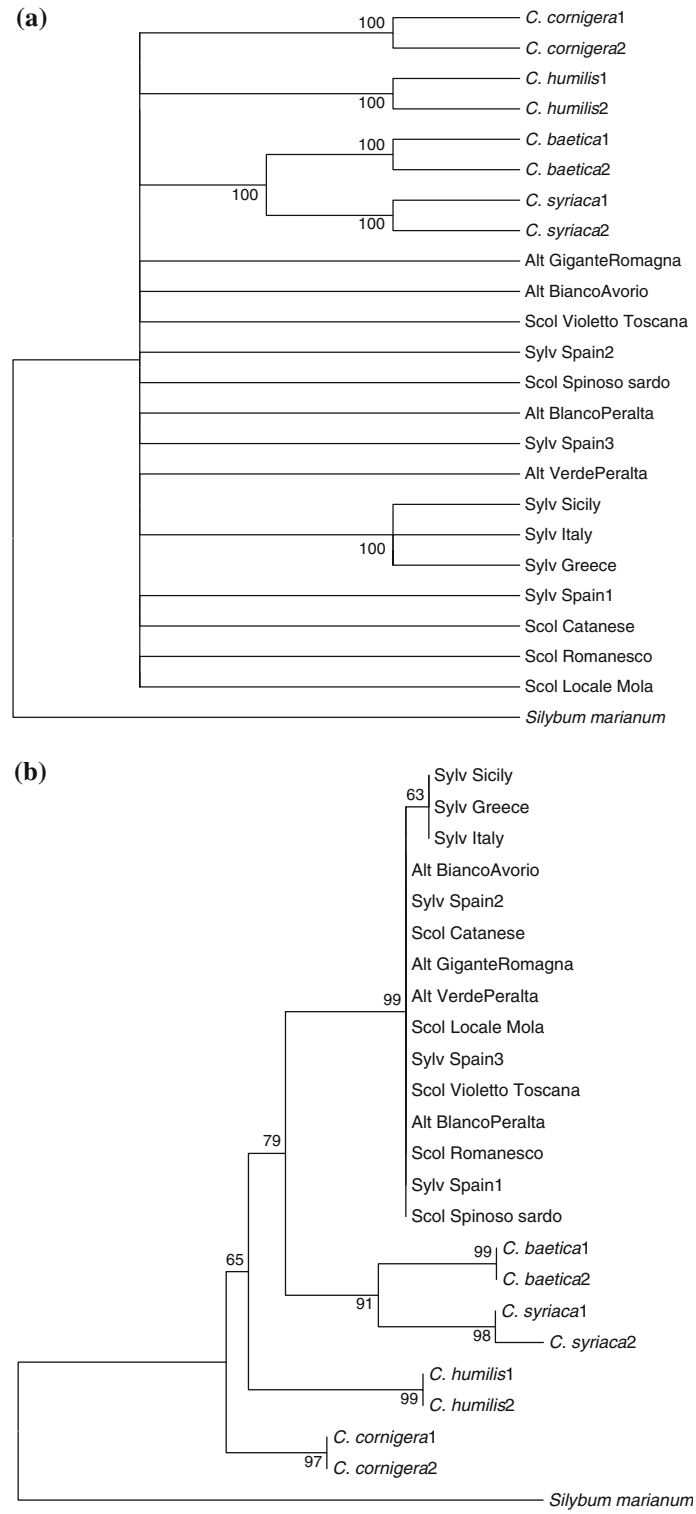


**Fig. 2** (**a**) ETS trees: parsimony consensus. (**b**) ETS trees: maximum likelihood

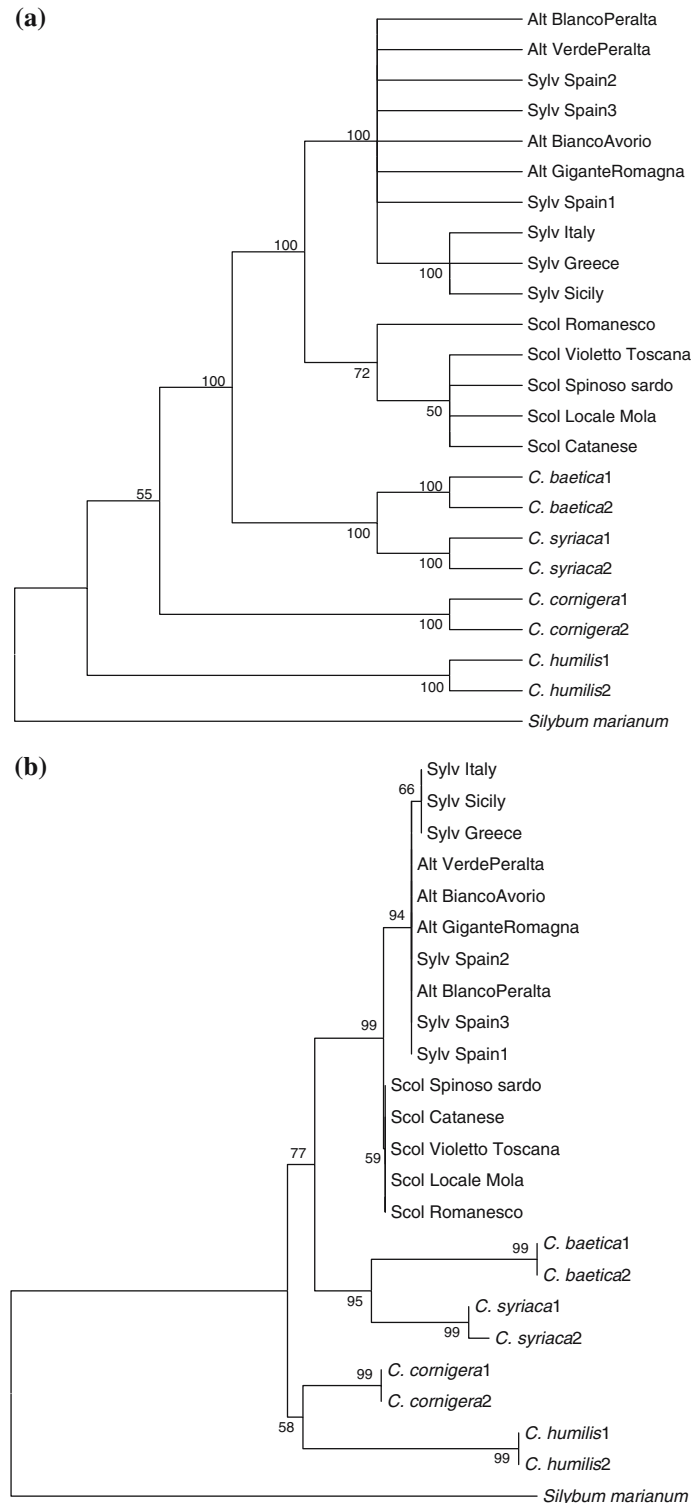


**Fig. 3** (**a**) ITS + ETS trees: parsimony consensus. (**b**) ITS + ETS trees: maximum likelihood

*Cynara syriaca* and *C. baetica* were clustered together and were closer to the cultigen group, whereas *C. cornigera* and *C. humilis* showed a greater differentiation. The Maximum likelihood tree (Fig 3b), with SBL = 0.1439, displayed a slightly different pattern, in which *C. cornigera* and *C. humilis* formed a more basal separated cluster. When implementing the molecular clock divergence time option, the separation of cultivated artichoke was set at 2,000 years before present (Foury 1989), resulting in comparative times for divergence of the other species (see discussion).

## Discussion

### On the origin of artichoke and cardoon

Within *C. cardunculus*, the combined ITS + ETS sequence was able to better describe the phyletic relationships among taxa respect to ITS and ETS separately. This enhancement of the resolution of phyletic relationships is congruent with previous reports (Markos and Baldwin 2002) that attribute ETS region a differential evolutionary behaviour as compared to ITS spacers.

*Cynara cardunculus* is subdivided into three main sub-groups: the most basal one is constituted by the cultivated artichokes, then from a collapsed branch, including all the cultivated cardoons, a sub-group is formed embracing var. *sylvestris* samples, except for those from Spain, which cluster with the leafy cardoon samples. This might imply three possibilities: (a) Spanish wild cardoons may belong to a genepool different from the eastern Mediterranean one, according to the Wiklund (1992) hypothesis, or (b) the gene flow between cultivated cardoon and Spanish wild *C. cardunculus* has continued after domestication, or (c) the samples collected in Spain might have naturalized after escaping from cultivation. Wiklund (1992) recognised two subspecies within wild *C. cardunculus*, i.e. ssp. *cardunculus*, with eastern Mediterranean distribution, and ssp. *flavescens*, distributed in the western range; the two subspecies being distinguished by some morphological characters. Our Spanish wild material is indeed quite different from eastern wild cardoons, mostly for the presence of a smaller number of spines and a more gentle growth habit; they resemble cultivated cardoons with spiny leaves, also in the head shape. Moreover, in the same report, Wiklund (1992) hypothesizes that both cultigens derived from the ssp. *flavescens*, while our observations based on the evolution of rDNA regions do not support her hypothesis. It is reported that in California and Australia, seeds escaped from artichoke fields have formed noxious weedy and invasive populations of ''wild'' cardoon (Robbins et al. 1970), attesting the high capacity of this species to naturalize in environments noncomprehended in its natural distribution range. Present data on the Spanish wild cardoons are not sufficient to propend for one hypothesis or the other and further evidence might derive from the use of additional markers.

The observation that the cultivated artichokes occupy a more basal position in the trees than the rest of the *C. cardunculus* gene pool might be explained by the disturbance to the reproductive cohesivity introduced by the practice of vegetative propagation (Birky 1996). In this view, the clonal reproduction might have fixed in the artichoke a set of characters and a level of heterozygosity which have undergone a strong selective pressure operated by man, the phenomenon known under the name of domestication syndrome (Hammer 1984): genetic isolation and human selection have enhanced the divergence from the rest of the gene pool. It has been suggested that the cultivation of artichoke started around the beginning of the Christian era, that is some 2 millennia before present (Foury 1989). This gives a date to the divergence time of this crop and allows to compare the divergence time of the other taxa. Applying a molecular clock model, the divergence of cultivated cardoon dates at the beginning of the second millennium AD; a more precise indication cannot be worked out due to the limited number of phylogeny informative characters and to the disturbance introduced by vegetative reproduction (Birky 1996). Sonnante et al. (2002, 2004) using other molecular markers, such as RAPDs and AFLPs, indicated that wild cardoon germplasm appears genetically closer to leafy cardoon than to artichoke. The present results are in agreement with those previous observations. The similarity of wild cardoon to

cultivated leafy cardoon is also indicated by a number of nucleotide substitutions that are synapomorphic to these two taxa, especially in the ITS regions.

All these results suggest that a second turn of domestication took place in the northern/western range of the Mediterranean, leading to a seed propagated crop, mostly utilised for its leaves (cultivated cardoon). The localization of this second domestication event might also explain the larger diffusion of this crop in northern Italy, northern Spain, and southern France (Dellacecca 1990). Moreover the lower number of varieties present in cultivated cardoon (Dellacecca 1990) as compared to artichoke (Bianco 1990) is a further indication of a more recent domestication.

Recently, it has been hypothesized that artichokes were domesticated in Sicily (Pignone and Sonnante 2004). This issue is quite controversial since the historical data, due to linguistic uncertainty, are not a sure font of information. As an example, the ancient Greek word *scolymos* relates to 'spiny' and the use of this word in ancient scripts might not refer unequivocally to *C. cardunculus* but also to other thistles. This may ingenerate errors while reading chapters of Pliny the Elder (23–79 A.D., in *Naturalis historia*) which are interpreted to report on cultivated artichoke in south Italy and south Spain. According to De Candolle (1890), it appears that artichoke was unknown to the Egyptians and to the Hebrews; Theophrastus (371–287 B.C.) wrote that this plant was cultivated in Sicily but not in Greece (Montelucci 1962). In the Middle Age artichokes are often associated with the Arabs, and as a matter of fact, the nowadays names for this plant largely derive from the Arabic '*al kharshuff*'. The Arabs dominated the Mediterranean from the 6th to the 14th century, and, in particular, Sicily for more than two centuries starting 827 AD; they surely helped the diffusion of artichoke cultivation. The first certain records of artichoke commerce relate to Filippo Strozzi trading artichokes from Sicily to Florence in the 15th century (Bianco 1990), or to the presence of artichokes in paintings by Vincenzo Campi (1536–1591; *L'ortolana*, ca. 1580) or Giuseppe Arcimboldo (1527–1593; *L'estate*, 1563; *Vertumnus*, 1590).

Also cardoons can be seen in painting at the beginning of the 17th century. Representations are present in Caravaggio's *Still Life of Flowers, Fruits, and Vegetables* (uncertain dating, after 1600) or in Juan Sánchez Cotán's still life paintings (ca. 1602). The fact that cardoons appear in Spanish and Italian paintings at the same time might relate to the Spanish domination in Italy ($16^{th}$–18th centuries).

## Some hypotheses on evolutionary patterns in *Cynara*

The evolution of the genus *Cynara* is still unclear, both with respect to other genera of the tribe Cardueae and with respect to the phyletic relationships of the species that constitute this genus. Several authors have tried to untie this matter with unlike results (Franco 1976; Wiklund 1992; Bremer 1994; Susanna et al. 1995; Häffner 2000; Garcia-Jacas et al. 2002; Robba et al. 2005). One possible reason is uncertainty in the taxonomical delimitation of the species, some of which are not completely accepted. Another possible reason is the relatively little amount of collections available to scientists, so that genetic studies based on living material are normally limited to few samples with little or no recognition of intraspecific variation.

In the present study, the chloroplast *trn*H-*psb*A spacer did not vary among the *Cynara* species, possibly as a combined consequence of low evolutionary rate even for a noncoding region of cpDNA (Small et al. 2004) and little time of divergence. When using ITS or ETS sequences separately as evolutionary markers, the general perception of phyletic relationships within *Cynara* is similar. The main differences regard the relative distance of *C. baetica* and *C. syriaca* from the cultigen and the distinction of the *C. cardunculus* intraspecific structure. The combined use of ITS and ETS sequences allowed a better discrimination. In the trees of Fig. 3, the species of *Cynara* appear to form two clusters, one including *C. cornigera* and *C. humilis*, the other the remaining species analysed. This pattern is consistent with the one drawn by Wiklund (1992) based on morphological characters. Conversely, the here outlined grouping is only partially

consistent with the tree presented by Robba et al. (2005), based on ITS molecular markers. In fact, one of the differences with their tree, is that their samples of *C. baetica* fall into two different clusters: one sample is grouped with *C. syriaca*, similarly to what we observe, and the other three cluster with *C. algarbiensis* and *C. tournefortii*, species that we have not analysed since no seeds were available to us. This incongruence is already discussed in the Robba et al (2005) paper.

For this reason, we decided to analyse our ITS sequences together with the other *Cynara* sequences present in the EMBL database, and relative to the study by Robba et al. (2005). The results (data not shown) indicate that our sequences are highly homologous to those present in the database, with the only exception of the three samples of *C. baetica* mentioned above. These latter samples cluster in a position that is inconsistent with the present understanding of evolutionary relationships in *Cynara*. Robba et al. (2005) attempted some explanations in order to justify this incongruence and the fact that three samples of *C. baetica* share an identical ITS sequence with *C. algarbiensis*. A possible explanation, not considered by Robba et al. is that they have compared nonhomologous sequences, but paralogous ones.

In our study, the combined use of ITS and ETS in phylogenetic analysis increases the resolution, possibly as a consequence of different evolutionary rates of the two regions (Baldwin and Markos 1998; Markos and Baldwin 2002). It is interesting to notice that, when a molecular clock divergence time analysis is applied, hypothesizing that the origin of artichoke dates around 2 millennia ago (Foury 1989; Pignone and Sonnante 2004), the branch including the species *C. cardunculus*, *C. baetica*, and *C. syriaca* appears to have originated some 16 millennia ago, while the cluster including *C. cornigera* and *C. humilis*, originated less than 20,000 years before present (ybp), around the time of the Würm glaciation (ca. 20,000–10,000 ybp; Cheddadi et al. 1998; Davis et al. 2003). It can be hypothesized that the common progenitor of *Cynara*, possibly originated in Northern Africa, was forced by the changing climatic conditions to move to different environments, thus starting the differentiation process. Subsequently, during the Holocene Climate Optimum (a warm period roughly in the interval 9,000 to 5,000 ybp; Cheddadi et al. 1997; Davis et al. 2003), the species spread to a wider environment, thus differentiating more consistently. The nowadays distribution of present species is consistent with this hypothesis. In fact, *C. baetica*, and *C. syriaca* occupy limited territories at, respectively, extreme western and eastern Mediterranean region; *C. humilis* and *C. cornigera* display relatively wider ranges again in the western and eastern Mediterranean area respectively; *C. cardunculus* occupies almost the whole basin of this sea (Wiklund 1992). This distribution possibly relates to differential thermophily of the different species. This view is further corroborated by the observation that *C. cardunculus* is a highly invasive species, able to adapt to a wide range of environments, as its widest geographic distribution among the species of *Cynara* and its rapid naturalization in other continents testify. Man has taken advantage of this plasticity to produce two crop species. It is even possible to hypothesize that domestication has been attempted also for other species (e.g. *C. humilis* or *C. cornigera*, still used as a food from the wild in north Africa or in Greece, respectively) with lesser success.

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