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Phylogenetic study some of *Crataegus L.* (Rosaceae, Pyreae) Species in Iran

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Abstract

Crataegus belongs to Pyreae tribe and Rosaceae family. *Crataegus,* has about 700 species, distributed mainly in temperate regions of northern Hemisphere. Diagnoses of these species rely on morphological features of leaves, flowers and fruits. We used nuclear (ribosomal ITS) DNA regions to estimate the phylogeny of *Crataegus* species of Iran. Maximum parsimony, maximum likelihood, and Bayesian analyses all corroborate the sister group relationship between *Crataegus* and *Mespilus*, and *Crataegus brachyacantha* is sister to the rest of that genus species. According to results, *Mespilus* is considered as sister group to the monophyletic *Crataegus* species. Trees are divided into two main brands which show separation of old world from new world species. Accordingly, we (1) suggest the separation of *Pentagynae* section from *Crataegus* section (2)recommend the presence of a new subspecies of *C. pentagyna* from Iran ; (3)relate that *C. persica* is the synonym of *C. meyeri* and also *C. aminii* is the synonym of C. *atrosanguinea* (4)report the appearance of *C. rhipidophylla* in North-West of Iran and (5)suggestion of change ranking of *C. zarreii* as variety of *C. azarolus*.

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Introduction

Crataegus is distributed in temperate regions of Iran (North, West, and Northwest) with 27 species in Iran and is one of known genus of Rosaceae family. *Crataegus* L. and *Mespilus* L. were previously in the same genus but based on leaf form, sect leaf, seed coating and so …….were separated and now *Mespilus* is considered as a sister group of *Crataegus*. Species of this genus have hard wood whose name due to this characteristic originated from greek term, Kratos, namely hard and rigid. This genus possesses three series in Iran, including ser. *Crataegus,* ser. *Orientales,* ser. *Pentagyna* and most of species belong to ser. *Crataegus*.(Christesen 1992). In flora of Iran this genus was divided into four parts comprising sect. *Pentagyna,* sect. *Azaroli,* sect. *Sanguinea* and sect. *Oxycantha* and in this classification most of Iranian species fit in sect. *Oxycantha*. In this flora, new division, sect. *Sanguinea,* was defined for Iran. (Khatamsaz 1992) A new taxonomic study was carried out on species of this genus. Of these studies, Donmez conducted researches on taxonomic characters of this genus and cited that Phenology, morphological characters, polymorphism and hybridization are major cause for abundance of synonymous species of *Crataegus*. He introduced phenology, fruit color, fruit flesh, number of pyrene and chromosome number as important features for detection of Crataegus species. According to Donmez, *C. azarolus* possess two varieties, namely var. *aronia* and var.*pontica* but to Browicz, these two varieties are distinct species. Also, *C.monogyna C. rhipidophylla,C. curvisepala* form one collection of species and *C. curvisepala* is *C. rhipidophylla* synonym. Donmez (2009) reported new species from West of Iran under the name of *C. zerrei* Donmez, this species is much like *C. azarolus* but he believes that in terms of toothed leaves, number of dents in each leaf lobe and small fruit with dark orange color is different from *C. azarolus*. Khatamsaz introduced and expounded three new species from Iran including C. babakhanloui. , C. assadii and C. aminii In this study, *C. atrosanguinea* is close relative of *C.aminii*. Regarding *C. babakhanloui* is also the closest species to it and belongs to Sanguinea section. Christensen (2008) mentioned *C. assadii*, considered as a species by Khatamsaz, is a variety of *C. azarolus* and introduced it as *C. azarolus* var. *assadii*. In *Crataegus* monograph Pentagyna Ser. Includes *C. pentagyna* with two subspecies; *C. pentagyna* subsp. *pseudomelanocarpa* and *pentagyna* subsp. *pentagyna* but in flora of Iran these two were presented as two discrete species; *C. pentagyna* from Gilan province and *C. pseudomelanocarpa* from Mazandaran (both of localities are in Hyrcanian zone). In flora of Iranica also *C. pseudomelanocarpa* was introduced distinct from *C. pentagyna*In the other word. *C. sanguine*, in flora of Iranica and *Crataegus* monograph was not designated for Iran but in flora of Iran the species was introduced as a species from north of Iran. According to Russia flora, this species and this section do not exist in Iran as well. And so *C. assadii*, *C. aronia* and *C. pontica* are independent species in Azaroli section in flora of Iran. In *Crataegus* monograph, these species are in Orientales Ser. and all as varieties of *C. azarolus* including *C. azarolus* var. *assadii, C. azarolus* var. *pontica* and *C. azarolus* var. *aronia*. Donmez (2004) mentioned that relationship between *C. pontica* and *C. azarolus* remains to be studied phylogenetically.

In Flora of Iran *C. atrosanguinea* from north and center and *C. aminni* from center of Iran are reported as separate species. In *Crataegus* monograph, *C. atrosanguinea* is synonymous with *C. ambigua* subsp. *ambigua*. On the other hand, in Flora of Iran and Flora Iranica *C. ambigua* is synonymous with *C. meyeri*. In *Crataegus* monograph, *C. meyeri* was introduced as a distinct species and so Khatamsaz in flora of Iran introduced *C. persica* from west of Iran, were presented as synonymous to *C. meyeri*. Khatamsaz believes that *C. atrosanguinea* is much similar to *C. aminii* except for fruit color,inflorescence, number of pyrene and hair of branch. *C. curvicepala* in flora of Iran was reported from west and northwest of Iran, however, in flora Iranica and monograph not cited for Iran.

Besides, in *Crataegus* monograph, Christensen does not consider *C. curvisepala* as a distinct species and deemed two subspecies of this species, *C. curvisepala* subsp. *curvisepala* Petaeur, *curvisepala* subsp. *carstica* Herabetova-Uhrova synonymous with *C. rhipidophylla* var. *rhipidophylla* and *C. curvisepala* subsp. *Colorata* Hraeetova with *C. monogyna* var. *monogyna* and *C. curvisepala* with *C. rhipidophylla* too and not necessary to apply this name, *C. curvicepala,* due to being older than *C. rhipidophylla*.

In flora of Iran, *C. monogyna* is not considered as a discrete species and solely two varieties, *C. monogyna* Jacq. Var. and monogyna Jacq. Var. *dolicocarpa* Somm are synonymous to *C. microphylla* whilst. In *Crataegus* monograph, *C. monogyna* with two varities *C. monogyna* var.*monogyna* and *C. monogyna* var. *lasiocarpa* is distinct from *C. microphylla* from north, North West and west of Iran. In flora Iranica, *C. microphylla* involves two varieties *C. microphylla* var. *dolicocarpa* and *C. microphylla* var. *microphylla*. In recent research, Arjomandi and his colleagues found that *C. monogyna* var. *lasiocarpa* from east north of Iran in contrast to flora of Iran and flora Iranica which believe that it is independent from *C. microphylla*.(Arjomand,2009) Donmez took into consideration that *C. microphylla* different from C.micro? Due to having certain morphological features such as erect sepal on the fruit and small leaves and he believes *C. curvispala* is synonymous with *C. rhipidophylla*. Furthermore, *C. rhipidophylla* and *C. monogyna* are very close to each other but it is considered to be separate from one another.

Moreover, *C. monogyna* and *C. microphylla* are diploid but *C. rhipidophylla* is tetraploid with apomixis in it. On the other hand *Crataegus* and *Mespilus* are distinguished from the *Amelanchier* group and most other Pyreae by (1) lateral short shoots modified as thorns; (2) collateral ovules that become superposed by the time of anthesis so that typically only the lower one is fertilized, (3) abundant endosperm in the mature seed (Aldasoro *et al.* 2005), and (4) a polypyrenous drupe (rather than a berry or "pome") that develops from the hypanthial ovary (Lo *et al.*, 2007).In this paper We use nuclear ribosomal internal transcribed spacers (ITS) that is part of a larger project on *Crataegus* systematics and evolution that has the following objectives: (1)to revision the classification of Iranian *Crataegus* species; (2) to evaluate the support for *Crataegus* species as monophyletic genera, (3) to discover the intragenetic taxonomic structure within Crataegus and find out to what extent the existing subgeneric classification represents distint clades.

Materials and methods

Taxon sampling

Plant material was collected in the field in spring for flowers, summer and at the beginning of autumn for fruits. Voucher specimens are deposited in the Islamic Azad University North Tehran Branch (AUNT) unless noted otherwise in Appendix 1. A total of 43 *Crataegus* and two *Mespilus* species were included, with in most cases a minimum of two individuals representing each species. In 13 cases only a single individual was available to represent a section or series (Appendix 1). In some other cases where more than one species was available to represent a section or series, some species were represented by a single individual (Appendix 1). Species of *Amelanchier, Malus* and *Aronia* were used as outgroups because they have been shown to be divergent to varying degrees from *Crataegus* and *Mespilus* (Campbell *et al.*, 2007).

DNA Extraction, PCR, and sequencing

Total genomic DNA was extracted from leaves that were dried in small packets and stored at room temperature. Dried leaves were extracted using the method of Tsumura *et al.* (1995) modified to a small scale. The nuclear ribosomal region encompassing ITS-1, 5.8S rRNA and ITS2 spacer was amplified using primers 18s and 28s (Muir & Schlotterer, 1999)(Table 1.).

Table 1. Primer sequences of nrDNA regions for PCR

Primers name	Designer	Primer sequences
18S	Schlotterer *et al.*, 1994	5'-CCTTMTCATYTAGAGGAAGGAG-3'
28S	Schlotterer *et al.*, 1994	5'-CCGCTTATTKATATGCTTAAA-3'

Each 25 µl PCR reaction contained 1 µl each of 5' and 3' primer, 1 µl dNTP, 0.5 µl Taq DNA polymerase (Fermentas), and 2.5 µl 10×PCR buffer. DMSO was added to a final 10% in ITS amplifications to increase the specificity of the PCR fragments and the intensity of the sequence peak profiles. All amplifications were carried out using a Thermocycler (Eppendorf, Authorized Thermal Cycler, and Germany). PCR cycles involved an initial denaturing step at 94°C for 3 min, then 35 cycles of 94°C for 45 s, 56°C for 1 min, and 72°C for2 min. An additional extension was performed at 72°C for 5 min, then cooled to 4°C. PCR products were checked on 1% agarose gels. Purification and sequencing of PCR products were performed in South Korea.

Sequence editing, alignment, and phylogenetic analyses

Sequence edditing were first performed using the Sequencher ver. 4.1.4 program and then alignment in Mesquite ver. 2.73. Gaps within the sequence data were treated as missing. Phylogenetic analyses were conducted using PAUP*4.0b (Swofford 2002) for maximum parsimony (MP) and maximum likelihood (ML), and Mr. Bayes version 3.0b4 (Huelsenbeck & Ronquist 2001) for Bayesian inference (BI). Nuclear data were analysed with the three methods. Heuristic parsimony searches were performed using equally weighted characters, tree-bisection-reconnection (TBR) branch swapping, random addition of sequence (1000 replicates), and with no limit to the number of trees saved. Character changes were interpreted with the ACCTRAN optimization. Branch support was assessed by bootstrap (BS) analyses (Felsenstein 1985) with full heuristic searches, 100 replicates using simple taxon addition and TBR swapping, MULTtrees option, and all trees saved. The substitution models for ML and Bayesian analyses were obtained using Modeltest (version 3.4, Posada & Crandall 1998) with both Hierarchical Likelihood Ratio Tests (hLRTs) and Akaike Information Criterion (AIC) methods. Maximum likelihood analysis of the combined nuclear data was conducted with Transitional (TVM+I+G) model (parameters: base frequencies A = 0.1626, C = 0.3554, G = 0.3138, T = 0.1682, proportion of invariable sites (I) 0.380, gamma 0.7160, Ti/Tv 1.3709, 6 rate parameters and molecular clock not enforced). Bayesian inference was initiated from a random starting tree and the program was set to run four Markov chain Monte Carlo (MCMC) iterations for 3,000,000 generations with trees sampling every 100th generation. The remaining trees were saved and imported into PAUP* for constructing the majority rule consensus trees. Posterior probability for each clad was obtained to evaluate branch support in the resulting trees.

Results

Sequences

The alignment sequences of *ITS* (nrDNA) in relation to 43 taxa were analyzed and it created a matrix with 645 nucleotides position. Of these, 467 had constant position. In point of view parsimony 84 bp were informative and 94bp were uninformative. Size variation was observed in nuclear regions (Table 2.)

Table 2. Comparison of sequence variation in *Crataegus*, *Mespilus*, and outgroups for nuclear regions (ITS). PI = parsimony informative; MPT = most parsimonious tree; C.I. = consistency index; R.I. = retention index.

	Nuclear sequence (ITS)
Number of sequences	156
Number of characters	645
GC content	66.3
Number of constant characters	467
Number of informative characters	84
Number of uninformative characters	94
C.I.	0.64
R.I.	0.727

Maximum parsimony analyses

Maximum Parsimony analysis is created a consensus tree with the informatic indices CI=0.64(Consistency Index) andRI=0.727(Retention index).

The cladogram showed *Amelanchier bartramiana, A. arborea* and *Malus angustifolia* as out group, including three species groups *Mespilus canecens, M. germanica* and *Crataegus brachycantha* as sister group to other *Crataegus sp.*

The remaining *Crataegus* taxa are divided into two large clades labeled as (I) and (II) with moderate bootstrap or Bayesian support (Fig. 1. and Fig.4.).

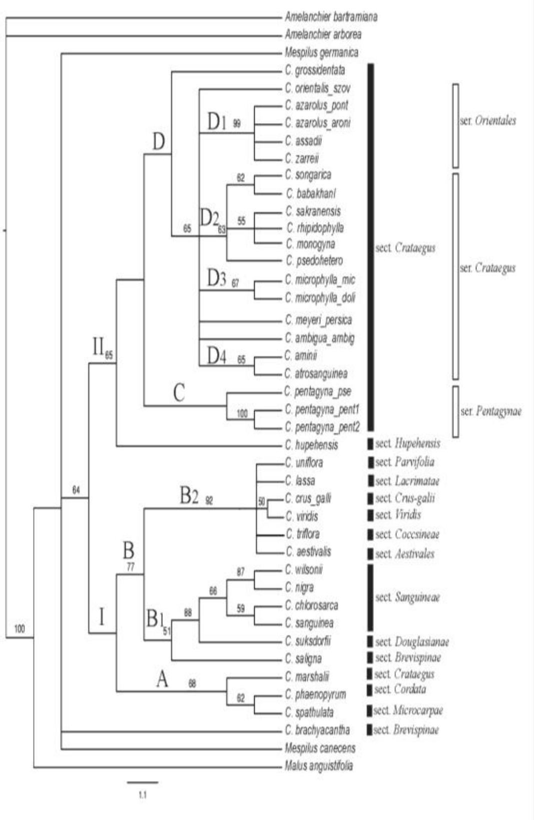

Fig.1. Strict consensus trees, from maximum parsimony (MP) analyses of ITS1-5.8SITS2. Nodes with bootstrap (BS; above branch) values >50% are indicated. Species, sections, and genera (Phipps and Robertson 1990) are listed on the right.

Large Clade (I) contains members of the new world and large clade (II) contains members of old world. Large clade (I) are divided in to two clades A and B. Clade A is a small group of three North American taxa: *C. marshallii* (sect. *Crataegus), C. phaenopyrum* (sect. *Cordatae*), and *C. spathulata* (sect. *Microcarpae*). Clade B is divided two sub clades. Sub clade B1 contains members of sections *Sanguineae* and *Douglasianae,* and *C. saligna* (sect. *Brevispinae*), and this whole group was sister to clade B2 which contains members of section *Coccineae, Crus-galli, Virides, Parvifolia*, *Lacrimatae* and *Aestivales* exclusively from eastern North America.

Other species are located in main clad II with 65% statistical support. This main clad includes old world species which belong to the section Crataegus. And C. *hupehensis* of China is as a sister group to the rest of the species clad (II).

Clad C includes Pentagynae Series means *C. pentagyna* subsp. *pentagyna*, *C. pentagyna* subsp. *pentagyna* (two accessions) and *C. penatgyna* subsp. *pseudomelanocarpa* seen the first two taxa with 100% statistical support are located in sub clad. Clad D includes *C. grossidentata* that is divided from other species, the rest of species are as polytomic state and monophyletic with different statistical support. D1 group is placed .after *C. orientalis* subsp. *szovitsii* with 99% statistical support and is monophyletic and includes *C. azarolus* var. *pontica*, C. *azarolus* var. *aronia*, *C. assadii* and *C. zarrei* , it seems this complex consist that are located in Oriental's series.

D2 groups also support 67% poor quality is one of a group that includes species *C. songarica, C. babakhanloui, C. sakranensis, C. rhipidophylla* var. *rhipidophylla C. monogyna* var. *monogyna* and *C. pseudoheterophylla* is that due to low clad support has certain situation is not much and it seems that contains of a complex of close species.

Taxa of the group D3 *C. microphylla,* var. *microphylla* and *C. microphylla* var. *dolicocarpa* that are supported by 67% in this group are located. Alongside the D3 group as C. meyeri is located.

According to the same ITS sequences of these species and C. persica, both have the same position. Then such *C. ambigua* subsp. *ambigua* is located. D4 group also contain two species *C. aminii* and *C. atrosanguinea*.

Maximum likelihood and Bayesian analyses and tests of alternative phylogenetic hypotheses

For nuclear data, ML analysis using TVM+I+G model (rAC = 1.15, rAG = 2.38, rAT = 1.34, rCG = 0.61, rCT = 2.48 and pinv = 0.625) recovered a single tree (Fig. 2.) with –lnL = 2713.2827. The topology found was similar to the MP (Fig. 1.) and Bayesian (Fig. 4.) results.

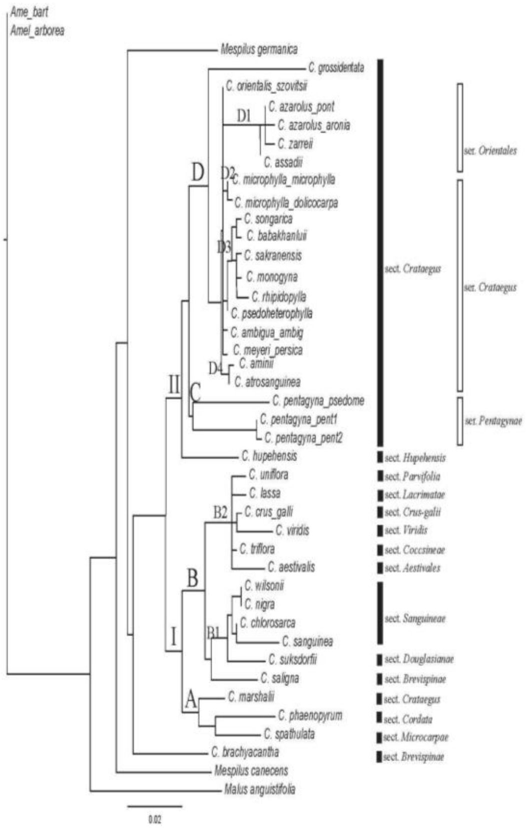

Fig. 2. Strict consensus trees of maximum likelihood (ML) analyses, using the TVM+I+G model with lnL: - 2713.2827, I= 0.38 and 0.7

In Bayesian analysis after removal of species *Amelanchier arborea, malus angustifolia* and *A. Bartramiana* as outgroup, A clad including *mespilus canescens* with 0.86 posterior probability is separated, it is a sister group to all *crataegus* species.(Fig.3. and Fig.4.). Then the species of *Crataegus brachycanta* is seen and it seems as ancestor of other *Crataegus* species. Two main clad(I) and (II) with 0.64 posterior probability are split taxa to the new world species(I) and old world(Europe and Asia) species(II),

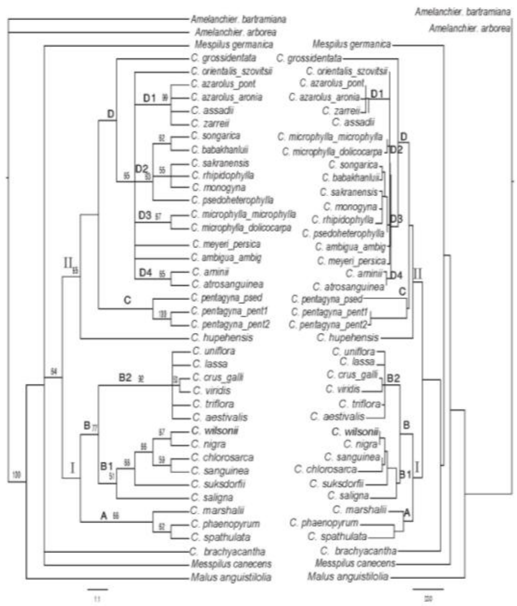

Fig. 3. Trees based on nuclear data generated by (a) maximum parsimony (MP) and (b) maximum likelihood (ML), using the TVM+I+G model with lnL: - 2713.2827, I= 0.38 and G= 0.72. In (a), bootstrap (BS; above branch) values >50% are indicated.

Species in category (I) with statistical support 0.88 where A, B are divided into two subcategories. A sub-category, with statistical support 0.97 species, including *C. phaenopyrum*, *C. spathulata* and *C. marshalii* which all species are in North America, two species, *C. phaenopyrum*, C. *spathulata* statistical support 0.88 are located in a directory. Subtype B were supported by 1.00 B1 and B2 is divided into two groups, one sibling group B1, which were supported by 0.84, including the species *C. sanguinea, C. chlorosarca* statistical support 0.96 and the two species *C. wilsonii* and *C. nigra* were supported by 1.00 from Sec. Sanguinea and *C. Suksdorfi* from Sec. *Dauglasianae* and *C. saligna* belongs Sec. *Brevispinaet*.

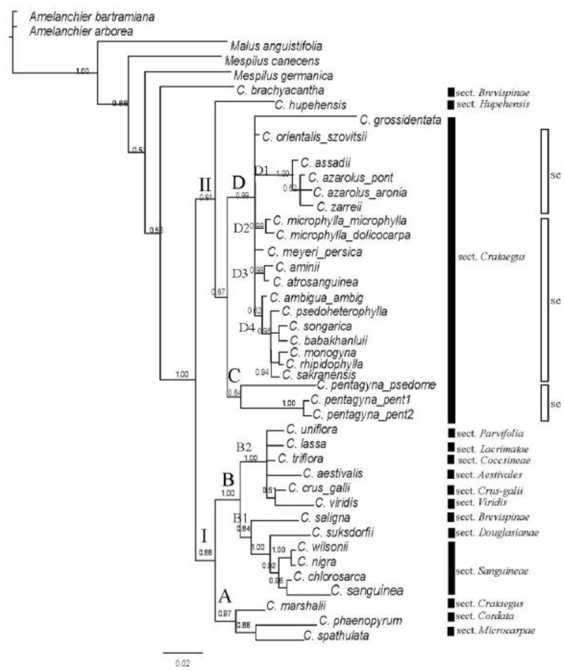

Fig. 4. Tree based on nuclear data generated by Bayesian method, using the TVM+I+G model with lnL: 2713.2827, I= 0.38 and G= 0.72. posterior probability (BI; above branch) values >50% are indicated.

The group B2 also also includes Polytomy of northeast America species.(*C. viridis* from Sec. *Viridis*, *C. crus-galli* of the Sec. *Crus-galli*, *C. aestivalis* from Sec. *Aestivalis*, *C. triflora* of Sec. *Coccinineae*, *C. lassa* of Sec. *Lacrimata* and *C. uniflora* from Sec. *Parvifolia* the status of these species in polytomy is not very clear.

Clad (II) were also supported by 0.91 of the Old World species and all taxa belong to Sec.*Crataegus*. In clad (II) the species of *C.hupehensis*is divided from other species and it is as sister group of other species. The other species by supporting 0.67 are located in C and D groups.

Group C includes the species *C. pentagyna* subsp. *pentagyna* 1 and *C. pentagyna,* subsp. *penatgyna*2 by statistical support 1.00 and *C. pentagyna* subsp. *pseudomelanocarpa*; all three taxa belong to the *pentagyna* ser.

Clad D includes of other species with different statistical supporting. The species of *C. grossidentata is* absolutly is divided and then *C. orientalis* subsp.*szovitsii* is placed.

The D1 group is monophyletic by statistical supporting 1.00 and includes *C. assadii, C. zarrei, C. azarolus* var.*ponica* and *C. azarolus* var.*aronia* that all of them belong to *la* D2 group also includes the species *C. microphylla* var.*microphy Orientalias* Ser. and *C. microphylla* var. *dolicocarpa* is statistically supported by 0.99. D3 the two species, *C. aminii* and *C. atrosanguinea* are located by statistical support 0.98. D4 groups with weak support 0.62 is a monophyletic group including *C. ambigua* subsp. *ambigua, C. pseudoheterophylla*, two species, *C. songarica* and *C. babakhanloui* statistical support 0.96, the two species *C. monogyna* var. *monogyna* and *C. rhipidophylla* var. *rhipidophylla* and in the end the species of *C. sakranensis with* statistical support 0.94 is located.

The results of ML analysis is similar to Bayesian analysis except that in Group D in the ML analysis, *C. grossidentata* is as sister group to other species of this group (D1, D2, D3 and D4), but in the Bayesian analysis, *C. grossidentata* in Group D, which is as a polytomy.(Fig.3.).

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Appendix 1. Locality and vouchers data for outgroup, *Mespilus*, and *Crataegus* taxa used for molecular analyses. Nomenclature follows that used by Talent and Dickinson (2005) , Monograph of *Crataegus*(Christensen, 1992) and Flora of iran (Khatamsaz,1992).

All collected species are saving in herbarium of Azad University branch of North- Tehran. The species that are labeled by (*) their sequences are taken from Genomic Bank site (NCBI).

Azad University North Tehran = AUNT

Species	(Voucher , Source)	GeneBank accession no. NrDNA ITS
Amelanchier arborea (Michx. f.)Fernald *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A.,Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"S. Nguyen 2003-1 (TRT)"	EF127041.1
Amelanchier bartramiana (Tausch)Roemer*	Lo, E.Y.Y., Stefanovic,S., Christensen, K.I.B. and Dickinson, T.A. ,Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada"B5"	EU500453.1
Malus angustifolia (Aiton) Michx. *	Robinson, J.P., Harris, S.A. and Juniper, B.E., Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK	AF186523.1
Mespilus canescens Phipps *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A., Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"S. Nguyen 2003-37-13 (TRT)"	EF127039.1
Mespilus germanica L.*	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A., Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"T.A. Dickinson 645-80 (MOR)"	EF127040.1
Crataegus brachycantha Sarg. & Engelm. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A., Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"T.A. Dickinson 2000-11 (TRT)"	EF127032.1
C. Pentagynae Waldstein & Kitaibel subsp. *Pentagynae*	Sharifnia, F. and Seyedipour N., Islamic Azad University - Tehran North Branch,12005(AUNT)	_
C. Pentagynae Waldstein & Kitaibel subsp. *Peseudomelanocarpa* Pojarkova Christensen	Sharifnia, F. and Seyedipour N., Islamic Azad University - Tehran North Branch,12007 (AUNT)	_
C. sakeanensis Hadac & Chrtek	Seyedipour , N. and Sharifnia, F., Islamic Azad University - Tehran North Branch,12017(AUNT)	_
C. rhipidophylla Gandoger var. *rhipidophylla*	Seyedipour, N. and Sharifnia, F., Islamic Azad University - Tehran North Branch,12038(AUNT)	_
C. monogyna Jacquin var. *monogyna*	Sharifnia, F. and Seyedipour N. , Islamic Azad University - Tehran North Branch,12012027 (AUNT)	_
C. babakhanloui Khatamsaz	Sharifnia, F. and Seyedipour N. , Islamic Azad University - Tehran North Branch,12014 (AUNT)	_
C. songarica Koch	Sharifnia, F. and Seyedipour N., Islamic Azad University - Tehran North Branch,12022 (AUNT)	_
C. pseudoheterophylla Pojark.	Sharifnia, F. and Seyedipour N., Islamic Azad University - Tehran North Branch,12012026(AUNT)	_
C. ambigua Meyer ex Becker subsp. *ambigua*	Seyedipour, N. and Sharifnia, F., Islamic Azad University - Tehran North Branch,12016(AUNT)	_

C. atrosanguinea Pojark.	Seyedipour, N. and Sharifnia F., Islamic Azad University - Tehran North Branch,12018 (AUNT)	_
C. aminii Khatamsaz	Seyedipour, N. and Sharifnia, F., Islamic Azad University - Tehran North Branch,12019 (AUNT)	_
C. meyeri Pojark.	Seyedipour, N. and Sharifnia, F. , Islamic Azad University - Tehran North Branch,12008 (AUNT)	_
C. persica Pojark.	Seyedipour, N. and Sharifnia, F., Islamic Azad University - Tehran North Branch,12013 (AUNT)	_
C. microphylla Koch var. *microphylla*	Sharifnia, F. and Seyedipour N., Islamic Azad University - Tehran North Branch,12023 (AUNT)	_
C. microphylla Koch var. dolichocarpa (Sommier & Levier) Hand.-Mzt.	Sharifnia, F. and Seyedipour N., Islamic Azad University - Tehran North Branch,12024(AUNT)	_
C. zarrei Donmez	Seyedipour , N. and Sharifnia, F., Islamic Azad University - Tehran North Branch,12034 (AUNT)	_
C. azarolus L. var. *aronia* L.	Seyedipour, N. and Sharifnia, F., Islamic Azad University - Tehran North Branch,12003 (AUNT)	_
C. azarolus L. var. *pontica* (Koch) Christensen	Seyedipour, N. and Sharifnia, Islamic Azad University - Tehran North Branch,12002 (AUNT)	_
C. assadii Khatamsaz	Asadi and Masoomi, Research Institute of Forests and Rangelands Herbarium, 50895 (TARI)	_
C. orientalis Pall. Ex Bieb. subsp. *szovitsii* (Pojark.) Christensen	Seyedipour, N. and Sharifnia, F., Islamic Azad University - Tehran North Branch,12004 (AUNT)	_
C. grossidentata	Sharifnia, F. and Seyedipour, N., Islamic Azad University - Tehran North Branch,12028 (AUNT)	_
C. spathulata Michx. *	Lo, E.Y.Y., Stefanovic,S. and Dickinson, T.A., Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"S. Nguyen 2003-34 (TRT)"	EF127033.1
C. phaenopyrum (L. f.) Medikus *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A., Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"T.A. Dickinson 99ME1 (TRT)"	EF127034.1
C. marshalii Egglest. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. ,Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"S. Nguyen 2003-05 (TRT)"	EF127037.1
C. sanguinea Pall. Ex Bieb. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. (20-NOV-2006) Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"T.A. Dickinson JBM1232-49 (TRT)"	EF127027.1
C. chloroscara Maxim. *	Lo, E.Y.Y., Stefanovic, S., Christensen, K.I.B. and Dickinson,T.A. , Ecology and Evolutionary Biology,University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2,Canada"2003-60"	EU683917.1
C. nigara Waldst. and Kit. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. , Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario	EF127007.1

	M5S 3B2, Canada="K.I. Christensen 294 (TRT)"	
C. wilsonii Sarg. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. , Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"T.A. Dickinson AA749-74A (TRT)"	EF127008.1
C. suksdorfii (Sarg.) Kruschke *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. , Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada "R. Love 2003-11 (TRT)"	EF127025.1
C. saligna Greene *	Lo, E.Y.Y., Stefanovic, S., Christensen, K.I. and Dickinson, T.A. , Ecology and Evolutionary Biology,University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2,"2001-7A"	EU683910.1
C. viridis L. *	Lo, E.Y.Y., Stefanovic, S., Christensen, K.I. and Dickinson,T.A. , Ecology and Evolutionary Biology,University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2,Canada "2003-63"	EU683922.1
C. crus-galii L. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. , Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada "N. Talent 213A (TRT)"	EF127010.1
C. aestivalis (Walt.) T. & G. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. , Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada "N. Talent 321 (TRT)"	EF127023.1
C. triflora Chapm. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. , Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"N. Talent 290a (TRT)"	EF127019.1
C. lassa Beadle *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. , Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada "S. Nguyen 2003-34 (TRT)"	EF127024.1
C. uniflora Munchh. *	Lo,E.Y., Stefanovic,S., Christensen, K.I. and Dickinson,T.A., Ecology and Evolutionary Biology,University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2,Canada "2003-52"	EU683923.1
C. hupehensis Sarg. *	Lo, E.Y.Y., Stefanovic, S. and Dickinson, T.A. ,Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario M5S 3B2, Canada"T.A. Dickinson AA356-81B (TRT)"	EF127038.1