

TAXONOMIC CIRCUMSCRIPTION AND RELATIONSHIP IN RHAMNUS L. AND ATADINUS RAF. (RHAMNACEAE) SPECIES IN IRAN BASED ON MORPHOLOGICAL CHARACTERS

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The genera *Rhamnus* and *Atadinus* have been dealt as a single genus, namely *Rhamnus* or two distinct genera. The genus *Atadinus*, includes unarmed (not spiny) species with 6 or more pairs of lateral veins on leaves. Moreover, relationship of the species and subspecific taxa needed further studies. In order to delimit and elucidate the taxonomic relationships of *Rhamnus* L. and *Atadinus* Raf. taxa (Rhamnaceae) in Iran, morphometric data analyses were performed. Studies were based on 30 morphological characters (8 quantitative and 22 qualitative) potentially belonging to two genera, 14 taxa, 52 populations and 749 individuals. Data analyses were performed using SPSS, Past and Excel softwares. Clustering and Ordination Analyses illustrated similarities and differences among the populations and taxa. The species referred to any of the genera *Rhamnus* and *Atadinus* were separated in clustering and ordination. Morphometric studies more or less confirmed former classification of the taxa in Iran. Some of the specimens having characters of 2 or more taxa supposed to be hybrids. Also, some of the collections seemed to be new subspecies.

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Key words: Morphometric characters; phenogram; *Rhamnus*; *Atadinus*; Iran

تعیین حدود و قرابت دو جنس *Rhamnus* و *Atadinus* از تیره *Rhamnaceae* در ایران بر اساس ویژگی‌های ریختی
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جنس‌های *Rhamnus* و *Atadinus* در منابع مختلف به عنوان یک جنس *Rhamnus* و یا دو جنس مستقل در نظر گرفته شده‌اند. جنس *Atadinus* به گونه‌های بدون خار با داشتن ۶ یا تعداد بیشتر رگه جانبی روی برگ‌ها اطلاق شده است. علاوه بر این قرابت و ارتباط گونه‌ها مطالعه بیشتری را می‌طلبد. در این مطالعه، مورفومتری به منظور تعیین حدود و رابطه بین آرایه‌های قابل انتقال به هر یک از جنس‌های *Rhamnus* و *Atadinus* از تیره *Rhamnaceae* انجام شده است. تعداد ۳۰ صفت (شامل ۲۲ صفت کیفی و ۸ صفت کمی) در دو جنس، ۱۴ آرایه، ۵۲ جمعیت و ۷۴۹ فرد مطالعه و بررسی شدند تا مرز آرایه‌ها و ارتباط آنها تعیین شود. تجزیه و تحلیل داده‌ها بوسیله نرم افزارهای SPSS, Past و Excel و با روش خوشه‌ای و رسته‌بندی انجام شده است که اغلب حدود و ارتباط تاکسون‌های این جنس‌ها تعیین شدند. گونه‌هایی که قابلیت انتقال به هر یک از دو جنس مذکور را داشتند در خوشه‌بندی و رسته‌بندی تفکیک شدند. نتایج این مطالعه تا حدودی طبقه‌های قبلی را تایید می‌نماید. وجود نمونه‌هایی با ویژگی‌های دو گونه مختلف ماهیت دورگه‌ای بودن آنها را تایید می‌نماید. همچنین یک زیرگونه جدید در بین گونه‌ها قابل تشخیص بود.

INTRODUCTION

Rhamnaceae is a family including 25 genera and about 925 species (Medan & Schirarend 2004), or based on Gandolfo & al. (2017) more than 50 genera and about 900 species. The genus *Rhamnus* L. is a genus of the Tribe Rhamneae, one of 12 recognized tribes in *Rhamnaceae* (Richardson & al. 2000). *Rhamnus* species varies from 100 (Medan & Schirarend 2004) to 125 (Mabberley 1997). *Rhamnus* species have a worldwide distribution in temperate and tropical zones of eastern and southwestern Asia, southwest and northern America (Mabberley 1987). Based on molecular systematic analyses (Fay & al. 2001; Richardson & al. 2000a, 2001), the family now contains 52 genera in 11 tribes (Richardson & al. 2000b). In Iran, the genus includes 8 or 6 species according to Flora of Iran (Sofian & Dinarvand 2007) or Flora Iranica (Browicz & Zielinski 1977) respectively, some of which are subspecific taxa. The genus *Atadinus* Raf. was introduced by Rafinesque (Rafinesque 1838) and it has been regarded as a distinct genus from *Rhamnus* with 11 species, one of

them with 2 hybrids in Iran (Hauenschild & al. 2016). The genus *Atadinus*, includes unarmed (not spiny) species with 6 or more pairs of lateral veins on leaves. Among the Iranian *Rhamnus* species, *Rh. cornifolia* Boiss. & Hohen. is unarmed plant with more than 6 lateral veins on leaves. The authors are going to transfer this species to the genus *Atadinus* in another taxonomic paper (submitted).

There are several phylogenetic and morphometric studies on *Rhamnaceae* taxa that includes Iranian members (Iqbal & al. 2019, Onstein & al. 2015, Islam & Guralnick 2015, Messina & al. 2010).

The aim of this paper is to classify the complex of *Rhamnus* and *Atadinus* taxa based on numerical taxonomy.

MATERIALS AND METHODS

This study is based on the the materials collected from the fields in Iran. The herbarium specimens are deposited in the herbarium of Research Institute of Forests and Rangelands (TARI), (table 1).

Table 1. Studied populations of *Rhamnus* L. and *Atadinus* Raf. taxa for the multivariate analyses of morphological characters (the taxa of first column of table 1., table 2. and table 3. are the same).

Taxa	Locality and collection data
1. <i>Rhamnus cathartica</i> L. var. <i>cathartica</i>	Kurdistan: From Marivan towards Saggez, opposite of Agje village, 1790 m, 23 May 2017, 46°25' 64" E, 35°47' 00" N, Alijanpoor & Sabzi 103815 (TARI).
2. <i>Rh. cathartica</i> var. <i>cathartica</i>	Golestan: From Gorgan towards Jelin village, Chahar Bagh, 26 May 2017, 2350 m, 54°33' 39" E, 36°40' 00" N, Alijanpoor & Alijanpoor 103859, 103860 (TARI).
3. <i>Rh. cathartica</i> var. <i>cathartica</i>	Gilan: Manjil, Jirindih road, Calishum village, 20 August 2017, 2260 m, 49°57' 46" E, 36°45' 51" N, Alijanpoor & Jalili 103805 (TARI).
4. <i>Rh. cathartica</i> var. <i>caucasica</i> Kusun.	Azerbaijan (W): From Urmia towards Oshnavieh, Darreh Khan, 1600 m, 29 May 2017, 45°60' 53" E, 37°18' 38" N, Alijanpoor & Mirzaloo 103852 (TARI).

Table 1. Continued.

5. <i>Rh. cathartica</i> var. <i>caucasica</i> Kusn.	Azerbaijan (E): Kaleybar, Arasbaran forest, Darre Makidi, 1400 m, 01 June 2017, 46°54'53" E, 38°51'14" N, Alijanpoor 103858 (TARI).
6. <i>Rh. cathartica</i> var. <i>caucasica</i> .	Mazandaran: From Karaj towards Mazanabad, Kojur road, Dasht-e Nazir village, opposite of Sand company, 980 m, 04 May 2017, 51°25' 638" E, 36°24' 96" N, forest habitat, Alijanpoor & Azizi 103814 (TARI).
7. <i>Rh. cathartica</i> var. <i>caucasica</i>	Semnan: From Shahmirzad towards Parvar Protected Area, after Kavard village, 27 April 2017, 2300 m, 53°27' 55" E, 36°27' 54" N, Alijanpoor & Hoseinii 103805 (TARI).
8. <i>Rh. cathartica</i> var. <i>cathartica</i>	Mazandaran: Pol Sefid, Sang Deh village, after Fagus forest (Mer SiSi), 27 September 2017, 2400 m, 53°13' 06" E, 36°00' 51" N, Alijanpoor & Mohamady 103855 (TARI).
9. <i>Rh. cathartica</i> var. <i>caucasica</i>	Mazandaran: Chalus road, Siah Bisheh dam, 1830 m, 04 May 1830, 51°18' 24" N, 36°14' 05" E, Alijanpoor 103874 (TARI).
10. <i>Rh. cornifolia</i> Boiss. & Hohen. var. <i>cornifolia</i>	Markazi: From Arak towards Tureh, after Farr, Dare Salehan, Rasvand Mt. 2300-2500 m, 15 May 2017, 49°12' 21" E, 33°22' 59" N, Alijanpoor & Godarzi 103854 (TARI).
11. <i>Rh. cornifolia</i> var. <i>denudata</i> Bornm.	Markazi: From Arak towards Tureh, after Farr, Dare Salehan, Rasvand Mt. 2300-2500 m, 15 May 2017, 49°12' 21" E, 33°22' 59" N, Alijanpoor & Godarzi 103854 (TARI).
12. <i>Rh. persica</i> × <i>Rh. kurdica</i> (hybrid 1)	Azarbayjan (w): From Urmia towards Oshnavieh, Darreh Khan, 1600 m, 29 May 2017, 45°60' 53" E, 37°18' 38" N, Southern slope, rocky habitat, Alijanpoor & Heidari 103841 (TARI).
13. <i>Rh. persica</i> × <i>Rh. kurdica</i> (hybrid 1)	Kermanshah: From Eslam Abad towards Gahvareh, ca.15 Km to Chenar village, 1490 m, 21 May 2017, 46°27' 07" E, 34°13' 04" N, Alijanpoor & karimi 103849 (TARI).
14. <i>Rh. persica</i> × <i>Rh. kurdica</i> (hybrid 1)	Kermansha: From Ilam towards Kermansh, Goaver road, Gholi Gholi village, On the Mts. 1010 m, 21 May 2017, 40°04' 02" E, 34°22' 47" N, Alijanpoor & karimi 103840 (TARI).
15. <i>Rh. kurdica</i> Boiss. & Hohen.	Kurdistan: From Sanandaj toward Marivan, Darband-e Dezli, 1340 m, 24 May 2017, 46°10' 19" E, 35°22' 13" N, Alijanpoor & Sabzi 103861 (TARI).
16. <i>Rh. kurdica</i>	Azarbayjan (w): From Urmia towards Oshnavieh, Darreh Khan, 1600 m, 29 May 2017, 45°06' 53" E, 37°18' 38" N, Alijanpoor & Mirzaloo 103895 (TARI)
17. <i>Rh. kurdica</i>	Kermanshah: From Ilam towards Kermansha, Goaver road, Gholi Gholi village, On the Mts. 1010 m, 21 May 2017, 40°04' 02" E, 34°22' 47" N, Alijanpoor & Karimi 103867 (TARI).
18. <i>Rh. oleoides</i> L.	Azarbayjan (w): From Urmia towards Oshnavieh, Darreh-ye Qasemlu, Darreh Khan, 1600 m, 29 May 2017, 45°60' 53" E, 37°18' 38" N, Alijanpoor & Mirzalo 103896 (TARI).
19. <i>Rh. oleoides</i>	Kermansh: From Eslam Abad towards Gahvareh, ca. 15 Km to Chenar village, 1490 m, 21 May 2017, 46°27' 07" E, 34°13' 04" N, Alijanpoor & Karimi 103868 (TARI).
20. <i>Rh. oleoides</i>	Azarbayjan (w): Naqade, Kouh-e Sultan yaqub, 1400 m, 29 May 2017, 45°36' 05" E, 36°93' 16" N, Alijanpoor & Mirzalo, 103824 (TARI).

Table 1. Continued.

21. <i>Rh. pallasii</i> subsp. 1	Gilan: From Manjil towards Lowshan, 5 Km from Jirindeh towards Calishom village, 1480 m, 03 May 2017, 1870 m, 49°46' 42" N, 36°42' 58" E, Alijanpoor & Jalali 103845 (TARI).
22. <i>Rh. pallasii</i> subsp. 1	Semnan: From Shahmirzad towards Kiasar, ca. 5 Km after Foulad Mahalle, 1870 m, 09 September 2017, 53°44' 14" N, 36°04' 59" E, Alijanpoor & Alijanpoor 103884 (TARI).
23. <i>Rh. pallasii</i> subsp. 1	Tehran: From Karaj towards Gachsar, Karaj Dam, 1830 m, 02 May 2017, 51°05' 36" N, 35°58' 13" E, Alijanpoor & Hosseini 103876 (TARI)
24. <i>Rh. pallasii</i> subsp. <i>iranica</i> Browicz & J. Zielinski	Ilam: from Ilam towards Eyvan Gharb, Mt. Renow, 1850 m, 29 August 2017, 46°14' 52" E, 33°25' 37" N, Alijanpoor 103857 (TARI).
25. <i>Rh. pallasii</i> subsp. <i>iranica</i>	Lurestan: Dorud, Oshtorankouh, 2220 m, 16 May 2017, 49°11' 21" E, 33°22' 59" N, Alijanpoor 103890 (TARI).
26. <i>Rh. pallasii</i> subsp. <i>iranica</i>	Markazi: From Arak towards Tureh, after Farr, Dare Salehan, Rasvand. 2320 m, 15 May 2017, 49°12' 21" E, 33°22' 59" N, Alijanpoor & Godarzi 103835 (TARI).
27. <i>Rh. pallasii</i> Fisch. & C. A. Mey. subsp. <i>pallasii</i>	Kurdistan: From Bijar towards Salavatabad village, Salavatabad, 1700-1900 m, 6 May 2017, 47°33' 41" E, 36°19' 02" N, Alijanpoor & Arabamer 103813 (TARI).
28. <i>Rh. pallasii</i> subsp. <i>pallasii</i>	Azərbayjan (w): From Urmia towards Noshin Shahr, Kouh-e Keshish, 1572 m, 28 May 2017, 46°31' 29" E, 38°18' 40" N, Alijanpoor & Mirzalo 103826 (TARI)
29. <i>Rh. pallasii</i> subsp. <i>pallasii</i>	Azərbayjan (w): From Salmas towards Taze Shar, before Kuzehrash village, 1750 m, 28 May 2017, 44°38' 21" E, 38°09' 49" N, Alijanpoor 103828 (TARI).
30. <i>Rh. pallasii</i> subsp. <i>pallasii</i>	Azərbayjan (E): Ca. 17 Km from Jolfa toward Marand, 1226 m, 02 Jun 2017, 46°31' 33" E, 38°07' 41" N, Western slope, rocky pasture habitat, Alijanpoor 103810 (TARI).
31. <i>Rh. pallasii</i> subsp. <i>pallasii</i>	Azərbayjan (E): From Jolfa towards Poldasht, Arass, Saintstepanos church, 800 m, 29 May 2017, 45°28' 18" E, 38°59' 19" N, Alijanpoor 103827 (TARI).
32. <i>Rh. pallasii</i> subsp. <i>pallasii</i>	Azərbayjan (E): Tabriz towards Ahar, Shahre Jadide Shahriar, 1590 m, 31 May 2017, 46°29' 16" N, 38°40' 40" E, Alijanpoor 103808 (TARI).
33. <i>Rh. pallasii</i> subsp. <i>pallasii</i>	Tabriz toward Zanjan, near to Terian willage, 1345m, 03 June 2017, 47°31' 53" N, 37°08' 12" E, Alijanpoor & Arabameri 103811 (TARI).
34. <i>Rh. pallasii</i> subsp. <i>pallasii</i>	Azərbayjan (E): Kaleybar, Arasbaran forest, Khoda Afarin area, Tu Ali village, 350 m, 01 June 2017, 46°49' 16" E, 39°05' 04" N, Alijanpoor 103809 (TARI)
35. <i>Rh. pallasii</i> subsp. <i>sintensis</i> Browicz & J. Zielinski	Mazandaran: Haraz road, Filband road, 510-710 m, 28 July 2017, 52°22' 08" E, 36°14' 45" N, Alijanpoor & Alijanpoor 103818 (TARI).
36. <i>Rh. pallasii</i> subsp. <i>sintensis</i>	Marzanabad: Kelardasht, before Pardangoun village, 830 m, 04 May 2017, 51°14' 31" E, 36°20' 38" N, Alijanpoor 103886 (TARI).
37. <i>Rh. pallasii</i> subsp. <i>sintensis</i>	Mazandaran: Pol Sefid, Sang Deh road, Sang Deh village, 1250 m, 02 May 2017, 53°10' 48" E, 36°05' 28" N, Alijanpoor 103852 (TARI).
38. <i>Rh. pallasii</i> subsp. <i>pallasii</i>	Azərbayjan (E): From Salmas towards Khoy, Pirkandi village, Gholi valley, 1470 m, 29 May 2017, 45° 60' 05" E, 38° 44' 09" N, Alijanpoor & Mirzalo (TARI).

Table 1. Continued.

39. <i>Rh. pallasii</i> (J)	Tehran: Jajrud, Mahi Sara of Jajrud, 1480 m, 09 May 2017, 42°51' E, 35°43'60" N, Alijanpoor & Atazade 103819 (TARI).
40. <i>Rh. pallasii</i> (L)	Tehran: Lavasan, Kalugan village, Mt. Kalugan, 1877 m, 09 May 2017, 51°34'26" E, 35°53'06" N, Alijanpoor & Zamannejad 103821 (TARI)
41. <i>Rh. pallasii</i> (SD)	Mazandaran: Chalus road, Siah Bisheh dam, 1830 m, 04 May 1830, 51°18' 24" N, 36°14' 05" E, Alijanpoor 103874 (TARI).
42. <i>Rh. pallasii</i> (SA)	Firuzkough: Seransa and Mahen villages, 2538 m, 29 April 2017, 52°53' 39" E, 35°42' 52" N, Alijanpoor 103878 (TARI).
43. <i>Rh. persica</i> Boiss.	Fars: Neyriz towards Estahban, Eich chroda, beside Eich Transcommunication tower, 2400 m, 13 April 2017, 54°11' 08" E, 29°04' 38" N, Alijanpoor & Sadeghi 103863 (TARI).
44. <i>Rh. persica</i>	Isfahan: Road Shahreza towards Samirom, before Kohrouyeh village, 2200 m, 07 May 2017, 51°48' E, 31°43' N, Alijanpoor 103839 (TARI).
45. <i>Rh. persica</i>	Isfahan: From Najafabd towards Teiran, Kurd-e Olya village, Osor (Abshar) towards Cheheldokhtran, 2420 m, 06 May 2017, 50°42' E, 32°55' N, Alijanpoor 103871 (TARI).
46. <i>Rh. persica</i>	Isfahan: From Najafabd towards Teiran, Kurd-e Olya village, Faridel, Tang-e Kolang, 2490 m, 06 May 2017; 50°38' E, 33°00' N, Alijanpoor 103872 (TARI).
47. <i>Rh. prostrata</i> Jacquem. ex Parker	Kerman: Cheshme Imam Reza village, Kouh-e Mehr 2380 m, 05 May 2017, 57°19' 12" E, 30°17' 16" N, Alijanpoor & Pourmirza 103894 (TARI).
48. <i>Rh. sp 1</i>	Mazandaran: From Ramsar towards Chalus, Jannatroudbar, ca. 500 m from Jannatroudbar towards Palhamjan village, 1530-1560 m, 11 May 2017, B. Alijanpoor & S. Hosseini 103846 (TARI).
49. <i>Rh. spathulifolia</i> Fisch. & C. A. Mey.	Mazandaran: 35 Km from Pol Sefid toward Firuzkough, Gadok, 1590 m, 29 April 2017, 52°57' 25" E, 35°51' 28" N, Alijanpoor 103887 (TARI).
50. <i>Rh. spathulifolia</i>	Mazandaran: From Babolsar towards Bahnamir, Mirod village, 45 m, 02 May 2017, 52°44' 31" E, 36°43' 13" N, Alijanpoor & Hosseini, 103844 (TARI).
51. <i>Rh. spathulifolia</i>	Golestan: 10 Km from Kalaleh towards Maraveh Tapeh, 210 m, 30 April 2017, 55°37' 15" E, 37°34' 20" N, Alijanpoor & Taheri 103891 (TARI).
52. <i>Rh. spathulifolia</i>	Mazandaran: Chalus road, after Siah Bisheh dam, 1500 m, 04 May 2017, 51°19' 12" E, 36°12' 48" N, Alijanpoor & Azizi, 103885, 103892 (TARI).

The morphometric data analyses was carried out by measuring 30 morphological features (22 qualitative and 8 quantitative features) belonging to the 2 genera of *Rhamnus* and *Atadinus* complex, 14 species or subspecific ranks, 52 populations and 749 individuals (tables 3 and 4). The mean of 8 informative quantitative characters and the mode of 22 informative qualitative characters were used and 52 populations were compared based on 30 characters. Standardized data (mean = 0, variance = 1) were used for multivariate statistical analyses. One Way ANOVA and 1- Sample K-S Test were used to

determine the signification in analysis of data.

Generally, 22 informative qualitative characters were coded as multistate characters (table 4). In order to examine the stability of morphological features 15 accessions were selected per taxon.

Individuals from different species and populations of *Rhamnus* were subjected to clustering analysis. Twenty-two quantitative and 8 qualitative morphological characters were evaluated (tables 2-4). After being standardized, the "mean" of quantitative characters and the "mode" of qualitative characters were used for clustering analysis using Unweighted

Pair Group Method with Arithmetic mean (UPGMA) with Ward method and ordination of OTUs was carried out using Principal Component Analyses (PCA) in PAST3, Excel and SPSS 16 with Varimax rotation. The reasons for choosing these methods are because of, UPGMA is used for the creation of

phenetic trees (phenograms) indeed, the tree aims at grouping the most similar data, Ward method as a hierarchical clustering method, was used to create groups and PCA is often used to visualize genetic distance and relatedness between populations.

Table 2. Qualitative characters and their abbreviations and coding in *Rhamnus* L. and *Atadinus* Raf. taxa:

Qualitative characters: **Spn**= spiny or not spiny, **BrTy**= branching type, **LeTe**= leaf texture, **PuLe**= pubescent leaf, **RaLe**= ratio length/width leaf, **MaTo**= margin tooth, **LaNeSh**= lateral nerve shape, **LaNeKi**= lateral nerve kind, **Ptco**= petal condition, **LaNecl**= lateral nerve clear or not., **LaNeAr**= lateral nerve arrangement, **Lemo**= leaf (monomorph, dimorph or polymorph), **Leeq**= leaf equal or unequal, **Leaf**= leaf alternate or fasciculate, **Ptpu**= petiole pubescence, **Blesh**= base leaf shape, **Alesh**= apex leaf shape, **Pepu**= peduncle pubescent, **Mafrco**= maturing fruit color, **Fufr**= furrow fruit, **Flar** = flower arrangement, **plha**= Plant habit.

Characters and Codes

Spn: spiny=1, not spiny=2, much spiny=3, medium to much=4, medium=5

BrTy: simple=1, simple & branch=2

LeTe: membranous =1, herbaceous=2, coriaceous=3, thick herbaceous =4, thick membranous =5, thin membranous =6

PuLe: a few =1, absent=2, many=3

RaLe: >2, 1.5-2=2, 1-1.5=1

MaTo: 0=1, 1-10=2, 11-20=3, 21-30=4, >30=5

LaNeKi: superficial=1, deep=2

Ptco= petal caduque=1, persistent=2

LaNeSh: curved=1, erect=2, erect to curved=3

LaNecl: conspicuous=1, inconspicuous=2, almost conspicuous=3

LaNeAr: alternate=1, opposite=2, mostly alternate rarely opposite=3, mostly opposite rarely alternate=4, opposite and alternate=5

Lemo: monomorph=1, dimorph=2, polymorph=3, almost monomorph=4

Leeq: equal=1, unequal=2, almost equal=3

Leaf: fasciculate =1, alternate and fasciculate =2, alternate=3, fasciculate and rarely alternate=4, fasciculate and alone=5

Ptpu: scarce =1, absent=2, many=3

Blesh: cuneate=1, rounded =2, narrow cuneate=3, angustifoliate=4, cuneate to narrow cuneate=5, cuneate to roundish=6

Alesh: acute=1, obtuse=2, mostly acute and rarely obtuse=3, mostly obtuse and rarely acute=4, acute and obtuse=5

Pepu: present=1, absent=2, sometime present=3

Mafrco: black=1, bright red=2, bright red and yellow=3

Fufr: open (deep)=1, close (superficial)=2, almost open=3

Flar: fasciculate =1, alternate=2, fasciculate and rarely single=3, single =4

Plha: shrub standing=1 shrub spreading=2, shrub runner and some time standing =3, shrub prostrate=4, shrub similar to arboreus=5, shrub standing and spreading=6

RESULTS

The examined taxa of *Rhamnus-Atadinus* and their morphological traits are summarized in tables 1-4. Spine is either absent (in *Rh. cornifolia*) or up to 2.34 mm (in *Rh. cathartica* var. *cathartica*, Clisium specimen) and 111.54 mm (in *Rh. pallasii* subsp. *iranica*, Oshtorankouh specimen).

The mean leaves length exhibit from 3.53 mm in *Rh. persica* (Tang-e Kolang) to 63.47 mm in *Rh. cathartica* var. *caucasica* (Darreh Makidi) or 62.50 mm

in *Rh. cornifolia* var. *denudata* (Rasvand) while in the mean leaves width exhibit from 1.35 mm in *Rh. pallasii* subsp. *pallasii* (Saintstepanos) to 46.00 mm in *Rh. cathartica* var. *caucasica* (Darreh Khan).

The mean peduncles length demonstrates from 2.00 mm in *Rh. persica* (Eich) to 15.00 mm in *Rh. cathartica* L. var. *caucasica* (Dasht-e Nazir) while in the mean petiole length is from 1.87 mm in *Rh. persica* (Tang-e Kolang) to 19.07 mm in *Rh. cathartica* L. var. *caucasica* (Darreh Makidi). The

mean marginal teeth number varies from no marginal teeth in *Rh. persica* to 1.3 marginal teeth in *Rh. oleoides* (Naqade) and 15 marginal teeth in *Rh. spathulifolia* (Siah Bisheh) while in the mean lateral nerve number varies from 2.60 in *Rh. prostrata* (Kouh-e Mehr) to 10.34 in *Rh. cornifolia* var. *denudata* (Rasvand). The mean flowers length is from 1.50 mm in *Rh. persica* (Eich) to 8.54 mm in *Rh.*

spathulifolia (Maraveh Tapeh) but the mean fruits length is from 2.70 mm in *Rh. pallasii* subsp. *sintensisii* (Sang Deh, rocky) to 5.60 mm in *Rh. pallasii* subsp. *iranica* (Renow) (tabl 3).

Relationships of taxa are represented in Ward method, UPGMA and PCA phenograms (fig. 1, fig. 2 and fig. 3).

Table 3. The mean quantitative characters of populations of *Rhamnus* L. and *Atadinus* Raf. taxa (values in mm), (the taxa of first column of table 1, table 3 and table 4 are the same as), (na=Non available data). Quantitative characters: **SL**= spine length, **LLFL**= long leaf in flowering, **WLFL**= width leaf in flowering, **PDLFL**= peduncle length in flowering, **LNN**= lateral nerve number, **PTL**= petiole length, **FLL**= flower length, **FRL**= fruit length.

Taxa	SL	LLFL	WLFL	PDLFL	LNN	PTL	FLL	FRL
1. cat1	11.00	28.67	21.78	6.78	3.50	12.33	4.33	4.41
2. cat2	na	31.30	22.33	4.37	4.77	12.87	3.13	4.77
3. cat3	2.34	42.33	23.50	6.37	4.56	12.90	4.90	5.17
4. cat4	43.33	60.00	46.00	11.40	4.00	18.20	na	na
5. cat5	55.57	63.47	38.33	5.82	3.69	19.07	4.58	5.22
6. cat6	29.17	36.67	17.61	15.00	4.86	10.39	3.70	4.61
7. cat7	15.00	16.00	18.75	3.25	4.50	6.00	3.00	4.33
8. cat8	na	49.40	31.67	5.93	4.53	14.03	na	5.40
9. cat9	40.91	28.83	12.30	6.53	5.10	7.87	3.00	4.75
10. corn1	0.00	30.02	20.20	3.48	9.70	7.84	3.00	5.22
11. corn2	0.00	62.50	34.00	na	10.34	8.75	na	na
12. meh1	50.00	17.07	7.47	4.10	4.60	4.77	3.00	4.50
13. meh2	36.36	13.33	6.78	3.43	4.43	2.25	2.17	3.60
14. meh3	47.62	15.03	9.13	3.23	4.30	2.90	2.00	4.60
15. kur1	53.89	21.22	9.06	8.00	5.33	8.06	na	4.00
16. kur2	47.14	18.60	8.30	4.43	4.90	4.87	3.00	4.50
17. kur3	66.36	19.83	7.48	3.60	4.53	3.15	3.60	na
18. ole1	58.06	15.87	42.00	2.43	4.97	3.92	na	3.60
19. ole2	39.17	13.47	40.63	2.56	4.88	2.58	na	3.50
20. ole3	54.29	13.06	44.76	na	5.03	3.39	na	3.63
21. palm1	86.33	11.47	3.02	3.00	3.60	2.45	2.50	4.20
22. palm2	51.72	18.60	2.43	3.37	4.40	2.67	na	5.00
23. palm3	75.38	16.17	8.27	3.60	4.93	3.60	na	5.47
24. pali1	70.00	23.87	6.10	5.03	4.17	4.83	3.00	5.60
25. pali2	111.54	25.20	7.33	7.43	4.43	7.30	3.60	3.60

Table 3. Continued.

26. pali3	87.50	22.97	6.10	6.50	3.63	6.43	3.60	3.50
27. palp1	5.75	23.35	3.19	4.93	4.52	4.23	2.56	4.00
28. palp2	na	25.33	2.52	4.80	4.60	4.65	2.90	2.86
29. palp3	52.00	26.80	2.05	3.36	4.63	4.09	2.45	2.90
30. palp4	4.70	27.90	2.33	4.37	3.70	4.37	2.33	3.31
31. palp5	50.00	20.83	1.35	3.97	3.97	3.07	na	3.97
32. palp6	na	24.00	1.93	4.70	3.97	4.23	2.30	4.23
33. Palp7	4.40	23.62	6.05	5.37	5.47	4.50	na	3.66
34. palp8	79.09	30.97	2.40	4.33	6.10	4.43	na	4.63
35. pals1	60.00	18.77	4.47	na	4.27	3.05	na	5.17
36. pals2	63.67	19.30	3.92	3.77	3.83	3.83	3.00	2.77
37. pals3	45.77	19.07	4.43	3.62	5.23	4.93	2.77	3.58
38. palp9	4.00	20.00	14.33	3.80	3.60	3.33	3.00	3.30
39. palpJ	0.00	21.13	4.65	4.67	4.20	3.57	2.57	na
40. palpL	77.22	17.60	4.43	3.67	4.50	4.57	3.00	4.53
41. palp10	44.62	12.27	3.37	3.66	3.80	2.90	2.42	na
42. palp11	na	17.27	2.93	3.20	4.47	3.27	na	3.60
43. per1	30.00	4.87	2.97	2.00	3.70	1.92	1.50	3.47
44. per2	42.78	8.57	5.82	3.92	3.00	2.28	1.80	3.60
45. per3	na	5.60	3.70	3.00	4.00	2.36	2.00	3.60
46. per4	49.33	3.53	2.47	2.43	4.00	1.87	2.00	3.60
47. pro	20.00	4.40	2.80	3.00	2.60	1.80	2.00	na
48. rah	25.28	11.39	7.00	2.71	3.67	3.00	2.71	4.67
49. spa1	50.00	26.88	7.50	4.67	11.21	8.63	2.67	4.33
50. spa2	140.00	21.90	6.90	4.50	4.10	6.93	2.80	4.50
51. spa3	63.57	24.27	8.67	6.43	5.57	10.20	8.54	4.60
52. spa4	49.44	19.70	7.50	3.60	4.00	7.67	2.50	4.60

Table 4. The scores of qualitative characters of populations of *Rhamnus* L. and *Atadinus* Raf. taxa (the taxa of first column of table 1, table 3 and table 4 are the same), (na=Non available data). Qualitative characters: **CoSt**= stem color, **YoBrCo**= young branch color, **Spn**= spiny or not spiny, **BrTy**= branching type, **LeTe**= leaf texture, **PuLe**= pubescent leaf, **RaLe**= ratio length/width leaf, **MaTo**= margin tooth, **LaNeSh**= lateral nerve shape, **LaNeKi**= lateral nerve kind, **Ptc**= petal condition, **LaNecl**= lateral nerve distinct or not., **LaNeAr**= lateral nerve arrangement, **Lemo**= leaf (monomorph, dimorph or polymorph), **Leeq**= leaf equal or unequal, **Leaf**= leaf alternate or fasciculate, **Ptpu**= petiole pubescence, **Blesh**= base leaf shape, **Alesh**= apex leaf shape, **Pepu**= peduncle pubescent, **Mafrco**= mature fruit color, **Fufr**= furrow fruit, **Flar**= flower arrangement, **Plha**= Plant habit

Taxa	Laneto	Pepe	Spn	BrTy	LeTe	PuLe	RaLe	MaTo	Lansh	Lanecl	LaneAr	Lemo	Leeq	Leaf	Ptpu	Blesh	Alesh	Pepu	Mafrco	Fufr	Flar	Plha
cat1	1	1	1	1	2	1	3	5	1	1	4	2	1	2	3	2	2	na	1	2	1	5
cat2	1	1	1	1	2	2	3	5	1	1	4	2	1	1	1	2	2	na	1	2	1	5
cat3	1	1	1	1	2	2	3	5	1	1	4	2	1	4	1	2	2	na	1	2	1	5
cat4	1	1	1	1	2	1	3	5	1	1	4	2	1	4	1	2	1	na	1	na	1	5
cat5	1	1	1	1	2	1	3	5	1	1	4	2	1	4	1	2	2	na	1	2	1	5
cat6	1	1	1	1	4	1	3	5	1	1	3	1	1	2	1	2	1	na	1	2	1	5
cat7	1	1	1	1	2	2	3	5	1	1	4	2	1	1	1	2	2	na	1	2	1	5
cat8	1	1	1	1	2	2	3	5	1	1	4	2	1	1	1	2	2	na	1	2	1	5
cat9	1	1	1	2	1	2	3	5	1	3	3	2	2	4	3	1	1	2	1	2	3	6
corn1	2	2	2	1	2	2	3	5	2	1	4	2	1	3	2	2	4	2	1	2	1	2
corn2	2	2	2	1	2	1	3	5	2	1	2	1	1	3	3	2	4	na	1	2	1	2
mrh1	1	1	4	2	1	1	2	2	3	3	3	2	3	4	3	1	2	1	2	2	1	6
meh2	1	1	5	2	1	1	2	1	3	3	3	2	3	4	1	1	1	1	2	2	1	6
meh3	1	1	4	1	1	1	2	2	1	1	3	2	3	4	1	1	2	3	2	2	1	6
kur1	1	1	1	1	2	2	2	2	1	1	4	2	1	2	2	2	2	2	2	1	4	1
kur2	1	1	1	2	6	1	2	2	3	3	5	2	3	4	1	1	2	1	2	2	1	6
kur3	1	1	?	1	1	1	2	2	1	1	3	2	3	1	1	1	4	3	2	1	1	6
ole1	1	1	4	2	1	1	2	2	2	3	5	1	2	1	1	1	2	1	2	2	na	6
ole2	1	1	3	1	1	1	2	2	3	3	5	1	2	4	1	1	2	1	2	na	na	6
ole3	1	1	3	2	1	1	2	2	3	3	5	2	2	4	1	1	2	1	2	1	na	6
palm1	1	1	1	1	2	2	1	3	1	1	1	1	2	2	1	3	4	2	1	1	1	1
palm2	1	1	4	1	1	2	1	2	1	2	1	1	2	4	1	4	4	2	1	1	1	6
palm3	1	1	4	1	1	2	1	3	1	2	1	1	2	4	2	4	4	2	1	3	1	1
pali1	1	1	5	2	1	1	1	2	1	3	1	1	2	4	1	1	1	1	1	1	1	1
pali2	1	1	4	1	1	1	1	2	1	3	1	1	2	1	1	1	2	2	1	1	1	1
pali3	1	1	5	1	1	1	1	3	1	3	1	1	2	1	2	1	2	2	1	1	1	1

Table 4. Continued.

palp1	1	1	1	1	1	1	1	3	3	3	1	1	2	1	1	4	3	1	1	1	1	1
palp2	1	1	1	1	1	1	1	2	1	2	na	1	2	1	1	4	3	1	1	2	1	na
palp3	1	1	1	1	1	1	1	2	1	2	3	1	2	1	1	4	4	1	1	1	1	na
palp4	1	1	1	1	1	1	1	3	1	2	1	1	2	4	1	4	5	1	1	1	1	1
palp5	1	1	1	1	2	1	1	2	1	1	5	1	2	4	1	1	2	1	1	1	1	1
palp6	1	1	1	1	1	1	1	3	1	2	5	1	2	3	1	4	4	1	1	1	1	1
palp7	1	1	1	1	1	1	1	3	1	2	5	2	2	4	1	4	4	1	1	3	1	1
palp8	1	1	1	1	1	1	1	3	1	2	na	1	2	4	1	4	3	1	1	1	na	1
pals1	1	1	3	1	1	1	2	2	1	2	1	1	3	5	1	1	1	3	1	1	3	2
pals2	1	1	3	2	1	1	2	2	1	2	5	2	3	5	1	1	3	2	1	2	4	1
pals3	1	1	3	1	1	1	2	2	1	2	1	4	3	4	1	5	3	3	1	2	3	6
palp9	1	1	3	2	1	1	1	2	na	2	1	1	2	4	1	4	4	1	1	1	3	2
palpJ	1	1	2	1	1	1	1	2	1	3	1	1	2	1	1	4	2	1	1	1	1	6
palpL	1	1	5	1	1	1	1	2	1	2	5	2	2	5	1	4	5	1	1	1	3	6
palp10	1	1	1	2	1	1	1	2	1	1	1	2	3	1	1	4	2	1	1	1	1	6
palp11	1	1	1	1	1	1	1	2	1	1	5	1	2	1	1	4	2	1	1	1	1	6
per1	1	1	3	2	3	1	3	1	1	2	1	2	1	1	1	6	2	2	2	1	1	2
per2	1	1	3	2	3	1	3	1	1	2	1	2	1	1	3	6	2	1	2	1	1	2
per3	1	1	3	2	3	1	3	1	1	2	1	2	1	1	3	6	2	1	2	1	na	2
per4	1	1	3	2	3	1	3	1	1	2	1	2	1	1	3	6	2	1	2	1	na	2
pro	1	1	3	2	1	1	3	3	1	2	1	2	1	1	3	1	2	na	na	na	1	4
rah	1	1	1	2	2	3	3	4	1	3	2	2	1	4	1	2	4	1	1	1	3	3
spa1	1	1	1	1	1	1	3	3	1	1	1	4	1	4	1	3	1	na	1	1	1	1
spa2	1	1	5	1	5	2	1	2	1	3	5	2	1	5	1	1	5	2	1	1	1	1
spa3	1	1	2	1	5	1	1	3	1	na	5	4	2	4	1	6	2	2	1	1	1	1
spa4	1	1	5	1	5	1	1	3	1	na	5	2	1	4	1	1	2	2	1	1	1	1

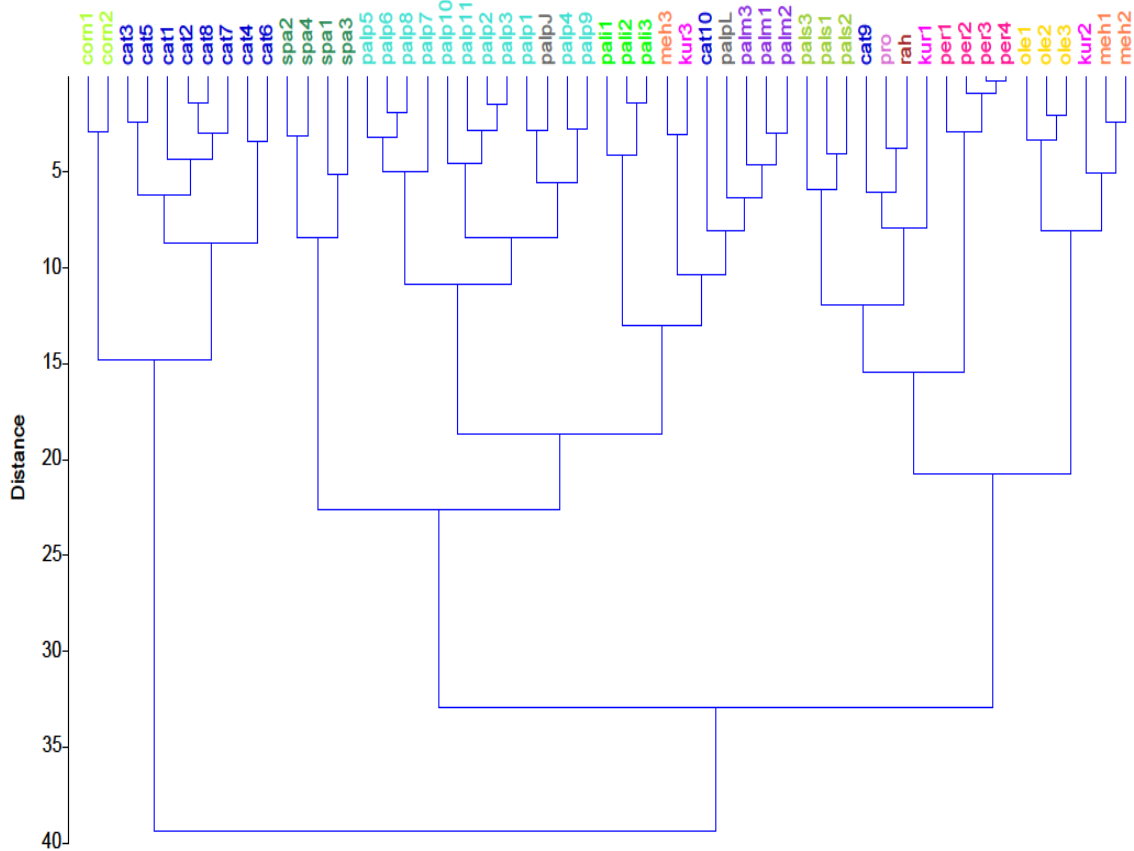


Fig. 1. Ward method phenogram of morphological characters. Populations of taxa abbreviations (the location of populations were mentioned in table 1): cat1, cat2, cat3 and cat6 = *Rh. cathartica* L. var. *cathartica*; cat4, cat5, cat7, cat8 and cat9 = *Rh. cathartica* L. var. *caucasica*; cat9 = *Rh. cathartica*; corn1 = *Rh. cornifolia* var. *cornifolia*; corn2 = *Rh. cornifolia* var. *denudata*; meh1, meh2 and meh3 = meh3 = hybrid 1; kur1, kur2 and kur3 = *Rh. kurdica*; ole1, ole2 and ole3 = *Rh. oleoides*; palm1, palm2, palm3 = subsp 1; pali1, pali2, pali3 = *Rh. pallasii* subsp. *iranica*; palp1, palp2, palp3, palp4, palp5, palp6, palp7, palp8 = *Rh. pallasii* subsp. *pallasii*; pals1, pals2, pals3, pals4 = *Rh. pallasii* subsp. *sintenisii*; palp9, palpJ, palpsd, palpsr, palpL = *Rh. pallasii*; per1, per2, per3 and per4 = *Rh. persica*; pro = *Rh. prostrata*; rah = sp1; spa1, spa2 and spa4 = *Rh. spathulifolia*.

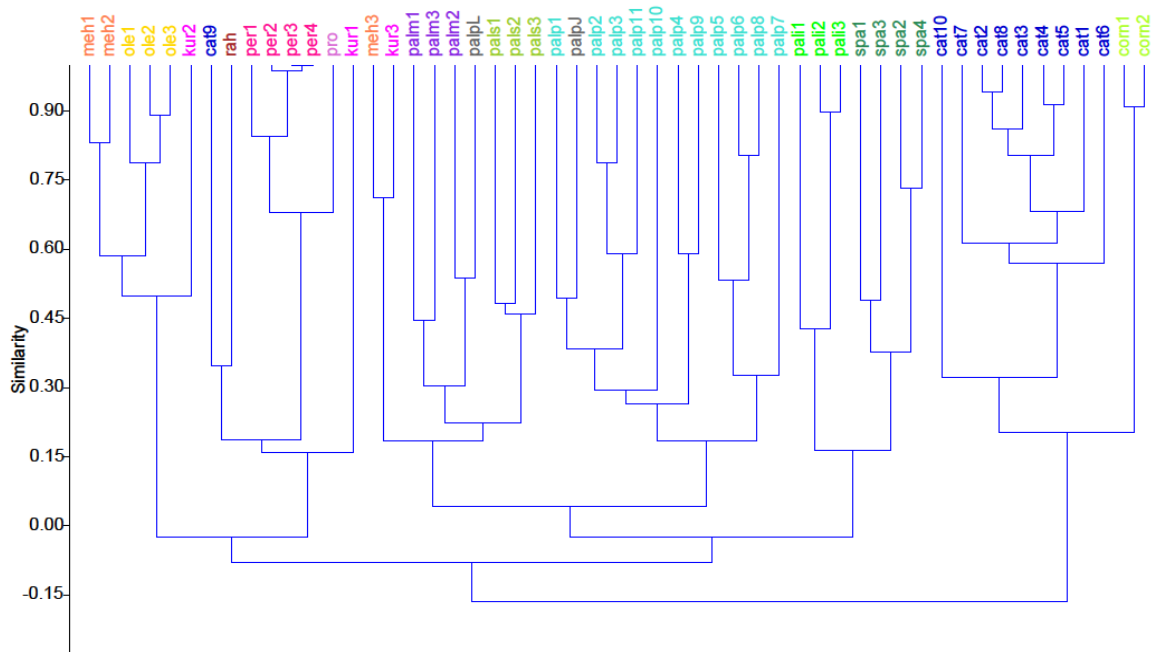


Fig. 2. UPGMA phenogram of morphological characters. Taxa abbreviations (populations were explained in table 1): cat1, cat2, cat3 and cat6 = *Rh. cathartica* L. var. *cathartica*; cat4, cat5, cat7, cat8 and cat9 = *Rh. cathartica* L. var. *caucasica*; cat9 = *Rh. cathartica*; corn1 = *Rh. cornifolia* var. *cornifolia*; corn2 = *Rh. cornifolia* var. *denudata*; meh1, meh2 and meh3 = meh3 = hybrid 1; kur1; kur2 and kur3 = *Rh. kurdica*; ole1, ole2 and ole3 = *Rh. oleoides*; palm1, palm2, palm3 = subsp 1; pali1, pali2, pali3 = *Rh. pallasii* subsp. *iranica*; palp1, palp2, palp3, palp4, palp5, palp6, palp7, palp8 = *Rh. pallasii* subsp. *pallasii*; pals1, pals2, pals3, pals4 = *Rh. pallasii* subsp. *sintenisii*; palp9, palpJ, palpsd, palpsr, palpL = *Rh. pallasii*; per1, per2, per3 and per4 = *Rh. persica*; pro = *Rh. prostrata*; rah = sp1; spa1, spa2, spa3 and spa4 = *Rh. spathulifolia*.

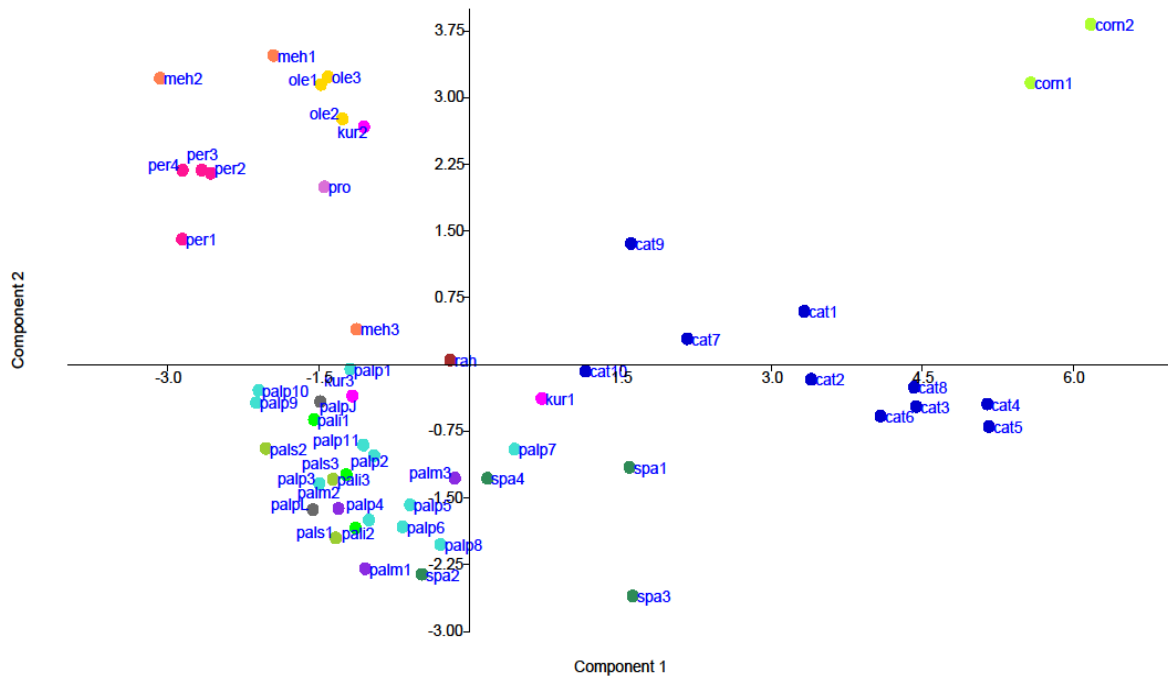


Fig. 3. PCA phenogram of morphological features. Taxa abbreviations (the location of populations is mentioned in table 1): cat1, cat2, cat3 and cat6 = *Rh. cathartica* L. var. *cathartica*; cat4, cat5, cat7, cat8 and cat9 = *Rh. cathartica* L. var. *caucasica*; cat9 = *Rh. cathartica*; com1 = *Rh. cornifolia* var. *cornifolia*; com2 = *Rh. cornifolia* var. *denudata*; meh1, meh2 and meh3 = meh3 = hybrid 1; kur1, kur2 and kur3 = *Rh. kurdica*; ole1, ole2 and ole3 = *Rh. oleoides*; palm1, palm2, palm3 = subsp 1; pali1, pali2, pali3 = *Rh. pallasii* subsp. *iranica*; palp1, palp2, palp3, palp4, palp5, palp6, palp7, palp8 = *Rh. pallasii* subsp. *pallasii*; pals1, pals2, pals3, pals4 = *Rh. pallasii* subsp. *sintenisii*; palp9, palpJ, palpsd, palpsr, palpL = *Rh. pallasii*; per1, per2, per3 and per4 = *Rh. persica*; pro = *Rh. prostrata*; rah = sp1; spa1, spa2, spa2 and spa4 = *Rh. spathulifolia*.

DISCUSSION

Qualitative characters and the mean of quantitative characters of morphological features were used to define 52 populations and recognized 14 taxa of *Rhamnus* L. in Iran (tables 1-4).

The result of Ward method, UPGMA clustering and PCA ordination were observed mostly similar among studied taxa and populations but rarely differences were detected among them (figs. 1-3).

The first principal component (PC1) scoring system demonstrated the highest variation (20.483) while PC2 indicated the 10.891 variation. Leaf shape, margin teeth, petiole length, long leaf in flowering, peduncle length in flowering, lateral nerve number, leaf form, lateral nerve arrangement, leaf shape, leaf pubescence, young branch color, stem color, spiny or not spiny characters separated the taxa of *Rhamnus*. *Rhamnus cornifolia* by having the characters of the

genus *Atadinus* is separated at generic level (fig. 3).

In Ward method clustering analysis, the populations and taxa grouped into three clusters. First cluster included two subclusters. One subcluster included the populations of *Rh. cornifolia* var. *cornifolia* and *Rh. cornifolia* var. *denudata* is mostly separated at generic level, referring to Hauenschild & al. (2016), Doweld A. B. (2017) and Rafinesque (1838), regardless of having the characters of the genus *Atadinus*. Second subclusters respectively, included the populations of *Rh. cathartica* L. var. *cathartica* (Chahar Bagh, Sang Deh, Agje, Calishum) and *Rh. cathartica* var. *caucasica* (Kavard, Siah Bisheh, Darre Makidi, Darreh Khan), are separated from the other taxa, forming a single cluster which are not mostly in agreement with previous studies (Sattarian & al. 2016).

Second cluster included four subclusters. First

subcluster included the populations of *Rh. spathulifolia* (Gadok, Mirod, Maraveh Tapeh, Siah Bisheh) and are separated from *Rh. pallasii* that has a relationship with it. This is in agreement with previous studies (Sofian, & Dinarvand 2007) and (Browicz & Zielinski 1977).

Second subcluster included the populations of *Rh. pallasii* subsp. *pallasii* (Bijar, Kouh-e Keshish, Kuzehrash, Marand, Saintstepanos, Gogje, Trian, Tu Ali, Gholi valley).

Third subcluster included the populations of *Rh. pallasii* subsp. *iranica* (Renow, Oshtorankouh, Foulad Mahalle).

Fourth subcluster included the populations of *Rh. pallasii* subsp.1 (Jirindeh, Foulad Mahalle, Karaj Dam) are separated from the populations of *Rh. pallasii* subsp. *iranica* forming a single cluster and it might be a new subspecies. *Rhamnus pallasii* subsp. *sintenisii* (Filband, Kelardasht, Sang Deh) forming a single cluster and illustrates affinity of them. The taxa of *Rh. pallasii* subsp. *sintenisii*, *Rh. pallasii* subsp. *iranica* and subsp. 1 show affinity to each other and there is a close relationship between them which is mostly in agreement with previous studies (Sofian, & Dinarvand 2007) and (Browicz & Zielinski 1977).

Third cluster included four subclusters. First subcluster included the species of *Rh. prostrata* which is closely related to *Rh. persica* and this is mostly in agreement with previous studies (Sofian, & Dinarvand 2007) and (Browicz & Zielinski 1977). Second subcluster included the populations of *Rh. persica* (Eich, Kohrouyeh, Osor, Faridel), showing a close relationship among them forming a single cluster and third subcluster included the populations of *Rh. oleoides* (Darreh Khan, Gahvareh, Naqade), showing a close relationship among them and the fourth subcluster included the populations of the hybrid 1 (Darreh Khan) and hybrid 1 (Gahvareh), that could well be new hybrids.

In fig. 2 different populations of a taxon are placed in separate clusters. This might be due to the presence of hybridisation in the population (Charles-Dominique & al. 2012) and (Gil-AD. & Reznicek 1997).

Rhamnus cornifolia var. *cornifolia* and *Rh. cornifolia* var. *denudata* having the characters of the genus *Atadinus* (Hauenschild & al. 2016; Doweld A. B. 2017; Rafinesque 1838), are separated from *Rh. cathartica* L. var. *cathartica* and *Rh. cathartica* L. var. *caucasica* forming a single cluster which is in agreement with previous studies (Sofian, & Dinarvand 2007; Browicz & Zielinski 1977).

In PCA phenogram, different populations of a taxon are placed in separate groups. There is a close relationship between *Rh. persica* and *Rh. kurdica* of

the region one, also there is a close relationship between them and specimens that could well be hybrid and we call it at present hybrid 1. It is evident from the region one in PCA phenogram, relationship between taxa is mostly in agreement with previous studies (Sofian, & Dinarvand 2007), (Browicz & Zielinski 1977) and (Hauenschild & al. 2016).

There is a close relationship between *Rh. cathartica* and *Rh. Cornifolia*, but, *Rh. cornifolia* is separated at generic level by having the characters of the genus *Atadinus*. (fig. 3).

In PCA phenogram, morphological variabilities separated the taxon defined as sp.1 from the other taxa to some extent.

Morphological characters could not group two samples of *Rh. cathartica* var. *caucasica*, i.e. Siah Bisheh (cat9) and Kavard (cat10) with the rest. All samples of *Rh. kurdica* scattered mostly into different branches, a phenomenon could imply inter-species hybridization (Charles-Dominique & al. 2012, Gil-AD. & Reznicek 1997). Samples named under sp.1, which could not be related to our known taxa, are morphologically distinct and distinguishable from the others. The results of this research are mostly in agreement with previous studies (Sofian, & Dinarvand 2007, Browicz & Zielinski 1977 and Hauenschild & al. 2016).

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