

PHYLOGENETIC RELATIONSHIPS BASED ON MORPHOLOGICAL AND ANATOMICAL CHARACTERS ON *RICOTIA* L. GENUS (BRASSICACEAE) GROWING IN TURKEY

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Abstract

The phylogenetic relation of *Ricotia* (Brassicaceae) genus, which is distributed throughout Turkey based on morphological and anatomic characters were investigated. A total of 36 characteristics of which 24 from morphological and 12 from anatomic studies were used in the cladistics analyses. Anatomical characters were studied from horizontal and superficial sections of the stems and leaves. Analysis of data set utilizing maximum parsimony criteria with Branch-and-Bound search algorithm yielded 87 parsimonious trees. Some of the branches obtained by majority rule consensus algorithm were supported by bootstrap analysis. Maximum parsimony tree formed based on morphological and anatomic data sets consisted of three clades. Clade 1 consisting of *R. tenuifolia*, *R. sinuata* and *R. carnosula* is a monophyletic group. This group was supported by a bootstrap value of 54%. Clade 2 is made up of *R. davisiana*, *R. aucheri* and *R. varians* and was supported with a bootstrap value of 54%.

Introduction

The Cruciferae (Brassicaceae) is one of the largest families of angiosperm comprising 338 genera and 3.709 species, distributed throughout the world, mainly in the temperate regions of the Northern Hemisphere (Al-Shehbaz 1984, Warwick *et al.* 2006, Kasem *et al.* 2011, Selvi and Paksoy 2013). The major centres of distribution of the family are in the Irano-Turanian, Mediterranean, and Saharo-Sindian regions (Hedge 1976). Turkey is one of the richest countries in the world in terms of the number of species of the Brassicaceae represented by 571 species, 65 subspecies, 24 varieties and 660 taxa belonging to 91 genera approximately (Al-Shehbaz *et al.* 2007). The genus *Ricotia* which includes nine species is distributed in the South East Europe, Eastern Mediterranean and adjacent Middle East (Burt 1951, Appel and Al-Shehbaz 2003; Özüdoğru *et al.* 2015). In the flora of Turkey, there are six species (*Ricotia tenuifolia* Sibth. & Sm., *Ricotia sinuata* Boiss. & Heldr., *Ricotia carnosula* Boiss. & Heldr., *Ricotia davisiana* B.L. Burt., *Ricotia varians* B.L. Burt. and *Ricotia aucheri* (Boiss.) B.L. Burt.). *Ricotia* has one of the highest rates of endemism within genera in Turkey, with five endemic species, thus a rate of endemism in the flora of Turkey of 83,3% (Davis 1985).

Previously, of the plants belonging to different families, phylogenetic studies were performed using morphological and anatomical characters. For example, Coşkun *et al.* (2010) identified phylogenetic relation on 15 *Crocus* (Iridaceae) taxa distributed throughout Turkey based on 29 morphological- and 4 anatomic characters. The analysis of the data performed using Branch-and-Bound algorithm indicated the majority of parsimonious trees that had parsimony in 32 equal ways. Some of the branches were supported by bootstrap analysis performed using majority compatibility method algorithm. Haghghi *et al.* (2014) investigated phylogenetic relation of 15 *Artemisia* taxa based on morphological characters. In the morphological studies, a cladogram was created using 17 characters. The branches found in cladogram were supported with the bootstrap

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analysis values. Sanön *et al.* (2011) performed phylogenetic analysis of the *Carlina* (Asteraceae) genus distributed throughout Turkey based on the morphological data. In the study, branch and bound trees were obtained based on the maximum parsimony criteria using 28 morphological characters and the branches were supported with the bootstrap value. Akbari *et al.* (2012) performed morphological cladistics analysis of *Astragalus* (Fabaceae) genus using species of *Dissitiflora*, *Erioceras*, *Onobrychoidei*, *Ornithopodium*, *Corethrum* Bunge, and *Cytisodes* sections that are distributed throughout Iran. They obtained strict consensus trees based on parsimony criteria using 28 morphological and vegetative characters. Later on, the tree branches obtained were supported by a bootstrap analysis. Aydın *et al.* (2013) conducted a taxonomic study on seven endemic *Centaurea* (Asteraceae) species distributed throughout Turkey using anatomic and molecular data. In the study, they obtained UPGMA tree with cluster analysis by using 13 anatomic characters. At the end of the study, it was revealed that the analyses performed with anatomical characters and molecular data were consistent with each other.

Six *Ricotia* taxa were analyzed using 24 morphological and 12 anatomical characters. Analysis of data utilizing maximum parsimony criterion with Branch-and-Bound search algorithm yielded 87 most parsimonious (MP) trees. This study provides information on phylogenetic relationships of *Ricotia* genus from Turkey utilizing morphological and anatomical characters. *Chrysochamela elliptica* (Boiss) Boiss and *Chrysochamela noeana* (Boiss) Boiss were used outgroups.

Materials and Methods

Plant specimens collected from different localities of Turkey and were submitted in Tunceli University Herbarium. Collection of data including localities and collector information for the examined specimens are given in Table 1.

Biometric measurements of vegetative and reproductive organs of six *Ricotia* taxa were performed over herbarium samples. Morphological characters obtained from biometric measurements are illustrated in Table 2.

Fresh plants were fixed in 70% alcohol. Stem and leaves of flowering plants were used in the anatomical study. Transverse sections of stem and leaves were cut manually. Tissues were stained with Phloroglucinol-HCL solutions (Yakar-Tan 1982) and chlorophyll in leaves was removed with chloral hydrate. Stomatal density on abaxial and adaxial surfaces of the leaf was counted under a light microscope. Stomatal index was calculated according to the method of Meidner and Mansfield (1968). Stomatal and leaf epidermal terminology were based on the classification proposed by Dilcher (1974) and Wilkinson (1979), respectively. Measurements and photographs were taken using Olympus BX 53 and Nikon Eclipse E200 binocular light microscopes.

Table 3 shows the characters and their stage used to form data matrix during phylogenetic analysis using PAUP* (Swofford 2001). In PAUP*, Branch-and-Bound search used the following settings: Optimality criterion = Parsimony (MP), addition sequence = furthest, multiple trees ('Multrees') option in effect, initial 'MaxTrees' setting = 100, branches collapsed (creating polytomies) if maximum branch length was zero, topological constraints were not enforced, trees were unrooted. The bootstrap analysis (Felsenstein 1985) was also performed to see how some branches were statistically supported during the phylogenetic analysis (Fig. 1B). Bootstrap support was categorized according to Kress *et al.* (2002) criteria, i.e., strong (85%), moderate (70 - 85%), weak (50 - 70%) or poor (50%) support.

Table 1. Specimens used for anatomical studies and localities of collected specimen.

Species	Collection areas and collector's number
<i>R. tenuifolia</i> Sibth & Sm.	Antalya, between Finike and Elmalı, Calcareus rocky, 390 m, 24.04.2010, Paksoy 1080
<i>R. sinuata</i> Boiss. & Heldr.	Antalya; Kemer, Tahtalı mount, Cableway station, roadsides, 100 m, 24.04.2010, Paksoy 1078
<i>R. carnosula</i> Boiss. & Heldr.	Antalya; Kemer, Göynük canyon, around Göynük creek, calcareus slopes, 10 m, 24.04.2010, Paksoy 1075
<i>R. davisiana</i> B.L. Burt	Antalya; Kemer, Tahtalı mount, Peynirlik location, 1600 m, 17.07.2010, Paksoy 1098
<i>R. varians</i> B.L. Burt	Isparta; Aksu, Dedegöl mount, Obruk plateau, 1350 m, 01.08.2010, Paksoy 1104
<i>R. aucheri</i> (Boiss.) B.L. Burt	Kahramanmaraş; Çağlayancerit, Öksüz mount, Akdut location, calcareous mobile slopes, 1200 m, 11.06.2011, Paksoy 1094
<i>C. elliptica</i> (Boiss.) Boiss	Ankara: Between Ayas and Beypazarı, before 2 km to Beypazarı, gypsum slopes, 590 m, 01.05.2005, Paksoy 1446.
<i>C. noeana</i> (Boiss.) Boiss	Sivas: Between Düzova and Dedeli viilage, Rock slopes, 1300 m, 08.06.2013, Paksoy 1482; Hafik, Tuzhisar village, rock slopes, 1000 m, 08.06.2013, Paksoy 1484

Table 2. Morphological and anatomical characters and their stages used in the phylogenetic analyses.

Characters	Character stages and comments				
	0	1	2	3	
Morphological characters					
1	Life cycle of plant	Annual	Perennial	-	-
2	Status of basal leaves	Fleshy	Not fleshy	-	-
3	Type of basal leaf	Simple	Compound	-	-
4	Shape of basal leaf	Ovate-elliptic	Pinnatifid	Trifoliate	-
5	Petiole of basal leaf	Presence	Absence (Sessile)	-	-
6	Basal leaf segments	Absence	Linear, linear-oblong to ovate	Linear, linear-oblong to ovate or trifid-pinnatisect	Elliptic-orbicular to obovate
7	Basal leaf hair	Presence	Absence	-	-
8	Stem hair	Presence	Absence	-	-
9	Status of stem leaves	Fleshy	Not fleshy	-	-
10	Type of stem leaf	Simple	Compound	-	-

(Contd.)

(Contd.)

11	Shape of stem leaf	Ovate-elliptic	Pinnatifid	Trifoliate	-
12	Petiole of stem leaf	Presence	Absence (Sessile)	-	-
13	Stem leaf segments	Absence	Linear, linear-oblong to ovate	Linear, linear-oblong to ovate or trifid-pinnatisect	Elliptic-orbicular to obovate
14	Stem leaf hair	Presence	Absence	-	-
15	Flower petal color	White	Lilac	Pale red- pink	-
16	Petal margin	Entire	Emarginate	-	-
17	Fruit types	Silicula	Siliqua	-	-
18	Shape of fruit (silicula type)	Absence	Oval	Elliptic-oval	Pear-shaped
19	Shape of fruit (siliqua type)	Absence	Linear-lanceolate	Linear-oblong	Oblong
20	Peak of the fruit	Rotund	Obtuse	-	-
21	Stylus residue	Clear	Unclear	-	-
22	Fruit hair	Presence	Absence	-	-
23	Number of seed in fruit	1	1 - 5	6 - 12	-
24	Shape of seed	Rounded	Rounded-elliptic	-	-
Anatomical characters					
25	Cortex parenchyma layers	3 - 5	6 - 10	-	-
26	Pericycle layers	1	1 - 2	2 - 4	-
27	Interfascicular bundles	Clear	Unclear	-	-
28	Pith region	Wide	Narrow	-	-
29	Mesophyll type	Bifacial	Equifacial	-	-
30	Anticlinal walls of adaxial epidermal surface	Straight	Undulate	Sinuate	-
31	Anticlinal walls of abaxial epidermal surface	Straight	Undulate	Sinuate	-
32	Trichomes	Absence	Presence	-	-
33	Sclerenchyma	Absence	Presence	-	-
34	Stomatal index (adaxial)	20 - 24	25 - 29	30 - 34	-
35	Stomatal index (abaxial)	15 - 20	21 - 26	27 - 32	-
36	Stomatal index ratio	0.6 - 0.79	0.8 - 0.99	1.0 - 1.99	-

Table 3. Data matrix of 24 morphological and 12 anatomical characters used in the cladistic analyses of 8 taxa (Ingroups and outgroups).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<i>R. tenuifolia</i>	0	1	1	1	0	1	1	1	1	1	1	0	1	1	0	0	1
<i>R. sinuata</i>	0	1	1	1	0	2	1	1	1	1	1	0	2	1	1	1	0
<i>R. carnosula</i>	0	1	1	1	0	2	1	1	1	1	1	0	2	1	1	1	0
<i>R. davisiana</i>	1	0	1	2	0	3	0	0	0	1	2	0	3	0	2	0	0
<i>R. varians</i>	1	0	1	2	0	3	1	1	0	1	2	0	3	1	2	0	1
<i>R. aucheri</i>	1	0	0	0	1	0	1	1	0	0	0	1	0	1	1	1	1
<i>C. elliptica</i>	0	1	0	0	1	0	0	0	1	0	3	1	0	1	3	2	0
<i>C. noeana</i>	0	1	0	0	1	0	0	0	1	0	3	1	0	0	3	2	0

Right side of the table

18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	0	0	1	1	1	1	0	1	1	0	0	1	1	0	0	0	0	2
0	1	0	0	1	2	0	0	1	1	0	0	2	2	1	0	0	1	1
0	2	1	0	1	2	0	0	0	1	1	0	0	1	0	0	0	1	1
0	3	1	0	0	2	1	1	0	0	0	1	0	1	1	0	0	2	0
2	0	1	1	1	1	0	1	1	0	1	1	0	0	0	0	2	2	2
3	0	0	1	1	0	0	1	2	0	0	1	0	0	0	1	2	2	2
2	0	2	1	1	2	2	0	0	0	0	0	2	2	1	0	1	1	2
2	0	2	1	1	2	2	0	1	0	1	0	1	2	0	0	1	3	1

Results and Discussion

Phylogenetic relationships among six *Ricotia* and two *Chrysochamela* taxa using 24 morphological and 12 anatomical characters were investigated. Analysis of data using maximum parsimony criteria with Branch-and-Bound search algorithm yielded 87 most parsimonious trees. Score of those MP trees are found as 87. Tree number 1 is shown in Fig. 1A. Indices and values obtained after the Branch-and-Bound search are as follows. Three variable characters were parsimony-uninformative whereas 33 characters were parsimony-informative. Branch- and Bound tree formed using 24 morphological and 12 anatomic characters consisted of 2 clades (Fig. 1A). Clade 1 consisted of *R. tenuifolia*, *R. sinuata* and *R. carnosula* (Fig. 1A). Bootstrap analysis supported this monophyletic group with a value of 54%. (Fig. 1B). Selvi and Paksoy (2013), conducted comparative and anatomy of stem and leaf of *Ricotia* growing in Turkey. As a result of study, mesophyll tissue is important anatomical character for *Ricotia* species. *R. tenuifolia*, *R. sinuata* and *R. carnosula* are bifacial (dorsiventral); while *R. davisiana*, *R. varians* and *R. aucheri* are equifacial (isobilateral). This result was supported by members of clade 1. Within clade 1, *R. sinuata* and *R. carnosula* created a sister group and this branch was supported with a value of 63% (Fig. 1A). *R. tenuifolia* was found to be close to this group (Fig. 1A, B). Clade 2 is a group consisting of *R. davisiana*, *R. varians* and *R. aucheri* species. This branch was supported with a low bootstrap value of 54% (Fig. 1B). According to Selvi and Paksoy (2013), mesophyll characters for *R. davisiana*, *R. varians* and *R. aucheri* are equifacial. This result was supported by

members of clade 2. Within the branch, *R. varians* and *R. davisiana* created a sister group, this branch was supported 52% and *R. aucheri* was found to be close to this group (Fig. 1A, B).

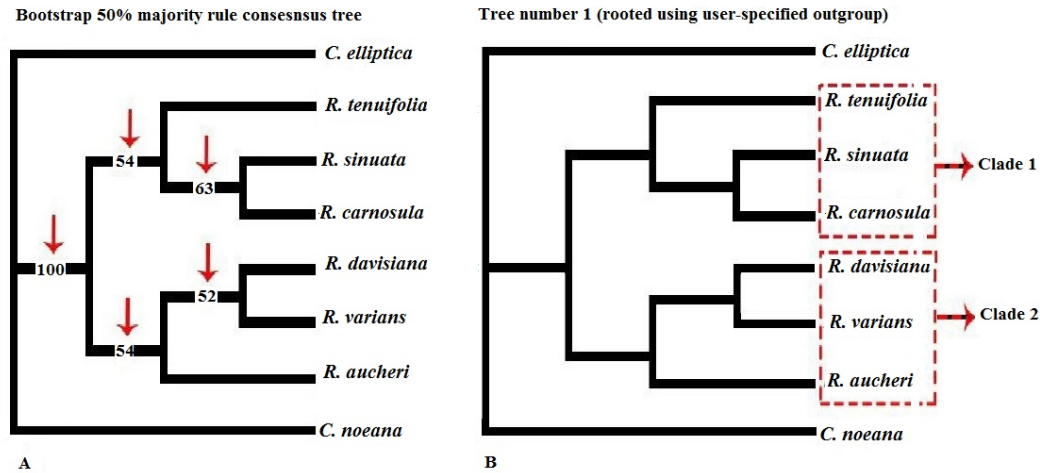


Fig. 1. A. Bootstrap method tree generated using trees based on morphological and anatomical characters of genus *Ricotia*. B. One of the 87 most parsimonious trees of *Ricotia* based on morphological and anatomical characters shown in Table 3 using Branch-and-Bound search algorithm.

The maximum parsimony trees based on morphological and anatomic data were found to be suitable and helped gain insight into the morphophylogenetics of *Ricotia* genus.

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