Anatolian Journal of Botany

Ant J Bot

e-ISSN 2602-2818

***2** (1) (2018) - Anatolian Journal of Botany*

Anatolian Journal of Botany

Anatolian Journal of Botany

e-ISSN 2602-2818

Volume 2, Issue 1, Year 2018

Published Biannually

Owner
Prof. Dr. Abdullah KAYA

Corresponding Address
Karamanoğlu Mehmetbey University, Kamil Özdağ Science Faculty, Department of Biology, 70100, Karaman – Turkey
Phone: (+90 338) 2262156
E-mail: anatolianjbot@gmail.com
Web: http://dergipark.gov.tr/ajb

Anatolian Journal of Botany

Anatolian Journal of Botany
e-ISSN 2602-2818
Volume 2, Issue 1, Year 2018

Contents

Anatolian Journal of Botany

Anatolian Journal of Botany
e-ISSN 2602-2818
Volume 2, Issue 1, Year 2018

Referees (of this issuue)

Prof. Dr. Ahmet AKSOY	Akdeniz University, Science Faculty, Dept. of Biology, Antalya
Prof. Dr. Erol YILDIRIM	Atatürk University, Agriculture Faculty, Dept. of Plant Protection, Erzurum
Prof. Dr. Ertuğrul SESLİ	Karadeniz Technical Univ., Fatih Ed. Fac., Dept. of Sci. and Math., Trabzon
Prof. Dr. Esra MARTİN	Necmettin Erbakan Univ., Science Faculty, Dept. of Biotechnology, Konya
Prof. Dr. Gıyasettin KAŞIK	Selçuk University, Science Faculty, Department of Biology, Konya
Prof. Dr. Hasan TÜRKEZ	Erzurum Techn. Univ., Science Faculty, Dept. of Mol. Bio. & Genetics, Erzurum
Prof. Dr. İbrahim TÜRKEKUL	Gaziosmanpaşa Univ., Science and Arts Fac., Dept. of Biology, Tokat
Assoc. Prof. Dr. Aslı DOĞRU KOCA	Hacettepe Univ., Science Faculuty, Dept. of Biology, Ankara
Assoc. Prof. Dr. Birol MUTLU	İnönü University, Science Faculty, Dept. of Biology, Malatya
Assoc. Prof. Dr. Feyza CANDAN	Celal Bayar Univ, Science & Arts Faculty, Dept. of Biology, Manisa
Assoc. Prof. Dr. Gökhan SADİ	Karamanoğlu Mehmetbey Univ., Science Faculty, Dept. of Biology, Karaman
Assoc. Prof. Dr. M. Bilgehan PEKTAŞ	Afyon Kocatepe Univ., Medicine Fac., Dept. of Pharmacology, Afyon
Assoc. Prof. Dr. Mesut KIRMACI	Adnan Menderes Univ., Science & Arts Fac. Dept. of Biology, Aydın
Assoc. Prof. Dr. Murat ÜNAL	Yüzüncü Yıl University, Education Faculty, Dept. of Science & Math., Van
Assist. Prof. Dr. Başak TOGAR	Bayburt Univ., Vocational Sch. Of Health Serv., Dept of Medical Serv., Bayburt
Assist. Prof. Dr. Eda GÜNEŞ	Necmettin Erbakan Univ., Tourism Fac. Dept. of Gastronomy, Konya
Assist. Prof. Dr. Hakan ALLI	Muğla University, Science & Arts Faculty, Dept. of Biology, Muğla
Dr. Barış ÖZÜDOĞRU	Hacettepe Univ., Science Faculuty, Dept. of Biology, Ankara
Dr. Hayrunnisa Baş SERMENLİ	Muğla University, Science & Arts Faculty, Dept. of Biology, Muğla
Dr. Mustafa Emre AKÇAY	Yüzüncü Yıl University, Science Faculty, Department of Biology, Van
Dr. Nur Banu BAL	Gazi Üniversity, Pharmacy Faculty, Dept. of Pharmacology, Ankara
Dr. Ömer Faruk ÇOLAK	Süleyman Demirel Univ., Isparta Vocational Sch. of Health Serv., Isparta

Anatolian Journal of Botany
2 (1): 1-9 (2018)
doi:10.30616/ajb.357059

Karyotype analysis of some lines and varieties belonging to *Carthamus tinctorius* L. species

Tuna UYSAL[1]*, Betül Sena TEKKANAT[1], Ela Nur ŞİMŞEK SEZER[1], Rahim ADA[2], Meryem BOZKURT[1]
[1]*Selçuk University, Science Faculty,Biology Department, Konya,Turkey*
[2]*Selçuk University, Agriculture Faculty,Biology Department, Konya,Turkey*
**tuysal@selcuk.edu.tr*

Received : 22.11.2017
Accepted : 06.01.2018

Carthamus tinctorius L. türüne ait bazı hat ve çeşitlerin karyotip analizi

Abstract: In this paper, karyology of thirty one accessions of Safflower (*Carthamus tinctorius* L.) were investigated in terms of their chromosome numbers and karyomorphology. The chromosomal counts confirmed the results of previous reports, that the *Carthamus* has same basic chromosome numbers. According to our results discussed all accessions of chromosome numbers have been identified 2n = 24, x=12 and also all have the diploid number of chromosomes. We found predominance of chromosomes being metacentric and sub-metacentric in karyotypes. Five quantitative asymmetry indices were used to evaluate our karyological results in all species and elucidate the chromosomal alterations of *Carthamus tinctorius* accessions. All karyotyping analyses were described for the first time in this report via KAMERAM programme. We hope that these findings would be contributed for *Carthamus* genetic and breeding studies.

Key words: Safflower, Karyomorphology, Asteraceae.

Özet: Bu çalışmada 31 Aspir (*Carthamus tinctorius* L.) çeşidine ait kromozom sayıları ve karyomorfolojileri araştırılmıştır. Kromozom sayımları, *Carthamus'un* aynı temel kromozom sayılarına sahip olduğunu gösteren daha önceki raporları doğrulamıştır. Elde edilen sonuçlara göre, kromozom sayıları tüm çeşitler için 2n = 24, x = 12 olarak tanımlanmış ve aynı zamanda tümünün diploid kromozom sayısına sahip oldukları bulunmuştur. Karyotiplerde metasentrik ve submetasentrik olan kromozomların baskın olduğu tespit edilmiştir. Tüm çeşitleri karyolojik açıdan değerlendirmek ve *C. tinctorius* çeşitleri arasındaki kromozomal değişikliklerini aydınlatmak için beş adet kantitatif asimetri indeksi kullanılmıştır. Tüm karyotip analizleri ilk kez bu raporda KAMERAM programı ile tanımlanmıştır. Çalışmada elde edilen bulguların *Carthamus* türünün genetik ve ıslah çalışmalarına katkıda bulunmasını umuyoruz.

Anahtar Kelimeler: Aspir, Karyomorfoloji, *Asteraceae*

1. Introduction

Carthamus L. genus consists of about 25 main species in the world. *Carthamus tinctorius*, commonly called safflower, is the only cultivated species of this genus (Anjali and Srivastava, 2012). Safflower, *C. tinctorius* L., is a member of the family Compositae or Asteraceae, cultivated mainly for its seed, which is used as edible oil and as birdseed. Traditionally, the crop was grown for its flowers, used for colouring and flavouring foods and making dyes, especially before cheaper aniline dyes became available, and in medicines (Dajue and Hans-Henning, 1996). Safflower is one of humanity's oldest crops, but generally it has been grown on small plots for the grower's personal use and it remains a minor crop with world seed production around 800.000 t per year (Gyulai, 1996). Oil has been produced commercially and for export for about 50 years, first as an oil source for the paint industry, now for its edible oil for cooking, margarine and salad oil.

As far as we know *Carthamus* species have different chromosome numbers. Cassini (1819) and De Candolle (1838) classified the species of *Carthamus* into two genera, *Carthamus* and Kentrophyllum and after Knowles (1958) divided the genus into four taxonomic sections on the basis of chromosome numbers. Section I, II, III and IV contained taxa with 2n = 24, 2n = 20, 2n = 44, and 2n = 64, respectively (Sehgal et al., 2009). The species having 10 pairs of chromosomes are characterized by a preponderance of purple, blue and pink flowers and include *C. boissieri*, *C. dentatus*, *C. glaucus*, *C. leucocaulos* and *C. tenuis* (López-González, 1989). The only species with 11 pairs of chromosomes is *C. divaricatus* which has a very restricted range in Libya (Knowles, 1988). *C. tinctorius*, *C. alexandrius*, *C.syriacus* and *C. tenuis* have diploid chromosome number as 2n = 24. *C. lanatus* L., a tetraploid species with 22 pairs of chromosomes, occurs naturally in Portugal, Spain, Morocco, Greece and Turkey (2n=44). In addition a hexaploid member of *Carthamus* species, *C. baeticus* has 2n = 64 chromosome number.

In plant taxonomy, chromosome karyotypes can be useful in genetic studies and breeding information about species identification and analysis of hybrid populations (Anjali and Srivastava, 2012). Further studies were therefore undertaken to elucidate chromosomal details with the following objectives: (i) to determining the karyotypes of the populations, according the shape, size and number of chromosomes, (ii) classification of the populations with regard to karyotype evolution, and (iii) determining the parents for intersections, to obtain maximum diversity for the next generation (Yazdani, 2013). As far as we know, there have been many studies about karyology of *Carthamus tinctorius* (Yenice and Bayraktar 1996; Anjali and Srivastava 2009a, 2009b, 2009c).

Within this study we aimed to carried out the chromosome number and calculate chromosomal indices of

economically important thirty one *Carthamus* lines and varieties.

2. Materials and Method

The research material, different lines and varieties of *C. tinctorius* L. were provided by Ass. Prof. Dr. Rahim ADA. Some varieties used in the study were derived from patented plant genetic resources. Thirthy one samples were genotyped in this study. Code of some lines and varieties of Safflower species are given in Table 1.

Sample number	Sample code	Sample ID
1	**Tcar 1**	Black sun 2
2	**Tcar 2**	J-41
3	**Tcar 3**	CB
4	**Tcar 4**	F4
5	**Tcar 5**	KS03
6	**Tcar 6**	KS07
7	**Tcar 7**	CT-8-3
8	**Tcar 8**	G8
9	**Tcar 9**	J-19
10	**Tcar 10**	A13
11	**Tcar 11**	A29
12	**Tcar 12**	H3
13	**Tcar 13**	E12
14	**Tcar 14**	C12
15	**Tcar 15**	J51
16	**Tcar 16**	G16
17	**Tcar 17**	Black sun 1
18	**Tcar 18**	F6
19	**Tcar 19**	C11
20	**Tcar 20**	Y 11-8-14-1
21	**Tcar 21**	A30
22	**Tcar 22**	F5
23	**Tcar 23**	J29
24	**Tcar 24**	C 2-8-1
25	**Tcar 25**	E5
26	**Tcar 26**	H7
27	**Tcar 27**	H14
28	**Tcar 28**	Dinçer
29	**Tcar 29**	Remzibey
30	**Tcar 30**	E1
31	**Tcar 31**	KS06

Table 1. The codes of *Carthamus* varieties and lines.

Particularly, mature seeds were selected and periodically germinated for chromosomal analyses. Somatic chromosomes were studied in root tips obtained from germinating seeds, which were pretreated in hydroxinoline for a while, then fixed in 3:1 absolute ethanol: glacial acetic acid overnight, stored in fixative and stained using the Feulgen technique, and squashed in 2% aceto-orcein. Slides were made permanent in Euparal by mean of Bowen's method (1956). Minimum of ten metaphase cells were selected for preparing the karyotype. The best metaphase plates were photographed (100 x) with a digital camera (Olympus DP-72) mounted on an Olympus BX53 microscope). Karyotyping analyses were carried out via KAMERAM chromosomal analyses software and to measure chromosome parameters such as Total haploid complement length (TCL), longest chromosome length (LC), shortest chromosome length (SC), mean length of the long short arm (p), mean length of the long arm (q), mean centrometric index (CI), karyotype asymmetry index (AI), coefficient of variation of chromosome length (CVCL) and coefficient of variation of centromeric index (CVCI). Chromosome nomenclature followed Levan et al. (1964), the symbols mc, smc and st designating metacentric, submetacentric and subtelocentric chromosomes, respectively (Table 2 and 3). The chromosomes were assorted into different categories on the basis of arm's ratio following Levan et al (1964) (m=1.0-1.7, sm=1.7-3.0, st=3.0-7.0).

3. Results and Discussion

Chromosomes usually give very important informations about plant taxonomy. As a result of our cytogenetic examinations, mitotic metaphase chromosome numbers, karyotype analysis, idiograms and chromosomal indices were determined for 31 *Carthamus* varieties. The karyotype formulas, asymmetry index (AI) values and the other karyotype parameters are given in Table 2 and 3.

All of the studied varieties and lines have a chromosome number of 2X=2n=24 as supported with the earlier studies (Knowles 1988, Anjali and Srivastava, 2012, Yazdani 2013). On the whole, karyotypes of the most analysed varieties had a predominance of metacentric (mc) chromosomes. The most common formula among analysed specimens was determined to be 18 m+ 6 sm (eleven species). The other karyotype formulas were various, followed in 20m + 4sm (eight species), 22m + 2sm (eight species) and 24m (three species) and 10m + 14sm (one species).

The karyotype analysis revealed that there was no sub-telocentric chromosome in any of the thirty one accessions of *C. tinctorius*. When we evaluate varieties in point of their chromosome sizes, it is seen that very small size chromosomes range from 1.14 µm – 1.9 µm. Sheidai et al. (2009), were performed karyotype and meiotic studies in thirty-seven cultivars of *C. tinctorius* grown in Iran and their findings are suitable with our results in terms of the predominance of metacentric chromosomes but their chromosome sizes ranged between 1.55 to 4.63 µm.

Total chromosome lengths (TCL) of the studied samples were between 27.304 and 45.534 µm. Tcar 13 (E12) had larger chromosomes as well as a larger genome than other samples. Anjali and Srivastava (2012) investigated karyomorphological features of twelve accessions of *C. tinctorius*. Their total chromosome lengths range from 38.79 – 55.56 µm. Their chromosome sizes were moderately larger than our samples. Among the different samples of *C. tinctorius* in the present study all the accessions having maximum number of metacentric chromosomes may be evaluated as the most primitive. In terms of chromosomal indices and analyses, Tcar 18 (F6), Tcar 21 (A30) and Tcar 29 (Remzibey) safflower types have the lowest chromosomal variation; all of them have only metacentric chromosomes. So, they considered as primitive types. At the same time these three varieties asymmetry index is quite low and in terms of selection they have high potential to be used as rootstock in the hybridization studies. Particularly, the commercial Remzibey variety may be evaluated a good rootstock for inbreedings. Both of them are quitely close to each other because of the based on the same genetic source from Turkey.

On the other hand karyotype asymmetry is one of the important standards for evaluating evolutionary relationships (Li and Chen, 1985). Karyotype asymmetry is a good expression of the general morphology of plant

karyotypes, which has been frequently related to evolution in higher plants (Stebbins, 1971). According to our results, AI values ranged from 0.443 to 2.401. When we evaluate

Table 2. Karyotype formula according to Levan et al. (1964) and characteristic parameters of the studied varieties. R-range; SC-the shortest chromosome length; LC- the longest chromosome length; p-mean length of the short arm; q- mean length of the long arm; CL-mean length of the chromosome; TCL- the total chromosome length; CI-mean centromeric index; CF- chromosome formula; m-metacentric; sm-submetacentric; SD-standard deviation.

Sample code	2n	R (SC – LC) (µm)	Ratio LC/SC	p (µm) Ort±SD	q (µm) Ort±SD	CL (µm) Ort±SD	TCL (µm)	CI Ort±SD	CF
TCAR1	24	1,19 - 1,86	1,556	0,63 (±0,10)	0,88 (±0,16)	1,51 (±0,22)	36,216	42 (±0,04)	20,m + 4,sm
TCAR2	24	1,12 - 1,51	1,345	0,56 (±0,04)	0,75 (±0,09)	1,31 (±0,10)	31,426	43 (±0,04)	20,m + 4,sm
TCAR3	24	1,11 - 1,83	1,641	0,57 (±0,08)	0,80 (±0,12)	1,37 (±0,17)	32,882	41 (±0,04)	18,m + 6,sm
TCAR4	24	0,94 - 1,89	2,005	0,58 (±0,07)	0,85 (±0,19)	1,43 (±0,25)	34,294	41 (±0,04)	18,m + 6,sm
TCAR5	24	0,97 - 1,41	1,465	0,49 (±0,06)	0,69 (±0,09)	1,18 (±0,13)	28,212	41 (±0,02)	22,m + 2,sm
TCAR6	24	1,11 - 1,73	1,561	0,58 (±0,07)	0,83 (±0,14)	1,42 (±0,16)	34,044	41 (±0,05)	18,m + 6,sm
TCAR7	24	0,95 - 1,86	1,952	0,62 (±0,14)	0,83 (±0,18)	1,45 (±0,25)	34,92	43 (±0,06)	20,m + 4,sm
TCAR8	24	1,17 - 1,70	1,455	0,60 (±0,07)	0,85 (±0,13)	1,45 (±0,15)	34,776	41 (±0,04)	20,m + 4,sm
TCAR9	24	1,00 - 1,54	1,537	0,54 (±0,08)	0,70 (±0,09)	1,24 (±0,16)	29,702	44 (±0,03)	22,m + 2,sm
TCAR10	24	0,90 - 1,42	1,57	0,50 (±0,08)	0,64 (±0,08)	1,14 (±0,14)	27,304	44 (±0,04)	22,m + 2,sm
TCAR11	24	1,19 - 1,59	1,338	0,57 (±0,07)	0,81 (±0,10)	1,38 (±0,12)	33,086	42 (±0,04)	18,m + 6,sm
TCAR12	24	1,35 - 1,96	1,454	0,66 (±0,09)	0,93 (±0,11)	1,60 (±0,17)	38,306	42 (±0,04)	20,m + 4,sm
TCAR13	24	1,56 - 2,33	1,487	0,73 (±0,09)	1,17 (±0,22)	1,90 (±0,24)	45,534	39 (±0,05)	10,m + 14,sm
TCAR14	24	1,26 - 1,82	1,44	0,66 (±0,09)	0,91 (±0,11)	1,57 (±0,17)	37,63	42 (±0,04)	20,m + 4,sm
TCAR15	24	1,37 - 2,08	1,525	0,71 (±0,11)	0,99 (±0,15)	1,69 (±0,21)	40,61	42 (±0,04)	22,m + 2,sm
TCAR16	24	0,99 - 1,61	1,617	0,53 (±0,08)	0,78 (±0,11)	1,31 (±0,16)	31,534	41 (±0,03)	18,m + 6,sm
TCAR17	24	1,11 - 1,50	1,358	0,55 (±0,09)	0,73 (±0,07)	1,28 (±0,13)	30,648	43 (±0,04)	18,m + 6,sm
TCAR18	24	0,96 - 1,47	1,539	0,51 (±0,08)	0,67 (±0,08)	1,19 (±0,14)	28,452	43 (±0,03)	24,m
TCAR19	24	1,06 - 1,86	1,76	0,64 (±0,12)	0,88 (±0,18)	1,52 (±0,25)	36,454	42 (±0,05)	18,m + 6,sm
TCAR20	24	1,56 - 2,20	1,413	0,76 (±0,11)	1,07 (±0,14)	1,83 (±0,19)	44,036	41 (±0,04)	18,m + 6,sm
TCAR21	24	1,04 - 1,51	1,456	0,57 (±0,06)	0,71 (±0,08)	1,28 (±0,14)	30,606	45 (±0,02)	24,m
TCAR22	24	1,18 - 1,74	1,47	0,61 (±0,10)	0,84 (±0,14)	1,45 (±0,15)	34,846	42 (±0,06)	20,m + 4,sm
TCAR23	24	0,91 - 1,82	2,002	0,62 (±0,12)	0,86 (±0,18)	1,48 (±0,22)	35,524	42 (±0,06)	18,m + 6,sm
TCAR24	24	1,02 - 1,59	1,566	0,55 (±0,06)	0,73 (±0,12)	1,28 (±0,15)	30,83	43 (±0,03)	22,m + 2,sm
TCAR25	24	1,03 - 1,58	1,537	0,55 (±0,06)	0,70 (±0,14)	1,25 (±0,16)	29,988	44 (±0,05)	22,m + 2,sm
TCAR26	24	1,16 - 2,14	1,836	0,71 (±0,11)	0,97 (±0,18)	1,68 (±0,26)	40,422	42 (±0,04)	22,m + 2,sm
TCAR27	24	1,12 - 1,61	1,437	0,60 (±0,07)	0,75 (±0,11)	1,35 (±0,14)	32,42	45 (±0,04)	22,m + 2,sm
TCAR28	24	1,36 - 2,12	1,563	0,71 (±0,13)	1,00 (±0,17)	1,71 (±0,24)	41,086	42 (±0,05)	18,m + 6,sm
TCAR29	24	1,02 - 1,59	1,555	0,55 (±0,07)	0,68 (±0,10)	1,24 (±0,17)	29,676	45 (±0,02)	24,m
TCAR30	24	1,22 - 2,12	1,73	0,65 (±0,12)	1,00 (±0,18)	1,65 (±0,24)	39,592	40 (±0,05)	18,m + 6,sm
TCAR31	24	1,07 - 2,06	1,932	0,67 (±0,10)	0,89 (±0,24)	1,55 (±0,30)	37,224	43 (±0,05)	20,m + 4,sm

Table 3. Karyotypes of *Carthamus* varieties using different methods of evaluating karyotype asymmetry. A1-intrachromosomal asymmetry index; A2-interchromosomal asymmetry index; CV_{CL}-relative variation in chromosome length; CV_{CI}-relative variation in centromeric index; AI-karyotype asymmetry index; DI-dispersion index; Stebbins' types-classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971).

Sample code	A_1	A_2	CV_{CL}	CV_{CI}	AI
TCAR1	0,27	0,147	14,656	10,375	1,52
TCAR2	0,244	0,08	8,001	8,367	0,669
TCAR3	0,285	0,126	12,575	9,117	1,147
TCAR4	0,286	0,172	17,239	9,614	1,657
TCAR5	0,294	0,114	11,383	5,537	0,63
TCAR6	0,283	0,113	11,304	10,872	1,229
TCAR7	0,226	0,172	17,244	13,924	2,401
TCAR8	0,29	0,105	10,52	9,445	0,994
TCAR9	0,222	0,129	12,947	6,529	0,845
TCAR10	0,208	0,125	12,498	7,975	0,997
TCAR11	0,281	0,091	9,063	10,241	0,928
TCAR12	0,283	0,107	10,74	8,696	0,934
TCAR13	0,355	0,127	12,733	13,67	1,741
TCAR14	0,272	0,108	10,76	9,089	0,978
TCAR15	0,272	0,124	12,382	10,414	1,289
TCAR16	0,312	0,123	12,289	7,497	0,921
TCAR17	0,243	0,101	10,132	10,038	1,017
TCAR18	0,232	0,119	11,931	7,068	0,843
TCAR19	0,26	0,164	16,376	12,236	2,004
TCAR20	0,283	0,103	10,31	10,563	1,089
TCAR21	0,191	0,108	10,767	4,117	0,443
TCAR22	0,241	0,106	10,586	13,595	1,439
TCAR23	0,252	0,15	14,96	14,787	2,212
TCAR24	0,241	0,121	12,052	6,795	0,819
TCAR25	0,192	0,129	12,883	10,914	1,406
TCAR26	0,257	0,154	15,408	9,155	1,411
TCAR27	0,186	0,101	10,065	9,926	0,999
TCAR28	0,278	0,143	14,262	11,493	1,639
TCAR29	0,183	0,137	13,719	3,366	0,462
TCAR30	0,334	0,143	14,312	13,858	1,983
TCAR31	0,216	0,194	19,385	11,74	2,276

the varieties in terms of AI, Tcar 7 (CT-8-3) had the most asymmetric chromosomes. This asymmetry index of wild accession is 2.401 and higher than patented types. High asymmetry index values indicate the high levels of karyotypic heterogeneity sourced from extensive chromosomal alterations and it correlates with the potential for yield. A similar situation applies in Tcar 31 (KS06) and Tcar 23 (J29), they have high asymmetry indices 2.276 and 2.212 respectively and their karyotype formulas contain various types of chromosomes. These two varieties could be evaluated as having high chromosomal variation but certainly they are not possible to use more for hybridisations or rootstock.

In addition, Tcar 13 (E12) has a unique karyotype formula with 10m + 14sm chromosomes. This type's asymmetry index is 1.741. Cross-cultivations can be made between varieties to make this variety most efficient in terms of all features.

According to the A1 index, while 28 species with symmetric karyotypes had A1 values ranging from 0.183 to 0.29, the remaining three species with asymmetric karyotypes had higher A1 values, varying from 0.312 to 0.355. When we consider the A2 values of studied samples, they displayed a low variation level ranging from 0.08 to 0.194. Our results compatible with the other studies related with karyomorphology of *C. tinctorius*. Yazdani et al., (2013) studied seven *C. tinctorius* populations and their A1 values, varying from 0.36 to 0.46 and A2 values ranging from 0.13 to 0.18 respectively.

The CV_{CI} index evaluates differences in centromere position for each chromosome in the karyotype and provides a measure of intrachromosomal asymmetry. In contrast the CV_{CL} gives a measure of interchromosomal asymmetry as it reflects how variable the chromosome sizes are in the karyotype (Peruzzi, 2009). Our CV_{CI} values are between 3.366- 14.787. CV_{CL} values range from 8.001 to 19.385. In both situations, the larger value indicate the greater the asymmetry in the karyotype. So when we evaluated our specimens in terms of these values, they consist of mostly symmetric chromosomes.

Population diversity existed with regards to the number of satellites and their positions on the chromosomes (Yazdani et al., 2013). In this study, satellites were observed at seventeen samples (Tcar 1, 7, 8, 10, 12, 14, 15, 19, 20, 22, 23, 25, 26, 27, 28, 30, 31) at various chromosome pairs (Table 4).

When we evaluate satellite locations of the studied species, Tcar 7, 23 and 30 have satellite at fourth chromosome pairs. They collected from same locality and asymmetry indices are close to each other. Therefore, we can say easily that these accessions are belonging to same origin or one species. On the other hand, Tcar 1 and 31 are American originated varieties and they share same chromosome formula. They also have satellites but at different chromosome pairs. We concluded that satellites are valuable chromosomal markers; however, they are not always to be held responsible in determining the chromosomal origin.

According to our karyological and karyotype analyses of *Carthamus* types and varieties we have been found cytotypes have a very high potential in terms of selection and hybridization studies.

Table 4. Satellite locations of the chromosomes.

Sample code	Sample ID	Satellite numbers	Satellite position	Chromosome ocation of satellite
Tcar 1	Black sun 2	1	Third chromosome	m
Tcar 7	CT-8-3	1	Fourth chromosome	m
Tcar 8	G8	1	Sixth chromosome	m
Tcar 10	A13	1	Seventh chromosome	m
Tcar 12	H3	1	First chromosome	m
Tcar 14	C12	2	First and sixth chromosomes	m
Tcar 15	J51	2	First and sixth chromosomes	m
Tcar 19	C11	1	Second chromosome	m
Tcar 20	Y 11-8-14-1	2	First and fifth chromosomes	m
Tcar 22	F5	1	Fifth chromosome	m
Tcar 23	J29	1	Fourth chromosome	m
Tcar 25	E5	2	Fourth and seventh chromosomes	m
Tcar 26	H7	1	Second chromosome	m
Tcar 27	H14	1	Third chromosome	m
Tcar 28	Dinçer	1	Third chromosome	m
Tcar 30	E1	1	Fourth chromosome	m
Tcar 31	KS06	1	Fifth chromosome	m

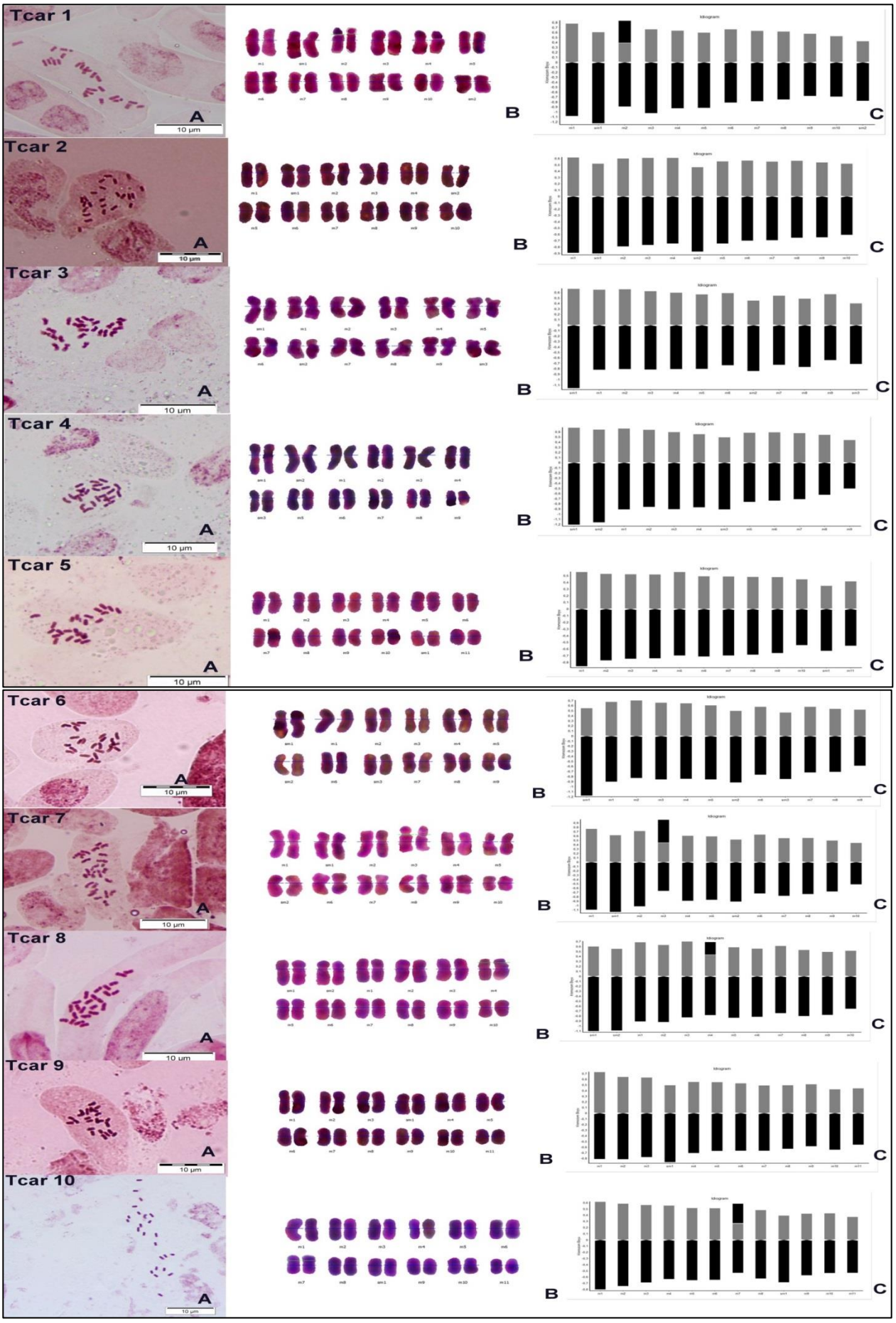

Figure 1. The metaphasis plates (A), karyograms (B) and idiograms (C) of studied samples

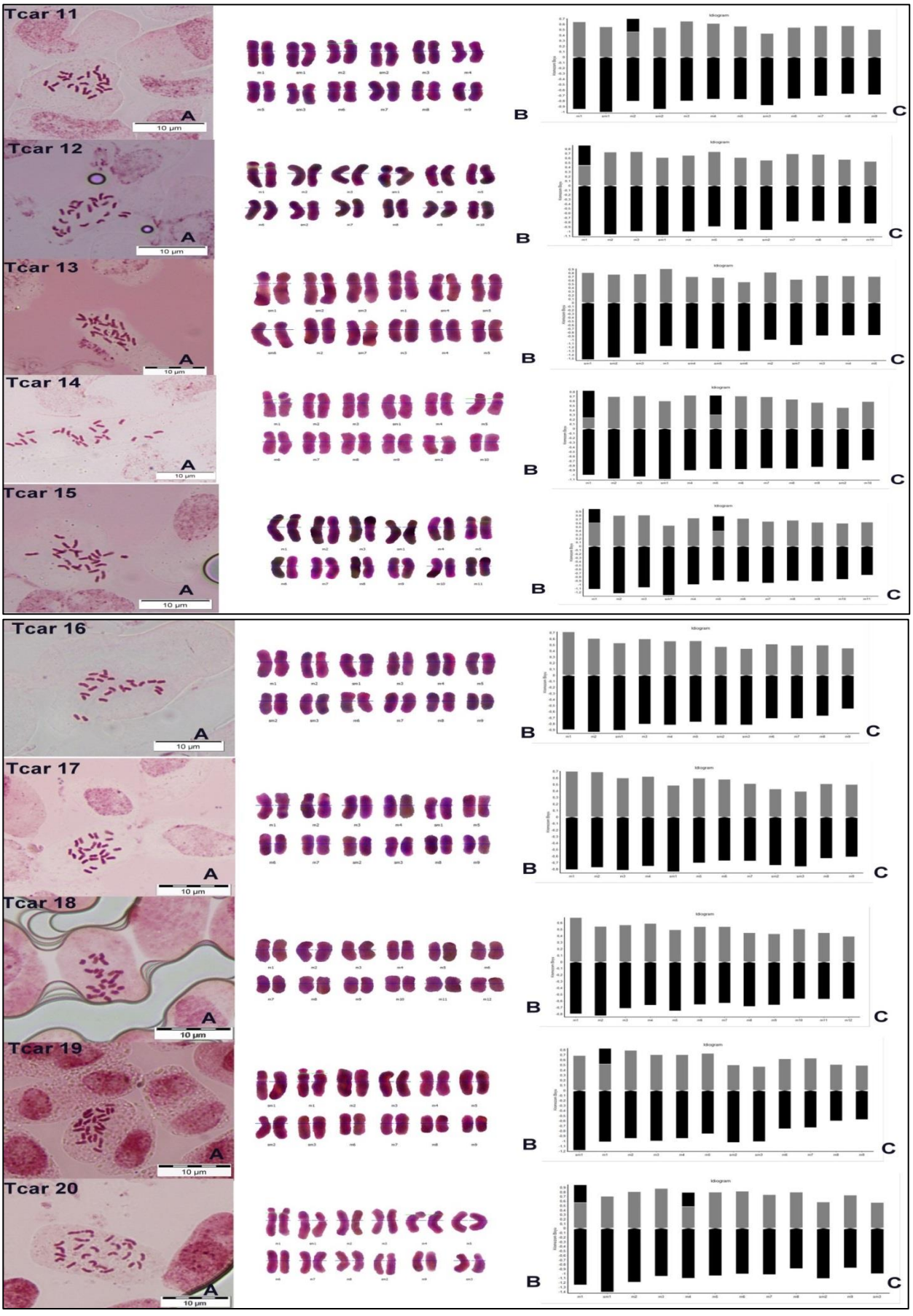

Figure 1(cont.). The metaphasis plates (A), karyograms (B) and idiograms (C) of studied samples

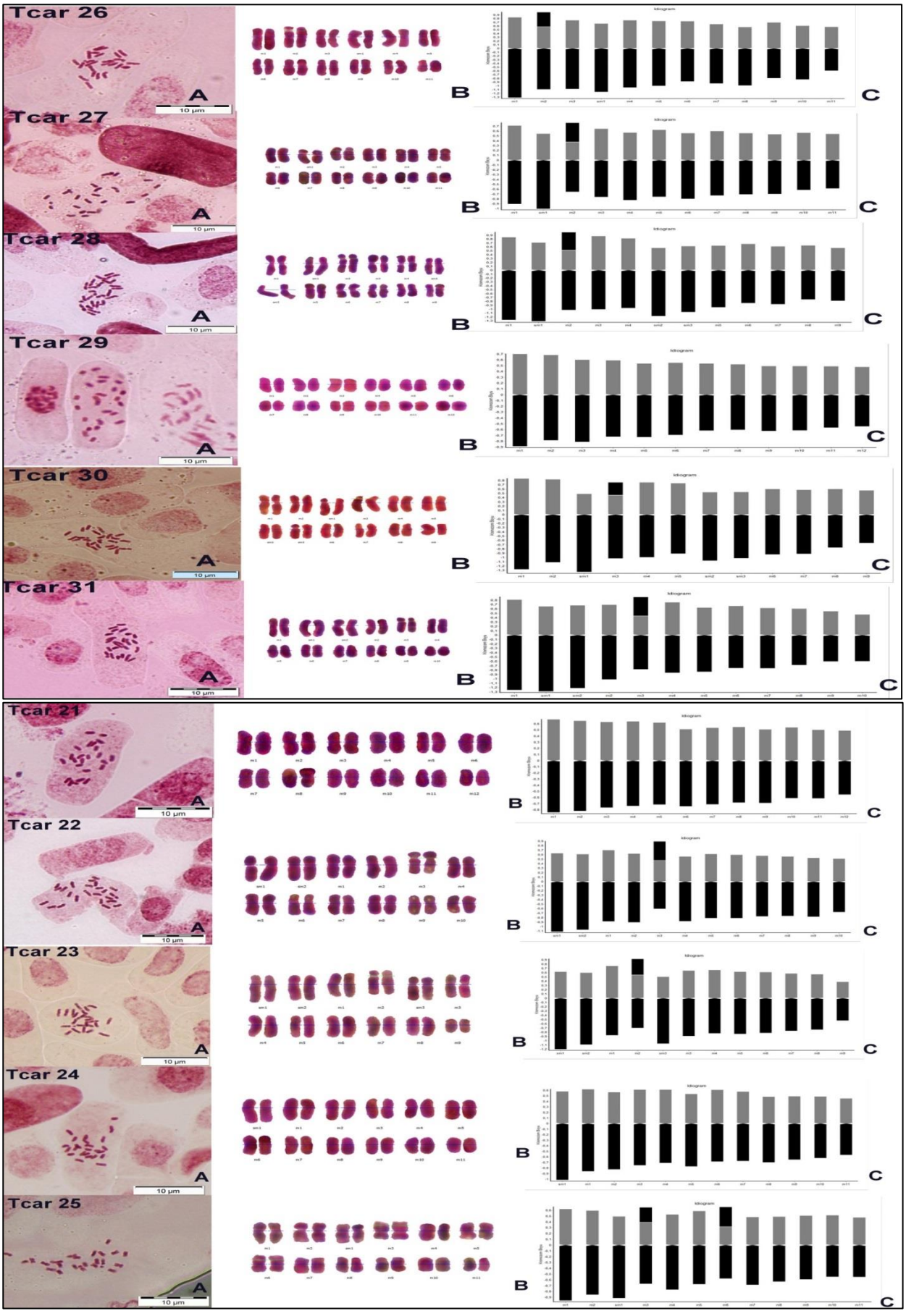

Figure 1 (cont.). The metaphasis plates (A), karyograms (B) and idiograms (C) of studied samples

4. Conclusion

The present study emphasizes very important information in terms of chromosomal variation and, in particular for improvement of new offspring's or genotypes. The findings coming from these 31 accessions indicate the presence of significant differences between their karyotypes, in terms of chromosome formula, total length and symmetry indices. From this information we can declared that the karyotyping has a big and important potencial for breeding and selection studies.

When we consider the karyological results obtained as a whole, we think that some of the varieties of ours can be evaluated commercially because they have close-karyological values with the patented types.

Finally, we can conclude that it is also thought that molecular markers will be important for the selection of *Carthamus* varieties with similar characteristics to be made and will contribute to breeding varieties in terms of increase seed yield. These studies are important sources for plant breeding and rehabilitation.

Acknowledgments

We would like to thank the Scientific Investigation Project Coordinator of Selçuk University [project number: 13201053] for its financial support.

References

Anjali M, Srivastava AK (2009a). 3D somatic karyotype analyses in *Carthamus tinctorius*. Cytologia 73(4): 365-370.

Anjali M, Srivastava AK (2009b). Karyotypic analysis of different populations of *Carthamus tinctorius* Linnaeus (Asteraceae). Comparative Cytogenetics 3(1): 51-55.

Anjali M, Srivastava AK (2009c). The fine structure of safflower (*Carthamus tinctorius* L.) chromosomes as revealed by Pachytene analysis. Cytologia 74(3): 289-293.

Anjali M, Srivastava AK (2012). Karyological studies in twelve accessions of *Carthamus tinctoriusi*. Caryologia 65(1): 1-6.

Bowen CC (1956). Freezing by liquid carbon dioxide in making slides permanent. Stain Technology 31(2): 87-90.

Cassini H (1819). Dictionaire de Sciences Naturelles, Paris. Cited by King R, Dawson HW, (1975) Cassini on Compositae. Oriole Editions, New York.

Dajue L, Mündel HH (1996). Safflower (Carthamus tinctorius L.). Promoting the conservation and use of under utilized and neglected crops, 7. Institute of Plant Genetics and Crop Plant Reasarch, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.

De Candolle AP, De Candolle A, Buek HW (1837). Prodromus Systematis Naturalis Regni Vegetabilis, Sive, Enumeratio Contracta Ordinum Generum Specierumque Plantarum Huc Usque Cognitarum, Juxta Methodi Naturalis Normas Digesta, 6: 611, Paris.

Gyulai J 1996. Market outlook for safflower. P. 15 in Proceedings of North American Safflower Conference, Great Falls, Montana, January 17-18 (H.-H. Mündel, J. Braun and C. Daniels, eds.). Lethbridge, AB, Canada.

Knowles PF (1958). Safflower. Advances in Agronomy 10: 289-323.

Knowles PF (1988). *Carthamus* species relationships. A lecture in Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences, Beijing, China.

Levan A, Fredga K, Sandberg AA (1964). Nomenclature for centromeric position on chromosomes. Hereditas 52(2): 201-220.

Li MX, Chen RY (1985). A suggestion on the standardization of karyotype analysis in plants. J Wuhan Bot Res 3(4): 297-302.

López González G (1989). Acerca de la clasificación natural del género *Carthamus* L., sl. in Anales del Jardin Botanico de Madrid 47(1): 11-34).

Peruzzi L, Leitch IJ, Caparelli KF (2009). Chromosome diversity and evolution in Liliaceae. Ann Bot Lond. 103(3): 459-475.

Sehgal D, Raina, SN, Devarumath RM, Sasanuma T, Sasakuma T (2009). Nuclear DNA assay in solving issues related to ancestry of the domesticated diploid safflower (*Carthamus tinctorius* L.) and the polyploid (*Carthamus*) taxa, and phylogenetic and genomic relationships in the genus *Carthamus* L. (Asteraceae). Molecular phylogenetics and evolution 53(3): 631-644.

Sheidai M, Sotoode M, Nourmohammadi Z (2009). Chromosome pairing and cytomixis in safflower (*Carthamus tinctorius* L., Asteraceae) cultivars. Cytologia 74(1): 43-53.

Stebbins GL (1971). Chromosomal evolution in higher plants. Edward Arnold Ltd., London.

Yazdani M, Sarrami M, Zeinali H (2013). Karyotype study of seven safflower (*Carthamus tinctorius* L.) populations native to Iran. The Nucleus 56(1): 31-36.

Yenice N, Bayraktar N (1996). Yerli Aspir (*Carthamus tinctorius* L.) Hatlarında Karyotip Analizi. Tarım Bilimleri Dergisi 2(2): 81-86.

Cite this article: Uysal T, Tekkanat BS, Şimşek Sezer EN, Ada M, Bozkurt M (2018). Karyotype analysis of some lines and varieties belonging to *Carthamus tinctorius* L. species. Anatolian Journal of Botany 2(1): 1-9.

Anatolian Journal of Botany
2(1): 10-12 (2018)
doi:10.30616/ajb.345385

A new *Inocybe* (Fr.) Fr. record for Turkish macrofungi

Yusuf UZUN[1], İsmail ACAR[2*]
[1]*Yüzüncü Yıl University, Pharmacy Faculty, Pharmacy Sciences Department, Van, Turkey*
[2]*Yüzüncü Yıl University, Başkale Vocational High School, Organic Agriculture Department, Van, Turkey*
**iacar2011@gmail.com*

Received : 20.10.2017
Accepted : 23.12.2017

Türkiye Makrofungusları için Yeni Bir *Inocybe* (Fr.) Fr. Kaydı

Abstract: *Inocybe* (Fr.) Fr. is a very complicated and mostly mycorrhizal genus with a large number of species. A typical member of the genus, *Inocybe decipiens* Bres., was collected from Hani (Diyarbakır) district and recorded for the first from Turkey. Short description and the photographs of the determined taxon was given and discussed briefly.

Key words: Macrofungus, *Inocybe decipiens*, New record, Hani

Özet: *Inocybe* (Fr.) Fr. çoğunlukla mikorizal ilişkide bulunan oldukça karmaşık ve geniş tür sayısına sahip bir cinstir. Cinsin tipik bir üyesi olan *Inocybe decipiens* Bres. Hani (Diyarbakır) yöresinden toplanmış ve Türkiye'den ilk kez rapor edilmiştir. Tespit edilen türün fotoğrafları ve kısa deskripsiyonu verilmiş ve kısaca tartışılmıştır.

Anahtar Kelimeler: Makrofungus, *Inocybe decipiens*, Yeni kayıt, Hani

1. Introduction

The genus *Inocybe* (Fr.) Fr. (*Inocybaceae, Agaricales*) is a prominent genus of lamellate macrofungi. The majority of the members of the genus have toxic properties beside the mycorrhyzal relationships. *Inocybe* species are generally characterized by Brown, lilac and sometimes purplish coloured basidiomata, squamose to fibrillate pileus and stipe, brownish lamellae, brown spore print and the growing mode on soil. Some members have cystidia with crystalline and ornamentations at the apex. *Inocybe* species have a distinctive and significant odour (Deepna Latha ve Manimohan, 2016).

In Turkey, many significant studies are being carried on macrofungi especially in last three to four decades. Regarding the diversity of the country, it can easily be understand that there are losts of macrofungi which are waiting to be determined. Some Turkish mycologist have periodically presented the studies which were carried out on Turkish macrofungi as checklists. Latest checklists were prepared by Solak et al. (2015) and Sesli and Denchev (2014).

Some studies were also carried out after the presentation of latest checklist. According to the checklists and the previous studies (Acar and Uzun, 2016; Akata and ark., 2016; Demirel and ark., 2016; Doğan and Kurt, 2016; Kaya et al., 2016; Sesli et al., 2016; Acar and Uzun, 2017; Allı et al., 2017; Demirel et al., 2017; Uzun et al., 2017a; Uzun et al., 2017b) 83 Inocybe species have been determined.

This study aims to make a contribution to the mycobiota of Turkey.

2. Materials and Method

Macrofungi specimens were collected within the boundaries of Hani (Diyarbakır) district in 2010. General and ecological properties of the samples were recorded to field notebook and they were photographed with a digital photograph machine. Later on the samples were transferred to the laboratory. Macroscopic and microscopic data related to the idendification of macrofungi were traced and the identifiation of the taxon were performed with the help of the relevant literature (Moser, 1983; Hoiland, 1978; Breitenbach ve Kränzlin, 2000). The identifed sample is kept in the fungarium of Yüzüncü Yıl University, Science Faculty, Department of Biology.

3. Results

The macroscopic and microscopic properties and the photographs of basidiocarps and basidiospores are provided.

Basidiomycota R.T. Moore

Agaricomycetes

Agaricales Underw.

Inocybaceae Jülich

Inocybe decipiens Bres.

Syn: *Astrosporina decipiens* (Bres.) Zerova, *Inocybe decipiens* Bres. var. *decipiens*, *Inocybe decipiens* var. *megacystis* J. Favre, *Inocybe decipiens* var. *mundula* J. Favre, *Inocybe favrei* var. *mundula* (J. Favre) Bon.

Macroscopic and microscopic features: Pileus15-35 mm in diameter, conical or convex, accentrically umbonate, with radial fibrils, waxy when young, with vellar remants at the margin, light Brown to dark brown or slightly reddish brown. Lamellae wide, dark whitish to light yellow when young, greyish brown when mature, adnately to adnexely connected to the stipe. Flesh thin, whitish, odour distinct. Stipe 17-40 × 4-7 mm, cylindrical, surface whitish when young, smooth or covered with white longitudinal fibrils on a brownish background, whitish at the base and distinctly bulbous. Spores 10-13.5 × 5.5-7.8 µm, elliptical to slightly rectanculate with slight tubercules, yellowish brown. Basidia 4-spored. Cheilocystidia 30-60 × 10-23 µm, usually urticoid. Pleurocystidia smilar to Cheilocystidia (Figure 1).

Specimen examined: Diyarbakır, Hani, city centre, along stream side, under *Populus-Salix* sp. trees, 38° 24'584"K, 40° 23'612"D, 881 m, 13.05.2010, A. 189.

4. Discussions

With this study, *Inocybe decipiens* was added to the mycobiota of Turkey as the 84th member of the genus *Inocybe*. Seventy nine of them were compiled within the checklists prepared by Sesli and Denchev (2014) and Solak et al. (2015). The other four taxa were recorded by Afyon et al. (2014), Solak et al. (2014) and Öztürk et al (2016).

Morphologically *Inocybe decipiens* show similarities with *I. dunensis*. But the habitat and the spore shapes of the two species differentiates them from each other (Breitenbach ve Kränzlin, 2000; Moser, 1983).

Acknowledgments

The work was supported by Yüzüncü Yıl University Reseacrh Fund (2009-FBE-YL065).

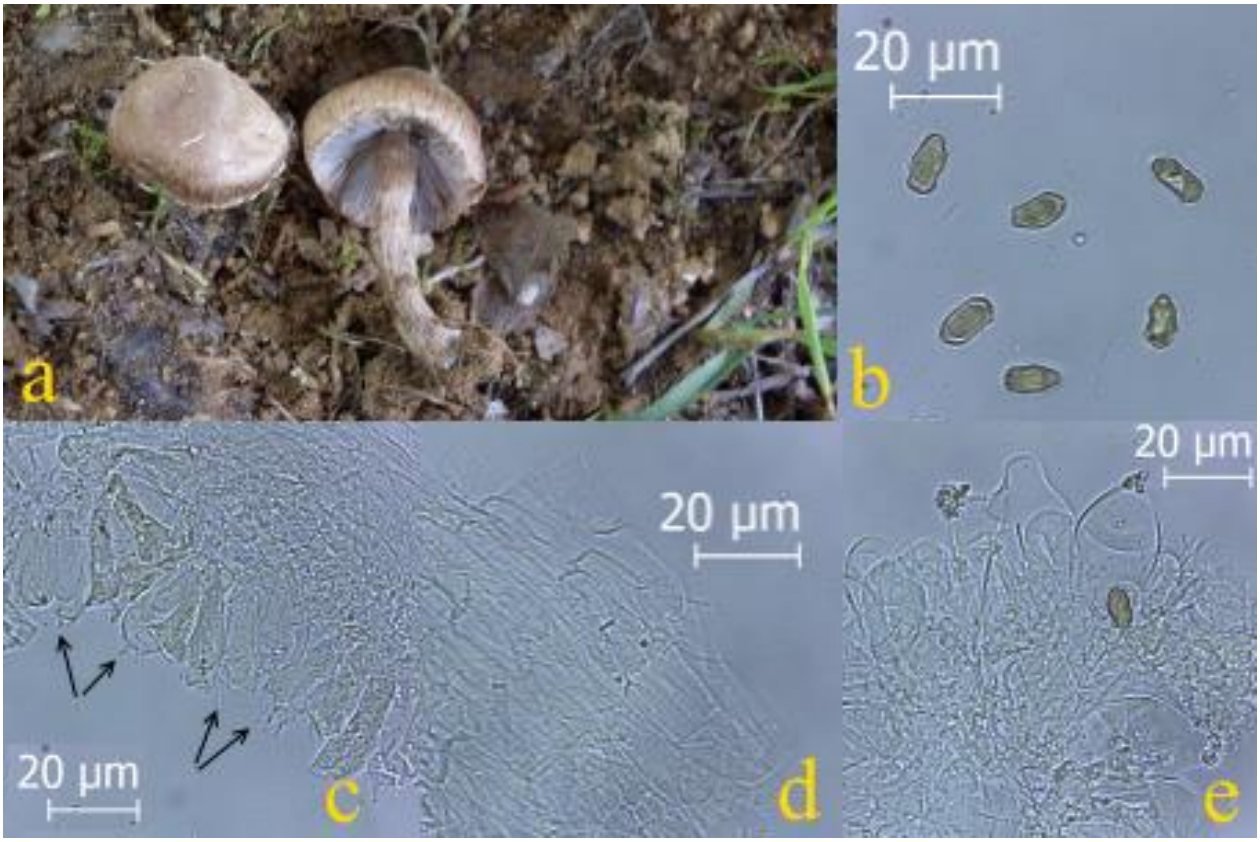

Figure 1. *Inocybe decipiens*, a. basidiocarps, b. basidiospores, c. basidia, d. hypae, e. cheilocystidia

References

Acar İ, Uzun Y (2016). *Peziza granularis* Donadini Türkiye Mikobiyotası için Yeni Bir Kayıt. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi 21(1): 39-42.

Acar İ, Uzun Y (2017). An interesting half-free Morel record for Turkish mycobiota (*Morchella populiphila* M. Kuo, M.C. Carter & J.D. Moore). The Journal of Fungus 8(2): 125-128.

Afyon A, Yağız D, Gezer K, Kaygusuz O (2014). Two New *Inocybe* (Fr.) Fr. Records for Macrofungi of Turkey. Biological Diversity and Conservation 7(3): 1-4.

Akata I, Uzun Y, Kaya A (2016). Macrofungal diversity of Zigana Mountain (Gümüşhane/Turkey). Biological Diversity and Conservation 9(2): 57-69.

Allı H, Çöl, B, Şen İ (2017). Macrofungi biodiversity of Kütahya (Turkey) province. Biodicon 10/1: 133-143.

Breitenbach J, Kränzlin, F (2000). Fungi of Switzerland. Vol.5, Verlag Mykologia Lucerne, Switzerland.

Deepna Latha KP, P Manimohan (2016). Five new species of *Inocybe* (Agaricales) from tropical India. Mycologia 108(1): 110-122.

Demirel K, Acar İ, Ömeroğlu Boztepe, G (2016).Lice (Diyarbakır) Yöresi Makrofungusları. Mantar Dergisi, 7(1): 29-39.

Demirel K, Uzun Y, Keleş A, Akçay ME, Acar İ (2017). Macrofungi of Karagöl–Sahara National Park (Şavşat-Artvin/Turkey). Biological Diversity and Conservation. 10/2: 32-40.

Doğan HH, Kurt F (2016). New macrofungi records from Turkey and macrofungal diversity of Pozantı-Adana. Turkish Journal of Botany 40: 209-217.

Hoiland K (1979). Studies in the genus *Inocybe* (Fr.) Fr. (Agaricales) of the westenl Canary Islands. Vieraea 8(1): 13-22.

Kaya A, Uzun Y, Karacan İH, Yakar S (2016). Contributions to Turkish Pyronemataceae from Gaziantep province. Turkish Journal of Botany 40: 298-307.

Moser, M (1983). Keys to Agarics and Boleti. Gustav Fiscer Verlag, Stuttart.

Öztürk Ö, Doğan HH, Şanda MA (2016). Some new additions to Turkish mycobiota from Sakarya region. Biodicon 9/1: 97-100.

Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6th edn. Mycotaxon Checklists Online. (http://www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf): 1-136.

Sesli E, Türkekul İ, Akata I, Niskanen T (2016). New records of Basidiomycota from Trabzon, Tokat, and İstanbul provinces in Turkey. Turkish Journal of Botany 40(5): 531-545.

Solak MH, Allı H, Işıloğlu M, Güngör H, Kalmış E (2014). Contributions to the macrofungal diversity of Antalya Province. Turk J Bot 38: 386-397.

Solak MH, Işıloğlu M, Erbil K, Allı H (2015). Macrofungi of Turkey, Checklist Volume II, Üniversiteliler Ofset, İzmir.

Uzun Y, Acar İ, Akçay ME, Kaya A (2017a). Contributions to the macrofungi of Bingöl, Turkey. Turkish Journal of Botany 41(5): 516-534.

Uzun Y, Kaya A, Karacan İH, Yakar S. (2017b). New additions to Turkish Hyaloscyphaceae. The Journal of Fungus 8(1): 13-19.

Cite this article: Uzun Y, Acar İ (2018). A New *Inocybe* (Fr.) Fr. Record for Turkish Macrofungi. Anatolian Journal of Botany 2(1): 10-12.

Anatolian Journal of Botany
2(1): 13-18 (2018)
doi:10.30616/ajb.358122

A morphometric study on *Draba cappadocica* Boiss. & Balansa and *Draba rosularis* Boiss.*

Nasip DEMİRKUŞ[1], Metin ARMAĞAN[2], Mehmet Kazım KARA[3]**
[1]*Van Yüzüncü Yıl University, Faculty of Education, Department of Science and Mathematics, Van, Turkey*
[2]*Adnan Menderes University, Buharkent Vocational School, Medicinal and Aromatic Plants Program, Aydın, Turkey*
[3]*IğdırUniversity, Faculty of Agriculture, Department of Zootechnics, Iğdır, Turkey*
***nasip@hotmail.com*

Received : 08.11.2017
Accepted : 06.01.2018

Draba cappadocica Boiss. & Balansa ve *Draba rosularis* Boiss. türleri üzerine morfometrik bir çalışma

Abstract: *Draba cappadocica* Boiss. & Balansa and *Draba rosularis* Boiss. samples were collected from the around of Van (Turkey) province, between 1997 and 2001, after performing population observations. Numerical data were obtained minimun about 10 dry samples collected from each locality and evaluated by SPSS. Special and common characteristics of the taxa were determined and detailed and reliable knowledge were gathered about the little-known properties of them. As a result of the statistical analyzes, depending on their range of variation, the new descriptions were obtained.

Key words: *Draba rosularis*, *Draba cappadocica*, morphometry, Van

Özet: Van (Türkiye) çevresinde 1997-2001 yılları arasında *Draba cappadocica* Boiss. & Balansa ve *Draba rosularis* Boiss. türlerine ait populasyon gözlemleri yapılarak, örnekler toplanmış ve her lokaliteden en az 10 kuru bitki örneğine ait numerik veriler derlenerek SPSS paket programı ile değerlendirilmiştir. Taksonların özgün ve ortak özellikleri saptanarak, literatürde az bilinen özellikleri hakkında daha ayrıntılı ve sağlıklı bilgi derlenmiştir. İstatistik analizleri sonucunda, karakterlerin değişim aralıklarına bağlı olarak yeni betimler ortaya konmuştur.

Anahtar Kelimeler: *Draba rosularis*, *Draba cappadocica*, morfometri, Van

1. Introduction

Draba L., the largest genus in the family Brassicaceae (Al-Shehbaz et al., 2006; Bailey et al., 2006; Koch et al., 2007), comprises more than 370 species in the world (Warwick et al., 2006). It has been spread in arctic, subarctic, alpine, and mountainous regions of the world. Members of *Draba* are annuals, biennials or perennials. Some molecular studies (Koch and Al-Shehbaz, 2002; Beilstein and Windham, 2003) showed that most of the sections of *Draba* are polyphyletic (*Drabella, Tylodraba, Calodraba, Adenodraba, Phyllodraba* etc.).

The computer has made it possible to consider large numbers of characteristics in classifying many phenomena, notably living organisms, fossil organisms and even imaginary organisms (Sokal, 1966). We chose *Draba rosularis* Boiss. and *Draba cappadocica* Boiss. & Balansa species in an attempt to fill the gap in the literature caused by their description which is based on fewer materials in terms of quantitative and qualitative properties, and to emphasize the importance of numerical taxonomy, though simple in this field.

The present basic information about these two species is bounded with the information provided by the first articles published by the Flora of Turkey (Coode and Cullen, 1965). However, information about the morphological characteristics and habitat-spreading habits of species is inadequate or incomplete (Vural and Aytaç, 2005; Adıgüzel et al., 2006; Kandemir and Türkmen, 2008; Jordan-Thaden and Koch, 2008; Başköse and Dural, 2011; Karaer, 2012; Yetişen et al., 2014; Moradkhani and Milan, 2015). Considering the current status of the taxa in the literature, this study was carried.

The study aims to make a contributionthe Flora of Turkey by obtaining and presenting much more reliable characteristics for the determinetion of the two *Draba* species.

2. Materials and Method

Field studies were carried out between the years 1997-2001, especially during 4th, 5th and 6th months, and minimum 10 plant specimens were collected for each species from different localities, 7 times for *D. rosularis*, and 2 times for *D. cappadocica* (Demirkuş et al., 2000). *D. rosularis* samples were collected from the localities around the fountain in Güzeldere Pass (Başkale-Van) for 6 times, and the end of Keşiş Lake, around Güvelek Village for 1.

Photographs of collected fresh and dry materials were taken, and they were scanned by using Işık Kutusu (Light Box) (Demirkuş et al., 2005). During field studies, observations related to the populations, flowering time, and the characteristics of the spreading areas of these species were also carried out.

Ten dry plant specimens were used for each locality to obtain numerical data. Firstly they were numbered from 1 to 10 and placed in small envelopes. Then stem (plant) size, number of flowers, stem leaves width and height

*The abbreviated form of this study was presented in 18th National Biology Congress;(p.26-27), Adnan Menderes University, Department of Biology, June 26-30, 2006, Kuşadası/AYDIN

(sub, middle and upper part), sepals width and height (sub, middle and upper branches), petals width and height (sub, middle and upper branches), pedicels width and height (sub, middle and upper branches), fruit width and height (sub, middle and upper branches), width × height ratio and stylus (sub, middle and upper branches) length measurements were carried out. Under a 10X, 20X, 80X magnification Lup-Microscope, measurement datapedicel length, sub-middle-upper rosette leaves length, base leaves width × height, width of sub-middle-upper sepals in inflorescence × height, width of sub-middle-upper petals in inflorescence × height, width of fruit in inflorescence × height, stylus and flowers were obtained.

SPSS demo version was used for evaluating the data, and reliability ratings, variation and change analyses of the numerical properties of each species were evaluated. By sorting the variants according to the ratio of variance coefficients of the analysed data from the most to the least variant characteristics, we drew graphs. Following the original English descriptions of the two specimens in the Flora of Turkey, we provided new descriptions based on the data obtained out of our research.

The samples (*ND5163*, *ND5336*, *ND5654*, *ND7130*, *ND7184*, *ND7184A*, *ND7697*, *MA1103*, and *MK230408*) are stored in VANF herbarium (ND= Nasip Demirkuş, MA= Metin Akpınar, MK= Mehmet Koyuncu).

3. Results

Population sample 1 (D. cappadocica): B9 Van; between Hoşap (Güzelsu) and Başkale, Güzeldere Pass, over the Fountain, 2650 m, 21.04.2000, ND7184A. We collected and named this population sample on the same date and in the same locality as Number 5 Population of *D. cappadocica* species, which was collected from Güzeldere Fountain.

Population sample 2 (D. cappadocica): B9 Van; between Hoşap (Güzelsu) and Başkale, Güzeldere Pass, Fedai Taşı surroundings, rocky slope, 2900 m, 27.06.1997, ND5654. We collected and diagnosed the fruited sample of this population on Fedai Taşı hill (rocky hill) which is located opposite Güzeldere Hill in the accompaniment of Prof. Dr. M. Koyuncu, and evaluated it as original *D. cappadocica* species.

Population sample 3 (D. rosularis): B9 Van; between Hoşap (Güzelsu) and Başkale, Güzeldere Pass, 2750 m. 24.05.1997. ND5163. Quantitative and qualitative characteristics of this population's specimens, pertaining to mature flowering time, generally overlaps with the limited definition in The Flora of Turkey.

Population sample 4 (D. rosularis): B9 Van; between Hoşap (Güzelsu) and Başkale, Güzeldere Pass, over the fountain, 2660 m. 21.04.2000, MK230408. Quantitative and qualitative characteristics of this population's specimens, which were collected in early flowering time, generally overlaps with the limited definition in The Flora of Turkey.

Population sample 5 (D. rosularis): B9 Van; between Hoşap (Güzelsu) and Başkale, Güzeldere Pass, 2750 m. 24.05.1997, ND7184. Quantitative and qualitative characteristics of this populations' specimens, which were collected in early flowering time, show many variations. As some plant specimens we collected in this area overlapped with *D. cappadocica* taxon (ND7184A), we evaluated them as population number 1 under the name of *D. cappadocica*, as for the other specimens (7184), however, we evaluated them as *D. rosularis* (population number 5). It generally overlaps with the limited definition in The Flora of Turkey.

Population sample 6 (D. rosularis): B9 Van; the end of Keşiş Lake (upper foothill of Erek mountain) Güvelek Village and surroundings, 2000 m, 19.05.1999, ND7697. Quantitative and qualitative characteristics of this population' specimens belonging to mature flowering time originally overlaps with the limited definition in The Flora of Turkey.

Draba rosularis samples were collected from the localities around the fountain in Güzeldere Pass (Başkale-Van) for 6 times, and the end of Keşiş Lake, around Güvelek Village for 1.

Population sample 7 (D. rosularis): B9 Van; between Hoşap (Güzelsu) and Başkale, Güzeldere Pass, rocky slopes, 2700 m. 19.05.2001. MA1103. Quantitative and qualitative characteristics of this population's specimens of mature flowering time generally overlaps with the limited definition in The Flora of Turkey.

Population sample 8 (D. rosularis): B9 Van; between Hoşap (Güzelsu) and Başkale, Güzeldere Pass, over the Fountain, 2680 m, 28.05.2000, ND7130. This population sample belonging to mature flowering time collected from this locality is different from the other 6 samples of the same locality. Quantitative and qualitative characteristics of this population's specimens generally overlaps with the limited definition in The Flora of Turkey.

Population sample 9 (D. rosularis): B9 Van; between Hoşap (Güzelsu) and Başkale, Güzeldere Pass, 2750 m, 13.06.1997, ND5336. Quantitative and qualitative characteristics of this population' specimens pertaining to mature flowering time, generally overlaps with the limited definition in The Flora of Turkey.

The descriptions of population samples;1 and 2 pertaining to *D. cappadocica*, and 3, 4, 5, 6,7, 8, 9 belonging to *D. rosularis* species. The 7th, 8th, and 9th population belongs to *D. rosularis*. Because of the data in these three populations are insufficient, they have not been evaluated.

Among the measured characteristics of the two species; descriptive characteristics used in Flora of Turkey (for them to show parallelism with ours in comparison) were preferred.

All studied descriptive characters and measurements results of them are given in Table 1.

4. Discussionand Conclusions

It seems to be more reliable to make a decision about the status of *D. cappadocica* species in Van basin after collecting specimens from B5 Kayseri: Erciyes Mountain, 2400 m, and B9Van: Ispiriz Mountain, 3400 m, D. 23685 localities again and compiling them with the current data.

Draba cappadocica Boiss. & Balansa in Boiss., Diagn. ser. 2(6): 14 (1859). Syn: *D. calycosa* Boiss. & Balansa in Boiss., Fl. Or. 1:299 (1867), excl. var. *aucheri* Boiss.

Table 1. Descriptive averages and limit values of population samples' numerical data pertaining to *Draba cappadocica* and *Draba rosularis* (The significant data pertaining to *D. cappadocica* were given bold and in red; those of *D. rosularis* were given bold)

Measured Plant Organ	Evaluated Taxa	Specimen Number	Mean	Standard Deviation	Minimum	Maximum
Stem (plant size) (cm)	1. *D. cappadocica*	64	**5,1**	**1,22**	**2,9**	**8**
	2. *D. rosularis*	231	**6,14**	**3,03**	**1,5**	**16,5**
	3. Total	295	5,92	2,77	1,5	16,5
Number of flowers	1. *D. cappadocica*	64	**8,34**	3,28	**1**	**19**
	2. *D. rosularis*	219	**9,42**	4,71	**1**	**28**
	3. Total	283	9,18	4,45	1	28
Sub-rossette leaves length (mm)	1. *D. cappadocica*	20	**8,33**	2,37	**4**	12
	2. *D. rosularis*	59	**11,82**	4,96	**4**	**30**
	3. Total	79	10,94	4,69	4	30
Middle-rosette leaves length (mm)	1. *D. cappadocica*	20	8,38	3,31	4	**15**
	2. *D. rosularis*	59	10,97	4,64	5	28
	3. Total	79	10,32	4,47	4	28
Upper-rosette leaves length (mm)	1. *D. cappadocica*	20	7,8	2,91	4	13
	2. *D. rosularis*	59	10,58	4,27	4	25
	3. Total	79	9,87	4,13	4	25
Sub-rosette leaves width (mm)	1. *D. cappadocica*	20	1,62	0,38	1	2,2
	2. *D. rosularis*	59	1,66	0,42	1	**2,8**
	3. Total	79	1,65	0,41	1	2,8
Middle-rosette leaves width (mm)	1. *D. cappadocica*	20	1,82	0,5	1,1	3,5
	2. *D. rosularis*	59	1,6	0,49	**0,75**	3
	3. Total	79	1,67	0,5	0,75	3,5
Upper rosette leaves width (mm)	1. *D. cappadocica*	20	1,8	0,44	**1**	**3**
	2. *D. rosularis*	59	1,5	0,45	0,5	2,2
	3. Total	79	1,58	0,46	0,5	3
Sub-branch sepals length (mm)	1. *D. cappadocica*	14	**2,82**	0,36	2	3
	2. *D. rosularis*	60	**2,54**	0,51	1,5	3,5
	3. Total	74	2,59	0,49	1,5	**3,5**
Middle-branch sepals length (mm)	1. *D. cappadocica*	14	2,85	0,29	2	3
	2. *D. rosularis*	60	2,49	0,48	1,5	3,2
	3. Total	74	2,56	0,47	**1,5**	3,2
Upper-branch sepals length (mm)	1. *D. cappadocica*	11	2,86	0,78	**2**	**5**
	2. *D. rosularis*	59	2,38	0,45	1,3	3,1
	3. Total	70	2,45	0,54	1,3	5
Sub-branch sepals width (mm)	1. *D. cappadocica*	14	1,61	0,39	1,2	2,5
	2. *D. rosularis*	60	1,43	0,28	1	**2**
	3. Total	74	1,46	0,31	1	2,5
Middle-branch sepals width (mm)	1. *D. cappadocica*	14	1,59	0,29	**1,2**	2
	2. *D. rosularis*	60	1,45	0,32	**0,8**	2
	3. Total	74	1,48	0,32	0,8	2
Upper-branch sepals width (mm)	1. *D. cappadocica*	11	1,66	0,51	1,2	**3**
	2. *D. rosularis*	59	1,39	0,33	0,7	2,8
	3. Total	70	1,44	0,37	0,7	3
Sub-branch petals length (mm)	1. *D. cappadocica*	13	**5,35**	**0,56**	4,5	6
	2. *D. rosularis*	58	**5,01**	**0,76**	3,5	**7**
	3. Total	71	5,07	0,73	3,5	7
Middle-branch petals length (mm)	1. *D. cappadocica*	14	5,2	0,71	4	6
	2. *D. rosularis*	58	4,82	0,9	3	7
	3. Total	72	4,89	0,88	3	7
Upper-branch petals length (mm)	1. *D. cappadocica*	11	4,45	0,78	**3**	**6**
	2. *D. rosularis*	56	4,48	0,77	**2,3**	6
	3. Total	67	4,47	**0,78**	2,3	6

Sub-branch petals width (mm)	1. *D. cappadocica*	13	2,82	0,4	2	3,3
	2. *D. rosularis*	58	2,26	0,37	1,5	3
	3. Total	71	2,37	0,43	1,5	3,3
Middle-branch petals width (mm)	1. *D. cappadocica*	14	2,69	0,61	1,8	**4**
	2. *D. rosularis*	58	2,25	0,34	1,7	3
	3. Total	72	2,33	0,44	1,7	4
Upper-branch petals width (mm)	1. *D. cappadocica*	11	2,41	0,58	**1,4**	3
	2. *D. rosularis*	56	2,16	0,35	**1,3**	**3**
	3. Total	67	2,2	0,4	1,3	3
Sub-branch pedicel length (mm)	1. *D. cappadocica*	20	**6,99**	**2,02**	4,2	**12**
	2. *D. rosularis*	60	**7,1**	**2,54**	2,5	**15**
	3. Total	80	7,07	2,41	2,5	15
Middle-branch pedicel length (mm)	1. *D. cappadocica*	20	5,63	1,32	4	10
	2. *D. rosularis*	60	5,79	2,33	2	12
	3. Total	80	5,75	2,11	2	12
Upper-branch pedicel length (mm)	1. *D. cappadocica*	17	4,64	1,07	**3**	7
	2. *D. rosularis*	59	4,99	1,94	**1**	10
	3. Total	76	4,92	1,78	1	10
Sub-branch fruit length (mm)	1. *D. cappadocica*	6	3,75	0,88	**2,5**	**5**
	2. *D. rosularis*	2	6	0	6	6
	3. Total	8	4,31	1,28	2,5	6
Middle-branch fruit length (mm)	1. *D. cappadocica*	6	**3,67**	**0,61**	3	4,5
	2. *D. rosularis*	2	**6**	**1,41**	**5**	**7**
	3. Total	8	4,25	1,31	3	7
Upper-branch fruit length (mm)	1. *D. cappadocica*	6	3,42	0,66	3	4,5
	2. *D. rosularis*	2	5	0	5	5
	3. Total	8	3,81	0,92	3	5
Sub-branch fruit width (mm)	1. *D. cappadocica*	6	3,33	0,82	**2**	**4**
	2. *D. rosularis*	2	4,25	**0,35**	4	**4,5**
	3. Total	8	3,56	0,82	2	4,5
Middle-branch fruit width (mm)	1. *D. cappadocica*	6	3,17	0,52	2,5	4
	2. *D. rosularis*	2	3,75	0,35	3,5	4
	3. Total	8	3,31	0,53	2,5	4
Upper-branch fruit width (mm)	1. *D. cappadocica*	6	2,77	0,54	2	3,5
	2. *D. rosularis*	2	3,5	0,71	**3**	4
	3. Total	8	2,95	0,63	2	4

The original description in the Flora of Turkey;

Perennial herb, forming rounded tufts. Caudiculi leafy only near the top. Scapes up to 2 cm, villous. Leaves linear to linear-obovate, soft, overlapping, canescent with stellate hairs. Petals yellow, c. 4 mm. Ovary with 16-24 ovules. Siliculae ovoid, as long as broad. Fl. 6. Rock crevices, slopes, 2400-2900 m.

The new description in respect of this study

Perennial plant, forming rounded tufts. Caudiculi leafy only near the top. Scapes 2.9-8 cm, villous. Leaves linear to linear-obovate; 4-15 × 1-3 mm, soft, overlapping, canescent with stellate hairs. Flowers 1-19. Pedicel 3-12 mm. Sepals, 2-5 × 1.2-3 mm. Petals yellow, 3-6 × 1.4-4 mm. Ovary with 16-24 ovules. Siliculae ovoid, as long as broad, 2-2.5 × 2-4 mm. Fl. 6. Rock crevices, slopes, 2400-2900 m.

Draba rosularis Boiss. in Ann. Sci. Nat. 17: 165 (1842). Syn: *D. calycosa* Boiss. & Balansa var. *aucheri* Boiss., Fl.Or.1:299(1867).

The original description in the Flora of Turkey;

Caespitose perennial. Scapes ascending-erect, up to 10 cm, pubescent. Leaves narrowly elliptic, soft, canescent with stellate hairs, 8-20 mm long. Petals yellow, 4-5 mm. Ovary with (12-)16-32 ovules. Siliculae ovoid-ellipsoid, inflated, with an indumentum of stellate hairs. Fl. 4-7. Rocks, up to 3200 m.

The new description in respect of this study;

Caespitose perennial, soft, stellate and branching hairy. Scapes ascending-erect, 1.5-17.5 cm, pubescent. Rosette leaves narrowly elliptic, oblong-lanceolate to linear, acute and acuminate at the apex soft, canescent with stellate and branching long hairy; 4-30 × 0.75-2.8 mm. Flowers 1-28. Pedicel 1-15 mm. Sepals 1.5-3.5 × 0.8-2 mm, with soft; stellate and branching hairy. Petals yellow, 2.3-7 × 1.3-3 mm. Ovary with (12-)16-32 ovules. Silicula ovoid-ellipsoid, inflated, 5-7 × 3-4.5 mm, with an indumentum of short stellate hairs. Fl. 4-7. Rocky slopes, 2400- 3200 m.

We obtained the following results based on this study;

1. We gave more detailed and reliable information about morphological characteristics, flowering time and spreadaltitude of *D. cappadocica* and *D. rosularis* species, known to Turkey alone, not the literature and the world.

2. We determined the statistical analysis and coefficient of variation of the two species studied in terms of stem (plant) size, leaf size / width, sepals' length / width, petals length / width, fruit length / width, and some other characteristics. We determined that the most reliable (the least changing) characteristics, according to these data, are the numerical data of fruits, petals, sepals, stem leaves and stem (plant) size respectively (Figure 1, 2).

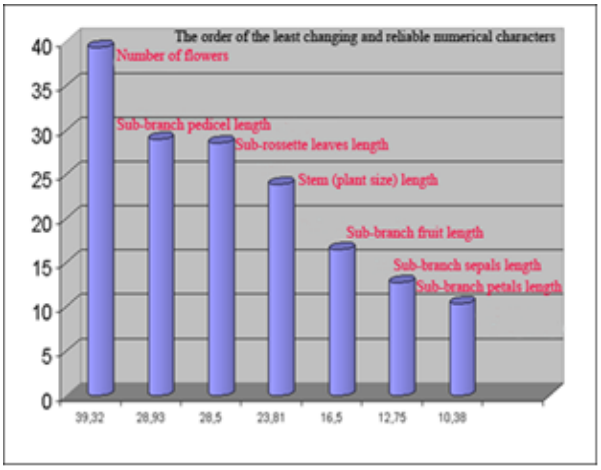

Figure 1. Variation coefficient ratios of *Draba cappadocica* characters.

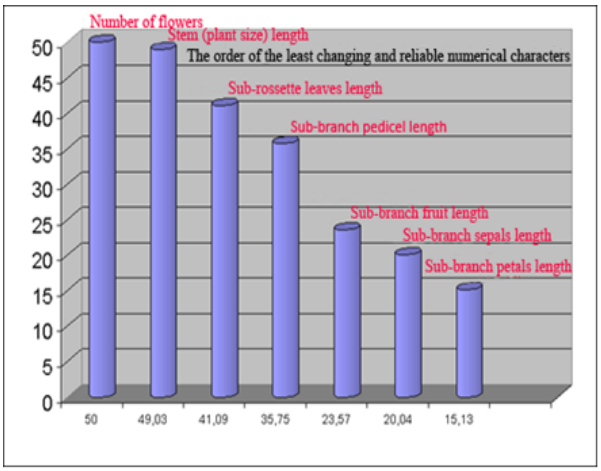

Figure 2. Variation coefficient ratios of *Draba rosularis* characters.

3. Based on numerical data, we gave substantial information about the method followed and the programmes used in determining-using similar, transitive and original characteristics of two close taxa.

References

Adıgüzel N, Bani B, Pınar SM (2006). Rare and endemic species from Van and Hakkari provinces, East Anatolia/Turkey and their threat categories. Plant, fungal and habitat diversity investigation and conservation. Proceedings of IV BBC Sofia: 229-233.

Al-Shehbaz IA, Beilstein MA, Kellogg EA (2006). Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. plant systematics and evolution 259: 89-120.

Bailey CD, Koch MA, Mayer M, Mummenhoff K, O'Kane SL, Warwick SL, Windham MD, Al-Shehbaz IA (2006). Toward a global phylogeny of the Brassicaceae. Mol. Biol. Evol. 23: 2142-2160.

Başköse İ, Dural H (2011). The Flora of Hasan (Aksaray Region, Turkey) Mountain, Biological Diversity and Conservation 4(2): 125-148.

Beilstein MA, Windham MD (2003). A phylogenetic analysis of western North American *Draba* (Brassicaceae) based on nuclear ribosomal DNA sequences from the ITS region. Systematic Botany 28: 584-592.

Coode MJE, Cullen J (1965). *Draba* L. In: Davis PH, editor. Flora of Turkey and The East Aegean Islands, Vol 1. Edinburgh, UK: Edinburgh University Press.

Demirkuş N, Tunç C, Okut H (2005). Numerical taxonomy study pertaining to *Thlaspi valerianoides* Rech. Fil. and *Thlaspi kurdicum* Hedge species. Yüzüncü Yıl University Sciences and Technology Journal, XVI. National Mathematics Symposium Special Issue pp. 277-287.

Demirkuş N, Koyuncu M, Gül M (2000). The endemic plants of Van province. Proceedings of the 2nd Balkan Botanical Congress, 16-18.05.2000. Plants of the Balkan Peninsula: into the next Millennium 1: 163-170.

Karaer F (2012). *Draba cemileae* (Brassicaceae), a new species from NE Anatolia, Turkey, Annales Botanici Fennici 49(1,2): 111-116.

Jordan-Thaden I, Koch MA (2008). Species richness and polyploid patterns in the genus Draba (Brassicaceae): a first global perspective. Plant Ecology and Diversity 1(2): 255-263.

Kandemir A, Türkmen Z (2008). The Flora of Üzümlü-Sakaltutan (Erzincan-Gümüşhane). Turkish Journal of Botany 32: 265-304.

Koch MA, Al-Shehbaz IA (2002). Molecular data indicate complex intra- and intercontinental differentiation of American *Draba* (Brassicaceae). Annals of the Missouri Botanical Garden 89: 88-109.

Koch MA, Dobeš C, Kiefer C, Schmickl R, Klimes L, Lysak MA (2007). SuperNetwork identifies multiple events of plastid trnF (GAA) pseudogene evolution in the Brassicaceae. Molecular Biology and Evolution 24: 63-73.

Moradkhani S, Milan BS (2015). Floristic study of the rangeland Gugerd region in Khoy city (West Azarbaijan Province, NW Iran). Journal of Biodiversity and Environmental Sciences (JBES) 6(6): 48-59.

Sokal RR (1966). Numerical Taxonomy, reprinted from Scientific American 215(6): 106-116.

Vural C, Aytaç Z (2005). The Flora of Erciyes Dağı (Kayseri, Turkey). Turkish Journal of Botany 29: 185-236.

Warwick SI, Francis A, Al-Shehbaz IA (2006). Brassicaceae: species checklist and database on CD-Rom. plant systematics and evolution 259: 249-258.

Yetişen K, Akyol Y, Özdemir C, Kocabaş O (2014). *Draba nemorosa* L. ve endemik *Draba rosularis* Boiss. türleri üzerinde morfolojik ve anatomik bir çalışma. Anadolu Doğa Bilimleri Dergisi 5(1): 44-51.

Cite this article: Demirkuş N, Armağan M, Kara MK (2018). A morphometric study on *Draba cappadocica* Boiss. & Balansa and *Draba rosularis* Boiss. Anatolian Journal of Botany 2(1):13-18.

Anatolian Journal of Botany
2(1): 19-21 (2018)
doi:10.30616/ajb.348740

Flammulina fennae Bas, A new record from Karz Mountain (Bitlis)

Cemil SADULLAHOĞLU[1]*, Kenan DEMİREL[2]
[1]*Yuzuncu Yıl University, Faculty of Science, Department of Biology, Van, Turkey*
[2]*Ordu University, Faculty of Education, Department of Mathematics and Science Education, Ordu, Turkey*
**csadullahoglu@yandex.com*

Received : 02.11.2017
Accepted : 18.12.2017

Flammulina fennae Bas, Karz Dağı'ından (Bitlis) bir yeni kayıt

Abstract: *Flammulina fennae* Bas (*Physalacriaceae)* is recorded for the first time from Turkey. Ecology, distribution, locality, and photographs related to macro and micromorphologies and a short description of the new record were given.

Key words: *Flammulina fennae*, *Physalacriaceae*, new record, Karz Mountain, Turkey.

Özet: *Flammulina fennae* Bas (*Physalacriaceae)* Türkiye'de ilk kez kaydedilmiştir. Yeni kaydın ekolojisi, yayılışı, lokalitesi, makro ve mikro fotoğrafları ve kısa bir deskripsiyonu verilmiştir.

Anahtar Kelimeler: *Flammulina fennae*, *Physalacriaceae*, yeni kayıt, Karz Dağı, Türkiye.

1. Introduction

Flammulina P.Karst. is a genus in the family *Physalacriaceae* Corner which has a cosmopolitan distribution, especially in temperate regions. Robert et al (2005; http://www. mycobank.org) and Index fungorum.org (accessed 1 November 2017) list 17 conformed species of *Flammulina*, most of which are edible (Ge et al., 2008).

Two *Flammulina* species, *F. ononidis* Arnolds and *F. velutipes* (Curtis) Singer, have been recorded from Turkey up to date. Although *F. velutipes* has been reported from many places of Turkey (Abatay, 1983; Demirel, 1998; Sesli, 1999; Solak et al., 1999; Öztürk et al., 2001; Uzun et al., 2004; Kaya et al., 2009), *F. ononidis* has been collected only from Samsun (Pekşen and Karaca, 2003).

During routine field studies in Karz Mountain (Bitlis-Turkey) some basidiomes were collected. *Flammulina fennae* Bas, was described as a new record according to the current checklists on Turkish macromycota (Sesli and Denchev, 2014; Solak et al., 2015) and the latest contributions to the basidiomycetous macrofungi of Turkey (Demirel and Koçak, 2016; Akata and Uzun, 2017; Aktaş et al., 2017; Demirel et al., 2017; Işık and Türkekul, 2017; Sesli and Vizzini, 2017; Uzun and Kaya, 2017; Uzun et al., 2017a,b).

The present study aims to make a contribution to the macrofungi of Turkey.

2. Materials and Method

Fungal specimens were collected from Obuz village, Karz Mountain (Tatvan-Bitlis-Turkey) in 2010. Morphological and ecological chracteristics of the samples were recorded during the field study and they were photographed in their natural habitats. Then, they were taken to the laboratory and microscopic investigations were carried out on them.

Microscopic investigation of the samples were done by using a Nikon light microscope. Reagents such as 5 % KOH and Congo red were used. Identification was performed with the aid of the relevant literature (Bas, 1983; Ripkovà et all., 2010; Schafer and Kibby, 2015).

3. Results

Fungi Bartling
Basidiomycota R.T. Moore
Agaricales Underw.
Physalacriaceae Corner
Flammulina P. Karst.
Flammulina fennae Bas, Persoonia 12(1): 52 (1983)

Macroscopic features: Pileus 20-45 mm in diameter, convex-parabolic, smooth, slightly viscid, pale ochre yellow, when moist short translucently striate at margin of mature basidiocarps, thick-fleshed, rather elastic. Lamellae adnexed to adnate, sinuate, moderately distant, with numerous intermediate gills, white to pale cream. Stipe 25-90 × 2-8 mm, cylindric-tapered, mostly solid, tough, densely subtomentose, concolorous with the pileus at apex, becoming darker reddish brown to dark brown below. In large basidiocarps there are a few remarkable longitudinal grooves (Fig. 1a).

Microscopic features: Spores 5.8-7 × 4-4.5 µm, ellipsoid to elongate-ellipsoid, thin-walled, smooth, hyaline, with small apiculus. Basidia 28-33 × 4.5-6.µm, 4-spored, clamped. Cheilocystidia 35-70 × 6-15 µm, scarce, utriform to lageniform, slightly thick-walled relatively to spore and basidium walls, hyaline. **P**leurocystidia similar to cheilocystidia (Fig. 1b,c,d).

Specimen examined: Bitlis–Tatvan, Karz Mountain, Obuz village, mixed woodland, on buried roots, 38° 26'625"K, 42° 22'467"D, 1788 m, 02.10.2010. S. 036.

4. Discussions

Flammulina fennae was added to Turkish mycobiota as the third member of the genus *Flammulina*. Macro and micromorphological properties of the newly recorded taxon agree with those described by Bas (1983) and Schafer and Kibby (2015).

Figure 1. *Flammulina fennae*: a- basidiomata; b- basidiospores; c- basidia and basidioles, d- cheilocystidia.

Among the *Flammulina* species, *F. velutipes* and *F. ononidis* are morphologically similar to *F. fennae*. But both of them have larger spores. Though *F. fennae* has a spore size of 6-8 × 4-4.5(-5) µm, spore sizes of *F. velutipes* and *F. ononidis* were reported as 7-11 × (2.5-)3-4 µm, and as 8.5-12.5 × 4.5-5.5 µm, respectively (Ripkova et al., 2010).

Field characteristics may also be used to distinguish these three species. Flammulina ononidis is known to associate with the roots of Ononis spinosa. Flammulina velutipes grows usually in winter season. But F. fennae has no association with Ononis and normally fruits outside winter season (Schafer and Kibby, 2015).

Acknowledgments

The authors are thankful to Yüzüncü Yıl University Research Fund (BAP Project No. 2011-FBE-YL-009) for the financial support.

References

Abatay M (1983). Studies on the fungus species infecting woody plants in the East Black Sea Region. Ormancılık Araştırma Enstitüsü Yayınları 118: 57-67.

Akata I, Uzun Y (2017). Macrofungi Determined in Uzungöl Nature Park (Trabzon). Trakya University Journal of Natural Sciences 18(1): 15-24.

Aktaş S, Öztürk C, Pamukçu D (2017). Nallıhan (Ankara) İlçesi Makrofungusları. The Journal of Fungus 8(1): 60-67.

Bas C (1983). Flammulina in western Europe. Persoonia 12: 51-66.

Demirel K (1998). Contributions to the macrofungi flora of West Black Sea Region. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi 5(1): 23-27.

Demirel K, Koçak MZ (2016). Zilan Vadisinin (Erciş-VAN) Makrofungal Çeşitliliği. The Journal of Fungus 7(2): 122-134.

Demirel K, Uzun Y, Keleş K, Akçay ME, Acar İ (2017). Macrofungi of Karagöl-Sahara National Park (Şavşat-Artvin/Turkey). Biological Diversity and Conservation 10(2): 32-40.

Ge ZW, Yang ZL, Zhang P, Matheny PB, Hibbett DS (2008). *Flammulina* species from China inferred by morphological and molecular data. Fungal Diversity 32: 59-68.

Index Fungorum (2017). http://www.indexfungorum.org/Names/Names.asp. Accessed 25 October 2017.

Işık H, Türkekul İ (2017). A new record for Turkish mycota from Akdağmadeni (Yozgat) province: *Russula decolorans* (Fr.) Fr. Anatolian Journal of Botany 1(1): 1-3.

Kaya A, Uzun Y, Karacan İH (2009). Macrofungi of Göksun (Kahramanmaraş) District. Turkish Journal of Botany 33: 131-139.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Dictionary of the Fungi. 10th ed. Wallingford, UK: CAB International.

Öztürk C, Doğan HH, Kaşık G (2001). Additions to the macrofungus flora of Ermenek (Karaman). Selçuk Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi 18: 61-66.

Pekşen A, Karaca G (2003). Macrofungi of Samsun Province. Turkish Journal of Botany 27: 173-184.

Ripková, S., Hughes, K., Adamčik, S., Kučera, V. & Adamčikova, K. (2010). The delimitation of Flammulina fennae. Mycological Progress 9: 469–484.

Robert V, Stegehuis G, Stalpers J (2005 Onward (Continuously Updated). The MycoBank Engine and Related Databases. Available online at http://www.mycobank.org.

Schafer D, Kibby G (2015). *Flammulina fennae* new to Britain. Field Mycology 16(3): 97-99.

Sesli E (1999). The macrofungi determined in A5 (Samsun-Bafra) and A6 (Ordu)]. The Herb Journal of Systematic Botany 6(1): 95-98.

Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6th edn. Mycotaxon Checklists Online. (http://www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf): 1-136.

Sesli E, Vizzini A (2017). Two new *Rhodocybe* species (sect. Rufobrunnea, Entolomataceae) from the East Black Sea coast of Turkey. Turkish Journal of Botany 41: 200-210.

Solak MH, Işıloğlu M, Gücin F, Gökler I (1999). Macrofungi of İzmir Province. Turkish Journal of Botany 23: 383-390.

Solak MH, Işıloğlu M, Kalmış E, Allı H (2015). Macrofungi of Turkey, Checklist, Vol. II. İzmir, Turkey: Üniversiteliler Ofset.

Uzun Y, Acar İ, Akçay ME, Kaya A (2017a). Contributions to the macrofungi of Bingöl, Turkey. Turkish Journal of Botany 41(5): 516-534.

Uzun Y, Kaya A (2017). A Hypogeous *Lactarius* sp., New to Turkish Mycobiota. The Journal of Fungus 8(2): 163-167.

Uzun Y, Kaya A, Karacan İH, Yakar S (2017b). New additions to Turkish Agaricales. Biological Diversity and Conservation 10(2): 8-13.

Uzun Y, Keleş A, Demirel K, Solak MH (2004). Some macrofungi from Bayburt Province ın Turkey. Bulletin of Pure and Applied Sciences 23(1): 47-55.

Cite this article: Sadullahoğlu C, Demirel K (2018). *Flammulina fennae* Bas, A New Record from Karz Mountain (Bitlis). Anatolian Journal of Botany 2(1):19-21.

Anatolian Journal of Botany
2(1):22-27 (2018)
doi:10.30616/ajb.371020

Use of lichens as natural insecticide

Bugrahan EMSEN[1]*, Ali ASLAN[2]
[1]*Karamanoğlu Mehmetbey University, Kamil Özdağ Science Faculty, Department of Biology, Karaman, Turkey*
[2]*Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmacology, Van, Turkey*
**bugrahanemsen@gmail.com*

Received : 26.12.2017
Accepted : 20.01.2018

Likenlerin doğal insektisit olarak kullanımı

Abstract: Agriculture has become one of the greatest sources of employment for mankind from the past to the present. The products obtained in this area provide a significant contribution to the national economies. However, the number of organisms causing the decline in crop yield is quite high. The preferred applications for combating harmful organisms are the use of chemical insecticides. However, the high level of side effects of these insecticides led researchers to alternative study areas. Insecticide production through natural products that is under the title of biological struggle, is within popular areas. Lichens are also materials for natural insecticide sources due to their unique constituents in the constructions. For many years, the toxic effects of lichens utilized in many fields on different insect species have been tested through their extracts and pure components. At this point, it is noteworthy that while high yield is obtained from lichens, the studies are predominantly carried out on the extracts. In the light of all these studies, it will be contributed to biological insecticide production stage by increasing of the studies performed on determination of the active components in lichens showing insecticidal activity on different species and on mechanisms of action in insects.

Key words: Extract, Insecticide, Lichen, Metabolite

Özet: Tarım, geçmişten günümüze kadar insanoğlu için en büyük istihdam kaynaklarından biri olmuştur. Bu alanda elde edilen ürünler ülke ekonomilerine önemli ölçüde katkı sağlamaktadır. Bununla birlikte, ürün veriminde düşüşlere sebep olan organizma sayısı bir hayli fazladır. Zararlı organizmalar ile mücadele konusunda tercih edilen uygulamaların başında kimyasal insektisit kullanımı gelmektedir. Fakat bu insektisitlerin yan etkilerinin yüksek düzeyde olması araştırmacıları alternatif çalışma konularına yönlendirmiştir. Biyolojik mücadele başlığı altında ele alınan, doğal ürünler aracılığı ile insektisit üretimi popüler alanlar içerisinde yer almaktadır. Likenler de yapılarında bulunan kendilerine özgü bileşenleri sayesinde doğal insektisit kaynaklarına malzeme olmaktadır. Yıllardır birçok alanda yararlanılan likenlerin gerek ekstraktları gerekse saf bileşenleri aracılığı ile farklı böcek türleri üzerinde toksik etkileri test edilmektedir. Bu noktada, likenlerden yüksek oranda verim elde edilmekle beraber çalışmaların ağırlıklı olarak ekstraktlar üzerinde gerçekleştirildiği dikkat çekmektedir. Tüm bu çalışmalar ışığında, farklı türler üzerinde insektisit aktivite gösteren likenlerdeki aktif bileşenlerin ve böceklerdeki etki mekanizmalarının tespiti üzerine gerçekleştirilecek çalışmaların artırılması ile biyolojik insektisit üretim basamağına katkı sağlanacaktır.

Anahtar Kelimeler: Ekstrakt, İnsektisit, Liken, Metabolit

1. Introduction

The goal in all past studies is to develop insecticides that can only be effective on the harmful target organism, taking care not to disturb the natural balance without affecting the environment and human health. In this direction, the researchers have been working in the last years and serious studies have started to be done on herbal insecticides (Pant et al., 2016). Natural herbicide insecticides constitute 1% of the world insecticide market. As more work is done on organic agriculture than in the past, annual sales increase by about 10-15% per year (Isman, 1997).

Many researchers have proved that plants are potential sources of insecticides. It was reported that many plant species affect various forms of insects which are harmful to agriculture. Despite the fact that so many of the plants are known to have the insecticidal effect, few have been used in practice. Isman (1997) cites the reasons for this, as natural resources are limited, standardization and licensing difficulties. Natural insecticides have some advantages and disadvantages compared to synthetic insecticides. Natural insecticides break down very quickly in sunlight, humidity and windy conditions, so they can be used just before harvesting. However, many of these insecticides stop feeding their insects very quickly, although they do not kill them immediately. Therefore, it sometimes takes a few days for the death of the insects, but it has a rapid effect in terms of prevention of damage (Oberemok et al., 2015).

If we exclude some highly toxic plant-based insecticides, it can be said that most of the plant-derived natural insecticides are not toxic to mammals and the environment. Rapid disintegration and stomach poisoning make natural insecticides more selective against plant-feeding harmful insects (Walia et al., 2017). Natural insecticides are not usually phytotoxic. They also have no negative effects on their germination, growth and product quality. However, nicotine may have a negative effect on some ornamental plants. When they are used in their natural form, the risks of creating durability are practically absent. They can be obtained cheaper and easier than synthetic insecticides in those areas, as they grow mostly in areas with less developed and lower labor costs (Sierro et al., 2013).

The compounds that plants cause to act on the insects are secondary metabolites that secrete at the time of stress (Rattan, 2010). Herbal substances used against insects are compounds which are obtained by various methods from plants and show insecticidal properties. These can be in various forms, such as unprocessed plant materials, plant extracts, and pure compounds isolated from plants. While some of the natural insecticides are used directly as lethal,

others have repellent and nourishment-inhibiting properties (Murugesan et al., 2016).

The action mechanisms of insecticides are generally on the nervous and digestive system of the organism. Insecticides of plant origin are said to have entered the body of insects through contact and poisoned the nervous-muscle system via secondary metabolites (Denecke et al., 2015). In addition, it has also been found that herbal insecticides affect physiology of the insect by acting in different forms on various receptor sites (Buckingham et al., 2017).

It is known that plant-based insecticides have effect a number of harmful species. Yildirim et al. (2005) have investigated the effect of three essential oil extracts on *Tribolium confusum* (du Val) which damages products such as flour and flour produced pasta, biscuits, bran, soup material, starch and some oil seeds and *Sitophilus granarius* (L.) damaging in stored seed known as wheat weevil. As a result of the experiment, mortality rate of 67% for *T. confusum* and 43% for *S. granarius* appeared. In another study, fumigant toxicity effect of essential oils from nine plant species on adults of *S. granarius* was investigated (Kordali et al., 2012).

Yıldırım et al. (2009) have evaluated effectiveness of plants grown from pesticide seeds against cabbage aphid (*Brevicoryne brassicae* L.), cabbage leaf fleas (*Phyllotreta atra* F., *Phyllotreta nigripes* F.), cabbage moth (*Plutella xylostella* L.) and large cabbage butterfly (*Pieris brassicae* L.). As a result, it was detected that the damage of leaf fleas in cabbage plants was considerably reduced and after six weeks, the loss was reduced by 90%. Many studies that demonstrate that some medical plants have the same effect as some chemical insecticides are also included in the literature (Tewary et al., 2005; Kordali et al., 2013; Yildirim et al., 2013). In recent years, insecticidal activity studies carried out through compounds derived from *in vitro* propagated plants have also gained popularity (Emsen et al., 2016; Dogan et al., 2017).

Since plant origin ones among the insecticides used in the struggle with harmful organisms act differently on the target organism without harming the environment, their usage areas have expanded.

2. General Characteristics of Lichens

Among the many species of organisms used for the purpose of obtaining insecticides, lichens are the most remarkable species. Lichens are very interesting livings with their forms of formation, their living habits, their diversity of uses, and their components that they have in their structure. According to Nash (2008), lichens are symbiotic organisms formed with a fungus partner called a mycobiont and by the photosynthetic partner which can be one or more algae or cyanobacteria and is known as a photobion. Lichens show a completely separate structure from the algae and fungi that form themselves in terms of shape and life. The photosynthetic organism (photobiont) involved in the structure of a colorless fungal hyphae is usually green algae or a cyanobacterium; but they are also known to be composed of some yellow-green algae and brown algae (Bačkor and Fahselt, 2008). Some of the genus belonging to Cyanophyta and Chlorophyta and some algae from Xanthophyta and Phaeophyta are observed. Mushrooms are usually Ascomycetes and rarely belonging to Basidiomycetes (Grube and Spribille, 2012; Millot et al., 2016).

In the periods when the lichens first began to be examined, although the fungus in the lichen symbiosis was thought to have benefited from algae such as parasites due to lack of chlorophyll, but later this explanation lost its significance. It has been understood that the fungus has been supplied with water and water-soluble minerals from the environment in common life with the help of hyphae (Brodo et al., 2001; Nash, 2008). Fungi that can not produce their own carbohydrates meet the needs for glucose from photosynthesizing algae and cyanobacteria thanks to the chlorophyll pigment contained. Starch is also used as a storage material in lichens (Rikkinen, 2015; Suzuki et al., 2016).

For years, many scientists have gone to the way of classifying lichens by adding their own interpretation. One of these classifications is based on the environment on which lichens live. According to this, lichens are divided as living on limestone rocks, siliceous soils, tree trunks and branch bark, leaves, overturned tree logs, and in water. Another classification has been made according to the type of fungus found in the lichen species. In this case, lichens are divided into three classes as Phycolichens, Ascolichens and Basidiolichens (Brodo et al., 2001). Nowadays, the most important classification accepted by many scientists is to group lichens according to thallus structures. The thallus structures found in lichens show the character of this lichen. Because, in many cases, a great deal of a lichen comes from the thallus, unlike the reproductive and reproduction processes. According to thallus structure, lichens are divided into three groups, namely crustose, foliose and fruticose (Sipman, 2002; Armstrong and Bradwell, 2010).

Among the lichens, the species that are short-lived are rare. Although there are environmental issues that reduce the lifespan of lichens like other life forms, they can survive for more than 1000 years under favorable conditions such as fresh air, adequate humidity and light (Armstrong, 2004). Lichen species are very susceptible to polluted air, so the lichen flora is very poor in industrial areas and near large cities. On the other hand, the lichens cover the rocks, tree trunks and branches in various colors and shapes in clean air areas. For this reason, they are indicator organisms for the cleanliness of a zone's air (Szczepaniak and Biziuk, 2003; Rikkinen, 2015).

Lichens are distributed almost everywhere in the world. They are able to develop even in the desert where there is enough humidity, Arctic and Antarctic regions, the freezing temperatures of high mountains, on the stones that other plants can not live on, inefficient soil, dry tree bark and tiles (Kranner et al., 2008). The feature that allows lichens to survive on extreme conditions is hidden in the acids in their structure. Lichen acids, the secondary metabolites of lichens, give the lichens the ability to become a preliminary organism in the primary succession in the nature. The sandy, rocky, clayey, marsh and gravel environments are primarily occupied by lichens. These are called leading populations. At this point, lichens increase the soil quality of the environment (Xu et al., 2005; Zambare and Christopher, 2012; Nguyen et al., 2013).

3. Different Usage Areas of Lichens

Due to the lichen acids produced by the fungi in the symbiotic life of lichens, since the past centuries it was benefitted from the lichens in many areas. For example; in the past, during scarcity in Scandinavia, *Cetraria islandica* (L.) Ach. was milled and added to wheat flour or potatoes to increase insufficient supplies. In the 1880s in Sweden, *Cladina rangiferina* (L.) Nyl. was used as a sugar source (Brodo et al., 2001). *C. islandica* and *Lobaria pulmonaria* (L.) Hoffm. have been used for skin tanning because of their shrunken properties. *L. pulmonaria* has also been used in the fermentation of beer. It has been found that the beer made with this lichen is exactly the same as the one made with hops (Tutel, 1986).

Most of the *Rocella* species from lichens were used in the dyeing of wool and clothes until the mid-19th century. Two different color substances were identified among *Rocella* species. There is erythrin acid in *Roccella fuciformis* (L.) DC., *Rocella fucoides* (Dicks.) Vain., *Rocella peruensis* Krempelh., *Lecanora montagnei* (Fr.) Schaer. and lecanorin acid was determined in *Roccella tinctoria* DC., *Roccella sinensis* Nyl. and *Cladonia portentosa* ((Dufour) Coem.) (Aslan, 1995; Culberson and Culberson, 2001). *Evernia prunastri* (L.) Ach., *Pseudevernia furfuracea* (L.) Zopf., *L. Pulmonaria*, *Anaptychia ciliaris* (L.) Körb. and some *Usnea* sp., *Physcia* sp. were used in the field of cosmetics. These lichens are used in perfume as a mixture, not alone. The lichens that increase the persistence of smell give a pleasant fragrance to the combination of perfume (Tutel, 1986).

The lichens were also used in the medical field. The lichen species in this area are generally used with meaning according to their morphological appearance. *L. pulmonaria* was used as a softening cream in the treatment of pulmonary disease for pulmonary resemblance. *Usnea Florida* (L.) Weber ex F.H. Wigg. and many other *Usnea* species, which are filamentous and resembling hair, were used to prevent hair loss and to make hair look louder and more vibrant. *Xanthoria parietina* (L.) Beltr. which has a yellow-orange color and *Peltigera apthosa* (L.) Willd. which is similar to the small warts in the thallus were used in the treatment of jaundice and canker diseases, respectively (Brodo et al., 2001).

Lichens are used in the form of laxatives, expectorants, tonic, internal or external pastes, decoction (boiling) or infusion. In various parts of the world, researches are being conducted for new pharmaceutical uses of lichens (Shukla et al., 2010). It has been found that some lichens have antitumor activity through components such as polysaccharide, glucan and glycoprotein. Antimicrobial activities against different bacterial species were identified in lichens where many antibiotic substances were detected (Shrestha and St. Clair, 2013; Shrestha et al., 2015).

4. Insecticidal Efficacies of Lichens

It is also necessary to add the insecticide activities to the above-mentioned and many other unused usage areas of lichens. It was reported that *Letharia vulpina* (L.) Hue ve *Vulpicida pinastri* (Scop.) J.-E. Mattsson species are toxic and these lichens have been used in some European and Scandinavian countries to kill wolves and foxes that have damaged to animals in winter . When these species are eaten by animals, they cause death by stopping breathing, particularly by influencing the respiratory system. Brodo et al. (2001) reported that a lichenologist who collected *L. vulpina* from these toxic species in large quantities severely undergone respiratory tract irritation since he was constantly in contact with this species and this person had a nosebleed. This event suggests that these lichen species may also be poisonous to humans. Some researchers, considering these properties of lichens, have gone to investigate the insecticidal effect of lichens.

Cetin et al. (2008) investigated the insecticidal effects of (-)-usnic acid and (+)-usnic acid secondary metabolites obtained from *Cladonia foliaceae* (Huds.) Wield. and *Ramalina farinacea* (L.) Ach. against *Culex pipiens* L. (mosquito) larvae in laboratory conditions and get 100% results in some biological periods of these organisms. 1440 samples from 50 lichen species with different chemical properties were analyzed to determine whether these characteristics of lichens-fed insects originated from lichen species, particularly lichen chemistry by Nimis and Skert (2006). Multivariate analyzes revealed a special negative correlation between the presence of grass-fed animals and some lichen substances.

Silva et al. (2009) examined potential insecticide effects of lectin isolated from *Cladonia verticillaris* (Raddi) Fries on *Nasutitermes corniger* Motschulsky termite. As a result of their work, it was stated that *C. verticillaris* preparations might be able to control termites (or other insects) that are economically related to agriculture and the wood industry. In another study, the effects of methanol, chloroform and water extracts of *L. pulmonaria* on the life span of *Drosophila melanogaster* Meigen (vinegar fly) were investigated (Uysal et al., 2009). They found that methanol extract was more effective than chloroform and water extracts and water extract was relatively weaker than methanol and chloroform extracts. Emmerich et al. (1993) investigated the antifeedant and lethal effects of four of the lichen metabolites, (-)- and (+)-usnic acid, vulpinic acid, and stictic acid on *Spodoptera littoralis* Boisduval larvae. They noted that both types of usnic acid as well as vulpinic acid showed a lethal effect at high levels and delayed growth. It was also detected that stictic acid did not show any effect.

In various treatments, it was reported that larval killer effect of usnic acid and vulpinic acid was tested on *Bemisia tabaci* Gennadius (white fly) and vulpinic acid was more effective than (-)- usnic acid. In addition, it was determined that hexane, ethyl acetate, methanol and water extracts of *Roccella montagnei* Bél. had a lethal effect on *Helicoverpa armigera* (Hubner) Among (green worm) (Balaji et al., 2007). Another lichen-insecticide related study was performed on *Usnea* sp., *Heterodermia diademata* (Taylor) D.D. Awasthi, *Roccella montagnei* Bél. and *Leproloma sipmanianum* Kümmerl & Leuckert. from lichen species. In the related work, high insecticidal effect of ambewelamide A compound isolated from aforementioned lichens was determined on the second period larvae of *Aedes aegypti* (L.). In another study performed with the same larvae, it was pronounced that cabraleadiol monoacetate and 3,6-dimethyl-2-hydroxy-4-methoxybenzoic acid from lichen acids had insecticidal activity (Tufan Çetin and Sümbül, 2008).

In recent years, efforts to create biological insecticides against storage pests through different lichen extracts and components have gained popularity. Emsen et al. (2012b) observed toxicity of *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale on grain weevil, *Sitophilus granarius* (L.) and reported 91 and 83% mortality rates for both species, respectively. Similarly, experiments carried out with *L. vulpina*, *Lecanora muralis* (Schreb.) Rabenh. and *Peltigera rufescens* (Weiss) Humb revealed other lichen species that was effective on *S. granarius* (Emsen et al., 2015). In that study where concentration-dependent insecticidal activity was observed, median lethal concentration (LC_{50}) values of *L. vulpina*, *L. muralis* and *P. rufescens* were 0.51, 0.67 and 0.33, respectively. Insecticidal activities caused by extracts of *L. vulpina*, *L. muralis* and *P. rufescens* were 100, 86 and 100%, respectively.

There are also studies in which insecticidal effects of active ingredients isolated from lichens against grain weevil are tested. Yildirim et al. (2012a) obtained diffractaic and usnic acid from *Usnea longissima* Ach. and measured insecticidal effect of these secondary metabolites. Although they found that both compounds had high activity, it was revealed that usnic acid was more effective than diffractaic acid. Another insect that causes loss in warehouse products is *Sitophilus zeamais* Motschulsky. Lichens were also used to combat with this pest through natural insecticide. Diffractaic acid and usnic acid from pure lichen compounds and *L. vulpina*, *P. rufescens* extracts were tested against *S. zeamais* and positive results achieved (Yildirim et al., 2012b). In previously mentioned study, the highest mortalities belonged to *L. vulpina* extract and diffractaic acid with 96.97%. *L. vulpina*, *L. muralis* and *P. rufescens* have taken place among lichens used for solution to potato beetle (*Leptinotarsa decemlineata* Say) that damages an important agricultural product, potato. The insecticidal activities of *L. vulpina* and *P. rufescens* after 120 hours was remarkable (Emsen et al., 2013). In the same way, when measured the toxicity efficacies of diffractaic and usnic acids isolated from *U.longissima* against 4th instar larvae and adults of *L. decemlineata*, it was determined that the activity levels of mentioned compounds were significant after 96 hours (Emsen et al., 2012a).

The lichens that are used in different areas due to their unique compounds also have a place in the field of biological insecticide. However, total extracts are noteworthy in the studies carried out so far for natural insecticide production field. In the future, increasing of the studies performed on detection of the active components that play insecticidal role in the lichens and on insect death mechanisms will contribute much more to the field of biological insecticide production.

Conflicts of interest

There is no conflict of interest in any form between the authors.

References

Armstrong RA (2004). Lichens, Lichenometry and Global Warming. In: Microbiologist. pp 32–35.

Armstrong R, Bradwell T (2010). Growth of Crustose Lichens: A Review. Geogr Ann Ser A, Phys Geogr 92: 3–17.

Aslan A (1995). Erzurum-Kars-Artvin Arasında Yer Alan Bölge Likenleri Üzerine Taksonomik İncelemeler. Doctorate Thesis, Uludağ University Institute of Science and Technology, Bursa, Turkey.

Bačkor M, Fahselt D (2008). Lichen Photobionts and Metal Toxicity. Symbiosis 46: 1–10.

Balaji P, Malarvannan S, Hariharan GN (2007). Efficacy of *Roccella montagnei* Extracts on *Helicoverpa armigera* (Lepidoptera: Noctuidae). J Entomol 4: 248–252.

Brodo IM, Sharnoff SD, Sharnoff S (2001). About the Lichens. In: Lichens of North America. Yale University Press, New Haven & London, pp 3–113.

Buckingham SD, Ihara M, Sattelle DB, Matsuda K (2017). Mechanisms of Action, Resistance and Toxicity of Insecticides Targeting GABA Receptors. Curr Med Chem 24: 2935–2945.

Cetin H, Tufan-Cetin O, Turk AO, Tay T, Candan M, Yanikoglu A, Sumbul H (2008). Insecticidal Activity of Major Lichen Compounds, (-)- and (+)-Usnic Acid, against the Larvae of House Mosquito, *Culex pipiens* L. Parasitol Res 102: 1277–1279.

Culberson CF, Culberson WL (2001). Future Directions in Lichen Chemistry. Bryologist 104: 230–234.

Denecke S, Nowell CJ, Fournier-Level A, Perry T, Batterham P (2015). The Wiggle Index: An Open Source Bioassay to Assess Sub-Lethal Insecticide Response in *Drosophila melanogaster*. Plos One 10: e0145051.

Dogan M, Emsen B, Aasim M, Yildirim E (2017). *Ceratophyllum demersum* L. Extract as a Botanical Insecticide for Controlling the Maize Weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Egypt J Biol Pest Control 27: 11–15.

Emmerich R, Giez I, Lange OL, Proksch P (1993). Toxicity and Antifeedant Activity of Lichen Compounds against the Polyphagous Herbivorous Insect *Spodoptera littoralis*. Phytochemistry 33: 1389–1394.

Emsen B, Bulak Y, Yildirim E, Aslan A, Ercisli S (2012a). Activities of Two Major Lichen Compounds, Diffractaic Acid and Usnic Acid against *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae). Egypt J Biol Pest Control 22: 5–10.

Emsen B, Yildirim E, Aslan A, Anar M, Ercisli S (2012b). Insecticidal Effect of the Extracts of *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale Against Adults of the Grain Weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Egypt J Biol Pest Control 22: 145–149.

Emsen B, Aslan A, Yildirim E, Ercisli S (2013). Toxicity Effects of Some Lichen Species Extracts against the Colorado Potato Beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Egypt J Biol Pest Control 23: 193–199.

Emsen B, Yildirim E, Aslan A (2015). Insecticidal Activities of Extracts of Three Lichen Species on *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Plant Prot Sci 51: 156–161.

Emsen B, Dogan M, Aasim M, Yildirim E (2016). Insecticidal Activity of *In Vitro* Propagated Aquatic Plant *Ceratophyllum demersum* L. against Granary Weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae). Egypt J Biol Pest Control 26: 619–624.

Grube M, Spribille T (2012). Exploring Symbiont Management in Lichens. Mol Ecol 21: 3098–3099.

Isman MB (1997). Neem and Other Botanical Insecticides: Barriers to Commercialization. Phytoparasitica 25: 339–344.

Kordali S, Yildirim E, Yazici G, Emsen B, Kabaagac G, Ercisli S (2012). Fumigant Toxicity of Essential Oils of Nine Plant Species from Asteraceae and Clusiaceae against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Egypt J Biol Pest Control 22: 11–14.

Kordali S, Emsen B, Yildirim E (2013). Fumigant Toxicity of Essential Oils from Fifteen Plant Species against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Egypt J Biol Pest Control 23: 241–246.

Kranner I, Beckett R, Hochman A, Nash TH (2008). Desiccation-Tolerance in Lichens: A Review. Bryologist 111: 576–593.

Millot M, Dieu A, Tomasi S (2016). Dibenzofurans and Derivatives from Lichens and Ascomycetes. Nat Prod Rep 33: 801–811.

Murugesan S, Senthilkumar N, Babu DS, Rajasugunasekar D (2016). Chemical Composition, Antifeedant and Insecticidal Activities of Tree Borne Oil Seeds. Pestology 40: 29–41.

Nash TH (2008). Lichen Biology. Cambridge University Press, New York, p 486.

Nguyen K-H, Chollet-Krugler M, Gouault N, Tomasi S (2013). UV-Protectant Metabolites from Lichens and Their Symbiotic Partners. Nat Prod Rep 30: 1490–1508.

Nimis PL, Skert N (2006). Lichen Chemistry and Selective Grazing by the Coleopteran *Lasioderma serricorne*. Environ Exp Bot 55: 175–182.

Oberemok VV, Laikova KV, Gninenko YI, Zaitsev AS, Nyadar PM, Adeyemi TA (2015). A Short History of Insecticides. J Plant Prot Res 55: 221–226.

Pant M, Dubey S, Patanjali PK (2016). Recent Advancements in Bio-Botanical Pesticide Formulation Technology Development. In: Herbal Insecticides, Repellents and Biomedicines: Effectiveness and Commercialization. pp 117–126.

Rattan RS (2010). Mechanism of Action of Insecticidal Secondary Metabolites of Plant Origin. Crop Prot 29: 913–920.

Rikkinen J (2015). Cyanolichens. Biodivers Conserv 24: 973–993.

Shrestha G, St. Clair LL (2013). Lichens: A Promising Source of Antibiotic and Anticancer Drugs. Phytochem Rev 12: 229–244.

Shrestha G, St. Clair LL, O'Neill KL (2015). The Immunostimulating Role of Lichen Polysaccharides: A Review. Phyther Res 29: 317–322.

Shukla V, Joshi GP, Rawat MSM (2010). Lichens as a Potential Natural Source of Bioactive Compounds: A Review. Phytochem Rev 9: 303–314.

Sierro N, Battey JN, Ouadi S, Bovet L, Goepfert S, Bakaher N, Peitsch MC, Ivanov NV (2013). Reference Genomes and Transcriptomes of *Nicotiana sylvestris* and *Nicotiana tomentosiformis*. Genome Biol 14: R60.

Silva MDC, Sá RA, Napoleão TH, Gomes FS, Santos NDL, Albuquerque AC, Xavier HS, Paiva PMG, Correia MTS, Coelho LCBB (2009). Purified *Cladonia verticillaris* Lichen Lectin: Insecticidal Activity on *Nasutitermes corniger* (Isoptera: Termitidae). Int Biodeterior Biodegrad 63: 334–340.

Sipman HJM (2002). The Significance of the Northern Andes for Lichens. Bot Rev 68: 88–99.

Suzuki MT, Parrot D, Berg G, Grube M, Tomasi S (2016). Lichens as Natural Sources of Biotechnologically Relevant Bacteria. Appl Microbiol Biotechnol 100: 583–595.

Szczepaniak K, Biziuk M (2003). Aspects of the Biomonitoring Studies Using Mosses and Lichens as Indicators of Metal Pollution. Environ Res 93: 221–230.

Tewary DK, Bhardwaj A, Shanker A (2005). Pesticidal Activities in Five Medicinal Plants Collected from Mid Hills of Western Himalayas. Ind Crops Prod 22: 241–247.

Tufan Çetin Ö, Sümbül H (2008). Liken Ekstraktları ve Sekonder Bileşiklerinin Böcek Kontrolünde Kullanımları. Türk Liken Topluluğu Bülteni 5: 8–10.

Tutel B (1986). Liken Biyolojisi ve Faydaları. Marmara Üniversitesi Eczac Fakültesi Derg 2: 185–194.

Uysal H, Altun D, Aslan A (2009). *Drosophila melanogaster*'de *Lobaria pulmonaria* (L.) Hoffm. Likeninin Ömür Uzunluğu Üzerine Etkisi. TÜBAV Bilim Derg 2: 271–276.

Walia S, Saha S, Tripathi V, Sharma KK (2017). Phytochemical Biopesticides: Some Recent Developments. Phytochem Rev 16: 989–1007.

Xu ZQ, Li WH, Bao WK, Xu Q (2005). A Review on Primary Succession of Vegetation. Acta Ecol Sin 25: 3383–3389.

Yildirim E, Kesdek M, Kordali S (2005). Effects of Essential Oils of Three Plant Species on *Tribolium confusum* Du Val and *Sitophilus granarius* (L.) (Coleoptera: Tenebrionidae and Curculionidae). Fresenius Environ Bull 14: 574–578.

Yıldırım E, Çalmaşur Ö, Kesdek M (2009). Imidacloprid, Thiamethoxam and Cyromazine Seed Treatments for the Control of Cabbage Insect Pests in Erzurum, in Turkey. Atatürk Univ J Agric Fac 40: 23–34.

Yildirim E, Aslan A, Emsen B, Cakir A, Ercisli S (2012a). Insecticidal Effect of *Usnea longissima* (Parmeliaceae) Extract against *Sitophilus granarius* (Coleoptera: Curculionidae). Int J Agric Biol 14: 303–306.

Yildirim E, Emsen B, Aslan A, Bulak Y, Ercisli S (2012b). Insecticidal Activity of Lichens against the Maize Weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Egypt J Biol Pest Control 22: 151–156.

Yildirim E, Emsen B, Kordali S (2013). Insecticidal Effects of Monoterpenes on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). J Appl Bot Food Qual 86: 198–204.

Zambare VP, Christopher LP (2012). Biopharmaceutical Potential of Lichens. Pharm Biol 50: 778–798.

Cite this article: Emsen B, Aslan A (2018). Use of lichens as natural insecticides. Anatolian Journal of Botany 2(1): 22-27.

Anatolian Journal of Botany
2(1): 28-38 (2018)
doi:10.30616/ajb.379549

New bryophillic *Pyronemataceae* records for Turkish *Pezizales* from Gaziantep province

Yasin UZUN[1]*, İbrahim Halil KARACAN[2], Semiha YAKAR[1], Abdullah KAYA[1]
[1]*Karamanoğlu Mehmetbey University, Kâmil Özdağ Science Faculty, Department of Biology, Karaman, Turkey*
[2]*Ömer Özmimar Religious Anatolian High School, Gaziantep, Turkey*
**yasinuzun_61@hotmail.com*

Received : 16.01.2018
Accepted : 03.02.2018

Türkiye *Pezizales*'leri için Gaziantep'ten yeni briyofilik *Pyronemataceae* kayıtları

Abstract: This study was based on fourteen bryophilous *Pyronemataceae* species. Thirteen of them (*Inermisia gyalectoides* (Svrček & Kubička) Dennis & Itzerott, *Lamprospora carbonicola* Boud., *Lamprospora dictydiola* Boud., *Lamprospora miniata* De Not., *Octospora areolata* (Seaver) Caillet & Moyne, *Octospora axillaris* (Nees) M.M. Moser, *Octospora coccinea* (P. Crouan & H. Crouan) Brumm., *Octospora excipulata* (Clem.) Benkert, *Octospora gemmicola* Benkert, *Octospora muscimuralis* Graddon, *Octospora orthotrichi* (Cooke & Ellis) K.B. Khare & V.P. Tewari, *Octospora polytrichi* (Schumach.) Caillet & Moyne and *Octospora rustica* (Velen.) J.Moravec) are given as new records for the macromycota of Turkey. *Inermisia* and *Lamprospora* are new at genus level. New localities are given for the 14th species, *Octospora leucoloma* Hedw. Brief descriptions about the macroscopic and microscopic characters of the species and their photographs are provided.

Key words: Biodiversity, bryoparasitic fungi, *Pyronemataceae*, new records, Turkey

Özet: Bu çalışma 14 briyofilik *Pyronemataceae* türü üzerinde temellendirilmiştir. Bunlardan 13 tanesi (*Inermisia gyalectoides* (Svrček & Kubička) Dennis & Itzerott, *Lamprospora carbonicola* Boud., *Lamprospora dictydiola* Boud., *Lamprospora miniata* De Not., *Octospora areolata* (Seaver) Caillet & Moyne, *Octospora axillaris* (Nees) M.M. Moser, *Octospora coccinea* (P. Crouan & H. Crouan) Brumm., *Octospora excipulata* (Clem.) Benkert, *Octospora gemmicola* Benkert, *Octospora muscimuralis* Graddon, *Octospora orthotrichi* (Cooke & Ellis) K.B. Khare & V.P. Tewari, *Octospora polytrichi* (Schumach.) Caillet & Moyne ve *Octospora rustica* (Velen.) J.Moravec) Türkiye makromikotası için yeni kayıttır. *Inermisia* ve *Lamprospora* cins düzeyinde yenidir. On dördüncü türe (*Octospora leucoloma* Hedw.) ilişkin yeni lokaliteler verilmiştir. Türlerin makroskobik ve mikroskobik karakterlerine ilişkin kısa betimlemeleri ve fotoğrafları verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, bryoparazitik mantarlar, *Pyronemataceae*, yeni kayıtlar, Türkiye

1. Introduction

Fungi are heterotrophic organisms growing on either dead organic material or on different organs of some other living organisms. Those growing on gametophytic or sporophytic organs of bryophytes are usually known as bryophilous fungi. Within the order *Pezizales* the members of the genera *Filicupula* Y.J.Yao & Spooner, *Hiemsia* Svrček, *Inermisia* Rifai, *Lamprospora* De Not, *Neottiella* (Cooke) Sacc., *Octospora* Hedw., *Octosporella* Döbbeler, *Octosporopsis* U.Lindem. & M.Vega and *Ramsbottomia* W.D.Buckley are known to have bryophilous mode of life (Felix, 1988; Benkert, 2007; Kirk et al., 2008). Except the genus *Filicupula* all of them are currently positioned in *Pyronemataceae*.

Since they require similar environmental conditions, moss and fungus could also live together. Bryophytes are capable of supporting fungal growth by providing a good microenvironment (Stephenson & Studlar 1985). Some fungi within the order *Pezizales*, especially the members of the genera *Octospora*, prefer small, acrocarpous mosses as substrates that can live in unfavorable conditions like a rocky or hot and frequently dry surfaces (Hughes, 1982).
Though research on bryoparasitic species has a long tradition in some European countries (Egertova et al., 2015) and the presence of about 300 species of ascomycetes growing on the gametophytes of mosses or hepatics were reported (Döbbeler, 1997), bryophilous fungi has received almost no attention in Turkey. According to the current checklists (Sesli and Denchev, 2014; Solak et al., 2015) 31 basidiomycete species growing on or around the bryophytes are known to exist in Turkey. But only six bryophilous ascomycete species, *Marcelleina persoonii* (P.Crouan & H.Crouan) Brumm. (*Pezizaceae*) (Yılmaz et al., 1997), *Neottiella rutilans* (Fr.) (Akata and Kaya, 2013), *Octospora itzerottii* Benkert (Uzun et al., 2017), *O. leucoloma* Hedw. (Çolak and Kaygusuz, 2017), *Scutellinia trechispora* (Berk. & Broome) Lambotte (*Pyronemataceae*) (Kaya et al., 2016) and *Pseudoplectania sphagnophila* (Pers.) Kreisel (*Sarcosomataceae*) (Türkoğlu and Yağız, 2012), have so far been recorded from Turkey yet. Compared to 81 octosporoid fungi existing in Europe (Benkert, 2007), there is still much to be done in Turkey.
The work aims to make a contribution to the mycobiota of Turkey by adding new bryophilous ascomycete species.

2. Materials and methods

Macrofungi samples were collected from the different localities within the boundaries of Gaziantep province between 2013 and 2017. Geographic positions were noted together with their necessary morphological and ecological characteristics, and they were photographed in their natural habitats. Olympus SZX7 trinocular stereomicroscope was used for some macrostructural properties of smaller fruit bodies. Nikon eclipse Ci-S trinocular light microscope, DS-Fi2 digital camera and Nikon DS-L3 displaying apparatus were used for microstructural investigation and photographing. Identification of the samples were performed with the help

of Seaver (1912, 1914, 1942), Le Gal (1940), Svrček and Kubička (1961), Dougoud and Roth (1972), Dennis and Itzerott (1973), Khare and Tewari (1975), Khare (1976, 2003), Breitenbach and Kränzlin (1984), Senn-Irlet (1988), Wang and Kimbrough (1992), Schumacher (1993), Fox et al. (1994), Benkert (1987, 1997, 1998), Yao and Spooner (1996), Hansen and Pfister (2006), Medardi (2006), Eckstein and Eckstein (2009, 2013), Eckstein (2014), Pradhan et al. (2013), Wieschollek (2013), Beug et. al. (2014), Eckstein et al., (2014) and Egertova et al., (2015).

3. Results

The systematics of the taxa are given in accordance with Cannon and Kirk (2007), Kirk et al. (2008), and the Index Fungorum (www.indexfungorum.org; accessed 02 November 2017). The taxa are listed in alphabetical order with brief descriptions, habitats, localities, collection dates and accession numbers.

Ascomycota Whittaker
Pezizales J. Schröt.

Pyronemataceae Corda

3.1. ***Inermisia gyalectoides*** (Svrček & Kubička) Dennis & Itzerott, Kew Bull. 28(1): 22 (1973) (Figure 1)

Syn. *Octospora gyalectoides* Svrček & Kubička.

Macroscopic and microscopic features: Apothecia 1-2 mm in diameter, more or less convex, pale orange to orange, sometimes with lighter margin. Asci 180-240 × 15-18 µm, cylindrical, eight spored, spores uniseriate. Paraphyses cylindrical, enlarged towards the apex, some forked above. Spores 16.5-22 × 9-12.5 µm, ellipsoid, smooth, hyaline, with a large oil drop. *Inermisia gyalectoides* grows in association with scattered small mosses, *Bryum argenteum* Hedw., *Funaria hygrometrica* Hedw, *Pterygoneurum ovatum* (Hedw.) Dixon and *Pottia* (Reichenbach) Fürnrohr sp. (Dennis and Itzerott, 1973; Benkert, 2007; Eckstein and Eckstein, 2009).

Specimen examined: Gaziantep, Şehitkâmil, city cemetery, on *Pterygoneurum ovatum*, 37°04′N, 37°23′E, 845 m, 04.01.2015, K.11164; Öğümsöğüt village, 37°06′N, 37′18°E, 1020 m, 07.03.2015, K.11409.

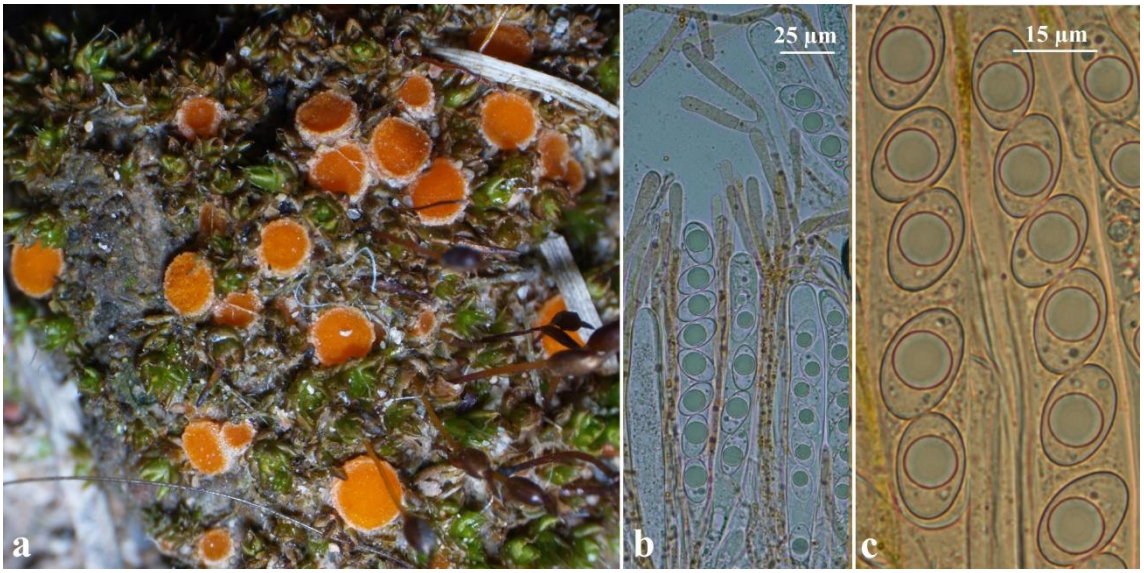

Figure 1. *Inermisia gyalectoides*: a- ascocarps, b- asci and paraphyses, c- ascospores.

3.2. ***Lamprospora carbonicola*** Boud., Hist. Class. Discom. Eur. (Paris): 68 (1907) (Figure 2)

Syn. *Barlaeina carbonicola* (Boud.) Sacc. & Traverso; *Octospora carbonicola* (Boud.) Yei Z. Wang.

Macroscopic and microscopic features: Apothecia 1-4 mm in diameter, broad, partly submerged in the soil among shoots of its host, hymenium orange to bright red, with conspicious membranaceous margin. Asci 180-260 × 16-19 µm, cylindrical, tapering towards the base. Paraphyses straight, septate, enlarged at the tip with orange, granular content. Spores 13-16 µm, globose to subglobose, with a ridged ornamentation forming a fine-meshed reticulum, and a large oil drop. *Lamprospora carbonicola* grows among the moss *Funaria* Hedwig, *Tortula* Hedw. (Wang and Kimbrough, 1992), *Pholia* Hedw., *Barbula* Hedw. sp. (Schumacher, 1993) on moist, burnt sandy gravelly forest floor in shade of trees (Pradhan et al., 2013).

Specimen examined: Gaziantep, Nizip, Sekili village, on cemetery wall with *Funaria hygrometrica* Hedw., 36°58′N, 37°40′E, 600 m, 28.02.2015, K.11340; Şehitkâmil, central city cemetery, 37°04′N, 37°23′E, 845 m, 06.03.2015, K.11383; Oğuzeli, cemetery, 36°57′N, 37°30′E, 670 m, 08.03.2015, K.11427; Nurdağı, Atalar village, 37°08′N, 36°54′E, 985 m, 22.03.2015, K.11497.

3.3. ***Lamprospora dictydiola*** Boud., Hist. Class. Discom. Eur. (Paris): 68 (1907) (Figure 3)

Syn. *Barlaeina dictydiola* (Boud.) Sacc. & Traverso; *Octospora dictydiola* (Boud.) Caillet & Moyne.

Macroscopic and microscopic features: Apothecia 1-2 mm in diameter, broad, shallow cupulate to turbinate, partly submerged among shoots of its host, hymenium orange to orange red, with prominent membranaceous margin. Asci 170-200 × 17-19 µm, cylindrical, gradually tapering towards the base. Paraphyses straight, sparsely branched from below, enlarged at the tip with orange, granular content. Spores 14-16 × 13.5-15.5 µm, globose to

subglobose, with a ridged ornamentation forming a fine-meshed reticulum, and a large oil drop. *Lamprospora dictydiola* was reported to grow on vertical parts of old garden wall among the moss *Tortula muralis* L. ex Hedw. (Benkert and Brouwer, 2004).

Specimen examined: Gaziantep, Şehitkâmil, central city cemetery, on *Tortula muralis*, 37°04′N, 37°23′E, 860 m, 27.03.2015, K.11503; Islahiye, Tandır village, Huzurlu high plateau, near the road, 36°58′N, 36°30′E, 1410 m, K.11899.

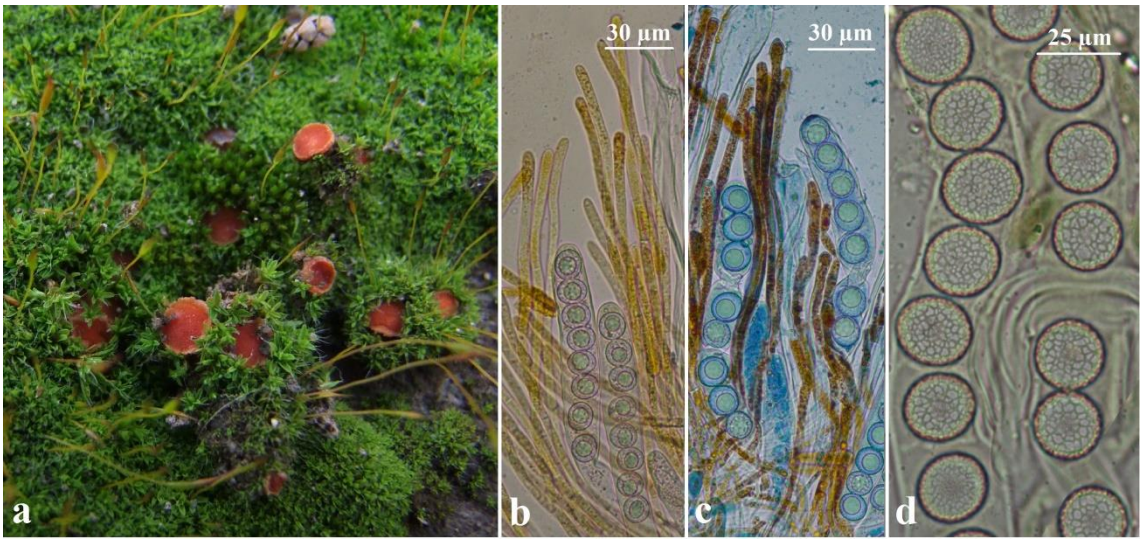

Figure 2. *Lamprospora carbonicola:* a- ascocarps, b,c- asci and paraphyses, d- ascospores.

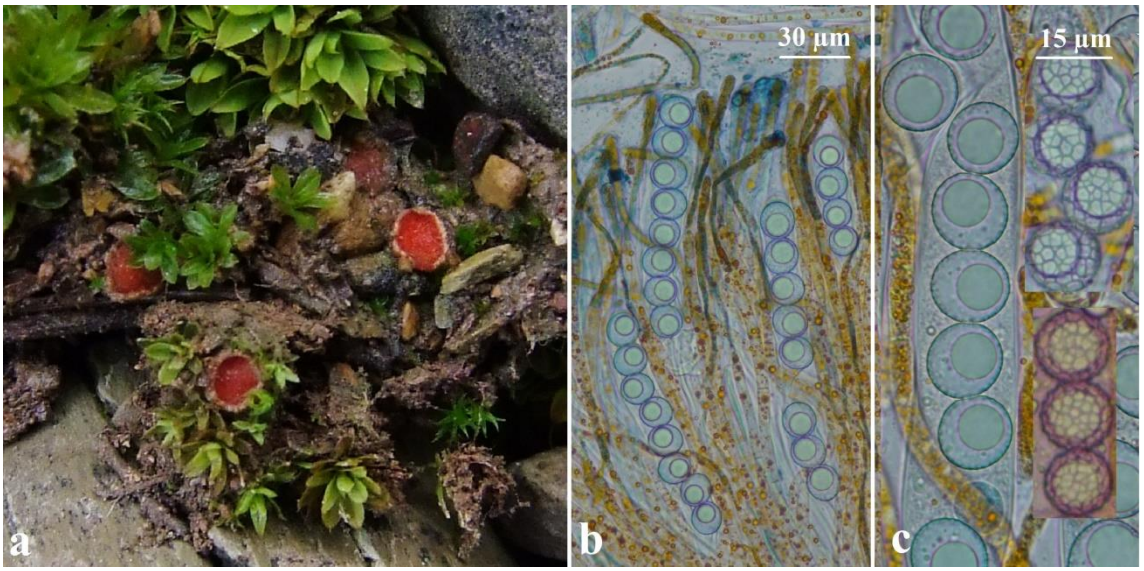

Figure 3. *Lamprospora dictydiola:* a- ascocarps, b- asci and paraphyses, c- ascospores.

3.4. ***Lamprospora miniata*** De Not., Comm. Soc. crittog. Ital. 1(fasc. 5): 388 (1864) (Figure 4)

Syn. *Ascobolus miniatus* P. Crouan & H. Crouan; *Barlaea crouanii* (Cooke) Massee; *Barlaea miniata* (De Not.) Sacc.; *Barlaeina miniata* (De Not.) Sacc. & Traverso; *Crouania crouanii* (Cooke) Lambotte; *Crouania miniata* (De Not.) Fuckel; *Humaria crouanii* (Cooke) Quél.; *Lamprospora crouanii* (Cooke) Seaver, *Lamprospora crouanii* (Cooke) Seaver f. *crouanii*; *Lamprospora crouanii* f. *magnihyphos*a J. Moravec; *Lamprospora miniata* De Not.; *Lamprospora miniata* De Not. f. *miniata*; *Lamprospora miniata* f. *parvispora* Benkert; *Lamprospora miniata* De Not. var. *miniata*; *Lamprospora miniata* var. *parvispora* Benkert; *Lamprospora miniata* var. *ratisbonensis* Benkert; *Octospora miniata* (De Not.) Caillet & Moyne; *Peziza crouanii* Cooke.

Macroscopic and microscopic features: Apothecia 1-5 mm in diameter, subglobose at first, expanding and becoming concave to almost plane with a slightly elevated margin when mature. Bright red, margin lighter. Asci 180-260 × 18-22 µm, cylindrical, eight spored, spores uniseriate. Paraphyses cylindrical, somewhat thickened above. Spores 15-17 µm in diameter, globose, hyaline, with a large oil drop and ridged ornamentation when mature. *Lamprospora miniata* grows in association with *Pottia bryoides* (Dicks.) Mitt. and *Phascum cuspidatum* Schreb. ex Hedw. (Eckstein and Eckstein, 2009).

Specimen examined: Gaziantep, Nizip, Sekili village, cemetery, on soil with *Phascum cuspidatum*, 36°58′N, 37°40′E, 600 m, 14.12.2014, K.11110; Oğuzeli, cemetery, 36°57′N, 37°30′E, 670 m, 05.04.2015, K.11664.

3.5. ***Octospora areolata*** (Seaver) Caillet & Moyne, Bull. trimest. Soc. mycol. Fr. 96(2): 199 (1980) (Figure 5)

Syn. *Lamprospora areolata* Seaver; *Lamprospora areolata* Seaver var. *areolata*; *Octospora areolata* (Seaver) Yei Z. Wang.

Macroscopic and microscopic features: Apothecia 0.5-1 mm in diameter, discoid to cupulate, hymenium orange red, light yellow when dry, margin slightly raised, outer surface darker and closely attached with moss. Asci 190-240 × 18-21 µm, clavate-cylindrical, eight spored, spores uniseriate. Paraphyses subclavate, septate, slightly enlarged at apex up to 6-7 µm. Spores 14-16 µm exluding ornamentation, globose, hyaline, marked with high ridges of 3-4 × 0.8 µm. *Octospora areolata* grows on soil among mosses (Wang and Kimbrough, 1992).

Specimen examined: Gaziantep, Nurdağı, Olucak village, on *Syntrichia ruralis* (Hedw.) F.Weber & D.Mohr, 37°10′N-36°40′E, 950 m, 10.04.2015, K.11684.

Figure 4. *Lamprospora miniata:* a- ascocarps, b- asci and paraphyses, c- ascospores.

Figure 5. *Octospora areolata:* a- ascocarps, b- asci and paraphyses, c- ascospores.

3.6. ***Octospora axillaris*** (Nees) M.M. Moser, in Gams, Kl. Krypt.-Fl., Edn 3 (Stuttgart) 2a: 110 (1963) (Figure 6)

Syn. *Helotium axillaris* (Nees) Boud.; *Humaria axillaris* (Nees) Sacc.; *Humaria carneola* (Saut.) Sacc.; *Humaria sublutea* Velen.; *Humarina axillaris* (Nees) Seaver; *Leucoloma axillaris* (Nees) Fuckel; *Octospora axillaris* (Nees) M.M. Moser var. *axillaris*; *Octospora axillaris* var. *dennisii* Itzerott; *Octospora axillaris* var. *tetraspora* Benkert; *Octospora carneola* (Saut.) Dennis; *Octospora sublutea* (Velen.) Svrček; *Peziza axillaris* Nees; *Peziza carneola* Saut.

Macroscopic and microscopic features: Apothecia 0.5-2 (2.5) mm in diameter, flat to convex, sessile, orange. Asci 140-190 × 18-22 µm, cylindrical, eight spored, somehow tapered toward the base, spores usually uniseriate. Paraphyses filiform, enlarged toward the apex up to 7-8 µm. Spores 21-28 × 10-12 µm, ellipsoid, hyaline, smooth, usually with two large oil drops. *Octospora axillaris* was reported to grow on soil among *Barbula unguiculata* Hedw., *Entosthodon fascicularis* (Hedw.) Müll. Hal., *Pottia davalliana* (Sm.) C.E.O.Jensen, *Pottia lanceolata* (Hedw.) Müll.Hal. (Caillet and Moyne, 1989), *Phascum cuspidatum* Schreb. ex Hedw., *Syntrichia ruralis* (Hedw.) F.Weber & D.Mohr, *Tortula ruralis* (Hedw.) Gaertn.

(Dennis and Itzerott, 1973; Benkert, 2007; Eckstein and Eckstein, 2009), and on the protonema of *Dicranella heteromalla* (Hedw.) Schimp. (Itzerott, 1977).

Specimen examined: Gaziantep, Islahiye, Hanağzı village, on soil among *Ptychostomum donianum* (Grev.) Holyoak & N.Pedersen, 37°03′N-36°36′E, 625 m, 08.11.2014, K.10515; K.10517.

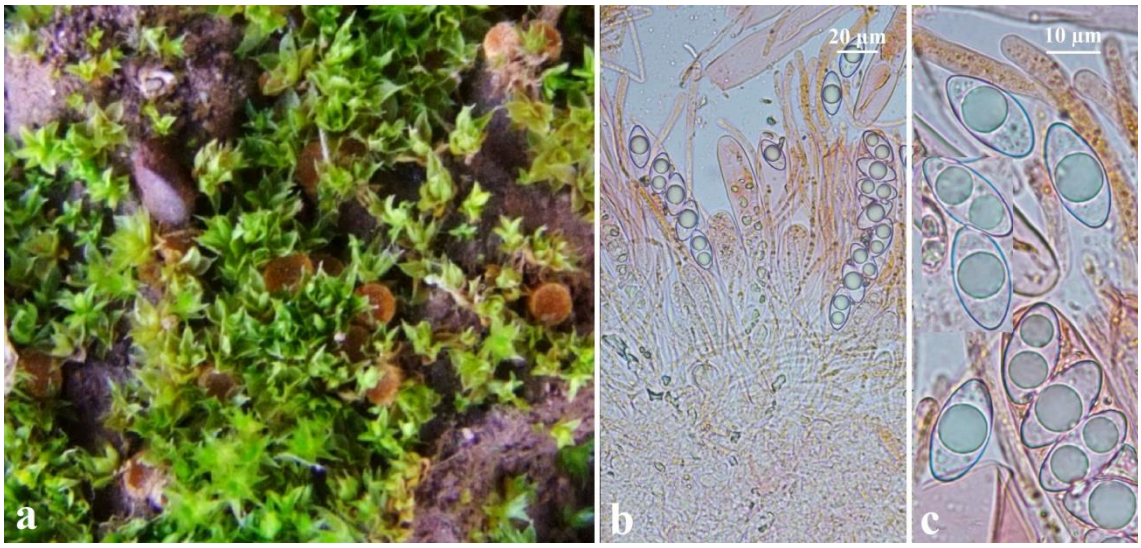

Figure 6. *Octospora axillaris:* a- ascocarps, b- asci and paraphyses, c- ascospores.

3.7. ***Octospora coccinea*** (P. Crouan & H. Crouan) Brumm., Persoonia, Suppl. 1: 213 (1967) (Figure 7)

Syn. *Ascobolus coccineus* P. Crouan & H. Crouan; *Byssonectria coccinea* (P. Crouan & H. Crouan) M. Torre; *Humaria coccinea* (P. Crouan & H. Crouan) Sacc.; *Humaria coccinea* var. *maritima* Grélet; *Humaria muralis* Quél.; *Humarina coccinea* (P. Crouan & H. Crouan) Seaver; *Humarina coccinea* (P. Crouan & H. Crouan) Seaver var. *coccinea*; *Humarina coccinea* var. *maritima* (Grélet) Cash; *Neottiella corallina* (Cooke) Massee; *Octospora coccinea* (P. Crouan & H. Crouan) Brumm. var. *coccinea*; *Octospora coccinea* var. *maritima* (Grélet) Parrett. & Gaggian.; *Octospora coccinea* var. *tetraspora* Benkert; *Peziza corallina* Cooke; *Peziza muralis* Quél.

Macroscopic and microscopic features: Apothecia 1-2 mm in diameter, cup to flat disk shaped, margin sometimes pubescent, hymenium orange. Asci 120-180 × 16-22 µm, cylindrical to clavate, eight spored, spores biseriate. Paraphyses cylindrical, very slim, enlarged towards the apex up to 3-7 µm, often slightly curved. Spores 25-27 × 7.5-9.5 µm, narrowly ellipsoid to narrowly fusiform, smooth, hyaline, with two big drops in the middle and often small drops at the poles. *Octospora coccinea* grows mainly among *Mniobryum* sp. but also with *Bryum argenteum* Hedw., *Bryum klinggraeffii* Schimp., *Ceratodon purpureus* (Hedw.) Brid., *Pottia* sp. (Dennis and Itzerott, 1973; Eckstein and Eckstein, 2009), *Encalypta vulgaris* Hedw. (Benkert, 2007), *Barbula unguiculata* Hedw. and *Oxyrrhynchium praelongum* (Hedw.) Warnst. (Caillet and Moyne, 1989).

Specimen examined: Gaziantep, Şehitkamil, Cerityeniyapan village, among *Bryum* sp., 37°10′N-37°08′E, 1070 m, 07.03.2015, K.11398; K.11402; Yavuzeli, Çimenli village, 37°16′N-37°34′E, 710 m, 29.03.2015, K.11548; Nurdağı, Kömürler village, roadside, 37°09′N-36°48′E, 535 m, 03.04.2015, K.11583; Atmalı village, 37°07′N-36°53′E, 700 m, 04.04.2015, K.11618.

Figure 7. *Octospora coccinea:* a- ascocarp, b- asci and paraphyses, c- ascospores.

3.8. ***Octospora excipulata*** (Clem.) Benkert, Mycologia Montenegrina 10: 10 (2008) (Figure 8)

Syn. *Leucopezis excipulata* Clem., *Octospora roxheimii* Dennis & Itzerott, *Octospora roxheimii* var. *aestivalis* Caillet & Moyne, *Octospora roxheimii* Dennis & Itzerott, var. *roxheimii.*

Macroscopic and microscopic features: Apothecia 0.5-5 mm in diameter, pale orange to orange, lighter at the margin. Asci 150-220 × 17-22 µm, cylindrical to clavate, eight spored, spores uniseriate. Paraphyses cylindrical, septate, enlarging towards the apex up to 5-7 µm. Spores 17-27 × 12-17 µm, ellipsoid, smooth, hyaline, with a central large drop. *Octospora excipulata* grows in association with the moss *Funaria hygrometrica* Hedw. (Benkert, 2007; Eckstein and Eckstein, 2009).

Specimen examined: Nurdağı, Atmalı village, roadside, among *Bryum dichotomum* Hedw., 37°08′N-36°52′E, 620 m, 04.04.2015, K.11619; Ataköy, roadside, among moss, 37°08′N-36°54′E, 985 m, 22.03.2015, K.11486; Şehitkamil, Öğümsöğüt village, roadside, 37°07′N-37°19′E, 1110 m, 01.03.2015, K.11371; Gaziantep, Şahinbey, Gaziantep Zoo, among moss, 37°02′N-37°17′E, 970 m, 15.11.2015, K.12788.

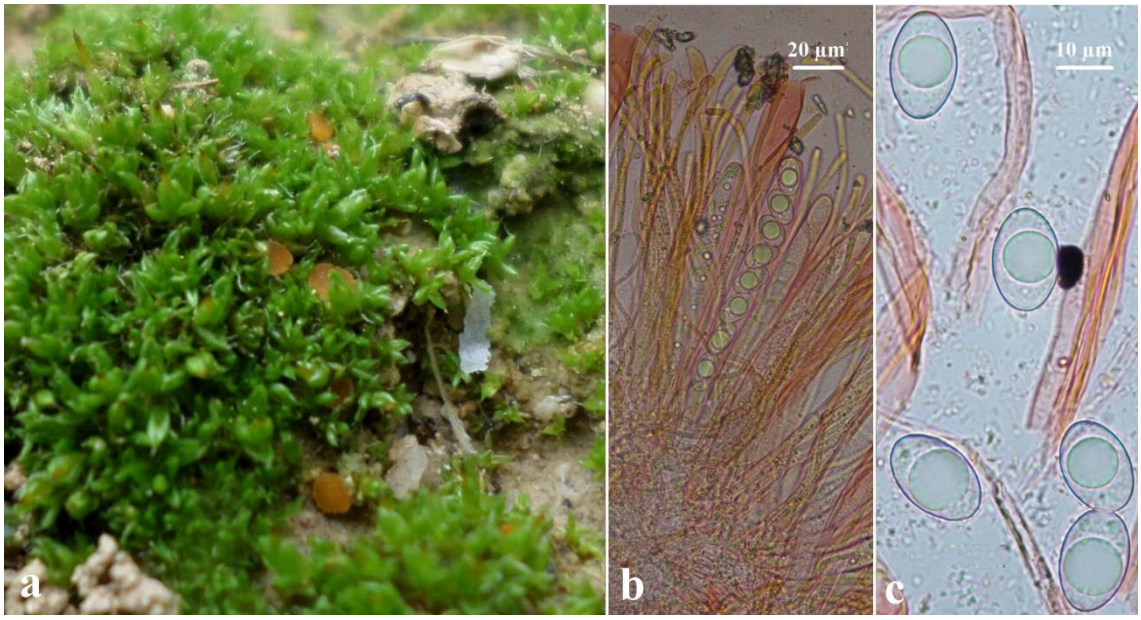

Figure 8. *Octospora excipulata:* a- ascocarps, b- asci and paraphyses, c- ascospores.

3.9. ***Octospora gemmicola*** Benkert, Öst. Z. Pilzk. **7**: 49 (1998) (Figure 9)

Syn. *Octospora gemmicola* Benkert, var. *gemmicola*, *Octospora gemmicola* var. *tetraspora* Benkert.

Macroscopic and microscopic features: Apothecia 1-2.5 mm in diameter, cup to disc shaped with a weak membranaceous margin, hymenium plane to cupulate, orange. Asci 160-240 × 16-23 µm, cylindrical to somewhat clavate, eight spored, spores mostly biseriate. Paraphyses cylindrical, enlarged toward the apex up to 5-8 µm. Spores 18-25 × 12-15 µm, narrowly ellipsoid to subfusiform, smooth, one to two (seldomly three) large and several small oil droplets. *Octospora gemmicola* was reported to grow among *Bryum dichotomum* Hedw., *B. rubens* Mitt., *B. ruderale* Crundw.& Nyholm and *B. radiculosum* Brid. (Eckstein and Eckstein, 2009).

Specimen examined: Gaziantep, Şehitkamil, Dülükbaba picnic area, on *Bryum* sp, 37°07′N-37°19′E, 1110 m, 01.03.2015, K.11367.

3.10. ***Octospora leucoloma*** Hedw., Descr. micr.-anal. musc. frond. (Lipsiae) 2: 13 (1789) (Figure 10)

Specimen examined: Gaziantep, Şahinbey, Yeşilkent cemetery, among *Pterygoneurum ovatum* (Hedw.) Dixon, 37°00′N-37°25′E, 865 m, 13.11.2015, K.12771; Nurdağı, Kömürler village, roadside, 37°09′N-36°48′E, 535 m, 03.04.2015, K.11593; Konya, city center, bus terminal, between the floor brick, on *Bryum argenteum*, 37°56′N-32°30′E, 1030 m, 04.12.2017, K.13944.

3.11. ***Octospora musci-muralis*** Graddon, Trans. Br. mycol. Soc. 58(1): 147 (1972) (Figure 11)

Syn. *Octospora musci-muralis* Graddon var. *musci-muralis*; *Octospora musci-muralis* var. *neglecta* (Dennis & Itzerott) Benkert; *Octospora neglecta* Dennis & Itzerott.

Macroscopic and microscopic features: Apothecia 1-4 mm in diameter, hymenium bright orange to orange-brown, margin paler and finely toothed. Asci 160-200 × 19-24 µm, cylindrical to clavate, attenuated at the base, 8 spored, spores biseriate. Paraphyses filiform, apically curved and enlarged up to 4-5 µm. Spores 20-25 × 9-10.5 µm, elliptical to subcylindrical, hyaline, with two or one large drops. *Octospora musci-muralis* was reported to grow on the walls on the pads of *Grimmia* Hedw. sp. (Dennis and Itzerott, 1973; Eckstein and Eckstein, 2009) and *Schistidium* Brid. sp. (Benkert, 2007).

Specimen examined: Gaziantep, Şehitkâmil, cemetery, on *Grimmia pulvinata* (Hedw.) Sm., 37°04′N-37°23′E, 845 m, 27.02.2015, K.11329; Cerityeniyapan village, 37°10′N-37°08′E, 1070 m, 07.03.2015, K.11395; Oğuzeli, cemetery, 36°57′N-37°30′E, 670 m, 08.03.2015, K.11428.

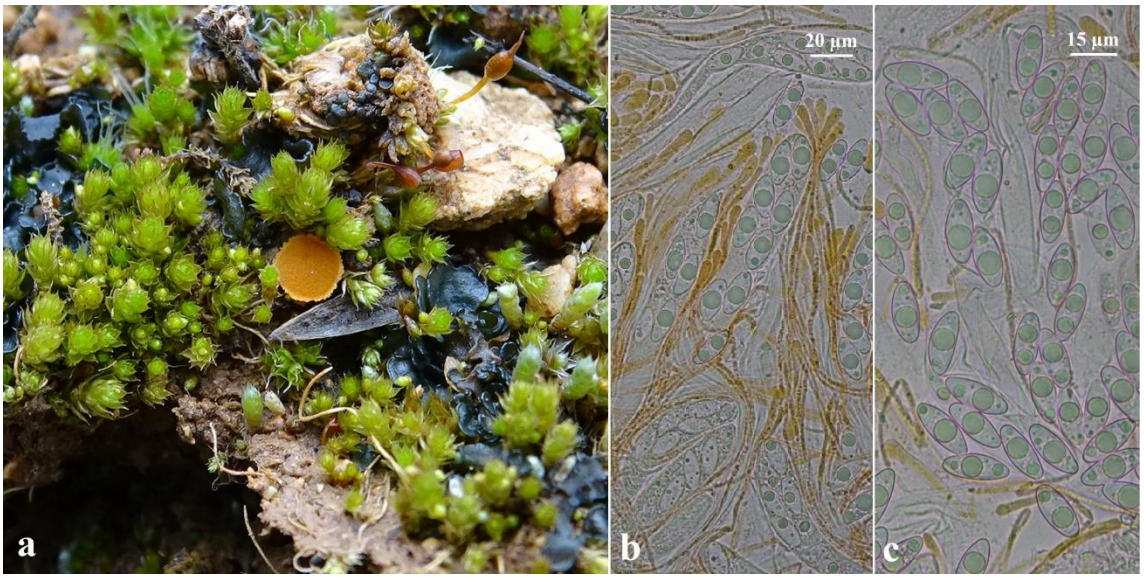

Figure 9. *Octospora gemmicola:* a- ascocarp, b- asci and paraphyses, c- ascospores.

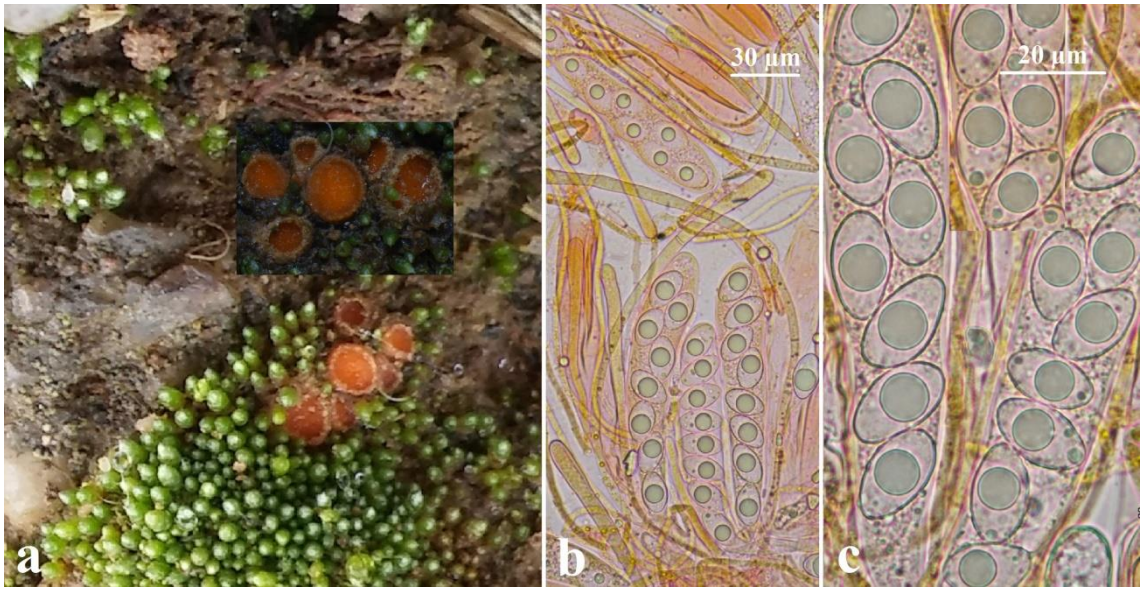

Figure 10. *Octospora leucoloma:* a- ascocarps, b- asci and paraphyses, c- ascospores.

3.12. ***Octospora orthotrichi*** (Cooke & Ellis) K.B. Khare & V.P. Tewari, Can. J. Bot. 56(17): 2118 (1978) (Figure 12)

Syn. *Humaria orthotrichi* (Cooke & Ellis) Sacc., *Humarina orthotrichi* (Cooke & Ellis) Seaver, *Peziza orthotrichi* Cooke & Ellis.

Macroscopic and microscopic features: Apothecia 0.5-2 mm in diameter, cup to disc shaped, with inconspicuous membranaceous margin, hymenium pale orange to orange, margin paler. Asci 140-240 × 17-24 µm, cylindrical, 8 spored, spores generally uniseriate. Paraphyses straight, septate, enlarged towards the apex. Spores 15-20 × 10-13 µm, ellipsoid to broadly ellipsoid, some with flattened one side, warted, with one or less often with two guttules. Though the exclusive association of *Octospora orthotrichi* with *Orthotrichum diaphanum* Brid. was reported (Benkert, 1998), it can also be found on the bark of trees or on stones, always between shoots of its host (Egertova et al., 2015).

Specimen examined: Gaziantep, Nurdağı, Olucak village, on *Orthotrichum diaphanum* Schrad. ex Brid., 37°10′N-36°40′E, 950 m, 10.04.2015, K.11678.

3.13. ***Octospora polytrichi*** (Schumach.) Caillet & Moyne, Bull. trimest. Soc. mycol. Fr. 96(2): 192 (1980) (Figure 13)

Syn. *Barlaea polytrichi* (Schumach.) Sacc.; *Lamprospora polytrichi* (Schumach.) Le Gal; *Neottiella polytrichi* (Schumach.) Massee; *Peziza polytrichi* Schumach.; *Peziza turfosa* Pers.; *Sarcoscypha polytrichi* (Schumach.) Höhn.

Macroscopic and microscopic features: Apothecia 1-3 mm in diameter, saucer to disc-shaped, resting stalkless on the ground. Hymenium orange to orange reddish. Outer surface lighter, margin smooth. Asci 230-250 × 19-21 µm, cylindrical, tapering towards the base. Paraphyses cylindrical, forket at the base, septate. Spores 12-14 µm in diameter, round, hyaline and reticulately ornamented. *Octospora polytrichi* was reported to grow in association with *Polytrichum* Hedw. sp. (Wang and Kimbrough, 1992).

Figure 11. *Octospora musci-muralis:* a- ascocarps, b- asci and paraphyses, c- ascospores.

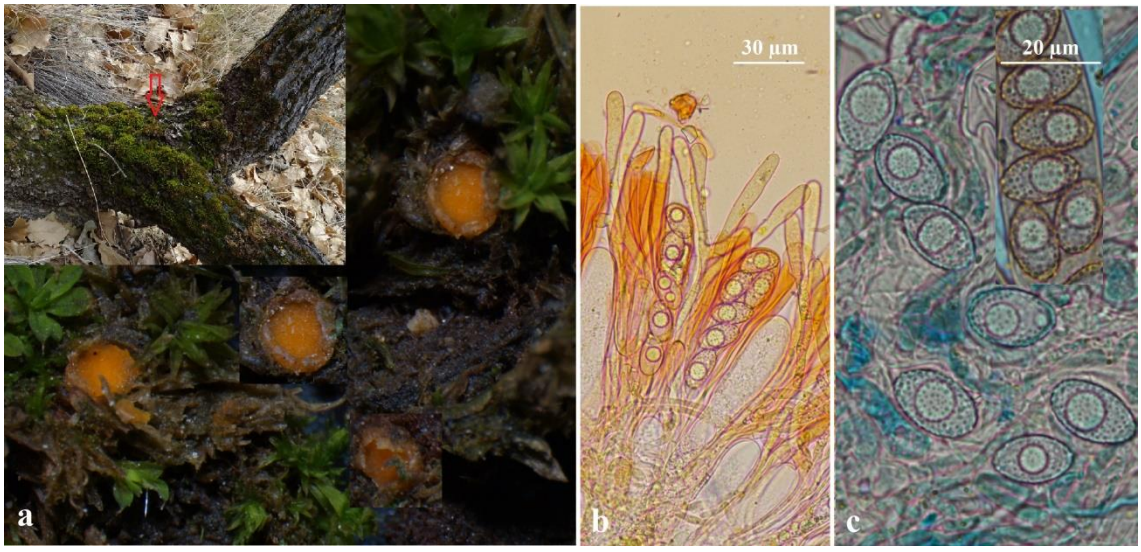

Figure 12. *Octospora orthotrichi:* a- ascocarps, b- asci and paraphyses, c- ascospores.

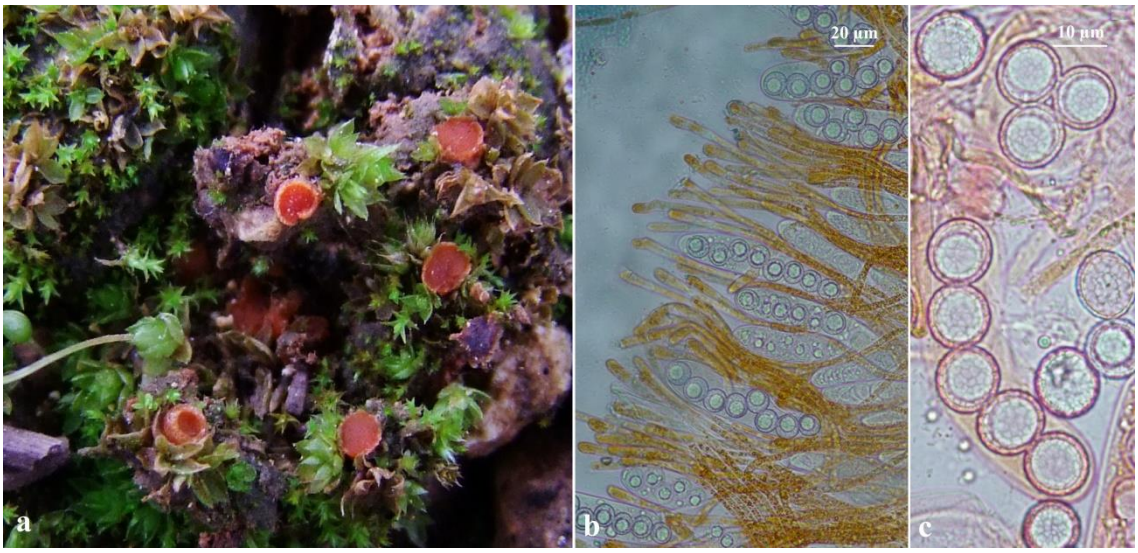

Figure 13. *Octospora polytrichi:* a- ascocarps, b- asci and paraphyses, c- ascospores.

Specimen examined: Gaziantep, Nurdağı, Kömürler village, roadside, among *Funaria hygrometrica* Hedw., 37°09′N-36°48′E, 535 m, K.11581.

3.14. ***Octospora rustica*** (Velen.) J. Moravec, Česká Mykol. 23(4): 226 (1969) (Figure 14)

Syn. *Humaria rustica* Velen.

Macroscopic and microscopic features: Apothecia 1-4 mm in diameter, at first cup shaped, soon becomes flat and disc shaped, hymenium orange, pale orange without a membranaceous margin. Asci 140-190 × 14-19 µm, cylindrical, eight spored, spores uniseriate. Paraphyses cylindrical and enlarged up to 4-7 µm towards the apex. Spores 15-18 × 10-12 µm, ellipsoid, smooth, hyaline with a large drop. *Octospora rustica* grows on sandy soil on burnt ground among *Ceratodon purpureus* (Hedw.) Brid. (Dennis and Itzerott, 1973; Cailet and Moyne, 1989).

Specimen examined: Gaziantep, Nurdağı, Atmalı village, among *Tortula acaulon* (With.) R.H. Zander, 37°08′N-36°52′E, 620 m, 12.04.2015, K.11728.

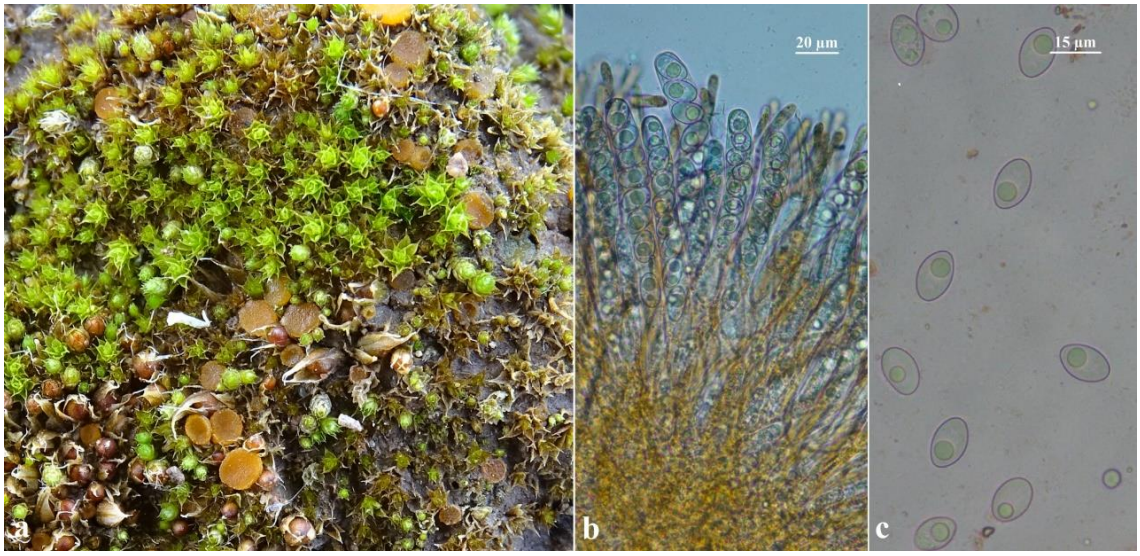

Figure 14. *Octospora rustica:* a- ascocarps, b- asci and paraphyses, c- ascospores.

4. Discussion

The habit is very similar in most bryophilous *Pezizales* with extremely small and more or less orange colored ascomata hidden between host plants or organs. That's why they are usually an easily overlooked group of fungi. Even for a keen and experienced observer it is a time-consuming and troublesome task to detect them in field (Döbbeler, 1997). Due to the close similarities between macroscopic features, microscopic characteristics, especially the spores as being globose, subglobose, ellipsoid, fusiform, smooth or ornamented, are generally used as important diagnostic characters for the identification of them.

Though *Lamprospora* and *Octospora* have generally been differentiated from each other by globose and ellipsoid spores (Seaver, 1914; Yao and Spooner, 1996), here the systematics of Kirk et al. (2008) and the Index fungorum (www.indexfungorum.org; accessed 2 November 2017) were followed.

As a result of this study, two bryophilous ascomycete genera (*Inermisia* and *Lamprospora*) and thirteen bryophilous species within the order *Pezizales* (*Inermisia* 1, *Lamprospora* 3 and *Octospora* 9) were added as new records for the macromycota of Turkey, increasing the number of bryophilous ascomycete species of Turkey from 6 to 19. Among the determined species, *Inermisia gyalectoides* resembles *Sepultaria semi-immersa* (P. Karst.) Massee, in terms of habitat and morphology. But it differs with colorless and thin-walled anchoring hyphae, and less fusoid and shorter ascospores. It also recalls *Inermisia pilifera* (Cooke) Dennis & Itzerott, from which it differs with larger and broder ascospores (Dennis and Itzerott, 1973).

Due to the spore similarity, *Lamprospora carbonicola* has generally been misinterpreted and frequently confused with *L. dictydiola*. However, the habitat and the host of latter species makes it easy to distinguish each other (Benkert, 1987). This species also has morphological similarities with *Pulvinula mussooriensis* (K.S. Thind, E.K. Cash & Pr. Singh) L.R. Batra. But *P. mussoriensis* differs with yellow apothecia and smaller spores (Pradhan et al., 2013; Eckstein and Eckstein, 2013).

Seaver (1914) has noted the similarity of *Octospora areolata* to the *Moravecia calospora* (Quél.) Benkert, Caillet & Moyne, but round and non-elliptic spores of *O. areolata* distinguishes it from the latter species.

Octospora musci-muralis may grow in association with cushions of *Grimmia* sp., where *O. grimmia* Dennis & Itzerott generally exists. However *O. music-muralis* is easily recognizable by its elliptic cylindric, smooth and biguttulate ascospores (Dennis and Itzerott, 1973).

Octospora orthotrichi has a special habitat, *Orthotrichum* sp., but *O. affinis* Benkert & L.G. Krieglst may also parasitize the mosses within the same genus. However, smaller, ellipsoid to subglobose spores and exclusive parasiticity of *O. affinis* on *Orthotrichum affine* Schrad. ex Brid. differs it from *O. orthotrichi* (Egertova et al., 2015).

Morphological and ecological characteristics of the determined species generally agree with those given in literature. But some ecological differences were also determined. For example, *O. areolata* were reported to

grow on some mosses belonging to the genera *Bryum* and *Pohlia*, but our sample was determined on *Syntrichia ruralis.* Here we determined the substrate of *Octospora exipulata* as *Bryum dichotomum*, while it was reported on *Funaria hygrometrica*. Likewise, *Ptychostomum donianum, Pterygoneurum ovatum* and *Tortula acaulon* were also determined as new hosts for *O. axillaris* and *O. rustica* respectively.

Acknowledgements

The authors would like to thank TÜBİTAK for supporting the project (212T112) financially, and Prof. Dr. Güray Uyar for identifying the bryophyte samples.

References

Akata I, Kaya A (2013). Three pyronemataceous macrofungi genera new to Turkish Mycota. Turkish Journal of Botany 37(5): 977-980.

Benkert D (1987). Contributions to the taxonomy of the genus *Lamprospora (Pezizales)*. Zeitschrift für Mykologie 53(2): 195-272.

Benkert D (1997). New Species of the Genus *Octospora*. Beiträge zur Kenntnis der Pilze Mitteleuropas XI: 35-42.

Benkert D (1998). Beiträge zur Kenntnis bryophiler Pezizales-Arten. 8. Viersporige Taxa der Gattung *Octospora*. Österreichische Zeitschrift für Pilzkunde 7: 39-63.

Benkert D (2007). Knowledge of the Occurrence of Bryophilous *Pezizales* (Ascomycotina) in South-East Europe. Mycologia Montenegrina 10: 7-21.

Benkert D, Brouwer E (2004). New Species of *Octospora* and Some Further Remarkable Bryoparasitic *Pezizales* From the Netherlands. Persoonia 18(3): 381-391.

Beug MW, Bessette AE, Bessette AR (2014). Ascomycete Fungi of North America. A Mushroom Reference Guide.

Breitenbach J, Kränzlin F (1984). Fungi of Switzerland Volume 1. Lucerne: Verlag Mykologia.

Caillet M, Moyne G (1988-89). Bull. Soc. Hist. nat Doubs 84: 9-24.

Cannon PF, Kirk PM (2007). Fungal Families of the World. Wallingford, UK: CAB International.

Çolak ÖF, Kaygusuz O (2017). *Octospora leucoloma* (*Pyronemataceae*): a new bryoparasitic genus record for Turkish mycobiota. Phytologia Balcanica 23(3): 345-348.

Dennis RWG, Itzerott H (1973). *Octospora* and *Inermisia* in Western Europe. Kew Bulletin 28: 5-23.

Döbbeler P (1997). Biodiversity of bryophilous ascomycetes. Biodiversity and Conservation 6: 721-738.

Dougoud R, Roth JJ (1972). *Octospora musci-muralis* Graddon. Trans. Brit. Mycol. Soc. 58: 97-103.

Eckstein J (2014). *Lamprospora hispanica* and *Lamprospora tuberculatella* - two bryoparasitic discomycetes (*Ascomycota, Pezizales*) new to Germany. Zeitschrift für Mykologie 80(1): 105-117.

Eckstein J, Eckstein G (2009). Bryoparasitic *Pezizales* (Ascomycetes) of the genera *Lamprospora*, *Octospora* and *Neottiella* in the Old Botanical Garden at Göttingen (Germany, Lower Saxony). Herzogia 22: 213-228.

Eckstein J, Eckstein G (2013). Noteworthy findings of bryoparasitic *Pezizales* (*Ascomycota*) from Germany. Boletus 34: 55-66.

Eckstein J, Eckstein G, Vega, M (2014). Bemerkenswerte Funde bryoparasitischer *Pezizales* (*Ascomycota*) aus Deutschland II. Boletus 35: 17-25.

Egertova Z, Eckstein J, Vega M (2015). *Lamprospora tuberculata*, *Octospora ithacaensis*, *O. orthotrichi* and *O. affinis* – four bryoparasitic ascomycetes new to the Czech Republic. Czech Mycology 67: 119-133.

Felix H (1988). Fungi on bryophytes, a review. Botanica Helvetica 98: 239-269.

Fox HF, Grant PJ, Moore CR (1994). *Octospora musci-muralis* Graddon, (*Ascomycotina: Humariaceae*) in Ireland. The Irish Naturalists Journal 24(9): 378-379.

Hansen K, Pfister DH (2006). Systematics of the *Pezizomycetes:* The Operculate *Discomycetes.* Mycologia 98(6): 1029-1040.

Hughes JG (1982). Penetration by rhizoids of the moss *Tortula muralis* Hedw. into well cemented oolitic limestone. International Biodeterioration Bulletin 18: 43-46.

Index Fungorum (2017). http://www.indexfungorum.org/Names/Names.asp. Accessed 2 November 2017.

Itzerott H (1977). The genus *Octospora* and an attempt to solve its taxonomic problems. Kew Bulletin 31: 497-500.

Kaya A, Uzun Y, Karacan İH, Yakar S (2016). Contributions to Turkish *Pyronemataceae* from Gaziantep province. Turkish Journal of Botany 40(3): 298-307.

Khare KB (1976). Some *Lamprospora* species from India. Norwegian Journal of Botany 23: 1-5.

Khare KB (2003). Descriptions of and comments on some species of *Octospora* and *Kotlabaea* (Pezizales, Humariaceae). Nova Hedwigia 77: 445-485.

Khare KB and Tewari VP (1975). The Genus *Octospora* and Its Typification. Mycologia 67(5): 972-979.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Dictionary of the Fungi. 10th ed. Wallingford, UK: CAB International.

Le Gal M (1940). *Lamprospora polytrichi* (Fr. ex Schum.). Bulletin trimestriel de la Société mycologique de France 56: 39-45.

Medardi G (2006). Atlante fotografico degli Ascomiceti d'Italia. A.M.B. Fondazione, Centro Studi Micologici, Italia.

Pradhan P, Dutta AK, Acharya K (2013). *Pezizales* of West Bengal, India I. *Pyronemataceae*: *Lamprospora* and *Miladina*. Asian Journal of Microbiological Biotechnology Environmenatl Science 15: 491-494.

Schumacher TK (1993). Studies in arctic and alpine *Lamprospora* species. Sydowia 45(2): 307-337.

Seaver FJ (1912). The Genus *Lamprospora*, with Descriptions of Two Species. Mycologia 4: 45-48.

Seaver FJ (1914). A Preliminary Study of the Genus *Lamprospora*. Mycologia 6: 5-24.

Seaver FJ (1942). The North American Cup-Fungi (Operculates). Supplementary Edition. New York, NY, USA.

Senn-Irlet B (1988). Zum Nachweis der Bryoparasitischen Lebensweise von *Octospora orthotricha* und *O. tetraspora* (*Pezizales, Ascomycetes*). Mycologia Helvetica 3: 173-181.

Sesli E, Denchev CM (2014). Checklists of the Myxomycetes, Larger Ascomycetes, and Larger Basidiomycetes in Turkey. 6th edn. Mycotaxon Checklists Online. (http://www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf): 1-136.

Solak MH, Işıloğlu M, Kalmış E, Allı H (2015). Macrofungi of Turkey, Checklist, Vol. II. İzmir, Turkey: Üniversiteliler Ofset.

Stephenson SL, Studlar SM (1985). Myxomycetes fruiting upon bryophytes: coincidence or preference? Journal of Bryology 13(4):537-548.

Svrček M, Kubička J (1961). Some operculate Discomycetes from the vicinity of the pond 'Dvoriste' in southern Bohemia. Ceská Mykologie. 15(2):61-77.

Türkoğlu A, Yağız D (2012). Contributions to the macrofungal diversity of Uşak Province. Turkish Journal of Botany 36(5): 580-589.

Uzun Y, Karacan İH, Yakar S, Kaya A (2017). *Octospora* Hedw., A new genus record for Turkish *Pyronemataceae.* Anatolian Journal of Botany 1(1): 18-20.

Wang YZ, Kimbrough JW (1992). Monographic studies of North American species of *Octospora* previously ascribed to *Lamprospora* (Pezizales, Ascomycetes). Special Publication no. 4, National Museum of Natural Science. Taiwan.

Wieschollek D (2013). *Octospora gemmicola* var. *tetraspora* Benkert, a bryoparasitic discomycete with four-spored asci new for Saxony-Anhalt. Boletus 34: 67-71.

Yao YJ, Spooner BM (1996). Notes on British species of *Octospora*. Mycologiacal Research 100: 175-178.

Yılmaz F, Öder N, Işıloğlu M (1997). The macrofungi of the Soma (Manisa) and Savaştepe (Balıkesir) districts. Turkish Journal of Botany 21(4): 221-230.

Cite this article: Uzun Y, Karacan İH, Yakar S, Kaya A (2018). New bryophillic *Pyronemataceae* records for Turkish *Pezizales* from Gaziantep province. Anatolian Journal of Botany 2(1): 28-38.

Anatolian Journal of Botany
2(1): 39-45 (2018)
doi:10.30616/ajb.379122

Anatomical, palynological, morphological, karyological, and ecological investigations on *Gypsophila davisii*

Metin ARMAĞAN[1*], Fevzi ÖZGÖKÇE[2]
[1]*Adnan Menderes University, Buharkent Vocational School, Department of Medicinal and Aromatic Plants, Aydın, Turkey*
[2]*Tuşba District Municipality Presidency, Van, Turkey*
**metinarmagan@gmail.com*

Received :16.01.2018
Accepted :07.02.2018

Gypsophila davisii üzerinde anatomik, palinolojik, morfolojik, karyolojik ve ekolojik araştırmalar

Abstract: *Gypsophila davisii* Barkoudah, an endemic species from Muğla (Turkey), was investigated for the first time as a whole in terms anatomy, morphology, ecology, karyology, and palynology. No druse crystals were observed in stems and roots of examined specimens in contrast to the known. Seeds are pyriform with shiny, smooth grooves on the surface. The pollen grains are polyporate, spheroidal, granulate-microechinate-microperforate. *G. davisii* has 30 somatic chromosomes (2n=30). The samples are compared morphologically with the description given in Flora of Turkey.

Key words: Anatomy, chromosome number, ecology, *Gypsophila davisii*, morphology, palynology.

Özet: Muğla'ya endemik bir tür olan *Gypsophila davisii* Barkoudah, anatomik, morfolojik, ekolojik ve palinolojik açıdan bir bütün olarak ilk kez incelenmiştir. Bilinenin aksine incelenen örneklerin kök ve gövdelerinde hiçbir druz kristaline rastlanmamıştır. Tohumlar armut şeklinde olup yüzeyinde parlak düz tüberküller vardır. Polen taneleri, çok porlu, küremsi, granül-mikroekinat-mikroperforattır. *G. davisii*'nin 30 somatik kromozomu vardır (2n = 30). Örnekler morfolojik açıdan Türkiye Florası'ndaki betim ile karşılaştırılmıştır.

Anahtar Kelimeler: Anatomi, kromozom sayısı, ekoloji, *Gypsophila davisii*, morfoloji, palinoloji.

1. Introduction

The genus name *Gypsophila* Barkoudah was derived with the combination of the Greek terms "gypsos=gypsiferous soil" and "philos=like, prefer", since the members of this genus prefers gypsiferous soils or limestones (Korkmaz et al., 2012). Seventy four *Gypsophila* species, 43 of which are endemic, currently exist in Turkey (Barkoudah, 1962; Huber-Morath, 1967; Davis et al., 1988; Ataşlar, 2000; Ekim, 2012; Armağan et al., 2017). The existence of all the three subgenera (*Gypsophila*, *Pseudosaponaria* and *Macrorhizaea*) of *Gypsophila* increases the importance of this genus in terms of Turkey. Among the 126 species of the subgenus *Gypsophila*, 75 species have distribution in the region containing Turkey, North Iraq, North Iran, Caucasus and Black Sea region (Barkoudah, 1962). The pollens of *Gypsophila* taxa are spheroid and polyporate, and generally have granulate-microechinate-microperforate ornamentations (Ataşlar et al., 2009). Members of *Gypsophila* contains excess amount of calcium oxalate crystals in the structure of their roots and stems (Ataşlar and Ocak, 2017). Although diploid number of the chromosomes (2n=34) is a typical representative of this genus, the differences at a ploidy level has been found between the *Gypsophila* species (Vettori et al., 2015). *Gypsophila* species generally prefer of gyps and erosive areas. They are widespread in arid and semiarid steppe areas and also distributed on dry and calcareous rocks, serpentine rocks, and stony–sandy lands (Korkmaz and Özçelik, 2013).

The aims of this study is to report of pollen morphological, anatomical, karyological and ecological characteristics of *G. davisii* for the first time and thus to provide a contribution to the taxonomy of the genus *Gypsophila*.

2. Materials and Method

The *Gypsophila davisii* specimens which are collected during PhD thesis and the herbarium specimens previously collected within the boundaries of Turkey constitute the materials of the study. The initial samples were collected from Muğla province (Figure 1). During field works ecological and morphological characteristics of the populations were recorded, photographed in their natural habitat and some of them were collected to prepare herbarium specimens and some of them keep in 70 % ethanol. For further ecological studies, soil samples were also collected from a deep of 20 cm. Morphological, studies was carried out in the herbarium of VANF, HUB, GAZI, K, and E (herbaria acronyms according to Thiers 2016+), while anatomical, ecological, palynological, and karyological studies were conducted in the laboratories of Yüzüncü Yıl University.

2.1. Morphological studies

Morphological findings were obtained from the field, herbaria and laboratories works. Macromorphological and some micromorphological measurements were performed from the herbarium samples. High resolution photographs of the specimens kept in different virtual herbaria were also investigated. The terminology of Stearn (1992) and Bittrich (1993) was adopted to describe the seed coat.

2.2. Anatomical studies

Anatomical findings were obtained by thin sections from the roots, stems and leaves of the samples kept in %70

ethanol. These sections were stained with Fast Green and Safranin dyes. Metcalfe and Chalk (1957) were followed for the anatomical terminology.

2.3. Ecological studies

Soil samples which were transported to the laboratory in cloth bags were used for further ecological studies. They were dried and passed through from 2 mm sieves. Then, the physical and chemical (texture, pH, salinity, CaCO3, N, P, K, Ca, Mg, Fe, Cu, Zn, Mn and Na) analysis of them were carried out by using standard techniques, in the laboratories of Agricultural Analysis Laboratory of Eğirdir Fruit Research Station.

2.4. Palynological studies

Pollen grains were investigated using both light and scanning electron microscopes (SEM). Wodehouse (1935) technique was followed for light microscopy preparations. Pollen determining terminology is in accordance with Hesse et al. (2009). Terminology for pollen morphology proposed by Hesse et al. (1966) and Ataşlar et al. (2009) was considered.

2.5. Seed surface studies

Seed surface investigations were carried out using a Leica EZ4 Stereo Microscope. Shape, colour, length, width, surface structure, and length/width ratio of the seeds were determined. For this purpose, more than 20 seeds were measured and photographed. A millimetric ruler was used to measure the length and the width of the seeds, and the smallest and the biggest average data were provided.

Figure 1. The distribution map of *Gypsophila davisii*. ▲ In Flora of Turkey, ● in this study.

2.6. Karyological studies

Mature seeds of the collected *G. davisii* samples were germinated in petri dishes and root tips of these plantlets were used for karyological investigations. The seeds were germinated within one week at room temperature. Though methods used to obtain chromosomes vary more or less according to the researchers and the species, the common first step for all the methods was fixation, hydrolysis and staining (Darlington and La Cour, 1976; Elçi, 1994, Atasagun et al, 2016).

Root tips were pretreated with 1% α-monobromonaphthalene at 4°C for 16-17 h. Root tips were fixed with Farmer fixative (3:1 ethyl alcohol: glacial acetic acid) for 24 h at 4°C. The material was hydrolyzed with 1N HCl for 13-14 minutes at room temperature after the alcohol-extracted root tips have been washed with distilled water several times. The chromosomes were stained with 2% acetic orcein and mounted in 45% acetic acid. Permanent slides were prepared using Canada balsam. Photographs were taken using a Leica EZ4 Stereo Microscope.

3. Results

Gypsophila davisii Barkoudah, Wentia 9: 62 (1962), (Figure 2, 3, and 6).

Type: Turkey-Muğla: Gökçeova near to Sandras mountain, 1700 m, 23.07.1947, Davis 13516a (holo. E, iso. K)

Perennial, caespitose, glabrous, minutely scabrous. Stem numerous, flowering stems 4-16 cm. Basal leaves rigid, linear, acute, subspiny, ciliate below, distinctly three veins, keeled in the middle vein, scarious papillous at margins, 7-10 × 0.8-1.2 mm, numerous. Stem leaves similar to basal leaves, 2-8 × 0.3-0.8 mm, 3-4 pairs. Inflorescence minutely scabrous somewhere, dichasial (from 2/3 of the body), 3-7 flowered. Bracts leafy, lanceolate-ovate, green, finely ciliate at the base, 1.2-2.3 × 0.4-0.5 mm. Pedicels 3-13 mm. Calyx campanulate-turbinate, 2.5-3.5 × 2-3 mm. Calyx teeth ovate, subobtuse, ciliate, 0.9-1.3 × 0.9-1.1 mm. Petals pink with darker veins, linear-cuneate, obtuse, 4.2-5 × 1-1.6 mm. Style 1.6-2.3 mm. Ovarium 1.3-2.0 × 1.0-1.3 mm. Capsules 3.8-4.6

× 2.5-2.6 mm. Number of ovules 8-11. Seeds pyriform, flat tubercles, dark brown to black, shiny, 0.98-1.17 × 1.08-1.39 mm.

Flowering-fruiting: July
Habitat: Subalpine meadows
Altitude: 1750-1770 m

Figure 2. Herbarium sample of *Gypsophila davisii* (VANF162322!).

Conservation status: Its habitat is located in the Sandras Mountain which is one of important Nature Areas in Turkey. This endemic species has a distributing area of about 10 km^2 just in Muğla. The picnickers, the creation of recreational areas and grazing threat its habitat. Based on the criteria B1a, B1b(iii), B2b(iii, v), we propose to assess *G. davisii* as Critically Endangered (CR) (IUCN 2014).

Specimens examined: Muğla: Around Gökçeova lake, on organic matter-rich (wet) soil deposited on the serpentine bedrock, 37°03'34.3"N 28°48'17.5"E, 1770 m, 10.07.2009, VANF162322 (Figure 2); subalpine meadows, 37°03'39.4"N 28°48'22.4"E, 1752 m, 18.07.2017, Armağan 7708 (Figure 3).

Anatomic properties:

Stem: Secondary enlargement was not observed. A thin cuticle layer exists as an outermost layer and an epidermal layer, which is composed of oval cells, takes place just under this cuticle. Intercellular space doesn't exist, but a single celled scabrid which was formed by the differentiation of epidermis, takes place. Cortex starts just under the epidermis and much more coloured sclerenchyma cells take place between them. Phloem cells lined up beside the cortex. Just after phloem, much larger xylem cells exist. Thee pith takes place at the innermost region where many large cells with hyaline appearance are seen. No druse crystals were observed in the stems of examined specimens (Figure 4c).

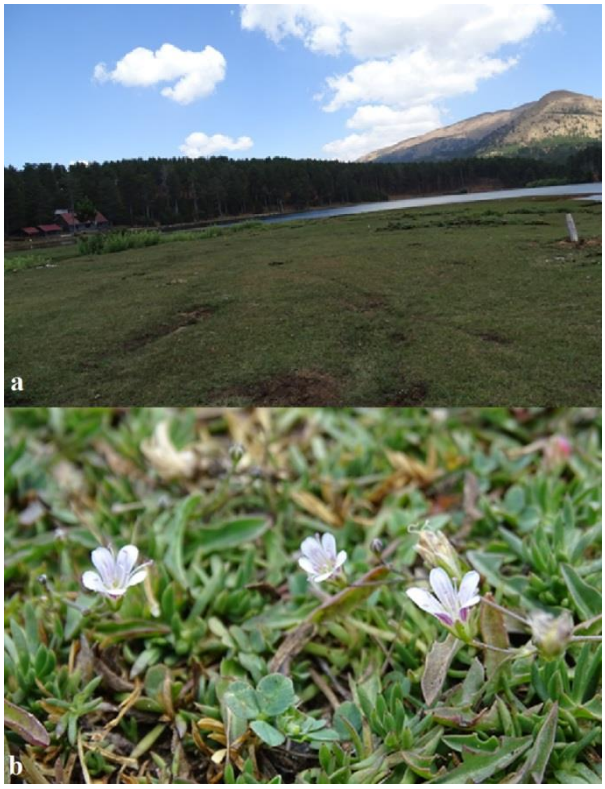

Figure 3. The habitat (Gökçeova lake and Sandras mountain) (a) and habit (b) of *Gypsophila davisii.*

Caudex and root; at the outermost layer, 6-7 layered cork exists as the protective tissue. Cortex takes place just under the cork layer. An endodermis layer which is composed of smaller cells compared to cortex cells exists at the innermost layer of the cortex. At the central cylinder which starts just under the endodermis, vascular bundles take place. No druse crystals were observed in the roots of examined specimens (Figure 4a, 4b).

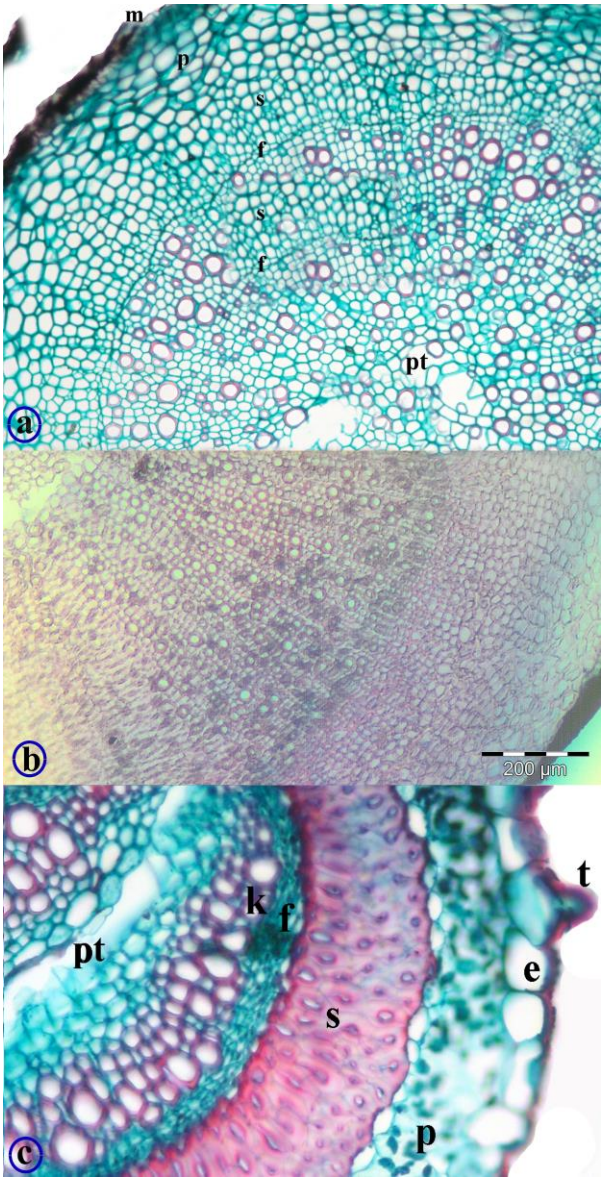

Figure 4. The root (a), caudex (b) and stem (c) anatomy of *Gypsophila davisii* (m. cortex, p. parenchyma, s. sclerenchyma, f. phloem, k. xylem, pt. pith, t. scabrid).

Leaf; an epidermis which is composed of ovate-to rectangular and single layered cell, forms the outermost layer. Mesophyll is composed of 9-13 layered cells which start from upper epidermis and extends till lower epidermis. There isn't a parenchymatic differentiation in mesophyll. Other than chloroplasts, druse crystals are seen seldomly. Venation is parallel and the central vein is larger compared to others (Figure 5a).

Through the sections taken from the surface of the leaves, amaryllis type stoma, the form belonging to the family Caryophyllaceae was observed. Neighbouring cells have a varying shape from ovoid to rectangular with more or less smooth cell walls (Figure 5b)

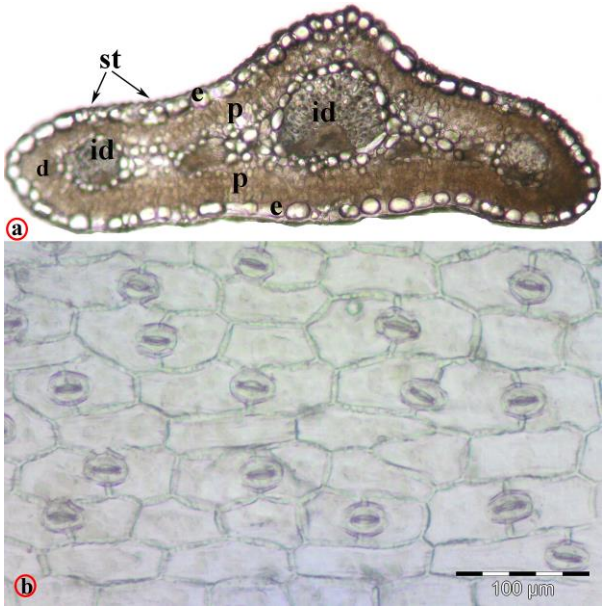

Figure 5. The leaf (a) and leaf surface (b) anatomy of *Gypsophila davisii* (e. epidermis, st. stoma, d. druse crystal, id. vascular bandle, p. palisade parenchyma).

Seed Morphology:

The seeds are pyriform, mature seeds dark brown to black, 0.98-1.17×1.08-1.39 mm. Unripe seeds red. Seed surface with flat tubercles (Figure 6).

Pollen Morphology:

The pollen grains are polyporate, spheroidal, granulate-microechinate-microperforate; the equatorial diameters are 25.87561-28.397911 µm (Figure 7).

Ecological properties:

Gypsophila davisii, prefers soils with medium texture; neutral; unsalted; low in terms of lime, phosphorus, potassium, calcium, sodium and zinc; medium in terms of magnesium and cupper; rich in terms of iron and organic matter (Table 1). Though it doesn't have a side preference, it grows on wet soils accumulated on serpentine bedrock.

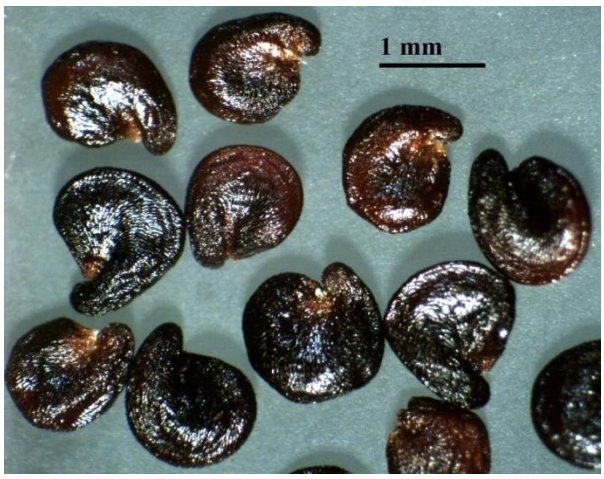

Figure 6. The seeds of *Gypsophila davisii*.

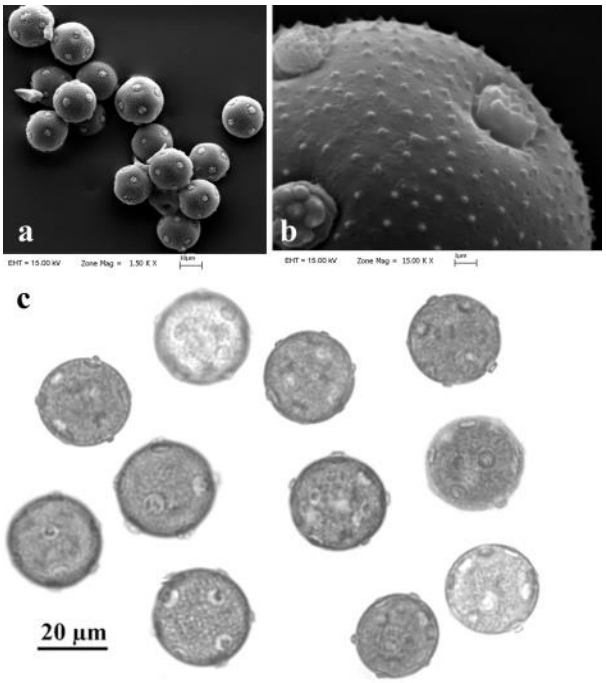

Figure 7. The pollen grains of *Gypsophila davisii* (a & b. SEM, c. LM).

It was determined to have a natural distribution around Köyceğiz (Muğla) district. It is generally distributed on red-brown Mediterranean soils, accumulated on mesosoic peridatit bedrock. Annual precipitations, summer time precipitations, precipitation regimes, annual average temperatures, maximum mean temperature of the hottest months, minimum average temperatures of the coldest months and the climate types of Köyceğiz, Dalaman and Muğla are given in Table 2 in accordance with Emberger (Akman, 2011). There have also been sedimentary or volcanic rocks around the region.

Karyological properties:

The chromosome numbers of *G. davisii* an endemic species in Turkey were examined. The results of our studies showed that the chromosome number of *G. davisii* was 2n = 30. The basic chromosome number of species was determined as x = 15 (Figure 8).

4. Discussions

The other species (*G. repens* L., *G. nana* Bory et Chaub., *G. spergulifolia* Grisebach, and *G. achaia* Bornm.) of the section *Gypsophila* have distributions in Europe. *G. davisii* is known from a few localities only within the boundaries of Muğla province in Turkey. It prefers wet meadows as habitat. The major difference of this species from the others is the smooth, bright and pyriform structure of the seeds. As a result of this study, the description of the species in Flora of Turkey was expanded by adding the missing properties (Table 2).

Table 1. Soil properties of *Gypsophila davisii*

Satur.	EC	pH	Lime	Org. Subs.	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
50	125	6.66	2.2	4.9	11	74.8	858	590	14	63.6	0.7	24.5	1.2

Table 2. Climatic data, precipitation regimes and climate type of Köyceğiz, Dalaman and Muğla (Anonymous 1).

Locality	Annual rainfall (mm)	Summer rainfall (mm)	Precipitation Regime	Average temperature (°C)	Max. av. temp. of the hottest month (°C)	Min. av. temp. of the coldest month (°C)	Climate type
Köyceğiz	1122.1	23.8	WinterAutumnSpringSummer	18.3	35.0	4.7	Humid sub-temperate Mediterranean
Dalaman	1080.9	6.2	WinterAutumnSpringSummer	18.0	33.7	5.1	Humid sub-temperate Mediterranean
Muğla	1209.2	38.4	WinterSpringAutumnSummer	15.0	33.4	1.6	Humid sub-cool Mediterranean

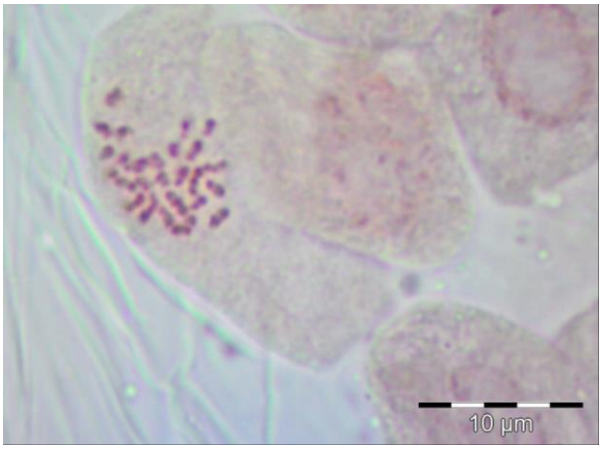

Figure 8. The metaphase chromosomes of *Gypsophila davisii* (2n=30).

None of the determined samples have a view preference. *G. davisii* prefers to grow at mountain meadows where excess amount of water exist. Peridotites belonging to mesozoic exist in the habitats of *G. davisii* and it lives on the soils formed by the metamorphosis of these rocks.

Druse crystals don't exist in the pith, pith branches and cortex of *G. davisii*. They were not observed in preparations from the cross section of the stem either. Although druses are present in woody species of the Caryophyllaceae family (Carlquist 1995), it was not found in stem and caudex of *G. davisii*. It may not have produced the druse crystals as *G. davisii*'s habitat is humid meadow. The epidermis of the stem has prominent protrusions. It is diacytic in terms of the number of cells surrounding the stoma.

Anatomy of root, stem and leaves well fit with the descriptions of Metcalfe & Chalk (1957), Barkoudah

(1962) and Ataşlar & Ocak (2017). Leaves of *G. davisii* are isolateral and have 2 or rarely 3 neighbouring cells. Stoma neighbouring cells rectangular underside while generally ovate to square shaped at upper side. Stomal cells are abundant at upper side of the leaves.

The chromosome number ranges between x=6 and 34 in the genus *Gypsophila*. The chromosome number of *G. perfoliata* L. was x=17, 18, 24, and 34 (Rice et al. 2014). It could be suggested that there is polyploidy in some species of *Gypsophila* based on these amounts. The chromosome number of *G. davisii* an endemic species in Turkey was examined. The results of our studies showed that the chromosome number of *G. davisii* was 2n = 30. The basic chromosome number of species was determined as x = 15 such as *G. cerastoides* D.Don (Sharma, 1970).

Table 3. Morphological comparison of our samples and the species description given in Flora of Turkey for *Gypsophila davisii*.

	Flora of Turkey	**Our samples**
Habitat	Subalpine meadows, 1700 m	Subalpine meadows, 1700-1800 m
Stem	Caespitose, 5-10 cm	Caespitose, 4-16 cm, minutely scabrous
Basal leaves	Linear, ciliate at the base, acute, subspiny, 5-10×0.6-1 mm, numerous	Linear, scarious papillous at the margins, acute, subspiny, distinctly three veins, keeled in the middle vein on the back, ciliate at the base, 7-10×0.8-1.2 mm, numerous
Stem leaves	-	2-8 × 0.3-0.8 mm, 3–4 pairs, linear, acute, scarious papillous at the margins, ciliate at the base
Inflorescence	-	Minutely scabrous somewhere, dichasial, branched 2/3 of stem
Flowers	2-7	3-7
Pedicel (mm)	5-13	3-13
Petals	Linear-cuneate, obtuse-retuse, pink with darker veins, 4-6 mm	Linear-cuneate, obtuse, pink with darker veins, 4.2-5×1.0-1.6 mm
Calyx	Campanulate-turbinate, 3-3.5 mm	Campanulate-turbinate, 2.5-3.5×2-3 mm
Calyx teeth	Ovate, subobtuse, ciliate	Ovate, subobtuse, ciliate, 0.9-1.3×0.9-1.1 mm
Bracts	Lanceolate-triangular, green, ciliate at the margins	Leafy, lanceolate-ovate, green, ciliate at the base, 1.2-2.3×0.4-0.5 mm
Style length	-	1.6-2.3 mm
Ovarium	-	1.3-2.0×1.0-1.3 mm
Capsules	-	3.8-4.6×2.5-2.6 mm
Ovules	-	8-11
Seeds	-	Pyriform, flat tubercles, dark brown-black, shiny, 0.98-1.17×1.08-1.39 mm

Acknowledgements

The study was supported by the Scientific Research Projects of Yüzüncü Yıl University (Project No. BAP 2011-FBE-D 030), and we would like to thank to Yüzüncü Yıl University for its financial support. We also thank to E. Martin for helping the karyological studies, to A. Çelik for sharing his samples of *G. davisii*, and to B. Muca for taking photos of pollen grains under SEM.

References

Akman Y (2011). İklim ve Biyoiklim (Biyoiklim Metodları ve Türkiye İklimleri). Palme Yayıncılık, Ankara, Turkey.

Anonymous (2018). https://tr.climate-data.org/location/19315/ date of acces: 11.01.2018

Armağan M, Özgökçe F, Çelik A (2017). Notes on the genus *Gypsophila* (Caryophyllaceae) in Turkey, with a description of *G. guvengork*i sp. nov. Phytotaxa 295 (3): 271-279.

Atasagun B, Aksoy A, Martin E (2016). Anatomical, palynological and karyological remarks of *Silene brevicalyx* and *Silene ozyurtii* (Caryophyllaceae). Phytotaxa 270 (2): 116-126.

Ataşlar E (2000). *Gypsophila* L. In: Güner A, Özhatay N, Ekim T, Başer KHC (Eds.). Flora of Turkey and the East Aegean Islands (Suppl. 2). Vol. 11, Edinburgh University Press, Edinburgh, UK.

Ataşlar E, Potoğlu Erkara İ, Tokur S (2009). Pollen morphology of some *Gypsophila* L. (Caryophyllaceae) species and its taxonomic value. Turkish Journal of Botany 33: 335-351.

Ataşlar E, Ocak A (2017). Anatomy and micromorphology of the endemic *Gypsophila osmangaziensis* (Caryophyllaceae) and taxonomic contributions for the genus Gypsophila. Phytotaxa 31(1): 84-92.

Barkoudah YI (1962). A revision of Gypsophila, Bolanthus, Ankyropetalum and Phryna. Wentia 9: 1-203.

Bittrich V (1993). Caryophyllaceae. Vol. 2: 206-236, The Families and Genera of Vascular Plants (Ed. Kubitzki at all.) Springer-Verlag, Berlin Heidelberg-Germany.

Carlquist S (1995). Wood anatomy of the Caryophyllaceae: ecological, habital, systematic, and phylogenetic implications. Aliso 14(1): 1-17.

Darlington CD, La Cour LF (1976). The handling of chromosomes. Sixth edition. George Allen and Unwin Ltd., London, UK.

Davis PH, Mill RR, Tan K (1988). Flora of Turkey and The East Aegean Islands, Vol.10, (Suplement I) Edinburgh University Press, Edinburgh, UK.

Ekim T (2012). *Gypsophila* L. In: Güner A, Aslan S, Ekim T, Vural M, Babaç MT (Eds.) Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi and Flora Araştırmaları Derneği Press, İstanbul, Turkey.

Elçi Ş (1994). Sitogenetikte Araştırma Yöntemleri ve Gözlemler. Yüzüncü Yıl Üniversitesi Yayınları, yayın no: 18, Van, Turkey.

Hesse M, Halbritter H, Zetter R, Weber M, Buchner R, Frosch-Radivo A, Ulrich S (2009). Pollen Terminology, an illustrated handbook. Springer-Verlag, Wien, Austria.

Huber-Morath A (1967). *Gypsophila* L. In: Davis PH (Ed.). Flora of Turkey and the East Aegean Islands. Vol. 2, Edinburgh University Press, Edinburgh, UK.

IUCN (2014). Guidelines for Using the IUCN Red List Categories and Criteria, Version 11. Prepared by the Standards and Petitions Subcommittee. Available from: http://www.iucnredlist.org/documents/RedListGuidelines.pdf

Korkmaz M, Özçelik H, İlhan V (2012). Türkiye'nin Bazı *Gypsophila* L. (Caryophyllaceae) Taksonlarının Habitat Özellikleri. Biyoloji Bilimleri Araştırma Dergisi 5(2): 111-125.

Korkmaz M, Özçelik H (2013). Soil-plant relations in the annual *Gypsophila* (Caryopyhllaceae) taxa of Turkey. Turkish Journal of Botany 37: 85-98

Linnaeus C (1753). Species Plantarum. Tomus I, Holmiae, Impensis Laurentii Salvii, Stockholm, Sweden.

Metcalfe CR, Chalk L (1957). Anatomy of the Dicotyledons. Leaves, Stem and Wood in Relation to Taxonomy with Notes on Economic Uses. Vol. 1, Clarendon Press, Oxford, UK.

Sharma AK (1970). Annual report, 1967-1968. Res. Bull. Univ. Calcutta Cytogenetics Lab. 2: 1–50.

Stearn WT (1983). Botanical Latin (Third edition, revised). David & Charles, London, UK.

Thiers B (2016). *Index herbariorum: A global directory of public herbaria and associated staff.* Available from: http://sweetgum.nybg.org/ih/ (accessed 23 Aug 2017)

Vettori L, Schiff S, Tani C, Pasqualetto P, Bennici A (2015). Morphological and cytological observations of wild species and hybrids of Gypsophila. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology, 149(2): 322-328.

Wodehouse RP (1935). Pollen Grains. McGraw-Hill, New York, USA.

Cite this article: Armağan M, Özgökçe F (2018). Anatomical, palynological, morphological, karyological, and ecological investigations on *Gypsophila davisii*. Anatolian Journal of Botany 2(1): 39-45.

Anatolian Journal of Botany
2(1): 46-51 (2018)
doi:10.30616/ajb.391985

Effects of *Acorus calamus* plant extract on prostate cancer cell culture

Halit Buğra KOCA[1]*, Tülay KÖKEN[1], Mete ÖZKURT[2], Gökhan KUŞ[3], Selda KABADERE[2], Nilüfer ERKASAP[2], Oğuzhan KOCA[3], Ömer ÇOLAK[4]
[1]*Afyon Kocatepe University, Faculty of Medicine, Department of Clinical Biochemistry, Afyon, Turkey*
[2]*Eskişehir Osmangazi University, Faculty of Medicine, Department of Physiology, Eskişehir, Turkey*
[3]*Karabük University, Education and Research Hospital, Biochemistry Laboratory, Karabük, Turkey*
[4]*Eskişehir Osmangazi University, Faculty of Medicine, Department of Clinical Biochemistry, Eskişehir, Turkey*
**bugrakoca@yahoo.com*

Received : 08.02.2018
Accepted : 03.03.2018

Acorus calamus bitki ekstraktının prostat kanser hücre kültürü üzerine etkileri

Abstract: In western countries, prostate cancer is the most frequently diagnosed cancer and the second most common cause of death from cancer in men. Vascular endothelial growth factor-A (VEGF-A), thought to be the single most important angiogenic factor in prostat cancer. Poly-(ADP-ribose) polymerase (PARP) involved in apoptotic process and cleavage of PARP serves as a marker of cells undergoing apoptosis. *Acorus calamus* have long been considered to have anti-carcinogenic and medicinal properties especially in Asia. We examined whether ethanolic extract of *A. calamus* root affects the survival of prostate cancer LNCaP cells and induces apoptosis and angiogenesis of these cells *in vitro*. Cells were incubated during 24 and 48 hours with various doses of extract. Extract with these concentrations reduced the number of LNCaP living cells up to 44 % as compared to the control at dose and time dependent manner at 24 and 48 hours. Significantly alterations were observed at cleaved PARP, VEGF-A protein and gene expression amounts after 24 and 48 hours. The present study reveals the possibility that ethanolic extract of *A. calamus* root posseses a dose and time dependent anticancer, apoptotic and anti-angiogenic properties.

Key words: LNCaP, *Acorus calamus*, anti-cancer, anti-angiogenic, apoptotic

Özet: Batılı ülkelerde erkekler arasında prostat kanseri en sık tanısı koulan kanser türüdür ve erkeklerde kanser nedeniyle ölümde ikinci sırada yer almaktadır. Prostat kanserinde vasküler endotelyal büyüme faktör-A'nın (VEGF-A) tek başına en önemli anjiogenik faktör olduğu düşünülmektedir. Apoptotik süreçte yer alan poli-(ADP-riboz) polimeraz'ın (PARP) yıkılımı apoptotik süreç markırı olarak kullanılmaktadır. *Acorus calamus*'un anti-kanserojen etkisi ile birlikte çeşitli tıbbi özelliklere sahip olduğu uzun yıllardır özellikle Asya'da kabul edilmektedir. Çalışmamızda *A. calamus* kökünün etanolik ekstraktının insan prostat kanser LNCaP hücre dizisinde hücre çoğalmasına, anjiogeneze ve apoptozise olası etkileri *in vitro* ortamda çalışıldı. Hücreler 24 ve 48 saat boyunca çeşitli ekstrakt konsantrasyonlarıyla muamele edildi. Bu konsantrasyonlardaki ekstraktlar 24 ve 48 saatte LNCaP hücre yaşayabilirliğini kontrolle karşılaştırıldığında doza ve zamana bağlı olarak %44'lere kadar azalttı. Bölünmüş PARP, VEGF-A proteini ve gen ekspresyon miktarlarında 24 ve 48 saat sonra belirgin değişiklikler gözlemlendi. Bu çalışma *A.calamus* kökünün etanolik ekstraktının zaman ve doz bağımlı olarak güçlü bir anti-kanser, anti-anjiogenik ve apoptotik etkileri olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: LNCaP, *Acorus calamus*, anti-kanser, anti-anjiogenik, apoptotik

1. Introduction

Traditional medicine (TM) is very popular not only in Asia or Africa, but also in Europe and in the United States (Zuba and Byrska, 2012). Historically, the majority of new medicines are either derived from natural products or from natural products (Jesse and Verdaras, 2009). In recent years, the use of traditional medicine has increased significantly in developed and developing countries. The use of *Acorus calamus* L. plant, in addition to foodstuffs in India and Far East countries, for medical purposes is based on a very long history. In studies conducted, these plants have been reported to have characteristics such as anticancer (Pandit et al., 2011; Bisht et al., 2011), anti-inflammatory (Kim et al., 2009), anti-microbial (Devi and Ganjewala, 2009), anti-inflammatory (Joshi, 2016), neuroprotective (Shukla et al., 2006), antioxidant (Acuna et al., 2002) and antiasthmatic effects (Shah and Gilani, 2010). Different parts of the plant showed the presence of large number of phenyl propanoids, sesquiterpenes and monoterpenes as well as xanthone glycosides, flavones, lignans, steroids and inorganic constituents (Raja et al., 2009). The most important ingredients of *A. calamus* are asarones, that is alpha- and beta-asarone (Zuba and Byrska, 2012).

Angiogenesis is the physiological processes through which new blood vessels form from pre-existing vessels. It leads to tumor expression of pro-angiogenic factors such as angiogenin, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), TGF-β, etc. and resulted in an increased tumor vascularization. These factors are reported to promote proliferation of new vascular structures from blood vessels. Inhibition of angiogenesis leads to suppression of "angiogenic switching" which prevents activation of pro-angiogenic factors and subsequently the tumor progression halts (Mahapatra et al., 2015). Angiogenesis has become an attractive target for anticancer chemotherapy due to its important role in the progression of solid tumors (Wei et al., 2011). It has been reported that tumor growth and metastasis are angiogenesis dependent processes and that inhibition of angiogenesis may be a strategy for the prevention of disease progression (Folkman, 1971). Tumor cells produce pro-angiogenic growth factors. Among these factors, vascular endothelial growth factor (VEGF) is the most

important and accelerates all stages of angiogenesis and shows pro-angiogenic properties. It has been shown that VEGF is overexpressed in high amounts in various tumors. Therefore, VEGF-targeted approaches to tumor angiogenesis are of great importance (Gerald, 2012).

Apoptosis, also known as programmed cell death or cell suicide, allows the death of the cell at the end of a particular series of molecular manipulations and is also required for the maintenance of embryonic development and adult tissue development. Apoptosis is characterized by unique phenotypic changes involving cell shrinkage, chromatin condensation, plasma membrane blistering and the formation of apoptotic bodies, and walking is an energy-dependent process (Bohm and Schil, 2003). A nuclear enzyme, poly (ADP-ribose) polymerase (PARP) plays an important role in the repair of DNA yarn breaks. This enzyme binds by recognizing DNA strand breaks that occur with oxidative damage or other causes and facilitates protection of the cell from DNA damage. The destruction of PARP, which is also a target of caspase-3, is one of the processes of apoptotic mechanism. (Brock et al., 2004).

In this study, we investigated the effects of *A. calamus* extracts on cell proliferation, apoptosis and angiogenesis in human prostate carcinoma LNCaP cells..

2. Materials and Method

2.1. Cell line and culture conditions

The LNCaP cell line was obtained from the Yeditepe University, Faculty of Medicine, Department of Physiology (Istanbul, Turkey). The cell line was cultured in Roswell Park Memorial Institute medium (RPMI) 1640 supplemented with 10% fetal bovine serum, 100 U/ml penicillin G and 100 mg/ml streptomycin and kept at 37 °C in a humidified incubator with an atmosphere of 5% CO2 and 95% air.

2.2. Preparation of ethanolic extract of *A. calamus*

Plant processing was done in the Anadolu University Medicinal Plants, Drugs and Scientific Research Center. The plant was chopped into small pieces and dried in the shade. The dried plant was crushed to coarse powder using a hand mill and sieved. Coarse powder was successively extracted in a soxhlet apparatus with 70% ethanol for 12 h. The ethanol extracts were concentrated by evaporating under vacuum. The yield of the ethanol extract was 28.29%. The ethanol extracts were freshly dissolved in dimethylsulphoxide (DMSO) just before administration.

2.3.Cell viability assay of LNCaP cells using XTT assay

Cells were seeded at $1x10^4$ cells per well in complete culture medium containing various concentrations of ethanolic extract of *A. calamus* (0, 250, 500, 750, 1000, 1250 µg/ml and 1% DMSO vehicle control). The cells were incubated for 24 and 48 h, respectively, to determine cytotoxic and apoptotic effects. Each concentration of ethanolic extract of *A. calamus* for each incubation period was incubated in four wells to identify the most efficient dose(s) and incubation period(s). Cytotoxic activity was measured using a colorimetric assay system (XTT Cell Proliferation Kit; Biological Industries), which measures the reduction of a tetrazolium component, XTT (sodium 3′ - [1- phenyl-aminocarbonyl)-3,4-tetrazolium] bis(4-methoxy-6-nitro)benzenesulphonic acid) into a soluble formazan product by the mitochondria of viable cells. Briefly, cells were cultured in 96-well plates containing 200 µl of complete medium. 100 µl of XTT solution was added into the wells and plates were incubated for additional 3 h. at 37 °C. The controls included native cells and medium only. Optical density (OD) of 96-well plates was measured at 450 nm in a microplate enzyme-linked immunosorbent assay (ELISA) reader (Trinity Biotech PLC, Bray CO. Wicklow, IRELAND). The percentage cytotoxicity was calculated with the formula: Percentage cytotoxicity (cell death) = [1-(absorbance of experimental wells/absorbance of control wells)] x 100%.

2.4. Apoptosis assay: Cleaved PARP (asp 214) sandwich ELISA

Cleaved PARP was assayed using the PathScan cleaved PARP (asp 214) sandwich ELISA kit (Cell Signaling Technology, Danvers, MA). Cells were seeded at $1x10^4$ cells per well in complete culture medium containing various concentrations of ethanolic extract of *A. calamus* (0, 250, 500, 750 µg/ml). The cells were incubated for 24 and 48 h, respectively, to determine apoptotic effects. Briefly, after treatment cells were washed twice with ice-cold PBS, lysed with 500 µl of ice-cold lysis buffer, scraped, and sonicated on ice. The cell lysate was then microcentrifuged for 10 min, 10.000 g at 4 °C, and the supernatant was stored at −80 °C before analysis. 100 µl of the soluble fraction was used in each well of the ELISA plate. The ELISA was performed according to the manufacturer's instructions. Absorbance was measured with a microplate reader.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR) to amplify VEGF mRNA

Total RNA was extracted from cultured LNCaP cells according to the manufacturer's instructions using the RNeasy Protect Mini Kit (Qiagen, Manheim, Germany). RNA concentration and purity was calculated after measuring absorbance at 260 nm on a UV spectrophotometer and then stored at -80°C. First-strand cDNA synthesis was performed with the QuantiTect Reverse Transcription cDNA Kit (Qiagen, Manheim, Germany). The synthesized cDNA was used as a template for PCR amplification. Quantitative PCR was performed on a LightCycler (Roche Diagnostics GmbH, Mannheim, Germany) using TaqMan technology with amplification of human glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA as a housekeeping standard. Oligonucleotide sequences of the cDNA primers were designed at Gene Research Laboratories, UK. The forward primer for VEGF was 5′-CCAGGAAAGACTGATACAGAACG-3′ and the reverse primer was 5′- GGTTTCTGGATTAAGGACTGTTC -3′. The forward primer for GAPDH was 5′-GAAGGTGAAGGTCGGAGT-3′ and the reverse primer was 5′-GAAGATGGTGATGGGATTTC-3′ The following Light Cycler conditions were used: initial denaturation at 95 °C for 10 min, followed by 50 cycles with denaturation at 95 °C for 20 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 20 s. Cycle threshold (CT) values were determined by automated threshold analysis. The primer quality (lack of primer-dimer amplification) was confirmed by melting curve analysis. Relative quantification of gene expression was performed using a

standard curve constructed with serial dilutions of control mRNA or RT-PCR amplicons. All experiments were carried out in triplicate. VEGF levels were standardized to GAPDH (ratio VEGF:GAPDH) to account for loading differences. Gene expression levels (mRNA) were reported using the median as a point estimator and the range of values.

2.6. Statistical analysis

In the cell proliferation assay, experiments were repeated three times, measurements within an experiment were done in six duplicates, and in cell cycle analysis, experiments were repeated three times. Data from three independent experiments were expressed as mean ± standard deviation (SD). Data were analyzed by a computerized probit analysis, which provided an estimate of the half maximal inhibitory concentration (IC_{50}). The level of significance between different groups was based on ANOVA test followed by Tukey test. All analyses were performed using SPSS 17.0 trial (IBM, USA) with $P < 0.05$ as the significance level.

3. Results

3.1. Effect of *A. calamus* on cell viability

The effect of *A. calamus* extract on the viability of LNCaP cells is shown in figure 1. The XTT assay showed that *A. calamus* extract decreased the viability of LNCaP cells.

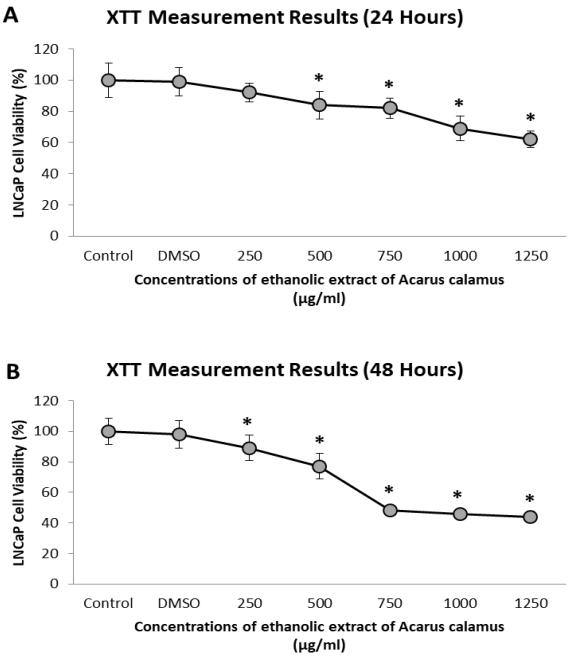

Figure 1. Effect of *A. calamus* treatment on the viability of prostate cancer cell lines LNCaP. (A) The cells were treated with various concentrations (0, 250, 500, 750, 1000, and 1250 µg/mL) of *A. calamus* for 24 h and (B) 48 h. Percentages of viable cells after treatment were determined by XTT assay. Error bars represent SD of triple samples (*P < 0.001 compared with control group). SD, standard deviation.

Acorus calamus concentrations ranging from 500 to 1250 µg/ml at 24 h and from 250 to 1250 µg/ml at 48 h showed a dose–response relationship of cell survival (24 h: control vs. 500, 750, 1000 and 1250 µg/ml; 48 h: control vs. 250, 500, 750, 1000 and 1250 µg/ml P < 0.001), while *A. calamus* concentrations ranging from 750–1250 µg/ml showed a time-dependent decrease (750 µg/ml: 24 vs. 48h, P < 0.001; 1000 µg/ml: 24 vs. 48 h, P < 0.001; 1250 µg/ml: 24 vs. 48 h, P < 0.001). The IC_{50} value was 923 µg/mL for the 48 hr treatment with extract. Based on this experiment, three concentrations (250, 500 and 750 µg/ml) lower than the IC_{50} were chosen for later experiments examining the effects of *A. calamus* on LNCaP cells.

3.2. Effect of *A. calamus* on Apoptosis of LNCaP Cells

A. calamus induces apoptotic cell death in LNCaP cells. The levels of cleaved poly (ADP-ribose) polymerase (PARP), one of the best biomarkers of apoptosis, were analyzed after 24 and 48 hours of treatment with *A. calamus*. Full-length active PARP is a 116 kDa molecule, which is cleaved to fragments of 86 and 30 kDa by the action of caspase-3 and related caspases. A marked increase in cleaved PARP was observed after 24 hours of exposure to *A. calamus* at concentration of 750 µg/ml ($p<0.01$); 48 hours at 500 and 750 µg/ml ($p<0.01$, $p<0.001$) (Fig. 2).

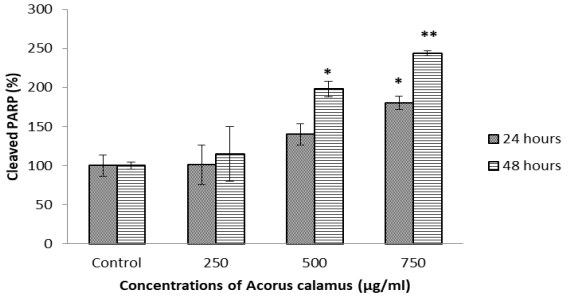

Figure 2. Effects of *A. calamus* treatment on PARP cleavage in prostate cancer cell lines LNCaP. The cells were treated with various concentrations (0, 250, 500, and 750 µg/mL) of *A. calamus* for 24 and 48 h. values represent mean ± SD (*P < 0.01; **P< 0.001 compared with control group). PARP, poly (ADP-ribose) polymerase.

3.3. Effect of *A. calamus* on VEGF mRNA expression

The dose-dependent effect of *A. calamus* extract on LNCaP cells showed a significant decrease in VEGF mRNA expression at 500 and 750 µg/ml when assessed at 48 hours post-treatment. No changes were observed after 24 hours of exposure to *A. calamus* (Fig. 3).

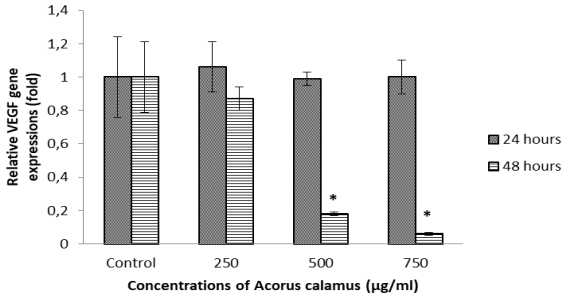

Figure 3. Effect of *A. calamus* treatment on VEGF mRNA expression in prostate cancer cell lines LNCaP. The cells were treated with various concentrations (0, 250, 500, and 750 µg/mL) of *A. calamus* for 24 and 48 h. values represent mean ± SD. (*P < 0.001 compared with control group). VEGF, vascular endothelial growth factor.

4. Discussions

Natural products have long been an important source of anticancer agents. More than 60% of drugs for cancer treatment are of natural origin, particularly derived from plants (Zhao et al., 2016). The aim of this study was to evaluate the anticancer effect of *A. calamus* extract on human prostate carcinoma LNCaP cells. We found that *A. calamus* extract inhibited the proliferation and viability of human prostate cancer LNCaP cells via induction of apoptosis. LNCaP cells showed clear apoptosis within 24 h after (750 µg/ml) and 48 h after *A. calamus* (500 and 750 µg/ml) treatment.

Apoptosis is a programmed cellular process that occurs in physiological and pathological conditions (Wu et al., 2014). Furthermore, the programmed cell death is disrupted in the cancer, which causes the malignant cells to grow too much. Induction of tumor cell apoptosis is the ultimate goal of many cancer therapies (Zhao et al., 2016). One of the cellular responses that preceded DNA repair mechanisms against DNA damage is PARP activation (Dungey et al., 2009). Anti-cancer therapies are being developed in combination with the inhibition of PARP activation and the combination of drugs that cause DNA damage (Penning et al., 2009). In the present study, we demonstrated that *A. calamus* treatment of LNCaP cells resulted in both a time and dose-dependent increase of the cleavage of PARP, which is considered a biochemical hallmark of apoptosis. However, it is not clear yet whether induction of apoptosis is mediated by pro-apoptotic factors, anti-apoptotic factors or a combined effect.

Regarding the chemical composition of *A. calamus* extract, we could hypothesize that its apoptotic effects might be dependent on the presence of lectins, volatile oils and flavonoids. Chemical analysis has demonstrated that the main constituents of *A. calamus* are monoterpenes, sesquiterpenes, phenlypropanoids, flavonoids, quinine (Kim et al., 2009). Two major active components in volatile oils of *A. calamus* are alpha-asarone and beta-asarone (Zuba and Byrska, 2012). Lectins from the rhizomes of *A. calamus* have mitogenic activity and inhibitory potential in murine cancer cell growth (Bains et al., 2005). Alfa-asarone shows radioprotective activity against lethal and sublethal doses of gamma-radiation by preventing radiation-induced production of free radicals and damage to DNA, membranes, and the hematopoietic system in animal models. Beta-asarone has anticancer effects, inhibiting survival of the human hepatoma cell line HepG2 (Kevekordes et al., 2001). Xenograft tumor models in mice showed similar results, with beta-asarone decreasing tumor size. Furthermore, survival of colorectal cancer cells *in vitro* was markedly inhibited by incubation with beta-asarone. They propose that it acts through inducing expression of lamin B1 and activating p53 (Liu et al., 2013). Beta-asarone significantly activated caspase-3, caspase-8, caspase-9, Bax, Bak and suppressed Bcl-2, Bcl-xL and survivin activity. Moreover, beta-asarone increased the expression of RECK, E-cadherin and decreased the expression of MMP-2, MMP-9, MMP-14 and N-cadherin. Beta-asarone effectively inhibits the proliferation of human gastric cancer cells, induces their apoptosis and decreased the invasive, migratory and adhesive abilities.(Wu et al., 2015). Beta-asarone can suppress the growth of colon cancer and the induced apoptosis is possibly mediated through mitochondria/ caspase pathways (Zou et al., 2012).

Angiogenesis, neovascularization from pre-existing vessels, is a key step in tumor growth and metastasis, and anti-angiogenic agents that can interfere with these essential steps of cancer development are a promising strategy for human cancer treatment (Kim et al., 2016). VEGF is the most potent known mitogen for endothelial cells, and is a specific of angiogenesis in several cancer types, including prostate cancer. Elevated VEGF levels have been observed in a variety of tumor cells (Lee et al., 2006). Prostate cancer cells produce VEGF that facilitates the growth of the tumor presumably through enhancing angiogenesis and perhaps migratory ability. VEGF promotes endothelial cell proliferation, survival, and migration (Kitigawa et al., 2005). Because prostate cancer tumors require angiogenesis for growth and metastasis, and angiogenesis is a complex multifactorial process, antiangiogenic therapy could offer a variety of avenues to arrest tumor growth, induce tumor regression, or block the ability of tumors to metastasize. Antiangiogenic therapy is a particularly attractive antitumor modality with many potential targets, considering that the regulation of tumor angiogenesis is profoundly multifactorial (Nicholson and Theodorescu, 2004).

The process of tumor angiogenesis is highly regulated by growth factors such as VEGF-A which is secreted by tumor cells and ECs and functions via paracrine and autocrine signaling pathways. After secretion VEGF-A primarily binds to its specific receptors located on ECs, triggering the process of angiogenesis (Ferrara et al., 2003). Therefore, we evaluated VEGF gene expression levels. Although we did not thoroughly investigate the mechanism, we demonstrated that VEGF expression was downregulated by treatment with *A. calamus*. Similar results indicated that *S. barbata D. Don* inhibits expression of VEGF in human colon carcinoma cells. *S. barbata D. Don* inhibits cancer growth via at least two mechanisms, inducing mitochondrion-dependent apoptosis of cancer cells and inhibiting tumor angiogenesis. Both apoptosis and angiogenesis are regulated by multi-pathways. In addition, *A. calamus* is composed of many chemical compounds including monoterpenes, sesquiterpenes, phenlypropanoids, steroides and flavonoids. It is unknown how many of these compounds contain anti-cancer activity, and we do not know which pathway(s) effected from these compounds (Wei et al., 2011).

Taken together, our study demonstrated the first evidence that *A. calamus* extract can effectively decrease proliferation, induce apoptosis and suppress VEGF-A expressions of prostate carcinoma LNCaP cells in a time- and dose-dependent manner. Therefore, although further studies are required, *A. calamus* extract may be a promising therapeutic agent for the treatment of prostate tumors.

Conflicts of interest

There is no conflict of interest in any form between the authors.

References

Acuna UM, Atha DE, Ma J, Nee MH, Kennelly EJ (2002). Antioxidant capacities of ten edible North American plants. Phytother Res 16(1): 63-65.

Bains JS, Dhuna V, Singh J, Kamboj SS, Nijjar KK, Agrewala JN. (2005). Novel lectins from rhizomes of teo Acorus species with mitogenic activity and inhibitory potential towards murine cancer cell lines. Int Immunopharmacol 5(9): 1470-8.

Bisht VK, Negi JS, Bhandari AK, Sundriyal RC (2011). Anti-cancerous Plants of Uttarakhand Himalaya: A Review. International Journal of Cancer Research 7: 192-208.

Bohm I, Schild H (2003). Apoptosis: the complex scenario for a silent cell death. Mol Imaging Biol 5: 2-14.

Brock WA, Milas L, Bergh S, Lo R, Szabó C, Mason KA (2004). Radiosensitization of human and rodent cell lines by INO-1001, a novel inhibitor of poly(ADP-ribose) polymerase. Cancer Lett 205(2): 155-160.

Devi SA, Ganjewala D (2009). Antimicrobial activity of *Acorus calamus* (L.) rhizome and leaf extract. Acta Biologica Szegediensis 53(1): 45-49.

Dungey FA, Caldecott KW, Chalmers AJ (2009). Enhanced radiosensitization of human glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition of heat shock protein 90. Mol Cancer Ther 8(8): 2243-2254.

Ferrara N, Gerber HP, LeCouter J (2003). The biology of VEGF and its receptors. Nat Med 9: 669-76.

Folkman J (1971). Tumor angiogenesis: therapeutic implications. N Engl J Med 285: 1182-1186.

Gerald WP, Poettler M, Unseld M, Zielinski CC (2012). Angiogenesis in cancer: anti-VEGF escape mechanisms. Transl Lung Cancer Res 1(1): 14-25.

Jesse W-H.L,Verdaras JC (2009). Drug discovery and natural products: end of an era or an endless frontier? Science 325: 161-165.

Joshi RK (2016). *Acorus calamus* Linn.: phytoconstituents and bactericidal property. World J Microbiol Biotechnol 32(10): 164.

Kevekordes S, Spielberger J, Burghaus CM, Birkenkamp P, Zietz B, Paufler P, Diez M, Bolten C, Dunkelberg H (2001). Micronucleus formation in human lymphocytes and in the metabolically competent human hepatoma cell line Hep-G2: results with 15 naturally occuring substances. Anticancer Res 21(1A): 461-469.

Kim H, Tan TH, Lee SG (2009). Anti-inflammatory activity of a water extract of *Acorus calamus* L. leaves on keratinocyte HaCaT cells. J Ethnopharmacol 122(1): 149-156.

Kim SH, Kim EC, Kim WJ, Lee MH, Kim SY, Kim TJ (2016). Coptis japonica Makino extract suppresses angiogenesis through regulation of cell cycle-related proteins, Bioscience, Biotechnology, and Biochemistry 80(6): 1095-106.

Kitagawa Y, Dai J, Zhang J, Keller JM, Nor J, Yao Z, Keller ET (2005). Vascular Endothelial Growth Factor Contributes to Prostate Cancer–Mediated Osteoblastic Activity. Cancer Res 65(23): 10921-10929.

Lee M, Hwang JT, Yun H, Kim EJ, Kim MJ, Kim SS, Ha J (2006). Critical roles of AMP-activated protein kinase in the carcinogenic metal-induced expression of VEGF and HIF-1 proteins in DU145 prostate carcinoma. Biochemical Pharmacology 72(1): 91–103.

Liu L, Wang J, Shi L, Zhang W, Du X, Wang Z, Zhang Y (2013). Beta-Asarone induces senescence in colorectal cancer cells by inducing lamin B1 expression. Phytomedicine 20: 512-520.

Mahapatra DK, Bharti SK, Asati V (2015). Anti-cancer chalcones: Structural and molecular target perspectives. Eur J Med Chem 98: 69–114.

Nicholson B, Theodorescu D (2004). Angiogenesis and Prostate Cancer Tumor Growth. Journal of Cellular Biochemistry 91: 125–150.

Pandit S, Mukherjee PK, Ponnusankar S, Venkatesh M, Srikanth N (2011). Metabolism mediated interaction of α-asarone and *Acorus calamus* with CYP3A4 and CYP2D6. Fitoterapia 82(3): 369-374.

Penning TD, Zhu GD, Gandhi VB, Gong JC, Liu XS, Shi Y, Klinghofer V, Johnson EF, Donawho CK, Frost DJ, Bontcheva-Diaz V, Bouska JJ Osterling DJ, Olson AM, Marsh KC, Luo Y. Giranda VL (2009). Discovery of the poly(ADP-ribose) polymerase (PARP) inhibitor 2-[(R)-2-methylpyrrolidin-2-yl]-1H-benzimidazole-4- carboxamide (ABT-888) for the treatment of cancer. J Med Chem 52: 514-523.

Raja AE, Vijayalakshmi M, Devalarao G (2009). *Acorus calamus* Linn.: Chemistry and Biology. Research Journal of Pharmacy and Technology 2(2): 256-261.

Shah AJ, Gilani AU (2010). Bronchodilatory effect of *Acorus calamus* (Linn.) is mediated through multiple pathways. J Ethnopharmacol 131(2): 471-477.

Shukla PK, Khanna VK, Ali MM, Maurya R, Khan MY, Srimal RC (2006). Neuroprotective effect of *Acorus calamus* against middle cerebral artery occlusion-induced ischaemia in rat. Hum Exp Toxicol 25(4): 187-94.

Wei L, Lin J, Xu W, Hong Z, Liu X, Peng J (2011). Inhibition of tumor angiogenesis by *Scutellaria barbata* D. Don via suppressing proliferation, migration and tube formation of endothelial cells and downregulation of the expression of VEGF-A in cancer cells. Journal of Medicinal Plants Research 5(14): 3260-3268.

Wu J, Zhang XX, Sun QM, Chen M, Liu SL, Zhang X, Zhou JY, Zou X (2015). β-Asarone inhibits gastric cancer cell proliferation. Oncol Rep 34(6): 3043-50.

Wu Q, Kang Y, Zhang H, Wang H, Liu Y, Wang J (2014). The anticancer effects of hispolon on lung cancer cells. Biochemical and Biophysical Research Communications 453: 385–391.

Zhao L, Wen Q, Yang G, Huang Z, Shen T, Li H, Ren D (2016). Apoptosis induction of dehydrobruceine B on two kinds of human lung cancer cell lines through mitochondrial-dependent pathway. Phytomedicine 23(2): 114-122.

Zou X, Liu SL, Zhou JY, Wu J, Ling BF, Wang RP (2012). Beta-asarone induces LoVo colon cancer cell apoptosis by up-regulation of caspases through a mitochondrial pathway in vitro and in vivo. Asian Pac J Cancer Prev 13(10): 5291-8.

Zuba D, Byrska B (2012). Alpha- and beta-asarone in herbal medicinal products. A case study. Forensic Sci Int 223(1-3): 5-9.

Cite this article: Koca HB, Köken T, Özkurt M, Kuş G, Karadere S, Erkasap N, Koca O, Çolk Ö (2018). Effects of *Acorus calamus* plant extract on prostate cancer cell culture. Anatolian Journal of Botany 2(1): 46-51.