www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 6/2 (2013) 134-141

Research article/Araştırma makalesi

Relationships among some endemic *Hieracium* L. s.str. (Asteraceae) taxa based on internal transcribed spacer (ITS)

Mutlu GÜLTEPE *[1], Kamil COŞKUNÇELEBI [1], Ali Osman BELDÜZ [1], Uğur UZUNER [2]

[1] Karadeniz Technical University, Faculty of Science, Department of Biology, 61080 Trabzon, Turkey
[2] Texas A&M University, Department of Plant Pathology & Microbiology, College Station, TX, 77843 USA

Abstract

In the current study, thirteen individuals from ten endemic *Hieracium* (Asteraceae) taxa were analyzed by using sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA in order to see whether the ITS could provide additional useful information for the relationships of the genus or not. Therefore, total genomic DNA was isolated from healthy leaves of each individual collected from different localities of North-east (NE) Anatolia (Turkey). Amplification of the entire ITS region was performed by using universal primers with the aid of the polymerase chain reaction (PCR). Neighbor Joining (NJ) and Maximum Parsimony (MP) trees were constructed based on the ITS sequences to elucidate the phylogenetic relationships among the investigated taxa. The aligned sequences comprised 710 characters. Divergence values among the individuals ranged from 0.0 to 2.1%. The ITS data provided sufficient variation to resolve interspecific relationships, but the topologies of the NJ and MP trees did not support conventional subgeneric classification of the species.

Key words: Asteraceae, *Hieracium*, ITS, nrDNA, Turkey

---------- * ----------

Türkiye' de yayılış gösteren bazı endemik *Hieracium* (Asteraceae) taksonlarının ITS dizilerine dayalı akrabalıkları

Özet

Bu çalışmada, 10 endemik *Hieracium* L. taksonuna ait 13 farklı birey, nüklear ribozomal DNA ITS bölgesinin cinsin akarabalık ilişkilerini ortaya koyabilecek yeni bilgiler sağlayıp sağlayamayacağını belirlemek amacıyla karşılaştırılmıştır. Bunun için öncelikle Kuzey Doğu Anadolu Bölgesi'nin değişik yerlerinden toplanan bireylerin sağlıklı yapraklarından genomik DNA'ları elde edilmiştir. Evrensel oligonükleotidler kullanılarak bireylere ait ITS bölgeleri polimeraz zincir reaksiyonu ile çoğaltılmıştır. Taksonlar arasındaki filogenetik ilişkiyi ortaya çıkarmak için Neighbour-Joining (NJ) ve Maksimum Parsimoni (MP) ağaçları çizilmiştir. Baz dizinlerinin hizalanması sonucu 710 karakterden oluşan bir veri seti elde edilmiş ve örnekler arasındaki divergens değerlerinin % 0.0-2.1 arasında değiştiği bulunmuştur. ITS bölgesi taksonlar arasındaki ilişkiyi açıklama da yeterli bilgi sağlamasına rağmen analiz sonucu elde edilen NJ ve MP ağaçlarının topolojileri, cinsin seri seviyesinde geleneksel taksonomik verileriyle uyum göstermemiştir.

Anahtar kelimeler: Asteraceae, *Hieracium*, ITS, nrDNA, Türkiye

1. Introduction

Hieracium L. s. str. (Asteraceae) is well known for apomictic and sexual members with different ploidy levels (Beaman, 1990; Koltunow, 2000). The genus has long been known for its taxonomic complexity, which is associated with a variation of ploidy level, apomixis and hybridizitation (Štorchová et al., 2002; Vladimirov, 2003). Apomixis is a

* *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +904623774248; Fax.: +904623253200; E-mail: mutlugultepe@yahoo.com
 BioDiCon. 294-0113

reproduction pathway that produces morphologically similar plants (Nogler, 1984) and usually, only a small amount of variation occurs within each species (Tyler, 2000; Rich et al., 2008).

Hieracium is one of the largest genera of flowering plants and includes 500-8000 species depending on taxonomic concept (Fehrer et al., 2009). In Turkey there are 113 species of *Hieracium* (Coskuncelebi and Beyazoglu, 2003a). More half of them are endemic and distributed in NE Anatolia, one of the important diversity centres of this genus in Turkey (Gottschlich et al., 2000). Some of these taxa have been investigated in terms of morphological (Coskuncelebi and Beyazoğlu, 2003b), cytotaxonomical (Coskuncelebi and Hayırlıoğlu-Ayaz, 2006; Coskuncelebi and Vladimirov, 2008), and total phenolic content (Ayaz and Coskuncelebi, 2002). Molecular data including nuclear and plastid DNA sequences have been frequently used to solve taxonomic problems in plant systematics (Baldwin, 1992; Kim et al., 1992; Anderberg et al., 2002), and might be of independent systematic value (Conti et al., 2000). The ITS is a powerful tool for phylogenetic reconstruction at species and generic levels (Baldwin, 1992; Anderberg et al., 2002).This region was used in Asteraceae (e.g. Cardueae at tribal (Susanna et al.,1995), subtribal (Häffner and Hellwig, 1999; Garcia-Jacas et al., 2000) and generic levels (Susanna et al., 1999; Vilatersana et al., 2000)) and provided some phylogenetic information. *Jacea* group in *Centaurea* L was revisited using ITS by Garcia-Jacas et al (2006) and the mojar lineages could be identified in the group. Sequence analysis of the ITS also provided useful information for the reconstruction of phylogeny of genus *Caylcadenia* DC. (Baldwin and Markos, 1998) and *Silphium* L (Clevinger and Panero, 2000) in tribus Heliantheae. In the last fifteen years, *Hieracium* species were investigated in terms of isozyme systems (Mráz et al, 2001), RAPDs (Shi et al., 1996; Štorchová et al., 2002), AFLP markers (Ronikier and Szeląg, 2008), and external transcribed spacer (ETS) (Fehrer et al., 2009) but only little variation has been recorded among the investigated specimens. The internal transcribed spacers (ITS) of nuclear ribosomal DNA (nrDNA) have been widely used to resolve phylogenetic relationships for many plant taxa in the Asteraceae (Baldwin, 1992; Baldwin et al., 1995; Susanna et al., 1995).

In spite of the above cited studies on *Hieracium* taxa from NE Turkey, there have been no molecular studies on Turkish representatives up to now. The *Hieracium* species are closely related, and it has not been possible to identify these apomictic taxa based on simple morphological characters. Consequently, the relationships among ten endemic *Hieracium* species have not previously been addressed by molecular methods.

2. Materials and methods

2.1. Specimens

Plant materials were collected from different regions of NE Anatolia during field work in the frame of the PhD thesis of the second author. Information about the investigated taxa and their localities were given in Table 1. Vouchers were identified according to Sell and West (1974) and were deposited in the herbarium of Biology at Karadeniz Technical University (KTUB).

2.2. DNA Extraction and PCR

Total genomic DNA was extracted from healthy leaves of herbarium materials following the modified CTAB extraction procedure of Doyle and Doyle (1987).

The ITS regions were amplified by the polymerase chain reaction (PCR) using a Biometra Personal Thermal Cycler. The amplification reactions were performed using universal ITS5 (forward:5′- GGAAGTAAAAGTCGTAACAAGG -3′) and ITS4 (reverse:5′- TCCTCCGCTTATTGATATGC- 3′) primers designed by White et al. (1990). In this case, the entire ITS region (ITS1, 5.8S and ITS2) was obtained from the each of the investigated individuals. The amplification process was performed in a 50 µl PCR reaction volume containing 10 mM *Taq* polymerase reaction buffer, 2 mM magnesium chloride ($MgCl_2$), 200 mM of each dNTP, 1 µM of each primer, 1-2 units of *Taq* DNA polymerase, 2-6 ng (1 µl of 2-6 ng/µl) of total template DNA and 14 µl of ddH_2O. Reaction mixtures were sealed with one or two drops of mineral oil to prevent evaporation during thermal cycling. Amplification was performed with an initial denaturation at 94°C for 4 minutes, followed by 35 cycles of strand denaturation at 94°C for 1 minute, annealing at 50°C for 45 seconds, and primer extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

2.3. Sequence analysis

Sequencing of ITS regions was performed by Macrogen Inc. (Seoul, Korea) using universal forward and reverse primers (ITS5 and ITS4), respectively. The sequencing results obtained from Macrogen were compared with previously published sequences data of *Hieracium* and submitted to GenBank. The accession numbers are given in Table 1.

Table 1. Locality information and GenBank accession numbers of the examined taxa

Pop. No	Taxa	Location	Accession Number
1	*H. artabirense* (Zahn) Juxip	A8 Rize: İkizdere, Acısu Köyü Kaplıca yolu, 31 v 2001, 700 m, Coşkunçelebi 297, KTUB	FJ613406
2	*H. cryptonaevum* (Bornm. & Zahn) Sell & West	A8 Gümüşhane: Kelkit, Heneke yaylası, açık alan, 11 vii 2003, 2200 m, Coşkunçelebi 366, KTUB	FJ613405
3	*H. karagoellense* (Bornm. & Zahn) Sell & West	A8: Trabzon: Çaykara, Yaylaönü, 1950 m, 12 viii 1998, Coşkunçelebi 36, KTUB	FJ613413
4	*H. karagoellense* (Bornm. & Zahn) Sell & West	A7 Gümüşhane: Tersundağı, alpin çayır, 03 viii 2004, 2000 m, Coşkunçelebi 516, KTUB	FJ613412
5	*H. hypopityforme* Juxip	A7 Trabzon: Maçka, Meryemana, Dilaver Balık Tesisleri, 27 vii 2004, 1800 m, Coşkunçelebi 499, KTUB	FJ613417
6	*H. sarykamychesense* Juxip	A7 Gümüşhane: Tersundağı, alpin çayır, 03 viii 2004, 2000 m, Coşkunçelebi 511, KTUB	FJ613410
7	*H. lazicum* Boiss. & Bal	A7 Gümüşhane: Tersundağı, alpin çayır, 03 viii 2004, 2000 m, Coşkunçelebi 514, KTUB	FJ613411
8	*H. onosmopsis* (Zahn) Sell & West.	A9 Artvin: Ardanuç'tan Kutul'a giderken, 17 vii 2003, 1800 m, Coşkunçelebi 420, KTUB	FJ613407
9	*H. mannagettae* Freyn.	A8 Rize: Anzer Yaylası, 2150 m, 09 viii 1998, Coşkunçelebi 106, KTUB	FJ613409
10	*H. mannagettae* Freyn.	A7 Gümüşhane: Tersundağı, 03 viii 2004, 2000 m, Coşkunçelebi 527, KTUB	FJ613408
11	*H. tamderense* Hub.- Mor.	A7 Trabzon: Maçka, Meryemana-Camiboğazı yolu, 2100 m, 25 vii 1999, Coşkunçelebi 205, KTUB	FJ613416
12	*H. tamderense* Hub.- Mor.	A7 Gümüşhane: Tersundağı, açık alan, 03 viii 2004, 2000 m, Coşkunçelebi 507, KTUB	FJ613415
13	*H. microtum* Boiss.	A7 Gümüşhane: Karamustafa Köyü, Yol şevi ve gevşek topraklar, 03 viii 2004, 1600 m, Coşkunçelebi 524, KTUB	FJ613414

2.4. Data Analysis

The nucleotide sequences were automatically aligned with ClustalW as implemented in the BioEdit v. 7.0 Software (Hall, 1999). Neighbor-Joining (NJ) and Maximum Parsimony (MP) trees were built using the Molecular Evolutionary Genetics Analysis (MEGA v. 3.1) program (Kumar et al., 2004). DNA sequences were analyzed based on Kimura's two parameter model (K2P). All characters were ordered and equally weighted. The topology of the consensus tree was constructed and evaluated by 1000 bootstrap replications (Felsenstein, 1985) for both MP and NJ (Saitou and Nei, 1987) analysis. Consistency index (CI=0.923077) and retention index (RI=0.833333) were found for MP analysis. *Hieracium pilosella* L. (*Pilosella officinarum* Vaill) (GenBank: AY879161) was used as an outgroup for the analysis.

3. Results

The total lengths of the ITS regions of the examined individuals were 710 bp; ITS1, 5.8S, and ITS2 were determined as 283, 161, and 266 bp, respectively. There was only a single 1 bp-deletion in the ITS1 region for *H. hypopityforme* (709 bp). The aligned data matrix based on the ITS region of 13 individuals included 710 characters of which 11 (1.5%) were parsimony informative, 30 (4.2%) variable, and 680 (95.7%) conserved sites. Variable sites as inferred by MEGA are given in Table 2. *Hieracium cryptonaevum* showed differences from the rest of the individuals at positions 166 and 607. Among the taxa represented by two individuals, *H. karagoellense* differed from the rest of the examined taxa at position 158, and *H. mannagettae* at positions 131 and 368. *Hieracium hypopityforme*, represented with only one individual in this study, displayed dissimilarities to the other species at positions 86, 592, 600, 618, and 637 plus the 1 bp-deletion mentioned above. *Hieracium sarykamychesense* contained autapomorphic substitutions at positions 51, 130, 284, and 484.

Table 2. Variable and parsimony informative sites of aligned sequences of *Hieracium* taxa

Pop. No	Taxa	Variable sites	Parsimony informative sites
		111111 1111222234 4556666666	111111246 6
		5789001233 3356236866 8990011345	7001235661 4
		8866563701 4586180488 4250728774	8563758082 7
1	*H. artabirense*	CTGATCCATC TCACGTATGA AGCTTAGGTC	TTCCACAAAA T
2	*H. cryptonaevum*	....ATTC.. .A.A..T... ....CT..C.	.ATTCA.T.T C
3	*H. karagoellense*	....G.....	......G... .
4	*H. karagoellense*	....G.TC....	......G... .
5	*H. hypopityforme*	..A.A..C.. ......T... .T.C.TAAC.	.A..C..T.T C
6	*H. sarykamychesense*	T.....TCG. ....A.T G.........	...TC...T. .
7	*H. lazicum*	...GC.TC.. C........T ..T......A	.C.TC...T. .
8	*H. onosmopsis*	.......C..	....C..... .
9	*H. mannagettae*	.C..C....T	CC........ .
10	*H. mannagettae*	.C..C..C.. ....A.	CC..C..... .
11	*H. tamderense*	....AT.C.. .A....T... .....T..C.	.AT.CA.T.T C
12	*H. tamderense*	....AT.C.. .A....T... .....T..C.	.AT.CA.T.T C
13	*H. microtum*	.C.....C.. ....C.	C...C..... C

The NJ and MP trees show that the examined taxa are composed of two groups (Figures 1 and 2). While Group A consisted of only *H. sarykamychesense* and *H. lazicum*, Group B included the rest of examined taxa, separated with moderate bootstrap support (63%) from Group A. In group B, taxa represented by two individuals were always clustered together. The MP tree (Figure 2) showed a very similar topology to the NJ tree (Figure 1).

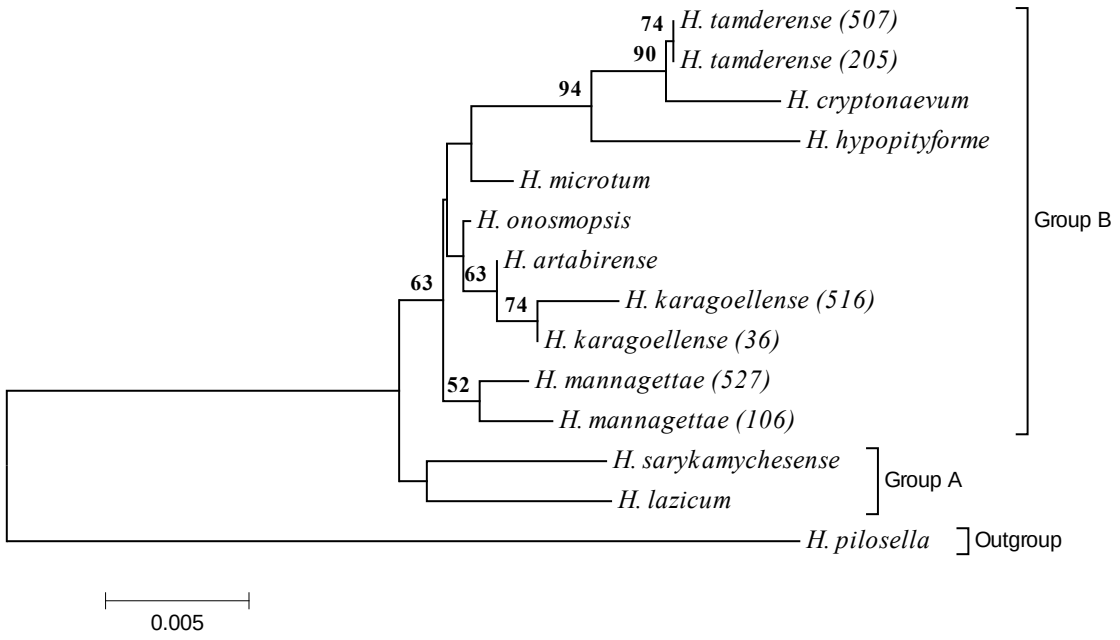

Figure 1. The dendogram shows the genetic relationships in *Hieracium* based on ITS1-5.8S-ITS2 (ITS) sequences, evaluated by the Neighbour Joining method, using outgroup *Hieracium pilosella* L. Values above branches indicate bootstrap values supporting the respective cluster. Bootstrap support higher than 50% is displayed. The numbers in brackets show the individual number (see Table 1)

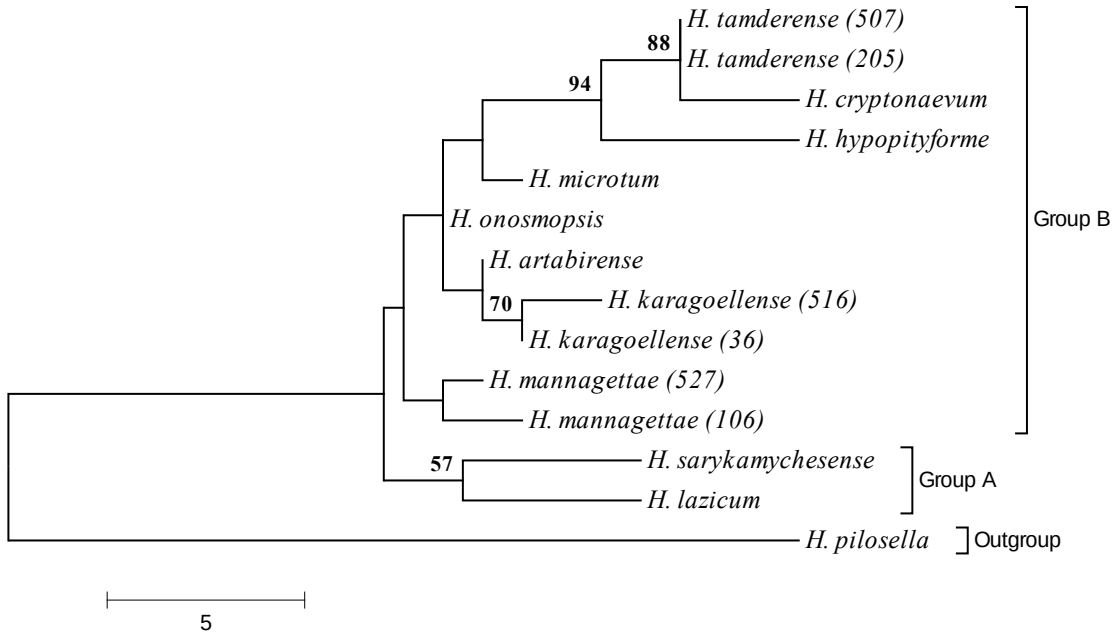

Figure 2. The dendogram shows the genetic relationships in the examined *Hieracium* taxa based on ITS sequences, evaluated by the Maximum Parsimony method, with *Hieracium pilosella* L. as outgroup. Values above the branches indicate bootstrap values supporting the respective cluster. Values higher than 50% are displayed

The pairwise distances obtained by Kimura's Two Parameter model varied from 0.0 to 2.1% in all investigated individuals (Table 3). The highest diversity occurred between *H. sarykamychesense* and *H. lazicum* compared to *H. hypopityforme* (2.1%) while the lowest interspecific differences occurred between *H. karagoellense*-36 and *H. onosmopsis* compared to *H. artabirense* with sequence divergence value of 0.1% (Table 3).

Table 3. Pairwise distance matrix of genetic divergence values among 13 individuals using Kimura's Two Parameter model

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *H. artabirense*													
2 *H. cryptonaevum*	0.014												
3 *H. karagoellense* (516)	0.004	0.019											
4 *H. karagoellense* (36)	0.001	0.016	0.003										
5 *H. hypopityforme*	0.014	0.014	0.019	0.016									
6 *H. sarykamychense*	0.010	0.019	0.014	0.011	0.021								
7 *H. lazicum*	0.011	0.019	0.016	0.013	0.021	0.013							
8 *H. onosmopsis*	0.001	0.013	0.006	0.003	0.013	0.009	0.010						
9 *H. mannagettae* (106)	0.006	0.016	0.010	0.007	0.016	0.013	0.011	0.004					
10 *H. mannagettae* (527)	0.004	0.017	0.009	0.006	0.017	0.014	0.013	0.006	0.004				
11 *H. tamderense* (507)	0.010	0.004	0.014	0.011	0.010	0.017	0.017	0.009	0.011	0.013			
12 *H. tamderense* (205)	0.010	0.004	0.014	0.011	0.010	0.017	0.017	0.009	0.011	0.013	0.000		
13 *H. microtum*	0.004	0.013	0.009	0.006	0.013	0.011	0.013	0.003	0.004	0.006	0.009	0.009	

4. Conclusions

Sequencing of the ITS for sytematic studies supplies useful data to solve the taxonomic problems among closely related plant species (Baldwin, 1993; Baldwin et al., 1995; Gielly et al., 1996; Gravendeel et al., 2001). ITS1 and ITS2 are currently the most useful and practical nuclear sequences for addressing lower-level relationships in plants because of their relatively small size, high mutation rate, and easy amplification (Baldwin et al., 1995). Sequences of the ITS region of nuclear ribosomal DNA were used to assess the relationships of some endemic *Hieracium* taxa in this study.

Hieracium lazicum and *Hieracium sarykamychsense* show morphological differences, and they were treated under different taxonomic ranks by Sell and West (1974). However, they shared three polymorphisms (at positions 113, 127, and 468), and their geographic distribution indicates a close relationship among these species. On the other hand, in the trees, they are grouped together with low bootstrap (Figures 1-2). When the member size of the genus considered, they may simply have grouped together because of the lack of more closely related species.

As seen in Figures 1-2, group B falls into two branches. These branches appeared as a consequence of same polymorphic sites as seen in Table 2, but do not correspond well with morphologically based series described by Sell and West (1974). *Hieracium tamderense*, *H. cryptonaevum*, and *H. hypopityforme* formed a strongly supported branch (94%) emerging from the other species in Group B (Figures 1-2), and *H. hypopityforme* has more similar sequences to *H. tamderense* than *H. cryptonaveum* as seen in Table 2. Additionally, the pair-wise distance matrix showed that *H. hypopityforme* is closer taxon than the *H. cryptonaevum* to *H. tamderense* (Table 3). The MP and NJ trees are also consistent with results mentioned above.

Despite of the apomictic mode of reproduction, some inter- and intraspecific genetic variation was found amongst the taxa. Most of this variation probably dates back before the advent of apomixis in *Hieracium*, i.e. it occurred at the diploid level (Fehrer et al., 2009). As expected, a higher level of interspecific sequence variation occurred than intraspecific (Table 3). Similarly, Štorchová et al. (2002) found low diversity among populations of sect. *Alpina* based on allozyme and RAPD studies. So, both individuals of each, *H. karagoellense*, *H. mannagettae*, and *H. tamderense*, appeared together in the resulting trees (Figures 1-2). But, there is intraspecific variation in individuals of *H. karagoellense* and *H. mannagettae*. Although it is known that the species are apomicts, the possible source of variation could be somatic mutations (Ronikier and Szelag, 2008) or due to multiple origin of the same taxa.

Hieracium tamderense, *H. cryptonaevum*, and *H. hypopityforme* formed a strongly supported branch (94%) emerging from the other species in Group B (Figures 1-2), and generally, ITS sequence variation were the highest values in the dataset (Table 3) which is obvius from the branch lengths in both trees. Although it is known that the species are apomicts, the possible source of variation may arised from diploid progenitors (Fehrer et al., 2009). Some *Hieracium* taxa used in this study (*H. tamderense*, *H. microtum*, *H. mannagettae*, *H.artabirense*, and *H. karagoellense*) produced pollen grains (Unpublished data). And, many *Hieracium* polyploids are still able to produce some amount of pollen and might therefore contribute to gene flow as pollen donors (Mráz et al. 2002, 2009; Slade and Rich 2007). In this case, hybridization via pollen from apomictic plants can be a source of variation (Rich et al., 2008). On the other hand, only very rare natural recent hybridization have been documented so far in the genus *Hieracium* (Mráz et al. 2005; Chrtek et al. 2006; Mráz et al. 2011). Moreover, in *Hieracium*, a strong mentor effect was described by Mráz (2003) which is usually preventing recent hybridization.

Nuclear DNA C-values are important genomic character and can be used in plant studies (Meriç and Güler, 2013). Among the examined species, only the chromosome number of *H. karagoellense* (*2n*=27) was known (Coskuncelebi and Hayırlıoglu-Ayaz, 2006). Additionally, C-values of *H. hypopityforme* (7.54 pg), *H. tamderense* (5.31 pg), *H. microtum* (7.22), *H. karagoellense* (5.50 pg), and *H. mannagettae* (6.09) were determined (Unpublished data). According to the C-values, *H. hypopityforme* and *H. microtum* are tetraploid, but *H. tamderense*, *H. karagoellense*, *H. mannagettae* are triploid. It was reported that most of the species (90%) of the genus from Turkey are polyploid (Schuhwerk and Lippert, 1998). Polyploidization as well as asexual propagation might have further contributed to the diversification and speciation of this genus (Coskuncelebi and Hayırlıoglu-Ayaz, 2006). Additionally, in many species, even asexually reproducing plants may reveal patterns of differentiation at a local or regional scale (Mes et al., 2002; Rich et al., 2008; Fehrer et al., 2009).

The results of the phylogenetic analyses are not corresponding to the serial level according to Sell and West (1974). The low sequence variation (2.1%) may also indicate that ITS is not sufficiently variable to resolve relationships in *Hieracium* (Fehrer et al., 2009). But, Vilatersana et al. (2000) stressed that results from ITS sequence analysis are congruent with the traditional usage of sections in the genus *Carthamus* L. Meanwhile, Noyes (2006) recorded low diversity, but distinct taxonomic delimitation in three varieties of *Erigeron* based on ITS and ETS sequences. In our study, *H. karagoellense*, *H. lazicum*, *H. sarykamychense*, *H. mannagettae*, and *H. tamderense* were collected from the same geographical region, and this region is one of the main centres of diversity of the genus *Hieracium* in Turkey. However, phylogenetic analysis, these five taxa did not cluster in the MP and NJ trees. It is much more likely that the ancestors of the investigated taxa were rather divergent and that at least some of them may be extinct nowadays. This would mean that the diversity is rather ancient instead of ongoing (Fehrer et al., 2009).

Hieracium consists of both apomictic and diploid sexual populations (Chrtek, 1997) and it is well known that apomictic taxa produce very similar offspring with their progenitor (Nogler, 1984; Shi et al., 1996). Although the genus has apomictic species that posses high phenetic similarities, they have enough stable morphological characters that enable taxonomical delimitation (Štorchová et al., 2002). In previous reports, it was declared that ITS sequences are useful for phylogeny reconstruction of different genus of Asteraceae e.g *Caylcadenia* DC. (Baldwin and Markos, 1998) and *Silphium* L (Clevinger and Panero, 2000). But, the result of our study is not efficient as comparable above mentioned studies. On the other hand, Fehrer et al. (2009) found relatively low interspecific variation (1.9%) among some diploid *Hieracium* taxa. In the same way, ITS sequence analysis of the thirteen specimens of *Hieracium* s.str.

investigated here revealed high similarity (Table 3) as well as moderate bootstrap values as seen Figures 1-2. This high similarities more likely causes are recent divergence and relatively young age of the genus.

The present study is a preliminary study in order to see whether the internal transcribed spacer can provide additional useful information for the relationships of the subgeneric level of the genus or not. However, additional molecular data and a broader taxon sampling are needed for better conclusions.

Acknowledgements

The authors would like to express their thanks to Dr. Sabriye Dulger and Dr. Fatih S. Beris for kindly helping with the laboratory studies, and to TUBITAK (106T204) and the Research Foundation of Karadeniz Technical University (KTU- 2003.111.004.08) for financial support.

References

Anderberg, A.A, Rydin, C., Källersjö, M. 2002. Phylogenetic relationships in the order Ericales s.l.: Analyses of molecular data from five genes from the plastid and mitochondrial genomes. American Journal of Botany, 89: 677-687.

Ayaz, F.A and Coskuncelebi, K. 2002. Total phenolics variation in leaves of some *Hieracium* L. and *Pilosella* Hill. species. Acta Biologica Cracoviensia Series Botanica, 44: 175-179.

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. Molecular Phylogenetics and Evolution, 1: 3-16.

Baldwin, B.G. 1993. Molecular Phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: Chromosomal and morphological evolution reexamined. American Journal of Botany, 80. 222-238.

Baldwin, B.G., Campbell, C.S., Porter, J.M., Wojciechowski, M.F., Donoghue, M.J. 1995. Utility of nuclear ribosomal DNA internal transcribed spacer sequences in phylogenetic analysis of angiosperms. Annals of the Missouri Botanical Garden, 82: 247-277.

Baldwin, B.G., Markos, S. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). Molecular Phylogenetics and Evolution, 10: 449-463.

Beaman, J.H. 1990. Revision of *Hieracium* (Asteraceae) in Mexico and Central America. In: Anderson C (ed.). Systematic botany monographs, pp. 1-76. Michigan: Michigan University Press.

Chrtek, J.Jr. 1997. Taxonomy of the *Hieracium alpinum* group in the Sudeten Mts and the West and Ukrainian East Carpathians. Folia Geobotanica and Phytotaxonomica, 32: 67-91.

Chrtek, J.Jr., Mráz, P., Sennikov, A.N. 2006. *Hieracium* × *grofae*—a rediscovered diploid hybrid from the Ukrainian Carpathians. Biologia (Bratislava), 61:365–373.

Clevinger, J.A., Panero, J.L. 2000. Phylogenetic analysis of *Silphium* and subtribe Engelmanniinae (Asteraceae: Heliantheae) based on ITS and ETS sequence data. American Journal of Botany, 87: 565-572.

Conti, E., Suring, E., Boyd, D., Jorgensen, J., Kelso, G.S. 2000. Phylogenetic relationships and character evolution in *Primula* L.: The usefulness of ITS data. Plant Biosystems, 134: 385-392.

Coskuncelebi, K., Beyazoglu, O. 2003a. Additional records of *Hieracium* L. and *Pilosella* Hill (Compositae : Lactueae) for the flora of Turkey. Turkish Journal of Botany, 27: 499-504.

Coskuncelebi, K.. & Beyazoglu, O. 2003b. Assessment of Phenetic Clustering in Hieracium L. s.str. (Asteraceae) Species Distributed in Black Sea Region of Turkey. Bulletin of The Polish Academy of Sciences 51: 17-24.

Coskuncelebi, K., Hayirlioğlu-Ayaz, S. 2006. Notes on chromosome numbers and karyotypes of five species in *Hieracium* L. s.str (Asteraceae) from Turkey. Caryologia, 59: 19-24.

Coskuncelebi, K., Vladimirov, V. 2008. IAPT/IOPB chromosome data 5: chromosome numbers of Turkish *Hieracia* & *Pilosella* (Asteraceae). Taxon, 57: 211.

Doyle, J.J., Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19: 11-15.

Fehrer, J., Krak, K., Chrtek, J. 2009. Intra-individual polymorphism in diploid and apomictic polyploid hawkweeds (*Hieracium*, Lactuceae, Asteraceae): disentangling phylogenetic signal, reticulation, and noise. BMC Evolutionary Biology, 9: 239.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39: 783-791.

Garcia-Jacas, N., Susanna, A., Moza arian, R., Ilarslan, R. 2000. The natural delimitation of Centaurea (Asteraceae: Cardueae): ITS sequence analysis of the Centaurea jacea group. Plant Systematics and Evolution 223: 185-199.

Garcia-Jacas, N., Uysal, T., Romaschenko, K., Suărez-Santiago, V.N., Ertuğrul, K., Suanna, A. 2006. CentaureaRevisited: A molecular survey of the Jacea group. Annals of Botany, 98: 741-753.

Gielly, L., Yuan, Y.M., Küpfer, P., Taberlet, P. 1996. Phylogenetic use of noncoding regions in the genus *Gentiana* L.: Chloroplast *trn*L (UAA) intron versus nuclear ribosomal internal transcribed spacer sequences. Molecular Phylogenetics and Evolution, 5: 460-466.

Gottschlich, G., Coskuncelebi, K., Beyazoglu, O. 2000. Four new species of *Hieracium* L. (Compositae) from NE Turkey. Willdenowia, 30: 279-291.

Gravendeel, B., Chase, M.W. De Vogel, E.F. 2001. Molecular phylogeny of *Coelogyne* (Epidendroideae; Orchidaceae) based on plastid RFLPs, *mat*K, and nuclear ribosomal ITS sequences: Evidence for polyphyly. American Journal of Botany, 88: 1915-1927.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95-98.

Häffner, E., Hellwig, F.H. 1999. Phylogeny of the tribe Cardueae (Compositae) with emphasis on the subtribe Carduinae: an analysis based on ITS sequence data. Willdenowia 29: 27-39.

Kim, K.J., Turner, B.L., Jansen, R.K. 1992. Phylogenetic and evolutionary implications of interspecific chloroplast DNA variation in *Krigia* (Asteracea-Lactuceae). Systematic Botany, 17: 449-469.

Koltunow, A.M. 2000. The genetic and molecular analysis of apomixis in the model plant *Hieracium*. Acta Biologica Cracoviensia series Botanica, 42: 61-72.

Kumar, S., Tamura, K., Nei, M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics, 5: 150-163.

Meriç, Ç and Güler, N. 2013. Nuclear DNA content of an endemic species for Turkey: *Silene sangaria* . Biological Diversity and Conservation, 6: 88-92.

Mes, T.H.M., Kuperus, P., Kirschner, J., Štěpánek, J., Štorchová, H., Oosterveld, P., Den Nijs, J.C. 2002. Detection of genetically divergent clone mates in apomictic dandelions. Molecular Ecology, 11: 253-265.

Mráz, P., Chrtek. J., Kirschner, J. 2001. Genetic variation in the *Hieracium rohacsense* group (*Hieracium* sect. *Alpina*). Phyton, 41: 269-276.

Mráz, P., Kovalčíkově, K., Marhold, K. 2002. Pollen shapes and size in *Hieracium* and related genera. In: Gutterman W (ed) Contribution abstracts (lectures and posters) from 6th *Hieracium* workshop, Hirschegg, Kleinwalsertal (Österreich). Institut für Botanik der Universität Wien, Wien, p 27.

Mráz P (2003) Mentor effects in the genus Hieracium s. str.(Compositae, Lactuceae). Folia Geobot 38:345–350

Mráz, P., Chrtek, J., Fehrer, J. Plačkově, I. 2005. Rare recent natural hybridization in the genus *Hieracium* s. str.—evidence from morphology, allozymes and chloroplast DNA. Plant Systematics and Evolution, 255:177–192.

Mráz, P., Chrtek, J. Šingliarově, B. 2009. Geographical parthenogenesis, genome size variation and pollen production in the arcticalpine species *Hieracium alpinum*. Botanica Helvetica, 119:41–51.

Mráz, P., Chrtek, J., Fehrer, J. 2011. Interspecific hybridization in the genus *Hieracium* s. str.: evidence for bidirectional gene flow and spontaneous allopolyploidization. Plant Systematics and Evolution, 293:237–245.

Nogler, G.A. 1984. Gametophytic Apomixis In: Johri B. M. (ed.), Embryology of Angiosperms, 475-518. Springer-Verlag, Berlin.

Noyes, R.D. 2006. Intraspesific nuclear ribosomal DNA divergence and reticulation in sexual diploid *Erigeron strigosus* (Asteraceae). American Journal of Botany, 93: 470-479.

Rich, T.C.G., McDonnell, E.J., Lledó, M.D. 2008. Conservation of Britain's biodiversity: the case of *Hieracium cyathis* (Asteraceae) and its relation to other apomictic taxa. Botanical Journal of the Linnean Society, 156: 669-680.

Ronikier, M., Szeląg, Z. 2008. Differentiation among disjunct population of agamospermous species of *Hieracium* section *Cernua* (Asteraceae) in Central European subalpine habitats. Botanical Journal of the Linnean Society,58: 93-105.

Saitou, N., Nei, M. 1987. The Neighbor-Joining method: A new method for reconstructing phylogenetic trees. Molecular Phylogenetics and Evolution, 4: 406-425.

Schuhwerk, F., Lippert, W. 1998. Chromosomenzahlen von *Hieracium* (Compositae, Lactuceae) Teil 2. Sendtnera, 5: 269-286.

Sell, P.D., West, C. 1974. *Hieracium* L. (Compositae). In: Davis PH (ed.) Flora of Turkey and the East Aegean Islands, Vol. 5, pp. 696-746. Edinburgh: Edinburgh University Press.

Shi, Y., Gornall, R.J., Draper, J. Stace, C.A. 1996. Intraspecific molecular variation in *Hieracium* sect. *Alpina* (Asteraceae), an apomictic group. Folia Geobotanica and Phytotaxonomica, 31: 305-313.

Slade, K., Rich, T.C.G. 2007. Pollen studies in British *Hieracium* sect. *Alpina* (Asteraceae). Watsonia, 26:443–450

Štorchová, H., Chrtek, J. Jr., Bartish, I.V., Tetera, M., Kirschner, J., Štěpánek, J. 2002. Genetic variation in agamospermous taxa of *Hieracium* sect. *Alpina* (Compositae) in the Tatry Mts (Slovakia). Plant Systematics and Evolution, 235: 1-17.

Susanna, A., Garcia-Jacas, N., Soltis, D.E., Soltis, P.S. 1995. Phylogenetic relationships in tribe Cardueae (Asteracea) based on ITS sequences. American Journal of Botany, 82: 1056-1068.

Susanna, A., Garnatje, T., Garcia-Jacas, N. 1999. Molecular phylogeny of Cheirolophus (Asteraceae: Cardueae-Centaureinae) based on ITS sequences of nuclear ribosomal DNA. Plant Systematics and Evolution 214: 147-160.

Tyler, T. 2000. Detecting migration routes and barriers by examining the distribution of species in an apomictic complex. Journal of Biogeography, 27: 979-988.

Vilatersana, R., Susanna, A., Garcia-Jacas, N., Garnatje, T. 2000. Generic delimitation and phylogeny of the *Carduncellus-Carthamus* complex (Asteraceae) based on ITS sequences. Botanical Journal of the Linnean Society, 221: 89-105.

Vladimirov, V. 2003. A new diploid *Hieracium* (Asteraceae Lactuceae) from Bulgaria. Botanical Journal of the Linnean Society, 143: 213-218.

White, T.J., Bruns, T., Lee, S. Taylor, J. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis M.A., Gelfand D., Sninsky, J., White, T.J. (eds.) PCR Protocols: A Guide to Methods and Application, 315-322. San Diego: Academic Press.

(Received for publication 04 January 2013; The date of publication 15 August 2013)