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*Original Article*

# Molecular Analyzing of Some Water Mite Species (Acari, Hydrachnidia) using DNA Barcodes

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**Keywords:**
Water Mites,
DNA Barcoding,
28S rDNA,
Karamik Lake,
Afyonkarahisar,
Turkey

**Abscract:** This study is the first DNA barcoding study with *Hydrachna globosa*, *Hydryphantes dispar*, *Georgella helvetica* and *Hydrodroma despiciens* which are very common species in the lakes (Acari, Hydrachnidia). Water mites samples used in the study were collected from Lake Karamik (Afyonkarahisar Turkey) between April to October 2015. Molecular analysis of the samples were carried out in the laboratory environment. The gDNA from each sample were isolated and the 28S rDNA gene region amplified. It was observed that the molecular analysis results obtained were in agreement with the classical systematic classification results for each species used in the study.

# DNA barkodları kullanılarak Bazı Su Akarı Türlerinin (Acari, Hydrachnidia) Moleküler Analizi

**Anahtar Kelimeler:**
Su Kenesi,
DNA Barkodlama,
28S rDNA,
Karamik Gölü,
Afyonkarahisar,
Türkiye

**Özet:** Bu çalışma, göllerde çok yaygın olan *Hydrachna globosa*, *Hydryphantes dispar*, *Georgella helvetica* ve *Hydrodroma despiciens* ile yapılan ilk DNA barkodlama çalışmasıdır. Araştırmada kullanılan su kenesi örnekleri Nisan-Ekim 2015 tarihleri arasında Karamık Gölü'nden (Afyonkarahisar Türkiye) alındı. Numunelerin moleküler analizi laboratuvar ortamında gerçekleştirildi. Her örnekten gDNA izole edildi ve 28S rDNA gen bölgesi amplifiye edildi. Elde edilen moleküler analiz sonuçlarının çalışmada kullanılan her tür için klasik sistematik sınıflandırma sonuçları ile uyumlu olduğu gözlendi.

## 1. INTRODUCTION

Water mites are one of the most important invertebrate groups found in inland waters. All the water ticks identified as Hydrachnidia (Hydrachnellae, Hydracarina, or Hydrachnidia) are all adapted to freshwater (Smith et al., 2009). Until now, there are more than 6000 species listed in the world, represented by 57 families, 81 sub-families and more than 400 genera (Di Sabatino et al., 2008, Smith et al., 2009). They have special significance in the determination of livelihoods, such as lakes, ponds and settlements and communities in rivers. The water mites who free-living live in underground waters, deposits, marsh, ponds, lakes and seas those who are parasitic live in the mantle of molluscs and in reptiles (Bader, 1975; Walter, 1922).

Although it seems that the studies on the water mites have been a classical systematic studies until recently, ecological, genetic, and other molecular studies have increased in the last few years (Dorda and

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Valdecasas, 2002; Bohonak et al., 2004; Ernsting, 2006; Martin, 2010; Więcek, 2013; Aşçı et al., 2015; Aşçı et al, 2016)

The analysis of DNA barcoding, which is the subject of this study, is the first study on these species. Both the molecular structures of the water mite species and the phylogenetic relationships between the species were determined in this study. This method has shown more accurate solutions as a new molecular taxonomic method that terminates the controversial classical definition of species.

At the basis of the DNA barcoding system, standardized gene regions are used. Analysis by polymerase chain reaction (PCR) has made a great contribution to the development of the molecular systematic. This PCR-based approach, which was first used in microbial studies, was later used in the taxonomy (Woese, 1996; Zhou et al, 1997). For this purpose, DNA barcoding is used specially for species, by special genomic regions in organisms.

DNA barcoding is based on the principle that standardized short sequences (600-700 bp) differ from genetic distance between species (Hebert et al. 2003). The method of DNA barcoding is based not only on identification of species but also on universal plant barcoding, including evolution, ecology, evolutionary biology and conservation studies, identification of gene flow between population dynamics and populations, detection of harmful insects in agriculture, detection of parasites and vectors, detection and conservation of endangered species, identification of different life stages in the same way, environmental analysis, determination of parasite-host relationship and illumination of symbiotic relationship are also used (Besansky et al., 2003; Ball and Armstrong, 2006; Bashasab et al., 2006; Kress and Erickson, 2008; Ramadan and Baeshen, 2012).

The application of molecular systematic studies is very simple and fast. The phylogenetic tree is constructed by calculating the genetic distances from the sequence data of the barcode region. Closely related individuals are clustered in this phylogenetic tree. Each type seems to be characterized by a single series. But there can be differences within species as there are important differences between species. However, the genetic distance between different species is usually larger than the in-species genetic distance. So phylogenetic tree is represented by clusters of closely related individuals and each cluster represents a species (Das Mahapatra and Mallet, 2006).

The PCR-based molecular systematic approach has many advantages. One of these is the rapid and easy access to molecular data. Sequence analysis data and PCR primers as well as species name, species label (locality, date, photograph, etc.) are included on barcode recordings. Verifiability of samples by predefined species is one of the advantages of DNA barcoding when morphological data is inadequate (Hebert et al., 2003, Jinbo et al., 2011).

In addition, molecular base classification is used to identify species that cannot be explained by classical taxonomic methods, especially parasitic species (Ernsting et al. 2008). Systematic studies based on the morphological characteristics of the water mites are faced with many problems, such as large and complicated literature, and variations arising from geographical differences, and waste of time (Sites and Marshall 2003, 2004).

In this study, the nucleotide sequence of the 28S ribosomal DNA (rDNA) gene region of *Hydrachna (Diplohydrachna) globosa*, *Hydrodroma despiciens*, *Georgella helvetica*, *Hydryphantes dispar* species collected from Karamik Lake was determined. Also intraspecific and interspecific relationships are determined. In addition, 28S rDNA gene region nucleotide sequences of the water mites that have large habitats in our country are defined,

as is the case in the world using molecular markers with DNA barcoding systematically.

## 2. MATERIALS and METHODS

The water mite samples used in this study were collected from Karamik Lake (38 ° 25'46.0 "K 30 ° 50'11.4" G) in Afyonkarahisar Province on April-October 2015 with 0.5 mm diameter aquarium scoops. An Olympus SZ61 stereo microscope was used to diagnose the water mite species.

### 2.1 DNA isolation

DNA isolation was performed from a single sample of each species and live samples were used. gDNA isolated from the genomes of *Hydrachna procesisifera, Hydrachna globosa, Georgella helvetica, Hydrodroma despiciens, Limnesia fulgida, Piona contraversiosa, Arrenurus affinis* and *Arrenurus maculator* for the COI gene were isolated for DNA isolation and .

In DNA isolation, gDNAs of the species of *Hydrachna procesisifera, H. globosa, Georgella helvetica, Hydrodroma despiciens, Limnesia fulgida, Piona contraversiosa, Arrenurus affinis*, and *A. maculator* were isolated for the COI gene region, and gDNAs of the species of *Hydrachna procesisifera, H. globosa, Eylais setosa, E. extendens, Hydryphantes dispar, Hydryphantes flexuosus, Georgella helvetica, Hydrodroma despiciens, Limnesia fulgida, Arrenurus affinis* and *Arrenurus maculator* were isolated for the 28S rDNA gene region.

Tissue samples were frozen in liquid nitrogen and powdered in a porcelain vat. The CTAB (Cetyl Trimethyl Ammonium Bromide) method was first applied to the powdered tissues in DNA isolation. Apart from the CTAB method, DNA isolation was performed according to the manufacturer's protocol with the QIAamp® DNA Mini Kit (Qiagen) and GeneJET Genomic DNA Purification Kits (Thermo Scientfic ™). The DNAs to be used as templates were used in the polymerase chain reaction (PCR) by storing them at -20 °C until used again.

### 2.2 Polymerase Chain Reactions (PCR)

Polymerase chain reaction (PCR) mixture was prepared in 0.2 mL ependorf tubes for each of the isolated gDNAs, a 50 µL. For this, 1.5 mM $MgCl_2$, 0.5 nM F primer, 0.5 nM R primer, 5 µL dNTP (25 µM dATP, dTTP, dGTP, and the like) were added to the tube (to amplify 28S rDNA and COI gene regions) dCTP), 5 µL gDNA and DNA polymerase (Thermo Scientific) were added and $dH_2O$ was added to the ependorf tube to give a final volume of 50 µL. Using the primers degenerate bcdF01 5'-CATTTTCHACTAAYCATAARGA TATTGG-3 'and bcdR04 5'-TATAAACYTCDGGATGNCCAAAAA-3' with LCO 1490-forward 5'-GGTCAACAAATCATAAAGATATTGG-3 'and HCO 2198-reverse 5'-TCAGGGT GACCAAAAATCA-3' for the COI gene different PCR mixes were prepared (Folmer et al., 1994). PCR conditions for the COI gene were denaturation at 98 °C for 3 minutes followed by denaturation at 35 °C for 20 seconds at 98 °C, 20 seconds annealing at 61.5 °C (primer binding), 72 °C (chain extension) for 30 seconds and finally for 8 minutes at 72 °C. The reaction was carried out using primers D2F-forward 5'-AGTCGTGTTGCTTGATAGTGCAG-3 'and D2R-reverse 5'-TTGGTCCGTGTTTCAAGACGGG-3' for the 28S rDNA region (Campbell et al., 1993, Goolsby et al. 28S rDNA PCR was carried out for 2 minutes at 98 °C (annealing and polymerization) for 35 cycles at 98 °C for 15 seconds and 72 °C for 3 minutes. One cycle for final polymerisation was set to 5 minutes at 72 °C. PCR cycles were performed in ProFlex ™ 3x32-Well PCR System.

After the obtained PCR products were run on a 1.8% agarose gel, DNA bands were imaged under UV light with GEN-BOX SDR Bio-imaging Systems.

Purification of PCR products before DNA sequence analysis reactions; 1/10 volume of cold NaOAc (sodium acetate) and 2.5 volumes of cold absolute ethanol were added to the PCR products in 1.5 mL ependorf tubes. The mixture was stirred gently by inverting the tubes and allowed to stand at -20 °C for 2 hours. After centrifugation at 15 000 rpm for 20 minutes at + 4 °C, the supernatant was removed. 500 ml of cold 70% ethanol was added to the DNA pellet and centrifuged at 15 000 rpm for 5 minutes at + 4 °C. After removal of the supernatant, the pellet was allowed to dry for 10 minutes at room temperature and dissolved in 30 μL of TE buffer.

**2.3 DNA Sequence Analysis**

The analyzes were carried out in Molecular Biology and Genetics Laboratory of Afyon Kocatepe University. Sequence analysis of purified PCR products was performed by Sangers Method. A two-way reading was done, forward and backward. The backreading sequence was translated using the Nucleic Acid Sequence Massager program and paired with the forward reading sequence. With this obtained data, the existing data recorded in NCBI were compared using BlastN and BlastP programs. In the comparison of sequences, phylogenetic relationship levels between species and interspecies of water mites species were evaluated using MEGA6 package program.

**3. RESULTS and DISCUSSION**

**3.1 Molecular Results**

**3.1.1 COI sequence**

In this study, amplification of COI gene regions was performed in PCR after extraction of genomic DNA samples from the water mites species. Although DNA isolation studies have been repeated many times, DNA region replication has not been achieved in PCR. In addition, although the PCR conditions were optimized, no successful results were obtained.

However, *Georgella helvetica* was amplified in the PCR fragment of COI gene only for one sample from the water mites. This PCR product was sequenced and found to have the following nucleotide protein sequence.

>GhCOI [*Georgella helvetica*] cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial, 657 nt
CTTGATTTAGCTTTTGGAGCATGGTCTG
GGATAGTGGGAGCTAGATTAAGAACAT
TAATTAGATTAGAATTAGGACAACCAG
GGACATTATTGGAGAGGATCAAGTATA
TAATACAACCTTTAACAGGCCACGCATT
TGTTATAATTTTCTTTATAGTTATACCAA
TAATCAGTGGTGGATGTGGGAATTGGTT
AGTTCCTTTAATAATTAGAGCCCCAGAC
ATAGCATTCCCTCTAACAAATAATATAA
GATTTTGGCTATTACCTCCTTCCTTAATT
CTATTGTTAACCAGATCATTTACATCAT
TGGGAACGGGAACGGGAGGAACAGTAT
ATCCTCCTCTCTCACGAAATTTAGCTCA
TTCAGGACCTTCAGTTGATTTAACAATT
TTTTCACTTCATTTAGCTGGTATTTCTTC
AATTTTAGGAGCTATTAATTTTATAGCA
ACTATTATTAATGTTAAACCTAAACATA
TAAAAATAGAACAAATTCCTTTATTTGC
TCGATCCATTTTCATTACAACAATTTTA
CTTCTTTTATCTCTTCCTGTACTAGCAGG
AGCCATTACTATACTTTTAACAGATCGA
AATTTTAATACATCATTCTTTGATCCTG
CTGGAGGTGGGGATCCCATTTTATATCA
ACATTTATTTTGA

>GhCOI [*Georgella helvetica*] predicted cytochrome oxidase subunit 1 (COI) protein, partial cds; mitochondrial, 218 amino acids
LDLAFGAWSGIVGARLRTLIRLELGQPGT
LLERIKYIIQPLTGHAFVIIFFIVIPIISGGCG
NWLVPLIIRAPDIAFPLTNNIRFWLLPPSLI
LLLTRSFTSLGTGTGGTVYPPLSRNLAHS
GPSVDLTIFSLHLAGISSILGAINFIATIINV
KPKHIKIEQIPLFARSIFITTILLLLSLPVLA
GAITILLTDRNFNTSFFDPAGGGDPILYQH
LF*

***3.1.2 28S rDNA***

In contrast to the COI gene sequence, the 28S rDNA gene region *Hydrachna procesisifera, H. globosa, E. setosa, E.*

*extendens H. dispar, H. flexuosus, G.helvetica, H. despiciens, L. fulgida, A. affinis, A. maculator, H. globosa*), *H. dispar* (1), *G. helvetica* (5) and *H. despiciens* (5) were successfully isolated and reproduced. A total of 14 28S rDNA genetic sequences ranging from 568 nt to 582 nt in length were obtained. The 100 bp region of 28S rDNA from 14 different samples (TAGCAAACAAGTACCGTGAGGGAAAG TTGCAAA GAACTTTGAAGAGAGAGTTCAAAAGGA CGTGAAACCGTATGCAGGTAAACAGAT GGACCCACGAAGT) are protected in large proportions, except for this region is protected as a species.

The 28S rDNA sequence has been successfully achieved from three different samples of *H. globosa*. The species numbers of these species are given as Hdg 01, Hdg 02 and Hdg 03.

Nucleotide alignments of these tags were made in the Clustal Omega Program and the nucleotide alignment is shown in Figure 1,2, and 3. As a result of alignment of the sequences, there was a gap in a region of nucleotide 525 in Hdg01 and Hdg02 tag samples and the total nucleotide number was determined as 568 nt. There was no gap in the Hdg03 tag sample and the total number of nucleotides for the same region was 569 nt. The three tagged samples of the 28S rDNA gene region *H. globosa* strain contained 42 variable nucleotides (7.4%). The nucleotide sequences of the 28S rDNA gene region of the Hdg01, Hdg02 and Hdg03 tagged samples are given below.

The 28S rDNA sequence has been successfully achieved from five different samples of *G. helvetica* (Grh01, Grh02, Grh03, Grh04 and Grh05). The numbers of the samples belonging to these species are Grh01, Grh02, Grh03, Grh04 and Grh05. The sequence alignment of these tags is shown in FIG. As a result of the sequence alignment, no gap was observed in the Grh01 and Grh02 tagged samples, and the total number of nucleotides was determined as 582 nt. In the Grh03 tag sample, there was a gap in two regions, nucleotides 538 and 582, and the total number of nucleotides was 580 nt. In the Grh04 tag example, there were gaps in three regions, nucleotides 536, 538 and 582, and the total number of nucleotides was 579 nt. A gap of 582 nucleotides in the Grh05 tag number was found and the total number of nucleotides was 581 nt. Five tag examples of the 28S rDNA gene region *G. helvetica* strain contain 20 variable nucleotides (3,4%).

The nucleotide sequences of the 28S rDNA gene region of the Grh01, Grh02, Grh03, Grh04 and Grh05 tagged samples are given below.

28S rDNA sequences were successfully achieved from five different samples of *H. despiciens* species. The species numbers are Hdd01, Hdd02, Hdd03, Hdd04 and Hdd05. The sequence alignment of these labels is shown in FIG 3. As a result of the alignment of the sequences, eight regions of nucleotides 538 and 545 were observed in the Hdd01 tag sample and the total number of nucleotides was 573 nt. There was no gap in the Hdd02 tag sample and the total number of nucleotides for the same region was determined as 581 nt. In the Hdd03 tag sample, four regions were identified as 1, 314, 552, and 581 regions, and the total number of nucleotides was 577 nt. In the Hdd04 tag sample, three regions were found in the 1 st, 2 nd and 581 th regions and the total nucleotide number was determined as 578 nt. In the Hdd05 tag sample, there were four regions in the 1 st, 2 nd, 445 th and 581 th regions, and the total nucleotide number was 577 nt. Five tag samples of the 28S rDNA gene region *H. despiciens* strain contained 113 variable nucleotides (19.5%). The 28S rDNA gene region nucleotide sequences of the Hdd01, Hdd02, Hdd03, Hdd04 and Hdd05 tagged samples are given below.

```
CLUSTAL O(1.2.1) multiple sequence alignment


Hdg0328S      AGTCCCCGAAGGGAAAAGATGCGTCCACCTAAGGCTAAATATCGCATAATGTGAGACACG      60
Hdg0128S      AGTCCCCGAAGGGAAAAGATGCGTATAGATGATGATAATATTCGAATAATAGAGGAGACC      60
Hdg0228S      AGTCCCCGAAGGGAAAAGATGCGTATAGATGATGATAATATTCGAATAATAGAGGACACG      60
              ************************  *  * * * ***   *** *****    ** **

Hdg0328S      AGTCGCAAACAAGTACCGTGAGGGAAAGTTGCAAAGAACTTTGAAGAGAGAGTTCAAAAG      120
Hdg0128S      GATAGCAAACAAGTACCGTGAGGGAAAGTTGAAAAGGACTTTGAAGAGAGAGTTCAACAG      120
Hdg0228S      AGTAGCAAACAAGTACCGTGAGGGAAAGTTGCAAAGAACTTTGAAGAGAGAGGTCAAAAG      120
                * ************************** **** *************** **** **

Hdg0328S      GACGTGAAACCGTTTGCAGGTAAACAGATGGACCCACGAAGTCTTGTTGAGTAGAAATTC      180
Hdg0128S      GACGTGAAACCGTTTGTAGGTAAACAGATGGACCCACGAAGTCTTGTTGAGTAGAAATTC      180
Hdg0228S      GACGTGAAACCGTTTGCAGGTAAACAGATGGACCCACGAAGTCTTGTTGAGTAGAAATTC      180
              **************** ******************************************

Hdg0328S      AATTTTTGATTGTGGTCGCTACTTTTTGAAGGATTGCATTGTCAAAAATTCAAATGTGGT      240
Hdg0128S      AATTTTTGATTGTGGTCGCTACTTTTTGAAGAATTGCATTGTCAAAGATTCAAATGTGGT      240
Hdg0228S      AATTTTTGATTGTGGTCGCTACTTTTTGAAGGATTGCATTGTCAAAAATTCAAATGTGGT      240
              ******************************** ************** *************

Hdg0328S      TGAACGCATGAGAAATGCATTTTTCTACTCATGAAAGAGCTCTGACTGCTTGGAAATAAG      300
Hdg0128S      TGAACGCATAAGAAATGCATTTTTCTACTCATGAAAGAGCTCTGACTGCTTGGAAATAAG      300
Hdg0228S      TGAACGCATGAGAAATGCATTTTTCTACTCATGAAAGAGCTCTGACTGCTTGGAAATAAG      300
              ********* ************************************************

Hdg0328S      TACCTCATTAAAACAATGTATTATCACTATCTTCGGAGGAGATTTTATATTGGAGTAAAC      360
Hdg0128S      TACCTCATTAAAACAATGTATTATCACTATCTTCGGAGGAGATTTTATATTGGAGTAAAC      360
Hdg0228S      TACCTCATTAAAACAATGTATTATCACTATCTTCGGAGGAGATTTTATATTGGAGTAAAC      360
              ************************************************************

Hdg0328S      TTAGTTTCGTTGCAGGTGAAATCGTTGCAATTCGCGGCGTTTCAGGTCATGTTTGTTTTT      420
Hdg0128S      CTAGTTTCGTTGCAGGTGAAATCGTTGCAATTCGCGGCGTTTCAGGTCATGTTTGTTTTT      420
Hdg0228S      TTAGTTTCGTTGCAGGTGAAATCGTTGCAATTCGCGGCGTTTCAGGTCATGTTTGTTTTT      420
               ************************************************************

Hdg0328S      CAGGGTGACAAATTAAGAAGTGTGATATATTTGTTACTTCGGTGATGAATTATTGTATCT      480
Hdg0128S      CAGGGTGACAAATTAGGAAGTGTGATATATTTGTTACTTCGGTGATGAATTATTGTATCT      480
Hdg0228S      CAGGGTGACAAATTAAGAAGTGTGATATATTTGTTACTTCGGTGATGAATTATTGTATCT      480
              *************** ******************************************

Hdg0328S      TTTTGAATTGTTGTCATGATAAATAAATGAGATGATCCAGTAGTAAGTAGGTCGTTTATC      540
Hdg0128S      TTTTGAATTGTTGCCATGATAAATAAATGAGGTGATCCAGTTTA-AGTAGGTCGGTTATC      539
Hdg0228S      TTTTGAATTGTTGCCATGATAAATAAATGAGATGATCCAGAGTA-AGTAGGTCGGTTATC      539
              ************* ***************** ********     ********* *****

Hdg0328S      CATCTGACCCGTCTTGAAACACGGACCAA     569
Hdg0128S      GTTCTCTCCCGTCTTGAAACACGGACCAA     568
Hdg0228S      CATCTGACCCGTCTTGAAACACGGACCAA     568
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**Figure 1.** Alignment of 28S rDNA (5'-3 ') DNA sequences of *H. globosa* specimens. The "*" sign indicates that the nucleotides at the same position are common; the "-" sign in the series refers to the space.

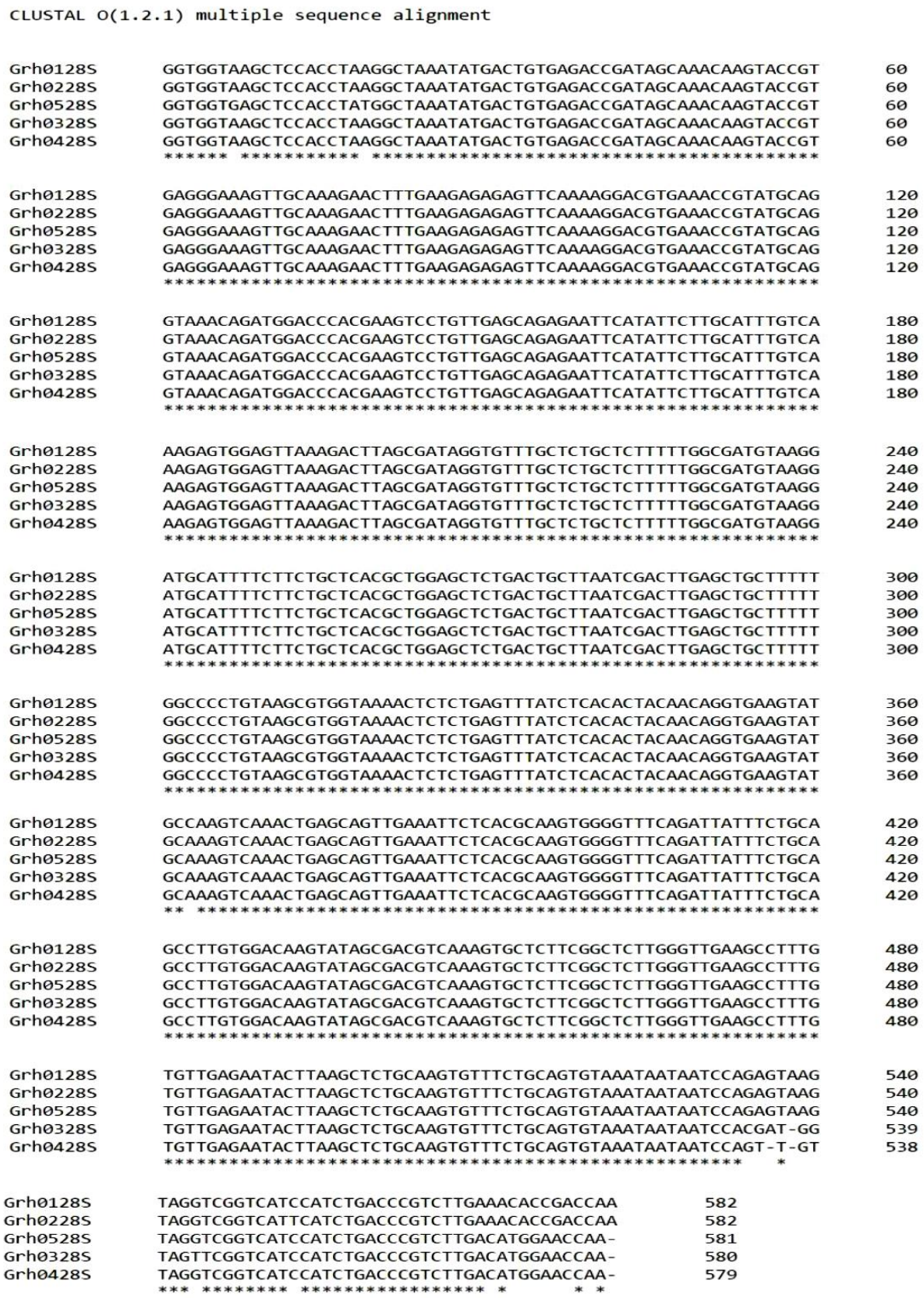


**Figure 2.** Alignment of 28S rDNA (5'-3 ') DNA sequences of *G. helvetica* specimens. The "*" sign indicates that the nucleotides at the same position are common; the "-" sign in the series refers to the space.

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CLUSTAL O(1.2.1) multiple sequence alignment

Hdd0328S        -GAGGGGGAGCTCACCTATGGCTATATATTACTGTGAGACCGATAGCAAACAAGTACCGT       59
Hdd0428S        --GGGTGCTAGCTCACCTAAGGCTAATATTACTGTGAGACCGATAGCAAACAAGTACCGT       58
Hdd0528S        --GGGTGCTAGCTCACCTAAGGCTAATATTACTGTGAGACCGATAGCAAACAAGTACCGT       58
Hdd0128S        GGTGGTAAGCTCCACTTAGGGCTAAATATTACTGTGAGACCGATAGCAAACAAGTACCGT       60
Hdd0228S        GGGGGGTAGCTCCACCTAAGGCTAAATATTACTGTGAGACCGATAGCAAACAAGTACCGT       60
                   **                *    **********************************

Hdd0328S        GAGGGAAAGTTGAAAAGGACTTTGAAGAGAGAGTTCAATAGGACGTGAAACCGTTTGCAG       119
Hdd0428S        GAGGGAAAGTTGAAAAGGACTTTGAAGAGAGAGTTCAATAGGACGTGAAACCGTTTGCAG       118
Hdd0528S        GAGGGAAAGTTGAAAAGGACTTTGAAGAGAGAGTTCAATAGGACGTGAAACCGTTTGCAG       118
Hdd0128S        GAGGGAAAGTTGAAAAGGACTTTGAAGAGAGAGTTCAATAGGACGTGAAACCGTTTGCAG       120
Hdd0228S        GAGGGAAAGTTGAAAAGGACTTTGAAGAGAGAGTTCAATAGGACGTGAAACCGTTTGCAG       120
                ************************************************************

Hdd0328S        GTAAACAGATGGACCCACGAAGTGCAGCTGAAAGGGCAGCAGAGATCTCGGTCTCAGTCT       179
Hdd0428S        GTAAACAGATGGACCCACGAAGTGCAACTGTGTGGGCAGCAGAGATCTCGGTCTCAGTCT       178
Hdd0528S        GTAAACAGATGGACCCACGAAGTGCAACTGTGTGGGCAGCAGAGATCTCGGTCTCAGTCT       178
Hdd0128S        GTAAACAGATGGACCCACGAAGTGCAACTGTGTGGGCAGCAGAGATCTCGGTCTCAGTCT       180
Hdd0228S        GTAAACAGATGGACCCACGAAGTGCAACTGTGTGGGCAGCAGAGATCTCGGTCTCAGTCT       180
                ************************* ***   *************************

Hdd0328S        GCTCCGTTGCAACTCTGACTGTTTAGGGACACTTTGATGTAGTCCTTTTCACAAAAAGGG       239
Hdd0428S        GCTCCGTTGCAACTCTGACTGTTTAGGGACACTTTGATGTAGTCCTTTTCACAAAAAGGG       238
Hdd0528S        GCTCCGTTGCAACTCTGACTGTTTAGGGACACTTTGATGTAGTCCTTTTCACAAAAAGGG       238
Hdd0128S        GCTCCGTTGCAACTCTGACTGTTTAGGGACACTTTGATGTAGTCCTTTTCACAAAAAGGG       240
Hdd0228S        GCTCCGTTGCAACTCTGACTGTTTAGGGACACTTTGATGTAGTCCTTTTCACAAAAAGGG       240
                ************************************************************

Hdd0328S        CAAGACGTCTTTAGCAGGAGTGATCCGCAAGGTTCGCCTACTAGAGAGCATCTTCATCAA       299
Hdd0428S        CAAGACGTCTTTAGCAGGAGTGATCCGCAAGGTTCGCCTACTAGAGAGCATCTTCATCAA       298
Hdd0528S        CAAGACGTCTTTAGCAGGAGTGATCCGCAAGGTTCGCCTACTAGAGAGCATCTTCATCAA       298
Hdd0128S        CAAGACGTCTTTAGCAGGAGTGATCCGCAAGGTTCGCCTACTAGAGAGCATCTTCATCAA       300
Hdd0228S        CAAGACGTCTTTAGCAGGAGTGATCCGCAAGGTTCGCCTACTAGAGAGCATCTTCATCAA       300
                ************************************************************

Hdd0328S        GTGATAGTCATGTA-ACAGATCTAATCGAGAATATCATATTGGGTACCAGTACTGAATGT       358
Hdd0428S        GTGATAGTCATGTAAACAGATCTAATCGAGAATATCATATTGGGTACCAGTACTGAATGT       358
Hdd0528S        GTGATAGTCATGTAAACAGATCTAATCGAGAATATCATATTGGGTACCAGTACTGAATGT       358
Hdd0128S        GTGATAGTCATGTAAACAGATCTAATCGAGAATATCATATTGGGTACCAGTACTGAATGT       360
Hdd0228S        GTGATAGTCATGTAAACAGATCTAATCGAGAATATCATATTGGGTACCAGTACTGAATGT       360
                ************** ******************************************

Hdd0328S        TGACTCTGCATTAGGTGCGTTCCCGCCTGTACTCTTCGGATACAGTGGAAGTACTTACAT       418
Hdd0428S        TGACTCTGCATTAGGTGCGTTCCCGCCTGTACTCTTCGGATACAGTGGAAGTACTTACAT       418
Hdd0528S        TGACTCTGCATTAGGTGCGTTCCCGCCTGTACTCTTCGGATACAGTGGAAGTACTTACAT       418
Hdd0128S        TGACTCTGCATTAAGCCCGTTCCCGCCTGTACTCTTCGGATACAGTGGAAGTACTTACAT       420
Hdd0228S        TGACTCTGCATTAGGTGCGTTCCCGCCTGTACTCTTCGGATACAGTGGAAGTACTTACAT       420
                ************* *  ******************************************

Hdd0328S        GTTCAGGGTACACCAGTACTGCTGGTCACAAATCACATCCCCTGAGCATTTCTGCAATTA       478
Hdd0428S        GTTCAGGGTACACCAGTACTGCTGGCCCCCAATTCCCTCCCATGTTCATTTTTCAAATCA       478
Hdd0528S        GTTCAGGGTACACCAGTACTGTGG-CCCCCAATTCCCTCCCATGTTCATTTTTCAAATCA       477
Hdd0128S        GTTCAGGGTACACCAGTACTGTTTGTCACCAAATCACTCCTGTGATCATATCTCGGATCA       480
Hdd0228S        GTTCAGGGTACACCAGTACTGTTTGTCACCAAATCACTCCCCTCATGAAATCTCGCATCA       480
                ********************     * * **     ***  *    *  * *   ** *

Hdd0328S        AATTAGTAGGACGGTAACCCTTCTAACCCGTCTTGAACCCAGGAACAAAAAAAAGTAAGT       538
Hdd0428S        AATTATAAGGCCGGCCTTCCTTCTAACCCGCCTGGAAAACGAACAACTAAAAAAGTAAGT       538
Hdd0528S        AATTATAAGGCCGGCCTTCCTTCTAACCCGCCTGGAAAACGAACAACTAAAAAAGTAAGT       537
Hdd0128S        GAGTAGTAGGTCGGTCATCCATCTGACCCGTCTTGAAAAAAAAGACCAATAGTGAGTA---       537
Hdd0228S        GAGTAGTGAGTACGTCATCCCTCTGAGCCGTCCTGCAAACAGACCAAAAAAAAAGTAAGT       540
                 * **    *   *    ** *** * *** *  * *              *   ****

Hdd0328S        AGGTCGGTCAC-CATCTGACCCGTCTTGAACACGGACCAA- 577
Hdd0428S        AGGTCGGTCATCCATCTGACCCGTCTTGAACACGGACCAA- 578
Hdd0528S        AGGTCGGTCATCCATCTGACCCGTCTTGAACACGGACCAA- 577
Hdd0128S        -----CGTCATCCCTCTGAGCCGTCCTGCAAACAGGAACAA 573
Hdd0228S        AGGTCGGTCATCCATCTGACCCGTCTTGAACACAAGACCAA 581
                     ****  * ***** ***** ** * **      *
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**Figure 3.** Alignment of 28S rDNA (5'-3 ') DNA sequences of *H. despiciens* samples. The "*" sign indicates that the nucleotides at the same position are common; the "-" sign in the series refers to the space.

### 3.2 Phylogenetic Analyzes

The dendrogram of the 28S rDNA gene sequences of the studied water mites species was constructed using the MEGA6 packet program (Figure 4). According to this dendogram, the species studied were separated by large genetic distances from other species and formed species clusters within themselves (P <0.5). *H. globosa* and *G. helvetica* species clustered within themselves to support morphological data, and according to the dendrogram, these two species were genetically close together. *H. despiciens* species differed and formed two different clusters. From these, the first cluster formed a cluster of *G. helvetica* and the other cluster in itself. In addition, individual samples belonging to the species *H. dispar* and *H. globosa* in the same family as *G. helvetica* were clustered independently in themselves. The findings have shown that existing samples can be separated from one another by molecular marking.

## 4. CONCLUSION

Nowadays, molecular-based systematic studies are needed in cases where morphological data are insufficient in defining the controversial species (Smith et al., 2006, Jinbo et al., 2011). Therefore, molecular based classification method is also used to help the classical systematic morphological basis (Hebert et al., 2003).

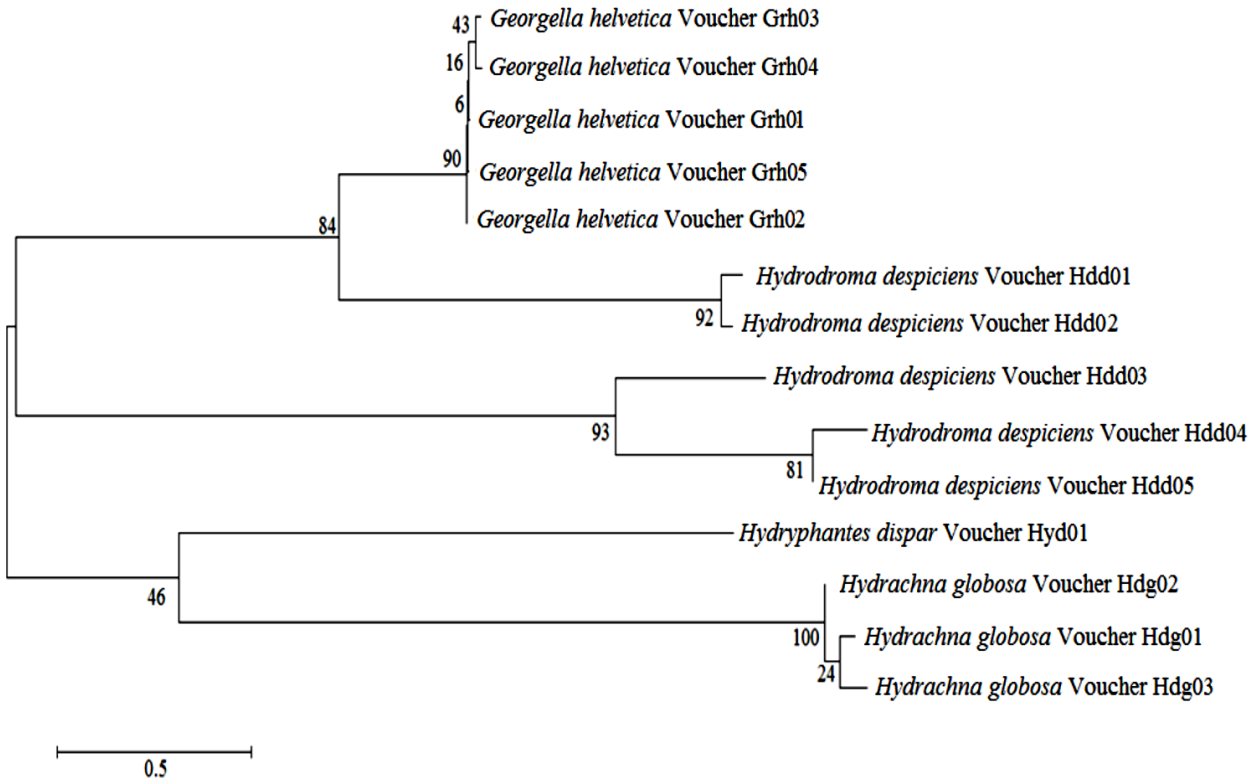


**Figure 4.** Phylogenetic relationships based on *H. globosa*, *H. dispar*, *G. helvetica* and *H. despiciens* rDNA sequences according to Neighbor-Joining method. The numbers at the branching points are the probability of clustering generated after 100 repetitive bootstrap tests.

In the DNA isolation of individual and multiple water mites, the CTAB method was first applied (Schäffer et al. 2008). To amplify the COI gene region of genomic DNA isolated from each sample, a universal COI primer pair and PCR reaction mixture were prepared. However PCR amplification of the desired PCR products could not be realized. It has been predicted that this problem may result from DNA isolation, universally accepted primers or PCR conditions. For this purpose, different DNA isolation methods were used, existing primer pairs were renewed, different primer pairs were used, PCR reaction content and cycle optimizations (annealing temperature) were tried. In addition to the CTAB method, genomic DNA isolation from individual water mites was performed with the QIAamp® DNA Mini Kit (Qiagen) and the GeneJET Genomic DNA Purification Kit (ThermoScientific, USA). In order to solve the problem which is thought to be caused by primers, Folmer et al. (1994) suggested that the degenerate primers were used. Furthermore, the binding temperature of the primers during the PCR cycle was regulated relative to the degenerate primers. After these changes, only amplification of *G. helvetica* species was successfully performed from the studied water mite species (*H. procesisifera, H. globosa, G. helvetica, H. despiciens, L. fulgida, P. contraversiosa, A. affinis, and A. maculator*). Whereas Dorda and Valdecasas (2002), Ernsting et al. (2006), Witt et al. (2006), Dabert et al. (2010), Martin et al. (2010), Asadi et al. (2012), Pešić et al. (2012), Young et al. (2012), Deiner et al. (2013) successfully performed molecular-based classification and phylogenetic analysis using the COI gene region. In our results, the fact that 28S rDNA gene fragments from the isolated DNAs were successfully amplified is indicative of no problem in DNA isolation. However, for unknown reasons, COI primers were not studied in PCR and the desired results could not be achieved.

It was recommended that a second gene region belonging to gDNA should be studied in addition to mtDNA, since the classification method based on a single gene region may occasionally cause problems (Wiemers and Fiedler 2007). The proposed gene regions such as Efα1, ITS1, ITS2 are not used in molecular based systematic studies since they do not have sufficient information for differentiation of species (Baxter and Barker 1999, Liyou et al. 1999). Similar results were obtained from the rDNA gene region with mtDNA in different animal groups (Martin et al. 2010, Lv et al. 2014). This study was continued with 28S rDNA due to glitch in the COI gene region. In the present study, the 28S rDNA gene region was successfully amplified from H. globosa, H. dispar, G. helvetica and H. despiciens species. The base sequences of the species were analyzed and a NJ-based phylogenetic tree showing the phylogenetic relationship between the species and the species. According to the phylogenetic tree generated according to the 28S rDNA gene region, H. dispar and H. globosa were found genetically close. However, H. dispar, G. helvetica is in the same family and is more morphologically similar to each other (Uysal 2005). In addition, two different clusters were seen in H. despiciens species. Similar results were recorded in terrestrial ticks as well as in Unionicola genus (Edwards et al., 1999) collected from the same host (Söller et al., 2001).

Variations based on the 28S rDNA gene sequence between the *H. globosa, H. dispar, G. helvetica* and *H. despiciens* species used in the present study are largely similar to other studies on different water-lines (Ernsting et al., 2006; Edwards et al., 2010; Pilgrim et al., 2011; Stalstedt et al., 2013). As a result of the obtained molecular findings, it has been seen that interspecies and intraspecific discrimination can be successfully performed with 28S rDNA.

As a result; DNA isolations were successfully accomplished from a few

milligrams of watery waters and the DNA isolation protocol from the water column was optimized in the laboratory.Reproduction of this gene region was inadequate, presumably because the primers used for the COI gene did not work in the PCR reaction. However, 28S ribosomal DNA regions have been successfully propagated, sequenced, and inter-sequence phylogenetic relationships established. After this study, the molecular system of morphologically troubled water mites will be made.

As the advantages and disadvantages of DNA Barcoding become clear, it is clear that this DNA sequence will be integrated with morphological and ecological studies to maximize the efficiency of species identification.

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