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RESEARCH ARTICLE

Genetic Structure of *Anopheles sacharovi* (Diptera: Culicidae) Populations from Türkiye

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Abstract

Objective: The *Anopheles maculupennis* complex is known to be a vector of malaria in Türkiye. *Anopheles sacharovi* is a member of the *Anopheles maculipennis* complex and is a major vector of malaria in Europe and the Middle East capable of transmitting both *Plasmodium vivax* and *P. falciparum*. The genetic diversity and population structure of the six populations of *Anopheles sacharovi* from Türkiye are studied here using the random amplified polymorphic DNA (RAPD) marker.

Materials and Methods: *Anopheles sacharovi* samples were collected from Muğla, Aydın, İzmir, Çanakkale, Balıkesir, and Denizli. Total DNA was extracted using the Lifton method, and a total of 120 individuals were screened using six RAPD primers.

Results: A total of 300 loci were observed in the six primers. Very close genetic diversity was observed in the studied populations. The number of alleles (n_a) observed for all populations was 1.55 ± 0.49, and the mean number of effective alleles (n_e) was 1.23 ± 0.31. The ratio of polymorphic loci for all populations ranged between 50.33%-60.33%, and Nei's genetic diversity (h) ranged between 0.1253-0.1576. The gene flow level (N_m) was 2.08, and the genetic differentiation value (G_{st}) was 0.19. The conclusions from the unweighted pair group arithmetic mean analysis (UPGMA) was inclined to be homogeneous on the whole, with the İzmir population being clearly separated from the rest.

Conclusion: Regional environmental conditions such as human-mediated transport, agricultural implementations, and discrepancies in vector control strategies might be considered agents in forming the genetic structure of this species in İzmir. Understanding the gene flow rates and phylogenetic relationships between vector species are very important for applying sustainable and effective pest management. This study ensures helpful knowledge for better understanding the population genetic structure of *An. sacharovi* populations in Türkiye.

Keywords: *Anopheles sacharovi*, RAPD, Population genetics, Genetic diversity

Introduction

Mosquitoes (Diptera: Culicidae) are organisms with serious importance for human health due to being vectors of many diseases such as yellow fever, dengue fever, West Nile virus, encephalitis, lymphatic filariasis, and most importantly malaria (Çağlar *et al*., 2008; Becker *et al*., 2010). The existence of 50 species belonging to eight genera has been reported in Türkiye. Under the genus *Anopheles* (*An.*), *An. algeriensis*, *An. clavier*, *An. hyrcanus*, *An. maculipennis*, *An. marteri*, *An. melanoon*, *An. pulcherrimus*, *An. messeae*, *An. plumbeus*, *An. superpictus*, and *An. sacharovi* species constitute the *Anopheles* fauna of Türkiye. Among these, *An. superpictus* and *An. sacharovi* are the most significant malaria vector species (Ramsdale *et al*., 2001; Çağlar *et al*., 2008). *Anopheles sacharovi*, a member of the *Anopheles maculipennis* complex, is one of the main malaria vectors in Türkiye and can be found in Europe and the Middle East (Sedaghat *et al*., 2003). Even though malaria cases have significantly been reduced in Türkiye, because of the irregular migrations through malaria-endemic countries such as Afghanistan, Pakistan, Iraq, and Syria, malaria still continues to be an endemic health threat. To control mosquitoes in Türkiye, strategies are usually designed to target larvae and adults (Yavaşoğlu *et al*., 2019). The adults of this species can be distinguished from other members of the complex by the lighter color of their mesonotum and wing characteristics. Larvae can be found in shallow sun-exposed fresh or saline water bodies. They are more tolerant to saline water than other members of the complex and also the most thermophilic (Becker *et al*., 2010).

The battle against mosquitoes goes back to the beginning of the battle against the diseases they cause. Since the early 1950s, humanity has used chemical methods against agricultural and medical pests to control arthropods (Taskin *et al*., 2016). Chemical methods of control grew with the discovery of DDT, and malaria cases during World War II dramatically decreased. After the discovery of DDT, the development of organochlorine insecticides and other groups of insecticides such as organophosphates, carbamates, and pyrethroids accelerated the processes of chemical control (Akıner, 2009). Since the 1980s after the introduction of biological methods of control, integrated control treatments have been carried out with the use of mechanical, chemical, biological, and sterilized insect techniques, and these have been widely applied throughout Türkiye and around the world (Alten & Çağlar, 1998).

Genetic markers based on nucleic acids can be used to study species characterization, genetic diversity, phylogenetic relationships, and insect identification (Rita *et al*., 2014). Random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) and other nonspecific amplification methods are quite useful for analyzing genetic variation within species due to the help they provide in quickly obtaining vast genetic information (Posso *et al*., 2003). RAPD-PCR is a method that allows one to amplify the template genomic DNA using random primers. The RAPD method has some advantages over gene mapping and gene tagging such as being inexpensive and easy to use, able to use universal primer sets and quickly scan genomes, able to produce or commercially acquire unlimited synthetic oligonucleotides, able to isolate the cloned DNA probes, able to eliminate the need to prepare hybridization filters. RAPD-PCR is also suitable for automation and highly polymorphic and only requires a small amount of DNA (Kelly, 1995).

Genetic diversity studies have been performed on *Anopheles* species using RAPD-PCR, internal transcribed spacer 2 (ITS2) markers, microsatellite loci, mitochondrial DNA, single nucleotide polymorphism genotyping, and isoenzyme analysis (Pinedo-Cancino *et al*., 2006). Many studies on *Anopheles* species are found to have been conducted with RAPD around the world in order to better understand the population genetic structure of this species. These species involve *An. gambiae*, *An. arabiensis*, *An. minimus*, *An. aconitus*, *An. pampanai*, *An. varuna*, *An. nuneztovari*, *An. darlingi*, *An. marajoara*, *An. triannulatus*, *An. halophylus*, *An. campestris*, *An. peditaeniatus*, *An. jamesii*, *An. maculatus*, *An. philippinensis*, *An. annularis*, *An. nivipes*, *An. sinensis*, *An. jeyporiensis*, *An. Vagus*, and *An. culucifacies* (Wilkerson *et al*., 1993; Favia *et al*., 1994; Dimopoulos *et al*., 1996; Kengne *et al*., 2001; Elisa Posso *et al*., 2003, 2006; Pinedo-Cancino *et al*., 2006; Silva-Do-Nascimento *et al*., 2006; Gonzalez *et al*., 2007; Rita *et al*., 2014; Tyagi *et al*., 2015). However, no study is found in

Figure 1. Sample locations of *An. sacharovi* from Turkey.

Table 1. The sequences of primers used for RAPD-PCR.

Number	Primer	Length of oligonucleotide	Nucleotide sequence	GC content %
1	OPL-02	10	TGGGCGTCAA	60
2	OPB-10	10	CTGCTGGGAC	70
3	OPE-03	10	CCAGATGCAC	60
4	OPC-07	10	GTCCCGACGA	70
5	OPN-08	10	ACCTCAGCTC	60
6	OPD-01	10	ACCGCGAAGG	70

the literature to have been conducted on the *An. sacharovi* species.

This study investigates the intra- and inter-population genetic diversity of natural *An. sacharovi* populations sampled from diverse geographical regions of Türkiye, which covers a large portion of the Eastern Mediterranean area.

Materials and Methods

Sample Collection

The study has collected *An. sacharovi* samples from the provinces of Muğla, Aydın, İzmir, Çanakkale, Balıkesir, and Denizli as the selected study area between 2018-2019 within the scope of the Scientific and Technological Research Council of Türkiye (TÜBİTAK) 1001 project. Larval and pupal collections were performed in six provinces in Western Türkiye, with the *Anopheles* specimens' species being identified using standard morphological keys (Glick, 1992; Becker *et al*., 2010). The *An. sacharovi* samples were also verified using the ITS2 sequencing on eight individuals for each province (a total of 48 samples). Analysis of the rDNA ITS2 region was amplified and directly sequenced following Djadid *et al*.'s (2007) method. The provinces from which all the samples were collected are presented in Figure1.

DNA Isolation and Amplification of the RAPD Loci

DNA isolation of the collected samples was carried out by modifying the Lifton method as used by Bender *et al*. (1983). This study uses the 10-base RAPD oligonucleotide primer

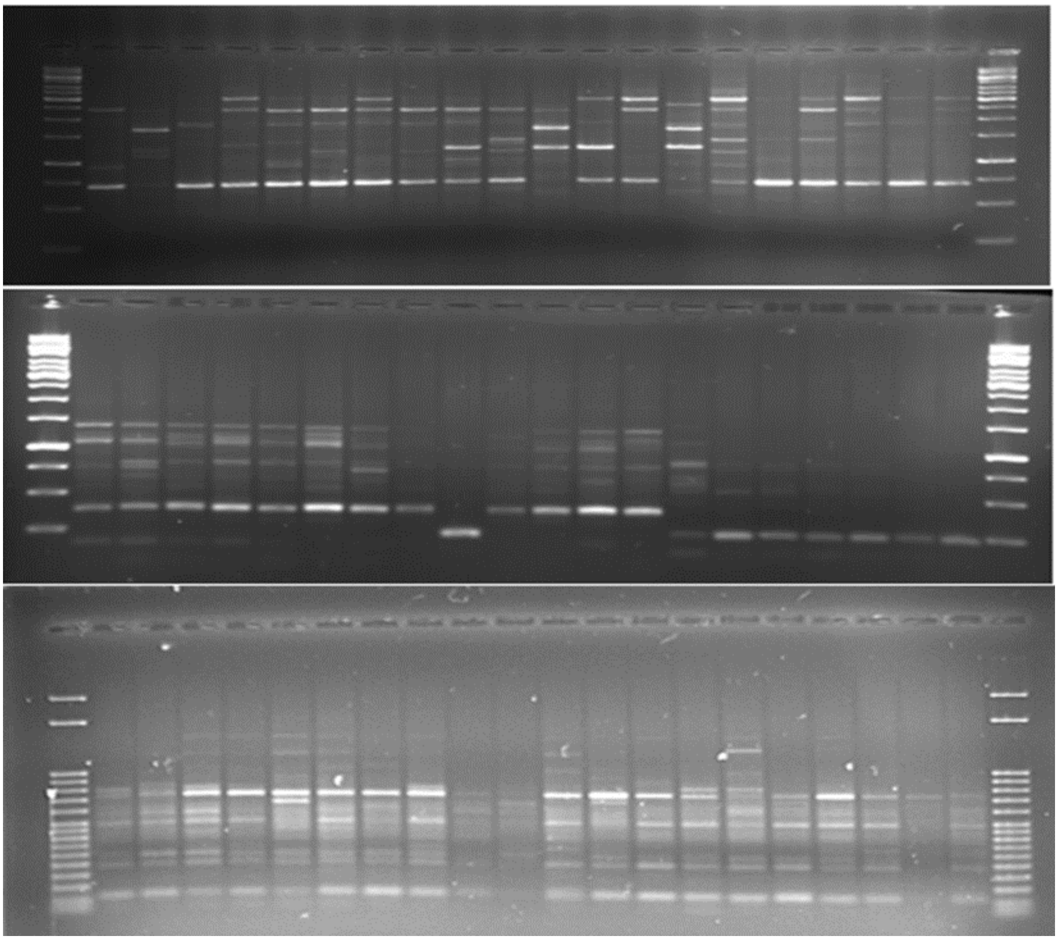

Figure 2. RAPD band patterns of Balıkesir, Aydın and Denizli samples with OPN-08, OPD-01 and OPC-07 primers, respectively.

Table 2. Genetic diversity parameters of *An. sacharovi*. Sample size (N), Observed Number of Alleles (n_a), Effective Number of Alleles (n_e), Gene Diversity (h), Percentage of Polymorphic Band ($P\%$).

Populations	N	n_a	n_e	h	$P\%$
Muğla	17	1.5033±0.5008	1.1991±0.2995	0.1253	50.33 %
Denizli	17	1.5467±0.4986	1.2581±0.3345	0.1576	54.67 %
Aydın	19	1.5333±0.4997	1.2447±0.3159	0.1525	53.33 %
İzmir	17	1.5100±0.5007	1.2344±0.3188	0.1450	51.00 %
Balıkesir	19	1.5933±0.4920	1.2249±0.2941	0.1444	59.33 %
Çanakkale	17	1.6033±0.4900	1.2100±0.2875	0.1361	60.33 %
Average	**17.66**	**1.55±0.50**	**1.23±0.31**	**0.14**	**54.83 %**

from OPERON Technologies (Alameda, California). These primers are OPL-02, OPB-10, OPE-03, OPC-07, OPN-08, and OPD-01. All of these primers are composed of random sequences and have a G + C content between 60%-70%. The PCR conditions used in this study were applied to the studied species as per Rita *et al*. (2014). Accordingly, the optimum PCR mix involve the 8 ng template DNA for a 25 µl reaction volume, 3 mM MgCl2, 11.5 pmol primer, 200 µM dNTP (for each dNTP), 1unit Taq DNA Polymerase, 2.5 µl PCR buffer (10x), and 0.6 mg/ml BSA. The optimum number of cycles for the PCR is 40, and after four minutes of pre-denaturing at 94^{o}C, each cycle was run for 1 minute at 94^{o}C (denaturation), 2 minutes at 37^{o}C (binding), and 2 minutes at 72^{o}C (extension). The PCR products were clarified by post-cycle stretching at 72^{o}C for 10 minutes. The products were run at a constant current of 70 V in a 1% agarose gel and viewed on a Vilber Lourmat imaging device.

Data Analysis

The RAPD band patterns of the populations were visualized with the help of the Vilber Lourmat gel imaging device, and these photographs were read in a computer environment. When evaluating the segregation data at this single locus, the presence of the band is measured as “1”, its absence as “0”, and no detection of the PCR product as “.”. The analyses of the data file created with these values were performed using computer-based programs such as POPGENE Version 1.32 (Yeh *et al*., 1999) and GenAlex (Peakall & Smouse, 2006).

Results

Genetic Structures and Population Genetic Diversity

As a result of the analysis of the six primers (i.e., OPL-02, OPB-10, OPE-03, OPC-07, OPN-08, and OPD-01) applied to a total of 120 individuals, a total of 300 loci were detected, all of which were polymorphic. The highest number of loci was obtained from the OPL-02 primer with 74, and the least number of loci were obtained from the OPE-03 primer with 35. The overall mean polymorphism rate was 40.93% (Table 1), with Figure 2 presenting the RAPD band patterns of the individuals belonging to the Balıkesir, Aydın, and Denizli populations.

The mean observed allele number (n_a), which is one of the components of genetic diversity, was found to be 1.55 ± 0.50 for all populations, with the mean effective allele number (n_e) being 1.23 ± 0.31. In the studied populations, Muğla was the population with the lowest n_a value (n_a = 1.5033 ± 0.5008), while the population with the highest n_a value was Çanakkale (n_a = 1.6033 ± 0.4900. In the studied populations, Muğla again was the population with the lowest n_e value (n_e = 1.1991 ± 0.2995), while the population with the highest n_e value was Aydın (n_e = 1.2447 ± 0.3159). The mean rate of polymorphic loci in the studied populations was found to be 54.83%, and the population with the lowest polymorphic locus ratio was Muğla (50.33%). Meanwhile the Çanakkale population had the highest rate of polymorphic loci (60.33%). In this study, the average Nei’s genetic diversity value (h) was found to be 0.14, with the Denizli population having the highest (h = 0.1576), and the Muğla population having the lowest (h = 0.1253; Table 2).

According to the G-statistic, the total genetic diversity (H_T) was found to be 0.18 ± 0.02 at 300 loci. Of this, 0.14 is intra-population genetic diversity (H_S), and 0.04 is inter-population genetic diversity (D_{st}). Also, the mean genetic differentiation coefficient (G_{st}) was found to be 0.19. The gene flow level (N_m), obtained as the average of the 300 loci in this study, was found to be 2.08.

The level of genetic variation between populations was determined by calculating Nei’s (1978) standard genetic distance (*DN*) values for the studied population pairs. *DN* values based on the 300 loci are given in Table 2. When considering the values, although all the populations had low genetic differentiation values, the lowest was found between Aydın and Muğla (*DN* = 0.0307) and the highest between İzmir and Çanakkale (*DN* = 0.0579; Table 3).

The visual expression of genetic distances between populations is given in the unweighted pair-group method with arithmetic average (UPGMA) dendrogram

Table 3. Genetic distance values between studied populations (Nei 1978).

Populations	Muğla	Denizli	Aydın	İzmir	Balıkesir	Çanakkale
Muğla	*****					
Denizli	0.0507	*****				
Aydın	0.0307	0.0569	*****			
İzmir	0.0539	0.0450	0.0568	*****		
Balıkesir	0.0400	0.0358	0.0432	0.0502	*****	
Çanakkale	0.0383	0.0410	0.0321	0.0579	0.0357	*****

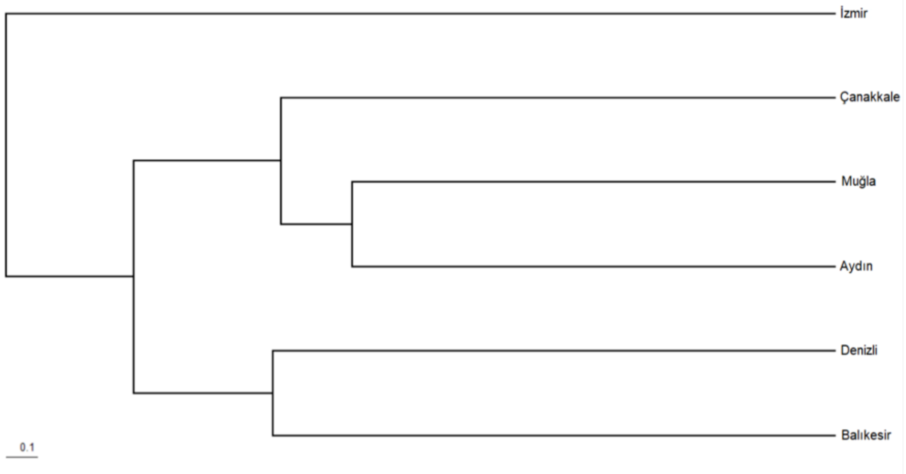

Figure 3. Dendrogram based on Nei 1972 genetic distance matrix.

and principal coordinates (PCoA) diagram (Figs. 3 and 4, respectively). When constructing the dendrogram based on genetic distances, the İzmir population was most clearly differentiated, while the Çanakkale, Muğla, Aydın, Denizli, and Balıkesir populations were clustered together. Consistent with the results of the UPGMA, the İzmir population was also separated from all other populations in the PCoA analysis. According to the results of the analysis of molecular variance (AMOVA), 83% of the genetic variation calculated for the 300 loci was observed to originate from within the population and 17% to originate from among the populations.

Discussion

When looking at mosquitoes as the carriers of various diseases that have great effects on human beings such as malaria in particular, the *Anopheles* genus has been the most interesting group in ecological, physiological, systematic, biochemical, genetic, and other fields. The

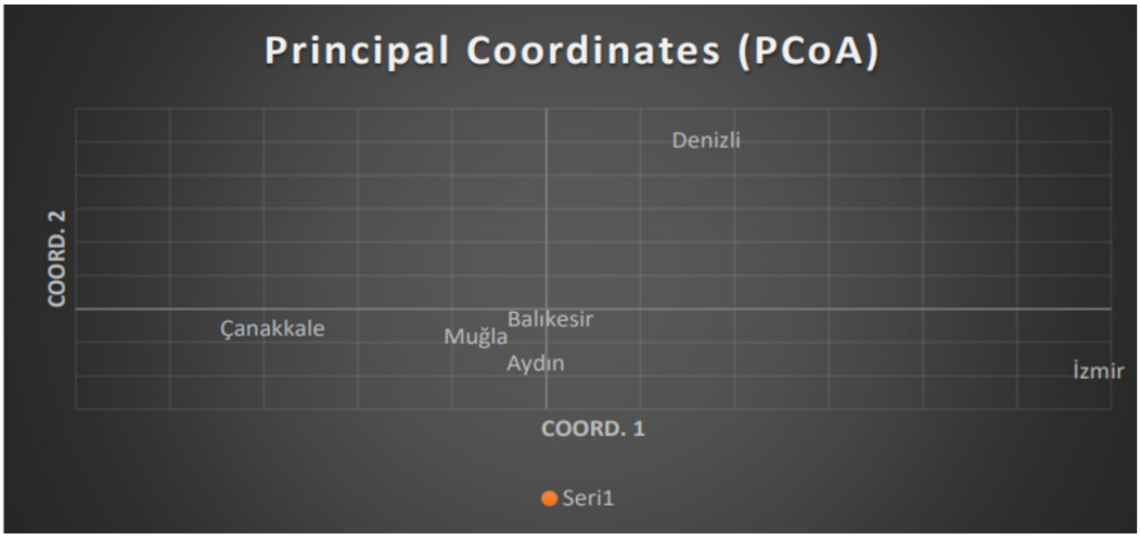

Figure 4. Principal coordinates (PCoA) diagram for studied populations.

Anopheles genus has 485 species, 80 of which are vectors of malaria, which causes the death of many people in various parts of the world, and is one of the species that people control the most (Harbach & Kitching, 2015). When considering the studies that have been conducted on the *Anopheles* species, these species have been shown to not only be vectors for malaria but also vectors for arboviruses such as the Rift Valley fever virus, the West Nile virus, and the O'nyong'nyong virus (Özbilgin *et al*., 2011). Although malaria has reached almost zero cases as a result of intense controls, it has remained the most important vectorial disease in Türkiye for a long time. Therefore, *Anopheles* species have been the main target organisms in control studies (Dogan *et al*., 2010; Özbilgin *et al*., 2011). Genetic methods for assessing the structure and differentiation of *An. sacharovi* populations have been used to provide information on population differentiation, diversity, and gene flow. Understanding these properties enables better adaptation of vector control methods. This study has determined the genetic diversity, population genetic structure, and inter-population relationships of *An. sacharovi* as an important vector pest in the Aegean regions of Türkiye using RAPD markers.

The number of studies aimed at understanding the genetic structure of *An. sacharovi* populations as an important vector species are very limited. No study other than the one conducted with the help of microsatellite markers is found in Türkiye (unpublished). The current study has determined the genetic diversity, population genetic structure, and inter-population relations of *An. sacharovi* in the western populations of Türkiye. In all the samples, which compared six provinces, genetic similarities were observed in terms of both the number of alleles as well as in the allele frequencies. The study used RAPD markers to elucidate the genetic structures of the *An. sacharovi* populations.

A total of 300 loci were identified based on the six primers scanned, all of which were determined to be polymorphic loci as a result of the analysis. The total genetic diversity (H_T) was found to be 0.18 ± 0.02 for the 300 detected RAPD loci. While 0.14 ± 0.01 of the genetic diversity constituting the total genetic diversity is due to intra-population genetic diversity (H_S), 0.04 is due to inter-population genetic diversity (D_{st}). The contribution of intra-population genetic diversity in total genetic diversity was calculated higher than the inter-population genetic diversity (77.8% vs. 22.2%). This situation might be a consequence of connected populations. Using the D_{st} and H_T values, the genetic differentiation coefficient (G_{st}) for all populations was calculated as 0.19. When considering the polymorphic loci rates for all analyzed populations, the location with the lowest polymorphic loci number is Muğla with 151 polymorphic loci and a polymorphism percentage of 50.33%. The location with the highest number of polymorphic loci is Çanakkale with 181 polymorphic loci and a polymorphism percentage of 60.33%. According to Nei's genetic diversity (h) values, the lowest genetic diversity is in Muğla ($h = 0.13$), while the highest genetic diversity was found in Çanakkale ($h = 0.16$).

Inter-population genetic diversity levels were analyzed for the *An. sacharovi* samples that were collected from the Denizli, Aydın, İzmir, Balıkesir, Çanakkale, and Muğla locations in the Aegean Region using six different primers. According to the obtained results in consideration of the genetic distances, İzmir is seen to have a different genetic structure than the other five locations. The reason for this may be that the *Anopheles* there were overexposed to environmental stress factors due to İzmir both being a gulf province and having a relatively metropolitan structure. These stress factors could involve insecticides, which are used extensively in the agricultural, tourism, and industrial sectors. This suggests that another factor might affect the change in the genetic pool of the *Anopheles* species from other geographies on the Aegean Sea coasts, one that originates from the gulf.

When examining the RAPD studies in the literature on *Anopheles* species, differences are seen in terms of various values. Posso *et al*. (2003) found a genetic diversity value of 0.34 in the results they obtained from the RAPD markers used to analyze the genetic diversity and structure of 119 samples from three *An. nuneztovari* populations they sampled from Colombia. They stated the gene flow values ($G_{st} = 0.035$, $N_m = 6.8$) to be higher than the genetic differentiation and gene flow values ($G_{st} = 0.08$, $N_m = 2.8$) among Northeastern populations. Posso *et al*. (2006) also reported the heterozygosity ratios of the *An. darlingi*, *An. nuneztoveri*, and *An. marajoara* species to range between 0.28-0.34 according to the results they obtained from RAPD markers. The current study saw the mean heterozygosity value to be 0.14, the genetic differentiation level (G_{st}) to be 0.19, and the gene flow value (N_m) to be 2.08. Pinedo-Cancino *et al*. (2006) investigated the genetic diversity of *An. darlingi*, an important malaria vector, using 5 RAPD primers on 270 samples collected from nine different locations around the city of Iquitos in Peru. They stated the intra-population genetic diversity values in *An. darlingi* to vary between 0.27-0.32, and their average genetic differentiation value to be 0.0017. Gonzalez *et al*. (2007) reported the genetic diversity value of the three *An. darlingi* populations they studied to be

0.374, with a low geographic separation present between the eastern and western populations. Rita *et al*. (2014) reported the population of 10 different *Anopheles* species (i.e., *An. campestris*, *An. peditaeniatus*, *An. jamesii*, *An. maculatus*, *An. philippinensis*, *An. annularis*, *An. nivipes*, *An. sinensi*, *An. jeyporiensis*, and *An. vagus*) to have a polymorphism rate of 65% and a genetic diversity value of 0.22. Although the results from the current show parallels to the results obtained in the literature, differences in genetic diversity were also able to be observed between different populations of the same species as well as between different species. This study used six RAPD primers for 120 samples in the *An. sacharovi* species and determined the genetic diversity value within the population to be 0.14 and the genetic differentiation value to be 0.19, which is due to the differences between the species.

The molecular analysis of variance (AMOVA) showed that most of the genetic variation (83% for *An. sacharovi*) was conserved within populations rather than between populations. The AMOVA and UPGMA dendrograms indicated no genetic structure variations to have occurred in the *An. sacharovi* species populations. A mechanism for mixing these mosquito populations from different geographical areas as well as a probable gene flow rate are evident from these observations, despite the high geographical distance present among the study sites. For this reason, the sites in the present study are attached by mosquito habitats, which permits gene flow among these locations. High intra-population genetic variation and migration rates can ensure the occasion for novel phenotypes to arise in pest populations (Zhou *et al*., 2000). In addition, the area, climatic alterations, and resistance to ecological factors (Willi *et al*., 2006) also have the ability to produce selection pressure on insects. Population genetic research ensures beneficial information concerning the potential for wide-ranging pest control, especially in species with a wide geographical spread (Alphey & Bonsall, 2018). Comprehending the phylogenetic relationships between insect populations is extremely significant for implementing a sustainable and effective *An. sacharovi* management. Nonetheless, information regarding the demographic history and population genetics of *An. sacharovi* in Türkiye remains limited.

The UPGMA dendrogram and PCoA diagram showed the *An. sacharovi* populations in Türkiye to not exhibit clustering. The possible reason for this situation is due to the rate of gene flow over time being considered suitable for estimating gene exchange between populations (Slatkin, 1985). The gene flow (N_m) values obtained by microsatellite studies for different *Anopheles* species have been reported to vary between 4-483 (Rongnoparut *et al*., 1999; Braginets *et al*., 2003; Michel *et al*., 2005; Ogola *et al*., 2019; Kaddumukosa *et al*., 2020). This current study was performed with RAPD markers and found a gene flow (N_m) value of $N_m = 2.08$. The likely reason for this low gene flow level is thought to be a result of the geographical distances between the populations. In addition, the intense control of these vector organisms may be another explanation for the low level of gene flow. The observed gene flow ($N_m = 2.08$) between populations appears to have been sufficient for homogenizing the mosquito populations in Türkiye. Variable effective population sizes, climate, and different collection times may also be some of the factors influencing differentiation.

In conclusion, the present study has characterized the *An. sacharovi* populations for the first time by using the RAPD marker. Future studies should investigate changes in mosquito populations over time, particularly as insecticide use and coverage evolve, new interventions emerge, and climate and land use changes. As a result, the examination of *Anopheles* populations in Türkiye from more locations, with more samples, and base sequence analyses from different parts of the genome or analysis of loci without any selection pressure will clearly make an important contribution to future studies in this field.

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RESEARCH ARTICLE

First Record of the Little Gulper Shark, *Centrophorus uyato* (Rafinesque, 1810), in the Turkish Marine Waters of the North Aegean Sea

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Abstract

Objective: Gulper sharks, are small to medium-sized benthopelagic deep-water sharks that occur worldwide along the outer continental shelves and upper continental and insular slopes. *Centrophorus uyato* (Rafinesque, 1810) is a rare species that has only been recorded twice in Turkish marine waters. Our study provides the first record of the species in the Turkish waters of the North Aegean Sea and the third record from the Turkish waters.

Materials and Methods: In April 2019, the male specimen of *C. uyato* was caught at 401 m with trawl commercial fish vessels. The little gulper shark, *C. uyato* was collected off the Gökçeada coasts, North Aegean Sea. Measurements of the caught specimen are provided and the geographical distribution of the *C. uyato* in the Mediterranean Sea is documented.

Results: The male specimen of *C. uyato* was caught off the coasts of Gökçeada. It was measured as 775 mm in total length and weighed 3040 g in total weight.

Conclusion: In the Turkish marine waters of the Mediterranean Sea, data on the species is lacking because it is hard to find the genus. Their K-selected life strategy makes them vulnerable, so in the Mediterranean region, they have a declining trend. Therefore, identifying the species across its distribution range is an important step for future conservation efforts.

Keywords: First Report, North Aegean Sea, Gökçeada, *Centrophorus uyato*, Centrophoridae

Introduction

Order Squaliformes comprise an abundant and diverse group of sharks specifically living in deep-sea marine environments (Compagno, 1984). This order contains six families: Centrophoridae, Dalatiidae, Etmopteridae, Oxynotidae, Somniosidae, and Squalidae (Straube *et al*., 2015).

In the Mediterranean Sea, the following species represent most of the deep-sea sharks; kitefin shark *Dalatias licha* (Bonnaterre, 1788) is a medium-sized (maximum total length: 1820 mm) (Springer, 1990) shark belonging to the family of Sleeper sharks, Dalatiidae. *D. licha* can usually be found at depths of 300 to 600 m (Compagno, 1984). The species has been recorded in the Aegean Sea (Kabasakal & Kabasakal, 2002; Kabasakal & Kabasakal, 2004; Turetken, 2009; Eronat & Ozaydin, 2014; Gonulal, 2016), Levantine Sea (Dalyan, 2012; Erguden *et al.,* 2017), Mediterranean Sea (Guven *et al*., 2012) and Marmara Sea (Meriç, 1995). Smaller in length (common length: 450 mm) (Compagno, 1984), the velvet belly lantern shark

Etmopterus spinax (Linnaeus, 1758) is a shark belonging to the family Etmopteridae. *E. spinax* can usually be found at depths of 200 and 600 m (Weigmann, 2016), recorded in the Aegean Sea (Meriç, 1994; Kabasakal & Kabasakal, 2004; Ismen *et al.*, 2007, 2009; Turetken, 2009; Bilge *et al.,* 2010, Eronat & Ozaydin, 2014; Gonulal, 2016, Öz & İşmen, 2017), Mediterranean Sea (Guven *et al*., 2012, Bayhan *et. al*., 2018, Akbora *et al*., 2020), and Levantine Sea (Dalyan, 2012; Başusta, 2016) and has shown a relatively stable population trend, however, continued research is required (Guallart *et al.,* 2016a). Another research-required shark is the little sleeper shark *Somniosus rostratus* (Risso, 1827) since it is classified as data deficient in the Mediterranean Sea (Guallart *et al.,* 2016b). *S. rostratus* is a medium-sized (maximum total length: 1430 mm) (Cox & Francis, 1997) shark belonging to the family of Sleeper sharks, Somniosidae. *S. rostratus* can be usually found at depths of 200 to 1330 m (Goren & Galil, 2015), and has only been recorded in the Aegean Sea (Irmak & Ozden, 2021). Information about records of these species in the Turkish Seas is presented in Table 1.

Family Centrophoridae, also known as the gulper sharks, are small to medium-sized benthopelagic deep-water sharks that occur worldwide along the outer continental shelves and upper continental and insular slopes (Compagno, 1984; Ebert & Winton, 2010; Kyne & Simpfendorfer, 2010). Centrophoridae contains two genera, *Centrophorus* (Müller & Henle, 1837) and *Deania* (Jordan & Snyder, 1902). Both genera have a complex taxonomic history. This identification problem is firstly due to poorly defined characteristics (Muñoz-Chápuli & Ramos, 1989). Secondly, sampling for these specimens is hard to find (White *et al*., 2008). In the Mediterranean Sea, only the genus *Centrophorus* can be found (Compagno, 1984; Ebert & Dando, 2021) and it is represented by *C. granulosus* (Bloch & Schneider, 1801) and *C. uyato* (Rafinesque, 1810). Both the gulper shark *C. granulosus* and the little gulper shark *C. uyato* can be found at depths of 50 to 1400 m (Compagno, 1984). *C. granulosus* has been recorded in the Aegean Sea (Ismen *et al*., 2009, Cengiz *et al*., 2011) Marmara Sea (Benli *et al*., 1993), and the Mediterranean Sea (Güven *et al*., 2012). The only records of *C. uyato* in Turkish waters are from the Marmara Sea (Meriç, 1995) and the Gulf of Antalya (Kabasakal *et al*., 2022). Both sharks have been included in several taxonomic checklists (e.g., Kabasakal, 2002; Bilecenoğlu *et al*., 2002; Papaconstantinou, 2014; Haroun *et al.,* 2017). However, recent molecular and morphometric studies have shown that the small-sized *Centrophorus* species could have been misidentified as *C. granulosus* (Kousteni *et al.*, 2021). This taxonomic issue dates back to 1906 (Garman, 1906) and has historically generated confusion over the identification of two species (White *et al*., 2013; Veríssimo *et al*., 2014).

Presently, the nomenclatural validity of *C. uyato* vs *C. granulosus* stays unsettled (White *et al*., 2013; Veríssimo *et al*., 2014; Serena *et al*., 2020). However, according to Compagno (1984) and White *et al*. (2013), these species can be specified by following the main characteristics: *C. uyato* is smaller in maximum total length (1100 mm) than *C. granulosus* (1700 mm); the denticles in *C. uyato* are flat while denticles in *C. granulosus* are flat with tear-drop shaped crowns that give the skin more granular composition; the free tip of the pectoral fin of *C. uyato* is not in line with the apex of the 1st dorsal fin whereas in *C. granulosus* they are in line. Therefore, we use the following characteristics of *C. uyato* until this taxonomical issue is resolved.

According to the IUCN Red List, (Finucci *et al.,* 2020) *C. uyato* is listed as Endangered (EN) which makes it important to protect the species. The purpose of this study is to provide the first record of the poorly known *C. uyato* from the Turkish waters of the North Aegean Sea and to make a contribution to the literature.

Materials and Methods

The North Aegean Sea has interactions with the Mediterranean and Marmara Seas, thereby northern parts of the sea show richness of nutrients via Dardanelles and Black Sea currents (Öztürk, 2009). Gökçeada, located in the North Aegean Sea (40°17'49.56"-40°17'47.40"N; 24°53'45.84"-25°52'54.60"E) and has a 92 kilometers coastline and is Turkey's biggest island (Fig. 1).

A male specimen of *C. uyato* was caught at 401 m depth in April 2019 in 3.6 miles off the Gökçeada coasts

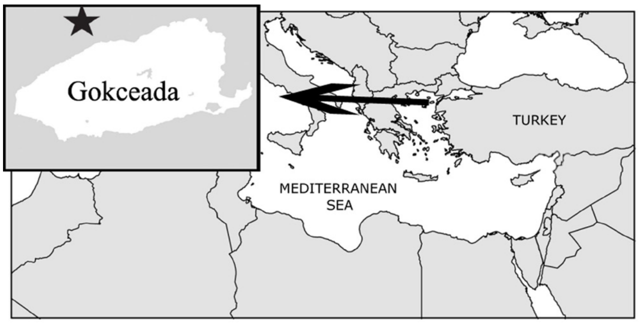

Figure 1. Map showing the coordinates of Gökçeada (40°17'49.56"-40°17'47.40"N; 24°53'45.84"-25°52'54.60"E).

(North Aegean Sea). Survey research is done with trawl commercial fish vessels. The necessary measurements were made in the laboratory, and the specimen was preserved in the freezer. All measurements and morphological features point to our specimen *C. uyato* (Fig. 2).

Figure 2. A male specimen of *C. uyato*, from Gökçeada, Turkey (TL. 775 mm).

Table 1. Records of Squaliformes deep water species in Turkey.

Familia	Species	Region	Depth Range	References
Centrophoridae	*Centrophorus granulosus*	Marmara Sea	-	(Benli *et al*., 1993)
Centrophoridae	*Centrophorus granulosus*	Aegean Sea (Saros Bay)	50-1400 m	(Cengiz *et al*., 2011)
Centrophoridae	*Centrophorus granulosus*	Aegean Sea (Saros Bay)	5-500 m	(Ismen *et al*., 2009)
Centrophoridae	*Centrophorus granulosus*	Levantine Sea (Antalya Bay)	200-800 m	(Güven *et al*., 2012)
Centrophoridae	*Centrophorus uyato*	Marmara Sea	-	(Meriç, 1995)
Centrophoridae	*Centrophorus uyato*	Levantine Sea (Antalya Bay)	140 m	(Kabasakal, 2022)
Dalatiidae	*Dalatias licha*	Aegean Sea (Gökçeada)	380 m	(Kabasakal, 2004)
Dalatiidae	*Dalatias licha*	Levantine Sea (İskenderun Bay)	40 m	(Ergüden, 2017)
Dalatiidae	*Dalatias licha*	Aegean Sea (Gökçeada)	500-800 m	(Gönülal, 2016)
Dalatiidae	*Dalatias licha*	Aegean Sea (İzmir and Sığacık Bay)	-	(Eronat & Özaydın, 2014)
Dalatiidae	*Dalatias licha*	Levantine Sea (Antalya Bay)	200-800 m	(Güven, 2012)
Dalatiidae	*Dalatias licha*	Marmara Sea	270 m	(Meriç, 1995)
Dalatiidae	*Dalatias licha*	Aegean Sea (Gökçeada)	380 m	(Kabasakal & Kabasakal, 2002)
Dalatiidae	*Dalatias licha*	Aegean Sea (Gökçeada)	-	(Türetken, 2009)
Dalatiidae	*Dalatias licha*	Levantine Sea (İskenderun Bay)	500-800 m	(Dalyan, 2012)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (Gökçeada, Saroz Bay)	280 m	(Kabasakal, 2004)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (Gökova Bay)	-	(Meriç, 1994)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (Saros Bay)	5-500 m	(Ismen *et al*., 2009)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (Saros Bay)	28-370 m	(Ismen *et al*., 2007)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (Gökçeada)	500-1000 m	(Gönülal, 2016)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (İzmir and Sığacık Bay)	-	(Eronat & Özaydın, 2014)
Etmopteridae	*Etmopterus spinax*	Levantine Sea (Antalya Bay)	200-800 m	(Güven *et al*., 2012)
Etmopteridae	*Etmopterus spinax*	Levantine Sea (İskenderun Bay)	360-400 m	(Başusta, 2016)
Etmopteridae	*Etmopterus spinax*	Levantine Sea (Mersin Bay)	300-601 m	(Bayhan *et al*., 2018)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (Gökçeada)	-	(Türetken, 2009)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (Sığacık Bay)	200-600 m	(Bilge *et al*., 2010)
Etmopteridae	*Etmopterus spinax*	Levantine Sea (Northern Cyprus)	274-641	(Akbora *et al*., 2020)
Etmopteridae	*Etmopterus spinax*	Aegean Sea (Saros Bay)	200-500 m	(Öz, 2017)
Etmopteridae	*Etmopterus spinax*	Levantine Sea (İskenderun Bay)	500-800 m	(Dalyan, 2012)
Somniosidae	*Somniosus rostratus*	Aegean Sea (Muğla)	2500 m	(Irmak & Özden, 2021)

Results and Discussion

A male specimen of *C. uyato,* measuring 775 mm in total length and weighing 3040 g in total weight, was caught off the coasts of Gökçeada. All measured morphometrics characters indicated to *C. uyato* (Table 2). Besides, the little gulper shark shows different features than its congeneric *C. granulosus*; a black tip on dorsal fins and a second dorsal fin origin over the pelvic inner fin margin (Ebert & Dando, 2021).

The first two records of *C. uyato* in Turkish waters are from the Sea of Marmara (Meriç, 1995) and the Gulf of Antalya (Kabasakal, 2022) respectively. This paper provides the third record of *C. uyato* in Turkish waters and the first record of the species in the Turkish waters of the North Aegean Sea.

The taxonomic classification of *C. uyato* has been complicated throughout history (White *et al.*, 2013). Günther (1870) expanded the diagnosis of the genus *Centrophorus* to include six additional squaloid species, as described by Barbosa du Bocage and de Brito Capello (1864). However, Garman (1906) argued that four distinct genera were involved, as originally designated by Barbosa du Bocage and de Brito Capello, and described two new species of *Centrophorus*. Garman also added *Squalus uyato* to the genus *Centrophorus*, in contrast to the previous classification by Müller and Henle (1839) and Bonaparte

Table 2. Morphometric measurements of male specimen of *C. uyato*, from Gökçeada.

Measurements mm	**Present Specimen**		**Kabasakal (2022)**		**Kousteni *et al.* (2021)**		**Bellodi *et al.* (2022)**	**White *et al.* (2022)**
	mm	**% of TL**	**mm**	**% of TL (TL = 663 mm)**	**% of TL (TL= 522 mm)**	**% of TL (TL= 483 mm)**	**Mean values of body length measurements (mm)**	**% of TL (TL= 983 mm)**
Pre-second dorsal-fin length (PD2)	550	70.98	431	65.01	63.51	63.69	69.72	64.6
Pre-first dorsal-fin length (PD1)	240	30.97	205	30.92	32.6	32.23	32.89	28.7
Head length (HDL)	170	21.93	150	22.62	24.82	23.82	21.93	22.5
Prebrancial length (PG1)	145	18.71	122.4	18.46	20.5	20.2	17.23	18.5
Prespiracular length (PSP)	85	10.97	86.9	13.11	14.66	14.1	-	12.1
Preorbital length (POB)	35	4.52	44.1	6.65	7.44	6.9	-	5.3
Prepectoral-fin length (PP1)	170	21.93	146	22.02	24.05	24.47	-	22.1
Prepelvic-fin length (PP2)	455	58.71	383	57.77	58.18	60.69	62.90	57.1
Interdorsal space (IDS)	205	26.45	161	24.28	20.27	20.04	31.28	23.2
Dorsal caudal-fin space (DCS)	55	7.10	53.5	8.07	7.89	6.9	-	6.4
Pectoral-fin pelvic-fin space (PPS)	260	33.55	211	31.83	28.49	29.82	-	31.3
Prenarial length (PRN)	30	3.87	21.5	3.24	4.7	4.29	-	3.7
Preoral length (POR)	70	9.03	63.7	9.61	10.33	9.29	-	9.5
Eye length (EYL)	40	5.16	31.6	4.77	6.3	6.54	-	5.3
Eye height (EYH)	20	2.58	15	2.26	1.73	1.77	-	1.4
Intergill length (ING)	25	3.22	34.1	5.14	4.32	3.62	-	-
First gill slit height (GS1)	20	2.58	13.8	2.08	1.82	1.8	-	-
Second gill slit height (GS2)	20	2.58	15.7	2.37	1.93	1.97	-	-
Third gill slit height (GS3)	20	2.58	18.1	2.73	2.07	2.07	-	-
Fourth gill slit height (GS4)	20	2.58	20.1	3.03	2.21	2.36	-	-
Fifth gill slit height (GS5)	30	3.87	20.2	3.05	2.38	2.57	-	-
Pectoral-fin anterior margin (P1A)	85	10.97	77.4	11.67	11.63	11.36	-	12.3
Pectoral-fin posterior margin (P1P)	85	10.97	59.7	9.00	10.46	10.53	-	-
Pectoral-fin height (P1H)	70	9.03	-	-	-	-	-	-
Pectoral-fin length (P1L)	115	14.84	-	-	-	-	-	-
Dorsal caudal-fin margin (CDM)	125	16.13	132.5	19.98	20.44	20.62	17.88	20.1
Preventral caudal-fin margin (CPV)	80	10.32	76.3	11.51	12.89	11.52	-	11.9
Subterminal caudal-fin margin (CST)	10	1.29	23.3	3.51	3.1	3.23	-	2.9
First dorsal-fin length (D1L)	120	15.48	119.2	17.98	17.39	16.7	-	19.0
First dorsal-fin anterior margin (D1A)	70	9.03	60.8	9.17	12.15	10.28	-	12.6
First dorsal-fin base (D1B)	75	9.68	69.4	10.47	11.54	11.49	-	13.5
First dorsal-fin height (D1H)	35	4.52	41.7	6.29	6.37	6.31	-	5.8
First dorsal-fin inner margin (D1I)	35	4.52	47.3	7.13	5.84	5.2	-	5.8
First dorsal-fin posterior margin (D1P)	70	9.03	64.7	9.76	8.68	8.31	-	9.3

Second dorsal-fin length (D2L)	70	9.03	75.4	11.37	12.36	12.69	-	12
Second dorsal-fin anterior margin (D2A)	50	6.45	45.9	6.92	9.23	9.13	-	8.6
Second dorsal-fin base (D2B)	40	5.16	42.9	6.47	8.1	8.53	-	8.3
Second dorsal-fin height (D2H)	30	3.87	32.9	4.96	3.86	4.28	-	4.7
Second dorsal-fin inner margin (D2I)	20	2.58	27.1	4.09	4.26	4.17	-	4.1
Second dorsal-fin posterior margin (D2P)	50	6. 45	43.6	6.58	6.36	5.44	-	6.3
Pelvic-fin length (P2L)	105	13.55	65.8	9.92	10.42	9.9	-	11.2
Pelvic-fin anterior margin (P2A)	55	7.10	40.7	6.14	6.52	6.71	-	-
Pelvic-fin base (P2B)	40	5.16	23.6	3.56	4.85	4.36	-	5.8
Pelvic-fin height (P2H)	35	4.52	-	-	-	-	-	-
Pelvic-fin inner margin (P2I)	75	9.68	49	7.39	5.84	5.86	-	5.8
Pelvic-fin posterior margin (P2P)	70	9.03	-	-	-	-	-	-
Head height (HDH)	90	11.61	150	22.62	24.82	23.82	21.93	22.5
Trunk height (TRH)	100	12.90	-	-	-	-	-	-
Abdomen height (ABH)	95	12.26	-	-	-	-	-	-
Tail height (TAH)	60	7.74	-	-	-	-	-	-
Caudal-fin peduncle height (CPH)	30	3.87	-	-	-	-	-	-
First dorsal-fin midpoint pectoral-fin insertion (DPI)	120	15.48	-	-	-	-	-	-
First dorsal-fin midpoint pelvic-fin origin (DPO)	165	21.29	-	-	-	-	-	-
Pelvic-fin midpoint first dorsal-fin insertion (PDI)	145	18.71	-	-	-	-	-	-
Pelvic-fin midpoint second dorsal-fin origin (PDO)	90	11.61	-	-	-	-	-	-
Mouth length (MOL)	10	1.29	-	-	-	-	-	-
Mouth width (MOW)	60	7.74	55	8.30	8.1	6.91	-	-
Upper labial-furrow length (ULA)	40	5.16	-	-	-	-	-	-
Lower labial-furrow length (LLA)	30	3.87	-	-	-	-	-	-
Nostril width (NOW)	55	7.10	-	-	-	-	-	-
Internarial space (INW)	30	3.87	-	-	-	-	-	-
Clasper outer length (CLO)	30	3.87	-	-	-	-	-	-
Clasper inner length (CLI)	86	11.10	-	-	-	-	-	-
Clasper base width (CLB)	10	1.29	-	-	-	-	-	-
Interorbital space (INO)	70	9.03	-	-	-	-	-	-
Spiracle length (SPL)	10	1.29	12.3	1.86	1.27	1.07	-	1.2
Eye spiracle space (ESL)	20	2.58	17.4	2.62	2	1.55	-	-
Head width (HDW)	90	11.61	-	-	-	-	-	-
Trunk width (TRW)	100	12.90	-	-	-	-	-	-
Abdomen width (ABW)	90	11.61	-	-	-	-	-	-
Tail width (TAW)	45	5.81	-	-	-	-	-	-
Caudal-fin peduncle width (CPW)	20	2.58	-	-	-	-	-	-

(1841). However, the original description and illustration of *S. uyato* by Rafinesque (1810) depict characteristics of a *Squalus* species rather than *Centrophorus*. Muñoz-Chápuli and Ramos (1989) also argued for the exclusion of *uyato* from the genus *Centrophorus*, while Böhlke (1984) regarded Bonaparte's treatment of *Spinax uyatus* as a new name proposal. It is clear that further investigations are needed to determine the validity of this classification.

A comprehensive synonymy analysis was conducted, revealing that *C. machiquensis*, *C. bragancae*, and *C. zeehaani* are junior synonyms of *C. uyato*. In order to maintain nomenclatural stability within the genus, the name *C. uyato* is retained for this species, with a neotype designated from the vicinity of the original type locality off Italy. (White *et al.*, 2013).

Further studies to clarify the taxonomic status of *C. uyato* are still being conducted (Bellodi *et al*., 2022, Kousteni *et al*., 2021, White *et al*., 2022). Bellodi *et al*. (2022) studied the genus through an integrated taxonomic approach including DNA sequencing and molecular analysis. Kousteni *et al*. (2021) provided the first record of *C. uyato* off the coast of Cyprus, examining both the mitochondrial and ribosomal DNA of the species. While White *et al*. (2022) focused more on detailed morphometric measurements providing a detailed synonym, aimed to persevere nomenclatural stability within the genus.

Deep sea habitats, characterized by their stability and isolation from human activity, provide a unique and fragile ecosystem that plays a vital role in the earth's biogeochemical processes (Ramirez-Llodra *et al*., 2010). These habitats are home to a diverse array of species, adapted to the extreme pressure and cold temperatures found at these depths. However, the long-life spans, slow growth rates, and low reproductive rates (Van Dover *et al.*, 2004) of deep-sea species make them particularly vulnerable to disturbances.

Information about these deep-sea sharks is still lacking (Simpfendorfer & Kyne, 2009; Pinte *et al*., 2020) even though they are apex predators (Heithaus *et al.,* 2008; Ferretti *et al*., 2010). It is known that sharks are, in general, slow-growing, late-maturing, and long-living beings (Musick, 1999) which potentially makes them vulnerable (Simpfendorfer & Kyne, 2009). This fact is not different for sharks that reside in the Mediterranean Sea since *D. licha*, *C. granulosus*, and *C. uyato* have shown a declining population trend.

The IUCN Red List assessment of the species for the Mediterranean Sea is as follows: *D. licha* is regarded as Vulnerable (VU), *E. spinax* is regarded as Least Concern (LC), *C. uyato* is regarded as Endangered (EN) while *C. granulosus* is categorized as Critically Endangered (CR). Although *S. rostratus* has been categorized as Least Concern (LC) globally, the Mediterranean population is considered Data Deficient (DD) for the species (Guallart *et al.*, 2016a; Finucci *et al.*, 2020).

The ongoing debate surrounding their definition and limited data availability regarding these endangered species in the Mediterranean highlights the precarious situation of these vulnerable animals. Accurately identifying the species across its distribution range may guide future conservation efforts by the IUCN to effectively protect their populations.

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RESEARCH ARTICLE

Medicinal Plant Diversity and Utilization in the Argo District of Badakhshan Province, Afghanistan

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Abstract

Objective: Plants have long been used for medicinal purposes and to treat various illnesses. Despite the rich potential of the Argo District in the Badakhshan province of Afghanistan, no studies have attempted to study its medicinal plant diversity. This study aims to fill this gap and identity the diversity of medicinal plants in the study area.

Materials and Methods: Field surveys and interviews were conducted between May and October 2021, by using semi-structured questionnaires. 136 informants, from 113 households in 16 villages, were interviewed and respondents were selected randomly. Additionally, transect walks in the field were used to collect medicinal plants in the study area and the collected specimens were stored in the laboratory of Biology Department at Badakhshan University.

Results: The study found 49 medicinal plant species, belonging to 44 genera and 26 families, to have medicinal properties. The largest percentage of herbal plants belonged to the families of Rosaceae 10%, Fabaceae 10%, Asteraceae 8%, Apiaceae 6%, Brassicaceae 6%, and Amaranthaceae 4% in reducing order of frequency of reported use. The highest genera were *Ziziphora* and the lowest genera were *Portulaca*. In terms of habit formation, herbs were the highest percentage at 80%, followed by trees at 14%, and shrubs at 6%. The highest percentage of utilizable plant parts were the leaves (24%), roots (23%), fruits (17%), flowers (14%), seeds (12%), and shoots (10%).

Conclusion: The local people used 49 different species of medicinal plants to treat a variety of ailments, including headaches, dysentery, cough, fever, gastritis, hypertension, diarrhea, anemia, and wounds.

Keywords: Argo district, Medicinal plant diversity, Conservation, Traditional knowledge, Diseases

Introduction

Medicinal plants are a significant source of healthcare and an economic component of biodiversity. Identifying and documenting medicinal plants is important for conservation and sustainable utilization (Rahman *et al*., 2012). Medicinal plants have been used all over the globe for thousands of years as natural medicines and palliatives. Today, traditional medicine serves as the primary healthcare of 80% of the world's population (Mi & Mach, 2012). Some of the rural population have traditional knowledge of medicinal plants, which is transmitted from generation to generation (Shrestha & Dhillion, 2003). Plants provide people with food, medicines, fodder for livestock, and also materials for the construction of houses (Hassan *et al*., 2019; Ishtiaq *et al*., 2012). Medicinal plants are vital raw materials for the production of traditional and modern medicines (Ali & Alamgir, 2014). Medicinal plants play a significant role in the economic and cultural lives of people around the world (Kamble *et al*., 2018).

People of the Argo District in Badakhshan province of Afghanistan utilize different medicinal plants to treat various diseases. Humanity has been utilizing plants for medicinal purposes since pre-history. For many centuries, herbs and their derived medicines have played an important role in health and ailments management (Okoye *et al*., 2014a), and more than 50,000 plant species are utilized for medicinal purposes around the globe, of which nearly

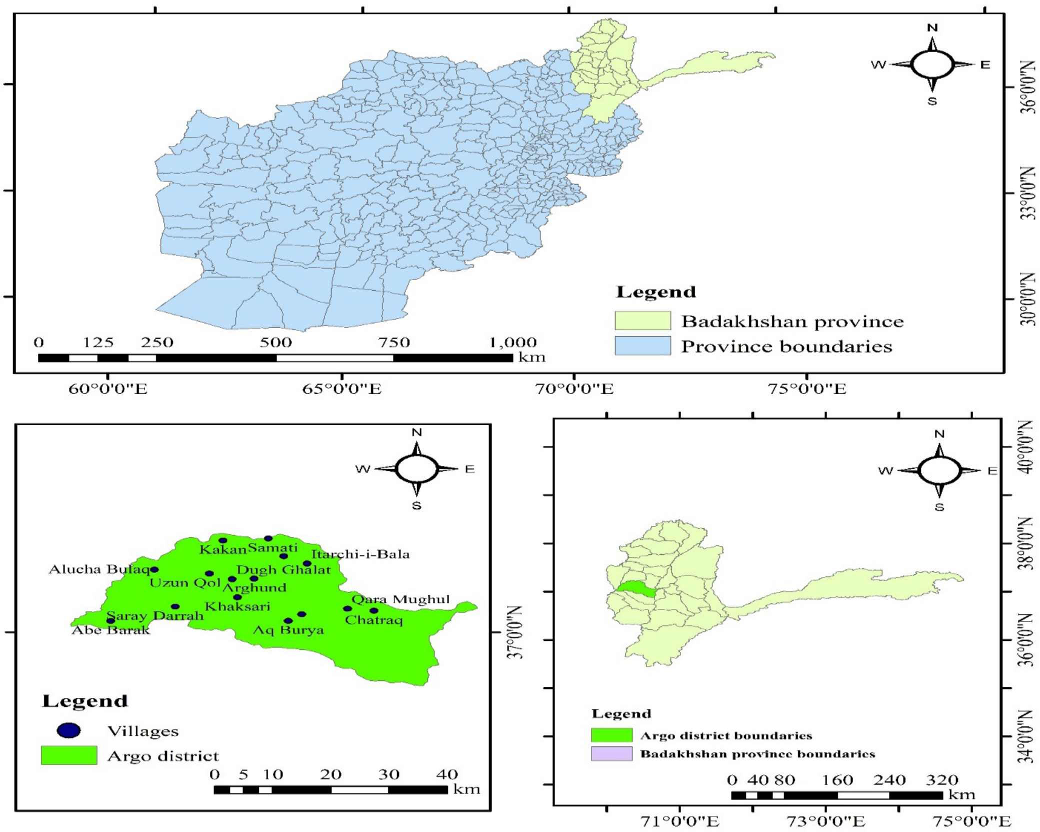

Figure 1. a) Indicates the whole of Afghanistan, b) shows Badakhshan Province and c) implies the exact research area.

13% are flowering plants. Local, national and international interest in medicinal plants has grown in recent years, leading to a rise in demand (Sakkir *et al*., 2012). Medicinal plant parts (roots, fruits, rhizomes, leaves, flowers, seeds, stem, and bark) can be utilized in different forms such as teas, fresh crude forms, and decoctions, powdered plant material, or extracted forms of medicinal agents (water or juices, extracts, essential oils, tinctures, resins, balsams). Furthermore, medicinal plants can also be significant sources of nutrition as they contain many biologically active substances like essential oils or vitamins (Mi & Mach, 2012). Medicinal plants are harvested from a vast range of plants, which are mostly wild. Certainly, the demand for wild resources has risen day by day (Chen *et al*., 2016). Medicinal plants and associated traditional knowledge are endangered in the study area. Continued utilization, habitat degradation, and unsustainable harvesting are the main threats to medicinal plants. Traditional knowledge is furthermore hampered by the loss of medicinal plant diversity. Medicinal plants play an important role in the health care and economy of the local people (Okoye *et al*., 2014b). This study aims to identify the medicinal plant diversity of the Argo District in the Badakhshan province of Afghanistan.

Materials and Methods

Study area

The current study was conducted in Argo District situated in Badakhshan Province, Afghanistan. Argo is one of 28 districts in the Badakhshan Province, and it covers an area of 1031 km^2 (Fig. 1). Its geographical coordinates are 70°44’6” to 70°6’22”E and 36°52’22” to 37°13’53”N. The area is very rich in medicinal plant species and topographically it is a mountainous region located to the west of the city of Faizabad. The climate of the Argo district is typically a continental interior mountainous region. The winter is characterized by cold temperatures (average 0.52°C), heavy snow and substantial amounts of precipitation (average 5.6). The summers are warm (average 21.67°C) and dry (precipitation 1.07 on average). Spring is a transition period, with increasing temperatures and some rain (NASA, 2021). The dominant plant species in the study area were pistachios, pears, and almonds. Most people in the Argo district are poor, with the majority being farmers, and more than 95% of the population depends on traditional agriculture. The majority of the population is dependent on sheep, goats, and cattle for milk production, and two tribes (Uzbek and Tajik) live in the area. The agricultural plants in the area are wheat, barley, peas, maize, etc. Overgrazing and unsustainable harvesting of plants are the main deleterious forces causing the loss of medicinal plant diversity.

Materials and Methods

Field surveys and interviews were conducted between May and October 2021, by using semi-structured questionnaires. 136 informants (89 males and 47 females) from 113 households in 16 villages (Itarchi, Afaqi, Taqcha Dara, Samad, Kakan, Qara Kamar, Alocha Bulaq, Uzon Qul, Dugh Ghalat, Khak Sari, Aq burya, Arghund, Sarai Darrah, Qara Mughul, Chatraq, and Ganda Chashma) were interviewed. The respondents were residents of the region aged 30 to 65 (Ssegawa & Kasenene, 2007; Uddin *et al*., 1970). The transect walks method was used to collect specimens. Some local people helped with the collection of plant species in the field. The collected specimens were stored in the laboratory of the biology department at Badakhshan University. During the interviews, the author asked the following questions. Which medicinal plant species are found in this area? Which part of the plant do you use? Which diseases do you treat with medicinal plants? Household informants were selected randomly in each village. The head of the household, as well as other knowledgeable adults, were interviewed. In each village, at least six respondents were interviewed. Every plant was recorded according to its local (Uzbek and Dari) names,

Table 1. Documentation of medicinal herbs by scientific name, local name, habit, parts used, and diseases that can be cured.

S. No	*Scientific name*	*Coll. no*	*Family*	*L. name*	*Habit*	*Part use*	*Diseases that can be cured*
1	*Achillea filipendulina* Lam	7	Asteraceae	Zarsarak	Herb	Flower	Gastritis
2	*Centaurea repens* L	13	Asteraceae	Talkha	Herb	Leaf	Fever and Diabetes
3	*Alhagi pseudalhagi* (M. Bieb.) Desv.	9	Fabaceae	Yantagh	Herb	Shoot, Root	Liver and Stomach ulcers
4	*Althaea officinalis* L	21	Malvaceae	Gulkhairy	Herb	Leaf, Flower	Kidney stone
5	*Amaranthus retroflexus* L	41	Amaranthaceae	Taj khoros	Herb	Flower	Intestine infection
6	*Anchusa azurea* Mill	8	Boraginaceae	Kokmaraz	Herb	Flower	Cough remedy
7	*Artemisia absinthium* L	38	Asteraceae	Irmashiwaq	Herb	Leaf	Intestine infection
8	*Astragalus sieversianus* Pall	43	Fabaceae	Pakhtawot	Herb	Fruit	Kidney infection
9	*Berberis vulgaris* L.	22	Berberidaceae	Zerk	Herb	Root	Indigestion, Traumatic pain
10	*Brassica oleracea*	47	Brassicaceae	Karam	Herb	Leaf	Hypertension
11	*Bunium persicum* (Boiss.) B. Fedtsch	39	Apiaceae	Gow zira	Herb	Seed	Stomach aches
12	*Biebersteinia multifida* DC	23	Biebersteiniaceae	Qantepar	Herb	Root	Hemostatic for post-natal bleeding
13	*Capparis spinosa* L	14	Capparidaceae	Kawer	Herb	Fruit	Gastrointestinal diseases
14	*Capsella bursa-pastoris* (L.) Medik	40	Brassicaceae	Mamabiti	Herb	Shoot	Dysentery and Uterine infection
15	*Carum carvi* L	24	Apiaceae	Zira	Herb	Seed	Dyspepsia and Diuretic
16	*Cichorium intybus* L	6	Asteraceae	Chachratqi	Herb	Root, Shoot	Fever, Improve digestion
17	*Conium maculatum* L	48	Apiaceae	Baldirghan	Herb	Seed	Headaches
18	*Crataegus songarica* K Koch	46	Rosaceae	Dolana sarigh	Tree	Flower, Fruit	Heart pain
19	*Crataegus altaica* (Loudon) Lange	25	Rosaceae	Dolana	Tree	Flower, Fruit	Heart diseases and laxative
20	*Crambe kotschyana* Boiss	5	Brassicaceae	Tatron	Herb	Root	Toothache
21	*Datura stramonium* L	35	Solanaceae	Bangi divana	Herb	Seed	Toothache
22	*Descurainia sophia* (L.) Webb ex Prantl	15	Brassicaceae	Khawaglan	Herb	Root, Seed	Diarrhea
23	*Elaeagnus angustifolia* L	33	Elaeagnaceae	Senjed	Tree	Fruit, Root	Wounded skins, Dysentery
24	*Ficus johannis* Boiss.	26	Moraceae	Anjir	Tree	Fruit, Leaf	Abdominal pains
25	*Fumaria officinalis* L	32	Fumariaceae	Shatara	Herb	Leaf, Flower	Skin diseases
26	*Glycyrrhiza glabra* L	4	Fabaceae	Biya	Herb	Root	Stomach diseases
27	*Glycyrrhiza uralensis* Fisch	27	Fabaceae	Biya qadkota	Herb	Root	Stomach diseases
28	*Hordeum vulgare* L.	16	Poaceae	Arpa	Herb	Seed	Urinary tract disorders
29	*Juglans regia* L	45	Juglandaceae	Yangaq	Tree	Fruit, Leaf	Cardiac, Gum disease
30	*Marrubium anisodon* K Koch	37	Lamiaceae	Telvateg	Herb	Leaf	Respiratory diseases
31	*Morus alba* L	44	Moraceae	Toot	Tree	Leaf	Urinary system diseases
32	*Peganum harmala* L	30	Zygophyllacea	Ispand	Herb	Leaf	Analgesic for sick person
33	*Plantago lanceolata* L	3	Plantaginaceae	Zof mida barg	Herb	Root	Gastritis
34	*Plantago major* L	2	Plantaginaceae	Zof kata barg	Herb	Leaf	Wound, Tumor
35	*Polygonum aviculare* L	29	Polygonaceae	Tarmaq	Herb	Shout	Intestinal infection
36	*Portulaca oleracea* L	17	Portulacaceae	Semiz wit	Herb	Shout	Dysentery
37	*Prunus sogdiana* Vassilcz	20	Rosaceae	Alocha	Tree	Fruit	Increase appetite
38	*Cullen drupacea* (Bunge) C.H. Stirt	18	Fabaceae	Aqourak	Herb	Seed	Skin diseases
39	*Ribes nigrum* L.	12	Grossulariaceae	Qorakat	Shrub	Leaf, Fruit	Hypertension
40	*Punica granatum* L	19	Punicaceae	Anar	Tree	Fruit Peel	Diarrhea
41	*Rosa canina* L	28	Rosaceae	Itburon	Shrub	Leaf, Root	Gastrointestinal diseases
42	*Rosa fedtschenkoana* Regel	31	Rosaceae	Qara tikan	Shrub	Fruit Root	Cough, Dysentery
43	*Rumex confertus* Willd	1	Polygonaceae	Shilkha	Herb	Leaf	Diarrhea
44	*Spinacia turkestanica* Iljin	34	Amaranthaceae	Palak	Herb	Shout	Anemia
45	*Leontodon taraxacum* L	42	Asteraceae	Qaquf	Herb	Leaf	Anemia
46	*Trichodesma incanum* (Bunge) A.DC	36	Boraginaceae	Kampirchpan	Herb	Root	Wounds
47	*Ungernia victoris* Vved. Ex Artjushenko	49	Amaryllidaceae	Amonqara	Herb	Bulb	Wounds
48	*Ziziphora clinopodioides* Lam	11	Lamiaceae	Kokwiti chaqili	Herb	Shout	Hypertension
49	*Ziziphora pedicellata* Pazij & Vved	10	Lamiaceae	Kokwiti	Herb	Shout	Hypertension

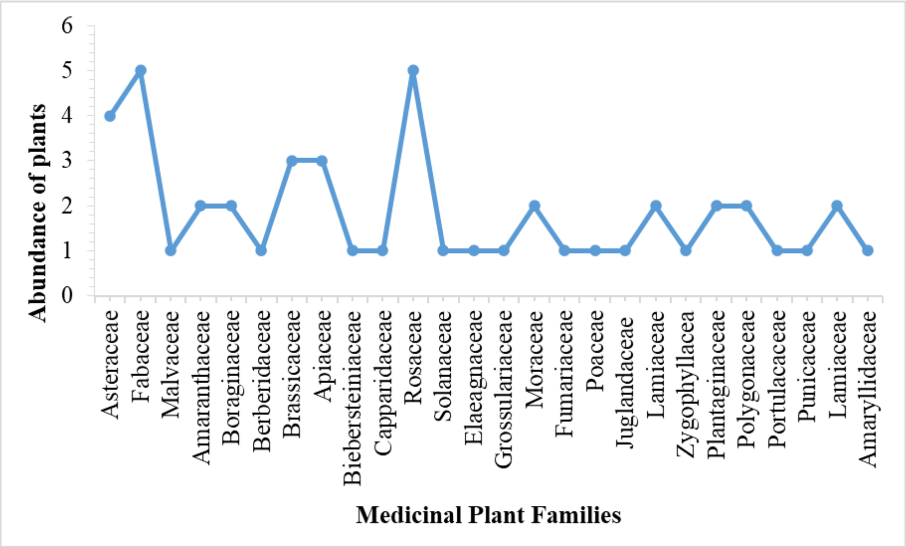

Figure 2. Illustrate plant families utilized as medicine for different ailments

Table 2. Documented genera of medicinal plants

Achillea	*Astragalus*	*Carum*	*Elaeagnus*	*Morus*	*Ribes*	*Ungernia*
Acroptilon	*Berberis*	*Cichorium*	*Ficus*	*Peganum*	*Punica*	*Ziziphora*
Alhagi	*Brassica*	*Conium*	*Fumaria*	*Plantago*	*Rosa*	
Althaea	*Bunium*	*Crataegus*	*Glycyrrhiza*	*Polygonum*	*Rumex*	
Amaranthus	*Biebersteinia*	*Crambe*	*Hordeum*	*Portulaca*	*Spinacia*	
Anchusa	*Capparis*	*Datura*	*Juglans*	*Prunus*	*Taraxacum*	
Artemisia	*Capsella*	*Descurainia*	*Marrubium*	*Psoralea*	*Trichodesma*	

and samples of the recorded trees, shrubs, and herbs were identified with the help of Medicinal Plants of Central Asia: Uzbekistan, and Kyrgyzstan, and other previous works (Eisenman *et al*., 2013). According to Jeppesen *et al*. (2012), “the flora of Badakhshan Mountains is in general related to the Tibetan and Central Asian floras”. An herbal shop survey was also carried out. Furthermore, we visited the shops in the city of Faizabad, and the center of Badakhshan province where traditional local healers (hakim) sell herbal medicines. MS. Excel 2013 was used in the analysis.

Result and Discussion

In the study, a total of 49 medicinal plant species belonging to 44 genera and 26 families were identified as having medicinal properties (Table 1). The highest percentage of medicinal plants belonged to the families of Rosaceae (10%), Fabaceae (10%), Asteraceae (8%), Apiaceae (6%), Brassicaceae (6%), Amaranthaceae (4%), and Boraginaceae (4%) in reducing order of frequency of reported utilization (Fig. 2). The percentage of medicinal plant diversity utilized for different ailments is summarized in (Fig. 2). In terms of species, herbaceous plants were the most abundant source of medicinal plants (80% of all species), followed by trees (14%), and shrubs (6%) (Fig. 3). The study found that 49 plant species have been used by local people to cure 17 diseases such as headache, dysentery, cough, fever, gastritis, hypertension, diarrhea, anemia, wounds, respiratory diseases, skin diseases, and gastrointestinal diseases, etc.

In this study, 136 informants were interviewed. Informants aged 50 and over generally knew medicinal plants better than younger informants. Some informants knew 12 medicinal plant names and utilized them to treat

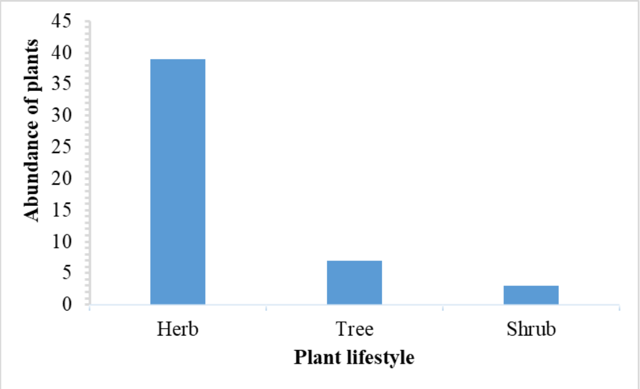

Figure 3. The lifestyle of medicinal plant diversity in the study area.

diseases; several of these plants were specifically utilized to treat gastritis and hypertension. The most mentioned medicinal plants were Ziziphora, Fern-leaf yarrow, Camel's thorn, Caraway, Liquorice, Syrian rue, and figs (Table 1). In this study, the author documented 49 species of medicinal plants belonging to 44 genera (Table 2) and 26 families in the Argo district of Badakhshan Province. The highest genera in the study region were Ziziphora, Crataegus, Glycyrrhiza, Plantago, and Rosa. Other genera have only one species. The majority of the medicinal plant species were found in the district's northern section and along the Kokcha river pathway.

The plant parts utilized for medical preparations were flowers, fruits, leaves, roots, seeds, and bulbs (Fig. 4). In some cases, the whole plant including the roots was utilized. The highest frequently utilized plants part were leaves (24%), roots (23%), fruits (17%), flowers (14%), seeds (12%), and shoots (10%). Roots generally contain a high concentration of bioactive compounds. It is worth mention that removing roots can have significant detrimental effects on plant regeneration and survival (Shrestha & Dhillion, 2003; Ssegawa & Kasenene, 2007; Uddin *et al*., 1970).

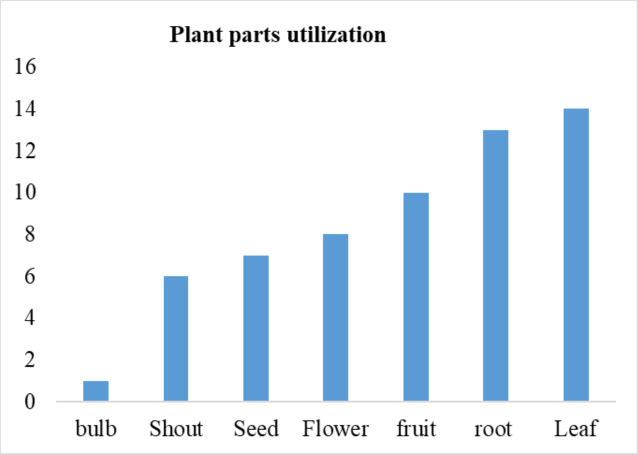

Figure 4. Illustrates medicinal plant parts used in various diseases in the study area.

The majority of those interviewed were familiar with the species' use in the treatment of several ailments such as gastritis, kidney infections, diarrhea, coughs, toothaches, fever, and headaches. Elders and close family friends or locals shared knowledge of the technique of collection, the portions used, and ailments that can be healed. However, other ailments, such as hypertension and abdominal problems, were mainly limited to elders and traditional medical professionals. During the survey, it was found that the Hakims (experts) in the region knew more about medicinal plants. Furthermore, elders, particularly women, are more informed than younger people, and indigenous knowledge about local plant species was passed down orally from one generation to the next. Local people collected medicinal plants mostly for their consumption (70%) followed by commercial purposes (30%) (Mukul *et al*., 2007; Sher *et al*., 2020). During the study we visited some herbalist shops in Faizabad city, where they sell hundreds of medicinal plant species, who shared information about tens of medicinal plant species inhabiting the Badakhshan region (Fig. 5).

Similar studies have been done in Afghanistan and other countries. Our outcomes correspond with research that reported that the people of the Shahgram valley in Pakistan's Swat district traditionally utilized 90 medicinal plants, of which (13) plants were utilized for diarrhea, and (11) for gastrointestinal diseases (Hassan *et al*.,

Figure 5. Displays herbalist shops in Faizabad City

2019); and a survey in the Phulbari Upazila region of Bangladesh that documented a total of 86 medicinal plant species used by the Santal community to treat a variety of diseases (Uddin *et al*., 1970). Furthermore, our findings agree with those reported by Amini (2017), who recorded 37 (54%) medicinal plants for the treatment of digestive system diseases from 68 medicinal plant species in the Guldara District of Kabul, Afghanistan. Another study documented that most of the medicinal plant species were found to have more than a single therapeutic utilization. In Southern Uganda, 163 medicinal plants (23.5%) were utilized to treat gastrointestinal diseases, and (0.5%) of the medicinal plant species were utilized for the treatment of cardiovascular, circulatory diseases, and hypertension (Ssegawa & Kasenene, 2007). Another study identified 144 medicinal plant species which are used to treat a total of 49 diseases. The highest numbers of medicinal plant species were recorded to cure coughs and fever (Pandey *et al*., 2017).

Afghanistan has varied geo-climatic conditions and possesses high medicinal plant diversity, hundreds of which are traditionally utilized. A comparison of medicinal plants in the region shows that the maximum medicinal plants were herbs, followed by trees and the lowest were shrubs (Table 3). Unfortunately, because of the continuous war and conflict, medicinal plant diversity was not surveyed in all provinces of Afghanistan. However, in 1987 it was reported that 215 medicinal plants were utilized traditionally in different regions of Afghanistan (Amini, 2017). Nonetheless, we were unable to collect medicinal plants from all provinces of Afghanistan. Hence, more efforts are required to identify and document medicinal plant diversity in Afghanistan. According to some experts, the Badakhshan province in Afghanistan has a vast range of medicinal plants.

Table 3. Comparison table of the medicinal plant used to treat different diseases.

The lifestyle of medicinal plants	Herb	Shrub	Tree	Total
Abundance of plants	**39**	**3**	**7**	**49**
Diseases treated by medicinal plants				
Stomach and Intestine infection	11	1	-	**12**
Hypertension	3	1	-	**4**
Kidney infection	3	-	1	**4**
Wound and skin diseases	5	-	1	**6**
Dysentery	2	1	-	**3**
Toothache	2	-	-	**2**
Heart diseases	-	-	3	**3**
Anemia	2	-	-	**2**
Diarrhea	3	-	1	**4**
Respiratory diseases	2	-	-	**2**
Fever	2	-	-	**2**
Headaches	1	-	-	**1**
Dyspepsia and diuretic	1	-		**1**
Hemostatic for post-natal bleeding	1	-	-	**1**
Analgesic for the sick person	1	-	-	**1**
Increase appetite	-	-	1	**1**
Total	**39**	**3**	**7**	**49**

In the study area, unsustainable harvest of medicinal plants, overgrazing, and habitat loss are very frequent activities that have a strong negative influence on medicinal plant diversity (Sakkir *et al*., 2012; Zlatković *et al*., 2014). Medicinal plants are a natural wealth and their conservation is important for ecological, economic, and scientific reasons. Currently, there are no previous reports on medicinal plant diversity from the northern part of Afghanistan. Therefore, these findings are critical for the conservation of medicinal plant species and future studies on traditional medicine.

Conclusion

In the current study, 49 medicinal plant species from 26 families were identified in the Argo district of Badakhshan Province. Herbs made up 80%, trees 14%, and shrubs 6%. Most of the medicinal plant species were found in the district's northern section and along the Kokcha river pathway. The parts of the medicinal plant utilized as medicine are leaves, roots, shoots, flowers, fruits, and seeds. The study found that 49 plant species have been used by locals in the study areas to treat a variety of ailments such as headaches and toothache, dysentery, cough, fever, gastritis, hypertension, diarrhea, anemia, respiratory diseases, skin diseases and gastrointestinal diseases, etc. However, unsustainable harvesting, overgrazing, and habitat degradation threaten the medicinal plant diversity in the study area.

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RESEARCH ARTICLE

Detection of Mediterranean Black Widow Spider, *Latrodectus tredecimguttatus* (Rossi, 1790) for the First Time in Jordan by DNA Barcoding and a Case of Envenomation Treated with *Ferula assa-foetida* L. (Apiaceae)

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Abstract

Objective: The Mediterranean black widow spider; *Latrodectus tredecimguttatus* (Rossi, 1790) could reasonably be regarded by many health practitioners as living solely in Southern Europe. However, its range extends well beyond the Mediterranean, into central Asia, and reaches parts of China. In this article, we detected this species in Jordan for the first time and confirmed the diagnosis by DNA barcoding. This is also the first clinically significant envenomation case in Jordan.

Materials and Methods: The spider was identified using DNA analysis which was extracted using a Qiagen DNeasy Blood and Tissue Kit following the standard protocol. PCR reactions were conducted in 20 µL volumes using 1 µL of DNA template, a final concentration of 0.5 µM of each primer. Cycling conditions consisted of an initial activation at 95°C for 15 min, then 35 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 30 s and elongation at 72°C for 1 min with a final elongation at 72°C for 10. Successful PCR reactions were cleaned using Clean NA Clean PCR magnetic beads (GC Biotech) using the standard PCR cleanup protocol. Bi-directional sequencing was conducted at Genewiz UK.

Results: The species was identified using morphological and molecular data. The clinical process was evaluated including the symptoms, medical treatment, and the use of *Ferula assa-foetida* as self-medicated traditional medicine.

Discussion: The first record of this spider is very important for the contribution of the biodiversity of Jordan. In addition, due to its medical importance, the envenomation case and its treatment with Freula are important for local practitioners. Such medically important animals and related cases need to be documented for public health. It is necessary to recognize the existence of venomous spiders in Jordan, and to study their patterns of bites. We also propose a set of recommendations for communities and local hospitals including the necessity of hospitalization and antivenin administration for patients exhibiting serious symptoms.

Conclusion: It is important to document envenomation accidents, symptoms, and treatment protocol. Furthermore, traditional medicine practices should be reported as they can interfere with or even hinder medical treatment. The results of this article will certainly apply to a wider range of countries in the region.

Keywords: Envenomation, Ethnopharmacology, Jordan, *Latrodectus tredecimguttatus*, Spider Bite DNA Barcoding

Introduction

Latrodectism is defined as the specific set of symptoms caused by alpha-latrotoxin and the other components present in the venom of *Latrodectus* spiders, commonly known as black widows (Nicholson & Graudins, 2002). Symptoms have been widely reported in the scientific literature and are well understood, varying considerably depending on the level of envenomation as well as other factors, and can begin within minutes after the bite, to several hours (Sutherland, 1983; White, 1987). Among the symptoms of latrodectism, the most severe cases can present skeletal muscle pain, constricting, diaphoresis, and hypertension (Kobernick, 1984), being potentially lethal due to respiratory and cardiac arrest in children (Vutchev, 2001).

Our knowledge about black widow spiders' diversity, their distribution, and human interactions in Jordan is very limited and is confined to a single record of the white widow, *Latrodectus pallidus* (El-Hennawy 2006, Shakhatreh *et al*., 2021). This manuscript reporting the first clinically significant envenomation of *L. tredecimguttatus* in Jordan is therefore also the first scientific record of this species in the country, despite its distinct morphological features. No research group has yet been established in Jordan focusing on spiders in general or the characterization of spider toxins in particular. This lack of information might have caused health practitioners in Jordan to overlook symptoms of latrodectism, potentially delaying effective treatment.

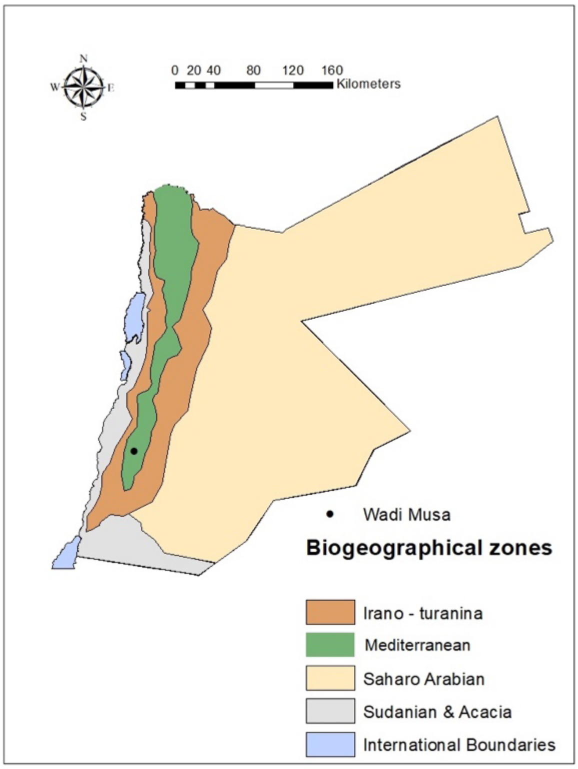

Figure 1. Location where the bite has occurred in relation to Jordan's biogeographical zones (Credit: Ehab Eid).

Materials and Methods

Site Description

The bite occurred in Wadi Musa, which is in the Ma'an governorate south of Jordan (Fig. 1), within the Mediterranean bio-geographical zone, which is characterized by annual rainfall ranges from 400 to 600 mm and a summer temperature rarely exceeding 30°C. The specimen of *L. tredecimguttatus* (Fig. 2) assumed to be responsible for the bite was found in Mediterranean vegetation consisting mostly of shrubs and bushes, dominated by *Rhamnus palaestinus*, *Artemisia herba-alba, Calycotome viliosa, Sarcopoterium spinosum,* and *Cistus* spp. This habitat type takes up a considerable portion of the country overlapping with multiple sites of sizable human presence and use.

Figure 2. *L. tredecimguttatus* collected from the envenomation study site (Credit: Hussein Alnasarat)

Case Study

A 29-year-old male was admitted to the Queen Rania hospital in Ma'an governorate located in the southern part of Jordan on the 25th of June 2020 with a self-diagnosed spider bite. The patient stated that around 08:00 pm Jordan time, as he was placing his shoes to leave a picnic on a field, he felt a sudden stinging pain lasting for around 5 seconds in the middle of his right toe. The patient did not witness the bite taking place, but when removing his foot from the shoe noticed a spider on that same toe. The spider was killed as it tried to escape and was collected by a friend who escorted him to the hospital along with the sample (analysis of this specimen is described below).

The patient described a clear sensation of pain starting immediately after the bite, but it was localized over the assumed bite area (the toe). However, the pain sensation gradually intensified and became very sharp, with the patient describing the feeling as if his foot had a fire ember on it. A tourniquet was self-applied below the knee and was in place for approximately 10 minutes, the time it took to reach the hospital after the incident. Once a nurse removed this ligament, the patient described the pain as spreading all over his leg and into the rest of the body.

Upon arrival, the patient was moved to the Intensive Care Unit as further systematic symptoms appeared, which included general weakness, dizziness, numbness, and generalized pain. Beyond the topical symptoms most probably because of the general pain situation, which included generalized pain in his body, there was complete paralysis and inability to move, slurred speech, numbness in the legs, and a constant tingling and heat in the foot.

Blood tests showed an increased level of liver enzymes including Aspartates aminotransferase and alanine aminotransferase, indicating toxicity and inflammation in the liver, while also displaying elevated levels of white blood cells, especially neutrophils. The patient also displayed an increased heart rate (90-125), high temperature (around 39°C), high blood pressure reaching 192/111 mmHg, and developing a skin rash that started 10 days after the bite.

The hospital treatment included administering glucose, antibiotics, and antipyretics. However, once the administered medications proved unable to mitigate the main symptoms, which remained severe even two days after the incident, the patient's family decided to resort to traditional medicine, specifically the use of *Ferula assa-foetida*. The resin of this plant was boiled in 200ml of water, and the patient drank from the entire dose only once, reporting intense sweat within 5 minutes, which lasted for six continuous days and decreased gradually. The patient continued his treatment at the hospital, where he left on the 28th of June 2020, while also reporting improvements in overall health and symptom mitigation daily. Ten days after the patient departed from the hospital, he presented a rash on his back, abdomen, and hands.

Analysis

The spider was promptly identified as a black widow, but the species identity was much harder to ascertain with accuracy, as no records of these species had been previously made and this was the first recorded bite of this group in the country. The animal that is inferred to have bitten the patient was killed on site, causing its morphology to be partially damaged, but its remains were collected and preserved in 70% ethanol, and later mounted with 5% glycerin in 50% ethanol on a microscope slide, to be analyzed and photographed. The specimen was not sexually mature and did not present any bifurcated spiders that are characteristic of *L. tredecimguttatus*.

An immature specimen sharing a similar habitus was later collected from the same site for molecular analysis. Similarly to the previous specimen, it did not present any dorsal bifurcated hairs. DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit following the standard protocol. PCR reactions were conducted in 20 µL volumes using 1 µL of DNA template, a final concentration of 0.5 µM of each primer (LCO1490, HCO2198 (Folmer *et al*., 1994) and 4 µL of 5×HOT FIREPol Blend Master Mix Ready to Load with 3.0 mM $MgCl_2$ (Solis BioDyne). Cycling conditions consisted of an initial activation at 95°C for 15 min, then 35 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 30 s and elongation at 72°C for 1 min with a final elongation at 72°C for 10. Successful PCR reactions were cleaned using Clean NA Clean PCR magnetic beads (GC Biotech) using the standard PCR cleanup protocol. Bi-directional sequencing was conducted at Genewiz UK.

Results

The specimen was not sexually mature and did not present any bifurcated spiders that were characteristic of *L. tredecimguttatus*. According to DNA barcoding study results the sample has a 100% BLAST ID with two previously sequenced *Latrodectus tredecimguttatus* specimens (GenBank codes: KC414085 and KC414084; Garb & Hayashi, 2013). Although we would question the geographical origin of KC414084, as it is labeled as Namibia/S. Africa border, a region where it seems unlikely for this species to have been introduced into, and therefore labeling error appears more likely."

Discussion

Knowledge about the black widow spiders in Jordan is still very limited and is confined to a single record of the white widow spider; *Latrodectus pallidus* (El-Hennawy, 2006; Shakhatreh *et al*., 2021). This article has expanded our knowledge on new species of widow spiders from Jordan and added details for the first time on an envenomation case and using folk medicine for treatment.

The incidents of envenomation by spiders are very limited worldwide. Watson *et al*. (2003) reported no lethal cases by *Latrodectus* envenomation according to the American Association of Poison Control Centers since its first annual report in 1983 until 2004. However, lethal cases involving *L. tredecimguttatus* have been reported in Spain (González *et al*., 2001), Greece (Pneumatikos *et al*., 2003), and through Albania (Hoxha, 2006). As the first case reported from Jordan, it should give healthcare practitioners pause to consider spiders as a potential source of envenomation, by terrestrial animals, on par with snakes and scorpions that have more commonly been reported in the region. This will require them to disambiguate among these species and recognize the signs and symptoms as quickly as possible to apply the appropriate health care for the patients. This process could be expedited through systematic data collection from patients that suspect to have been bitten, including accompanying escorts, and by requesting to observe the spider, ideally, as a preserved specimen, when it is safe to collect, or via photographic record. Although preserved specimens can be an ideal source of reliable information for health practitioners, black widow spiders can be difficult to identify at the species level, particularly in their immature stages, when some diagnostic characters are missing. While some characters that have been considered distinctive in the literature, i.e., the presence of bifurcated dorsal hair in *L. tredecimguttatus*, might be less reliable than once assumed. As seen in the case presented here, DNA analysis has proven to be an effective way to overcome some of these taxonomic challenges, but it will unlikely provide a timely answer to aid treatment. Fortunately, black widows can be quite distinctive from other spiders and several antivenoms have been developed against *Latrodectus* spp. in various parts of the world (Bildik *et al*., 2021). Furthermore, a case reported in Italy showed that the antivenom of *L. mactans* (Antivenom *L. mactans*, Merck Sharp & Dohme®) is effective to treat the bite of *L. tredecimguttatus* (Di Paola *et al*., 2020). Therefore, a species-specific identification might not be necessary from a medical standpoint to initiate treatment.

Best practices for first aid and medical treatment of latrodectism are relatively well understood, however, the application of tourniquets can still be in use (as in the case study presented here) despite having no scientific evidence to support this practice and having been discouraged since the early 80’s (Sutherland & Duncan, 1980). Meanwhile, health practitioners in several regions of the world likely over diagnose spider bites (i.e., misidentifying a range of cutaneous reactions as bites), while at times failing to recognize the symptoms of latrodectism, potentially delaying targeted treatment (Rochlin *et al*., 2021). Additionally, medical treatment efficacy may be further confounded using traditional medicine, information that patients might potentially not always share.

The plant *Ferula assa-foetida* (commonly known as Asafetida) is mostly grown in Iran and Afghanistan (Mahendra & Bisht, 2012), but its use has been reported in several regions of the world, mostly in Asia, Southern Europe, and Northern Africa (Iranshahy & Iranshahi, 2011), used for its aromatic properties, as a food condiment (particularly in India and Iran) and in traditional medicine (Amalraj *et al*., 2017). The current traditional medicine literature reports *F. assa-foetida* being used in a wide variety of ways for a vast range of medical issues (Iranshahy & Iranshahi, 2011; Javaid *et al*., 2012; Mahendra & Bisht, 2012). These include mixing it with garlic to treat snakebites (Bhattacharjee, 2004), its gum being dissolved in olive oil as a topical treatment for insect and snake bites (Mohammadhosseini, 2016) or reported to treat scorpion stings (Javaid *et al*., 2012), often misreported as scorpion bites. However, our bibliographical search only found recorded uses of *F. assa-foetida* in Jordan, consumed as a sugar reduction to induce abortion (Lev & Amar, 2002).

Ferula assa-foetida has never been reported for the treatment of spider bites, but its overuse has been reported to cause diarrhea, tympanites, headaches, and dizziness (Eigner & Scholz, 1999), as well as prevent platelet adhesion, lowering blood pressure (Mohammadhosseini *et al*. 2019). Side effects that confuse diagnosis as they overlap with latrodectism and might mislead health practitioners about the effectiveness of treatment. We recorded for the first-time black widow spider as a cause of envenomation in Jordan, while recording the self-prescribed use of *F. assa-foetida* as traditional medicine in its treatment, hoping it will help communities and local hospitals to take appropriate steps, potentially including targeted anti-venom, while triggering efforts at regional level toward this group of animals.

Conclusions

It is very important that healthcare facilities recognize the existence of venomous spiders, recognize their patterns of bites, and distinguish the symptoms of *Latrodectus* envenomation. This will help to provide immediate care to *Latrodectus* bite victims. In addition, understanding the distribution range is important since it helps to develop precautionary measures and reduce the risks of accidents to people, especially in rural communities, and tourists. Hospitalization and antivenin administration should be secured and reserved for patients exhibiting serious symptoms. This is important as people who report these factors should receive antivenin as soon as possible to avoid suffering envenomation complications.

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SHORT COMMUNICATION

A Possible Biomass Contribution to Fauna of Finike Seamounts by Terrestrial Insects

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Abstract
Although insects are defined as terrestrial organisms, they can occasionally be encountered in samples taken from the sea. What makes it special in our study is that terrestrial insects were caught from the open sea while sampling the planktons. As a result of our study, insect samples were collected from 8 different stations by using the plankton traps. These specimens found in the fauna of Finike Seamounts were not aquatic insects but terrestrial and were considered specimens that accidentally drifted away into the sea. This causes us to evaluate the possibility that they may have the potential to make a biomass contribution to the marine ecosystem. Further investigation of the subject and revealing possible insect biomass contributions in the sea constitute an important horizon for future studies.

Keywords: Marine insects, Diptera, Homoptera, Biodiversity, Finike, SEPA, Plankton trap

Introduction

Insects are a kind of special animal group that contains the largest amount of biodiversity on earth. However, they are dominantly known in terrestrial habitats. Only 25,000-30,000 species of insects (nearly 3% of all insect species) have aquatic life stages (Cheng, 1976). Among these, only several hundred species are associated with marine habitats (Cheng, 1976; Springer, 2009). However, many of the tiny insects can be drifted away even by weak breezes and they may travel hundreds of kilometers. (Bowden & Johnson, 1976). Moreover, some storms and other atmospheric events may also help some insects to be dispersed among continents or islands (Peck, 1994). Such passive dispersal of insects may have caused them to sink into marine habitats so that insects can be sampled from the sea (Bowden & Johnson, 1976). Even though it has not been thoroughly evaluated or calculated, Bowden and Johnson (1976) have reported that this type of biomass contribution may have a significant impact on the marine ecosystem.

In the eastern Mediterranean Sea (Levantine Basin), Finike (Anaximander) Seamounts are located off the southern coast of Turkey and west to Cyprus Island. The peaks are 700-1100 m deep, elevating from the sea floor at 1500-2000 m deep. The Finike Seamounts have peculiar ecosystem characteristics, such as cold seeps, hydrothermal chimneys, and mud volcanoes, where chemosynthesis takes

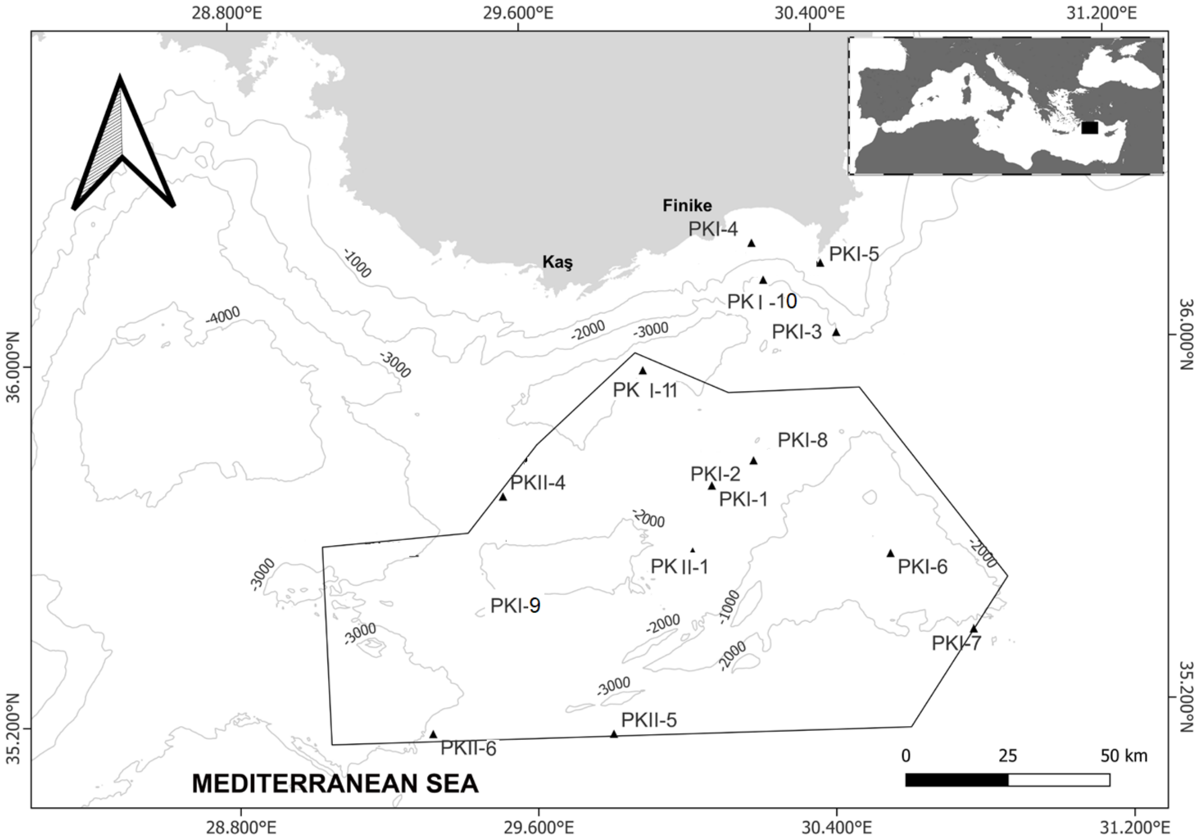

Figure 1. The map of the sampling stations for the plankton traps.

place in the dark deep sea. This is particularly important for the biodiversity of the eastern Mediterranean Sea but there are limited studies available about the marine biodiversity of the Finike Seamounts. The area was declared a "Finike Seamounts SEPA (Special Environment Protected Area)" on 16 August 2013 by the Turkish government due to its biological and ecological importance. Some cetaceans, benthic and other pelagic species such as sea birds, sea turtles, and large migratory fishes were recorded previously in the Finike Seamounts SEPA (Öztürk, 2022; Onmuş *et al*. 2022). To elaborate an effective management plan for the protection of this SEPA, research, and monitoring have been initiated in the area as well as in its surrounding water by the Turkish Ministry of Environment, Urbanization and Climate Change (TMEUC).

Table 1. The data and the coordinates of sampling stations.

Date	Sampling stations	Coordinates	
14.05.2021	PK(I)-1	35^{0}42’46.20”K	30^{0}05’60.60”D
14.05.2021	PK(I)-2	35^{0}45’45.10”K	30^{0}12’41.64”D
14.05.2021	PK(I)-3	36^{0}02’11.50”K	30 26’58.44”D
15.05.2021	PK(I)-4	36^{0}14’31.40”K	30^{0}13’24.18”D
16.05.2021	PK(I)-5	36^{0}11’36.91”K	30^{0}24’38.46”D
19.05.2021	PK(I)-6	35^{0}32’54.10”K	30^{0}34’14.00”D
19.05.2021	PK(I)-7	35^{0}22’14.00”K	30^{0}47’16.26”D
20.05.2021	PK(I)-8	35^{0}46’24.00”K	30^{0}15’21.00”E
20.05.2021	PK(I)-9	35^{0}10’58.60”K	30^{0}12’28.40”D
24.05.2021	PK(I)-10	35^{0}55’44.22”K	30^{0}12’11.00”D
26.05.2021	PK(I)-11	36^{0}01’23.10”K	30^{0}54’18.20”D
16.09.2021	PK(II)-1	36°40’00.39”K	28°02’73.92”D
18.09.2021	PK(II)-2	35°39’30.44”K	29°04’96.27”D
25.09.2021	PK(II)-3	35°10’33.30”K	29°19’26.04”D
25.09.2021	PK(II)-4	35°34’07.38”K	29°03’63.88”D
26.09.2021	PK(II)-5	35°09’75.11”K	29°48’45.23”D
27.09.2021	PK(II)-6	35°01’61.89”K	30°27’36.31”D

Figure 2. Terresterial insects that found in plankton traps. A: Aphididae (Homoptera), B: Pentatomidae (Hemiptera), C-D: Hymenoptera, E: Chrysomelidae (Coleoptera) F: Carabidae (Coleoptera). Scale represent 1 mm.

We present here some very limited but sporadic data on terrestrial insects that were blown out to sea and eventually deposited in the Finike Seamounts (Fig. 1). Insects sampled in this study are reported for the first time from the area. The accumulation of such records would provide us with information about a potential biomass contribution to marine ecosystems.

Materials and Methods

In the framework of monitoring studies in the Finike Seamounts as a “Special Environmental Protection Area” in the Mediterranean Sea, two surveys on a research vessel YUNUS-S, Faculty of Aquatic Sciences, Istanbul University, were carried out in May and September 2021.

Plankton samplings were carried out in the pelagic zone by RV YUNUS-S in May and September 2021 (Table 1). The plankton net had a mesh size of 300 µ, 133 cm in diameter, and 280 cm in length. Terrestrial insect samplings were obtained from the surface with the plankton net in the pelagic zone. Sampled insects were separated from other arthropods and were identified to the order or family categories.

Results

Among 22 sampling stations (Table 1), seven of them were containing insect specimens (Table 2). The most encountered insect groups were Homoptera members (some aphids), Coleoptera members (some carabids), Lepidoptera members, Diptera members (some culicids), and Hymenoptera members (Fig. 2). The abundance of encountered taxa did not been considered. Only the presence of the groups was recorded (Table 3).

Table 2. The list of the insects and arachnids observed among the research area.

Sampling Stations	Samples
PK(I)-4	Diptera
PK(I)-9	Homoptera
PK(I)-10	Coleoptera, Homoptera
PK(1)11	Coleoptera, Homoptera, Hymenoptera, Diptera, Lepidoptera
PK(II)-1	Diptera
PK(II)-4	Arachnida, Hemiptera, Homoptera
PK(II)-6	Diptera, Homoptera

Table 3. The presence data of the insects that was found from the sampling stations.

Sampling Stations	Number of different groups	Homoptera	Coleoptera	Hymenoptera	Arachnida	Hemiptera	Diptera	Lepidoptera
PK(I)-2	0							
PK(I)-3	0							
PK(I)-4	1						X	
PK(I)-5	0							
PK(I)-6	0							
PK(I)-7	0							
PK(I)-8	0							
PK(I)-9	1	X						
PK(I)-10	2	X	X					
PK(I)-11	5	X	X	X			X	X
PK(II)-1	1						X	
PK(II)-4	3	X			X	X		
PK(II)-5	0							
PK(II)-6	2	X					X	

Discussion

The result of our study was parallel with the findings of Cheng & Birch (1977). It was reported that the terrestrial insect samples from the sea comprise the members of Homoptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera. The majority of the successful samplings consist of the same insect orders.

Insects blown out to sea may provide a considerable amount of organic matter to the surface water. Bowden & Johnson (1976) estimated that at a time of maximum diurnal insect activity, 560 million aphids, or about 2200 million insects per km^2, may be available over the land to be blown over the western part of the North Sea. We cannot have concrete data to make a comparison with this estimation however these insects, especially aphids and bugs (Fig. 2A-B) can be abundant on land and have the potential to be drifted away by the winds. Springtime in Mediterranean habitats is the bloom season for many insect groups. Especially aphids may colonize host plants with thousands of members at that period. And since they are very tiny, they can be easily dispersed by strong wind currents and thus might throw them into the sea. Afterward, sea currents may distribute this organic biomass thorough hundred kilometers away from the shore. So, they can be collected with a plankton net. The distribution of terrestrial insects over the sea may depend on the season, the wind speeds, the marine currents, and the size of the insects as well. It is also possible that these insect species, which were dragged into the sea by various factors, were eaten or fragmented by different living communities. Considering this possibility, it is obvious that the diversity of insects drifting into the seas and affecting the ecosystem may be more than we found. However, lack of data concerning the contribution of such biomass content, more systematic attempts to measure such deposition should be made in the future.

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