Isolation and Identification of Copepods Indivuals from Kufa River Molecular Diversity Study

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Abstract:
Copepods are key components of aquatic ecosystems and can help regulate the global carbon cycle. Much attention has been paid to the species diversity of copepods worldwide, but the genetic diversity of copepods in Al kufa river is unexplored. Random amplified polymorphic DNA (RAPD) technique was used as a tool for assessing genetic diversity and species relationships among four species from Cyclopedia family Cyclops, Cyclops strenuus , Macrocyclops and Microcyclop. and Two species from the Diaptomidae family ,Diaptomus sp ,Nauplius . These samples were collected from a different region in the Kufa River in Najaf - Iraq . Three primers were used (OPC2 ,OPC8 and BH11) in Copepods species and the value of Jaccard's coefficient ranged from 0.46 to 0.06. Based on the bivariate (1-0) data and genetic similarity with the use of the UPGMA cluster method, the derogram separated the studied species. Our findings explored a high species diversity of copepods that was detected over a small geographic sampling range .Results from this study contribute to a better understanding of copepod diversity of Al Kufa river by using RAPD technique, an efficient technique for studying the molecular characterization and used for resolving relationships among copepod populations.

Keywords: Jaccard's coefficient, Diaptomidae family ,Diaptomus sp ,Nauplius

Introduction

Copepods are one of the most taxonomically diverse groups of crustaceans, containing approximately 14,000 described species globally (1). Copepods can be found in most kinds of aquatic habitats because of their remarkable evolutionary adaptability (1, 2). They are key components in aquatic ecosystems, playing an important role in food webs (3, 4) and living as endo- or ectoparasites associated with aquatic animals (2, 5, 6). Many previous studies have shown that copepods are sensitive to climate change (7, 8), because the range of copepods could track the rate of climate change (7). Copepods can also help regulate the global carbon cycle (9, 10), and they can be used as indica- tors to natural and anthropogenic environmental stressors by tracing their responses to the elevation of atmospheric CO_2 levels (11). Thus, much attention has been paid to the biodiversity of copepods in aquatic ecosystems (12, 13). However, there are some problems related to the classification of certain species and species within the tribes and subfamilies. Genetic divergence and convergence between two genotypes using biotechnologies, which provided modern methods of detection and differentiation between genotypes and show the extent of genetic divergence between them. Molecular taxonomy is one of the most important aspects of evolution in the last decade, with the application of DNA or RNA data to help solve most taxonomic problems by diagnosing or inferring the relationship between living organisms.

Molecular taxonomists believe that molecular data are more likely than phenotypic data to know the true ethnic origin of organisms because they reflect changes at the gene level and didn't directly affected by environmental changes such as those with phenotypic traits (14).

Several molecular technologies have been implemented in the Copepods Classification, such as protein techniques, which include amino. acid sequencing techniques and the electrical transfer of Enzyme electrophoresis, as well as DNA-related technologies, including Restriction Fragment Length Polymorphisms (RFLPS), Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), and the sequencing of the DNA (DNA Sequences). For this reason, molecular taxonomy has made tremendous achievements through all the data that have become available over the last 50 years in classification (15). In this study, RAPD based on polymerase chain reaction (PCR) was adopted because this technique is fast, easy and requires less time (16) to study the molecular variations.Due to the lack of molecular studies in Iraq for the different species under study. This study aims to study genetic diversity and determine the genetic relationship between species based on the degree of genetic similarity between them and determine the DNA of each species under study using RAPD technology.

Genetic diversity

Genetic diversity is a study undertaken to classify an individual or population, compared to other individuals or populations. It quantifies the magnitude of genetic variability within a population which is a fundamental source of biodiversity. Genes are the fundamental unit of biodiversity, the raw material for evolution, and the source of the enormous variety of plants, animals, communities, and ecosystems that we seek to protect, admire and use. Genetic variation shapes and defines individuals, populations, subspecies, species, and ultimately the kingdoms of life on earth. Genetic diversity among individuals reflects the presence of different alleles in the gene pool, and hence different genotypes within populations. Genetic diversity should be distinguished from genetic variability, which describes the tendency of genetic traits to vary within populations (17). Since the beginning of the 20th century, the study of genetic diversity has been the major focus of core evolutionary and conservation biology. The theoretical metrics developed, such as genetic variance and heritability (18),(19), provided the quantitative standards necessary for the evolutionary synthesis. Further research has focused on the origin of genetic diversity, its maintenance and its role in evolution. Simple questions such as "who breeds with whom" initiated studies on the relatedness of populations.

These investigations led to the formation of 'metapopulation' theory, where a group of spatially separated populations of the same species interact at some level and form a coherent larger group (20). The discovery of spatial structure in populations was a key element in the early concepts and models of population ecology, genetics and adaptive evolution (21). How different levels of genetic variation affect the rate of evolutionary change within populations has also been intensively studied. Subsequently, the detection of genetic variation has become more sensitive, firstly, through the utilization of variations in enzymes (allozymes) and then through PCR-based marker systems, allowing direct examination of DNA sequence variations. The precise detection of genetic variation/diversity has greatly enhanced the studies of evolution. There is no doubt that the genetic variation influences the fitness of individuals, and that this is reflected in natural selection. In this regard, individual genotypes must vary in ecologically important ways. Ecological adaptation is a significant factor for example, in range expansion of different species. Species with different genotypes conferring the highest levels of fitness are expected to survive and reproduce better, shifting the gene pool over time towards higher frequencies of the alleles making up the more successful genotypes (22).

Fisher (1930) reported that when an increase in fitness is allowed, genetic diversity can increase the population growth rate, but only if the population is not regulated by other factors and if it is experiencing directional selection. Despite the presence of genetic variation in ecologically important traits, relatively little is known about the range of potential ecological effects of genetic

diversity on population dynamics, species interactions and ecosystem processes (23). This has led to the rise of the field molecular ecology, which integrates the application of molecular population genetics, phylogenetics and genomics to answer ecological questions. Information about genetic diversity is necessary for the development of appropriate strategies in conservation biology as well as in many other applied fields. From a basic evolutionary standpoint, genetic diversity is assumed to be crucial for the evolutionary potential of a species. Research programs that aim to investigate population structure provide evolutionary insights into the demographic patterns of diverse organisms (24). Furthermore, knowledge of population structure of genetic resources is necessary for the development of strategies for appropriate conservation of genetic diversity. Molecular phylogenetics and genetic diversity analysis can help to clarify the taxonomic identity and evolutionary relationships of the wild species. These methods can also help prevent missidentification and can carefully plan effective germplasm management strategies. Variability and genetic diversity are important factors in evolution and also in applied sciences because they determine the responses of a given organism to, for example, environmental stress, natural selection and susceptibility to different diseases.

Importance of Genetic Diversity

Genetic diversity is a trait for both for an individual or a population and is characterized by the percentage of heterozygous alleles in diploid organisms (25). Genetic blueprint is the fundamental of all living organisms that carry a specific genetic fingerprint. This is true irrespective of them being plants, animals, or fungi, whether they are short or long-lived and whether they reproduce sexually or asexually. Therefore, conservation, genetics and conservation genetics play a role to a large extent for the restoration of living organisms. Although the basic design underlying the conservation genetics may be very familiar, a very little attention has been made to genetic considerations with respect to conservation genetics. The genetic variability has an immense effect and has a great importance on the survival and reproduction of any organism as well as populations .

Genetic diversity helps to adapt to environmental variability

Organisms live in complex environment that vary in spatial and temporal scale and is characterized by several factors such as weather, disturbance events, resource availability, population sizes of competitors, etc. (26). If a group of organisms were to live in a completely stable physical and biological environment, then a relatively narrow range of phenotypes might be optimally adapted to those conditions. Under these circumstances, organisms would benefit more by maintaining a narrow range of genotypes adapted to prevailing conditions, and allele frequencies might eventually attain equilibrium. By contrast, if the environment is patchy, heterogeneous, unpredictable over time, or includes a wide and changing variety of diseases, predators, and parasites, then subtle differences among individuals increase the probability that some individuals against others, will survive to reproduce i.e., the traits of the organisms are exposed to natural selection. Since differences among individuals are determined at least partly by genotype, population genetic theory predicts that in variable environments a broader range of genetic variation or higher heterozygosity will persist (27, 28 ,29 ,30). Any population can tolerate the stochastic environmental variations through genetically controlled traits. These traits are important from the stand point of resistance and resilience ability of the population to tolerate freezing conditions, drought or inundation, high or low light availability, salinity, heavy metals, soil nutrient deficiencies, extreme soil pH values, fluctuating temperature, dissolved oxygen, novel diseases in all groups of organisms (31).

Plant populations often include individuals with a range of phenological calendar. For instance, Great Basin shrub populations include individuals that leaf out and flower over a period of

weeks, increasing the likelihood of persistence of the population through periods of unusually early or late growing conditions (32). Knapp et al. (2001) documented flowering periods in a population of individual blue oak trees and found that trees initiated flowering over a period of a month in the spring. A diverse array of genotypes appears to be especially important in disease resistance (33 ,34). Genetically uniform populations are occasionally vulnerable to diseases and pathogens and such uniformity also predisposes a population to transmit disease from one individual to another by direct contact or proximity. More diverse populations are more likely to include individuals resistant to specific diseases. Moreover, infected individuals occur at lower density, and thus diseases or pathogens may move more slowly through the population. Finally, genetic variation is a factor in competition among individuals in real ecological communities. Among animals, behavioral traits may regulate inter-specific competition. Since organisms make energetic or life history trade-offs among traits (for example, allocating energy between growth and reproduction), genetic variability is an important factor with regard to how populations function (35 ,36 ,37 ,38 ,39 ,40) .

Ecology and Genetic diversity

Genetic diversity is a measure that quantifies the magnitude of genetic variability in the natural populations and is the fundamental source of biological diversity in nature. Over the nine decades the study of genetic diversity is considered as a principal domain for evolutionary and conservation biologists (41 , 18). The genetic diversity provides the raw material for evolution by natural selection, influences the fitness of individual genotypes and vary in ecologically important ways (18 ,42 ,43). However, the simple presence of heritable trait variation does not mean that different levels of genetic diversity will have predictable ecological consequences. For example, by increase in fitness, genetic diversity can increase in population but only if the population is not regulated by other evolutionary factors (18). Thus, despite the obvious presence of genetic variation for ecologically important traits, we know relatively little about the range of potential ecological effects of genetic diversity for population dynamics, species interactions and ecosystem processes (Figure 1). have reported the short term ecological effects of genetic diversity in small or endangered populations (44). Several agricultural practices has been carried out where genetically modified. crops have been harvested for better yield and production, as well as decreased risk of herbivores and pathogens (45). Mainly three lines of evidences lend foundation of the study of the ecological effects on genetic diversity. First, the ecological consequences of genetic diversity has focused on how the number of species and functional groups e.g. trophic structure within communities affects the stability and functioning of the ecosystem (46).

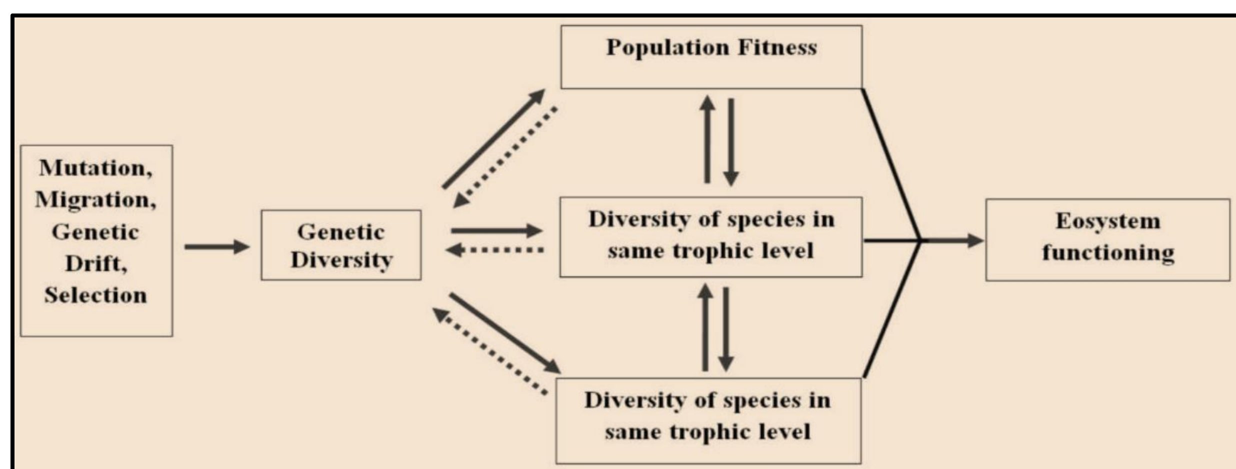

Figure 1: Processes underlying potential direct and indirect impacts of genetic diversity on the ecological functioning. Solid black lines indicate direct ecological consequences of genetic diversity; dotted lines indicate effects of natural selection, which depend on genetic diversity.

Secondly, there is a growing interest on the ecological effects of the variance component around the mean within the experimental or observable units of a particular describable variable (47). Several lines of research provide detailed information regarding how the genetic differences between individuals have influenced the species interaction and the interplay between the genetic and ecological dynamics (48). Finally, the community genetics has bridged the fields like evolutionary biology,

population genetics and community ecology (49). Community genetics focuses the genetic diversity as a hierarchical concept and is not limited to single taxonomic and genetic level. Therefore, the variation in ecologically important traits such as growth rate, competitive ability, immune function, virulence etc., the amount of genetic diversity at any level of population can have important ecological effects.

Molecular tools to assess Genetic Diversity

The most powerful catalyst in the field of conservation has been the advances in genetic and molecular technologies, leading to a wide variety of molecular methodologies for application in conservation and population genetic studies. To date, molecular methods have been applied vastly in conservation biology primarily as selectively neutral molecular tools for resolving the empirical questions of conservation and evolutionary relevance (50). The first step of molecular biological technologies in the field of conservation genetics was taken up in 1960 with the introduction of protein polymorphism analysis (51), followed by the mitochondrial DNA (mtDNA), Restriction Fragment Length Polymorphism (RFLP)-based methodologies (52), Randomly Amplified Polymorphic DNA (53), and more recently by microsatellite marker-based technologies (54), Inter Simple Sequence Repeat (ISSR) markers (55), Diversity Array Technology (DArT) (56) and other high throughput platforms. Therefore, the applications of particular types of genetic markers are becoming more and more specialized to achieve a particular goal to solve the specific questions of conservation concern (57). The ‘genomic era’ was started when the genome of the evolutionary, ecologically and commercially important model organisms were successfully sequenced (58). There are several benefits like larger number of molecular markers of a wide variety of organisms, SNP and microsatellite marker’s high sophistication and additional use of neutral markers that enhance the fidelity of conservation study, enabling researchers to look deep into the problems regarding conservation concern (59 ,58). Expressed Sequence Tag (EST) libraries are the valuable resource for the study of conservation and evolutionary genetics using bioinformatic tools by CASCADE databases, called in silico SNP mining pipeline (60). More recently, genomic technological advances like Next Generation Sequencing (NGS) technologies and “deep sequencing” or “ultra-high throughput sequencing” technologies (61) provide a wider path to understand the empirical questions regarding the conservation genetics of model as well as non-model organisms.The Randomly Amplified Polymorphic DNA (RAPD or AP-PCR) method was adopted to this study.

Randomly Amplified Polymorphic DNA (RAPD or AP-PCR)

RAPD was the first PCR-based molecular marker technique developed and it is by far the simplest method for genetic diversity analyses (53), especially when other sophisticated markers are unavailable. Short PCR primers (approximately 10 bases in length) are randomly and arbitrarily selected to amplify random DNA segments throughout the genome, hence the “Randomly” or “Arbitrarily Primed PCR (AP-PCR)”. The resulting amplification product is

generated at the region flanking a part of the 10 base pair priming sites in the appropriate orientation. RAPD products are usually visualized on agarose gels, after staining with ethidium bromide in a particular concentration. RAPD markers are easily developed and because they are based on PCR amplification followed by agarose gel electrophoresis, they are quickly and readily detected in very short time. RAPD technique was used extensively in studying genetic diversity within/ between plant species and animal species. Most RAPD markers are dominant and therefore, heterozygous individuals cannot be distinguished from the homozygotes. This contrasts with RFLP markers which are co-dominant and therefore, can distinguish among the heterozygote and homozygotes. Thus, in contrast to standard RFLP markers and especially VNTR loci, RAPD markers generate less information per locus examined. One disadvantage of using RAPD technique is the reproducibility between different gel runs which is due to the short primer length and low annealing temperature. However, if carefully chosen and PCR conditions standardized, RAPD gels can be of much value in certain situations.

Material and Methods

2.1: The Study Area

The Euphrates River is one of the important rivers for water supply and irrigation .It is formed by union of two rivers Kara and Murad in Turkey, then flows through Syria into Iraq, in Iraq, the river enters its delta between Hitt and Ramadi. It is divided into two main channels, Shatt Al-Hindiya and Shatt AlHilla. At Al-Kifl city, the Euphrates is subdivided into two parts: Al-Abassia and Al-Kufa River, the last one extends from Al-Kifl city via Al-Najaf province to Al-Diwania city. The water level in this river undergoes large fluctuations, there are domestic, municipal wastewater and agriculture drainage discharged to the river; in addition to the industrial wastes that come from: the industrial region in Al-Najaf city, the leather industry, and the cement factory, all of above have affecting on aquatic organisms (62).

2.1.1: General Description of the Stations

For the purpose of this study three stations have been chosen (Table 1 ,Figure 2):

1. Station One(St1) :

It is located near of Al-Emam Ali Bridge ,this station is characterized by the absence of any industrial or human activity north of it, except for agricultural activities.

2. Station two (St2) :

It is located near Al-Kufa Iron Bridge, about 2.5 km away from station one This station is characterized by the presence of a chain of restaurants that throw their waste into the river .

3. Station three (St3):

It is located near Al-Kufa old Bridge, about 566 m away from station two (close to the guest house of the governorate) before the river forks and pours into this station a stream to drain rainwater, which is part of the old network of the Kufa city .

Table 1: Geographical positions of the studied stations in Al-Kufa-River. GPS coordinates

Station	Longitude(East)			Latitude (North)		
	°	'	''	°	'	''
1- St1	44	23	33.3	32	03	20.0
2- St2	44	24	28.9	32	02	24.9
3- St3	44	24	44.6	32	02	13.0

Figure 2: Map represents study stations on Al-Kufa River. Source Google earth .

2.3.Work Strategy

Samples were collected from each station monthly in November 2022 during the morning hours (9-12), the work was divided into two parts, field work and laboratory work.

2.3.1.Fild Work

2.3.1.1 Sampling of Copepods

Copepod specimens were collected from three Locations on the Kufa River. Samples were collected using a 125-µm zooplankton net hauled vertically through the water column at two different sites per location. Samples collected from different sites in the same location were pooled together and preserved in Iodine. All specimens were identified morphologically according to the morphological description of copepods, which also worked as taxonomic keys in this paper.

2.3.2. Laboratory Work

2.3.2.1. Identification of Copepods

The samples were transported to the laboratory for identification. As much as possible, identification was made up to species level or genus level using dissecting microscopes with the aid of many references (63 ,64 ,65 ,66) .Temporary slides were made by using glycerol and pictures of the specimens were taken by camera with magnification of ×4 under binocular microscope.

2.3.2.2. DNA extraction

The cephalosome portion of the prosome was obtained from each individual Copepod to avoid DNA contamination from prey items in the gut, using a microscopic tweezer and a sharp blade under the stereo- microscope. Total genomic DNA was extracted from the head using H3 buffer with proteinase K (30 µL), containing 10 mM Tris-HCl, 0.05 M KCl, 0.005% Tween 20, 0.005% NP-40 and 10 mg/ml proteinase K (MERCK, Germany). Samples were incubated overnight at 55 °C in a water-bath with mild shaking. The proteinase K was ir- reversibly denatured after a 12 min incubation at 95 °C. The homogenate was centrifuged briefly and stored at 4°C before use (67) .

2.3.2.3. Interaction of random multiplication of DNA fragments using PCR-RAPD technology

The amplification reaction method was adopted according (68) to Three random primers were chosen: OPC2 (GTGAGGCGTC),OPC8 (TGGACCGGTG) and BH11 (GTGTGTGTGTGTCC), The polymerase chain reaction was performed in a volume of 25 microliter per sample consisting of 50 ng of DNA and 250 µm of each of the four nucleotides (dTTP/ dATP dCTP/dGTP) and 10 picomol from each one of the Taq DNA polymerase polymerase, The thermal cycler PCR System (Verity, Applied Biosystem) was amplified according to the following programs and by type of prefix. The DNA amplification products obtained from the use of the above prefixes for the species under study were carried out in the place assigned to the 1.3% agarose gel, DNA Ladder was carried and the samples were carried under 75 volt for 3-4 hours. With a special camera in the Gel documentation system.

2.3.2.4. Statistical analysis

Statistical analysis of the degree of genetic similarity between the samples studied by reading the DNA bands by binary characters where the appearance of a band was given the number (1)while the absence of the band was given the number (0) then analyzed the results using the statistical program past software ver. 1.92. The phylogenic tree was plotted between the studied samples of RAPD markers according to the Jaccard coefficient of genotype similarity (UPGMA) in the unweighted pair-group method using an arithmetic average (69).

Results and discussion

3.1. Copepods

Six species of the copepods order was identified by examining them with a Dissecting microscope and photographing them. They belong to two families, Two species from Diaptomidae family ,Diaptomus sp, Naupilus sp. and four species from Cyclopedia family ,Cyclops sp, Cyclops strenuus ,Macrocyclops and Microcyclops sp. (Table 2 ,Figure 3).

Table (2): list of copepods that diagnostic in stations of study

Group	Family	Genus and species	Stations of study		
			S1	S2	S3
Copepoda	Cyclopedia	*Cyclops*	+	+	+
		Cyclops strenuus	+	-	+
		Macrocyclops	+	-	+
		Microcyclops	+	-	-
	Diaptomidae	*Diaoptomus sp*	-	+	-
		Napilus	+	-	-

(+ = Exist , - = Absent)

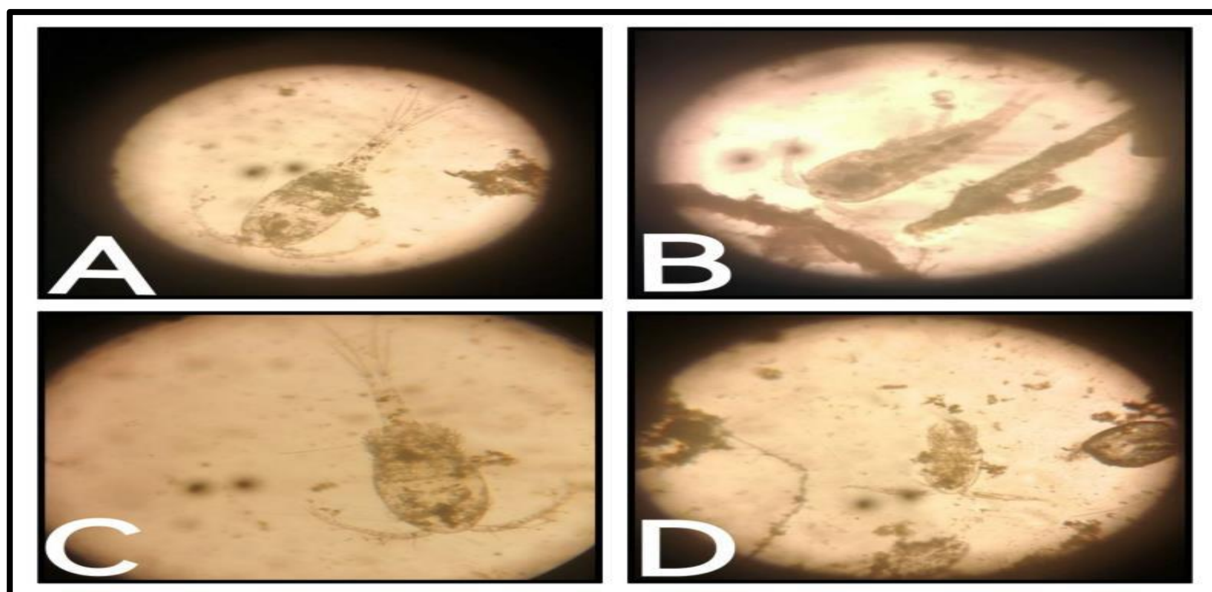

Figure (3): Some species of copepods (A: Cyclops, B: Diaoptomus sp, C: Cyclops sp, D: Microcyclops .X4).

Cyclops recorded at all study stations and with high frequencies arrived stability degree in the environment of the Al-Kufa River commensurate with the precedent Iraqi Studies (70, 71 ,72), they recorded high frequencies for these species in Iraqi environment ,while the rest of the species are distributed differently at the stude stations ,we also noticed that the first stop contains most of the species probably because it doesn't have any human or industrial activity on this station . In the present study, Copepods distribution was different from one region to another and in the same area from, because of different environmental conditions. Factors such as food availability, predation, dissolved oxygen salinity and temperature effect the population dynamics of copepods. The appearance of this group and its disappearance depends on many environmental factors which can adapt itself to different environmental conditions such as high or low temperature or lack of food, their ability to select prey, avoiding contaminated food (73), also copepodid stages and adults are to undergo diapauses and this also explains the disappearance in some areas. The Copepods community composition in shallow water systems are not only influenced by predation(74) but also by water chemistry and water cover are the major factors responsible for formation of the various ecological communities (75) .

3.2. RAPD-PCR Analysis

Previous studies have shown that molecular marker techniques can overcome many of the limitations of morphological and biochemical techniques and can detect DNA-level variations (76). Although there are many copepods encyclopedias, many of which have been described morphologically and chemically characters, some species are still ambiguous, so random samples from two different copepod families have been selected in an attempt to find genetic affinity and divergence between them by using three random primers as shown in Table (3).

Table (3) Details of RAPD amplifications between six species of the copepods**.**

Primer	Primer sequences 5' to 3'	Size range (in bp)
OPC2	GTGAGGCGTC	464 - 1336
OPC8	TGGACCGGTG	547 - 1173
BH11	GTGTGTGTGTGTCC	312 - 467

RAPD profile obtained by OPC8 primer as shown in the Figure(4). The size of the amplified products ranged from 547-1173 bp. The smallest fragment belongs to Napilus and the biggest one belongs to Cyclops strenuus .

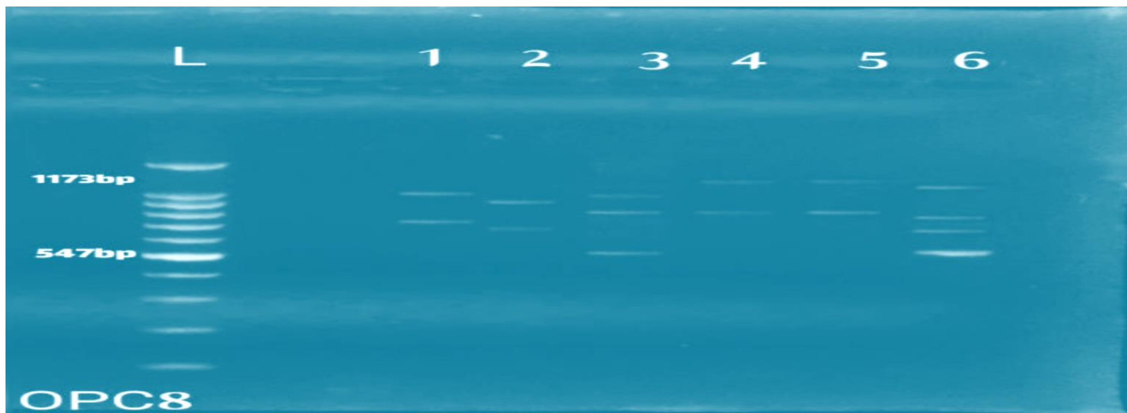

Figure (4): RAPD profile obtained by OPC8 primer of Cyclopedia and Diaptomidae individuals .Lane (L) molecular size marker one step 100 bp ladder .1: Diaoptomus sp 2:Cyclops sp 3:Macrocyclops sp. 4:Microcyclops sp. 5:Cyclops strenuus 6:Napilus sp. .

RAPD profile obtained by OPC2 primer as shown in Figure (5). The size of the amplified products ranged from 463-1336 bp. The smallest fragment belongs to Microcyclops whereas both Microcyclps had the largest fragment .

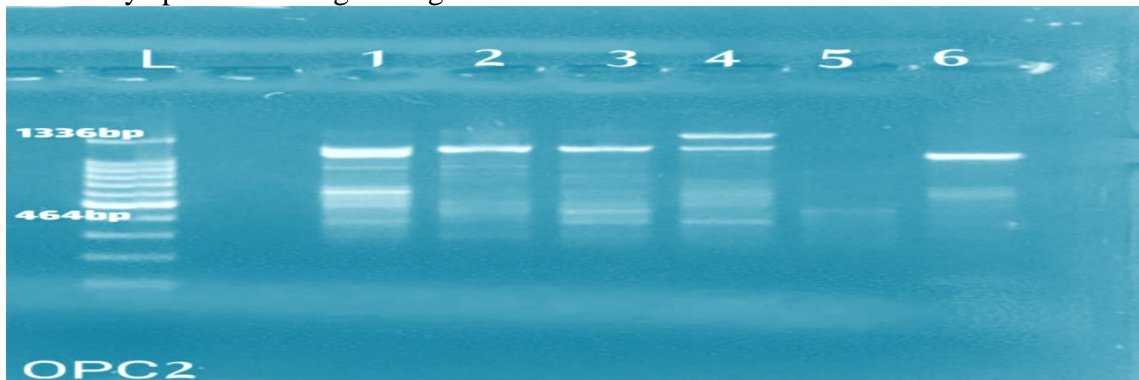

Figure (5): RAPD profile obtained by OPC2 primer of Cyclopedia and Diaptomidae individuals .

RAPD profile obtained by BH11 primer as shown in Figure (6). The size of the amplified products ranged from 312-467 bp. The smallest fragment belongs to cyclops and the biggest one belongs to Napilus .

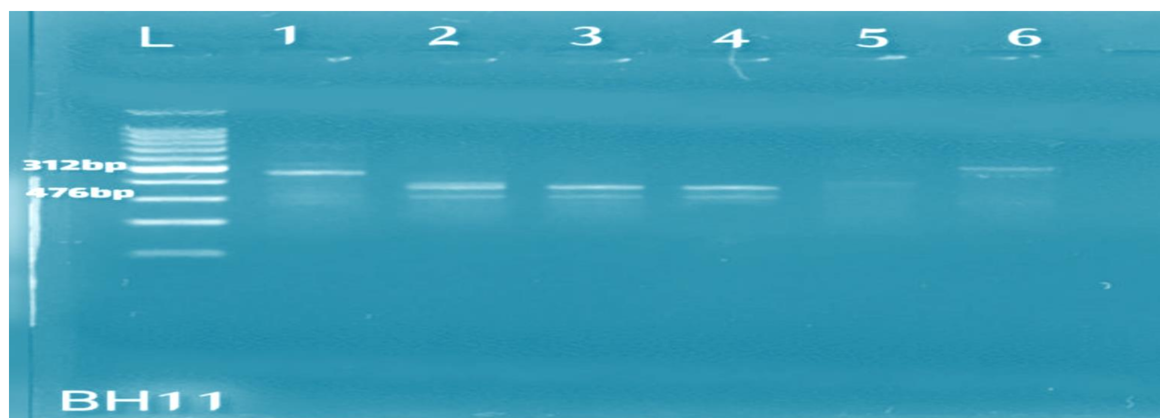

Figure (6): RAPD profile obtained by BH11 primer of Cyclopedia and Diaptomidae individuals **.**

It is clear that the primer OPC2 has the highest percentage of polymorphism in both the Cyclopedia and Diaptomus families, as well as the absence of monomorphic bands between the two families, indicating that each family has a genetic imprint that differs from the other family. This study agrees with the results of other research (77) which pointed out that the high efficiency and discriminatory power of primers are important in obtaining fingerprints for each taxon. It is worth mentioning that the distance or proximity to the genetic structure is determined by the number of joint bands. The more the number of band leads to a less genetic dimension, and the smaller the number of bands, leads to the greater the distance between the genotypes. The common band indicates a similarity in the genetic material in that region of the studied genome, which may represent similarities in phenotypic or anatomical characteristics or similarities in the environment (78). Dendrogram diagram was derived from the results obtained from the PCR-RAPD, indicated by the convergence and divergence of genotypes between species. Table (4)and Figure (7) shows that the copepoda species had the highest similarity between Macrocyclops and Microcyclops at 0.461, while the lowest value of the similarity is 0.066 between the two types Cyclops strenuus and Napilus. Also some other research (79) noted that the genetic distance between species or different species has increased during the evolutionary diversity, while the distance between species within the Intera-species have increased because of geographical isolation, the geographical location may also be attributed to the reason for the existence of variations between taxa in one species.

Table (4) Similarity Matrix computed with the Jaccard coefficient

Species	*Diaoptomus sp*	*Cyclops*	*Macrocyclops*	*Microcyclops*	*Cyclops strenuus*	*Napilus*
Diaoptomus sp	1	0.1894	0.35294118	0.29411765	0.1875	0.4375
Cyclops		1	0.33333333	0.25	0.3875	0.23076923
Macrocyclops			1	0.461653846	0.33333333	0.17647059
Microcyclops				1	0.36363636	0.1875
Cyclops strenuus					1	0.06666666
Napilus						1

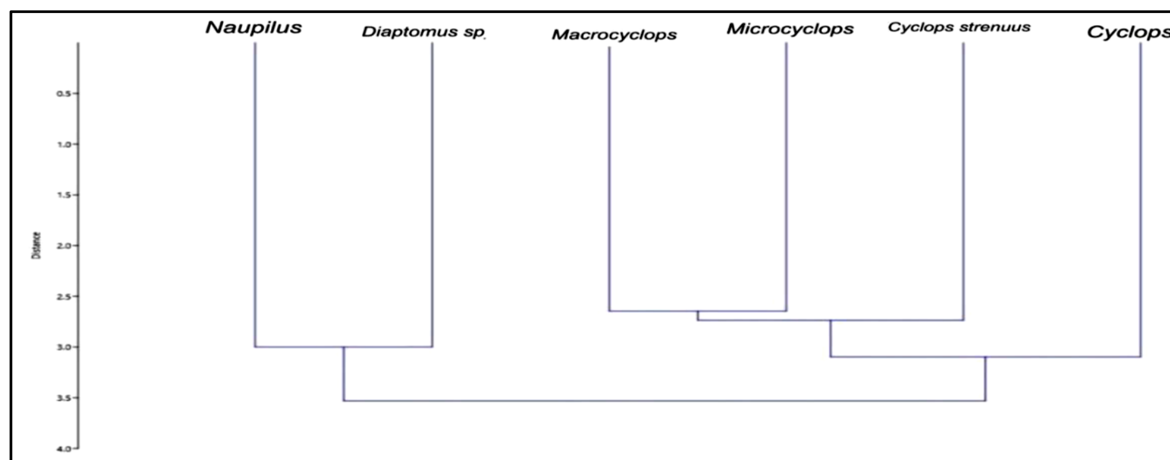

Figure (7) UPGMA dendrogram indicating the genetic relationships among copepods species based on RAPD markers

References

1- Boxshall GA, Halsey SH. An introduction to copepod diversity. London: Ray Society; 2004.
2- Huys R, Boxshall GA. Copepod Evolution. London: The Ray Society; 1991.
3- Roemmich D, Mcgowan J. Climatic warming and the decline of zooplankton in the California current. Science. 1995;267(5202):1324-6.
4- Schminke H. Entomology for the copepodologist. J Plankton Res. 2007;29: i149–62.
5- Ho J, Lin C, Liu W. High diversity of Caligus species (Copepoda: Siphonostomatoida: Caligidae) in Taiwanese waters. Zootaxa. 2016;4174: 114–21.
6- Humes AG. How many copepods. Hydrobiologia. 1994;293:1–7.
7- Chivers WJ, Walne AW, Hays GC. Mismatch between marine plankton range movements and the velocity of climate change. Nat Commun. 2017;8: 14434.
8- Beaugrand G, Reid PC, Ibanez F, Lindley A, Edwards M. Reorganization of North Atlantic marine copepod biodiversity and climate. Science. 2002;296: 1692–4.
9- Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill R, Paruelo J, et al. The value of the world's ecosystem services and natural capital. Nature. 1997;387:253–60.
10- Pond D, Tarling G. Phase transitions of wax esters adjust buoyancy in diapausing Calanoides acutus. Limnol Oceanogr. 2011;56:1310–8.
11- Hays GC, Richardson AJ, Robinson C. Climate change and marine plankton. Trends Ecol Evol. 2005;20(6):337–44.
12- Blanco-Bercial L, Bradford-Grieve J, Bucklin A. Molecular phylogeny of the Calanoida (Crustacea: Copepoda). Mol Phylogenet Evol. 2011;59(1):103–13.
13- Bradford-Grieve JM, Boxshall GA, Ahyong ST, Ohtsuka S. Cladistic analysis of the calanoid Copepoda. Invertebr Syst. 2010;24(3):291–321
14- Judd, W.S.; C.S. Campbell; E.A. Kellogg and P.F. Stevens. 1999. Plant Systematics. A phylogenetic Approach. Sinauer Associates, USA .
15- Crawford, D. J. 2000. macromolecular Systematics in the past 50 years: one view. Taxon, 49: 479-501
16- Williams, J.G.K.; A.R. Kubelik; K.J. Livak; J.A. Rafalski; and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6536
17- Laikre, L., Allendorf, F.W., Aroner, L.C., Baker, C.S., Gregovich, D.P., Hansen, M.M., Jackson, J.A., Kendall, K.C., McKelvey, K., Neel, M.C., Olivieri, I., Ryman, N., Schwartz, M.K.,Bull, R.S., Stetz, J.B., Tallmon, D.A., Taylor, B.L., Vojta, C.D., Waller, D.M. and

Waples, R.S. 2010. Neglect of genetic diversity in implementation of the conservation on biological diversity. Conservation Biology 24: 86–88.

18- Fisher, R.A. 1930. The Genetical Theory of Natural Selection. Oxford University Press, Oxford, UK.

19- Wright, S. 1931. Evolution in Mendelian populations. Genetics 16: 97– 159.

20- Hanski, I. 1998. Metapopulation dynamics. Nature 396: 41–49.

21- .Andrewartha, H.G. and Birch, L.C. 1954. The distribution and abundance of animals. University of Chicago Press, Chicago.

22- Ward, S.M., Gaskin, J.F. and Wilson, L.M. 2008. Ecological genetics of plant invasions: what do we know? Invasive Plant Science and Management 1: 98–109.

23- Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N. and Vellend, M. 2008. Ecological consequences of genetic diversity. Ecology Letters 11: 609–623.

24- Milligan, B.G., Leebens-Mack, J. and Strand, A.E. 1994. Conservation genetics: beyond the maintenance of marker diversity. Molecular Ecology 3: 423–435.

25- Kasnazany, S. A. S. ., Barznjy, L. G. K. ., Fatih, A. A. ., Mirza, A. N. ., & Krbchna, S. J. J. . (2021). Comparative study related to physico-chemical properties of four tomato cultivars grown in Kurdistan region, Iraq. Kufa Journal for Agricultural Sciences, 13(2), 41–52. Retrieved from https://journal.uokufa.edu.iq/index.php/kjas/article/view/3654

26- Nei, M. 1973. Analysis of Gene Diversity in Subdivided Populations. Proceedings of National Academy of Science USA 70: 3321-3323.

27- White, P.S., and Walker, J.L. 1997. Approximating nature's variation: selecting and using reference information in restoration ecology. Restoration Ecology 5 (4): 338-349.

28- Cohen, D. 1966. Optimising reproduction in a randomly varying environment. Journal of Theoretical Biology 12: 119-129.

29- Chesson, P.L. 1985. Coexistence of competitors in spatially and temporally varying environments: A look at the combined effects of different sorts of variability. Theoretical Population Biology 28: 263-287.

30- Tuljapurkar, S. 1989. An uncertain life: Demography in random environments. Theoretical Population Biology 35: 227-294.

31- Tilman, D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. Ecology 80: 1455-1474.

32- Huenneke, L.F. 1991. Ecological implications of genetic variation in plant populations. In: Falk, D.A. and Holsinger. K.E. (Eds.), Genetics and conservation of rare plants. New York: Oxford University Press, UK.

33- Hind A A., Samira K H. (2023). Isolation and identification of Proteus mirabilis bacteria from different clinical sources and investigation of some of their virulence factors., Journal Alharf ., Issue August, No. 18, P: 148-160.

34- Falk, A.D., Knapp, E.C. and Guerrant, E.O. 2001. Introduction to restoration genetics. Society for Ecological Restoration, US Environmental Protection Agency.

35- Schoen, D.J. and Brown, A. H. D. 1993. Conservation of allelic richness in wild crop relatives is aided by assessment of genetic markers. Proceedings of National Academy of Science, USA. 90: 10623-10627.

36- McArdle, B.H. 1996. Levels of evidence in studies of competition, predation, and disease. New Zealand Journal of Ecology 20: 7-15.

37- Thompson, J.D. and Plowright, R.C. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to Diervilla lonicera. Oecologia 46: 68-74.

38- Fowler, N. 1981. Competition and coexistence in a North Carolina grassland. II. The effects of the experimental removal of species. Journal of Ecology 69: 843-854.

39- Rusul Najm ., Jinan M H . (2023). Detection of Virulence Genes of Pathogenic Escherichia coli Isolated from Different Sources., Journal Alharf ., Issue August, No. 18, P: 130-138.

40- Gurevitch, J. 1986. Competition and the local distribution of the grass Stipa neomexicana. Ecology 67 (1):46-57.
41- Goldberg, D.E. 1987. Neighborhood competition in an old-field plant community. Ecology 68 (5): 1211-1223.
42- Manning, S.J. and Barbour, M.G. 1988. Root systems, spatial patterns, and competition for soil moisture between two desert subshrubs. American Journal of Botany 75: 885-893.
43- Welden, C.W., Slauson, W.L. and Ward, R.T. 1988. Competition and abiotic stress among trees and shrubs in northwest Colorado. Ecology 69: 1566-1577.
44- Wright, S. 1920. The relative importance of heredity and environment in determining piebald pattern of guinea-pigs. Proceedings of National Academy of Sciences U.S.A. 6: 320– 332.
45- Ford, E.B. 1964. Ecological Genetics. Chapman and Hall, London, UK.
46- Endler, J.A. 1986. Natural Selection in the Wild. Princeton University Press, Princeton.
47- Frankham, R., Ballou, J.D. and Briscoe, D.A. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK
48- Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J. et al. 2000. Genetic diversity and disease control in rice. Nature 406: 718–722.
49- Hooper, D.U., Chapin III, F. S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., SetSlS, H., Symstad, A.J., Vandermeer, J. and Wardle, D.A. 2005. Effects of Biodiversity on ecosystem functioning: a consensus of current knowledge. Ecology Monogram 75: 3–35.
50- Inouye, B.D. 2005. The importance of the variance around the mean effect size for ecological processes: comment. Ecology 86: 262 .
51- Turkington, R. and Harper, J.L. 1979. The growth, distribution, and neighbor relationships of Trifolium repens in a permanent pasture. II. Inter- and intra-specific contact. Journal of Ecology 67: 219–230.
52- Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweizer, J.A. and Shuster, S.M. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. Ecology 84: 559–573.
53- Primmer, R.C. 2009. From conservation genetics to conservation genomics. Annals of New York Academy of Science 1162: 357–368.
54- Ridgeway, G.J. 1962. The application of some special immunological methods to marine population problems. American Naturalist 96: 219– 224.
55- Bowen, B.W. and J.C. Avise. 1990. Genetic structure of Atlantic salmon and Gulf of Mexico populations of sea bass, menhaden, and sturgeon— Influences of zoogeographic factors and life-history patterns. Marine Biology 107: 371–381.
56- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalsk, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research 18: 6531-6535.
57- Bruns,T.D., White, T.J. and Taylor, J.W. 1991. Fungal molecular systematics. Annual Review in Ecology and Systematics 22: 525-564.
58- Zietkiewicz, E., Rafalski, A. and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)–anchored polymerase chain reaction amplification. Genomics 20: 176–183.
59- Kilian, A., Huttner, E., Wenzl, P., Jaccoud, D., Carling, J., Caig, V., Evers, M., HellerUszynska, K., Cayla, C., Patarapuwadol S., Xia, L., Yang, S. and Thomson, B. 2003. The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution. Bologna 2005: 443-461.
60- Schlotterer, C. 2004. The evolution of molecular markers—Just a matter of fashion? Nature Review of Genetics 5: 63–69.

61- Kohn, M.H., Murphy, J.W., Ostrander, E.A. and Wayne, R.K. 2006. Genomics and conservation genetics. Trends in Ecology and Evolution, 21(11): 629-637.

62- Sanchez, J.J. and Endicott, P. 2006. Developing multiplexed SNP assays with special reference to degraded DNA templates. Nature Protocol 1: 1370–1378.

63- Guryev, V., Berezikov, E. and Cuppen, E. 2005. CASCAD: A database of annotated candidate single nucleotide polymorphisms associated with expressed sequences. BMC Genomics 6: 10-13.

64- Holt, R.A. and S.J.M. Jones. 2008. The new paradigm of flow cell sequencing. Genome Research 18: 839–846.

65- Arab, K.A. (2012) Evaluation of some chemical ,physical properties and some metallic contents of drinking water in Kufa City Center. M.Sc. thesis. Univ. of Kufa.

66- Pontin, R. M.(1978) A key to the Freshwater Planktonic and Semiplanktonic Rotifera of the British Isles. Freshwater Biological Association Scientific Publication No.38.

67- Jordi, D. M. B. (2000) The rotifers of Spanish reservoir: ecological, systematical and zoogeographical remarks. Limnetica, 19:91-167.

68- Smith , D. G. (2001) Pennaks, freshwater invertebrates of the united states. 4th Ed. Porifera to crustacean

69- Thorp, J and Covich, A. (2010) Copepoda. ecology and classification of North American freshwater invertebrates. Elsevier, New York.

70- Yijun Ni. (2020) Phylogeography and genetic diversity of the copepod family Cyclopidae (Crustacea:Cyclopoida) from freshwater ecosystems of Southeast Nigeria .

71- Williams, J.G.K.; A.R. Kubelik; K.J. Livak; J.A. Rafalski; and S.V. Tingey.1990.DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6536 .

72- Hammer, Ø.; D. A. T. Harper and P .D. Ryan 2001. PAST: palaeontological statistics software package for education and data analysis. Paleontologia Eletronica. 4(1):1-9.

73- Al-Lami, A.A.; Mangalo, H. H.; Abdul -Kareem,T.K. and Abbas, E.K.(1999) Zooplankton occurrence in Euphrates river.

74- Sabri, A. W. Ali, Z. H. Shawkat, S. F. and Rasheed K. A. (1993).Zooplankton population in the Tigris river: Effects of Samarra impoundment , Regulated Rivers. 8: 237- 250.

75- Rabee, A.M.(2010). The effect of Al-Tharthar-Euphratescancl on the quantitative and qualitative composition of zooplankton in Euphrates river. J. Al-Nahrain University,13 : 120128.

76- Gretchen, K. B.; Martin, G. and Kevin, V. B. (2006) Toxicity of silver, zinc copper, and nickel to the copepod Acartia tonsa exposed via a phytoplankton diet .J. Env. Sci. Tech., 40 : 2063– 2068.

77- Walter S, Ewing G, von Haeseler A. The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. Mol Ecol.2011;20:1952–63.

78- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3(5):294–9.

79- Ezard T, Fujisawa T, Barraclough T. Splits: SPecies ' LImits by threshold statistics R package; 2009.

80- R Development Core Team: R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 2009.