Texas Journal of Agriculture and Biological Sciences
https://zienjournals.com
ISSN NO: 2771-8840
Date of Publication: 14-06-2022

Molecular classification for some species of ground beetles to the Carabidae family spread in Diyala Governorate

Ammar Abdulwahid Jbarah Al-Karawi[1], Ammar Ahmed Sultan[2]
[1,2]Department of Biology, Faculty of Education for Pure Sciences, University of Diyala, Diyala, Iraq.
[1]email: ammar.abdulwahid.jbara@uodiyala.edu.iq.

Abstract. The existence of millions of species for living organisms and the different stages of their life transformation made the classification of the animal kingdom very difficult, Insects are considered the most numerous in animals and their classification depends primarily on phenotypic traits. In recent years, molecular methods have been adopted to distinguish between species and genera of common descent that have not been distinguished by phenotypic methods. In the past ten decades, molecular methods, especially mitochondrial and nuclear DNA sequence data, have been used to classify insect species and genera of common descent. This study aims to use the technique of random polymerase chain reaction with the presence of ten random primers to distinguish among *Distichus planus*, *Harpalus rufipes, Chlaenius nigricornis* and *Brachinus bayardi*, the results showed that the total number of bundles is 36 and the number of polymorphic bundles is 32 for all ten primers. Primers OP-A04, OP-A08, OP-C06, OP-C10 and OP-C18 showed the highest percentage of polymorphism which is 100%, while primer OP-C15 showed the lowest percentage of polymorphism which is 60%, while primers OP-A15 and OP -B09 and OP-B14 No bundle appeared, so the polymorphism percentage was 0%. In addition, the highest percentage of primer efficiency was 25% at OP-A04 and the lowest percentage was 5.55 at OP-A08. Finally, the primer enabled OP-A04 was able to distinguish between the *Distichus planus*, *Harpalus rufipes*, *Chlaenius nigricornis* and *Brachinus bayardi* where the discriminating ability was 28.125%, while the primers OP-A15, OP-B09 and OP-B14 were not able to distinguish between the four types because of their discriminatory ability was 0%.

Key words: *Distichus planus, Harpalus rufipes, Chlaenius nigricornis, Brachinus bayardi*, RAPD-PCR.

Introduction

Beetles constitute about 40% of insects, as they are the most described species. The known species are estimated between 5-8 million species. To this day, new species are still discovered from time to time, as well as the species found in fossils (Kesdek, 2012). Beetles are found in most environments, from fields to forests and deserts, although some species are associated with specific ecosystems such as meadows or crop fields, and they can be used as biomarkers to assess land changes between different ecosystems (Certini, 2021). Ground beetles are a diverse group of insects with numbers of more than 40,000 species all over the world, 2,000 species of which are in North America, where the sizes of adult beetles range from 2 mm to more than 35 mm (Raupach et al, 2020), Its nocturnal species are black or brown in color, located under an earthen mass, a rock, or tree trunks. Ground beetles can be distinguished from other types of other beetles by the speed of their movement during the day, and they tend to be brightly colored or embossed and have long legs, which allows them to move quickly To catch prey and to avoid other predators (Atamehr, 2013), Ground beetles are one of the most important components of natural and human ecosystems, and their importance comes from their diverse biology and presence in most environments, and their importance comes from the primary role in organizing the numbers of insects, mollusks and other invertebrates (Prosser et al, 2016). Some ground beetles are opportunistic species that consume a variety of foods; however, it has been noted that the majority of species are primarily predators and feed on other insects (De Heij, 2020). Most species locate food by random search, although some of the day-active species hunt by sight. It has been observed that females tend to follow a more varied diet than males, due to an increase in the size and number of eggs (Raupach et al, 2012). Also, some of the ground beetles feed on stored materials such as rice and wheat, and some are predators that devour and nibble trees and invertebrates, and thus they are considered a domestic and agricultural pest that gnaws furniture from the inside and destroys crops (Rusynov et al, 2019), Including the species *Distichus planus* and the species *Harpalus rufipes*, which is a common seed predator and is widespread

ISSN NO: 2771-8840
Date of Publication: 14-06-2022

as it was introduced to North America in 1937 (Harrison and Gallant, 2012) , It is characterized by an elongated oval body and reddish legs, varying from 1-25 cm in length (Reshetniak et al, 2013) , They are highly mobile beetles and are more active at night, and this type of ground beetles stores seeds in burrows under plant residues, how much they are more active in areas with vegetation cover (Daria and Viktor, 2016), It is characterized by the diversity of its life cycle in the fall season and causes damage to crops of wheat, millet, barley, oats, and to a lesser extent on leguminous plants such as peas, beans and industrial crops such as beets, potatoes and sunflowers (Raupach et al, 2012). While Chlaenius is a large and diverse genus of ground beetles found in Europe, the Near East, North Africa, near the North Pole and throughout the world, there are about 1000 species of them, including the type *Chalenius nigricornis* found in the eastern regions and the African tropics (Bousquet, 2012), While the species *Brachinus bayarii* is known as the slanderer beetles because of its strange defense system, it can release chemical sprays in the form of rapid pulsed bursts, and the ejection is accompanied by a popping sound as the slinging beetles produce and store two types of chemical compounds in a separate tank at the back end of the abdomen represented by hydroquinone and peroxide Hydrogen, when the insect is threatened, the beetle constricts the muscles that push the two reactants through valved tubes into a mixing chamber containing water and a mixture of catalytic enzymes When mixed, the reactants undergo a violent chemical reaction, which raises the temperature to nearly the boiling point of water (Tian et al, 2015). The larvae of this species are external parasites and their adult body size depends on the size of their hosts. They are widely varied in geographical areas except for mainland Australia (Melania and Paul, 2015). Molecular taxonomic studies began in 1970 when rRNA was used to classify bacteria (Gholamzadeh and Incekara, 2016). During the past 25 years, molecular methods have been widely used to classify different organisms (Patwardhan et al, 2014) . It is possible that the complete classification of the animal kingdom includes at least 10 million species divided into more than one million genera. Due to this great diversity in living organisms, researchers have resorted to using molecular methods to classify organisms because they are more accurate and depend on DNA data. (Ragan et al, 2014). The use of DNA data in classifying living organisms has led to a great deal of controversy among researchers, but there is a general opinion that genetic clues are very useful in identifying the different stages of the evolution of organisms and diagnosing preserved samples, which may not be suitable for phonotypical study because they may be damaged. (Briggs and Summons, 2014). DNA data provide a character system universal to all life stages with the potential to overcome the problems of working with different *semaphoronts*. A DNA-based approach has already been used to associate different developmental stages in order to identify agricultural pests and invasive species (Cock et al, 2017), forensically important insects (Moemenbellah et al. 2018), larval parasitoids (Poyet et al, 2013) and endangered species in their early life stages (Kueneman et al, 2016). Initial attempts have also been made to survey larval or mixed larval and adult assemblages with DNA methods (Smith et al, 2018). The increasing taxonomic content of DNA databases and rapid sequencing technology now permit tree construction at ever larger scales (Huson et al, 2016). However, traditional phylogenetic methodologies struggle to accommodate these huge data sets, whilst newly developed techniques, more capable of coping with largescale analyses, have not become generally established. In the last decade, technical progresses in molecular biology have allowed evolutionary biologists to collect large DNA sequence data sets in a reasonably short amount of time. This has opened the way for extensive studies on the pattern of evolution of several mitochondrial and nuclear genes and for using DNA sequences to reconstruct phylogenetic relationships at different taxonomic levels (Gauthier et al, 2015). Molecular markers can be divided into DNA markers and protein markers. DNA markers have been widely used due to the disadvantages of allozymes and isozymes which can be referred as protein markers. The thousands of protein-coding genes in the eukaryotic nuclear genome present the richest untapped source of genetic data for phylogenetic research. These genes show a number of favorable properties for phylogenetic analysis (Khlestkina, 2014). Species identification represents a pivotal component for biodiversity studies and conservation planning, but represents a challenge for many taxa when using morphological traits only (e.g., the correct identification of juveniles or larval stages). As a consequence of tremendous technological advances in molecular biology during the last 20 years, molecular data have become increasingly popular in species identification (Krees et al., 2015). Random Amplified Polymorphic DNA (RAPD) gained importance due to its simplicity, efficiency and non-requirement of sequence information and hence, it has been successfully used for the generation of genetic similarities and phylogenetic analysis (Jain et al., 2010). RAPD markers are successfully used for the

ISSN NO: 2771-8840
Date of Publication: 14-06-2022

identification of different insect species (Wells et al., 2001). Moreover, it provides an opportunity to estimate relatedness within and among various species based on DNA variation. DNA barcoding represents the central component in the modern diagnostic toolbox of molecular biodiversity assessment studies and taxonomic research (Hajibabaei et al., 2016). For animals, a 658-base pair (bp) fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene has been selected as a DNA barcode (Hebert et al., 2003a, b). The utility of DNA barcoding relies on the assumption that genetic variation within a species is much smaller than variation between species a (Ratnasingham and Hebert 2005).

Given the importance of ground beetles from an economic and environmental point of view, the current research aims to use the RAPD-PCR technique to distinguish among *Distichus planus*, *Harpalus rufipes*, *Chlaenius nigricornis* and Brachinus bayardi because it is a technique that depends on DNA data and is able to separate species of common origin.

Materials And Methods

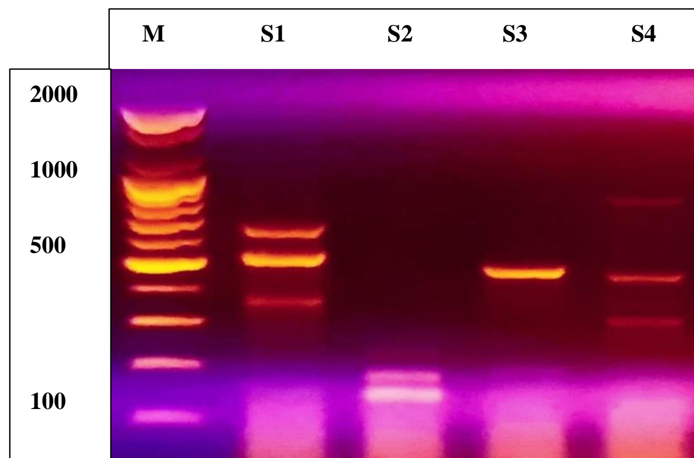
This work was carried out in the Molecular Genetics Laoratory of the College of Education for Pure Sciences/ University of Diyala – Iraq. Insect specimens were collected from different areas in Diyala Governorate - Iraq, with a total of 44 spec-imens, 11 samples for each species using light traps and bait traps. The DNA was extracted using the extraction kit (Genomic DNA Mini Kit (Tissue) Protocol), Equipped by Bioneer company in Korea. The purity and concentration of extracted DNA was measured using a spectrophotometer and by analyzing optical density ratio at 280 nm and 260 nm. The quality of DNA extracted was assessed by running 5µl of DNA per specimen on 1% agarose gel. The extracted DNA was stored at -20 ° C until use in amplification processes. The primer sequences used in this research were designed by Bioneer company- Korea, as shown in Table 1. The reaction mixture is prepared at a temperature of 4C° and the quantity is 25µl, consisting of 5 µl from PCR Pre-Mix, 4 µl of primer 5 µl DNA and 11 µl Deionized water. The amplification process was carried out in PCR device American-made. The amplification conditions for all primers were shown in Table1. In order to a negative control reaction was performed without the DNA template to detect any DNA contamination for each primer. The amplification reaction was repeated twice per sample and per primer to determine the range of consistency and frequency in the bands. The amplification result was then loaded in parallel with the volumetric guide of DNA on the 1% agarose gel and electrophoresed (Sambrook et al, 1989). Gels were filmed under ultraviolet radiation; band and their molecular weights were determined using a gel analysis program. Based on the appearance and absence of bands in each specimen for each insect species, the data matrix was then prepared. Randomly Amplified Polymorphic DNA is the marker of dominant expression, and thus is detected dominant allele in a certain position by the presence of the band while the absence of the band is the appearance of a recessive allele symmetric in this place. Genetic software analysis tools were used to calculate the genetic identity (Miller, 1997). average heterozygosity and clustering of the samples. The following equation was used to calculate the primer efficiency = Number of bands per primer / number of bands for all primers X 100, While the primer discriminatory ability was calculation by the following equation = number of polymorphic bands per primer / number of polymorphic bands for all primers X 100 (Grumman et al, 1995).

Table 1. Random primers used in the research with their nucleotide sequences and amplification reaction conditions.

Primer Name	Nucleotide sequences (5''- 3'')	Amplification reaction conditions	Reference
OP-A04	AATCGGGCTG	Initial denaturation at 94 C° for 5 min (1 cycle), 45 cycles of denaturation at 94 C° for 1 min annealing at 36 C° tor I min, extension at 72 C° for 2 min and a final extension at 72 C° for 7 min (1 cycle)	Hamad (2022)
OP-A08	GTGACGTAGG		
OP-A15	TTCCGAACCC		
OP-B09	TGGGGGACTC		
OP-B14	TCCGCTCTGG		
OP-B18	CCACAGCAGT		
OP-C06	GAACGGACTC		
OP-C10	TGTCTGGGTG		

OP-C15	GACGGATCAG
OP-C18	TGAGTGGGTG

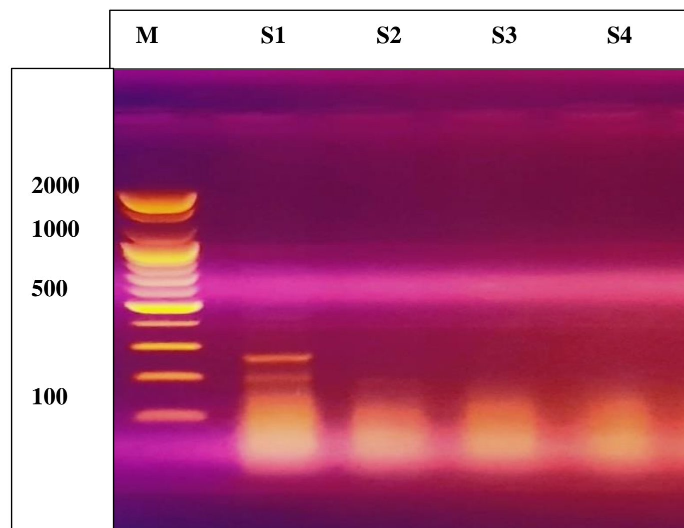
Results And Discussion



S4	S3	S2	S1	M.W.	NO.
0	0	1	0	150	1
0	0	1	0	170	2
1	0	0	0	300	3
0	0	0	1	360	4
1	0	0	0	490	5
0	1	0	0	500	6
0	0	0	1	550	7
0	0	0	1	690	8
1	0	0	0	900	9

0: absence band, 1: presence band

Figure 1. PCR amplification product of the primer OP-A08, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.

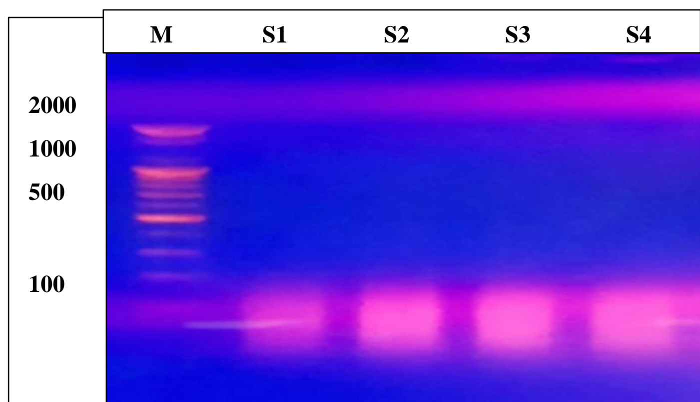


S4	S3	S2	S1	M.W.	NO.
0	0	0	1	200	1
0	0	0	1	280	2

0: absence band, 1: presence band

Figure 2. PCR amplification product of the primer OP-A04, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.

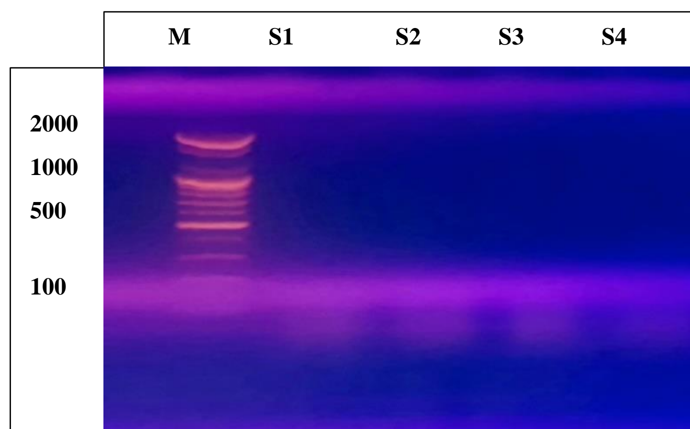
ISSN NO: 2771-8840
Date of Publication: 14-06-2022



S4	S3	S2	S1	M.W.	NO.
0	0	0	0	100	1
0	0	0	0	500	2
0	0	0	0	1000	3
0	0	0	0	2000	4

0: absence band, 1: presence band

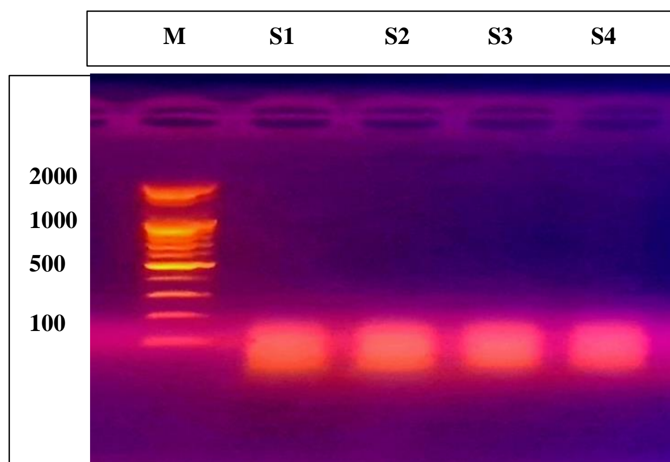
Figure 3. PCR amplification product of the primer OP-A15, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.



S4	S3	S2	S1	M.W.	NO.
0	0	0	0	100	1
0	0	0	0	500	2
0	0	0	0	1000	3
0	0	0	0	2000	4

0: absence band, 1: presence band

Figure 4. PCR amplification product of the primer OP-B09, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.

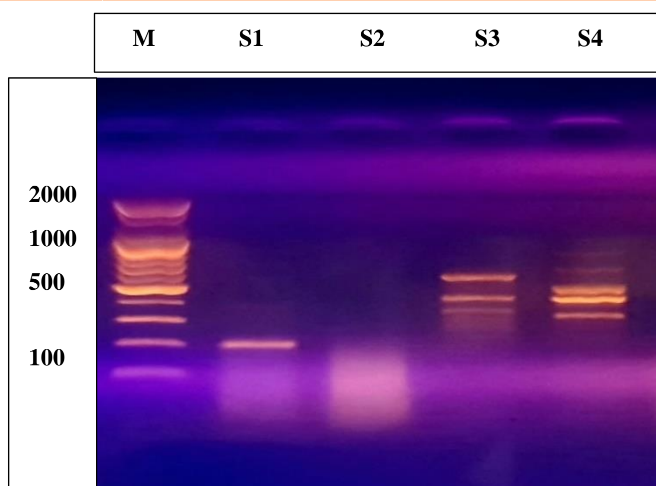


S4	S3	S2	S1	M.W.	NO.
0	0	0	0	100	1
0	0	0	0	500	2
0	0	0	0	1000	3
0	0	0	0	2000	4

0: absence band, 1: presence band

Figure 5. PCR amplification product of the primer OP-B14, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.

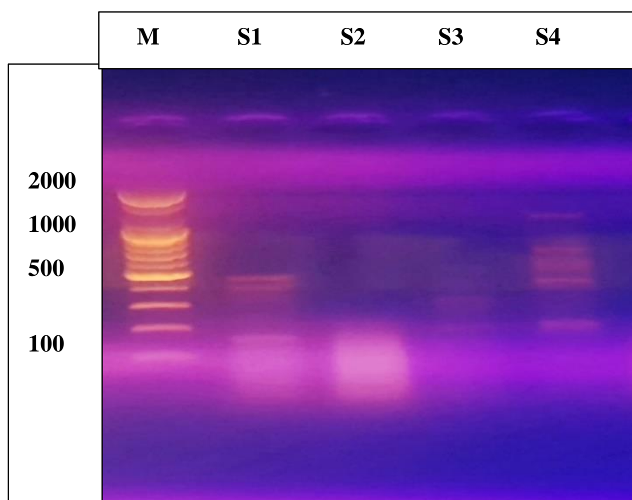
ISSN NO: 2771-8840
Date of Publication: 14-06-2022



S4	S3	S2	S1	M.W.	NO.
0	0	0	1	200	1
1	0	0	0	350	2
0	1	0	0	390	3
1	1	0	0	500	4
1	0	0	0	580	5
0	1	0	0	700	6
1	0	0	0	740	7

0: absence band, 1: presence band

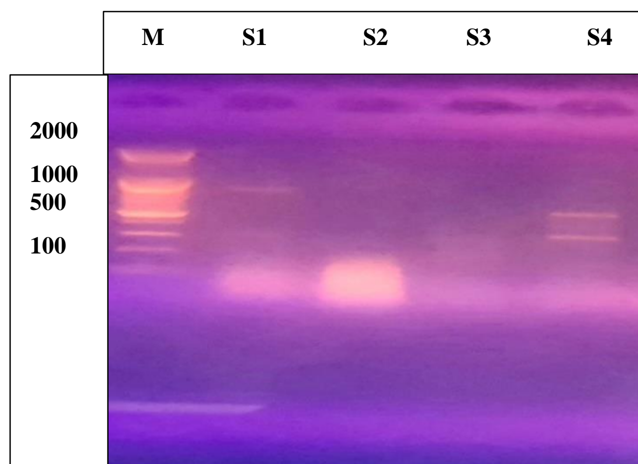
Figure 6. PCR amplification product of the primer OP-B18, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.



S4	S3	S2	S1	M.W.	NO.
0	0	0	1	200	1
1	0	0	0	380	2
1	0	0	0	420	3
1	0	0	0	690	4

0: absence band, 1: presence band

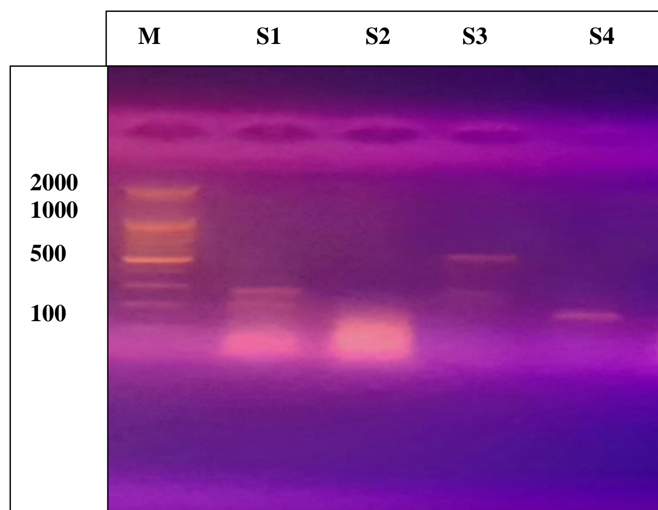
Figure 7. PCR amplification product of the primer OP-C06, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.



S4	S3	S2	S1	M.W.	NO.
1	0	0	0	320	1
1	0	0	0	540	2
0	0	0	1	900	3

0: absence band, 1: presence band

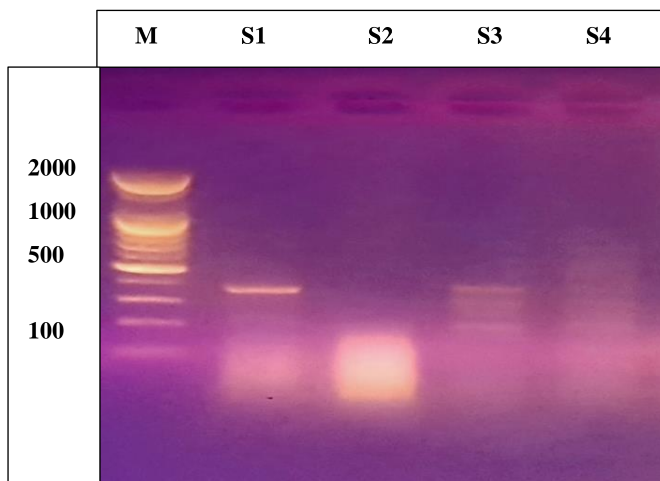
Figure 8. PCR amplification product of the primer OP-C10, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.



S4	S3	S2	S1	M.W.	NO.
1	0	0	0	100	1
0	0	0	1	120	2
1	0	0	1	200	3
1	0	0	0	500	4

0: absence band, 1: presence band

Figure 9. PCR amplification product of the primer OP-C15, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.



S4	S3	S2	S1	M.W.	NO.
1	0	0	0	190	1
1	0	0	0	230	2
1	0	0	0	300	3
0	0	0	1	350	4
1	0	0	0	390	5

0: absence band, 1: presence band

Figure 10. PCR amplification product of the primer OP-C18, M: Marker DNA (100 – 200bp), S1: *Distichus planus* sample, S2: *Harpalus rufipes,* S3: *Chlaenius nigricornis*, S4: *Brachinus bayardi*.

Table 2. Outputs of random primers from total and differentiated bundles with their efficiency and discriminatory ratios.

Primer discriminative ability Percentage	Primer efficiency percentage	Primer polymorphism percentage	Number of polymorphic fragments	Total number of amplified fragment	Primer name
28.125	25	100	9	9	OP-A04
6.25	5.555	100	2	2	OP-A08
0	0	0	0	0	OP-A15
0	0	0	0	0	OP-B09
0	0	0	0	0	OP-B14
18.75	22.222	75	6	8	OP-B18

ISSN NO: 2771-8840
Date of Publication: 14-06-2022

12.5	11.111	100	4	4	OP-C06
9.375	8.333	100	3	3	OP-C10
9.375	13.888	60	3	5	OP-C15
15.625	13.888	100	5	5	OP-C18
			32	36	Total

Discussion

The technique of randomized polymerase chain reaction (RAPD-PCR) is an effective technique in analyzing genetic polymorphisms and finding genetic affinity be-tween species and genera of common origin for different organisms because it depends on DNA data (Skoda et al, 2013). This technique uses a one-way random primer that binds to its complement sequence at different locations in the organism's genome. The patterns of amplicons produced are characteristic of the template DNA (Poczai et al, 2013). The presence and absence of a specific PCR product is diagnostic for the primer binding sites on genomic DNA, therefore, can serve as useful molecular markers for genetic characterization as well as assessment of genetic relationships (Hajibarat et al, 2015). The results in Table 2 showed that the amplification product of the OP-A04 primer gave 9 different molecular weight bundles, and this primer was able to distinguish among *Distichus planus, Harpalus rufipes, Chlaenius nigricornis* and *Brachinus bayardi* at the bundles (150bp, 170bp ,300bp, 360bp, 500bp, 490bp, 550bp, 690bp, 900bp). As for the amplification product of the primer OP-A08, it gave 2 bundles of different molecular weight, and this primer distinguishes *Distichus planus* from the species at the two bundles (200bp, 280bp).

The amplification result of each of the primers OP-A15, OP-B09 and OP-B14 is not presence any bundles and therefore the discriminative ability of these primers is 0, While the OP-B18 primer amplification product gave 8 bundles of different molecular weight and was able to distinguish between species at the bundles (200bp, 350bp, 390bp, 500bp, 580bp, 740bp and 700bp). The product of the OP-C06 primer amplification is 4 different molecular weight bundles and it was able to distinguish between the four species in the molecular weight bundles (200bp, 380bp, 690bp and 420bp), As for the amplification product of the OP-C10 primer, it is 3 bundles of different molecular weight, and this primer was also able to distinguish between the four species when the bundles of molecular weight appeared (320bp, 540bp and 900bp). The product of amplification of the primer OP-C15 is 5 bands of different molecular weight and this primer was able to distinguish between the four species when the bands of different molecular weight appeared (100bp, 120bp, 200bp and 500bp), As for the amplification product of the primer OP-C18, it was 5 bundles of different molecular weights, and it was able to distinguish between the four species when the bundles (190bp, 230bp, 300bp, 350bp and 390bp) appeared. The results of Table 2 showed that the primer OP-A04 gave the most number of resulting bundles, which numbered 9, and the most number of bundles of poly-morphism, which numbered 9, and the highest percent-age of polymorphism, which is 100%, and the highest percentage of initiator efficiency, which is 25%, and the highest percentage of discriminatory ability among *Distichus planus, Harpalus rufipes, Chlaenius nigricornis* and *Brachinus bayardi* which are 28.125% , While the primer OP-A08 gave less number in the resulting bundles, which is 2 bundles, and less number in the bundles with polymorphism, which is 2 bundles. The percentage of polymorphism was 100%, the percentage of the efficiency of the primer was 5.55, and the per-centage of the discriminating ability between the four species was 6.25. In this regard, the researcher Sultan (2020) was able to distinguish between *Tribolium castaneum* and *Tribolium confusum* using the random polymerase chain reaction (RAPD-PCR) technique, as it is very difficult to distinguish between these two types phenotypically because they are very similar. The researcher Baupch et al., (2010) was able to make a molecular diagnosis of species of ground beetles in Central Europe belonging to the Coleoptera order and the family *Carabidae* using expanded segments of ribosomal DNA and a DNA barcode. Species of terrestrial beetles, which are one of the most diverse invertebrates in Europe, by means of these molecular marker, namely 18SrDNA:V7, 28SrDNA:D3 and 18SrDNA:V4 , the expansion segments of the ribosomal DNA of 344 samples from 75 species, they were able to analyze 37 species, or 97% of the carefully studied species, and determine their genetic identity using the nucleotide sequence of the COI gene, As for the three molecular markers, by means of which they were able to distinguish between 71 species, 95%, of the studied ground beetles samples. The researcher Zahoor et al., (2013) were able to molecularly characterize the species of scarab beetles belonging to the (*Coleoptera: Scarabaeidae*), which were collected from different regions in

Pakistan using the random polymerase chain reaction technique (RAPD-PCR) and with the presence of 15 random Primers. Through the data that was generated from the phylogenetic tree diagram, two main groups were obtained: A and B, where group B was divided into three subgroups, and the similarity coefficient of group A with group B was 57.2%, and the genetic similarity between all types of scarab beetles from 44% to 71%, while the similarity of *Scarabaeous pithecius* and *Onthophagus atroplitus* beetles were larger at 61% but The similarity coefficient of *Schizonycha rufficollis* and *Onthophagus atroplitus* were the lowest one (44%). Since DNA polymorphisms among the collected species were high as revealed through RAPD analysis, therefore, it is concluded that the genetic diversity in scarab beetle species is high in Pakistan.

Conclusion

Some random primers used in this research gave diagnosable parameters in each of the DNA samples of the species of ground beetles studied in this research. Amplified DNA bands showed genetic polymorphisms among species of ground beetles. The random polymerase chain reaction technique gave stable results, and it was noted from these results that the ground beetles have a high level of polymorphism and high genetic diversity. The technique of random polymerase chain reaction can be adopted to distinguish between species and genera of common origin for all living organisms because it depends on DNA data and is very accurate in diagnosis. Adoption of the primer OP-A04 as a genetic fingerprint in the diagnosis among species and genera of ground beetles due to its high discriminatory ability compared with other primers obtained in this research.

References

1. Atamehr A. 2013. Ground beetles (Coleoptera: Carabidae) of Azarbaijan, Iran. Turkish Journal of Zoology 37(2):188-94. DOI:10.3906/zoo-1206-32
2. Raupach MJ, Astrin JJ, Hannig K, Peters MK, Stoeckle MY, Wägele JW. 2010. Molecular species identification of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes. Frontiers in zoology 7(1): 1-15. DOI:10.1186/1742-9994-7-26
3. Bousquet Y. 2012. Catalogue of Geadephaga (Coleoptera, Adephaga) of America, north of Mexico. ZooKeys, 245:1. DOI: 10.3897/zookeys.245.3416
4. Briggs DE, Summons RE. 2014. Ancient biomolecules: their origins, fossilization, and role in revealing the history of life. BioEssays 36(5): 482-490. DOI:10.1002/bies.201400010
5. Brygadyrenko V, Reshetniak D. 2016. Morphometric variability of Clitellocephalus ophoni (Eugregarinida, Gregarinidae) in the intestines of Harpalus rufipes (Coleoptera, Carabidae). Archives of Biological Sciences 68(3):587-601. DOI:10.2298/ABS150923049B
6. Certini G, Moya D, Lucas-Borja ME, Mastrolonardo G. 2021. The impact of fire on soil-dwelling biota: A review. Forest Ecology and Management 488:118989. DOI:10.1016/j.foreco.2021.118989
7. Cock MJ, Beseh PK, Buddie AG, Cafá G, Crozier J. 2017. Molecular methods to detect Spodoptera frugiperda in Ghana, and implications for monitoring the spread of invasive species in developing countries. Scientific reports 7(1):1-10. DOI:10.1016/j.foreco.2021.118989
8. Considine DM. 2012. Foods and food production encyclopedia. Springer Science & Business Media.
9. De Heij SE, Willenborg CJ. 2020. Connected carabids: Network interactions and their impact on biocontrol by carabid beetles. BioScience 70(6):490-500. DOI:10.1093/biosci/biaa039
10. Gauthier J, Vincent AT, Charette SJ, Derome N. 2019. A brief history of bioinformatics. Briefings in bioinformatics 20(6):1981-1996. DOI: 10.1093/bib/bby063
11. Gholamzadeh S, Incekara Ü. 2016. Review of molecular taxonomy studies on Coleoptera aquatic insects. International Journal of Entomological Research 4(1):25-36.
12. Grudman H, Schneider C, Hartung D, Daschner F, Pith T. 1995. Discriminatory power of three DNA typing techniques for P. aeruginosa. Clinical Microbiology 33(3):528-32. DOI:10.1128/jcm.33.3.528-534.1995
13. Hajibarat Z, Saidi A, Hajibarat Z, Talebi R. 2015. Characterization of genetic diversity in chickpea using SSR markers, start codon targeted polymorphism (SCoT) and conserved DNA-derived

polymorphism (CDDP). Physiology and molecular biology of plants 21(3):365-73. DOI:10.1007/s12298-015-0306-2

14. Hajibabaei M, Baird DJ, Fahner NA, Beiko R, Golding GB. 2016. A new way to contemplate Darwin's tangled bank: how DNA barcodes are reconnecting biodiversity science and biomonitoring. Philosophical Transactions of the Royal Society B: Biological Sciences 371(1702):20150330. DOI:10.1098/rstb.2015.0330
15. Hammed SS. 2022. Molecular genetic study and gene expression of cytochrome P450 between Tibolium castaneum and Tribolium confusum responsible for different peceticides. [Dissertation]. college of education for pure sciences,University of Diyala. [Iraq].
16. Harrison S, Gallandt ER. 2012. Behavioural studies of Harpalus rufipes De Geer: an important weed seed predator in northeastern US agroecosystems. International Journal of Ecology 2012. DOI:10.1155/2012/846546
17. Hebert PD, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences 270(1512):313-21. DOI:10.1098/rspb.2002.2218
18. Hebert PD, Ratnasingham S, De Waard JR. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. Series B: Biological Sciences 270(suppl_1):S96-S99. DOI:10.1098/rsbl.2003.0025
19. Hebert PD, Gregory TR. 2005. The promise of DNA barcoding for taxonomy. Systematic biology 54(5):852-9. DOI:10.1080/10635150500354886
20. Huson DH, Beier S, Flade I, Górska A, El-Hadidi M, Mitra S, Ruscheweyh HJ, Tappu R. 2016. MEGAN community edition-interactive exploration and analysis of large-scale microbiome sequencing data. PLoS computational biology 12(6):e1004957. DOI:10.1371/journal.pcbi.1004957
21. Jain SK, Neekhra B, Pandey D, Jain K. 2010. RAPD marker system in insect study: A review. Indian Journal of Biotechnology 9: 7-12.
22. Kesdek M. 2012. A contribution to the knowledge of the Carabidae (Coleoptera) fauna of Turkey. Acta Biologica Universitatis Daugavpiliensis 12(1):55-62.
23. P Khlestkina EK. 2014. Molecular markers in genetic studies and breeding. Russian Journal of Genetics: Applied Research 4(3):236-44. DOI:10.1134/S2079059714030022
24. Kueneman JG, Woodhams DC, Van Treuren W, Archer HM, Knight R, McKenzie VJ. 2016. Inhibitory bacteria reduce fungi on early life stages of endangered Colorado boreal toads (Anaxyrus boreas). The ISME journal 10(4):934-44. DOI:10.1038/ismej.2015.168
25. Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015. DNA barcodes for ecology, evolution, and conservation. Trends in ecology & evolution 30(1):25-35. DOI:10.1016/j.tree.2014.10.008
26. Miller MN. 1997. Tools for population genetic analysis (TFPGA) Narang, S.(1980). Genetic variability in natural populations, evidence in support of the selectionists view. Experientia 36:50-1.
27. Moemenbellah-Fard MD, Keshavarzi D, Fereidooni M, Soltani A. 2018. First survey of forensically important insects from human corpses in Shiraz, Iran. Journal of forensic and legal medicine 54:62-68. DOI:10.1016/j.jflm.2017.12.016
28. Patwardhan A, Ray S, Roy A. 2014. Molecular markers in phylogenetic studies-a review. Journal of Phylogenetics & Evolutionary Biology 2014. DOI:10.4172/2329-9002.1000131
29. Poczai P, Varga I, Laos M, Cseh A, Bell N, Valkonen J, Hyvönen J. 2013. Advances in plant gene-targeted and functional markers: a review. Plant methods 9(1):1-32. DOI:10.1186/1746-4811-9-6
30. Poyet M, Havard S, Prevost G, Chabrerie O, Doury G, Gibert P, Eslin P. 2013. Resistance of Drosophila suzukii to the larval parasitoids Leptopilina heterotoma and Asobara japonica is related to haemocyte load. Physiological Entomology 38(1):45-53. DOI:10.1111/phen.12002
31. Prosser RS, Anderson JC, Hanson ML, Solomon KR, Sibley PK. 2016. Indirect effects of herbicides on biota in terrestrial edge-of-field habitats: a critical review of the literature. Agriculture, Ecosystems & Environment 232:59-72. DOI:10.1016/j.agee.2016.07.009
32. Ragan MA, Bernard G, Chan CX. 2014. Molecular phylogenetics before sequences: oligonucleotide catalogs as k-mer spectra. RNA biology 11(3):176-185. DOI:10.4161/rna.27505

ISSN NO: 2771-8840
Date of Publication: 14-06-2022

33. Raupach MJ, Hannig K, Morinière J, Hendrich L. 2020. A DNA barcode library for ground beetles of Germany: the genus Pterostichus Bonelli, 1810 and allied taxa (Insecta, Coleoptera, Carabidae). ZooKeys 980:93-117. DOI:10.3897/zookeys.980.55979
34. Reshetniak DY, Brygadyrenko VV. 2021. Optimizing of Harpalus rufipes (Coleoptera, Carabidae) diet under laboratory conditions. Vìsnik Dnìpropetrovsʹkogo Unìversitetu: Serìâ Bìologìâ, Ekologìâ 21(1):318-324.
35. Rusynov VI, Martynov VO, Kolombar TM. 2019. Coleoptera pests of stored food supplies and field crops. Current problems of agrarian industry in Ukraine.
36. Denman AM. 1983. Molecular cloning: a laboratory manual. Immunology.