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RAPD Markers Distinguish Ground Beetle Species Using Genetic Polymorphism

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Abstract. *This study focuses on differentiating between two ground beetle species, Oryctes rhinoceros and Allomyrina dichotoma, using RAPD markers due to the limitations of phenotypic methods. Ten RAPD primers were employed to identify polymorphic markers. Results showed a total of 41 polymorphic bands, with primers OP-C06 and OP-C15 displaying significant variation in polymorphism percentages, 100% and 16.7% respectively. Primer OP-A08 demonstrated the highest efficiency rate (17.07%) and effectively distinguished between the two beetle species, unlike primers OP-B09, OP-B14, and OP-C10, which showed zero polymorphism. This study underscores the utility of RAPD markers, particularly OP-A08, in accurately identifying insect species, thereby advancing genetic classification techniques.*

Keywords - *RAPD markers; genetic polymorphism; ground beetles; insect classification; molecular genetics*

I.INTRODUCTION

Beetles, the most well-known bug species, account for more than 40% of all insect species. Currently, five to eight millions genera are thought to exist. New genera are occasionally identified in the present, in addition to the species described in fossils [1]. Even though some beetle species are linked to particular habitats, such pastures and cultivating land, and can therefore be utilized as biological indicators for assessing land changes across several environments, beetle can be found in a variety of places, including farms, wooded areas, and deserts [2]. Ground beetles are one of the 2,000 species that can be found in North America and are among a variety of beetles with over 40,000 distinct species globally The nocturnal ground beetle species are darkly colored, black or brown in appearance, and they reside beneath an earthen construction mass, stone, or bark from trees. A mature earth beetle may reach lengths of up to 35 millimeters, ranging in size from 2 millimeters. Ground beetles can be distinguished from other beetle species when they are traveling quickly during the day by their vibrant colors or embossing, as well as their big legs, which enable them to move quickly. According to [3], ground beetles' main job is to organize the populations of insects, mollusks, and other invertebrates. Because of their complex biology and prevalence in most habitats, The ground beetle is one of the most crucial elements of ecosystems that are both natural and created by humans [4]. Although, it is observed that most of beetles were normally predatory organisms and eat on different insects, certain species of ground-dwelling beetles are promiscuous type that eat many kinds of diets [5]. Even while certain species that are active throughout the day hunt by sight, the bulk of species look for food randomly. Due to an increase in egg size and quantity, it has been determined that females often consume a wider variety of meals than ale do [6]. In addition, some ground beetles are predators that feed on trees and invertebrates while others eat crops like wheat and rice that have been stored. Because they chew on crops and furniture from the inside, some genera and species of ground beetles, such as *Oryctes rhinoceres* and *Allomyrina dichoma*, are considered household and agricultural pests. The rhinoceros beetle, *Oryctes rhinoceros* (L.), one of the most significant pests of coconuts in India and other countries that grow coconuts, causes both direct and indirect harm to the crop. Infestations of beetles decrease productivity and, in some situations, can be fatal to young or old palm trees as well as seedlings. The mature stage of the beetle causes damage. It excavates burrows, hangs around at the leaf's crown and in the spaces between the leaf sheath, and if the leaf isn't opened, it will cut it. Unopened spathes feature rounded to rectangular openings, whereas unopened spindle leaves contain holes. When completely opened, the injured frond shows striking geometric wounds. The rhinoceros beetle attack on coconuts, according to [7], reduced the yield by 5.5 to 9.1%. [8] claim that a severe rhinoceros beetle infestation led

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to inflorescence damage and a production decline of up to 5.7%. In addition to direct harm, insect damage creates a route for the red palm weevil or other illnesses to spread. The Japanese rhinoceros beetle, *Allomyrina dichtoma*, which [9] first described in (1771), It is spread out over China, Japan, the Korean Peninsula, Vietnam, Myanmar, Laos, India, and Thailand, claim [10]. It frequently grows in broad-leaved forests in steep tropical and subtropical regions. The Japanese *rhinoceros* beetles has been traditionally utilized in Chinese traditional medicine for practically two thousand years. Recent studies [11]. has supported this. A previously unidentified lectin that was extracted from the larval stage of *Japanese rhinoceros* beetle could inhibit HeLa, mouse fibroblast cells (L929), and mouse L1210 cells that are leukemic [12]. Japanese *rhinoceros* beetles have compounds that may be employed in human medicine, Also accessible are immune-modulating agents and cancer development inhibitors [13]. The first molecular systematic study was initiated in 1970 with the use of ribosomal RNA to categorize bacteria [14]. In a number of organism groups, molecular tools have been extensively used for this purpose over the past 20–25 years [15]. It is anticipated that there would be a minimum of ten million types in the animal kingdom's final taxonomic taxonomy, distributed across over one million genera. Due of its enormous diversity, technology is becoming more and more important for both its original characterization and later identification [16]. It is generally accepted that genetic data is useful for identifying specimens that are too partially preserved for morphological study and for associating different developmental stages of organisms [17], even though there is much debate surrounding the use of DNA data in taxonomy and species diagnosis. DNA data provide a character system that can be used at all phases of life and has the power to address the difficulties that come with working with different semaphoronts.

Due to the importance of these two genera from a medical and economic standpoint as well as the challenges associated with discriminating between them in phenotypic terms, the research intended to use random genetic characteristics to distinguish between the two genera in genotype.

II.METHODS

This study was conducted at the Molecular Genetic Laboratory of the College of Education for Pure Sciences at the University of Diyala in Iraq. Using light traps and bait traps, 20 insect specimens—10 samples of each species—were collected from various places throughout the Iraqi province of Diyala. The DNA was extracted using the genomic DNA mini kit (tissue) procedure, which was made available by the Korean company Bioneer. A spectrophotometer and an analysis of the optical density to wavelength ratio at (280) nm and (260) nm were used for assessing the concentration and purity of the isolated DNA. The quality of the extracted DNA was assessed by running (5µL) of DNA from each specimen on a (1%) agarose gel. In order to be used for amplification procedures, the isolated DNA kept in a freezer at (-20) °C. The Korean company Bioneer designed the primer sequences used in this experiment, according to Table (1.) At a temperature of (4) °C, a reaction mixture made up of (5µL) of PCR PreMix, (4µL) of primer, (5µL) of DNA, and (11µL) of deionized water is created. The PCR apparatus that was used for the amplification was produced in the US. The amplification conditions for each primer are presented in Table (1.) A negative control reaction was carried out for each primer without using the DNA template in order to look for any evidence of DNA tampering. The amplification procedure was repeated twice for each sample and each primer in order to determine the range of consistency and frequency in the bands. The results of the amplification were then electrophoresed after being put on a (1%) agarose gel alongside a volumetric DNA guide. Under UV illumination, gels were captured, and a gel analysis program was used to determine the molecular weights of the bands. Then, depending on the presence or lack of banding for each sample for each species of insect, the information matrix was created. The existence of the band signifies the presence of a dominant allele at a specific site, but its absence suggests the creation of a symmetric recessive allele at this spot, as Randomly Amplification Polymorphous DNA is the distinctive property of dominant gene expression. The genetic identification was determined using genetic software analysis approaches [18][19]. Clustering and average heterozygosity of the sample. The primer discriminating ability was calculated using the formula: Number of polymorphic bands per primer divided by the Number of polymorphic bands for all primers X 100 [20]. The efficiency of the primer was calculated using the formula shown below: The number of bands for each primer divided by the sum of all bands X 100.

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Table 1. The nucleotide sequences and amplification reaction conditions for each of the study's random primers.

Primer Symbol	Nucleotide sequences (5"- 3")	Random polymerase chain reaction conditions	Reference
(OP-A04)	5"AATCGGGCTG3"	Initial denaturation at 94°C for 1 cycle lasting 5 minutes, 45 cycles of denaturation at 94°C for 1 cycle lasting 45 minutes, annealing at 36°C for 1 cycle lasting 1 minute, extension at 72°C for 2 min, and a final extension at 72°C for 1 cycle lasting 7 minutes.	[21]
(OP-A08)	5"GTGACGTAGG3"		
(OP-A15)	5"TTCCGAACCC3"		
(OP-B09)	5"TGGGGGACTC3"		
(OP-B14)	5"TCCGCTCTGG3"		
(OP-B18)	5"CCACAGCAGT3"		
(OP-C06)	5"GAACGGACTC3"		
(OP-C10)	5"TGTCTGGGTG3"		
(OP-C15)	5"GACGGATCAG3"		
(OP-C18)	5"TGAGTGGGTG3"		

III. RESULTS AND DISCUSSION

A. Results

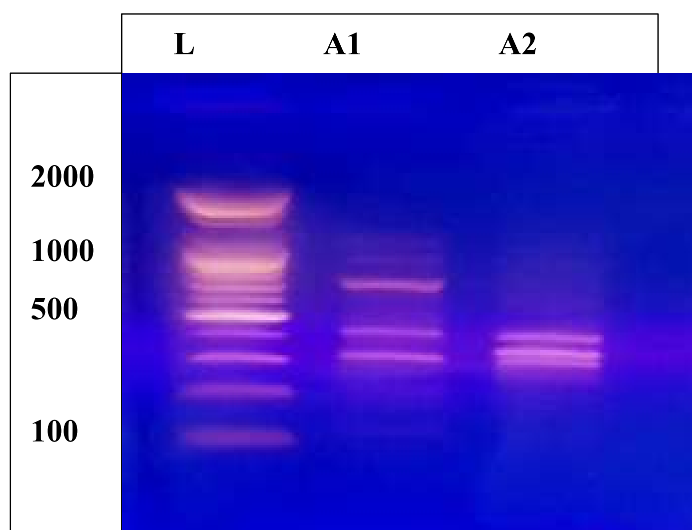

Figure 1. The amplification result from the PCR utilizing the primer (OP-A04), L: Ladder DNA (100–2000bp), A1: Oryctes rhinoceres, and A2: Allomyrina dichotoma

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Table 2. 1: Presence band , 0: Absence band

No.	M.W	A1	A2
1	300	1	1
2	350	0	1
3	400	1	1
4	700	1	0

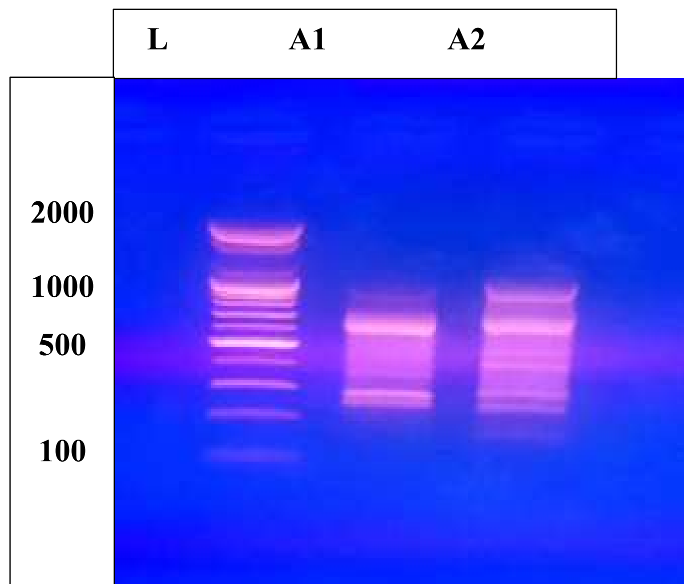

Figure 2. The amplification result from the PCR utilizing the primer (OP-A08), L: Ladder DNA (100–2000bp), A1: Oryctes rhinoceres, and A2: Allomyrina dichotoma

Table 3. 1: Presence band , 0: Absence band

NO.	M.W.	A1	A2
1	150	0	1

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2	250	1	1
3	280	1	1
4	400	0	1
5	500	0	1
6	600	1	1
7	800	0	1

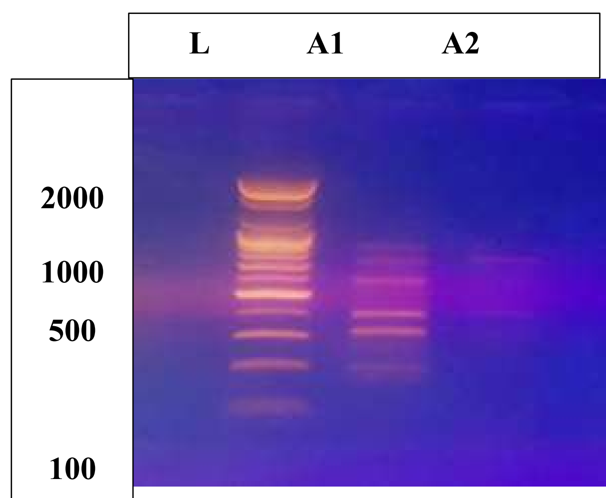

Figure 3. The amplification result from the PCR utilizing the primer (OP-A15), L: Ladder DNA (100–2000bp), A1: Oryctes rhinoceres, and A2: Allomyrina dichotoma

Table 4. 1: Presence band , 0: Absence band

NO.	M.W.	A1	A2
1	200	1	0
2	300	1	0
3	400	1	1
4	600	1	0
5	800	1	1
6	1000	1	0

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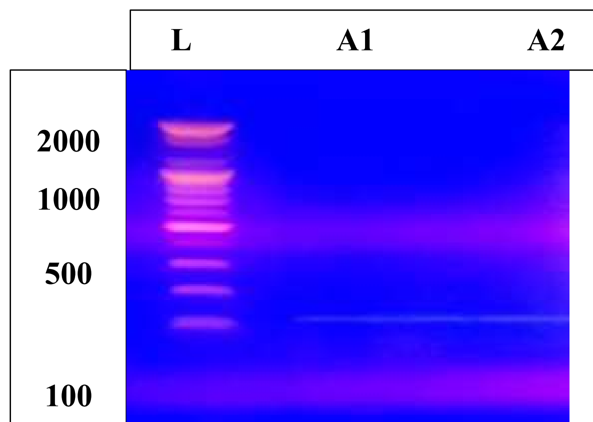

Figure 4. The amplification result from the PCR utilizing the primer (OP-B09), L: Ladder DNA (100–2000bp), A1: Oryctes rhinoceres, and A2: Allomyrina dichotoma

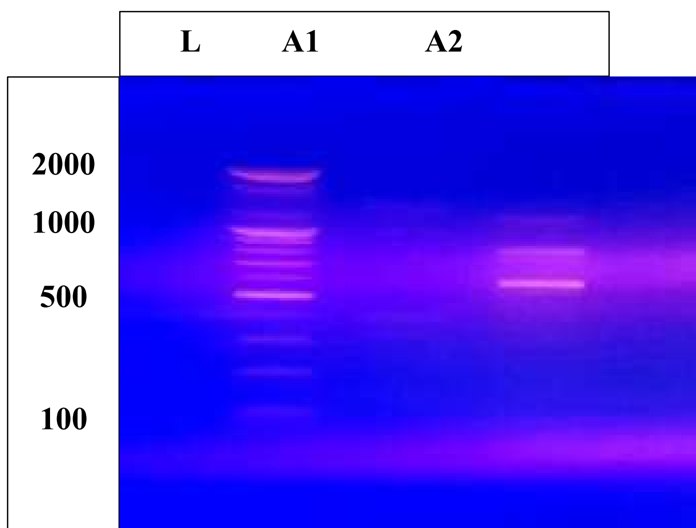

Figure 5. The amplification result from the PCR utilizing the primer (OP-B14), L: Ladder DNA (100–2000bp), A1: ***Oryctes rhinoceres***, and A2: ***Allomyrina dichotoma***

Table 5. 1: Presence band , 0: Absence band

NO.	M.W.	A1	A2
1	500	0	1
2	800	0	1

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3	1200	0	1

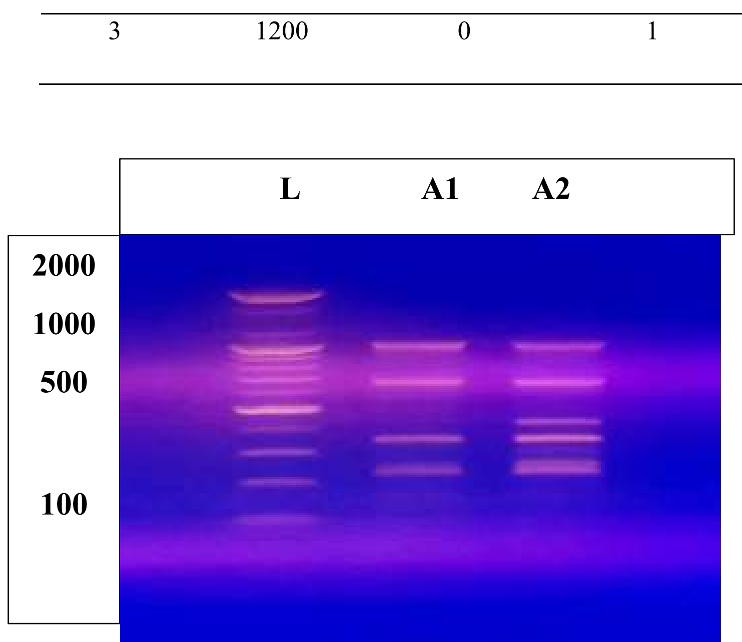

Figure 6. The amplification result from the PCR utilizing the primer (OP-B18), L: Ladder DNA (100–2000bp), A1: *Oryctes rhinoceres*, and A2: *Allomyrina dichotoma*

Table 6. 1: Presence band , 0: Absence band

NO.	M.W.	A1	A2
1	220	1	1
2	230	0	1
3	340	1	1
4	400	0	1
5	700	1	1
6	1000	1	1

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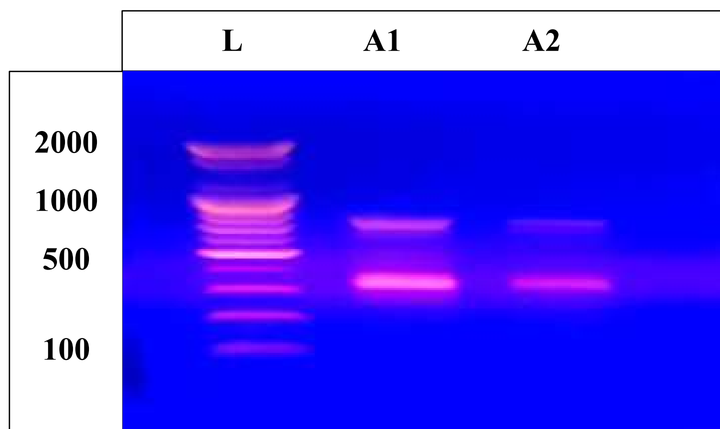

Figure 7. The amplification result from the PCR utilizing the primer (OP-C06), L: Ladder DNA (100–2000bp), A1: *Oryctes rhinoceres*, and A2: *Allomyrina dichotoma*

Table 7. 1: Presence band , 0: Absence band

NO.	M.W.	A1	A2
1	300	1	1
2	750	1	1

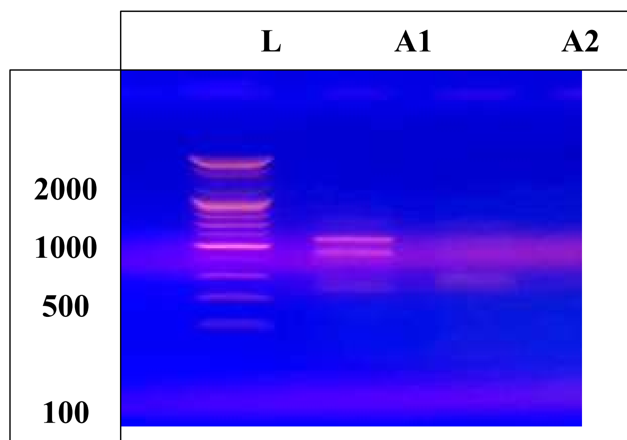

Figure 8. The amplification result from the PCR utilizing the primer (OP-C10), L: Ladder DNA (100–2000bp), A1: *Oryctes rhinoceres*, and A2: *Allomyrina dichotoma*

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Table 8. 1: Presence band , 0: Absence band

NO.	M.W.	A1	A2
1	**250**	**1**	**0**
2	**290**	**0**	**1**
3	**400**	**1**	**0**
4	**600**	**1**	**0**

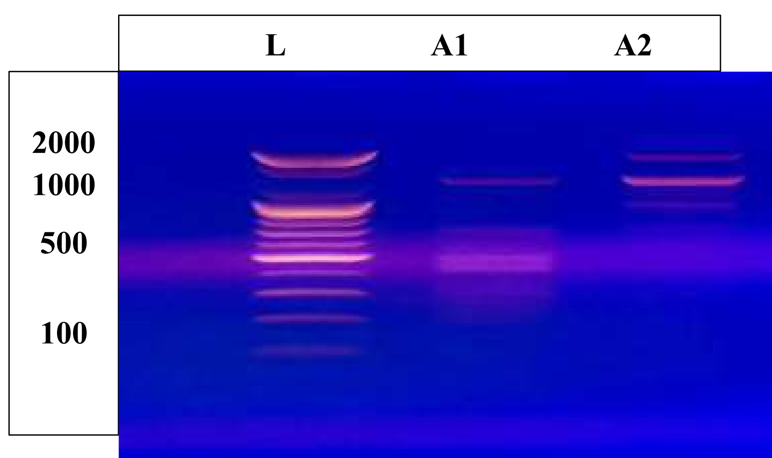

Figure 9. The amplification result from the PCR utilizing the primer (OP-C15), L: Ladder DNA (100–2000bp), A1: ***Oryctes rhinoceres***, and A2: ***Allomyrina dichotoma***

Table 9. 1: Presence band , 0: Absence band

NO.	M.W.	A1	A2
1	300	1	0
2	400	1	0
3	500	1	0
4	1000	0	1
5	1500	1	1
6	2000	0	1

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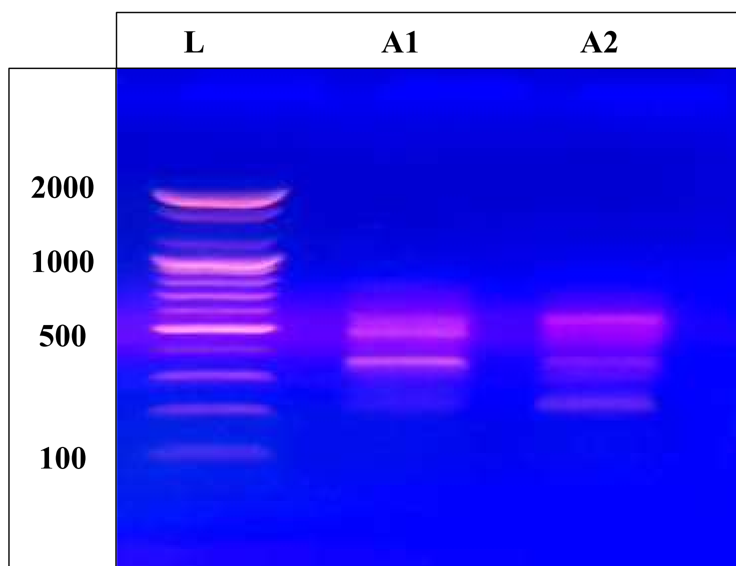

Figure 10. The amplification result from the PCR utilizing the primer (OP-C18), L: Ladder DNA (100–2000bp), A1: *Oryctes rhinoceres*, and A2: *Allomyrina dichotoma*

Table 10. 1: Presence band , 0: Absence band

NO.	M.W.	A1	A2
1	200	0	1
2	350	1	1
3	500	1	1

Table 11. Shows the results of random primers with their efficiency and discriminating ratios from whole and differentiated bundles.

Primer name	Total number of amplified fragment	Number of polymorphic fragments	Primer polymorphism percentage	Primer efficiency percentage	Primer discriminative ability Percentage
OP-A04	4	2	50	9.8	12.5
OP-A08	7	3	42.9	17.07	18.8
OP-A15	6	2	33.3	14.6	12.5
OP-B09	0.0	0.0	0.0	0.0	0.0
OP-B14	3	0.0	0.0	7.3	0.0
OP-B18	6	4	66.7	14.6	25

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OP-C06	2	2	100	4.9	12.5
OP-C10	4	0.0	0.0	9.8	0.0
OP-C15	6	1	16.7	14.6	6.3
OP-C18	3	2	66.7	7.3	12.5
Total	41	16			

B. Discussion

The RAPD-PCR is an effective tool for identifying genetic variation and determining hereditary similarity between species and genus of shared ancestry from a variety of animals since it uses DNA information. [22]. This technique makes use of a single-directional randomized primer that binds to complimentary sequences dispersed across the genome of the organism [23] claimed that the amplicon patterns depict the traits of the template DNA. Since PCR may be used to identify the genomic DNA's primer binding sites, its presence or absence can be utilized to characterize a gene's genetic make-up and pinpoint its genetic connections [24]. According to the data in Table (2) , by creating four distinct molecular weight bundles in the amplified product, the (OP-A04) primer was able to differentiate between *O. rhinoceres* and *A. dichtoma* at the bands (350 bp and 700 bp). The primer (OP-A08) produced (7) bundles of varied molecular weights as a result of amplification, and at the bands (150, 400, 500, and 800 bp), it can differentiate between *O. rhinoceres* and *A. dichtoma*. As a result of amplification, the (OP-A15) primer generated (6) bundles with various molecular weights. This primer can differentiate between *O. rhinoceres* and *A. dichtoma* at the bands (200bp, 300bp, 600bp, and 1000pb). Although there were no presence bands created by the (OP-B09) primer's amplification, it was unable to differentiate between *O. rhinoceres* and *A. dichtoma*. The (OP-B18) primer amplified products allowed for the separation of *O. rhinoceres and A. dichtoma* at the bases of the three bundle (500), (800), and (1200), respectively. The (OP-B18) primer amplification yielded six distinct molecular weight bundles, (230 bp) and (400 bp), and it was able to differentiate between *O. rhinoceres* and *A. dichtoma* in these bundles. Because the (OP-C06) primer produced identical bands in both genus, it was unable to differentiate between *O. rhinoceres* and *A. dichtoma*. Instead, it created two bundles of different molecular weight as its amplification output. While *O. rhinoceres* and *A. dichtoma* could be differentiate at the bundles' molecular weights of (250), (290), (400) and (600) base pairs, respectively, the (OP-C10) primer's amplification result produced (4) bundles with different molecular weights. This primer was able to differentiate between *O. rhinoceres* and *A. dichtoma* when the bands of varying molecular weight (300, 400, 500, 1000, and 2000 bp) formed. The result of primer (OP-C15) amplification was (6) bunds with various molecular weights. Three bundles of varying molecular weights that were created by the (OP-C18) primer's amplification process were able to differentiate between *O. rhinoceres* and *A. dichtoma* at the band (200bp).

The primer (OP-A08) created the most bundles, (7), but the primer (OP-B18) produced the most bundles of polymorphism, (9), and the primer (OP-C06) produced the most polymorphism overall, (100%), according to Table (2.) Results, and the (OP-A08) demonstrated the highest percentage of discriminatory ability (18.8) between *O. rhinoceres* and *A. dichtoma* as well as the best percentage of primer efficiency (17.07). While the polymorphism percentage was (100%), the primer efficiency was (4.9), and the capacity to differentiate between the two genus was (12.5%), the primer (OP-C06) produced less bundles, or (2) bundles, and fewer bundles with polymorphism, or (2) bundles. *Tribolium castaneum* and *Tribolium confusum* have phenotypical characteristics that are highly similar, making it difficult to differentiate between the two species. [25] differentiate between the two species using the Randomly Amplifying DNA Polymerase Chain Reaction (RAPD-PCR) method.

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IV. CONCLUSION

The primer (OP-B14) can be used to discriminate between the two species since it produced three separate bands with different molecular weights in the genus *Allomyrina dichtoma* but not in the genus *Oryctes rhinoceres*.

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