Effective Primer Selection for Differentiating Periplaneta Species via PCR

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Abstract. *This study utilized random polymerase chain reaction (PCR) with 10 random primers to differentiate between two insect species, Aegyptica Periplaneta and Periplaneta japonic. The results revealed a total of 29 bands, with 23 being polymorphic. The classification of the animal kingdom, especially insects, is complex due to the abundance and diversity of organisms. Traditional phenotypic methods often fall short in identifying closely related species, necessitating molecular approaches. Primers OP-A04, OP-A08, OP-B14, OP-B18, OP-C10, OP-C15, and OP-C18 showed the highest polymorphism (100%), while OP-A15 and OP-B09 had the lowest (40%). Primer OP-C06 exhibited no bands, indicating 0% polymorphism. Primers OP-A08 and OP-A15 had the highest efficiency (17.241%), whereas OP-C06, OP-C10, and OP-C15 had the lowest (3.448%). Notably, primer OP-A15 successfully discriminated between the species, while OP-C06 failed entirely. These findings underscore the importance of primer selection in molecular identification and suggest OP-A15 as a reliable primer for distinguishing between these Periplaneta species.*

Keywords - *insect classification; PCR; random primers; species differentiation; eriplaneta*

I.INTRODUCTION

The cockroach order includes cockroaches as members. Out of the approximately 4,600 species, only 30 species are known to feed on human flesh, and only four are considered pests. Cockroaches are found all over the world and are regarded as one of the insects that can adapt to a variety of environments, whether in hot tropical regions or the cold polar regions, where they can withstand temperatures as low as -122 C (-188 F) by producing glycerin-based antifreeze. [1]. A large number of them also reside among the decomposing plant stems. They have adapted to live in dry places without access to water sources by developing mechanisms in decaying wood, holes, tree trunks, and debris. Because they can dive for food, some of them reside close to bodies of water; in this instance, they can breathe When they dive, some of them carry an air bubble beneath their chest armor because their abdomen tips pierce the water's surface [2]. They can be seen everywhere they are used or handled, making them one of the common household insects that live in human environments in contact with their food and tools. [3][4] . Cockroaches are pathogen-carrying mechanical organisms. They spread dangerous bacteria, like salmonella, to every surface they come into contact with. In children, they can also result in allergies and asthma. Because homes provide heat, the right amount of humidity, and food sources, they are ideal places for cockroaches to reproduce. As a result, they have become In these kinds of settings, it is frequently present [5]. Including Periplaneta aegyptica, the tiny, straight-winged insect with six legs and translucent, brown wings known as the Egyptian cockroach. Its oval, flat body is colored tan [6]. The eyes are big, and the antennae are long. Because it tears, chops, and chews food with its front jaws—also referred to as its grinding jaws—cockroaches are categorized as biting insects. The posterior jaws are another set of jaws that are used to handle food and force it down the throat. They are weaker than the front jaws. Additionally, he has deux lips : the upper lip, which is a flap that covers the front of the mouth by hanging down over the oral cavity. The lower lip, on the other hand, conceals the mouth from behind [7]. It is regarded as a benign and tranquil insect. The female can be identified by what appears to be a shield on her body, while the male can be distinguished by his ability to fly and his quick movements. It only eats decomposing plant remnants [8]. One kind of small cockroach known as the Yamato cockroach is the Japanese cockroach, Periplaneta japonica. Its shiny black to brownish-black body is uniformly colored [9]. Its ability to withstand the bitter cold of northern climates is what makes it unique. This particular cockroach is usually found in human-inhabited areas, such as restaurants, food manufacturing facilities, hotels, and assisted living facilities. This kind of cockroach is uncommon to see during the day, especially in areas where there are a lot of them. Since they are thought to be among the insects that are most active at night, people or noisy people are frequently observed in the evening. When this kind of cockroach is disturbed or scared, it may release an unpleasant smell [10]. Its capacity to adapt to life sets it apart. In dwellings and structures where food is kept, cooked, or served [11]. It is unique in that, in contrast to most cockroaches, males have large amounts of cis-9-nonacosene, the primary hydrocarbon in skin fat, which is absent in females. Additionally, it was discovered on trehalose and glucose compounds in overwintering nymphs, and it is

thought to play a significant role in their ability to withstand freezing temperatures [12]. .For the first time, systematic molecular research using ribosomal RNA to categorize bacteria started in 1970 [13]. Over the last twenty to twenty-five years, molecular tools from a variety of organism groups have been used extensively for this purpose [14]. There will probably be at least 10 million species in the final animal kingdom classification scheme, spread across more than a million genera. Because of their immense diversity, more people are realizing that in order to describe them initially and then recognize them, technology support is required [15]. It is regarded as a benign and tranquil insect. The male can be distinguished by his ability to fly and his swift movements, while the female can be recognized by what looks to be a shield on her body. It consumes only decaying plant matter [16]. With the ability to solve issues with adjusting to various cues, DNA data offer a universal personality system for all life stages.

The current study used the RAPD-PCR technique, which is based on DNA data and can distinguish between species of common origin, to perform a molecular genetic comparison between the two species, Periplaneta aegyptica and Periplaneta japonica.

II. METHODS

This study was conducted at the University of Diyala in Iraq, in the Molecular Genetics Laboratory of the College of Education for Pure Sciences. Using light traps and bait traps, two specimens of insects total—one sample for each species—were collected from various locations within the Diyala Governorate in Iraq. The genomic DNA mini kit (tissue) protocol, provided by the Korean company Bioneer, was used to extract the DNA. A spectrophotometer was used to measure the concentration and purity of the extracted DNA, and the optical density ratio at 280 and 260 nm was examined. By running 5µl of extracted DNA per specimen on a 1% agarose gel, the quality of the extracted DNA was evaluated. The extracted DNA was kept cold until it was needed for amplification procedures. As indicated in Table 1, the primer sequences utilized in this study were created by the Korean company Bioneer. A temperature of 4C˚ is used to prepare a 25µl reaction mixture, which is made up of 5µl of the PCR Pre-Mix, 4µl of primer, 5µl DNA, and 11µl deionized water. An American-made PCR device was used for the amplification process. Every primer's amplification conditions were displayed [17]. Gels were exposed to ultraviolet light during filming, and a gel analysis program was used to calculate the bands and their molecular weights. The data matrix was then created based on the presence or lack of bands in each specimen for every insect species. Suddenly Enhanced Since polymorphic DNA is a marker of dominant expression, the presence of a band indicates the presence of a dominant allele in a particular location, whereas the absence of a band indicates the presence of a symmetric recessive allele in that same location. Utilizing genetic software analysis tools, the genetic identity was determined. sample average heterozygosity and clustering. The primer discriminatory ability was calculated using the following equation: number of polymorphic bands per primer / number of polymorphic bands for all primers X 100. The primer efficiency was calculated using the following formula: number of bands per primer / number of bands for all primers X 100. [18].

Table 1. The study employed arbitrary primers along with their corresponding nucleotide sequences and amplification reaction parameters.

Primer Name	Nucleotide sequences (5"- 3")	Amplification reaction conditions	Reference
OP-A04	AATCGGGCTG	Initial denaturation at 94C° for 5 min (1 cycle), 45 cycles of denaturation at 94C° 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)	[19]
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		

III. RESULTS AND DISCUSSION

A. Results of the randomized controlled molecular study

Two samples of the cockroach species used in this investigation were used: one from the Periplaneta japonica family and one from the Periplaneta aegyptica family. Using Pioneer's Genomic DNA mini kit, DNA was extracted from the samples of both species; all samples had extracted DNA with a purity of 1.8–2 nanometers. A comparison of the two species' molecular genetics was also conducted using ten randomly selected primers because of their high degree of phenotypic similarity. The amplification product of primer OP-A04 produced three bands with distinct molecular weights, as indicated by Table 1 results. This primer was capable of differentiating between the two species, Aegyptica Periplaneta and Periplaneta japonica. Table 3's results demonstrated that primer OP-A15's amplification product produced five bands with various molecular weights, and that this primer was capable of differentiating between the two types. Aegyptica periplanita and Periplanita japonica were able to be distinguished from one another using the primer OP-B09, which produced three bands with distinct molecular weights at the bands (1200 bp, 1100 bp, 520 bp, 320 bp, and 300 bp) as well as the amplification product. Between 1760 bp, 1000 bp, and 500 bp, as shown in Table 4). Table 5's findings demonstrated that three bands at the ranges of (480 bp, 400 bp, and 480 bp) appeared as a result of primer OP-B14 amplification. base, 150 bps, 400 bps), Although the OP-B18 primer produced four bands with varying molecular weights as the amplification result, this primer was able to discriminate between the two types at those bands (500 bp, 640 bp, 700 bp, and 980 bp, Table 6). The table's findings revealed (7) Primer OP-C06 produced an amplified product consisting of a single band that was similar to the other, and it was unable to differentiate between the two types (100 bp). Primer OP-C10 produced an amplification product with a single distinct band that allowed for the molecular weight (400 bp) difference between the two types (Table) (8) .Table 9's results demonstrated that primer OP-C15's amplification product is a single, similar band, and that primer was unable to differentiate between the two types (primer OP-C18's amplification product, at 100 bp, is three bands with distinct molecular weights). According to Table 10, this primer was able to discriminate between the two types in bands with varying molecular weights (150 bp, 400 bp, and 520 bp). Table 11 displays the results, which indicate that for all 10 primers, there are 29 total bands and 23 polymorphic bands. The highest polymorphism percentage of 100% was displayed by primers OP-A04, OP-A08, OP-B14, OP-B18, OP-C10, OP-C15, and OP-C18, while the lowest polymorphism percentage was displayed by primers OP-A15 and OP-B09. It is 40%, and the polymorphism percentage was 0% because primer OP-C06 did not display any bands. Furthermore, the primers OP-A08 and OP-A15 had the highest efficiency rate (17.241%), while the primers OP-C06, OP-C10, and OP-C15 had the lowest efficiency rate (3.448%). At last, primer OP-A15 demonstrated the ability to discriminate between Periplaneta japonic and Aegyptica Periplaneta. The OP-C06 primer's discriminatory ability was 0%, making it incapable of differentiating between the two types, whereas the other primer's was 27.586%.

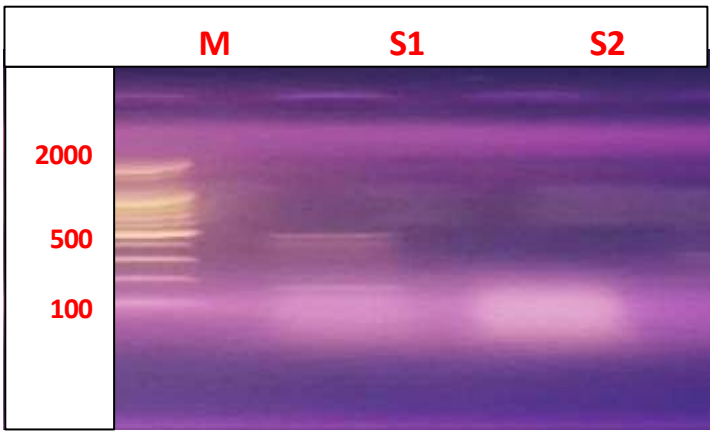

Figure 1. PCR amplification product of the primer OP-A04, M: Marker DNA (100 – 2000bp), S1: Periplaneta aegyptica ,S2: Periplaneta japonica

Table 2. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	190	1	0
2	380	1	0
3	520	1	0

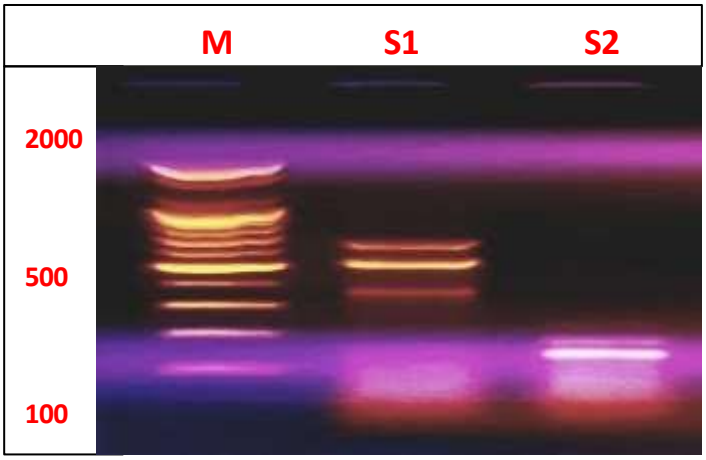

Figure 2. PCR amplification product of the primer OP-A08, M: Marker DNA (100 – 2000bp), S1: Periplaneta aegyptica ,S2: Periplaneta japonica

Table 3. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	190	0	1
2	210	0	1
3	350	1	0
4	500	1	0
5	730	1	0

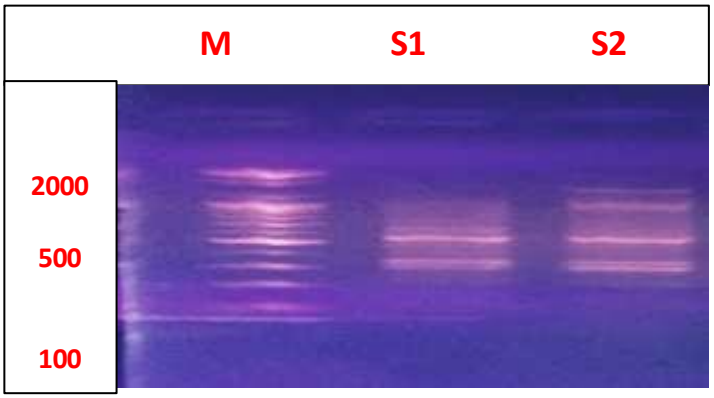

Figure 3. PCR amplification product of the primer OP-A15, M: Marker DNA (100 – 2000bp), S1: *Periplaneta aegyptica* , S2: *Periplaneta japonica*

Table 4. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	300	1	1
2	320	1	1
3	520	1	1
4	1100	1	0
5	1200	1	0

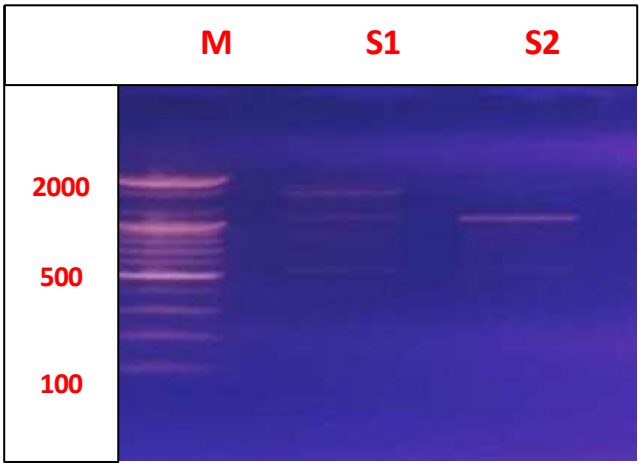

Figure 4. PCR amplification product of the primer OP-B09, M: Marker DNA (100 – 2000bp), S1: *Periplaneta aegyptica* , S2: *Periplaneta japonica*

Table 5. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	500	1	0
2	1000	1	1
3	1760	1	0

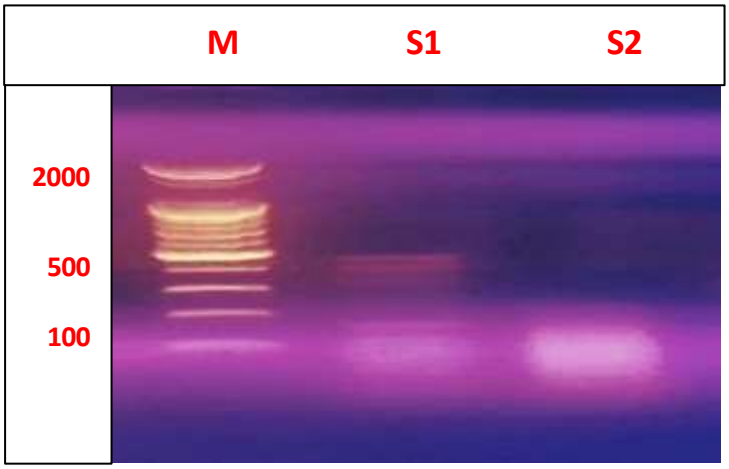

Figure 5. PCR amplification product of the primer OP-B14, M: Marker DNA (100 – 2000bp), S1: *Periplaneta aegyptica* , S2: *Periplaneta japonica*

Table 6. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	150	1	0
2	400	1	0
3	480	1	0

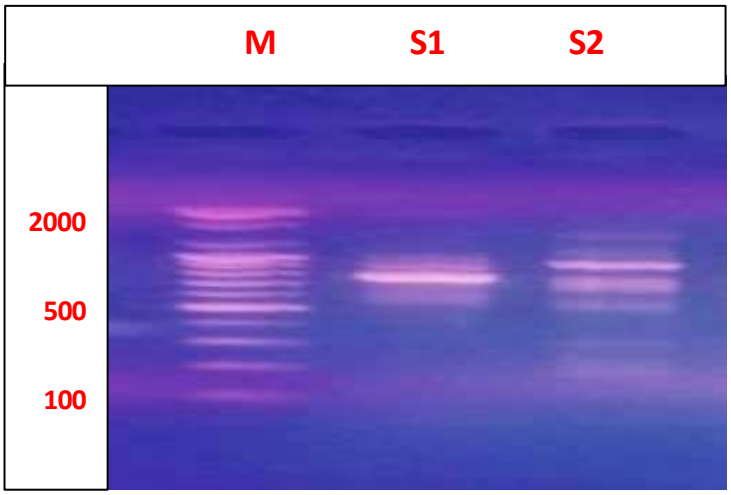

Figure 6. PCR amplification product of the primer OP-B18, M: Marker DNA (100 – 2000bp), S1: *Periplaneta aegyptica* , S2: *Periplaneta japonica*

Table 7. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	500	0	1
2	640	1	0
3	700	0	1
4	980	0	1

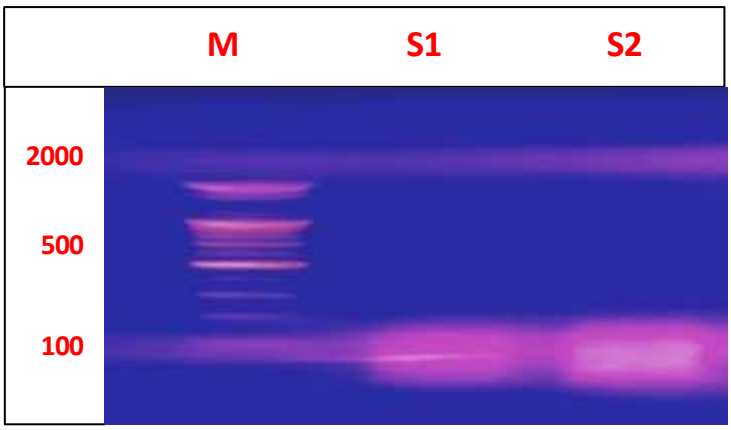

Figure 7. PCR amplification product of the primer OP-C06, M: Marker DNA (100 – 2000bp), S1: *Periplaneta aegyptica* , S2: *Periplaneta japonica*

Table 8. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	100	1	1

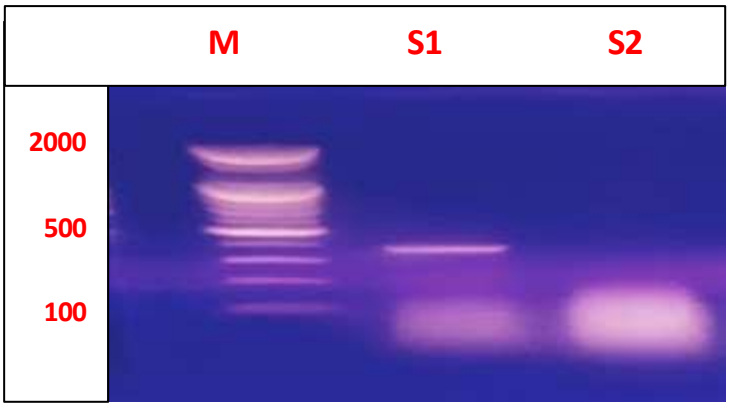

Figure 8. PCR amplification product of the primer OP-C10, M: Marker DNA (100 – 2000bp), S1: *Periplaneta aegyptica* , S2: *Periplaneta japonica*

Table 9. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	400	1	0

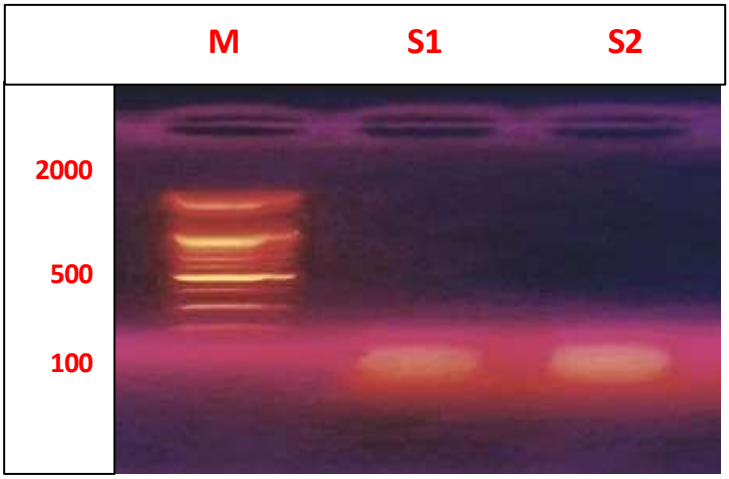

Figure 9. PCR amplification product of the primer OP-C15, M: Marker DNA (100 – 2000bp), S1: *Periplaneta aegyptica* , S2: *Periplaneta japonica*

Table 10. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	100	1	1

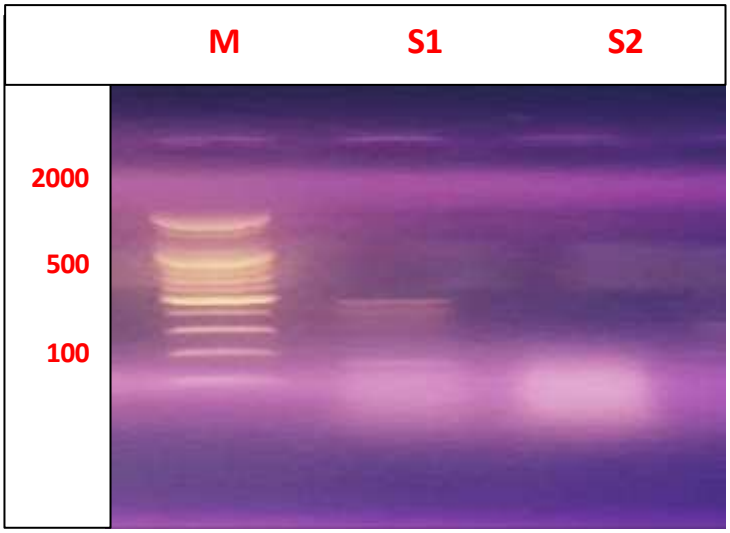

Figure 10. PCR amplification product of the primer OP-C18, M: Marker DNA (100 – 2000bp), S1: *Periplaneta aegyptica* , S2: *Periplaneta japonica*

Table 11. 0: absence band, 1: presence band

NO.	M.W.	S1	S2
1	150	1	0
2	400	1	0
3	520	1	0

Table 12. Random primer outputs from differentiated and total bundles, along with their discriminatory ratios and efficiency.

Primer discriminative Percentage ability	Primer % efficiency percentage	Primer polymorphism percentage	Number of polymorphic fragments	Total number of amplified fragment	Primer name
%13.043	%10.344	%100	3	3	OP-A04
%21.739	%17.241	**%100**	5	5	OP-A08
%27.586	%17.241	%40	2	5	OP-A15
%4.347	%10.344	%33.333	1	3	OP-B09
%13.043	%10.344	%100	3	3	OP-B14
%17.391	%13.791	%100	4	4	OP-B18
%0	%3.448	%0	0	1	OP-C06
%4.347	%3.448	%100	1	1	OP-C10
%4.347	%3.448	%100	1	1	OP-C15
%13.043	%10.344	**%100**	3	3	OP-C18
			23	29	**المجموع**

Based on object data, random polymerase chain reaction, or RAPD-PCR, is a useful method for X-ray analysis and electronic tools for convergent species and genera of origin of distinct facultative

organisms [20][21]. This method makes use of a single primer that is joined to its sequences at various points throughout the living genome. In addition to being useful for officials in monitoring relationships, the amplicons produced are template DNA. There is a specific PCR product, and its absence is diagnostic for the southern sites of primers on child protection. As such, they can be temporarily protected [22]. Based on Table 1's results, which show that the OP-A04's amplification output produced four distinct Greek weight bands at bands (290, 300, 330, and 750 bps), the amplification of the principles produced by OP-A08 resulted in two packs of distinct kings, and this starter could only train between one type when packing (250, 550, and 490 bps) Table (4-2). Outcomes Table (4–3) Principles of Amplification Output OP-A15 Overview Amplification of the OP-B09 primers resulted in 6 different weight bands blessed with Aegyptica Periplaneta and Periplaneta japonica at the bands (150bp, 170bp, 340pb, 40 0B pb, 500pb, 700pb) Table (4 - 4). These bands corresponded to 8 different geographic loss bands, aegyptica Periplaneta, and Periplaneta japonica, at the beams (200bp, 300bp, 420pb, 500bp, 750bp, 1200bp, 2000bp, 1600bp). Table (4 -5) demonstrated that whereas the amplification of the principles OP-B18 produced four bands with distinct targets, the amplification of the principles OP-B14 produced six bands with the same starting weight and did not select to distinguish between the three types at the bands (300 bp, 700 bp).

The three types at the bands (220 bp, 460 bp, and 520 bp) were all successfully trained by this primer (Table (4-6)). Amplification of the OP-C10 primers produced no Bands (4–8) appear with negligible results (4–9) among the three types at the bands (310 bp, 750 bp). The OP-C15 primers are amplified to create four distinct welcome bands, which allow this primer to discriminate between the three types in the 200 bp, 290 bp, and 500 bp bands. The OP-C18 primers were amplified, producing five distinct packages of blessed color. Table 4-10 lists the principles for differentiating between the two types in the various packages with welcome weights: 200 bp, 300 bp, 410 bp, 500 bp, and 700 basis points.

After analyzing the data in Table (4–11), I came to the conclusion that there are 36 multiple packages overall and 29 multiple packages for each of the ten principles. The hydration OP-A04, OP-B18, and OP-C06oOP-C15 showed the lowest percentage of polypharmacy, which is 70%, while the prefixes OP-B14 and OP-C10 have no beam and a diameter multiplicity ratio of 0%. The prefixes OP-A08, OP-A15, OP-B09, and OP-C18 recorded the highest percentage of polypharmacy, which is 100%. Furthermore, in OP-A15, the highest PRI was 22.222%, while in OP-B14 and OP-C06, the lowest PRI was 5.555. Ultimately, Principles OP-B14 and OP-C10 were unable to train between Periplaneta aegyptica and Periplaneta japonic, as their ability to train was 0%. In contrast, Excellence OP-A15 was able to train between the two types with a 27.586% ability.

The same random principle sequences were employed by [23] in the urban classification of certain regional competition types that are part of the Carabidae classification in the Diyala Governorate.

IV. CONCLUSIONS

Because random polymerase chain reaction technology is highly accurate in diagnosing diseases, it can be used to differentiate between the species and genera that all living things have in common with one another.

Because the OP-A15 primer has a higher discriminatory ability than the other primers obtained in this research, it should be used as a genetic fingerprint for the diagnosis of cockroach species and genera.

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