# Genetic Variation among Nine Egyptian Gecko Species (Reptilia: Gekkonidae) Based on RAPD-PCR

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**Abstract:** The RAPD-PCR in the present study was used to determine the genetic variation among nine Egyptian gekkonid species; *Tropiocolotes tripolitanus*, *Tropiocolotes nattererii*, *Hemidactylus turcicus*, *Cyrtopodion scaber*, *Stenodactylus petrii*, *Ptyodactylus guttatus*, *Ptyodactylus hasselquistii*, *Tarentola mauritanica* and *Tarentola annularis*. The animals were captured from several localities from Egypt (Giza, Sinai and Matruh governorates). A total of 94 bands were amplified by the four primers OPAO4, OPBO3, OPB18 and OPCO1 with an average of 23.5 bands per primer at molecular weights ranged from 1267 to 112 bp. The polymorphic loci between species were 91 with percentage 96.8 %. The similarity coefficients value between the nine gekkonid species are ranged from 0.313(31.3%) to 0.576 (57.6%) with an average of 0.42 (42%). The genetic distance between the nine species was ranged from 0.424 (42.4%) to 0.687 (68.7%) with an average of 0.58 (58 %). The dendrogram showed that, the nine gekkonid species separated from each other into two clusters. The first cluster includes *Tropiocolotes tripolitanus*; *Tropiocolotes nattererii*; *Hemidactylus turcicus*; *Cyrtopodion scaber*; *Stenodactylus petrii*. The second cluster includes the rest of gekkonid species. The clade *Tarentola annularis* is sister taxon to *T. mauritanica* and the clade *Ptyodactylus guttatus* is sister taxon to *P. hasselquistii*. It is also noted that, the genus *Tropiocolotes* is closer to the genus *Cyrtopodion* than the other genera and the genus *Tarentola* is closer to the genus *Ptyodactylus* than the other genera. It is concluded that, the less similarity coefficient and the high genetic distance value between the 9 gekkonid species indicates that, the nine gekkonid species are not identical and separated from each other.
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**Key Words**: *Gekkonidae*, RAPD-PCR, Phylogenetic Relationship, Dendrogram

## 1. Introduction

The order Squamata includes 4900 lizard species, 3070 snake species and 200 amphisbaenians species (**Vidal and Hedges, 2009**). Lizards are cosmopolitan and geographically distributed over a wide range of habitats and have a striking range of morphological characteristics, ecological habitats and body sizes. In Egypt, most of the gekkonid species are living in and around human habitation however, some species are free living in Egyptian deserts (**Goodman and Hobbs, 1994**).

Many studies carried out to classify and determine the phylogenic relationships among members of the family Gekkonidae on the bases of morphological and environmental characteristics (**Anderson, 1898; Marx, 1968; Baha El Din, 1994 and 1997; Goodman and Hobbs, 1994; Saleh, 1997**), chromosomal karyotyping (**Chen, *et al*., 1986; Castiglia, 2004; Kawai *et al*., 2009**), biochemical investigations (**Macey *et al*., 2000; Qin *et al*., 2006**), molecular DNA variation (**Carranza *et al*., 2000, 2002 and 2006; Han *et al*., 2004; Rato *et al*., 2010; Fujita and Papenfus, 2011**), RAPD-PCR (**Qin *et al*., 2005**) and mitochondrial DNA sequences (**Jesus *et al*., 2001 and 2005; Vences *et al*., 2004; Rocha *et al*., 2005; Carranza and Arnold, 2006; Bansal and Karanth, 2010; Busais and Joger, 2011**).

The genus *Hemidactylus* is one of the most diverse and widely distributed genus of the family *Gekkonidae* in the world (**Baha El Din, 2003 and 2005; Baldo *et al*., 2008**).

The genus *Tarentola* comprises 21 species (**Baha El Din, 1997; Sprackland and Swinney, 1998; Carranza *et al*., 2002; Diaz and Hedges, 2008**), most of which show low interspecific morphological variations. The species have been distributed in Libya, Sinai, Ethiopia and Somali land, Countries and Islands bordering the Mediterranean (**Marx, 1968; Baldo *et al*., 2008**). Molecular genetic of *Tarentola* have been demonestated by several studies (**Carranza, *et al*., 2000 and 2002; Harris *et al*., 2009; Rato, *et al*., 2010**).

The *Ptyodactylus* species distribute in and around human habitations, and therefore are known to be commensal with humans (**Goodman and Hobbs, 1994**). They are found from wet tropical forest to arid deserts and tropical Asia and Africa and Algerian Sahara, Egypt (**Anderson, 1898; Marx, 1968; Goodman and Hobbs, 1994; Ibrahim, 2001**).

The genus *Stenodactylus* contains 13 recognized species. The species *Stenodactylus stenodactylus* and *S. petrii* allocate from Egypt, Sudan to Mauritania (**Marx, 1968; Goodman and hobbs, 1994; Baha El Din, 2006**); Iran, Iraq, Syria, Jordan and Arabian Peninsula (**Anderson, 1999**).

The *Tropiocolotes* species allocate in and around

human habitations and are distributed from wet tropical forest to arid deserts and tropical Asia and Africa, Egypt to Tunisia and Sudan (**Anderson, 1898; Marx, 1968; Goodman and Hobbs, 1994**).

The genus *Cyrtodactylus* (*Cyrtopodion*) is a topic of taxonomic controversy (**Macey *et al*., 2000**). **Masroor (2008 and 2009) and Nazarov and Rajabizadeh (2007)** considered *Cyrtopodion* as a distinct genus with two subgenera *Cyrtopodion* and *Mediodactylus*, while **Shi and Zhao (2011)** considered that the *Cyrtopodion* and *Mediodactylus* are Subgenera of the genus *Cyrtodactylus*. **Macey *et al*. (2000)** used the allozymic data to determine the phylogenetic relationships among the Asian genus *Cyrtodactylus* and found that, the subgenera of *Cyrtopodion* and *Mediodactylus* are separate monophyletic groups of the genus *Cyrtodactylus*. The mitochondrial and nuclear DNA sequences have used to resolve the phylogeny of *Cyrtodactylus* gecko species (**Kasapidis *et al*., 2005; Carranza and Arnold 2006; Bansal and Karanth, 2010**).

Hence, it is necessary to study the RAPD- PCR analysis of the members of this family that may help in understanding the phylogeny of this primitive lacertilian family. Therefore, the present study aimed to discuss the phylogenetic relationships among nine Egyptian gekkonid lizard species belong to six genera based on RAPD-PCR technique.

## 2. Material and Methods

Animal dealer collected samples of nine Egyptian Gekkonid species from different localities (Giza, Sinai and Matruh governorates, Egypt). The nine species are belonging to six genera. Morphological identification and classification of the animals as well as scientific and common names of these species identified according to previous works (**Anderson, 1898; Marx, 1968; Baha El Din, 1994**).

The studied species: -

1-*Tropiocolotes tripolitanus*
Common names: Tripoli gecko, Tripoli pigmy gecko, Bors Taht El Hagar
2- *Tropiocolotes nattereri*
Common names: Natterer's gecko, Bors Taht El Hagar
3- *Hemidactylus turcicus*
Common names: Turkish gecko, warty gecko, Mediterranean gecko
4- *Cyrtopodion scaber*
Common name: Rough-skinned gecko, Rough-scaled gecko, Keeled rock gecko
5- *Ptyodactylus guttatus*
Common names: Fan-footed gecko, Bors Abu Kaff Sinai
6-*Ptyodactylus hasselquistii*
Common names: Fan-footed gecko, Bors Abu Kaff Cairo
7- *Stenodactylus petrii*
Common name: Petrie's gecko , Bors Abu Ain Wasa'h.
8- *Tarentola mauritanica*
Common name: Moorish gecko, Moorish wall gecko
9- *Tarentola annularis*
Common name: Egyptian gecko, white-spotted Gecko, Bors Abu Arba'a Noqat

### Genomic DNA extraction

Samples of muscle tissue from the nine gekkonid species taken and stored at -20 $^{o}$C. DNA extracted according to the method of **Yue and Orban (2005)** with slight modifications. DNA quality and concentration determined by spectrophotometric analysis and run in 0.7 % agarose gel. Each sample of DNA examined by optical density values at 260 and 280 nm and only good quality DNA samples used in RAPD-PCR reaction.

### RAPD-PCR reaction

Eight primers from Kits OP-A, OP-B and OP-C (Operon Technologies, Alameda, CA, USA) used for RAPD-PCR analysis (OPA-04, OPA-10, OPB-03, OPB-05, OPB-18, OPC-01, OPC-06 and OPC-10). Only four primers (OPA-04, OPB-03, OPB-18 and OPC-01) were reacted well and used to amplify DNA from all species (table 1). RAPD-PCR reactions carried out as described by **Williams *et al*. (1990)**. PCR cycles performed with 60 s, 94°C initial denaturation and 35 cycles of 20 s, 94°C; 20 s, 35°C; and 30 s, 72°C. Final extension performed at 72°C for 5 min. PCR amplifications were carried out in 96 well Thermal Cycler (Eppendorf Master Cycler) and all amplifications were carried out at two times. A PCR mixture without template DNA placed in each analysis as a control. The PCR products separated on 1.5 % agarose gels (Sigma) containing ethidium bromide in 0.5 X TBE buffer at 100 V constant voltages. For evaluating the base pair length of bands, DNA ladder (Fermentas) was loaded with each gel.

### Data and statistical analysis:-

The RAPD banding patterns scored for the presence (1) and absence (0) of bands for each sample. The scores obtained using all primers in the RAPD analysis combined to create a single data matrix. The statistical analysis of the data performed using the free software, Popgene version 1.31, computer program (**Yeh *et al*., 1999**) including the calculation of allele frequencies according to **Nei (1987)**. This program estimated the number and percentage of polymorphic loci and the genetic diversity according to **Nei (1973)**. For constructing

the dendrogram, the data resulted from RAPD markers banding patterns was introduced to NTSYS-pc package program by Unweighted Pair Group Method using Arithmetic Averages (UPGMA) method (**Rohlf, 2000**).

**3. Results**

Figures 1, 2, 3 and 4 showed the PCR bands produced by four random primers (OPA-04, OPB-03, OPB-18 and OPC-01) for the investigation of the genetic variation between the nine studied gekkonid species. The four primers yielded a sufficient and variable number of bands for comparison between the gekkonid species. The primer OPB-03 produces the highest number of bands (32 bands) in comparison to the other primers.

As shown in tables 2 and 3 the primers demonstrated 94 RAPD-PCR bands among the nine gekkonid species. The RAPD profile generated from these primers and the RAPD scoring bands have utilized to estimate the band frequency .

Primer OPA-04 generated 19 polymorphic bands with molecular weight ranged from 1267 to 227 bp. Band frequency ranged from 0.1 to 0.89 with mean value 0.491 (49.1%). The bands at 460bp, 407bp and 227bp were present only in *Tropiocolotes tripolitanus*, *Stenodactylus petrii* and *Hemidactylus turcicus* respectively. Primer OPB-03 produced 32 bands. Band frequency ranged from 0.1 to 1.0 with mean value 0.597(59.7%). Unique bands at 1256 bp and 112 bp are specific for *Tropiocolotes tripolitanus* *Ptyodactylus hasselquistii* respectively. The nine species have a common shared band at molecular weights 329 bp and 400 bp. Primer OPB-18 created 23 bands with a common band at molecular weight 452bp. The band frequency ranged from 0.1-1.0 with mean value 0.521(52.1%). Primer OPC-01 amplified 20 bands with band frequency ranged from 0.1- 0.9 with mean value 0.421(42.1%). Bands at 1048bp and at 955bp are unique bands in *Ptyodactylus hasselquistii* while the band at 304bp for *Ptyodactylus guttatus*.

Table 3 showed 94 scorable amplified bands with an average 23.5 bands/primer at molecular weights ranged from 1267 to 112bp between the 9 Gekkonid species and 91 of them were polymorphic (96.8%) with an average 22.75 bands/ primer. The polymorphic bands were19 (100 %), 30 (93.75%), 22(95.65%) and 20 (100%) for primers OPA-O4, OPB-O3, OPB-18 and OPC-O1, respectively. Table 4 showed the similarity coefficient value between the 9 gekkonid species, which ranged from 0.313 (31.1%) to 0.576 (57.6%) with an average of 0.42 (42%). The genetic distance between the 9 species was ranged from 0.424 (42.4%) to 0.687 (68.7%) with an average of 0.58 (58 %).

As shown in figure 5 the UPGMA dendrogram constructed to show phylogenetic relationships and pointed out that, the nine gekkonid species separated from each other into two clusters. The first cluster includes two clades. The clade *Tarentola annularis* is sister taxon to *T. mauritanica* and the clade *Ptyodactylus guttatus* is sister taxon to *P. hasselquistii*. The second cluster contains the rest of the gekkonid species; *Tropiocolotes tripolitanus*, *T. nattereri*, *Hemidactylus turcicus*, *Cyrtopodion scaber* and *Stenodactylus petrii*. In this cluster, the gekkonid species *Tropiocolotes tripolitanus* is sister taxon to *T. nattereri* and the remaining species are represent a further subclades from these taxa and separate from each other.

**4. Discussion**

In this study, the inter-specific genomic polymorphisms in nine gekkonid species, *Tropiocolotes tripolitanus*; *Tropiocolotes nattereri*; *Hemidactylus turcicus*; *Cyrtopodion scaber*; *Ptyodactylus guttatus*; *Ptyodactylus hasselquistii; Stenodactylus petrii; Tarentola mauritanica*; *Tarentola annularis* were analyzed by using RAPD-PCR technique. The molecular technique RAPD-PCR analysis is currently used to differentiate between the genomes of the closely related species in order to determine the genetic distance and genetic diversity (**Williams *et al*., 1990; Camargo *et al*., 2010**). The primer OPB-03 has a high G+C content (70 %) and produces the highest number of amplified fragments (32 bands) of genomic DNA in the studied gekonid species (**Dinesh *et al*., 1995**).

The results of this study showed high inter and intra- specific genetic variation among gecko species. This genetic variations among gecko species proved by protein polymorphism, mitochondrial DNA and nuclear DNA sequences (**Jesus *et al*., 2002; Harris *et al*., 2004; Kasapidis *et al*.,2005; Arnold *et al*.,2008; Perera and Harris, 2010**). **Qin *et al*. (2005)** found high genetic diversity in the same species, *Gekko geck* from six different localities of china with genetic distance (0.011-0.963) and similarity coefficient (38.17% – 98.88%) in relation to animal groups.

The results showed that the number of amplified bands for the 9 gekkonid species were 94 bands, 91 (96.8%) of them were polymorphic (Table 3). The genetic similarity between the 9 gekkonid species are ranged from 0.313 (31.3%) to 0.576 (57.6%) with average 0.42 (42%) and the genetic distance are ranged from 0.424 (42.4%) to 0.687 (68.7%) with average 0.58 (58 %). The low genetic similarity and the high genetic distance between the nine gekkonid species indicate that the nine species are separated from each other. According to **Baker *et al*. (2006)**,

these species are considered distinct and separate from each other if they have a genetic distance greater than 5%.

The UPGMA dendrogram (Fig. 5) and table 4 showed that, the species *Tarentola annularis* and *T. mauritanica* are sister to each other but they have high genetic distance (0.431) and low genetic similarity (0.569). Therefore, these two species separated from each other. This observation is similar to that presented by **Carranza *et al.* (2002).** They found that, *T. annularis* (subgenus, *Sahelogecko*) and *T. mauritanica* (subgenus, *Tarentolas*) are separated from each other by using molecular study. In addition, they found that, the *Tarentola mauritanica* is paraphyletic with *T. angustimentalis* in the Canary Islands by using mitochondrial DNA and nuclear sequences. Although, *Tarentola mauritanica* species is characterized by a conservative morphology and shows intraspecific high genetic diversity (**Carranza *et al*., 2000; Jesus *et al*., 2002; Harris *et al*., 2004 and 2009; Rato *et al*., 2010**). Therefore, *Tarentola mauritanica* is clearly a species complex. Moreover, the North African (Tunisia, Libya and Egypt) *Tarentola mauritanica fascicularis* and *Tarentola mauritanica mauritanica* show high genetic distinct polymorphism (8%) by using gene sequences (**Harris *et al*., 2004 and 2009**). The species *T. mauritanica*, *T. deserti* and *T. angustimentalis* are paraphyletic groups of the genus *Tarentola* (**Harris *et al*., 2009**). **Gubitz, 2005** found that the *Tarentola boettgeri* was monophyletic to *T. delalandii* by using cytochrome b and nuclear sequences. **Carranza *et al*. (2000 and 2002)** recorded that, the *Tarentola americana* is the sister taxon to remaining *Tarentola* species. In the present work, the genus *Tarantola* is closer to the genera *Ptyodactylus and Tropiocolotes* than the other gekkonid species. According to UPGMA dendrogram, the genus *Tarantola* is sister to the genus *Ptyodactylus*. **Gamble *et al*. (2008 and 2011)** previously postulated this observation. They found a strong sister relationship between *Ptyodactylus* and *Tarentola* genera by using molecular analyses. In addition, they postulated that, the genera *Ptyodactylus* and *Tarantola* are belong to Phylodactylidae family but the other Gekkotan genera are belong to the Gekkonidae family. Moreover, they observed that, the family Phylodactylidae is sister to the family Gekkonidae. Members of the genera *Tarentola* and *Geckonia* are more closely related to each other than to genera *Stenodactylus* and *ptyodactylus* and the species *Geckonia chazaliae* is evidently a member of the *Tarentola* clade by using mitochondrial and nuclear DNA (**Carranza *et al*., 2002**).

In the present work, the genus *Tropiocolotes* was closer to the genus *Cyrtopodion* than the genus *Stenodactylus* and *Hemidactylus*. **Fujita and Papenfuss (2011)** found that, the *Tropiocolotes Tropiocolotes* from Niger and *T. somalicus* from Djibouti were sister clade to a clade of *Stenodactylus* samples and some other species of the genus *Stenodactylus* is not monophyletic to *Tropiocolotes*. In addition, they found high genetic variation between the species of the genus *Stenodactylus* that found the genetic distance ranged from 14.6% to 43.2% by using mitochondrial DNA but the genetic distance was ranged from 0.60% to 6.80% by using nuclear data.

*Hemidactylus* species is one of the most diverse and widely distributed genera of reptiles in the world. Sometimes, very similar anatomical features *Hemidactylus* species show great genetic variation (1-2% variation) in mitochondrial DNA but most populations of *Hemidactylus mabouia* and *H. turcicus* are very uniform (**Carranza and Arnold, 2006**). Molecular study revealed that the *Hemidactylus robustus* and *H. turcicus* from Egypt have 14% genetic diversity (**Baha El Din, 2005**). Also, the morphological conservativeness of *Hemidactylus brooki, H. mabouia and H. frenatus* have been separated by using molecular data (**Jesus *et al*., 2005**). Recently, molecular work showed that *Hemidactylus anamallensis* was basal to all the *Hemidactylus* suggesting that *Hemidactylus anamallensis* was genetically very distinct from other *Hemidactylus* (**Bansal and Karanth, 2010**). In the present study, the genus *Hemidactylus* is closer to the genus *Tropiocolotes* than the genera *Cyrtopodion and Stenodactylus*. In the present work, the genetic variation between *Hemidactylus turcicus* and *Cyrtopodion scaber* is 0.532 (53.2%) and these two genera are not sister to each other but they have existed in the same cluster. This result is participated with the previous study for *Cyrtopodion kotschyi* and *Hemidactylus turcicus* (**Bauer *et al*., 2008**) and disagreement for **Kasapidis *et al*., 2005** and **Carranza and Arnold, 2006**. Also, the present work is in agreement with the results obtained by **Han *et al*. (2001)** who noticed that, the *Cyrtopodion elongates*, *C. russawi*, *Hemidactylus bowringii*, and *H. frenatus* were monophyletic lineage by using sequence of 12srRNA gene fragment. According to **Bauer *et al*. (2008)** although all gekkonidae are well studied ecologically and taxonomically, the phylogenetic relationship within and between the Gekkota have not been well established yet.

The conclusion derived from this work, within the Gekkonidae species from the Egyptian fauna shows that, the intergeneric relationships are poorly resolved

and the results suggest additional work is needed to clarify the taxonomy and monophyly of gecko genera.

**Table 1: Sequence of primers employed in molecular phylogenetic relationship among the nine Gekkonid species.**

| Primer | Sequence | G C % |
|---|---|---|
| OPA-04 | 5'-AATCGGGCTG-3' | 60 |
| OPB-03 | 5'-CATCCCCCTG-3' | 70 |
| OPB-18 | 5'-CCACAGCAGT-3' | 60 |
| OPC-01 | 5'-TTCGAGCCAG-3' | 60 |

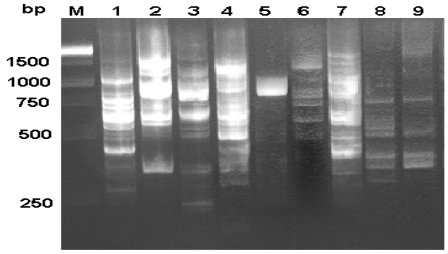


**Figure 1. RAPD amplifications showing diagnostic markers for gekkonid species, with primer OP-A04. M, DNA size standard (1kb ladder).1,*Tropiocolotes tripolitanus* ; 2,*Tropiocolotes nattereri* ; 3,*Hemidactylus turcicus* ; 4,*Cyrtopodion scaber* ; 5,*Ptyodactylus guttatus*; 6,*Ptyodactylus hasselquistii* ;7, *Stenodactylus petrii* ; 8,*Tarentola mauritanica* ; 9,*Tarentola annularis.***

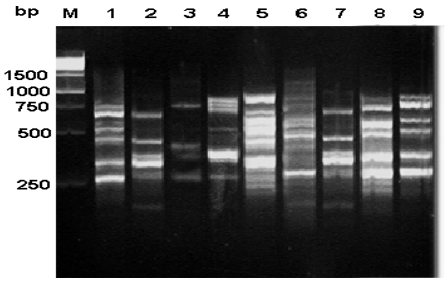


**Figure 2. Gel electrophoresis represents RAPD - PCR products for DNA from gekkonid species (Lanes 1 to 9) with OP-CO1 primer. 1,*Tropiocolotes tripolitanus* ; 2,*Tropiocolotes nattereri* ; 3,*Hemidactylus turcicus* ; 4,*Cyrtopodion scaber* ; 5,*Ptyodactylus guttatus*; 6,*Ptyodactylus hasselquistii* ;7, *Stenodactylus petrii* ; 8,*Tarentola mauritanica* ; 9,*Tarentola annularis.* M, DNA marker.**

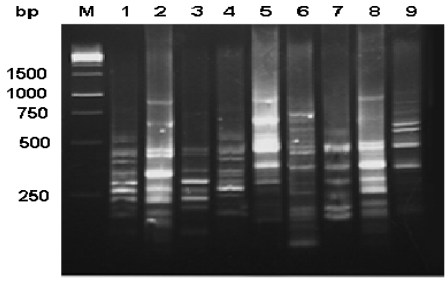


**Figure 3. Gel electrophoresis represents RAPD - PCR products for DNA from gekkonid species (Lanes 1 to 9) with OP-B18 primer. M, DNA size standard (1kb ladder).1,*Tropiocolotes tripolitanus* ; 2,*Tropiocolotes nattereri* ; 3,*Hemidactylus turcicus* ; 4,*Cyrtopodion scaber* ; 5,*Ptyodactylus guttatus*; 6,*Ptyodactylus hasselquistii* ;7, *Stenodactylus petrii* ; 8,*Tarentola mauritanica* ; 9,*Tarentola annularis.***

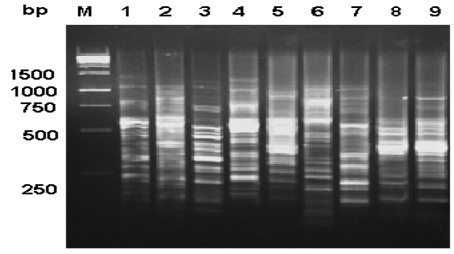


**Figure 4. RAPD profile showing DNA fingerprint patterns generated from DNA from 1 of 9 for gekkonid species with primer OP-B03. M, DNA size standard (1kb ladder). 1,*Tropiocolotes tripolitanus* ; 2,*Tropiocolotes nattereri* ; 3,*Hemidactylus turcicus* ; 4,*Cyrtopodion scaber* ; 5,*Ptyodactylus guttatus*; 6,*Ptyodactylus hasselquistii* ;7, *Stenodactylus petrii* ; 8,*Tarentola mauritanica* ; 9,*Tarentola annularis***

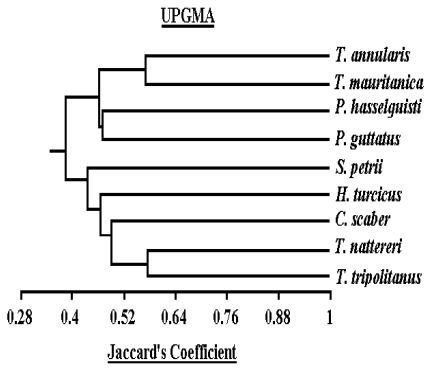


**Figure 5. UPGMA based Dendrogram showing phylogenetic relationships among the eight Gekkonid species (1-9) based on RAPD-PCR by OP-A04, OP-B03, OP-B18 and OP-C01 primers.**

**Table 2. RAPD-PCR bands produced by A4, B3, B18 and C1 primers in 9 Gecko species.**

| Primer | Band number | Molecular weight (bp) | *Tropiocolotes tripolitanus* | *Tropiocolotes nattereri* | *Hemidactylus turcicus* | *Cyrtopodion scaber* | *Ptyodactylus guttatus* | *Ptyodactylus hasselquistii* | *Stenodactylus petrii* | *Tarentola mauritanica* | *Tarentola annularis* | Band frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OPA-04 | 1 | 1267 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0.56 |
| | 2 | 1116 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0.33 |
| | 3 | 1035 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0.44 |
| | 4 | 959 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0.44 |
| | 5 | 912 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0.44 |
| | 6 | 825 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0.78 |
| | 7 | 784 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.22 |
| | 8 | 709 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0.67 |
| | 9 | 625 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0.89 |
| | 10 | 565 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0.89 |
| | 11 | 523 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0.89 |
| | 12 | 473 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0.33 |
| | 13 | 460 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.11 |
| | 14 | 428 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0.33 |
| | 15 | 407 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.11 |
| | 16 | 368 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0.44 |
| | 17 | 332 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0.78 |
| | 18 | 278 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0.56 |
| | 19 | 227 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.11 |
| | Total bands | | 13 | 10 | 11 | 10 | 2 | 7 | 14 | 8 | 9 | |
| OPB-03 | 1 | 1256 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.11 |
| | 2 | 1139 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0.44 |
| | 3 | 1067 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.44 |
| | 4 | 1032 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0.33 |
| | 5 | 967 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0.44 |
| | 6 | 906 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0.44 |
| | 7 | 849 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0.89 |
| | 8 | 769 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0.56 |
| | 9 | 721 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0.67 |
| | 10 | 675 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0.33 |
| | 11 | 612 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0.78 |
| | 12 | 573 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0.56 |
| | 13 | 555 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0.78 |
| | 14 | 503 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0.78 |
| | 15 | 471 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0.89 |
| | 16 | 456 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0.44 |
| | 17 | 427 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0.22 |
| | 18 | 400 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 19 | 375 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0.89 |
| | 20 | 329 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 21 | 308 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0.89 |
| | 22 | 289 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.22 |
| | 23 | 271 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0.67 |
| | 24 | 262 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0.67 |
| | 25 | 245 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.33 |
| | 26 | 230 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0.78 |
| | 27 | 208 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0.67 |
| | 28 | 189 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0.89 |
| | 29 | 171 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0.44 |
| | 30 | 155 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0.89 |
| | 31 | 128 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0.56 |
| | 32 | 112 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0.11 |
| | Total bands | | 21 | 21 | 20 | 20 | 16 | 23 | 15 | 14 | 19 | |
| OPB-18 | 1 | 866 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0.56 |
| | 2 | 793 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0.33 |
| | 3 | 704 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0.67 |
| | 4 | 626 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0.56 |
| | 5 | 556 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0.22 |
| | 6 | 524 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0.44 |
| | 7 | 494 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0.33 |
| | 8 | 452 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 9 | 426 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0.89 |
| | 10 | 390 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0.67 |
| | 11 | 347 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0.78 |
| | 12 | 337 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0.44 |
| | 13 | 317 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0.44 |
| | 14 | 291 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0.78 |
| | 15 | 274 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0.33 |
| | 16 | 258 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0.56 |
| | 17 | 243 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0.44 |
| | 18 | 229 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.33 |
| | 19 | 204 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0.67 |
| | 20 | 187 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0.78 |
| | 21 | 171 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0.33 |
| | 22 | 147 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0.33 |
| | 23 | 123 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0.11 |
| | Total bands | | 12 | 12 | 9 | 11 | 15 | 15 | 7 | 14 | 13 | |
| OPC-01 | 1 | 1048 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0.11 |
| | 2 | 955 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0.11 |
| | 3 | 818 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0.56 |
| | 4 | 745 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0.44 |
| | 5 | 701 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0.78 |
| | 6 | 582 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0.56 |
| | 7 | 547 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0.89 |
| | 8 | 498 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0.44 |
| | 9 | 483 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0.22 |
| | 10 | 454 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0.44 |
| | 11 | 427 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0.44 |
| | 12 | 389 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0.22 |
| | 13 | 366 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0.33 |
| | 14 | 333 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0.78 |
| | 15 | 304 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0.11 |
| | 16 | 285 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0.44 |
| | 17 | 268 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0.56 |
| | 18 | 237 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0.22 |
| | 19 | 216 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0.44 |
| | 20 | 179 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0.33 |
| | Total bands | | 8 | 7 | 3 | 7 | 12 | 14 | 7 | 9 | 9 | |

**Table (3): Total and averages of bands number, polymorphic bands, % of polymorphic bands, mean band frequency, unique bands and their size range (bp) for different primers in the nine gekkonid species .**

| Primer | Total No. of bands | No. of polymorphic bands | % of polymorphic bands | Band frequency | Mean sharing band frequency | Unique band | Size range (bp) |
|---|---|---|---|---|---|---|---|
| OPA-04 | 19 | 19 | 100% | 0.1-0.89 | 0.491(49.1%) | 3 | 1267-227 |
| OPB-03 | 32 | 30 | 93.75% | 0.1-1 | 0.597(59.7%) | 2 | 1256-112 |
| OPB-18 | 23 | 22 | 95.65% | 0.1-1 | 0.521(52.1%) | 1 | 866-123 |
| OPC-01 | 20 | 20 | 100% | 0.1-0.89 | 0.421(42.1%) | 3 | 1048-179 |
| Total (average) | 94 (23.5) | 91 (22.75) | 96.8% | 0.1-1 | 0.501(50.1%) | 9(2.25) | 1267-112 |

**Table ( 4 ) : The similarity matrix among the ten Gekkonid species according to Jaccard's Coefficient**

| G. S. \ G. D. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | --- | 0.424 | 0.508 | 0.50 | 0.662 | 0.654 | 0.574 | 0.544 | 0.529 |
| 2 | 0.576 | --- | 0.569 | 0.515 | 0.623 | 0. 603 | 0.524 | 0. 60 | 0.567 |
| 3 | 0.492 | 0.431 | --- | 0.532 | 0.687 | 0.60 | 0.542 | 0.581 | 0.631 |
| 4 | 0.50 | 0.485 | 0.468 | --- | 0.632 | 0.627 | 0.621 | 0.569 | 0.657 |
| 5 | 0.338 | 0.377 | 0.313 | 0.368 | --- | 0.529 | 0.646 | 0.57 | 0.518 |
| 6 | 0.346 | 0. 397 | 0.40 | 0.373 | 0.471 | --- | 0.62 | 0.592 | 0.534 |
| 7 | 0.426 | 0.476 | 0.458 | 0.379 | 0.354 | 0.38 | --- | 0.625 | 0.631 |
| 8 | 0.456 | 0. 40 | 0.419 | 0.431 | 0.43 | 0.408 | 0.375 | --- | 0.431 |
| 9 | 0.471 | 0.433 | 0.369 | 0.343 | 0.482 | 0.466 | 0.369 | 0.569 | --- |

**G. D., Genetic distance; G. S., Genetic similarity**

**1,*Tropiocolotes tripolitanus*; 2,*Tropiocolotes nattereri*; 3,*Hemidactylus turcicus*; 4,*Cyrtopodion scaber*; 5,*Ptyodactylus guttatus*; 6,*Ptyodactylus hasselquistii*; 7, *Stenodactylus petrii*; 8,*Tarentola mauritanica*; 9,*Tarentola annularis.***

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