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Phylogenetic relationship among five geckos from Egypt based on RAPD-PCR and protein electrophoresis (SDS–PAGE)

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Abstract Genetic variations between five gekkonid species from Egypt; *Tropiocolotes tripolitanus*, *Tropiocolotes steudneri*, *Tropiocolotes nattereri*, *Tarentola mauritanica* and *Tarentola annularis* were analyzed by SDS–PAGE for water soluble proteins and random amplified polymorphic DNA (RAPD) analysis. Based on SDS–PAGE of water soluble proteins for all species, the obtained results revealed a total of 17 bands at molecular weights that ranged from 95 to 16 kDa. The polymorphic bands among species were 11 (64.7%) and the mean similarity matrix value between them was 70.7%. Using RAPD-PCR, the results showed eight total amplified bands at molecular weights that ranged from 1408 to 360 bp. The polymorphic bands between species were 7 (87.5%) and the mean similarity matrix between them was 44.6%. The dendrogram showed that, the five gekkonid species are separated from each other into two clusters. The first cluster contains three species of the genus *Tropiocolotes*. The second cluster includes the two species of the genus *Tarentola*. Based on SDS–PAGE and RAPD-PCR results, *T. nattereri* is sister to *T. steudneri* with higher genetic similarity than with *T. tripolitanus*. It is concluded that, the similarity coefficient and the genetic distance values between the five gekkonid species indicate that the five gekkonid species are not identical and are separated from each other. From these results, it is indicated that the protein and RAPD analysis are useful molecular tools to indicate genetic variation between the species in the same genus or in the different genera.

Introduction

The squamates are the most diversified group containing lizards and snakes (Vidal and Hedges, 2009). Gekkonidae is one of the largest vertebrate group among squamates that are distributed throughout the world (Vidal and Hedges, 2005). There are four subfamilies of the Gekkonidae (Diplodactylinae, Gekkoninae, Eublepharinae and Sphaerodactylinae) with 1130 species and 108 genera (Han et al., 2004).

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Geckos have high diversity in arid and semiarid habitats in Africa and Australia (Baha El Din, 2006). Several investigations have been recorded on the herpetofauna of reptiles in Egypt (Anderson, 1898; Marx, 1968; Goodman and Hobbs, 1994; Baha El Din, 2006). The phylogenic relationship among the species of the family Gekkonidae was based on morphological (Anderson, 1898; Marx, 1968), karyological (Kawai et al., 2009) and molecular studies (Joger, 1985; Rato et al., 2010; Busais and Joger, 2011).

The genus *Tarentola* comprises about 20 morphologically similar species (Baha El Din, 1997; Harris et al., 2004a,b), which live mainly in semi-arid to arid habitats. They are distributed in North Africa, coastal regions of Mediterranean Sea, Macaronesian islands and also Cuba and the Bahamas. *Tarentola mauritanica* is distributed in Mediterranean regions (North Egypt) and extends across Northern Libya and Southern Tunisia. The *Tarentola annularis* is distributed in a wide area across Africa, central Sudan, to the north along the Nile to the Nile Delta and Sinai Peninsula (Egypt) (Joger, 1984a; Baha El Din, 2006). The phylogenetic studies of the genus *Tarentola* based on molecular analysis such as serum protein electrophoresis, quantitative precipitin tests of serum albumin (Joger, 1984b, 1985), RAPD-PCR analysis (Ali, 2012), DNA sequences from mitochondrial and nuclear genes (Carranza et al., 2002; Jesus et al., 2002; Harris et al., 2004a,b; Perera and Harris, 2008) indicated that morphologically similar species exhibit genetic variation.

The genus *Tropiocolotes* (Peters, 1880) comprises a group of small, nocturnal and ground dwelling geckos. Several authors (Baha El Din, 2001; Rastegar-Pouyani et al., 2007; Wilms et al., 2010) studied the morphological characters of *Tropiocolotes steudneri*, *Tropiocolotes nattereri* and *Tropiocolotes tripolitanus*. The distribution of *Tropiocolotes* ranges from the Western Sahara across Northern Africa to Israel, Sinai, the Arabian Peninsula, Iran, eastern Afghanistan and Pakistan (Anderson, 1898; Rastegar-Pouyani et al., 2007). Molecular studies on the genus *Tropiocolotes* (Ali, 2012) are very scarce.

Molecular markers proven by analysis of proteins and RAPD-PCR have shown excellent potential to analysis of genetic structure of germplasm for several varieties of organisms (Studer, 1992). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) is the most inexpensive, simple and important tool that is widely used to differentiate the evolution among the species (Ferguson, 1980; Avise, 1994; Mishra et al., 2010). The RAPD-PCR analysis is used to differentiate the closely related species in order to determine the genetic diversity between and within the species and is a useful method for showing of breeding populations (Welsh and Mc Clelland, 1990; Williams et al., 1990, 1993).

The present study was targeted to assess the genetic diversity among five gekkonid species by using biochemical (protein) and molecular (RAPD-PCR) markers.

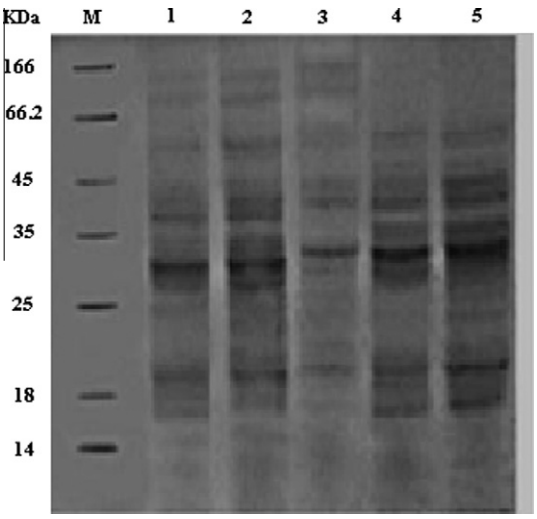

Figure 1 Gel electrophoresis represents protein bands from five Gekkonid species (lanes 1–5). M, protein marker with molecular size (1 kDa). 1, *Tropiocolotes steudneri*; 2, *Tropiocolotes nattereri*; 3, *Tropiocolotes tripolitanus*; 4, *Tarentola mauritanica*; 5, *Tarentola annularis*.

Materials and methods

Five gekkonid species from Egypt were collected from different localities (Table 1). The five species are belonging to two genera. Morphological identification and classification of animals as well as scientific and common names of these species were carried out according to previous works (Anderson, 1898; Marx, 1968; Goodman and Hobbs, 1994).

Protein electrophoresis (SDS–PAGE)

Protein electrophoresis was carried out with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Muscles from the five geckos were taken and stored at −20 °C. Muscles were grounded with 1 ml of 1× extraction buffer; (10% SDS, 10 ml glycerol, 1 M Tris–HCl and 0.25 M EDTA, pH 8.8) and left overnight in refrigerator. The samples were centrifuged and the clear supernatants containing water-soluble proteins were used for electrophoresis. Muscle soluble protein fractions were separated exclusively on a vertical slab gel (19.8 cm × 26.8 cm × 0.3 cm) using the gel electrophoretic apparatus (manufactured by APPEX) according to the method of Laemmli (1970). The final monomer concentration in the 0.75 mm-thick slab gels was 12% (w/v) for the separating gel and 4% (w/v) for the stacking gel. Prior to loading, all samples were incubated in the presence of 1% (w/v) SDS and 100 mM

Table 1 Scientific name, common name and localities of five gekkonid species from Egypt.

Scientific name	Common name	Locality
Tropiocolotes steudneri (Peters, 1869)	Steudner's Gecko, Steudner's Pigmy Gecko	Sinai
Tropiocolotes nattereri (Steindachner, 1901)	Natterer's Gecko, BorsTaht El Hagar	Sinai
Tropiocolotes tripolitanus (Peters, 1880)	Tripoli Gecko, Tripoli pigmy Gecko, BorsTaht El Hagar	Sinai
Tarentola mauritanica (Linnaeus, 1758)	Moorish Gecko, Moorish wall Gecko, Crocodile Gecko	Abu Rawash
Tarentola annularis (Geoffroy, 1809)	Egyptian Gecko, white spotted Gecko, Bors Abu ArbaNoqat	Abu Rawash