Molecular phylogeny of the genus *Eumeces* Wiegmann, 1834 (Reptilia: Scincidae) in Iran, inferred from 16s mitochondrial DNA

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Abstract. Phylogenetic relationships among the *Eumeces schneiderii princeps* and *Eumeces schneiderii pavimentatus* investigated using 509 bp partial sequences of 16S mitochondrial gene. Analyses were done by maximum-likelihood (RAxML) criteria on 52 specimens from over 20 geographically distinct localities. Our molecular results proposed two well-supported major clades by their phylogenetic positions, genetic differences and unique characterizations in their morphology and habitats including: (1) *Eumeces schneiderii princeps* *Eumeces schneiderii pavimentatus* (2) *Eurylepis (3) Scincus and (4) Scincopus*. However, the phylogenetic affinities of *Eumeces sp.* in the *Scincus* clade were not resolved. Phylogenetic analyses of the genus did not grouped *Eurylepis* with *Eumeces* and clustered it in a completely separate group. In addition, phylogenetic results revealed a monophyletic status for *Eumeces schneiderii*.

Keywords. biogeography, *Eumeces*, Iran, mitochondrial genes, Scincidae, phylogeny
INTRODUCTION

The name *Eumeces schneiderii*, initially proposed by Wiegmann in honor of Johann Gottlob Schneider (1750-1822), German zoologist in Herpetology Mexicana (1834) in that study, three species *E. pavimentatus*, *E. rufescens*, and *E. punctatus* recorded under the name of the genus for the first time. In a recent study by Griffith et al. (2000), the genus *Eumeces* divided into four groups based on a series of morphological characteristics analysis and radical changes were proposed for the genus. The paraphyletic genus *Eumeces* divided into four separate genera:

*Eurylepis* (“*E. taeniolatus*” group), *Mesoscincus* (“*E. schwartzei*” group), *Novoeumeces* (“*E. schneiderii*” group that includes *E. pavimentatus* as the type species of *Eumeces* sensu lato) and *Eumeces* (sensu stricto) includes all the other remaining species, and mainly distributed in East Asia and North America.

Placement of the genus *Eumeces* for the species of North America has emphasized in Griffith work and *Lacerta fasciata* Linnaeus 1758 chosen as type species of the genus *Eumeces*. In addition, based on cranial traits, Griffith and his colleagues (2000) recognized *Pariocela* species group as members of the genus *Eumeces* as the most basic group of all skinks throughout the world. In addition, a new subfamily Eumecinae proposed for this group of species. The proposed new generic name, *Pariocela* Fitzinger, 1843, for North American skinks of *Eumeces* (s. 1.), later replaced with the older available generic name *Plestiodon* Duméril & Bibron, 1839 (Brandley et al., 2005; Schmitz et al., 2004).

Moreover, the name *Eumeces* (sensu stricto) retained for the group surrounding the type species that is part of the African-Central Asian clade and there are currently only five recognized species in this clade has left.

These include *Eumeces algeriensis* Peters, 1864; *Eumeces blythianus* (Anderson, 1871); *Eumeces cholsitanensis* Masroor, 2009; *Eumeces indothalensis* Khan and Khan, 1997; *Eumeces schneiderii* (Daudin, 1802).

The other (old *Eumeces*) are now classify in *Eurylepis* (two species), *Mesoscincus* (three species, in North America) and *Plestiodon* (47 species, in North America). So far, five subspecies is known for *Eumeces schneiderii* (Daudin, 1802) includes *E. s. barani* (Kumlutas et al., 2007) in Turkey (Anatolia); *E. s. pavimentatus* (Geoffroy St. Hilaire, 1827) in Syria, Lebanon, Jordan; *E. s. princeps* (Eichwald, 1839) in Armenia, Azarbayegan, Caucasus, Iran; *Eumeces schneiderii* Zarudnyi (Nikolsky, 1900) in SE Iranian Plateau (Kerman, Sistan and Baluchistan provinces), Helmand Basin and southern desert districts of Afghanistan, Baluchistan and Mekran Coast of Pakistan. In this study, the intraspecific phyloge-netic relationships of Iranian subspecies *Eumeces schneiderii princeps* are extracted and will compare with another subspecies extracted from genbank dataset.

MATERIAL AND METHODS

Sampling localities for the 52 used specimens in this study presented in Figure 1 and Table 1. *Eumeces schneiderii princeps* in its natural habitat showed in Figure 2 We used *Eurylepis, Scincus* and *Scincopus* as close and distant relatives of the genus as outgroup comparisons to constructing phylogenetic trees and the relevant sequences downloaded from gene-bank data center (Table 1).

Original DNA and tissue samples deposited in the Department of Biology, Hakim Sabzevari University. All specimens are deposited in Razi University Zoological Museum (RUZM) collection in 95% alcohol.

In order to laboratory protocols (DNA extraction, PCR and Sequencing), DNA was extracted from preserved tissue samples using non-organic DNA Extraction Procedure (Proteinase K and Salting out). 16S gene was amplified with polymerase chain reaction (PCR) procedure using 16SL 5’-CCGCTGTGTTATCATAAACACAT-3’, and 16SH 5’-CCGTTCTGAACTCAG ATCACG-3’, as primers for 16S mitochondrial gene.

PCR reactions were performed in 30µl with the following conditions: Initial denaturation stage of 95° C (04:00) followed by the 35 cycles with denaturation at 95°C (00:40), annealing at 49°C (00:40) and extension at 72°C (01:20) then single extension cycle at 72° C (10:00).

The amplified genes were then sequenced with an automatic DNA sequencer in BIONEER Company, South Korea following the manufacture procedure and protocols. Construction of multiple alignments done by Clustal W (Thompson, 1994) program as implemented in Bioedit software program version 7.0.0 (Hall, 1999); all sequences adjusted their ends manually.
Fig. 1. Sampling localities of *Eumeces schneideri princeps* in this study.

Assessing substitution saturation done by DAM-BE software (Xia & Lemey, 2009). The genetic divergences of nucleotide sites among the species as p-distance in a matrix of pairwise sequences and the percentage of variable sites and parsimony informative sites calculated using MEGA5 (Kumar et al., 2008). Maximum likelihood phylogenetic analysis conducted using RAXMLGUI11.5b1 (Silvestro & Michalak, 2012).

Support for the estimated tree assessed using 1000 bootstrap replicates. Bayesian analyses using MrBayes v.3.1.2 used for inferring phylogenetic relationships among studied taxa and to compare the resulted tree with ML tree. J Model Test 2.1.1 (Posada, 2008) was used for inferring best fitting evolutionary model required for BI analyses. The resulted trees visualized using MrEnt-V.2.4 (Zuccon & Zuccon, 2012).

Fig. 2. *Eumeces schneiderii princeps* and its natural habitats in Kordestan and Zanjan provinces, Iran.
Table 1. Specimens used in this study, collection numbers, GenBank accession numbers, collecting localities and their exact coordination.

| Species                     | Collection number | GenBank accession | Coordinates | Locality                                      |
|-----------------------------|-------------------|-------------------|-------------|-----------------------------------------------|
| Scincopus fasciatus         | SCP2              | AY64917           | -           | 30km NW Rosso, Mauritania                     |
| Scincopus fasciatus         | SCP3              | AY30830           | -           | 30km NW Rosso, Mauritania                     |
| Scincus scincus             | SC4               | AY21799           | -           | Unknown                                       |
| E. pavimentatus             | ES10              | JF931189          | -           | Al Jaboul lake, Syria                         |
| E. pavimentatus             | ES11              | JF931190          | -           | Azraq, Jordan                                 |
| E. pavimentatus             | ES13              | JF931191          | -           | Um Quays, Jordan                              |
| E. pavimentatus             | ES14              | JF931192          | -           | Dana Nature Reserve, Jordan                   |
| E. pavimentatus             | ES17              | JF931193          | -           | Lattakia Beach, Syria                         |
| E. pavimentatus             | ES18              | JF931194          | -           | Petra, Jordan                                 |
| Eumeces sp.                 | -                 | JQ344224          | -           | Unknown                                       |
| Eumeces sp.                 | -                 | JQ344226          | -           | Unknown                                       |
| Eumeces sp.                 | -                 | JQ344228          | -           | Unknown                                       |
| Eumeces pavimentatus        | GB7               | EU27807           | -           | Coastal dunes after Karatas, Turkey           |
| Eumeces pavimentatus        | GB8               | EU27806           | -           | Karaotlak in the Euphrates, Turkey            |
| E. princeps                 | RUZMHF1           | This study        | 248431      | 4030619 Halab, Zanjan, Iran                   |
| E. princeps                 | RUZMHF2           | This study        | 244632      | 4024891 Halab, Zanjan, Iran                   |
| E. princeps                 | RUZMHF3           | This study        | 638788      | 3774751 Eslamabad, Amirabad Village, Iran     |
| E. princeps                 | RUZMHF4           | This study        | 677666      | 3921234 Between Sanandaj & Divandareh, Iran   |
| E. princeps                 | RUZMHF5           | This study        | 677670      | 3921280 Between Sanandaj & Divandareh, Iran   |
| E. princeps                 | RUZMHF6           | This study        | 727258      | 3649410 Ilam, Dinarkouh, Iran                 |
| E. princeps                 | RUZMHF7           | This study        | 680544      | 3872244 Around Gawshan Dam, Iran              |
| E. princeps                 | RUZMHF8           | This study        | 659158      | 3790907 Kermanshah, Lalabad, Iran            |
| E. princeps                 | RUZMHF9           | This study        | 668533      | 3831940 Kamyaran, Kordestan, Iran             |
| E. princeps                 | RUZMHF10          | This study        | 244632      | 4024289 Mahneshan, Zanjan, Iran               |
| E. princeps                 | RUZMHF11          | This study        | 659158      | 3790907 Kermanshah, Lalabad, Iran            |
| E. princeps                 | RUZMHF13          | This study        | 639068      | 3851876 Kermanshah, Javanroud                |
| E. princeps                 | RUZMHF14          | This study        | 723659      | 3823819 Kermanshah, Baghcheh, Iran           |
| E. princeps                 | RUZMHF15          | This study        | 693314      | 3807800 Kermanshah, Razi University, Iran     |
| E. princeps                 | RUZMHF16          | This study        | 244632      | 4024891 Halab, Zanjan, Iran                   |
| E. princeps                 | RUZMHF18          | This study        | 673955      | 3821022 Kermanshah, Harsin, Iran             |
| E. princeps                 | RUZMHF19          | This study        | 749281      | 3827092 Kermanshah, Harsin, Iran             |
| E. princeps                 | RUZMHF20          | This study        | 538512      | 3963007 Iraq, Soulaymani, Iran                |
| E. princeps                 | RUZMHF21          | This study        | 638788      | 3774751 Eslamabad, Amirabad Village, Iran     |
| E. princeps                 | RUZMHF22          | This study        | 734947      | 3794894 Kermanshah, Harsin, Iran             |
| Eumeces sp.                 | RUZMHF23          | This study        | -           | Tehran, Iran                                  |
| E. princeps                 | RUZMHF24          | This study        | 411936      | 3860850 Around Saveh, Iran                    |
| E. princeps                 | RUZMHF25          | This study        | 411936      | 3860850 Around Saveh, Iran                    |
| E. princeps                 | RUZMHF26          | This study        | 290439      | 4016052 Between Damghan and Semnan, Iran      |
| E. princeps                 | RUZMHF27          | This study        | 551204      | 3950031 E Tehran, Sorkhe Hesar, Iran          |
| E. princeps                 | RUZMHF28          | This study        | 548818      | 3950542 E Tehran, Sorkhe Hesar, Iran          |
| E. princeps                 | RUZMHF29          | This study        | 516021      | 3962733 Karaj, Verdi, Iran                    |
| E. princeps                 | RUZMHF30          | This study        | 290439      | 4016052 Between Damghan and Semnan, Iran      |
| E. princeps                 | RUZMHF31          | This study        | 411936      | 3860850 Around Saveh, Iran                    |
| E. princeps                 | ERP914            | This study        | 334430      | 4045232 Khorassan Razavi, Sarakhs, Iran       |
| E. princeps                 | ERP1443           | This study        | 631134      | 3900473 Khorassan Razavi, Kashmar, Iran       |
| E. princeps                 | ERP1462           | This study        | 562262      | 3995191 Sabzevar, Iran                        |
| E. princeps                 | ERP1987           | This study        | 665038      | 3415948 Fars, 15 km West of Eghlid, Iran      |
| E. princeps                 | ERP1965           | This study        | 557248      | 3387574 Fars, 25 km NW of Yasoj, Iran         |
| E. princeps                 | ERP1966           | This study        | 542588      | 3400838 Fars province, 25 km NW of Yasoj      |
RESULTS
Statistical test for substitution saturation analyses by DAMBE (Xia & Lemey, 2009) showed no significant saturation in the dataset. In our dataset alignments of 509bp of 16s gene, 108 positions (21.2%) are variable and 89 positions (17.5%) are parsimony informative. The estimated value of the shape parameter for the discrete Gamma Distribution is 0.14. Substitution pattern and rates estimated under the Tamura-Nei (1993) model (+G). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, [+G]).

Mean evolutionary rates in these categories were 0.00, 0.00, 0.04, 0.42, 4.54 substitutions per site. The nucleotide frequencies are A = 32.78%, T = 21.81%, C = 24.68%, and G = 20.74%. The estimated Transition/Transversion bias (R) is 3.21. Uncorrected p-distance for combined genes was 3% as intersubspecific genetic divergence between E.s.princeps and E.s.pavimentatus (Table 2) and 12% as maximum genetic divergence between Eumeces sp and Eurylepis taeniolatus.

Pairwise uncorrected genetic divergence (p-distance) among all species is presented in Table 2. The phylogenetic analyses did not grouped Eumeces sp with the remaining species of Eumeces schneideri group and clustered it along with Scincus scincus in a fully separate clade (Fig. 3).

In addition, phylogenetic results revealed a monophyletic status for Eumeces schneiderii group. The phylogenetic analyses revealed two distinct clades in-group taxa include one main clad (E.s.-princeps) and the next is E.s. pavimentatus with 95% bootstrap value (Fig. 3). Correspondingly, three major out-group clades resulted from analyses include Eurylepis taeniolatus, Scincus scincus and Scincopus fasciatus (Fig. 3). Selected evolutionary model by J Model Test 2.1.1 for combined dataset using AICc and BIC criteria was (TrN + I + G) model as the best-fitting model. The proportion of invariable sites (I) = 0.3440, for among site rate variation, its Gamma distribution shape parameter (a) = 0.7800.

The setting for selecting the best fitting model by J Model Test was as follows: number of substitution schemes = 11; thereby, candidate evolutionary models = 88 distinct models; base tree for likelihood calculations = ML tree; tree topology search operation = BEST. Phylogenetic analysis using BI methods yielded almost the same tree topologies with ML resulted from RAxMLGUI. Therefore, we chose the topology derived from ML analyses infer phylogenetic relationships in dataset, which had relatively similar branching patterns particularly referring to the deep lineages and major clades.

DISCUSSION
We have produced the first detailed and well-supported molecular phylogeny for the Iranian subspecies Eumeces schneideri princeps. Existing very little genetic divergence (about 1-1.4%) among different populations of the Eumeces schneiderii princeps as intrasubspecific genetic variations in the study can be signs of stable population genetic and existing gene flow among them in a cline way along Zagros Mountains.

Main clade of Eumeces schneiderii is a central and western Zagros clade, but the Eumeces sp. is a central Iranian plateau and eastern Zagros clade that seems to be affected by Zagros orogeny process during long last times.

The phenomenon of Zagros Mountains uplifting acted as a strong physical barrier against distribution of this genus towards central Iranian plateau around 10-12.4 MYA (Sborschchikov et al., 1981; Moutheraue, 2011). Additionally, the new species is located at lowlands with lower elevations (1100-1250 m asl) where is desert like habitats are more prominent unlike most of E.schneideri taxa which specified to mountainous and foothills.
The genera *Scincus* and *Scincopus* strongly supported as being nested within *Eumeces* s.s. (Perera *et al*., 2012) with phylogenetic affinity of *E. algeriensis* to *Scincopus* and *E. schneiderii* to *Scincus*. As it clear in Figure 3 the unknown taxon (here *Eumeces* sp.) is closer to *Scincus* than any other *E. schneiderii* group species. Most studies based on karyological analyses (Kupriyanova, 1986; Caputo *et al*., 1993, 1994) and morphological/molecular studies (Carranze *et al*., 2008) showed the paraphyly of *E. schneiderii* group species and argued that *Scincus* may be derived from an *E. schneiderii*-like stock. The *Scincus Eumeces* clade considered basal to other members of the family (Giovannotti *et al*., 2009).

Phylogenetic affinity of *Scincus* to *E. schneiderii* species group in this analysis is very noticeable. This can represent the paraphyly of *E. schneiderii* species group as presented by Pyron *et al*., (2013), Figure 6E extracted from squamate tree relationships, in phylogenetic relationships among *Eumeces*, *Scincus* and *Scincopus*. The results of molecular-
ar studies by Carranza et al., 2008 using mitochondrial genes (12S + Cyb), showed that common ancestors of *E. schneiderii* and *E. algeriensis* along with species of *Scincus* and *Scincopus* been divided around 13.7 mya. Very wide distribution range within the *E.schneiderii* (Punjab Pakistan to the West and North Africa) led to forming about 5 to 6 subspecies within this species. Placing three species *Eumeces blythianus*, *Eumeces cholistaneus* and *Eumeces indothalensis* in *Eumeces schneiderii* species group is only because of occurring at the same geographic range and same distribution pattern with this group of species. Since *Eurylepis taeniolatus* is in the same geographic region with the mentioned species group, there is also possibility and probability of the above three species belonging to the monotypic *taeniolatus* species group instead of *Eumeces schneiderii* species group.

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