فیلوژنی مولکولی جنس *Eumeces* Wiegmann, 1834 (خزندگان: سینسیده) در ایران، بر اساس DNA میتوکندریایی ژن 16S

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چکیده. روابط فیلوژنتیکی بین زیرگونه های *Eumeces schneiderii princeps* و *Eumeces schneiderii pavimentatus* با استفاده از ۵۰۹ جفت باز از توالی ژن 16S میتوکندریایی مورد بررسی قرار گرفت. آنالیزها با استفاده از رویکرد حداکثر احتمال (ML) به کمک نرم افزار RAxML بر روی ۵۲ نمونه جمع آوری شده از حدود ۲۰ منطقه مجزا صورت گرفت. نتایج مطالعات مولکولی ما چهار کلاد کاملا مجزا و به خوبی حمایت شده ای از لحاظ موقعیت فیلوژنتیکی، تفاوت ژنتیکی و ویژگی‌های مشخص در مورفولوژی و ویژگی‌های زیستگاهی آنها تشخیص داد. این کلادها شامل گروه‌هایی از (1) *Eumeces schneiderii princeps+Eumeces schneideri pavimentatus* (2) *Eurylepis* (3) *Scincus* و (4) *Scincopus* هستند. همچنین، رابطه فیلوژنتیکی تاکسون ناشناخته *Eumeces sp.* با کلاد *Scincus* بطور کامل مشخص و آشکار نیست. آنالیزهای فیلوژنتیکی صورت گرفته جنس *Eurylepis* را در کنار کلادهای حاوی جنس *Eumeces* قرار نداد. به علاوه، نتایج این آنالیزهای فیلوژنتیکی یک وضعیت مونوفایلتیک را برای گونه *Eumeces schneiderii* نشان داد.

واژه‌های کلیدی. ایران، بیوجغرافیایی، ژن میتوکندریایی، سینسیده، فیلوژنی، یومسس

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. *Acanthodactylus*, biogeography, cytochrome b, Iran, Lacertidae, mitochondrial genes, ND4, phylogeny

INTRODUCTION

The name *Eumeces schneiderii*, initially proposed by Wiegmann in honor of Johann Gottlob Schneider (1750-1822), German zoologist in Herpetologia 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. schneideri*" 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 works 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. l.), 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 cholistanensis* 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, Azarbayejan, Caucasus, Iran; *Eumeces schneiderii* Zarudnyi (Nikolsky, 1900) in SE Iranian Plateau (Kerman, Sistan and Baluchistan provinces), Helmand Basin and southern desert districts of Afganistan, 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´-CGCCTGTTTATCAAAAACAT-3', and 16SH 5'-CCGGTCTGAACTCAG ATCACG-3', as primers for 16s mitochondrial gene.

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

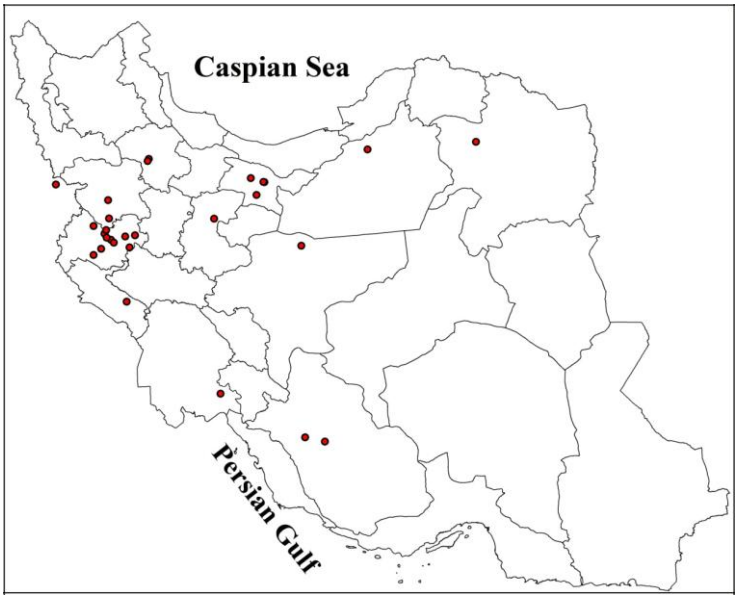

Fig. 1. Sampling localities of *Eumeces schneideri princeps* in this study.

Fig. 2. *Eumeces schneiderii princeps* and its natural habitats in Kordestan and Zanjan provinces, Iran.

Assessing substitution saturation done by DAMBE 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 RAxMLGUI1.5b1 (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).

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	SC3	AY71294	-	-	Unknown
Scincus scincus	SC4	AY21799	-	-	Unknown
E.s.pavimentatus	ES10	JF931189	-	-	Al Jaboul lake, Syria
E.s.pavimentatus	ES11	JF931190	-	-	Azraq, Jordan
E.s.pavimentatus	ES13	JF931191	-	-	Um Quays, Jordan
E.s.pavimentatus	ES14	JF931192	-	-	Dana Nature Reserve, Jordan
E.s.pavimentatus	ES17	JF931193	-	-	Lattakia Beach, Syria
E.s.pavimentatus	ES18	JF931194	-	-	Petra, Jordan
Eurylepis taeniolatus	-	JQ344224	-	-	Unknown
Eurylepis taeniolatus	-	JQ344226	-	-	Unknown
Eurylepis taeniolatus	-	JQ344228	-	-	Unknown
E.s.pavimentatus	GB7	EU27807	-	-	Coastal dunes after Karatas, Turkey
E.s.pavimentatus	GB8	EU27806	-	-	Karaotlak in the Euphrates, Turkey
E.s.princeps	RUZMHF1	This study	248431	4030619	Halab, Zanjan, Iran
E.s.princeps	RUZMHF2	This study	244632	4024891	Halab, Zanjan, Iran
E.s.princeps	RUZMHF3	This study	638788	3774751	Eslamabad, Amirabad Village, Iran
E.s.princeps	RUZMHF4	This study	677666	3921234	Between Sanandaj & Divandareh, Iran
E.s.princeps	RUZMHF5	This study	677670	3921280	Between Sanandaj & Divandareh, Iran
E.s.princeps	RUZMHF6	This study	727258	3649410	Ilam, Dinarkouh, Iran
E.s.princeps	RUZMHF7	This study	680544	3872244	Around Gawshan Dam, Iran
E.s.princeps	RUZMHF8	This study	659158	3790907	Kermanshah, Lalabad, Iran
E.s.princeps	RUZMHF9	This study	668533	3831940	Kamyaran, Kordestan, Iran
E.s.princeps	RUZMHF10	This study	244632	4024289	Mahneshan, Zanjan, Iran
E.s.princeps	RUZMHF11	This study	659158	3790907	Kermanshah, Lalabad, Iran
E.s.princeps	RUZMHF13	This study	639068	3851876	Kermanshah, Javanroud, Iran
E.s.princeps	RUZMHF14	This study	723659	3823819	Kermanshah, Baghcheh, Iran
E.s.princeps	RUZMHF15	This study	693314	3807800	Kermanshah, Razi University, Iran
E.s.princeps	RUZMHF16	This study	244632	4024891	Halab, Zanjan, Iran
E.s.princeps	RUZMHF18	This study	673955	3821022	Kermanshah, Harsin, Iran
E.s.princeps	RUZMHF19	This study	749281	3827092	Kermanshah, Harsin, Iran
E.s.princeps	RUZMHF20	This study	538512	3963007	Soulaymanieh, Iraq
E.s.princeps	RUZMHF21	This study	638788	3774751	Eslamabad, Amirabad Village, Iran
E.s.princeps	RUZMHF22	This study	734947	3794894	Kermanshah, Harsin, Iran
Eumeces sp.	RUZMHF23	This study	-	-	Tehran, Iran
E.s.princeps	RUZMHF24	This study	411936	3860850	Around Saveh, Iran
E.s.princeps	RUZMHF25	This study	411936	3860850	Around Saveh, Iran
E.s.princeps	RUZMHF26	This study	290439	4016052	Between Damghan and Semnan, Iran
E.s.princeps	RUZMHF27	This study	551204	3950031	E Tehran, Sorkhe Hesar, Iran
E.s.princeps	RUZMHF28	This study	548818	3950542	E Tehran, Sorkhe Hesar, Iran
E.s.princeps	RUZMHF29	This study	516021	3962733	Karaj, Verdij, Iran
E.s.princeps	RUZMHF30	This study	290439	4016052	Between Damghan and Semnan, Iran
E.s.princeps	RUZMHF31	This study	411936	3860850	Around Saveh, Iran
E.s.princeps	ERP914	This study	334430	4045232	Khorassan Razavi, Sarakhs, Iran
E.s.princeps	ERP1443	This study	631134	3900473	Khorassan Razavi, Kashmar, Iran
E.s.princeps	ERP1462	This study	562262	3995191	Sabzevar, Iran
E.s.princeps	ERP1987	This study	665038	3415948	Fars, 15 km West of Eghlid, Iran
E.s.princeps	ERP1965	This study	557248	3387574	Fars, 25 km NW of Yasoj, Iran
E.s.princeps	ERP1966	This study	542588	3400838	Fars province, 25 km NW of Yasoj, Iran

Table 2. Pairwise uncorrected genetic divergence (p-distance) within studied taxa derived from the 16s mitochondrial gene.

Species	*Eurylepis*	*Scincus*	*Scincopus*	*E.s.pavimentatus*	*E.s.princeps*	*E.sp*
Eurylepis						
Scincus	0.09					
Scincopus	0.10	0.08				
E.s.pavimentatus	0.11	0.07	0.11			
E.s.princeps	0.10	0.07	0.11	0.03		
E.sp	0.12	0.10	0.11	0.11	0.10	

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 am- ong 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 schneiderii* 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 invari able 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 schneiderii 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 (Sborshchikov *et al*., 1981; Mouthereau, 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.

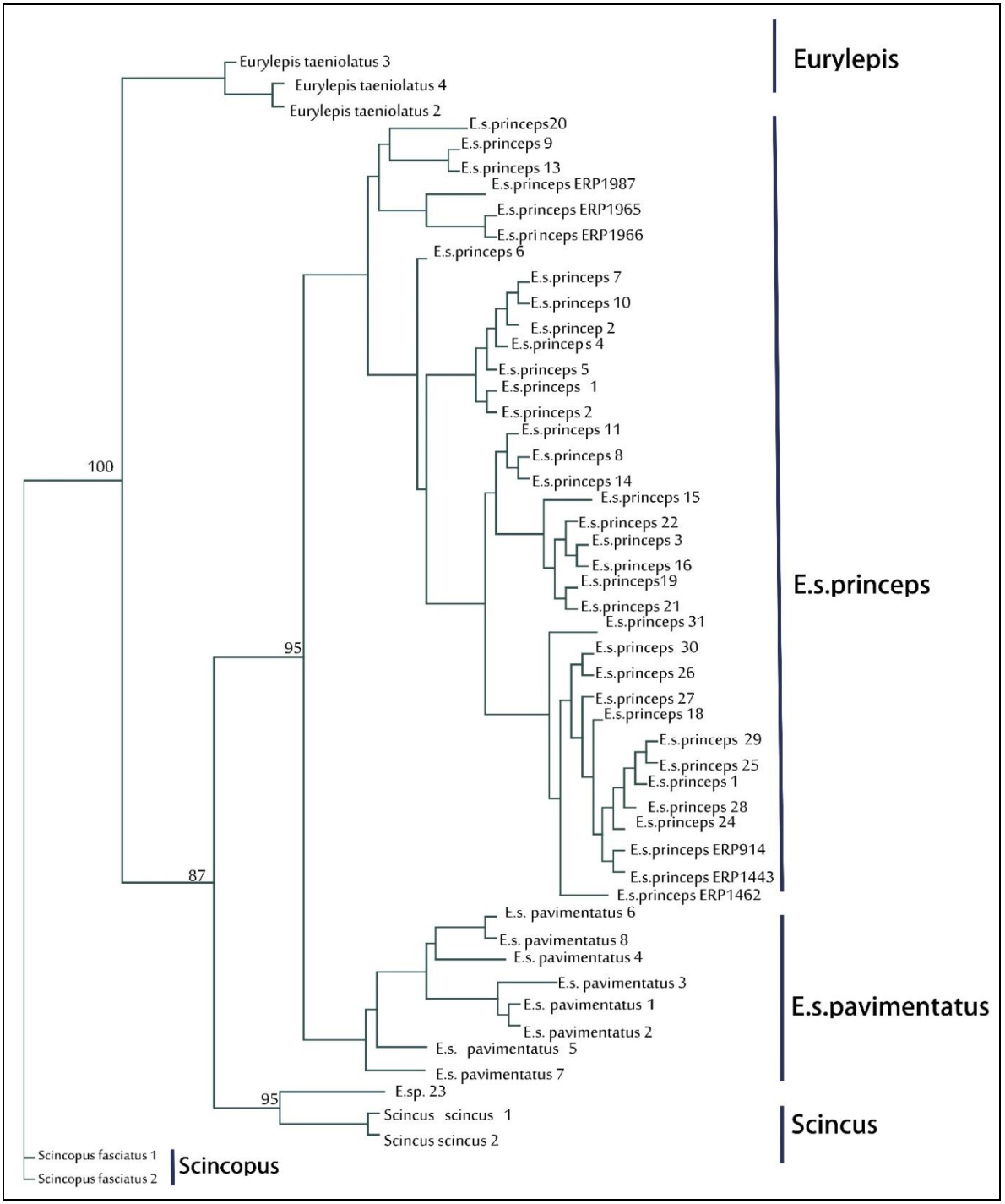

Fig. 3. Maximum-likelihood tree of 52 specimens based on mitochondrial data set (509bp of 16s gene). Numbers above branches are bootstrap support values (1000 replicates).

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 molecul-

ar studies by Carranza *et al*., 2008 using mitochondrial genes (12S + Cytb), 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 cholistanensis* 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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