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Molecular Phylogenetic Relationships of Exemplars of Four Spider Families from Ha'il Region, Northern Saudi Arabia and a Preliminary List of Spiders of Ha'il

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ABSTRACT

Knowledge about spider fauna in Saudi Arabia is hugely inadequate compared to adjacent countries. The present work is a preliminary study of Hai'l's spiders. Fourteen genera, in addition to five unidentified spider species, belonging to 16 families were recorded in Ha'il Region. This is the first study of spider's fauna in Northern Saudi Arabia and may provide a basis for future studies. In addition molecular markers including Histone (H3) and 12S rDNA sequences were used to examine the phylogenetic relationships of exemplars of the most common four spider families. The constructed tree based on both maximum parsimony and maximum likelihood of the two studied sequences revealed that the studied representatives of family Agelenidae and family Sparassidae were grouped in one supported clade.

However, the relationship of this clade with the other two studied families shows controversy in both H3 and 12S trees. Results for each of the studied genes, support their reliability for phylogenetic analysis of spiders. Ha'il, Spiders list, Molecular Phylogenetics, 12S rDNA, H3.

Key words: Molecular Phylogenetic- Spider Families - Ha'il Region - Saudi Arabia

INTRODUCTION

Spiders are one of the most diverse groups of animal species and occupy practically all terrestrial ecosystems. These predatory arthropods generally attack insects non-specifically, and may stabilize arthropod populations (Wise, 1995). The World Spider Catalog includes 42,751 described species of 110 families (Platnick, 2012). Study of spiders of Saudi Arabia is still preliminary and fragmented. In addition to the few records of spiders from Arabia in old literatures, different families of spiders were also studied including Linyphiidae (Jocqué, 1981), Zodariidae (Ono and Jocqué, 1986), Thomisidae (Dippenaar-Schoeman, 1989), Salticidae (Prószyński, 1989; 1993; Al Ghamdy and Faragallah, 1999), Lycosidae (Faragallah and Al Ghamdy, 2001), Theridiidae (Knoflach and van Harten, 2002), and Miturgidae (El-Hennawy, 2011).

Hai'l Region is located in the center of the northern part of Saudi Arabia. Explorations for oil in the eastern and southern Arabia attracted people of professional interest to the oil fields and they contributed in gathering some biota. Ha'il area which does not have oil fields gained little attention. The Region comprises diverse ecosystems that provide interesting aspects for species diversity investigations. A comprehensive contribution to the Flora of Ha'il Region was found in literatures (Alshammari and Sharawy, 2010; El-Ghanim *et al.,* 2010). On the other hand, the investigation of Ha'il's fauna is still infrequent and most studies focused on insects

(Hölzel, 1982; Cranston and Judd, 1989; Albarrak, 2009) and reptiles (Dekinesh, 1991). Although arachnids represent a major component of desert's fauna, they have only recently attained little consideration in the region. In this respect, Sharawy and Alshammari (2009) recorded two highly poisonous scorpion species. Moreover both Al-Asmary *et al*. (2009) and Desouky and Alshammari (2011) reported eight scorpion species in the Region. However, to the best of our knowledge no studies have dealt with spider's fauna in Northern Saudi Arabia.

The present work is a preliminary study of Hai'l's spiders. The study's aim is to evaluate the diversity of spider species in this highly neglected region. In addition the phylogenetic relationships of exemplars of four recorded spider families are to be investigated using molecular markers including Histone (H3) and 12S rDNA sequences that represent nuclear and mitochondrial genes, respectively.

MATERIAL AND METHODS

(a) Collection of spiders

Spiders of 16 families were collected from different habitats in the Ha'il Region during the periods of April-August 2010 and July-August 2011. Most of them were found under stones and sometimes on plants. All specimens were collected alive by hand. They were preserved in absolute ethanol and deposited in the Central Laboratory, Faculty of Science, Ha'il University. Adult specimens were identified to species. Juvenile and subadult specimens were, when possible, identified to genus level. Spider taxa were identified by the second author.

(b) DNA extraction

Four species representing the most common four families in the region were chosen for molecular phylogenetic studies. For this purpose, the whole organism was homogenized in 1 ml of DNA extraction buffer (50 mM Tris–HCl, pH 8.5, 10 mM EDTA, 100 mM NaCl and 2% SDS). The homogenate was centrifuged at 8,000 rpm for 5 minutes. The resulting supernatant was mixed with equal volume of phenol/chloroform to remove proteins. The mixture was centrifuged at 10,000 rpm for 10 minutes. DNA was precipitated from the aqueous layer with two volumes of absolute ethanol at -20 °C for overnight. DNA was collected via centrifugation at 12,000 rpm for 10 minutes. After that, DNA was washed with 70% ethanol and recollected with centrifugation at 12,000 rpm for 10 minutes. The supernatant was decanted and the DNA pellet was air dried and then suspended in 50 µl of Tris-EDTA (TE) buffer (10 mM Tris–HCl, pH 8, 1mM EDTA) and kept at -20 °C till use. The extracted DNA from all spiders was analyzed by running an aliquot of each DNA sample on 1% agarose gel electrophoresis containing 0.5 µg of ethidium bromide per ml of agarose. The applied voltage was 5-8 V/cm. After termination of electrophoresis process, DNA was visualized under UV to determine its quality.

(c) Amplification of a partial fragment of H3 gene

A partial fragment of the gene encoding for H3 was amplified via PCR using the primer set (H3aF and H3R, Table 1). The reaction mixture contained 5 µl of DNA, 25 µl of Dream Taq Master Mix (Fermentas Co.), 1 µl of each primer (10 pmol) and 19µl of nuclease free water. The PCR conditions of the thermocycler (pEQ lab.) were 95°C for 5 minutes for initial denaturation, 35 cycles, each cycle (94°C, 1 minute for denaturation, 50 °C, 1 minute for annealing and 72°C, 1 minute for extension) and 72°C , 10 minutes for final extension. After termination of PCR run, 5 µl of the PCR product was checked on 2% agarose gel using electrophoresis. The expected PCR product was almost 350 bp in length.

(d) Amplification of a partial fragment of 12S rDNA

A partial fragment of 12S rDNA was amplified via PCR using the primer set (12S-ai and 12S-bi, Table 1). The reaction mixture contained 5 µl of DNA, 25 µl of Dream Taq Master Mix (Fermentas Co.), 1 µl of each primer (10 pmol), 1 µl of 25 mM $MgCl_2$ and 18 µl of nuclease free water. The PCR conditions of the thermocycler (pEQ lab) were 95 °C for 5 minutes for initial denaturation, 35 cycles, each cycle (94 °C, 1 minute for denaturation, 45 °C, 1 minute for annealing and 72 °C, 30 seconds for extension) and 72 °C, 10 minutes for final extension. After termination of PCR run, 5 µl of the PCR product was checked on 2% agarose gel electrophoresis. The expected PCR product was almost 370 bp.

Table 1: Primers used for PCR.

Gene	Primer name	Sequence	Reference
H3	H3aF	5-ATGGCTCGTACCAAGCAGACVGC-3	Colgan *et al*., 1998
	H3aR	5-ATATCCTTRGGCATRATRGTGAC-3	
12S rRNA	12S-ai (12SR-N-14594)	5-AAACTAGGATTAGATACCCTATTAT-3	Simon *et al*., 1994
	12S-bi	5-AAGAGCGACGGGCGATGTGT -3	Folmer *et al*., 1994

(e) Purification and Sequencing of PCR products

PCR products were purified to remove all components of PCR that would have an inhibitory effect on the sequencing reaction. Each PCR product was mixed with two volumes of absolute ethanol and kept at -20°C for an overnight. The amplified DNA was collected via centrifugation at 10,000 rpm for 10 minutes. After that, the PCR product was washed with 70% ethanol and recollected via centrifugation. It was then allowed to air dry and was suspended in 30 µl of nuclease free water. Approximately 30 µg of the purified products were then used as templates for direct sequencing of both forward and reverse strands, using the same amplification primers in an automatic ABI 310 DNA Sequencer with Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin Elmer. All sequences generated in this study were deposited in the National Center for Biotechnology Information (NCBI) GenBank.

(f) Determination of phylogenetic relationships

The programs PHYLIP package and MEGA version 5.0 were used for the determination of phylogenetic relationships. A Neighbor-Joining (NJ) tree based on uncorrected p-distances was constructed with MEGA version 5.0. Maximum Likelihood (ML) was performed in order to check consistency in the results using different algorithms based on different assumptions of molecular evolution. The ML analysis was performed using online PHYLIP package (www.atgc-montpellier.fr/phyml/) with model parameters fitted to the data by likelihood maximization. Maximum Parsimony (MP) analysis was performed in MEGA version. 5.0. Branch swapping with 500 random stepwise additions of taxa were used.

RESULTS

(a) Study area

The Ha'il Region of Saudi Arabia, located between 25°35` & 29°00` N longitudes and 39°01` & 44°45` E latitudes and covers an area of approximately

118,322 km^2. It is named after the large Wade Ha'il (formerly called Al-Adair Valley) in the Shamir Mountain region. According to Chapman (1978) the area belongs to the Arabian shield and the great An-Nafud (Nafud Al-Kabir), which is connected by Dahma, to the Rub Al-Khali to the South of Saudi Arabia. Ha'il Region is characterized by its variation in topography and geomorphology. It is characterized by several landscape units, such as isolated mountains, escarpments, wadis (valleys) and sand-seas. The Ha'il valley extends to the North-East through a narrower corridor linking the Capital City with the Shammar Mountain. The Shammar Mountains form a major feature of Ha'il Region and consist of two great Granitoid mountain ranges namely Aja and Salma. The entire region is about 1000 m. The principal part of Ha'il is An-Nafud Desert. It forms the northern part of the Ha'il Region, covering about 64,000 km^2 and is composed of reddish sand and lies at an elevation of 900 m above sea level. Topographic map of Ha'il Region as obtained from the satellite SRTM DEM (2000) is shown in Fig. 1. According to Hereher *et al.* (2012) the region is topographically classified into four classes: plains, sand dunes, mountains and cultivated lands occupying 47.77%, 35.13%, 15.88% and 1.17% respectively of the total area.

Ha'il Region is generally arid to extremely arid zone (Al-Turki and Al-Olayan, 2003). Air temperature typically rises as high as 50 °C in summer and falls to freezing point in winter, especially at higher altitudes. Rainfall is irregular reaching a few tens of centimeters and occurs mostly between November and March.

The region is recently subjected to climatic and topographic changes. The draught increases heavy sand storms, which are most frequent during spring and winter seasons. Therefore, the sand dunes from the Northern Nafud Sand Sea occasionally encroach upon cultivated lands and people settlements. Moreover, agriculture is significantly growing in this arid region. Agricultural development projects and reclamation of new desert land increasedd the area of cultivated land from 9,500 ha in 1972 to 139,300 ha in 2010 (Hereher *et al.,* 2012).

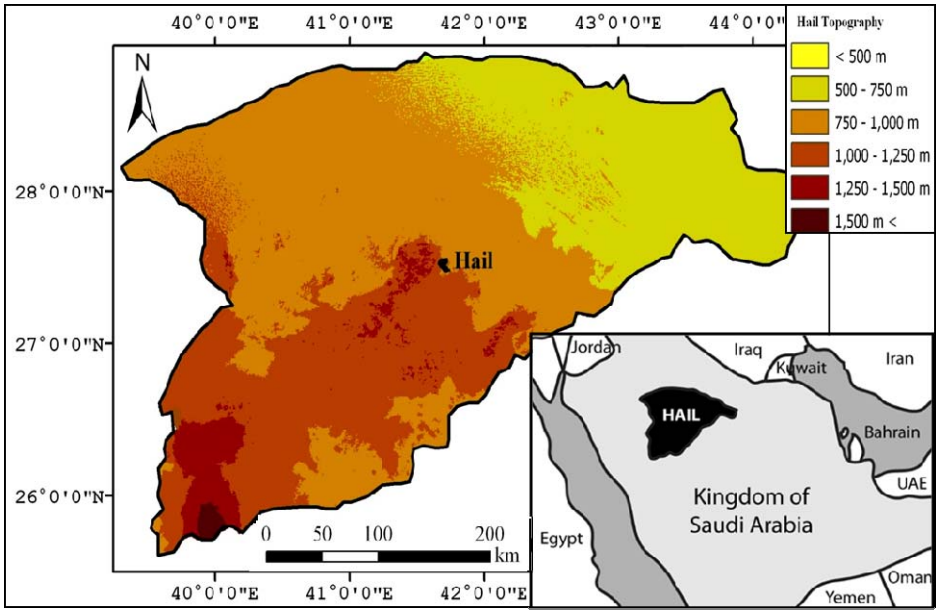

Fig.1: Location and topographic map of Ha'il Region as obtained from the satellite SRTM DEM (2000), 1 km spatial resolution. (Courtesy of Mohamed E. Hereher).

(b) Species account

All collected spider specimens, Order Araneae, (Figs. 2-14) are listed below with their identification, localities, and collecting dates.

1. Family Agelenidae

7♀, 1s♂, 3j *Benoitia lepida* (O.P.-Cambridge, 1876), Ha'il City, 22 July 2010, N 27°32'35" E 41°42'15"; Rujama Village, 13 August 2011, N 27°55'09" E 42°08'25"; Sofaitt, 14 August 2011, N 25°37'50" E 40°38'10".

Remarks

Family Agelenidae are called Funnel-web Spiders because they make funnel-shaped webs, which they use to trap insects. They are also known as Grass Spiders because they construct their webs in tall grass, heavy ground cover and the branches of thick shrubs. Members of this family can be easily recognized by the elongated apical segment of the posterior lateral spinnerets and the paired colulus. Only three Agelenid species were reported in Yemen of which *Benoitia lepida* was new record (Santos and van Harden, 2007). As a result, the fauna of the large continental areas as the Arabian Peninsula are still in great need for revision.

2. Family Eresidae

5♀ *Stegodyphus lineatus* (Latreille, 1817), Great Nofood, 21 August 2010, N 27°49'05" E 40°54'00"; Om Sanman, 21 August 2010, N 28°05'18" E 40°54'00"; Rujama Village, 13 August 2011, N 27°56 59" E 42°08'25".

3. Family Gnaphosidae

-1♂, 2♀, 1s♂ *Pterotricha dalmasi* Fage, 1929, Rujama, 13 August 2011, N 27°56'10" E 42°07'45".

-1♀; 1♀; 1♀, 1j; 1j Unidentified species, Rujama, 13 August 2011, N 27°56'10" E 42°07'45"; Setehaat Juppa, 29 April 2010, N 27°58'19" E 40°49'13"; Moraifig Village, 13 August 2011, N 27°21'44" E 41°38'34"; Mogeg, 31 July 2011, N 27°22'45" E 41°10'48".

4. Family Lycosidae

2♀, 1j; 1♀, 1j; 1j ; 1s♀; 1s♀; 1j Unidentified species, Jubla Mountain, 29 April 2010, N 25°29'07" E 40°42'10"; Ash Shamly, 30 July 2010, N 26°34'25" E 40°04'32"; Bag'a, 30 July 2010, N 27°47'34" E 41°51'32"; Shannan, 21 August 2010, N 27°10'02" E 42°25'58; Ha'il City, 1 August 2011, N 27°33'12" E 40°41'08".

5. Family Miturgidae

1. *Cheiracanthium* sp., Al-Bed'e, 22 July 2010, N 27° 26'39" E 40°49'32".

Remarks

The taxonomic situation of genus *Cheiracanthium* C.L. Koch, 1839 is controversy. It was recently placed in family "Chiracanthiidae" by Ono (2009) instead of family "Clubionidae". However, Jäger and Dankittipakul, (2010) placed the Eutichurinae with *Cheiracanthium* in the Clubionidae". This genus includes 178 species, 42 of them are African (El-Hennawy, 2010). *Cheiracanthium molle* L. Koch, 1875 was recorded for the first time outside Africa from Al-Baha, Saudi Arabia by El-Hennawy (2011).

6. Family Oecobiidae

1s♂, 1s♀, 1j *Uroctea sp*. Toula Mountain, 1 June 2010, N 25°44'00" E 41°05'42"; Al-Asfar Mountain, 14 August 2011, N 25°59'27" E 40°32'55".

7. Family Philodromidae,

1♀ Unidentified species, Juppa, 24 August 2011, N 40°42'10" E 25°29'07".

8. Family Pholcidae

3♂, 4♀, 1s♂, 3j *Artema atlanta* Walckenaer, 1837, Ha'il City, 22 July 2010, N 27°32'35" E 41°42'15"; Gafar, 15 August 2010, N 27°24'58" E 41°36'15"; Hulaifa, 15 August 2010, N 25°59'38" E 40°49'01"; Samiraa, 15 August 2010, N 26°29'29" E 42°07'28".

9. Family Salticidae

-1♂ *Mogrus mirabilis* Wesolowska and van Harten, 1994, Mo'Arrash, 1 June 2010, N 25°59'31" E 40°58'15".

-1j(s♀) *Plexippus* sp.?; 1♀; 1j Unidentified species, Om Sanman, 25 May 2010, N 40°53'35" E 28°07'12"; Aga Mountain, 1 August 2011, N 27°28'10" E 40°15'05".

Remarks

The jumping spider (Family Salticidae) contains 575 described genera and 5,423 described species, (Platnick, 2012) making it the largest family of spiders with about 13% of all species. Jumping spiders have some of the best vision among invertebrates and use it in courtship, hunting, and navigation. Though they normally move quietly and fairly slowly, most species are capable of very agile jumps, notably when hunting, but sometimes in response to sudden threats. Jumping spiders are easily distinguished from other spiders by their four big eyes on the face and four smaller eyes on top of the head.

Family Salticidae are notably studied in Saudi Arabia and adjacent countries of the Arabian Peninsula. In Yemen, Family Salticidae was extensively studied by Wesolowaska and van Harten (1994; 2002; 2007). Such studies increased the number of Salticid species in Yemen to 81. However, only 30 Salticid species containing one new genus and ten new species were described in Saudi Arabia (Prószyński, 1989; 1993).

Five species of the Salticid spider of the genus *Morgus* were recorded in different regions of Saudi Arabia by Prószyński (1989). *Morgus mirabilis* was recorded in Yemen as new species by Wesolowaska and van Harten (1994).

10. Family Scytodidae

2j *Scytodes* sp. Rujama Village, 13 August 2011, N 27°56 40" E 42°07'12".

Remarks

Family Scytodidae is also known as 'Spitting Spiders' because these spiders spit a sticky silken substance over its prey. It is a relatively small family including only five genera (Rheims *et al*., 2006). The most common genus of this family is *Scytodes*. Four species from this genus were recorded in Yemen by Rheims *et al.* (2006).

11. Family Selenopidae

1♀ 1j *Selenops* sp., Ash-Shuwayms, 30 July 2010, N 26°13'13" E 40°24'17".

12. Family Sicariidae,

2 ♂ *Loxosceles rufescens* (Dufour, 1820), Rujama Village, 13 August 2011, N 27°56'40" E 42°07'12".

13. Family Sparassidae

-2♀, 3j *Eusparassus walckenaeri* (Audouin, 1825), Gulaib, 29 April 2010, N 28°36'40" E 42°24'34"; Ha'il City, 29 April 2010, N 27°33'11" E 41°41'11"; Setehat Juppa, 29 April 2010, N 27°58'18" E 40°49'13"; Great Nofood, 1 June 2010, N 27°39'25" E 40°57'54"; NADEC Co, 1 June 2010, N 27°30'16" E 42°40'15".

-1♂, 2j *Cerbalus* sp. ?, NADEC Co, 1 June 2010, N 27°30'16" E 42°40'15".

14. Family Theraphosidae

1j, Unidentified species. Rujama Village, 13 August 2011, N 27°56'40" E 42°07'12".

15. Family Theridiidae

2♀ 1s♀ *Latrodectus tredecimguttatus* (Rossi, 1790), Salma Mountain, 25 May 2010, N 42°35'15" E 27°15'02"; Aga Mountain, 21 August 2010, N 27°31'20" E 40°16'02".

Remarks

The mature Black Widow Spider of the genus *Latrodectus* has a characteristic glossy black colour and sometimes an hourglass configuration on its ventral surface. Despite its medical importance, the taxonomic situation is still unsatisfactory

(Knoflach and van Harten 2002). Currently, 31 species of *Latrodectus* are known worldwide (Platnick, 2012), two of them (*L. cinctus* Blackwall, 1865 and *L. reinvulvatus* Dahl, 1902) were reported in the southern part of Saudi Arabia by Knoflach and van Harten (2002) for the first time.

Latrodectus tredecimguttatus, commonly known as the Mediterranean Black Widow or Steppe Spider, is commonly found throughout the Mediterranean region. In Arabian Peninsula, this species was early recorded in Socotra (Pocock, 1903). According to Platnick (2012) the distribution of the species extends from the Mediterranean across Central Asia to China.

Species of the genus *Latrodectus* are poisonous and dangerous to mammals including humans. In Saudi Arabia, nine cases of *Latrodectus* bite envenemation were recorded in Al-Baha Region (Bucur and Obasi, 1999).

16. Family Zodariidae

5j (unidentified species), Setehaat Juppa, 29 April 2010, N 27°58'17" E 40°49'14"; Samiraa, 30 April 2010, N 42°09'25" E 26°09'28"; Ghazala, 14 August 2011, N 26°19'23" E 43°19'42".

Molecular studies

A representative from the common four families was used for molecular studies. This include *Stegodyphus lineatus* (Family: Eresidae), *Artema atlanta* (Family: Pholcidae), *Benoitia lepida* (Family: Agelenidae) and *Eusparassus walckenaeri* (Family: Sparassidae). Comparison of some major morphological characteristics of these families is shown in Table (2).

Table 2: Comparison of some major morphological characteristics of the four studied families, taking in consideration that they are all araneomorph spiders. This table is based on Jocqué and Dippenaar-Schoeman (2006).

	Family: Eresidae C. L. Koch, 1851 (Velvet spiders)	**Family: Pholcidae C. L. Koch, 1851 (Daddy-long-legs spiders)**	**Family: Sparassidae Bertkau, 1872 (Huntsman spiders)**	**Family: Agelenidae C. L. Koch, 1837 (Funnel-web spiders)**
Eyes	Four median eyes close together, with lateral eyes wide apart	Anterior median eyes smallest or absent, other eyes in two triads or on tubercles	Median eyes usually largest	Equal in size
Labium	Rounded apically	Fused to sternum, wider than long	Free, short, never more than half length of endites	As wide as long
Legs	**Prograde**, short and stout	**Prograde**, often extremely long and slender, with pseudosegmented flexible tarsi	Laterigrade, long	**Prograde**, long, spiny
Tarsal claws	**Three**	**Three**	**Two**	**Three**
Tracheal spiracle	Close to spinnerets	Absent	Close to spinnerets	Close to spinnerets
Cribellum	Present, well-developed	Ecribellate	Ecribellate	Ecribellate
Male palp Tibial apophysis	Absent	Present	Present	Present
Female genitalia	Entelegyne, epigyne usually simple	Haplogyne (without epigyne)	Entelegyne, epigyne sclerotized and conspicuous	Entelegyne, epigyne variable

347bp sequences were obtained from H3 gene and 308bp from 12S rDNA of the studied spider species. All sequenced gene segments were deposited in NCBI GenBank. To the best of our knowledge, 12S rDNA and H3 sequences were found to be new records for three from the studied four species (Table 3). Data about these sequences and their GenBank Accession Numbers (submitted) are shown in Table (3).

Table 3: Data for gene sequences of the studied spider species

Gene	Species	Length	GenBank Accession Number	Remarks
H3	*Stegodyphus lineatus*	350 bp	JQ955680	submitted
	Artema atlanta	352 bp	JQ955681	New record, submitted
	Benoitia lepida	347 bp	JQ955682	New record, , submitted
	Eusparassus walckenaeri	352 bp	JQ955683	New record, , submitted
12S	*Stegodyphus lineatus*	317 bp	JQ955684	New record
	Artema atlanta	316 bp	JQ955685	
	Benoitia lepida	310 bp	JQ955686	New record
	Eusparassus walckenaeri	308 bp	JQ955687	New record

For the H3 gene fragment, the tree revealed two maximally supported clade in ML analysis (Fig. 15), one containing the families Pholcidae, Sparassidae and Agelenidae which represented by species, *A. atlanta, B. lepida.* and *E. walckenaeri*. 96 % bootstrap value in ML but only 59% in MP analysis was recorded for this clade. The clade of *Benoitia lepida* and *Eusparassus walckenaeri* had a very strong support in both ML and MP analyses (100%).

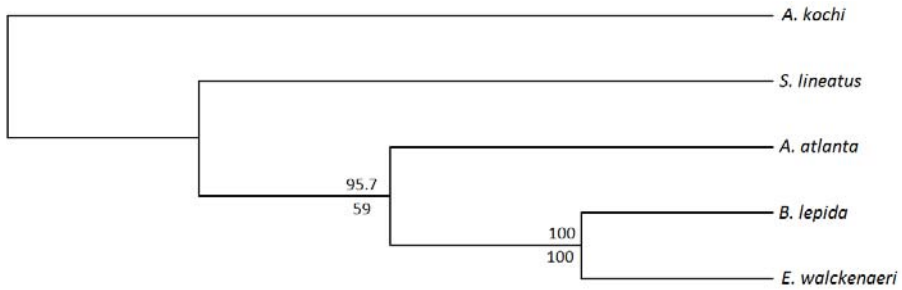

Fig.15: Phylogenetic tree for the partial H3 sequences of the studied spider species obtained with PHYML. Numbers by the nodes indicate: ML bootstrap values (>50%) are given above the nodes, and Maximum Parsimony (MP) are given below the nodes. H3 sequence from *Alopecosa kochi*. (Family: Lycosidae) (GenBank Accession Number, DQ628635.1) was used as an outgroup.

The 12S rDNA phylogenetic tree revealed only one maximally supported clade (Fig. 16) which was between the family Sparassidae, represented by *E. walckenaeri* and Agelenidae represented by *B. lepida* with 91% bootstrap value in ML but with low bootstrap support in MP analysis.

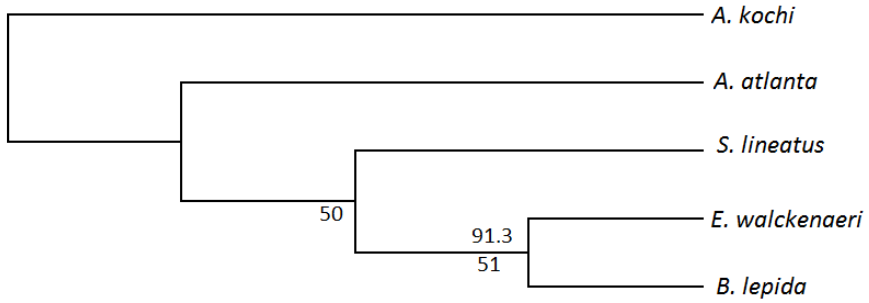

Fig.16: Phylogenetic tree for the partial 12S sequences of the studied spider species obtained with PHYML. ML bootstrap values (>50%) are given above the nodes, and Maximum Parsimony (MP) are given below the nodes. 12S sequence from Alopecosa kochi. (Family: Lycosidae) (GenBank Accession Number, DQ 019755.1) was used as an outgroup.

In the combined mitochondrial and nuclear gene fragments (682 bp), the tree revealed two maximally supported clade in ML analysis. (Fig. 17), one containing the families Pholcidae, Agelenidae and Sparassidae which represented by species, *A. atlanta, B. lepida* and *E. walckenaeri* respectively, with 97% bootstrap value in ML

and only 60% in MP analysis. The clade of *B. lepida* and *E. walckenaeri* had a very strong support in both ML and MP analyses.

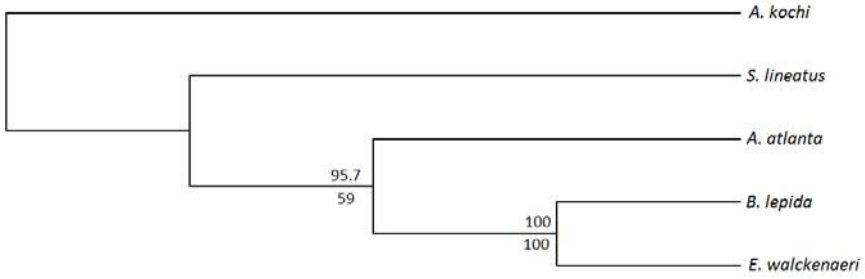

Fig. 17: Phylogenetic tree for the partial H3 sequences of the studied spider species obtained with PHYML. ML bootstrap values (>50%) are given above the nodes, and Maximum Parsimony (MP) are given below the nodes. *Alopecosa kochi*. (Family: Lycosidae) was used as an outgroup.

In order to clarify where the studied spiders are placed within their representative families, the phylogenetic tree was constructed for each family separately using gene sequences already published whenever these data are available. The only available sequences on data base are 12S rDNA and H3 sequences for considerable numbers of representatives of family Pholcidae and family Eresinidae respectively.

ML Phylogenetic tree of pholcid species based on partial 12S rDNA sequences retrieved from data base is shown in Fig. 18. Maximum likelihood under the GTR+G+I model of evolution revealed that *A. Atlanta* from Saudi Arabia forms a maximally supported sister clade with the same species from with 97% bootstrap value. The most relative species to *A. atlanta* are *Trichcyclus sp.* and *Ninetis subtillissima.*

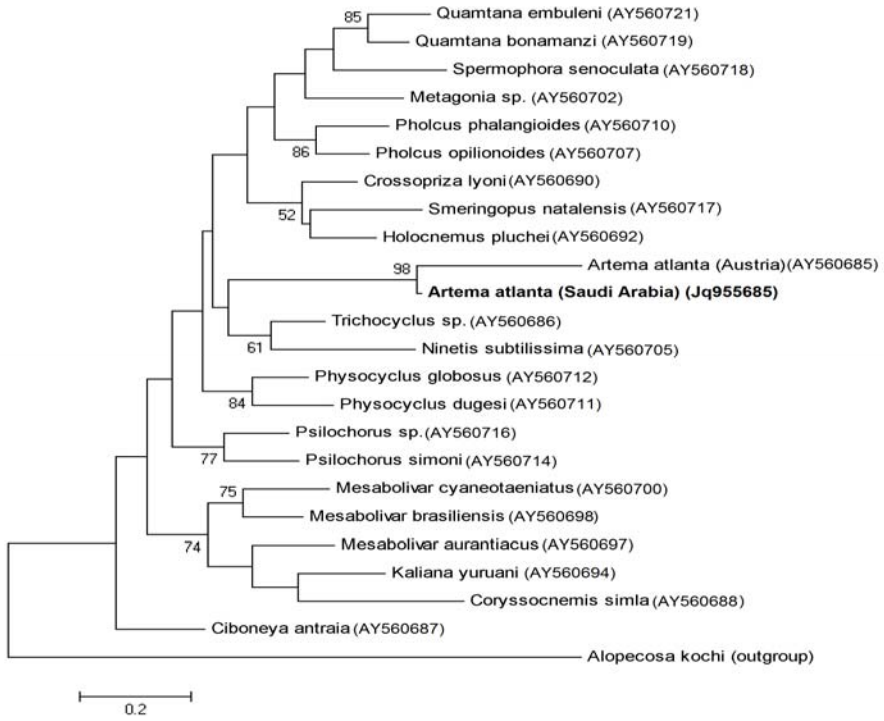

Fig. 18: ML Phylogenetic tree of pholcid species based on partial 12S rDNA sequences retrieved from data base (with their GenBank Accession Numbers) showing the place of *Artema Atlanta* within the Family: Pholcidae. ML bootstrap values (>50%) are given below the nodes,. 12S rDNA sequence from *Alopecosa kochi*. (Family: Lycosidae) (GenBank Accession Number, DQ 019755) was used as an outgroup.

On the other hand, ML Phylogenetic tree of Arsenid species based on partial H3 sequences retrieved from data base (Fig., 19) revealed that *S. lineatus* from the present study forms maximally supported sister clade with the same species from Netherland with 87% bootstrap value in ML analysis. Moreover, the tree showed that *S. lineatus* appears as sister to all other studied arsenids.

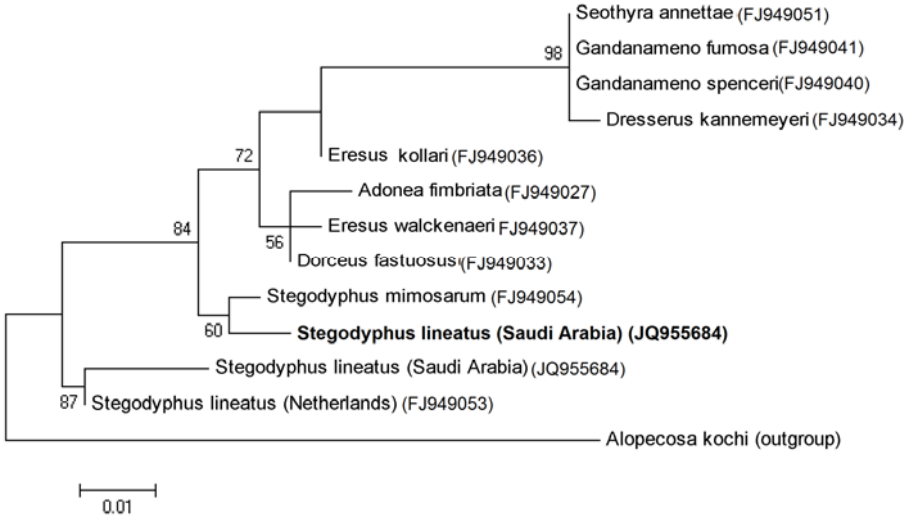

Fig.19: ML Phylogenetic tree of Arsenid spider species based on partial H3 sequences retrieved from data base (with their GenBank Accession Numbers) showing the place of *Stegodyphus lineatus* within the Family: Arsenidae. ML bootstrap values (>50%) are given below the nodes. H3 sequence from *Alopecosa kochi*. (Family: Lycosidae) (GenBank Accession Number, DQ628635) was used as an outgroup.

DISCUSSION

Knowledge about the spider fauna in Saudi Arabia is hugely inadequate compared to adjacent countries. For example, More than 80 Salticid species were recorded in Yemen (Wesolowaska and van Harten 2007) compared with only 30 species from Saudi Arabia (Prószyński, 1989; 1993) which is considerably larger country. This fact underlines the necessity for further studies on the spider's fauna in this highly neglected country. In the present study, 14 genera, in addition to five unidentified spider species, belonging to 16 families were recorded in Ha'il Region. This is the first study of spider's fauna in Northern Saudi Arabia and may provide a basis for future studies.

Biogeographically, the Arabian Peninsula is classified as belonging to Palaearctic region. However, spiders of African origin were recorded from regions south of Baha till Yemen (Knoflach and van Harten, 2002; El-Hennawy, 2011). Therefore, Grashoff and van Harten (2007) conclude that there is close biogeographical relationship between the Arabian Peninsula and Africa. Many people think of Arabian Peninsula as a part of Asia. From the viewpoint of geological history, this is erroneous; because the geological history of this area leads to the conclusion that the Arabian Peninsula was a part of Africa (Thompson, 2000).

In recent times, after the discovery of advanced methods utilizing DNA it has become of great significance to use these modern techniques in association with classical taxonomy which relies on measurements and description of morphological characters. Recently, DNA techniques, specifically sequencing, have been introduced into systematic and phylogeny of spiders (e.g. Bruvo-Madaric *et al.*, 2005; Bond *et al.*, 2012; Griswold *et al.*, 2012). In this way, sequence comparisons provide clues to clarify the view of relationships among species. In the present study, exemplars of

four different spider families, which are the most common in the Region, were examined for gene composition. According to Flook *et al.,* (1999) combination of data sets with varying rates of evolution (e.g., mitochondrial and nuclear genes), can efficiently accommodate for variation in results of individual analyses. Therefore, nucleotide sequence data were generated from one mitochondrial gene (12S rDNA) and one nuclear (H3) gene for which we assumed they might be informative. This assumption was based on the fact that these genes were previously used with success in a range of phylogenetic analyses in spiders (e.g., Fang *et al.,* 2000; Maddison and Hedin, 2003; Arnedo *et al.,* 2004).

The constructed tree based on both Maximum Parsimony and Maximum Likelihood revealed that the studied representatives of family Agelenidae and family Sparassidae were grouped in one supported clade. However, the relationship of this clade with the other two studied families appears controversy in both H3 and 12S trees. Results for each of the studied genes support their reliability for phylogenitic analysis of spiders. In this respect, sequence data from a portion of the mitochondrial 12S rDNA have yielded large data sets for phylogenetic analysis of spiders than the nuclear gene (e.g. Fang *et al.,* 2000; Vink *et al.,* 2002; Bruvo-Madaric 2005). Zehethofer and Sturmbauer (1998) found that 12S rRNA was especially suitable for resolving relationships higher than the species level. Generally, studying the mitochondrial DNA is very important to build the phylogenetic tree of living organisms, as it is the most useful molecule to infer the phylogeography. The mtDNA size is smaller compared to genomic DNA, and its sequence evolution rate is generally high; this high rate is the product of both high mutation rate and a high mutation fixation rate. The high mutation rate results in part from the mtDNA's lack of protective histones, inefficient DNA repair systems and continuous exposure to mutagenic effects of the oxygen radicals. The high mutation fixation rate is due to the efficient intracellular sorting of mutant molecules in the female germ line and the rapid genetic drift of mtDNAs in the general population (Wallace, 1994; Hartl, 1998). In spite of these facts, the current results revealed that the H3 nuclear gene sequence is also highly reliable for phylogenetic analysis of spiders which may be due to the high conservative nature of molecular gene.

In an effort to gain a better understanding of intergeneric and subfamilial relationships of some of the studied families, we used nucleotide sequence data previously published to construct phylogenetic tree for representatives of families Pholcidae and Arsenida separately. Regarding the studied representatives of these two families, it was found that *A. Atlanta* was derived species relative to the other pholcids. On the other hand, *S. lineatus* was found to basal clades which represent sister clades to all other arsenids with high support. However, the current molecular study is a preliminary step in phylogenetic analysis of spiders' fauna in Saudi Arabia and may represent valuable basis for further studies including a greater taxon sampling. Our knowledge about gene sequences of spiders is so limited that molecular data about many families are completely lacking in database. Further gene sequencing of other representatives of the studied families will hopefully allow spider Systematists to get a stable and more detailed idea about phylogenetic relationships both within and among spider families.

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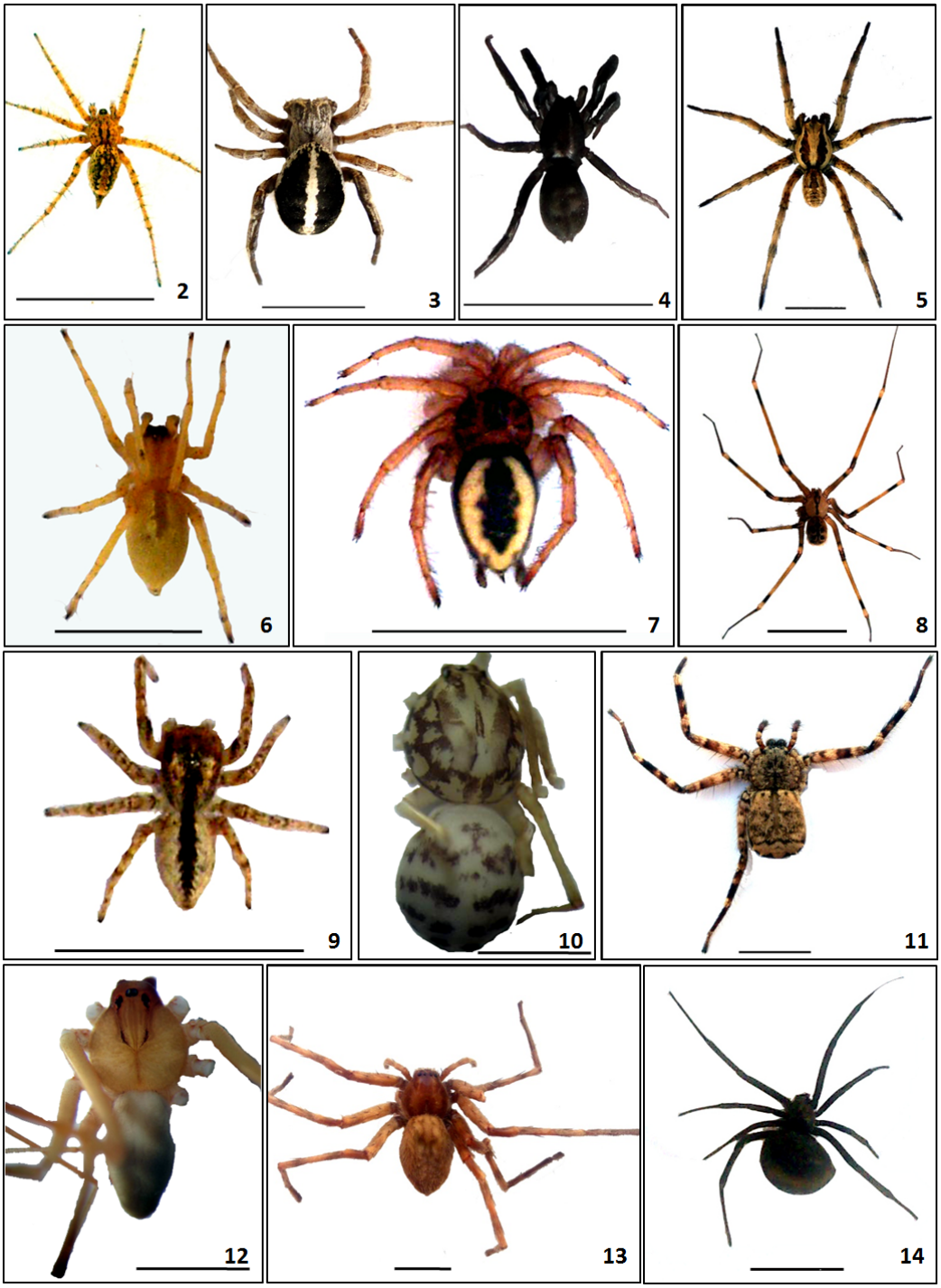

Figs. 2–14: Habitus of spiders from Hai'l Region. 2 *Benoitia lepida; 3 Stegodyphus lineatus; 4 Pterotricha dalmasi; 5* Unidentified species of family Lycosidae; 6 *Cheiracanthium* sp; 7 *Uroctea* sp.; 8 *Artema atlanta; 9 Mogrus mirabilis; 10, Scytodes* sp.; *11 Selenops* sp.; 12 *Loxosceles rufescens; 13 Eusparassus walckenaeri; 14 Latrodectus tredecimguttatus.* Scale *line* = 10 mm except nos. 10 and 12 = 2mm.

ARABIC SUMMARY

العلاقات التطورية الجزيئية بين أمثلة من أربع فصائل من العناكب من منطقة حائل بشمال السعودية ، مع قائمة أولية لعناكب المنطقة

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3-41 المنطقة الرابعة- مصر الجديدة- القاهرة

تشير الدراسات إلى قلة المعلومات المتاحة عن عناكب السعودية مقارنةً بما تمت دراسته من أنواع العناكب بالدول المجاورة. والعمل الحالي هو دراسة أولية لعناكب حائل، تم من خلاله تسجيل أربعة عشر جنساً، بالإضافة إلى خمسة أنواع غير معرَّفة من العناكب تنتمي لستة عشر فصيلة من منطقة حائل. وهذه هي الدراسة الأولى لعناكب شمال السعودية مما يجعلها أساساً للدراسات المستقبلية المتعلقة بالعناكب في هذه المنطقة البكر.

بالإضافة إلى ذلك فقد تم اختيار أربعة أنواع ممثِلة لأربعة من العائلات الأكثر انتشاراً في المنطقة لدراسة العلاقات التطورية بينها باستخدام التقنيات الجزيئية الحديثة. ولهذ الغرض تم تعيين التتابع الجيني التسلسلي لجينين أحدهما نووي وهو جين الوحيدة subunit الهستونية الثالثة (H3) والآخر ميتوكوندري وهو جين الوحيدة الريبوسومية الصغيرة (12S rDNA). وقد سُجلت هذه الجينات ببنك الجينات (NCBI GenBank) واتضح أن ثلاثة أنواع من العناكب الأربعة المدروسة لم يسجل بها تتابعات هذين الجينين من قبل. وقد تم رسم الشجرة التطورية للأنواع المدروسة وذلك اعتماداً على تحليل فروقات التتابعات الجينية بالطرق الجزيئية المتبعة، وبيان مدى التقارب بين هذه العائلات. وتدعم نتائج الجينات المدروسة أنه يمكن الاعتماد عليها في التحليل الوراثي-التطوري للعناكب. كما تم رسم الشجرة التطورية لعائلتين من العائلات المدروسة باستخدام تتابعات جينية للجينات المدروسة والمتوفرة بقواعد البيانات، وذلك بغرض تعيين وضع النوع المدروس بكل من العائلتين وعلاقاته بالأنواع المنتمية لنفس العائلة.

وتكمن أهمية الدراسة في كونها المحاولة الأولى لدراسة عناكب المنطقة سواء، بالطرق التقليدية أو الجزيئية ، مما يجعلها قاعدة يمكن البناء عليها مستقبلاً لإجراء المزيد من الدراسات المكملة في هذا المجال.