IDENTIFICATION OF THE ORB WEAVING SPIDER (ARANEAE: ARANEIDAE) FAUNA OF DIR LOWER (PAKISTAN) THROUGH DNA BARCODING

M. Sajid^{1*}, M. Zahid¹, M. Shah², M. Rasool³, I. Ullah¹, R. Ahmad¹, P. Habibullah¹ and N. Majeed⁴

¹ Department of Zoology Islamia College University, Peshawar, Pakistan; ² Department of Zoology Government Superior Science College Peshawar; ³ Government Degree College Kabal Swat, Pakistan; ⁴ Department of Zoology, University of Punjab, Lahore-Pakistan

*Corresponding author's email: muhammadsajid541@gmail.com

ABSTRACT

As a result of faunistic study on the Orb weaving spider fauna of Pakistan one genus, *Gibbaranea* Archer, 1951 and six species, *Argiope versicolor* (Dolleschall, 1859), *Argiope lobata* (Pallas, 1772), *Neoscona polyspinipes* (Yin, Wang, Xie and Peng, 1990), *Neoscona scylla* (Karsch, 1879), *Gea spinipes* (C. L. Koch, 1843) and *Gibbaranea ullrichi* (Hahn, 1835) are recorded from Pakistan for the first time and the presence of five species are confirmed. *Eriovixia excelsa* (Simon, 1889) and *Araneus mitificus* (Simon, 1886) are recorded for the first time since 1935. COI sequences >200 bp recovered from 23 specimens were analyzed using neighbor-joining trees and Barcode Index Numbers (BINs). Nucleotide alignment for each species is provided with Sample id, Process id, match process id and match BIN. A map showing the local distribution of each species is provided.

Key words: Dir Lower, Aranei, new records, distribution, taxonomy.

<https://doi.org/10.36899/JAPS.2021.4.0318>

Published online ~~December-January 1504~~, 20201

INTRODUCTION

The family Araneidae Clerck (1757) consists of 178 valid genera and 3051 species (WSC, 2020). Distribution of araneids is cosmopolitan. They are mostly found in damp places and rarely in arid places. Nearly all the species form orb web to live in. They construct web in herbs, shrubs, foliage, grasses and in trees. If disturbed, they fall down and use the same thread for climbing again. Generally smaller size is observed in the males. Males are rarely seen in the web as compared to females (Gajbe, 2005). Members of the family are reported poor and a relatively small number of new species has been described from Pakistan (Mukhtar, 2012). Simon (1889, 1907, 1918), Caporiacco (1934), Dyal (1935) described new species of the family from the country. Arshad *et al.* (1984), Khatoon (1986) recorded some species of the genus *Neoscona* from Pakistan and placed them in genus *Araneus* which were later on transferred to genus *Neoscona*. Tikader (1982), Qadir (1997), Ghafoor (2002), Ghafoor and Beg (2002), Parveen (2003), Butt and Siraj (2006), Mukhtar and Mushtaq (2005), Mukhtar (2012), Mukhtar and Tahir (2013), attempted to provide taxonomic description of Pakistani Araneid. Razzaq (2002) an unpublished data and Kazim *et al.* (2015) reported the family from Northern Areas of Pakistan.

Morphological identification of spiders has many drawbacks i.e. a time consuming and challenging task due to polymorphism, sexual dimorphism and lack of keys to identify juveniles. To consider this obstacle and to clarify all the ambiguities, the use of additional tools for rapid

species identification is indispensable (Tyagi *et al.*, 2019). DNA barcode provides an alternate approach to identify specimens (Ashfaq *et al.*, 2019). For accurate species identification, to resolve taxonomic questions, population estimation, detection of cryptic species and species complexes, DNA barcode has been widely used in the last decade in biodiversity research (Tyagi *et al.*, 2019). BOLD greatly facilitate the use DNA barcode for species identification. It enables species discrimination by assigning COI sequence clustered to BIN (Ashfaq *et al.*, 2019).

Present study was carried out with the aim to find out the diversity of family Araneidae at Dir Lower Pakistan. Phylogenetic alignment was carried out for the 1st time for new data of the family Araneidae from the country.

MATERIALS AND METHODS

Collection was made from 2017 to 2019. Different localities and habitats were searched out for the collection process at District Dir Lower Pakistan. Spiders were hand collected and preserved in 80% ethanol for morphological study and in 95% ethanol for molecular work. Specimens were examined under a LABOMED, INC. Los Angeles, USA (LB-344) stereomicroscope with 2x and 4x zoom lens. Nomenclature follows the World Spider Catalog (2020). The studied specimens are kept in Museum of Zoology Department Islamia College University Peshawar. Some specimens were sent for DNA barcoding to the Centre for Biodiversity Genomics,

Biodiversity Institute of Ontario, University of Guelph, Canada. DNA extraction, PCR and sequencing were performed with using Standard protocol (<http://ccdb.ca/resources/>) (Ashfaq *et al.*, 2019). Nucleotide sequence alignments of COI-5P are generated by using Codon Code aligner version 8.02.

Abbreviations: MICU- Museum Islamia College University Peshawar; m-meter; WSC- World Spider Catalog; ICUP- Islamia College University Peshawar; SPKPK- Spider Project Khyber Pakhtunkhwa; BIN- barcode index number.

RESULTS

Argiope lobata (Pallas, 1772)

Fig 2, Map

Aranea lobata Pallas, 1772: 46, figs 14-15 (D♀).

Argiope lobata Thorell, 1873: 520; *Argiope lobata* Jäger, 2012c: 288, figs 44-48 (♂♀).

Sample id: ICUP-MS-0052

Process id: SPKPK052-20

Match BIN: BOLD: AAZ8817

Material Examined: PAKISTAN: 1 ♀ (MICU, MP-170), Banda Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.77111°N, 71.95761°E), 1374 m a.s.l., 05.07.2018, coll. M. Sajid. ; 4 ♀ (MICU, MP-144 a,b; MP-156 a,b), Zombaqy Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.73737°N, 71.94780°E), 1012 m a.s.l., 03.07.2018, coll. M. Sajid.

Distribution: This species is distributed from Southern Europe to Central Asia and China, Northern Africa, South Africa, Israel, India, from Myanmar to New Caledonia and Northern Australia (WSC, 2020). The species is recorded for the first time from Pakistan.

Figure 2: Homology Dendrogram showing genetic similarity among *Eriovixia excelsa* (Simon, 1889), *Araneus mitificus* (Simon, 1886), *Gibbaranea ullrichi* (Hahn, 1835), *Argiope versicolor* (Dolleschall, 1859), *Cyrtophora citricola* (Forsskal, 1775), *Neoscona polyspinipes* (Yin, Wang, Xie & Peng, 1990), *Argiope lobata* (Pallas, 1772), *Neoscona theisi* (Walckenaer, 1841), *Gea spinipes* (C. L. Koch, 1843) and *Neoscona scylla* (Karsch,

1879).

Map. Araneidae records from District Dir Lower, Khyber Pakhtunkhwa, Pakistan.

Argiope versicolor (Dolleschall, 1859)

Fig 1, Map

Epeira versicolor Dolleschall, 1859: 31, figs 10 (D♀).

Argiope versicolor Jäger & Praxaysombath, 2009: 38, figs 69-73 (♂♀).

Sample id: ICUP-MS-0040 and ICUP-MS-0045

Process id: SPKPK040-20 and SPKPK045-20

Match BIN: BOLD: ACG0576

Material Examined: PAKISTAN: 1 ♀ (MICU, MP-08), Barcharay Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.71647°N, 71.91671°E), 1268 m a.s.l., 10.05.2018; 1 ♀

(MICU, MP-82), Barcharay (Jarjoray Hills), Talash, Dir Lower, Khyber Pakhtunkhwa (34.71667°N, 71.91701°E), 1310 m a.s.l., 18.06.2019, all. coll. M. Sajid.

Distribution. The species is distributed from China to Indonesia (WSC, 2020). Recently recorded from India (Tyagi *et al.*, 2019). Present study confirms its existence in Pakistan (Northern areas) for the first time.

Figs 1-14. 1—*Argiope versicolor*; 2—*Argiope lobata*; 3—female *Gea spinipes*; 4—Male *Gea spinipes*; 5—*Gibbaranea ullrichi*; 6—*Neoscona polyspinipes*; 7—*Neoscona Scylla*; 8—*Neoscona theisi*; 9—female *Araneus mitificus*; 10—male *Araneus mitificus*; 11—*Cyrtophora citricola*; 12—*Eriovixia excels*; 13-14—female & male *Cyclosa hexatuberculata*.

1-70 Nucleotide sequence alignment

A. Eriovixia excels.txt	GACTTTATATCTGATTTTTGGGGCTTGGGCTGCTATAGTAGGTACGGCGTTAAGTGTTTAATTCGGGTG
B. Araneus mitificus.txt	GACTTTATATCTAATTTTTGGAGCTTGGTCAAGCTATAGTGGGTACTGCAATGAGAGTATTAATTCGAGTG
C. Gibbaranea ullrichi.txt	TACTTTATATTTAATTTTTGGGGCTTGGGCTGCTATGGTAGGTACAGCAATAAGAGTTTAATTCGAATT
E. Cyrtophora citricola.txt	GACATTATATTTAATTTTTGGTGTCTTGAAGCTGCTATAGTGGGACAGCTATAAGAGTATTGATTCGAATT
F. Neoscona polyspinipes.txt	AACTTTATATTTAGTTTTTGGTGTCTTGAAGCTGCTATAGTAGGAACAGCTATAAGAGTATTGATTCGAATT
H. Neoscona theisi.txt	AACTTTATATTTGATTTTTGGAGCATGGGCTGCAATAATAGGAACAGCTATAAGAGTATTAATTCGAATT
J. Neoscona scylla.txt	GACTTTATATTTAATTTTTGGTGCATGAGCTGCTATAATAGGACAGCTATAAGAGTATTAATTCGAATT
G. Argiope lobata.txt	TACTTTATATTTGATTTTTGGAGCTTGAAGCTGCTATAGTAGGAACAGCAATAAGAGTTTAATTCGAATT
D. Argiope versicolor.txt	TACGTTATATTTAATTTTTGGTGTCTTGGGCTGCCATAGTAGGGACAGCAATAAGAGTGTTAATTCGAATT
I. Gea spinipes.txt	AACTTTATATTTAATTTTTGGGGCTTGAAGCTGCTATAGTAGGAACAGCAATAAGAGTATTAATTCGAGTG
Contigl:	GACTTTATATTTAATTTTTGGTGCTTGRGCTGCTATAGTAGGAACAGCAATAAGAGTATTAAATTCGAATT
	1 10 20 30 40 50 60

71-140 Nucleotide sequence alignment

A. Eriovixia excels.txt	GAATTAGGACAGCCGGGTAGATTTCATTGGGGACGATCAATTATACAATGTAATTGTTACTGCTCATGCGT
B. Araneus mitificus.txt	GAATTGGGTCAGTCAAGGAAGTTTATTGGAGATGATCAACTATATAATGTAATTGTAACGCTCATGCTT
C. Gibbaranea ullrichi.txt	GAATTAGGACAAATCGGGAAGGTTTATTGGTGATGATCAAAATTTATAACGCTGATTGTAACAGGCTCATGCTT
E. Cyrtophora citricola.txt	GAAC TAGGACAGCCAGGAGGTTTATAGGTGATGATCAATTGTATAATGTGATTGTTACTGCTCATGCTT
F. Neoscona polyspinipes.txt	GAATTAGGTCACCCAGGAGGTTTATTGGAGATGATCAACTTTATAATGTAATTGTAACGGGCTCATGCTT
H. Neoscona theisi.txt	GAGTTAGGTCAGCCTGGGAAGATTTATAGGTGATGATCAATTATATAATGTAATTGTTACTGCTCATGCTT
J. Neoscona scylla.txt	GAGTTAGGTCAGCCAGGTAGATTTATAGGTGATGATCAATTATATAATGTAATCGTTACTGCTCATGCTT
G. Argiope lobata.txt	GAGTTAGGACAGCCTGGGAGATTTATAGGAGATGACCAAGTTATATAACGTAATTGTAACGGGCTCATGCTT
D. Argiope versicolor.txt	GAATTAGGTCACCTGGGAAGGTTTATGGGGGATGATCAATTATATAATGTGATTGTAACAGCACATGCTT
I. Gea spinipes.txt	GAATTAGGACAACTGGGAGTTTATTGGAGATGACCAATTATATAATGTAATTGTTACAGCTCATGCTT
Contigl:	GAATTAGGWCAGCCWGGFRAGRTTATTGGWATGATCAATTATATAATGTAATTGTTACTGCTCATGCTT
	80 90 100 110 120 130

141-210 Nucleotide sequence alignment

A. Eriovixia excels.txt	TTGTAATAATTTTTTTTATAGTTATACCTATTTTAAATTGGGGGTTTGGGAATTGATTAGTTCCTTAAAT
B. Araneus mitificus.txt	TTGTAATAATTTTTTTTATGGTTATACCTATTTTAAATTGGTGGGTTTGGGAATTGTTAGTACCTCTAAT
C. Gibbaranea ullrichi.txt	TTGTAATAATTTTTTTTATAGTAATACCTATTTTGAATTGGGGATTGGTAATTGTTAGTTCCTTAAAT
E. Cyrtophora citricola.txt	TTGTGATAATTTTTTTTATAGTAATACCAATTTTAAATCGGAGGATTGGAAATTGATTAGTTCCTCTAAT
F. Neoscona polyspinipes.txt	TTGTAATAATTTTTTTTATAGTAATACCGATTTTGAATTGGTGGTTTGGAAATTGATTGGTACCTTAAAT
H. Neoscona theisi.txt	TTGTTATAATTTTTTTTATGGTAATACCTATTTTGAATTGGTGGATTGGAAATTGATTGGTTCCTCTAAT
J. Neoscona scylla.txt	TTGTAATAATTTTTTTTATGGTAATGCCTATTTTGAATTGGTGGATTGGAAACTGACTAGTCCCTCTAAT
G. Argiope lobata.txt	TTGTAATAATTTTTTTTATGGTTATGCCTATTTCTGATTGGAGGTTTGGAAATTGTTAGTTCCTTTGAT
D. Argiope versicolor.txt	TTGTAATAATTTTTTTTATAGTAATGCCTATTTTAAATTGGGGGATTGGAAATTGTTAGTGCCTCTTAT
I. Gea spinipes.txt	TTGTTATAATTTTTTTTATAGTAATGCCTATTTTAAATTGGGGGATTGGGAAGTGGTTAGTTCCTTAAAT
Contigl:	TTGTAATAATTTTTTTTATAGTAATACCTATTTTATTTGGGKGGATTGGAAATTGTTAGTTCCTCTTAAAT
	150 160 170 180 190 200

211-280 Nucleotide sequence alignment

A. Eriovixia excels.txt	ATTGGGTGCGCCTGACATAGCTTTTCCTCGAATAAATAATTTAAGGTTTTGGTTACTTCCCTTCTTTA
B. Araneus mitificus.txt	GTTGGGAGCTCCTGATATAGCTTTTCCTCGAATAAATAATTTAAGATTTTGGTTGCTTCCCTCCATCTCTT
C. Gibbaranea ullrichi.txt	GTTAGGTGCTCCTGATATAGCTTTTCCTCGTATAAATAATTTAAGTTTTTGATTATTACCCCATCTTTA
E. Cyrtophora citricola.txt	ATTAGGGGCTCCTGATATAGCTTTTCCTCGAATAAATAATTTAAGATTCTGATTATTACCCCATCTTTA
F. Neoscona polyspinipes.txt	ATTGGGGGCTCCAGATATAGCTTTTCCTCGAATAAATAATTTGAGATTTTGGTTATTACCTCCTTCGTTA
H. Neoscona theisi.txt	ATTAGGAGCTCCTGATATGGCATTTTCCTCGAATAAATAATTTAAGATTTTGAATTATTACCTCCATCTTTA
J. Neoscona scylla.txt	ATTAGGGGCTCCTGATATGGCTTTTCCTCGAATAAATAATTTAAGATTTTGAATTACTACCTCCTTCATTA
G. Argiope lobata.txt	ATTAGGAGCTCCTGATATAGCTTTCCACGAATAAATAATTTGAGATTTTGAATTATTACCTCCTTCGTTA
D. Argiope versicolor.txt	ATTAGGAGCCCAAGATATAGCTTTCCACGAATAAATAATTTAAGGTTTTGATTATTACCTCCTTCGTTA
I. Gea spinipes.txt	ATTAGGTGCCCCCTGATATAGCTTTTCCTCGAATAAATAATTTAAGTTTTTGATTATTACCTCCTTCATTA
Contigl:	ATTAGGAGCTCCTGATATAGCTTTTCCTCGAATAAATAATTTAAGATTTTGAATTATTACCTCCTTCCTTA
	220 230 240 250 260 270

281-350 Nucleotide sequence alignment

A. Eriovixia excels.txt	TTTCTGTTATTAATTTCTTCAATAGTCGAGATGGGGTGGGGTCCGGTTGAACTGTGTATCCCCCTCTTG
B. Araneus mitificus.txt	TTTCTTTTAGTAATTTCTTCTATAGTAGAAATAGGGGTTGGTGGCTGGTTGAACAGTATATCCCCCTTTAG
C. Gibbaranea ullrichi.txt	TTTCTTTTGATTGTCTCATCTTTGGTTGAAATAGGTGTTGGGGCTGGTTGAACTATTATCCCCCTATTAG
E. Cyrtophora citricola.txt	TTTCTTTTAATTATTTTCATCTATAGTAGAGATAGGAGTAGGGGGCTGGGTGAACTGTATATCCCCCTTTAG
F. Neoscona polyspinipes.txt	TTTTTATTAATTATTTTCATCAATAGTAGAAATAGGTGTAGGTGCTGGGTGAACAGTATATCCTCCTCTGG
H. Neoscona theisi.txt	TTTTTATTAAGTAATTTTCATCATTAGTAGAAATAGGAGTTGGGGCTGGTTGAACAGTGTATCCTCCACTGG
J. Neoscona scylla.txt	TTTTTACTAATTACATCATCATTAGTAGAAATAGGAGTAGGGGCAGGTTGAACAGTATATCCTCCATTAG
G. Argiope lobata.txt	TTTCTTTTAATTATTTCTTCGATAGTAGAGATAGGAGTTGGTGGGGATGAACGTGTTATCCTCCATTGG
D. Argiope versicolor.txt	TTTCTTTTAATTGTTTTCTTCAATAGTAGAAATAGGAGTAGGGGCAGGATGAACGTGTATATCCTCCTTTAG
I. Gea spinipes.txt	TTTCTTTTAATTGTTTTCTTATAGTAGAAATAGGGGTTGGAGCAGGATGAACGTGTTATCCTCCTTTGG
Contigl:	TTTCTTTTAATTATTTCTCAATAGTAGAAATAGGAGTTGGGGCTGGTTGAACTGTATATCCTCCTTTAG
	290 300 310 320 330 340

351-420 Nucleotide sequence alignment

A. Eriovixia excels.txt	CGAGGTTGGAGGGTCATAGTGGGAGTTCTGTAGATTTTGTCTATTTTCTTTGTCATCTCGCGGGGGGCATC
B. Araneus mitificus.txt	CAAGTTTAGAGGGGCATGCTGGGAGTTCAAGTTGATTTTGGCATTTTTCTTTACATTGGCGGGTGCTTC
C. Gibbaranea ullrichi.txt	CTGGGTTGGAAGGCCATGCTGGTAGATCAGTTGATTTTGTCTATTTTCTTTACATTAGCTGGGGCGTC
E. Cyrtophora citricola.txt	CTAGACTAGAAGGGCATGCTGGGAGATCAGTTGATTTTGCATTTTCTTTACATTAGCTGGAGCTTC
F. Neoscona polyspinipes.txt	CTGGATTAGAAGGTCATGCTGGGAGATCAGTTGATTTTGTCTATTTTCTTTACATTAGCTGGAGCTTC
H. Neoscona theisi.txt	CTGGATTAGAAGGTCATGCTGGTAGATCTGTAGATTTTGCATTTTCTTTACATTAGCTGGTGCTTC
J. Neoscona scylla.txt	CGGGTTTAGAAGGTCATGCTGGGAGATCTGTAGATTTTGTCTATTTTCTTTACATTGGCGGGAGCATC
G. Argiope lobata.txt	CTGGATTAGAAGGACATGCTGGGAGGTCAGTTGATTTTGTCTATTTTCTTTACATTAGCTGGGGCTTC
D. Argiope versicolor.txt	CTGGATTAGAAGGTCATGCTGGGAGATCTGTGATTTTGCATTTTCTCTTCATTGGCAGGTGCTTC
I. Gea spinipes.txt	CTGGGTTAGAAGGTCATGCGAGGCAGATCTGTAGATTTTGTCTATTTTCTCTTCATTGGCGGGAGCTTC
Contigl:	CTGGATTAGAAGGTCATGCTGGGAGATCTGTGATTTTGTCTATTTTCTTTACATTAGCTGGAGCTTC
	360 370 380 390 400 410

421-490 Nucleotide sequence alignment

A. Eriovixia excels.txt	TTCTATTATAGGGGCAATAAATTTTATTCTACTATTATTAATATACGATTTTATGGGCTAACTATAGAA
B. Araneus mitificus.txt	TTCTATTATAGGGGCAATTAATTTTATTCTACTATTATTAATATACGATTTTATGGAATAACCATAGAA
C. Gibbaranea ullrichi.txt	TTCTATTATAGGGGCTATTAATTTTATTCTACTATTATTAATATACGGTCTTATGGAATGTCTATAGAA
E. Cyrtophora citricola.txt	TTCAATTATAGGAGCTATTAATTTTATTCAACTATTTTAAATATACGATTTTATGGAATAACAATGGAA
F. Neoscona polyspinipes.txt	ATCTATTATGGGGCTATTAATTTTATTCAACAATTATTAATATACGATTTTATGGAATGACTATAGAA
H. Neoscona theisi.txt	GTCAATTATAGGGGCTATTAATTTTATTCAACTATTATTAATATACGATTTTATGGGATGACTATAGAG
J. Neoscona scylla.txt	TTCAATTATAGGTGCTATTAATTTTATTCAACTATTATTAATATACGTTTTTATGGAATAACTATGGAA
G. Argiope lobata.txt	TTCAATTATAGGAGCTATTAATTTTATTCAACTATTATTAATATACGATTTTATGGTATGACCATGGAA
D. Argiope versicolor.txt	TTCTATTATAGGAGCAATTAATTTTATTCTACAATTATTAATATACGATTTTATGGAATAACTATAGAA
I. Gea spinipes.txt	TTCTATTATAGGGGCAATTAATTTTATTCTACAATTATCAATATACGATTTTATGGAATAACTATGGAA
Contigl:	TTCTATTATAGGGGCTATTAATTTTATTCTCACTATTATTAATATACGATTTTATGGAATAACTATAGAA
	430 440 450 460 470 480

491-560 Nucleotide sequence alignment

A. Eriovixia excels.txt	AAGGTTCCCTTTATTTGTATGATCGGTTTTGATCAGGGCTGTACTATTATTATTGTCATTGCCTGCTTTGG
B. Araneus mitificus.txt	AAAGTACCTTTATTTGTATGATCAGTTTTAATTACAGCAGTTTACTTTTACTCTCTTTACCTGTATTAG
C. Gibbaranea ullrichi.txt	AAGGTTCCCTTTATTTGTTGATCTGTGTTAATTACAGCAGTTTATTATTACTTTCTTTACCGGTTTTAG
E. Cyrtophora citricola.txt	AAAGTTCCTTTATTTGTTTGGTCTGTTCTAATTACAGCTGTTCTTCTATTATTATCTTTACCGGTTTTAG
F. Neoscona polyspinipes.txt	AAGGTTCCCTTTATTTGTATGATCAGTTTTGATTACTGCTGTTTATTATTATTATCATTACCTGTATTAG
H. Neoscona theisi.txt	AAGGTACCTTTATTTGTATGATCTGTATTAATTACTGCAGTATTATTATTATTATCTTTACAGTATTAG
J. Neoscona scylla.txt	AAGGTACCTTTATTTGCTGCTGTCAGTATTAATTACTGCAGTTTATTATTGTTATCTTTACCTGTATTAG
G. Argiope lobata.txt	AAGGTTCCCTTTATTTGTTTGGTCTGTCTTAATTACAGCTGTACTTTTACTTTTATCATTGCCTGTATTAG
D. Argiope versicolor.txt	AAGGTTCCCTTTATTTGTTTGGTCTGTCTTAATTACTGCTGTTCTTTTACTTCTCTGTTACCGGTTTTAG
I. Gea spinipes.txt	AAGGTTCCCTTTATTTGTTTGGTCTGTCTTAATTACTGCTGTTCTTCTCTTTTGTCTTTACCTGTATTAG
Contigl:	AAGGTTCCCTTTATTTGTTTGAATCTGTTTTAATTACTGCTGTTTATTATTATTATCTTTACCTGTATTAG
	500 510 520 530 540 550

561-610 Nucleotide sequence alignment

Figure 1: Nucleotide sequence alignments result of COI-5P generated by codon code aligner version 8.02 of the *Eriovixia excels* (Simon, 1889), *Araneus mitificus* (Simon, 1886) *Gibbaranea ullrichi* (Hahn, 1835), *Argiope versicolor* (Doleschall, 1859), *Cyrtophora citricola* (Forsskal, 1775), *Neoscona polyspinipes* (Yin, Wang, Xie & Peng, 1990), *Argiope lobata* (Pallas, 1772), *Neoscona theisi* (Walckenaer, 1841), *Gea spinipes* (C. L. Koch, 1843) and *Neoscona scylla* (Karsch, 1879).

Gea spinipes (C. L. Koch, 1843)

Figs 3, 4, Map

Gea spinipes C. L. Koch, 1843: 101, figs 823 (D♀).

Gea spinipes Yin *et al.*, 1997d: 90, figs 21a-f (♂♀); *Gea spinipes* Chakrabarti, 2009: 128, figs 1-7 (♀).

Sample id: ICUP-MS-0064, ICUP-MS-0087 and ICUP-MS-0088

Process id: SPKPK064-20, SPKPK087-20 and SPKPK088-20

Match Process id: GACS1650-19

Material Examined: PAKISTAN: 1♂ 2♀ (MICU, G-32), Goro stream, Talash, Dir Lower, Khyber Pakhtunkhwa (34.44707°N, 71.49292°E), 768 m a.s.l., 09.07.2018, M. Sajid.

Distribution: The species was earlier known from India, China, Taiwan to Indonesia (WSC, 2020). The species is reported for the 1st time in Northern Pakistan.

Gibbaranea ullrichi (Hahn, 1835)

Fig. 5, Map

Epeira ullrichii Hahn, 1835: 66, figs 159 (D♂♀).

Araneus ullrichi Simon, 1929: 699, 765, figs 1091 (♂♀);

Gibbaranea ullrichi Heimer & Nentwig, 1991: 82, figs 186 (♂♀).

Sample id: ICUP-MS-0022

Process id: SPKPK022-20

Match BIN: BOLD: AAD6909

Material Examined: PAKISTAN: 1 ♀ (MICU, 469), Redar Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.77247°N, 71.96441°E), 1868 m a.s.l., 21.09.2018, M. Sajid.

Distribution: This species has distribution from Europe to Central Asia (WSC, 2020). Northern areas of Pakistan are connected to Central Asian countries and the species has distribution pattern in Pakistan. There is no record of the species from India, Iran and Afghanistan the neighboring countries.

Neoscona polyspinipes Yin, Wang, Xie & Peng, 1990

Fig 6, Map

Neoscona polyspinipes Yin *et al.*, 1990: 104, figs 254-261 (D♂♀).

Sample id: ICUP-MS-0042, ICUP-MS-0044, ICUP-MS-0082 and ICUP-MS-0083

Process id: SPKPK042-20, SPKPK044-20, SPKPK082-20 and SPKPK083-20

Match BIN: BOLD: AAO1983

Match Process id: AMSPK140-15

Material Examined: PAKISTAN: 2 ♀ (MICU, MP-77), Barcharay Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.71637°N, 71.91691°E), 1198 m a.s.l., 18.06.2018; 2 ♀

(MICU, S-63a,b), NagriPayeen, Talash, Dir Lower, Khyber Pakhtunkhwa (34.74637°N, 71.93991°E), 1001 m a.s.l., 13.06.2018, all. coll. M. Sajid.

Distribution: The species was endemic to China (WSC, 2020). The present record from Pakistan represents the westernmost limit of the species distribution.

Neoscona scylla (Karsch, 1879)

Fig 7, Map

Epeira scylla Karsch, 1879g: 71 (D♀); *Neoscona scylla* Yaginuma, 1960: 56, fig 52.1(♂♀); *Neoscona scylla* Baba & Tanikawa, 2015: 66, fig 7 (♂♀).

Sample id: ICUP-MS-0089, ICUP-MS-0090, ICUP-MS-0091, ICUP-MS-0092, ICUP-MS-0093 and ICUP-MS-0094

Process id: SPKPK089-20, SPKPK090-20, SPKPK091-20, SPKPK092-20, SPKPK093-20 and SPKPK094-20

Match BIN: BOLD: AAO1997

Match Process id: MASPD004-10

Material Examined: PAKISTAN: 2♀ (MICU, SW-01, 02), NagriPayeen, Talash, Dir Lower, Khyber Pakhtunkhwa (34.74767°N, 71.94041°E), 1010 m a.s.l., 14.08.2018, M. Sajid.

Distribution: The species is known from Russia (Far East), China, Korea and Japan (WSC, 2020). Present record from Northern areas of Pakistan represents the westernmost distributional limit of the species. This species is not recorded yet from adjacent India, Afghanistan and Iran but is likely to occur.

Neoscona theisi (Walckenaer, 1841)

Fig 8, Map

Epeira theisi Walckenaer, 1841: 53, pl. 18, f. 4 (D♀).

Neoscona theisi Butt & Siraj, 2006: 215, figs 1A-G (♂♀).

Sample id: ICUP-MS-0061

Process id: SPKPK061-20

Match BIN: BOLD: ACM3489

Match Process id: GBMIN114644-17

Material Examined: PAKISTAN: 1 ♀ (MICU, MP-276), Pato Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.76277°N, 71.92491°E), 1238 m a.s.l., 21.07.2018, coll. M. Sajid.

Distribution: This species has a wide range of distribution from Cyprus, Israel, Russia, Iran, Pakistan, India, Philippines, China, Indonesia, Japan to Australia (WSC, 2020). The present record in Pakistan is the northernmost record in the country.

Araneus mitificus (Simon, 1886)

Figs 9, 10, Map

Epeira mitifica Simon, 1886a: 150 (D♀). *Zilla nawazi* Dyal, 1935: 186, figs 6, pl. 16, fig 124 (D♀);

Araneus mitifica Biswas & Raychaudhuri, 2013a: 158, figs 1-7 (♂♀).

Sample id: ICUP-MS-0018 and ICUP-MS-0038

Process id: SPKPK018-20 and SPKPK038-20

Match BIN: BOLD: AAV1598

Match Process id: MTSPD326-15

Material Examined: PAKISTAN: 1 ♀ 1 ♂ (MICU, 804, 805), Kamranay Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.45974°N, 71.49475°E), 885 m a.s.l., 07.04.2019, M. Sajid.

Distribution: The species is distributed from India to Philippines, Japan, New Guinea (WSC, 2020). It was recorded from Eastern Pakistan (Dyal, 1935) and our record provides additional distributional data in the country.

Eriovixia excelsa (Simon, 1889)

Figs 12, Map

Glyptogona excelsa Simon, 1889i: 337 (D♀).

Araneus excelsus Dyal, 1935: 179, pl. 16, figs. 116 (♀)

Eriovixia excelsa Tso & Tanikawa, 2000: 129, figs 17-22 (♂♀)

Eriovixia excelsa Caleb, 2020b: 15717, figs 3C, 24K (♀).

Sample id: ICUP-MS-0005

Process id: SPKPK005-20

Match BIN: BOLD: AAQ0105

Match Process id: GBMIN114597-17

Material Examined: PAKISTAN: 2♀ (MICU, 107, Fn-09), Goro Hills, Dir Lower, Khyber Pakhtunkhwa (34.44761°N, 71.49451°E), 818 m a.s.l., 02.09.2018, M. Sajid.

Distribution: This species has a wide distribution range from Pakistan, India, China, Taiwan, Philippines to Indonesia (WSC, 2020). It was previously recorded from eastern part of Pakistan (Lahore) (Dayal, 1935). The present record is the northernmost in the country.

Cyrtophoracitricola (Forsskål, 1775)

Figs 11, Map

Araneacitricola Forsskål, 1775: 86 (D).

Cyrtophoracitricola Dyal, 1935: 175, figs 111-115 (♀);

Cyrtophoracitricola Nentwig et al., 2019: 35.

Sample id: ICUP-MS-0003 and ICUP-MS-0043

Process id: SPKPK003-20 and SPKPK043-20

Match BIN: BOLD: AAO2032

Match Process id: MASPD022-10

Material Examined: PAKISTAN: 5♀ (MICU, MP-78a,b; MP-79 a,b,c), Barcharay Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.71661°N, 71.91693°E), 1290 m a.s.l., 18.06.2018, M. Sajid; 4♀ (MICU, MP-134 a,b; MP-148 a, b), Zombaqy Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.73717°N, 71.94790°E), 1028 m a.s.l., 01.07.2018, M. Sajid; 2♀ (MICU, MP-308), Pato Hills, Talash, Dir Lower, Khyber Pakhtunkhwa (34.76297°N, 71.92481°E), 1248 m a.s.l., 21.07.2018, all. coll. M. Sajid.

Distribution: This species is known from Southern Europe, Africa, Middle East, Pakistan, India, China, Japan, Introduced to Dominican Rep., Costa Rica, Colombia and Brazil (WSC, 2020). The species is recorded for the first time from the study area.

***Cycloahexatuberculata* Tikader, 1982**

Figs 13, 14, Map

Cycloahexatuberculata Tikader, 1982a: 197, figs 382-387 (D♂♀).

Material Examined: PAKISTAN: 1♂, 1♀ (MICU, 819, Fn-820), Goro Hills, Dir Lower, Khyber Pakhtunkhwa (34.44761°N, 71.49451°E), 818 m a.s.l., 02.09.2018, M. Sajid.

Distribution: The species is distributed in Pakistan and India (WSC, 2020). It is recorded for the first time from the northern regions in Pakistan which is also the westernmost distribution limit of the species.

DISCUSSION

A total 35 araneid specimens were collected from Dir Lower Pakistan out of which 8 genera and 11 species were identified. Of which 23 were sent for DNA barcode to the Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph, Canada. All the eleven species were recorded for the first time from study area (Dir Lower), while six species, *Argiope versicolor*, *Argiope lobata* Pallas, 1772, *Neoscona polyspinipes* Yin, Wang, Xie & Peng, 1990, *Neoscona scylla* Karsch, 1879, *Gea spinipes* C. L. Koch, 1843 and *Gibbaranea ullrichi* Hahn, 1835 are new to the araneid fauna of Pakistan.

Table 1: Checklist of family Araneidae from Pakistan.

1	<i>Araneus alboquadratus</i>	Dyal, 1935
2	<i>Araneus altitudinum</i>	Caporiacco, 1934a
3	<i>Araneus camilla</i>	Dyal, 1935
4	<i>Araneus formosus</i>	Dyal, 1935
5	<i>Araneus fulvus</i>	Dyal, 1935
6	<i>Araneus obscurissimus</i>	Caporiacco, 1934
7	<i>Araneus pontii</i>	Caporiacco, 1934
8	<i>Chorizopes rajanpurensis</i>	Mukhtar and Tahir, 2013
9	<i>Cyclosa chichawatniensis</i>	Mukhtar and Mushtaq, 2005
10	<i>Cyclosa kashmirica</i>	Caporiacco, 1934
11	<i>Cyclosa krusa</i>	Barrion and Litsinger, 1995
12	<i>Cyclosa mohini</i>	Dyal, 1935
13	<i>Cyclosa punjabiensis</i>	Ghafoor & Beg, 2002
14	<i>Cyclosa saismarka</i>	Barrion and Litsinger 1995
15	<i>Cyclosa spirifera</i>	Simon, 1889

16	<i>Cyrtarachne nagasakiensis</i>	Strand, 1918
17	<i>Cyrtophora cicatrosa</i>	Stoliczka, 1869
18	<i>Cyrtophora citricola</i>	Forsskal, 1775
19	<i>Eriovixia excelsa</i>	Simon, 1889
20	<i>Gasteracantha dalyi</i>	Pocock, 1900
21	<i>Neoscona huzaifi</i>	Mukhtar, 2012
22	<i>Neoscona pavida</i>	Simon, 1906
23	<i>Neoscona theisi</i>	Walckenaer, 1841,
24	<i>Nephila pakistaniensis</i>	Ghafoor and Beg, 2002
25	<i>Araneus nympha</i>	Simon, 1889
26	<i>Argiope anasuja</i>	Thorell, 1887
27	<i>Cyclosa hexatuberculata</i>	Tikader, 1982
28	<i>Neoscona bengalensis</i>	Tikader and Bal, 1981
29	<i>Argiope versicolor</i>	Dolleschall, 1859
30	<i>Argiope lobata</i>	Pallas, 1772
31	<i>Neoscona polyspinipes</i>	Yin, Wang, Xie and Peng, 1990
32	<i>Neoscona Scylla</i>	Karsch, 1879
33	<i>Gea spinipes</i>	C. L. Koch, 1843
34	<i>Gibbaranea ullrichi</i>	Hahn, 1835

The spider fauna of Pakistan is not yet fully explored due to lack of expertise and interest. Dyal [1935] reported, *Cyrtophora citricola* (Araneidae) from Lahore but did not record *Neoscona theisi* from the area which is very common now (Butt and Siraj, 2006). WSC (2020) lists only 32 species from Pakistan in which *Araneus alboquadratus* Dyal, 1935, *Araneus altitudinum* Caporiacco, 1934a, *Araneus camilla* Dyal, 1935, *Araneus formosus* Dyal, 1935, *Araneus fulvus* Dyal, 1935, *Araneus obscurissimus* Caporiacco, 1934, *Araneus pontii* Caporiacco, 1934, *Chorizopes rajanpurensis* Mukhtar and Tahir, 2013, *Cyclosa chichawatniensis* Mukhtar and Mushtaq, 2005, *Cyclosa kashmirica* Caporiacco, 1934, *Cyclosa krusa* Barrion and Litsinger, 1995, *Cyclosa mohini* Dyal, 1935, *Araneus nympha* Simon, 1889, *Argiope anasuja* Thorell, 1887, *Cyclosa hexatuberculata* Tikader, 1982, *Neoscona bengalensis* Tikader and Bal, 1981, *Cyclosa punjabiensis* Ghafoor and Beg, 2002, *Cyclosa saismarka* Barrion and Litsinger 1995, *Cyclosa spirifera* Simon, 1889; *Cyrtarachne nagasakiensis* Strand, 1918, *Cyrtophora cicatrosa* Stoliczka, 1869, *Cyrtophora citricola* Forsskal, 1775; *Eriovixia excelsa* Simon, 1889, *Gasteracantha dalyi* Pocock, 1900, *Neoscona huzaifi* Mukhtar, 2012, *Neoscona pavida* Simon, 1906; *Neoscona theisi* Walckenaer, 1841, *Nephila pakistaniensis* Ghafoor and Beg, 2002 are confirmed from various localities of Pakistan. While *Neoscona chrysanthusi* Tikader and Bal, 1981, *Neoscona mukerjei* Tikader, 1980, *Neoscona shillongensis* Tikader and Bal, 1981 and *Neoscona sinhadensis* Tikader, 1975

have a doubtful record from the country because most of these species were recorded from India and there is no data about Pakistan.

With the addition of present data, the araneid list of Pakistan reaches to 38 species. Existence of 34 species were confirmed from the published literature including the new records of the present study while 4 species cited above has no evidence of existence in the country from the published literature at WSC. Further study and exploration efforts are necessary for documentation of the entire araneid fauna of Pakistan. There are great possibilities for the presence of many undescribed species. Moreover, species described from neighboring countries like China and India whose fauna are relatively well explored are likely to be present in Pakistan as well. An updated checklist is attached.

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