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Research Article

Diversity of Spiders in Rice Ecosystems of Punjab, Pakistan

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SM, HMT and SA designed the study and wrote the manuscript, SM, HMT and AN conducted the experimentation, MS, SMS and HMT analyzed the data.

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Spiders, Barcoding, Cytochrome c Oxidase I, DNA extraction, Morphological identification

Abstract | Present study was outlined to evaluate the authenticity of morphological identifications of spiders from rice ecosystems of district Lahore, Sialkot and Mandi Bahauddin, Punjab, Pakistan by using DNA barcoding. A total of 2736 spiders were collected from July to October during 2018-19. They were brought to the laboratory for subsequent morphological identification, DNA extraction and sequencing. We identified 38 morph-species, representing 22 genera and 8 families. Accuracy of morphological identification was confirmed by barcode analysis of tissue samples. A standard barcode sequence of COI (Cytochrome c Oxidase I) was recovered from 90 specimens of spiders. Percentage accuracy of morphological identification was 92.10%. Four morphologically misidentified spiders were allotted to correct taxon after molecular identification. To describe the species diversity and richness in numerical structure, diversity indices (Shannon's and Simpson's) and evenness indices (Margalf's index, Chao 1) were applied. No overlap was found between inter-specific and intra-specific divergence values. Neighbor joining tree clearly separated the species into different clusters. Present study concluded that although morphological identification of spiders works convincingly, but it becomes more authentic and reliable when merged with DNA barcoding.

Novelty Statement | Current study encompasses the use of novel molecular technique of DNA barcoding to authenticate the morphological identification as compared to the conventional taxonomy. Moreover, the barcodes of the spiders has generated the reference molecular library which would help in establishing the phylogenetic relationships among various spider species for diversity analysis in future. Authentic molecular evaluation will also help to correct the misidentified specimens.

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Introduction

Rice, *Oryza sativa* L. (Poales: Poaceae) is a vital food source for more than half of the global population with an annual yield of more than 700 tons approximately (Center, Africa Rice, 2011; Seck *et al.*, 2012). India, China, Thailand, Bangladesh, Philippines, Pakistan, Indonesia, USA and Vietnam are among major rice producers (Calpe, 2006). Moreover, total rice harvesting area of Pakistan is over 2.57 million ha, contributing 17% of annual cereals production. Annual rice production of Pakistan, being eleventh largest country for rice production, is 6.5 million tons making a share of 4.9% in agriculture sector and 1.3% in GDP (Bashir *et al.*, 2019).

Global productivity of rice is now at risk from the past few years due to different crop diseases and pest infestations (Oerke, 2006; Xie and Yang, 2018; Savary *et al.*, 2019). Furthermore, insect pests of rice cause an

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annual loss of 200 million tons to the crop (Nasiruddin and Roy, 2012; Singh and Singh, 2017). To control the pest outbreaks, synthetic pesticides are widely used (Karunamoorthi and Mohammed, 2012; Weng and Black, 2015). However, their excessive use cause negative impacts on environment, human health and agriculture production by severely damaging the fauna and flora (Houbraken *et al.*, 2016; Rosic *et al.*, 2020). Furthermore, the natural balance between pests and predators has been disturbed due to injudicious use of pesticides which are responsible for non-target killing of natural enemies (John and Shaike, 2015).

To minimize the hazardous effects of chemical pesticides, world agro analysts intend to shift their insect pest management strategies solely towards biological control, a method which encompasses the broad range of concepts from using the different cultural practices and introduction of living organisms to control the pest (Bakera *et al.*, 2020). Arthropod predators act as pollinators, nutrient recyclers and decomposers (Thrupp, 2000). Spiders are most abundant natural predators in rice ecosystem and restrict the population of insect pests below economic injury level (Maloney *et al.*, 2003; Thomson and Hoffmann, 2010; Radermacher *et al.*, 2020). They are excellent predators due to wasteful killing, high reproductive rate, functional and numerical response and their ability to survive under conditions of food shortage (Nyffeler and Birkhofer, 2017; Michalko *et al.*, 2019).

Accurate identification of spiders is necessary to discriminate the native spider fauna from the invasive species by establishing a barcode reference library (Tyagi *et al.*, 2019). Species level identification is dependent on different diagnostic characters like eye and epigynal patterns (Barrett and Hebert, 2005). Morphology based identification is a hectic task due to unavailability of literature and time consumption (Ball and Armstrong, 2006). Absence of clear differentiating characters and sexual dimorphism challenge the authenticity of morphological identification of spiders (Robinson *et al.*, 2009; Hamilton *et al.*, 2011). So, there is an unmet need to introduce the quick, economical and undisputed approaches to analyze the spiders taxonomically (Hebert and Gregory, 2005; Fontaneto *et al.*, 2009; Iftikhar *et al.*, 2016).

Different researchers and scientists across the world are using molecular techniques like DNA barcoding for precise identification (Bond *et al.*, 2001; Puillandre *et al.*, 2012; Palandacic *et al.*, 2017; Jalajakshi and Usha, 2019). DNA barcoding use short standardized genomic site (Cytochrome C oxidase subunit I, COI) of 658 base pairs of mitochondrial DNA to produce fast species evaluation results (Hebert *et al.*, 2003; Hajibabaei *et al.*, 2006; Sudhikumar and Kashmeera, 2015; Gotoh and Arabuli, 2019). This genomic region is referred as biological barcode and commonly known as species tag or barcode tag for specific animal taxa (Xing *et al.*, 2018; Gotoh and Arabuli, 2019). COI region is ideally considered for DNA barcoding as it is a basic part of genome of all eukaryotic organisms (Sudhikumar and Kashmeera, 2015). Moreover, different forms of chromosomal mutations like inversion, deletions and insertions are very rare in this region of mitochondrial DNA. Eventually this biological marker contains significant sequence divergence to evaluate the closely related eukaryotic species. Due to the short sequence length and efficacious annealing through universal primers make COI gene a best choice for molecular analysis (Folmer *et al.*, 1994; Zhang and Hewitt, 1997; Simmons and Weller, 2001).

DNA barcoding has gained a lot of fame by not only correct molecular identification of misidentified species but also help in new species discovery (Hebert *et al.*, 2003, 2004). Organisms belonging to multiple taxa like crustaceans, ants, algae, amphibians, birds and fishes have been successfully identified using DNA barcode analysis (Ward *et al.*, 2005; Saunders, 2005; Vences *et al.*, 2005; Witt *et al.*, 2006; Kerr *et al.*, 2007; Schoch *et al.*, 2012; Gotoh and Arabuli, 2019). Although this molecular analysis is reliable but sometimes sequences of biological barcode (COI gene) are not retrieved successfully, in such situations, alternative barcodes like ITS-2 (Internal transcribed spacer region 2), 12s rDNA, 16s rDNA and cytochrome b (Cytb) are preferred but it is hard to find all the required specifications in a single marker (Vences *et al.*, 2005; Steinke *et al.*, 2005; Kumar *et al.*, 2009; White *et al.*, 2014; Jalali *et al.*, 2015; Cao *et al.*, 2016). The future and success of DNA barcoding based on the hypothesis that genomic variations within a species are less than between the species (Smith *et al.*, 2005; Hajibabaei *et al.*, 2006). Currently, taxonomists and researchers globally working on the limiting aspects of DNA barcoding technique and come up with the solution in the form of "integrated barcoding" (Rubinoff, 2006). This novel concept involves the combined use of morphological and molecular approaches to discriminate a species.

The objective of current study was to document the undescribed fauna of spiders from three districts of Punjab (Lahore, Sialkot and Mandi Bahauddin), Pakistan. Evaluation of accuracy and authenticity of DNA barcoding was another motive behind this research. Finally, the present study was undertaken to generate genomic reference library of spiders of rice ecosystems of Pakistan.

Materials and Methods

Collection of arthropods

Live arthropods were sampled from rice-wheat fields across the three districts including Lahore, Mandi Bahauddin and Sialkot of Province Punjab, Pakistan.

Collection was done during 2018 and 2019 using different sampling techniques like visual search and sweep netting (Robinson *et al*., 2009; Tahir *et al*., 2016; Tyagi *et al*., 2019). This study did not involve any endangered species. Sampling was conducted from June through December during rice growing season and from January through May in wheat growing season. At each trapping date, sampling was done from randomly selected area of 25m^2. From the selected area 20 plants were randomly selected for visual search and sweep netting. Insects from selected area were also collected by sweep net using 10 sweeps from an area of 25m^2. Collection was done twice in a month. Searching method was consistent throughout the sampling period for all study sites.

Sampling sites

These sampling sites were Jallo (R-1) and Seed Research Area from University of the Punjab (R-2) in Lahore district (31.499N; 74.2994E), Rasul (R-3) and Shana Look (R-4) in district Mandi Bahauddin (32.6768N; 73.5632E) and Duska (R-5) and Pasrur (R-6) in district Sialkot (32.494N; 74.5229E). There were minor variations in the agronomic practices at sampling sites. All sampling sites were monoculture rice fields surrounded with grassy strips/bunds.

Storage and preservation

Insects were collected in plastics jars (4x6 inches) containing 75% ethanol. Collected specimens were brought to the laboratory in the Department of Zoology, Government College University, Lahore. After washing with alcohol arthropods were transferred to clean glass vials (20ml) with the help of forceps for morphological study containing 95% ethanol. Selected specimens of each species were preserved in adequate volume of 95% ethanol for subsequent DNA isolation and preserved at -20°C.

Morphological identification

Insects and spiders were identified upto lowest possible rank by carefully examining different morphological characters under stereozoom dissecting microscope (BCVS 121 and BIOCOM UK) with the help of available keys and catalogues, such as Barrion and Litsinger (1995), Whiting (2017) and data available on BOLD. Identified specimens were photographed with the help of dissecting microscope and digital camera (Canon power shot G9 digital camera).

Diversity indices and inventory completeness

As there was non-significant difference in data of two years, therefore it was pool together for further analysis. Species accumulation curves were prepared using SPDIVERS.BAS program. The richness of the spiders, non-spider predators and insect pests of rice-wheat ecosystems was computed using the non-parametric estimator Chao1 and Margalf's index. To check the completeness of inventories, ratio between Chao1 and observed richness was calculated. Shanon-wiener index was used for comparing the diversity. However, evenness Hill's ratio (E5) was used to compute evenness. Repeated measure ANOVA was used to compare the richness, diversity and evenness among trapping sites and trapping session. For the analysis program STATISTICA (Statsoft, 1999) was used.

DNA barcoding

Authenticity of morphological identification of insect pest and their natural enemies was confirmed by sequencing of mtCOI barcode region of 658 base pairs. Barcode sequences were generated by using following steps.

DNA extraction

DNA from the insects and spiders was extracted by using Thermo Scientific Gene JET DNA Purification kit following the company specified protocols. Left leg of the first pair of insects was preferred to get the DNA. It was extracted by following procedure. A portion of insect leg was removed and cut into pieces with the help of autoclaved blade. These small pieces were transferred to an autoclaved eppendroff (1.5ml). Digestion solution (180 µl) was added to the eppendroff containing tissue samples. Lately 20 µl of Proteinase K solution was added to the eppendroff and mixture was thoroughly vortexed on vortex machine (SCILOGEX MX-S) to get uniform solution. The solution was then incubated overnight at 56°C to allow complete tissue lysis.

After incubation, 20 µl of RNAase solution was added to lysate and then vortexed. After incubation at room temperature for 10 minutes 200 µl of lysis solution was added in the eppendroff, then 400µl of 50% ethyl alcohol was added and mixture was thoroughly mixed by vortex. The Lysate was transferred to the autoclaved Gene JET DNA Purification Column, placed in collection column. The column was centrifuged for one minute at 6000g and flow through was discarded. Gene JET DNA Purification Column was shifted in a new collection tube.

Wash buffer I with added ethanol (500ml) was propend to the purification column and centrifuged for one minute at 8000g. Lately wash buffer II solution was added and centrifuged for three minutes at 12000g. Flow through solution was discarded and column was shifted to a new eppendroff (1.5ml). Genomic DNA was then eluded by adding elution buffer (200 µl) to the column membrane. The column mixture was incubated for two minutes at room temperature and then centrifuged for one minute at 8000g. Finally, the purification column was discarded and purified DNA obtained after elution was stored at -20°C.

PCR amplification of Mt COI gene

The PCR amplification was done in total volume of 25 µl. Reaction mixture was containing 12.5 µl of PCR master mix, 1 µl of both forward and reverse primers, DNA sample (2 µl) and injection water 8.5 µl. Amplification of Barcode gene region was carried out with universal primer pairs (HCOOUTOUT and LCO1490). The main steps of PCR include denaturation of DNA sample at 94°C for one minute, 35 multiplication (amplification) cycles (94°C. for 45 seconds, 48°C. for 45 seconds and 72°C. for 30 seconds). Final step of elongation was carried out at 72°C for 5 minutes in thermocycler.

Verification of PCR products through agarose gel electrophoresis

Verification of PCR products was carried out through 1% agarose (TAE) gel electrophrosis. Agarose gel was prepared by pouring 0.5g of agarose in a conical flask and then adding 50ml of IX TAE buffer and contents were mixed thoroughly. Contents of the mixture were dissolved completely by heating in microwave oven. Agarose solution was then allowed to cool down and Ethidium bromide (Et Br) was added in leukwarm agarose solution. Et Br anneals the DNA and allowes DNA visualization when viewed under UV light. Leukwarm agarose solution was poured on agarose gel casting tray. After inserting comb into gel, it was allowed to polymerize at room temperature for about 30 minutes. After polymerization comb was carefully removed to avoid any rupturing of wells. Gel casting tray containing agarose gel was then placed in gel electophrosis tank containing IX TAE buffer. Samples of DNA (2 µl) along with loading dye (3µl) were loaded into wells by using micropipette. Tank was provided with voltage 120mV for 40 minutes. After that gel was removed from electrophrosis tank and bands were observed under UV light. PCR products with quality bands were further processed for sequencing and photographed.

Sequencing of PCR products

Purified DNA samples were sent to Canadian Centre for Biodiversity Genomics, University of Guleph, Canada, for sequencing.

Data analyses

Sequence evaluation tool in BOLD (www.Barcodinglife.org) was used to calculate genetic distance summaries. MUSCLE, a multiple sequence alignment programs was used for sequence alignment. Aligned sequences were converted into FASTA format. Distance histograms and distance ranks were generated using online version of Automatic Barcode Gap Discovery (ABGD) available at https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html.

Phylogenetic analysis

Phylogenetic tree, using the maximum parsimony (MP) method, was constructed with the help of MEGA5.2. Sequenced data has been submitted in BOLD (Barcode of Life Data System) databases.

DNA barcode reference library

Barcode sequences of sampled specimens with necessary details are available online in the BOLD projects MTINS and MTSPD in the database of BOLD and freely available.

Results

Morphological identifications of spiders

A total of 2736 spiders representing 38 species, 22 genera and 8 families were collected from study areas. Out of the total catch, 286 spiders were immature and identified upto genus level due to the unavailability of keys for juvenile identification. However, remaining 2450 specimens were mature. Highest number (1286) of spiders was captured from district Mandi Bahauddin followed by Lahore (910) and Sialkot (540). List of species identified on the basis of morphological characters is given in Table 1. Family Araneidae (37.569%) was found to be the most abundant followed by family Tetragnathidae (20.244%), Lycosidae (18.126%), Oxyopidae (13.516%), Salticidae (6.065%), Thomisidae (2.669%), Gnaphosidae (1.461%) and Clubionidae (0.584%). *Neoscona theisi* Walckenaer (Araneae: Araneidae) of family Araneidae was the most dominant species that represented 11.403 % of the total catch (Table 1). Family Araneidae was also represented by highest number of species (*n*=10). Figure 1 is showing the family composition spiders collected from rice-wheat ecosystems (combined for all sampling sites).

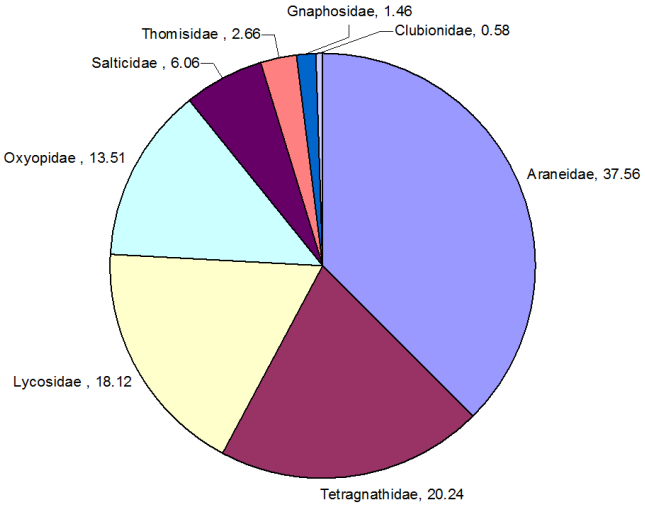

Figure 1: Percentage composition of spider families recoded from rice-wheat ecosystems of Punjab, Pakistan.

Figure 2 is showing the pooled species accumulation curves (combined for three districts) for spiders. The number of trappable spider species increased as the number of individuals increased. Initially number of species increased rapidly but after sample count of 2400

individuals, increase in the species became slow. Estimated species richness was 33, 33 and 39 for Lahore, Sialkot and Mandi Bahauddin respectively on the basis of Chao 2 estimates. The ratio of observed to estimated number of species was 92 % (combined for three districts) which showed that eight percent more species are present in the area than were actually collected (Table 2).

Figure 3 is showing the seasonal dynamics of spiders in rice-wheat ecosystem. Two activity density peaks for spiders were recorded. First peak was observed in October and second peak in March. The density of spiders was declined at the start of November in rice crop and start of April in wheat fields. Results showed non-significant differences in the richness ($F_{2,11}$ = 1.97; P = 0.11), diversity

Table 1: List of morphologically identified spiders collected from rice-wheat ecosystem.

Family	Species	Total	Relative abundance (%)
Salticidae	*Plexippus paykulli* (Audouin, 1826)	38	1.388
	Telamonia dimidiata (Simon, 1899)	48	1.754
	Salticidae sp.	80	2.923
Araneidae	*Neoscona theisi* Walckenaer, 1841	312	11.403
	Eriovixia excelsa (Simon, 1889)	204	7.456
	Cheiracanthium inclusum (Thorell, 1878)	128	4.678
	Argiope trifasciata (Forskål, 1775).	96	3.508
	Cryptophora citricola (Forsskål, 1775).	68	2.485
	Cryptophora parangexanthematica Barrion & Litsinger, 1995	64	2.339
	Neoscona mukergi Tikader, 1980	80	2.923
	Argiope ameula (Walckenaer, 1841)	68	2.485
	Araneus mitificus (Simin 1886)	4	0.146
	Araneidae sp.1	4	0.146
Tetragnathidae	*Tetragnatha javana* (Thorell, 1890)	108	3.947
	Tetragnatha caudata Emerton, 1884	96	3.508
	Tetragnatha vermiformis Emerton, 1884	88	3.216
	Leucauge decorata (Walckenaer, 1841)	70	2.558
	*Tetragnatha virescens (*Okuma, 1979)	40	1.461
	Tetragnatha nitens (Audouin, 1826)	86	3.143
	Tetragnatha sp.1	56	2.046
	Tetragnatha sp.2	6	0.219
	Tetragnatha sp.3	4	0.146
Oxyopidae	*Oxyopes tiengianensis* Barrion & Litsinger, 1995	128	4.678
	Oxyopes aspirasi Barrion & Litsinger, 1995	44	1.681
	Oxyopes javanus Thorell,1887	52	1.900
	Oxyopes oryzae Mushtaq & Qadar, 1999	128	4.678
	Oxyopes hindostanicus Pocock, 1901	6	0.219
Lycosidae	*Pardosa apostolic* (Clerck, 1757)	144	5.263
	Trochosa alviolai Barrion & Litsinger, 1995	64	2.339
	Pardosa birmanica Siman 1884	76	2.777
	Aractosa sp.	80	2.923
	Hippasa partita (O. P.Cambridge, 1876)	64	2.339
	Lycosa terrestris Butt, Anwar & Tahir 2006	60	2.339
	Pardosa sp.	4	0.146
Gnaphosidae	*Gnaphosidae* sp.	40	1.461
Clubionidae	*Clubiona* sp.	16	0.584
Thomisidae	*Runcinia insecta* (C. L. Koch, 1875)	76	2.777
	Thomisus okinawensis Strand, 1907	6	0.219
Total		2736	100

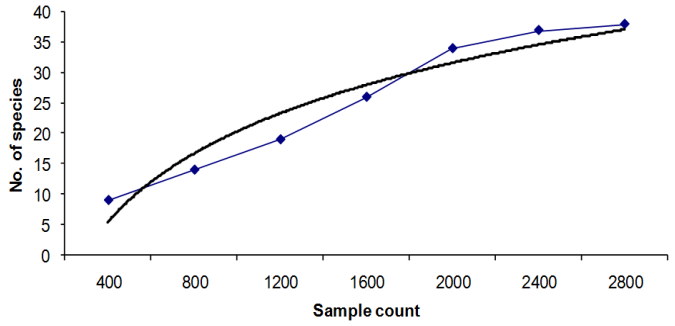

Figure 2: Species accumulation curve for spiders collected from rice-wheat ecosystems (combined for three sites).

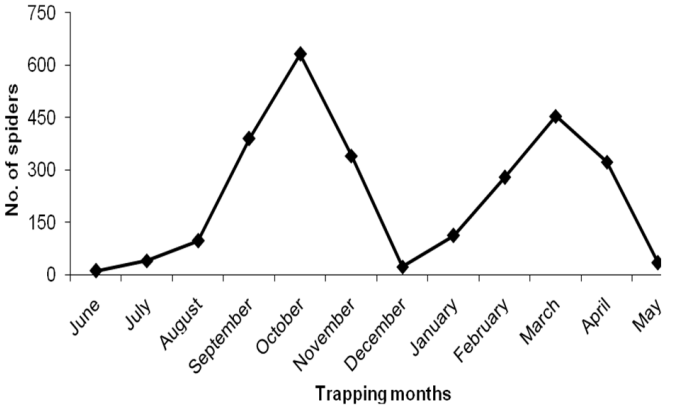

Figure 3: Seasonal dynamics of spiders (predators) collected from rice-wheat ecosystem.

($F_{2,11}$ = 1.97; P = 0.11) and evenness ($F_{2,11}$ = 1.97; P = 0.11) between study areas. However, analysis of trapping sessions showed statically significant different in richness ($F_{2,11}$ = 4.79; P < 0.01), diversity ($F_{2,11}$ = 3.97; P < 0.01) and evenness ($F_{2,11}$ = 5.78; P < 0.01).

Table 2: Species diversity and inventory completeness for spiders collected from three study areas.

Spiders	Study areas		
	Lahore	Sialkot	Mandi Bahauddin
No of specimens	898	538	1300
Observed richness	32	29	36
No of singletons	4	7	5
No of Doubletons	6	6	5
Chao 1	33	33	39
% completeness	97	88	92

mtCOXI based species discrimination

Based on the results of barcode sequence analysis two species which were not identified upto species level (*Clubiona* sp. and *Salticidae* sp.) were correctly identified to the species level (*Clubiona drassodes* O. Pickard-Cambridge (Araneae: Clubionidae) and *Thynes imperilalis* (Rossi) (Araneae: Salticidae). Furthermore, barcode analysis also assisted to correct identification of four morphologically misidentified specimens i.e., *Neoscona mukergi* to *N. theisi*, *Oxyopes oryzae* to *Oxyopes aspirasi*, *Pardosa* sp. to *Wadicosa fidelis* and *O. aspirasi* to *Oxyopes hindostanicus*. Detail of the morphological (misidentified specimens) and barcode-based identifications (corrected) are given in the Table 3.

Table 3: Summary of statistics for nucleotide frequency distribution for spider species.

Nucleotide %	Min	Mean	Max	SE
G	17.17	18.8	24.01	0.28
C	11.85	12.85	15.05	0.15
A	21.28	25.59	29.17	0.36
T	39.36	42.76	44.98	0.29

Inter-specific and intra-specific divergences and Neighbor joining tree

The divergence values for spiders of same species (Figure 4A) are small as compared to the spiders which belonged to different genera (Figure 4B). The maximum intraspecific genetic distance was smaller than the minimum interspecific distance between species which indicate that there is no overlapping among genetics distances. Table 3 is showing the summary statistics for nucleotide frequency distribution for spider species. It is evident from table that mean % of nucleotide Thymine was highest (42.76%) followed by Adenine (25.59%), Guanine (18.8%) and Cytosine (12.85%).

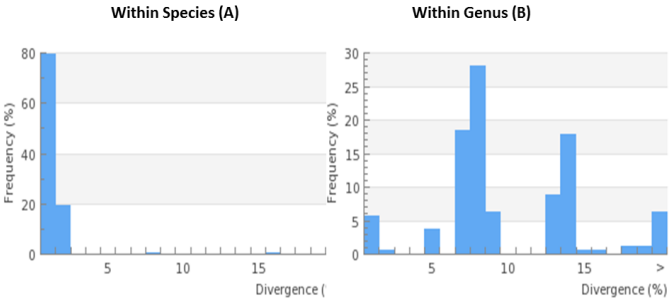

Figure 4: Both the bargraph with sequence divergences at different species (A) and genus level (B) shows how much the organisms belonging to various taxonomic levels are genetically disimilar from each other. Organisms at genus level containg the more significant barcode distances as compared to the species level.

The results of ABGD and BGA analysis revealed a clear gap between conspecific and congeneric species (Figure 5). Neighbor joining (NJ) tree also clearly separated the species into separate clusters (Figure 6). Barcode sequences of some species were not retrieved; therefore, these species could not be included in the Neighbor joining (NJ) tree.

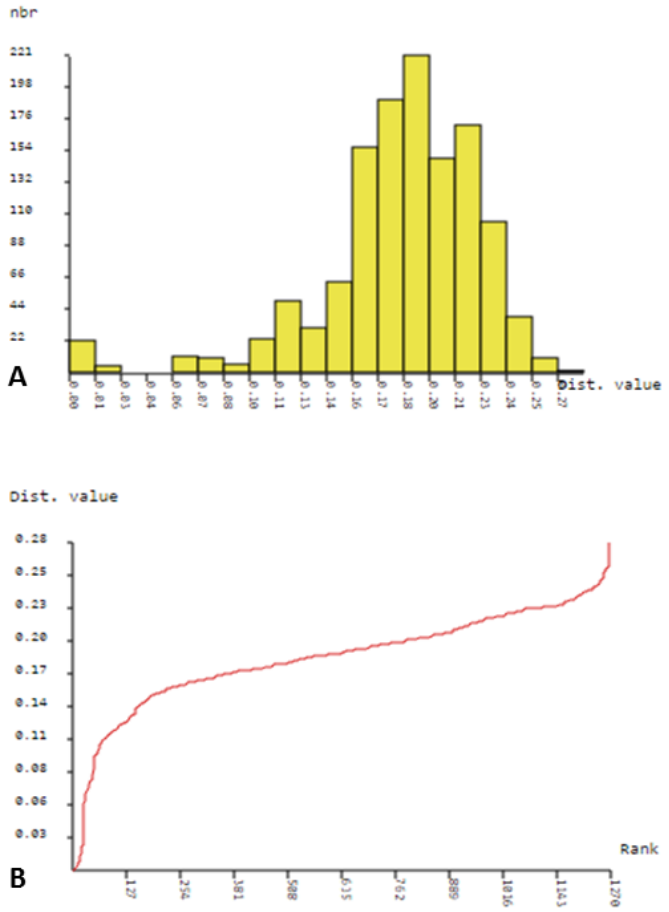

Figure 5: Histogram of genetic distances (A) and ranked distances (B) of spiders generated using ABGD and BGA analyses.

Discussion

During current study we compared the authenticity molecular identification of spiders from three districts of Punjab, Pakistan with the morphological approaches and to generate a barcode reference library for spider fauna of Punjab, Pakistan. Examination and probing of the genetic variations among the 38 distinguished morphospecies of spiders was another rationale behind this study. There are limited published studies on spiders of Pakistan (Ashfaq et al., 2019).

Percentage accuracy of morphological evaluation was 96.8% as compared to molecular method (DNA barcoding). In a similar study on spiders of agricultural crops, Tahir *et al.* (2019) have reported 88% accuracy of morphologically description of species. Unavailability of identification keys and deprivation of diagnostic characters of juvenile spiders could be the possible reasons for less accuracy of morphological evaluation. In the present study, three specimens were not described by molecular method due to the non-retrieval of barcode sequence and the possible argumentation could be the primer mismatching, contamination or inappropriate spider preservation. The primer mismatching and inexpedient preservation could affect the results of barcoding (Dean and Ballard, 2001; Kress and Erickson, 2008).

In current study we recorded 38 species of spiders from rice-wheat ecosystems. In the current study emphasis of spider sampling was on visual reaching and collection was done only during the day timing. Number of total species could be higher if sampling should have been done during night as well as pitfall traps would have been used. Tahir *et al.* (2019) have identified 49 spider species from agricultural fields of district Layyah belonging to the family Araneidae, Thomisidae, Oxyopidae, Lycosidae, Sparassidae, Oecobiidae, Gnaphosidae, Salticidae, Scytodidae, Tetragnathidae, and Hahnidae. Furthermore, Ashfaq *et al.* (2019) have recorded a total of 239 species of spiders from different regions of Pakistan.

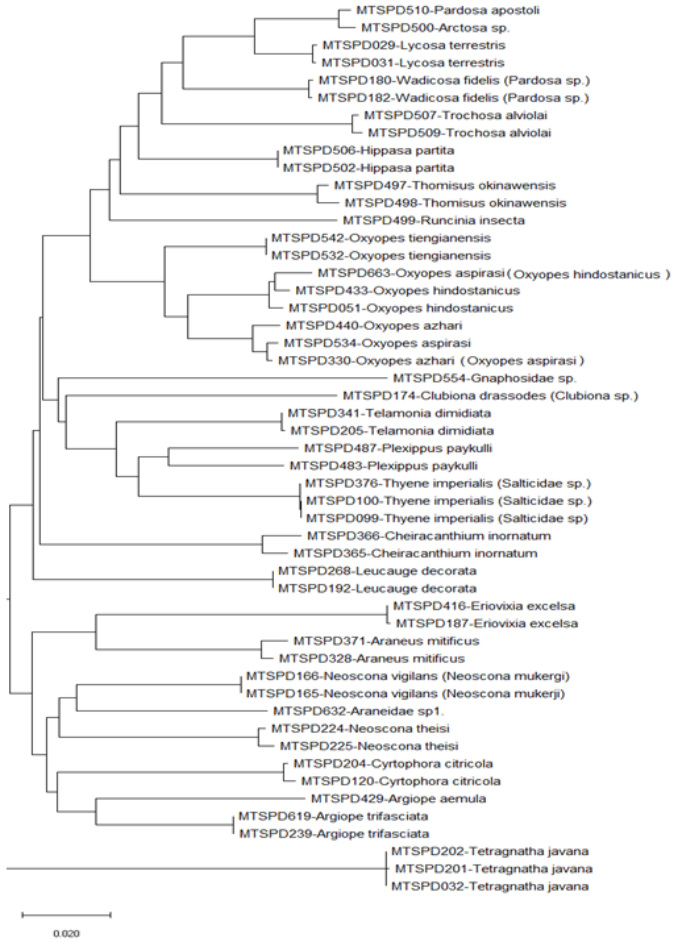

Figure 6: Neighbor joining tree for spiders collected from rice-wheat ecosystem fields.

Note: The names of the species in brackets are misidentified or incomplete morphological names.

The 5 end of COI marker was selected to identify the species via DNA barcoding because the primers for obtaining the DNA fragments from a range of taxa were conveniently available for this region (Hebert *et al.*, 2003). According to barcode results, three species were misidentified during morphological evaluation, so corrected and their relevant taxa were allotted. This correct

evaluation account for the importance of DNA barcoding in identifying the specimens with few diagnostic characters. Prosser *et al.* (2016) reported DNA recovery from century old specimens by using molecular techniques. It is deduced from the various taxonomic related studies that, for precise and complete species identification the molecular methods like DNA barcoding are requisite (Barco *et al.*, 2016). DNA barcoding is a technique that gives 100% accuracy in identification (Hebert *et al.*, 2003, 2004). In current studies specimens were segregated into different species with genetic difference of 2% or more, by using neighbor joining (NJ) tree.

Out of total catch of 2736 specimens, most dominant family was Araneidae in current study followed by Tetragnathidae and Lycosidae. Araneidae has also been reported as most abundant family of spiders from agricultural fields by Sharma *et al.* (2010) and Tahir *et al.* (2019). Tahir *et al.* (2015, 2019) proclaimed Lycosidae as dominant family on ground. There were strong correlations between the molecular and morphological identifications. While identifying spiders, a strong correspondence was also observed in morphological and molecular data by Blagoev *et al.* (2013) and Raso *et al.* (2014). The current results suggest that though DNA barcoding is a powerful technique for species identification.

For the families Thomisidae, Pholcidae and Salticidae it was not possible to generate the intra and inter-specific genetic divergences and distances to the nearest neighbors (NN). The small size of these families is the possible justification behind this phenomenon. Significant intra and inter specific genetic differences were observed among the members of family Tetragnathidae which ranges from 0 to 0.32 and 0.64 for *Leucauge decorata* and *Tetraganatha javana* respectively while the distance to NN value was found to be 22.81. Findings of Blagoev *et al.* (2009) are also in accordance with present study who described distinct genetic divergences for family Tetragnathidae.

Family Araneidae depicted remarkable mean and maximum intraspecific barcode values ranging from 0.55-0.82 for *Argiope trifasciata* to 0.71-1.55 for *Neoscona theisi* along with notable NN distance. Results of Blagoev *et al.* (2009), Slowik and Blagoev (2012) and Čandekand Kuntner (2015) were also in agreement with our study. Furthermore, barcode analysis of family Lycosidae revealed the significant genetic gaps values i.e. 0 for *Hippasa partita* to 13.15 for *Pardosa apostoli* with NN distance with maximum value of 12.61 for *Pardosa apostoli*. No overlap of the mean and maximum inter and intraspecific distance was found in family Lycosidae which are evidently supported by the outcomes of the Čandekand Kuntner (2015) study. During the taxonomic evaluation of family Oxyopidae, uncertainty was came across due to the less morphological dissimilarities like indistinguishable body patterns among different species. Bond *et al.* (2001) reported the ambiguity due to the presence of the similar characters in different species and suggested the DNA barcoding for authentic identification. Phenomenon of introgression and quick morphological divergences could be the possible argumentation behind this vagueness as described by Robinson *et al.* (2009).

Furthermore, seasonal dynamics in current study like the high abundance of pests as well as predators during the months of September-October in paddy fields and during February-March in wheat fields can be related to increased availability of food sources and micro-habitats for breeding sites in agro ecosystems (Tahir and Butt, 2008). Moreover, estimated insect and predator's species richness showed that many species which were actually present in the study areas were not sampled. This was not surprising as sampling was performed from foliage using visual research methods and sweep netting. The number of collected species would definitely be high if other sampling techniques would have been used especially pitfall traps which are considered as one of the best methods for the capturing of ground-dwelling arthropods (Sabu and Shiju, 2010). Furthermore, there may be an arbitrary number of undetected rare or less active species in the habitat (Deng *et al.*, 2015). Such rare or less active species can only be the part of collector sample if he/she is lucky enough.

During the last few years, DNA barcoding appeared as a supplementary tool for species accurate identification and novel research in biodiversity. However, Collins and Cruickhank (2013) stated that, due to the deficit of indecorous experimental design, often this technique (DNA barcoding) shows defects to solve the several biological questions. To get over the methodological problems, broad range sampling was made in the different areas of the province Punjab, Pakistan and identified on the base of morphology. Large scale study on the spiders of rice ecosystem could provide the open access reference library for the scientific community worldwide for assistance in research in different aspects of biodiversity like ecology and taxonomy (Tyagi *et al.*, 2017).

Conclusions and Recommendations

In conclusion, to overcome the shortcomings of conventional and molecular taxonomy, Slowik and Blagoev (2012), Franzini *et al.* (2013) and Čandek and Kuntner (2015) and Tyagi *et al.* (2017) suggested the combined use of morphological and barcode data (Integrated barcoding) for authentic identification of spiders, description of species complex and cryptic species.

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Conflict of interest

The authors have declared no conflict of interest.

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