



## Morphometry, principle component analysis and DNA barcoding of cox gene in *Leucauge decorata* (Decorative Spider) of south Bangalore, Karnataka

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### Abstract

The present study aims at attempting to describe morphology, morphometry, PCA BLAST analysis and phylogenetic construction of *Leucauge decorata* spider of suburban area of south Bangalore from Turahalli forest region. The five males and six female *L.decorata* spiders were collected and preserved in 70% alcohol for morphological study. Few were stored in -80°C freezer for DNA extraction and for further analysis. The morphological study revealed that females were larger in size (5.60mm) compared to males (1.13mm). The PCA data revealed that 1<sup>st</sup> principle component accounts for 83.873 % of total variance (0.271 weightage) on opisthosoma breadth ventral view as its dominant term and the 2<sup>nd</sup> principle component whose dominant term was the total body breadth ventral view with a 7.891 % of total variance (weightage 0.035). The points filled as two obvious clusters confirming the succinct sexual dimorphic feature of the spiders. The DNA extraction was carried over by cTAB method. cytochrome oxidase I gene (CoxI) has a significance of exhibiting fast mutation rate hence, forward and reverse primers were constructed and PCR analysis was performed. BLAST programme was employed to find the similarity between the closely related species and maximum likelihood method was used to construct phylogenetic tree. The branch point or internal nodes represented the most common ancestor. At each branch point lies the most recent common ancestor of all the groups descended from that branch point. Finally, the BLAST analysis gave 100% similarity matching to *Leucauge decorata* spider from the already existing database.

**Keywords:** *leucauge decorata*, morphology, DNA extraction, pca, blast, coxi, phylogenetic tree

### 1. Introduction

*Leucauge decorata* (Blackwell 1864) belongs to phylum Arthropoda, Subphylum: Chelicerata, Class:Arachnida, Order: Araneae, Family:Tetragnathidae. It is a common orb web spider in agro ecosystem of Punjab, Pakistan (Tahir and Butt 2008) [4]. These species are pantropical distribution and for about 165 to 170 species have been recorded. *L.decorata* spiders are commonly found in shaded vegetation in forests. Different spider species can co-exist simultaneously in the same habitat (Dzulhelmi Muhammad Nasir 2016) [3]. They are well known as decorative spiders found close to the ground and garden also. *Leucauge decorata* construct orb web like a wagon wheel shaped structure in an open hub. It builds oblique to horizontal orb web, remains hung in its web. Ventral surfaces face upwards. Hafiz Muhammad Tahir, Abida Butt and Imtiaz Alam (2010) [4]. concluded that for prey capture *L. decorata* construct webs of different sizes and at different heights but always maintain the basic web architecture

### 2. Materials and Methods

#### 2.1. Study area

The study area selected was surroundings of Turahalli forest located in south Bengaluru off Kanakapura road. The Turahalli forest is commonly known as mini forest with total area of 590 acres, and 888mt elevation. The flora and fauna of the area include figs (*Ficus tinctoria*) neralemara (*Syzygium cumini*). The most common herb is the *Byttneria herbacea*. Animals spotted were Hares Jackals, Lizards, Mongoose, etc. The forests bird population includes great horned Owls, Mynas, Babblers, and more. The variety of

spider diversity was found in this region hence the area was selected

#### 2.2. Collection and Identification Methods

Male and female *Leucauge decorata* spiders were collected by hand collection method. The specimens were brought to laboratory and stored in 70% alcohol. They were identified, by referring to "Indian Journal of Arachnology" "Identification to Indian Spiders". Six females and five male spiders' were collected their morphometric measurements were measured using calibrations under stereo zoom microscope (Labomed). All variables were recorded in mm. The specimens were photographed by using digital camera Nikon coolpix6300.

#### 2.3. Statistical method

The common statistical tool Principle component analysis was executed to draw the interrelationship among the variables between the male and female *Leucauge decorata* sixteen morphometric features were considered. Six Leg features included were coxa, trochanter, femur, patella, tibia and tarsus. Dorsal view (length and breadth) of prosoma and opisthosoma were represented as Prosoma length dorsal view (PLDV), prosoma breadth dorsal view (PBDV), opisthosoma length dorsal view (OLDV), and opisthosoma breadth dorsal view (OBDV). Similarly ventral view (length and breadth) of prosoma and opisthosoma were represented as prosoma length ventral view (PLVV) prosoma breadth ventral view (PBVV), opisthosoma length ventral view (OLVV), opisthosoma breadth ventral view (OBVV). The total body measurement indicated as total body length

dorsal view (TBLDV) and total body breadth dorsal view (TBBDV) similarly, total body length ventral view measurement indicated as total body length ventral view (TBLVV), and total body breadth ventral view (TBBVV).

#### 2.4. DNA extraction and PCR analysis

DNA extraction and PCR analysis 100mg of spider tissue was weighed and frozen in dry ice and the thigh was added with 200  $\mu$ l of cTAB homogenize 0.5ml cTAB was vortexed vigorously and incubated at 60°C for 1h. To the lysate, 0.5 ml of phenol - chloroform, Iso-amyl alcohol was added and mixed for 2-3 minutes. It was centrifuged at 10000 rpm for 15 min at 4°C. The upper aqueous layer was taken in a new tube, to which double the volume of cold 100% ethanol was added and 3M sodium acetate was added and was incubated for 1h minutes at 4°C, Centrifuged at 10000 rpm for 15 min. The supernatant, was removed and DNA pellet was washed in 70% ethanol and centrifuged at 5000 rpm for 10 minutes. Again, the supernatant was removed, the DNA pellet was air dried and was finally dissolved in TE buffer. The PCR mixture (final volume of 20  $\mu$ L) contained 2  $\mu$ L of DNA, 10  $\mu$ L of Red Taq Master Mix 2x (Amplicon) and 1 $\mu$ M of each complementary primer specific forward and reverse. The samples were denatured at 94°C for 5 minutes, and amplified using 40 cycles of 94°C for 30 seconds, 44°C for 30 seconds, and 72°C for 1 minute for followed by a final elongation at 72°C for 10 minutes. The optimal numbers of cycles have been selected for amplification of the mitochondrial cytochrome oxidase (Mt-Cox) gene.

#### 2.5. Sequencing

The PCR product was sequenced using the LCO F primers. Sequencing reactions were performed using a ABI PRISM® Big Dye TM Terminator Cycle Sequencing Kits with Ampli Taq® DNA polymerase (FS enzyme) (Applied Biosystems). The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were re suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Bio systems). Based on BLAST analysis the Mt-Cox sequence, of these species has been matched, compared and phylogenetic tree was constructed. PCR primers for Cox gene in DNA barcoding used are Lco1490FP GGTCACAAATCATAAAGATATTGG and hco2198RP TAACTTCAGGGTGACCAAAAATCA(Tm, 51. 3 & 53.2. product size 710bp)

### 3. Observation

#### 3.1 General Female: Description: (measurement in mm)

Total Body Measurement (DV): 5.60 long and 9.31 wide. Total Body Measurement (VV): 6.39 long and 9.75 wide. D.V- pr: 3.99 long & 3.93 wide. op: 5.53 long & 56.03 wide. V.V-pr: 9.52 long & 9.93 wide, op: 3.71 long & 3.36 wide. Leg 1- 16.57long, Leg 2- 12.77 long, Leg 3- 6.88 long & Leg4 – 11.39 long

#### 3.2 Prosoma

Dorsal side is was shaped greenish yellow in colour. Cephalic region narrowed anteriorly and broader posteriorly. Thoracic region is provided with a deep groove. Ocular quadrangle is slightly long. Eyes are arranged in two rows and subequal in size. The two eyes in front row and two eyes in behind row (at central position) are equal in size

whereas lateral eyes at behind row are larger than others. Ventrally sternum is heart shaped dark brownish in colour, pointed behind, coloured with hairs. Labium is blackish brown longer and wider. Chelicerae greenish, yellow and very strong. All four pairs of legs are scarcely clothed with hairs. Coxa is greenish yellow. The other parts are fluorescent green in colour.

#### 3.3 Opisthosoma

Dorsal side elongated abdomen decorated with fluorescent silvery yellow and blackish patches. The abdomen is broad at thoracic end and pointed at caudal end. It is bulged and prominent hump is seen. Ventrally abdomen is narrowed at cephalic end, broader at centre and pointed at caudal end. Three longitudinal greenish patches are prominently seen arising from the epigastric furrow. The greenish patches are surrounded by blackish lines and patches which extends near to the caudal end and surroundings of spinnerets. One upward directed hump is more prominent at caudal end. A pair of elongated silvery white patches in between the epigastric furrow and a slightly small silvery white patch at the epigastric furrow. Three silver white spots around the spinnerets are clearly seen.

#### 3.4 Epigynum

Situated mid ventrally on the epigastric furrow. It is simple and chitinous in structure with sclerotised plate. Epigynum is H shaped brownish yellow with small pointed projections at the edges. There is a pair of external openings to receive the palps of the male during copulation. These openings are round in shape and light brownish in appearance. (Fig 06a)

#### 3.5 General Male

Total Body Measurement (DV): 1.13 long and 0.92 wide. Total Body Measurement (VV): 2.11 long and 1.66 wide. D.V- pr: 1.19 long & 1.17 wide. op: 1.26 long & 0.95 wide. V.V-pr: 2.45 long & 2.12 wide, op: 0.98long & 0.74 wide. Leg 1- 6.96 long, Leg 2- 6.99 long, Leg 3- 4.05 long & Leg4 – 4.87 long

#### 3.6 Prosoma

Dorsal side is was shaped greenish yellow in colour. Cephalic region narrowed anteriorly and broader posteriorly. Thoracic region is provided with a deep groove. Ocular quadrangle is slightly long. Eyes are arranged in two rows and subequal in size. The two eyes in front row and two eyes in behind row (at central position) are equal in size whereas lateral eyes at behind row are larger than others.

#### 3.7 Opisthosoma

Long elongated, broad at cephalic region. Round and curved at caudal end. Covered with thick hairs. Silvery whitish color. Ventral side is thickly covered with hairs, long and elongated. The region towards thoracic end has a pair of triangular shaped yellow patch. The caudal end is blackish in color.

#### 3.8 Pedipalp

It is of haplogyne type ie simple in structure. Too long pedipalp measuring a length of a leg. Highly covered with hairs. Elongated resembling to a painting brush. The tarsus region extends as a bulb shaped palpals called cymbium. Cymbium is pear shaped broad at tarsal end and pointed at the terminal end acts as a reservoir of the sperms. The

cymbium is covered with thick hairs with unequal length. The palpi functions as a pipette allowing the sperms to deliver the in to the female genital organ epigynum through seminal receptacles. The bulb bears a duct, which opens at the far end and leads to the outside region called embolus. (Fig 06b)

#### 4. Result and Discussion

The morphometric measurements of five males and six females were performed total of sixteen morphometric features were considered and compared between males and females. The six features included were for the legs. The different leg features were coxa, trochanter, femur, patella tibia and tarsus. Body morphometric features included were prosoma length, and prosoma breadth for both dorsal view and ventral view. Similarly, opisthosoma length and opisthosoma breadth of both dorsal view and ventral view were also considered. Finally, the total body length and total body breadth of dorsal view and ventral view were measured, and measurements were recorded. The five males were collected and all for the sixteen features an average measurement were calculated with standard deviations. The six females were collected and for all the sixteen features an average measurement were calculated with standard deviations. Among all female's leg 1 was the longest measured with 12.48mm and leg 3 was smallest with 8.57 mm in length. Further each leg feature was compared among all the four legs. Coxa of leg 1, leg2, leg3, and leg 4 measured on an average of 0.835mm in length. Trochanter of leg 1, leg2, leg3, and leg 4 measured on an average of 0.21mm in length. Similarly, femur measurement was 2.90mm, patella measured around 0.79mm, tibia measured around 2.72mm and tarsus measured around 3.85mm. each leg morphometric feature were compared and found that tarsus part was longest with 3.85mm and trochanter was smallest part with 0.21mm.(Table No: 01, Fig No: 05) The female body morphometric measurements of dorsal view and ventral view revealed the total body length of dorsal view was 7.10mm and ventral view was 6.01mm. Total body breadth of dorsal view was 3.66mm and ventral view was 2.73mm. The prosoma length, prosoma breadth of dorsal view of all females were measured and gave an average value of 2.49mm and 1.9mm respectively. Similarly, prosoma length and prosoma breadth of ventral view of all six females were measured and gave an average value of 1.9mm and 1.1mm respectively. Further opisthosoma length of dorsal view measured around 4.51mm and opisthosoma length of ventral view around 1.76mm. opisthosoma length and opisthosoma breadth of ventral view measured 3.98 mm and 1.63mm respectively. (Table No: 03, Fig No: 05) Among all male's leg 1 was the longest measured with 11.49mm and leg 3 was smallest with 8.75 mm in length. Further each leg feature was compared among all the four legs. Coxa of leg 1, leg2, leg3, and leg 4 measured on an average of 0.5mm in length. Trochanter of leg 1, leg2, leg3, and leg 4 measured on an average of 0.25mm in length. Similarly, femur measurement was 2.71mm, patella measured around 0.51mm, tibia measured around 2.47mm and tarsus measured around 3.90mm. Each leg morphometric feature was compared and found that tarsus part was longest with 3.90mm and trochanter was smallest part

With 0.25mm. (Table No: 02, Fig No: 05)

The male body morphometric measurements of dorsal view and ventral view revealed the total body length of dorsal view was 5.18mm and ventral view was 5.15mm. Total body breadth of dorsal view was 2.89mm and ventral view was 1.97mm. The prosoma length, prosoma breadth of dorsal view of all males were measured and gave an average value of 2.44mm and 1.9mm respectively. Similarly, prosoma length and prosoma breadth of ventral view of all males were measured and gave an average value of 1.9 and 0.96 respectively. Further opisthosoma length of dorsal view measured around 2.74mm and opisthosoma length of ventral view around 2.88mm. Opisthosoma length and opisthosoma breadth of ventral view measured 0.99 mm and 1.01mm respectively. (Table No: 03, Fig No: 05) The morphometric measurements of male and females were compared for all 16 features. The PCA performed with the data on morphometric measurements of five males and six females were presented. The 1<sup>st</sup> principle component accounts for 83.873 % of total variance (0.271 weightage) on opisthosoma breadth ventral view as its dominant term and the 2<sup>nd</sup> principle component whose dominant term is the total body breadth ventral view with a 7.891 % total variance of (weightage 0.035)

Since the first two components together accounts for the total 91.764 variances, each individual is represented as a point in the co-ordinate space of the associated amplitudes of the two principle components. The points filled as two obvious clusters confirming the succinct sexual dimorphic feature of the spiders. The morphological details and PCA analysis reveal that females are much larger than males may be because of environmental factors or natural selection acting on females to exhibit female dominance than males. (Table No: 04, Fig No: 04) The cytochrome oxidase I gene sequence was submitted to NCBI database. The n BLAST contained three sections. Graphical summary, a list of blast hits and corresponding alignments. The top three hits matched to the highest level than the other hits. The distribution of 100 top BLAST hits on 100 subject sequences were displayed. The maximum likelihood method represented the sequence similarity of Cox gene for different organism with different families raised from a common ancestor. The branch point or internal node represented the most recent common ancestor at each branch point lies the most recent common ancestor of all the groups descended from that branch point. The accession number was assigned for all the organisms from existing NCBI database. The result concluded that *Leucauge decorata* (Accession No from database KU836873.1) and *Larinioides sclopetarius* (Accession No from database KC849077.1) raised from a common ancestor. The specimen identity was indicated to *L.decorata* (0918201024) (Fig No 05)

**Table 1:** *Leucauge decorata* Female Leg Morphometric Measurement In mm

	Leg 1	Std	Leg2	Std	Leg3	Std	Leg4	Std
Coxa	0.6	±0.14	0.6	±0.15	0.51	±0.13	0.56	±0.08
Trochanter	0.31	±0.03	0.27	±0.04	0.25	±0.04	0.3	±0.07
Femur	4.61	±0.40	3.68	±0.31	2.04	±0.21	3.6	±0.34
Patella	0.89	±0.11	0.8	±0.07	0.58	±0.21	0.6	±0.11
Tibia	4.18	±0.35	2.8	±0.30	1.2	±0.19	2.44	±0.25
Tarsus	5.98	±0.57	4.63	±0.45	2.3	±0.25	3.89	±0.38
Total	16.57	±1.41	12.77	±1.04	6.88	±0.73	11.39	±1.14

**Table 2:** *Leucauge decorata* Male Leg Morphometric Measurement In mm

	Leg 1	Std	Leg2	Std	Leg3	Std	Leg4	Std
Coxa	0.31	±0.12	0.32	±0.09	0.30	±0.15	0.34	±0.13
Trochanter	0.20	±0.09	0.15	±0.05	0.14	±0.05	0.16	±0.07
Femur	1.90	±0.48	2.12	±0.59	1.21	±0.66	1.52	±0.35
Patella	0.53	±0.15	0.55	±0.16	0.31	±0.19	0.38	±0.11
Tibia	1.37	±0.32	1.37	±0.32	0.68	±0.25	0.82	±0.15
Tarsus	2.65	±0.63	2.47	±0.56	1.41	±0.48	1.65	±0.38
Total	6.96	±1.71	6.99	±1.59	4.05	±1.67	4.87	±1.14

**Table 3:** *Leucauge decorata* Comparison of Female and Male Body Morphometric Measurement in mm

Sl.No	Features	Male	Std	Female	Std
1	Leg 1	6.96	±1.71	16.57	±1.41
2	Leg 2	6.99	±1.59	12.77	±1.04
3	Leg 3	4.05	±1.67	6.88	±11.39
4	Leg4	4.87	±1.14	11.39	±1.14
5	P.L(D.V)	1.19	±0.35	3.99	±0.31
6	P.B(D.V)	1.17	±0.29	3.93	±0.53
7	O.L(D.V)	1.26	±0.41	5.53	±0.71
8	O.B(D.V)	0.95	±0.24	6.03	±1.22
9	P.L(V.V)	2.45	±1.21	9.52	±1.02
10	P.B(V.V)	2.12	±0.76	9.93	±1.75
11	O.L(V.V)	0.98	±0.43	3.71	±0.93
12	O.B(V.V)	0.74	±0.24	3.36	±0.59
13	T.B.L(D.V)	1.13	±1.53	5.60	±0.76
14	T.B.L(V.V)	0.92	±0.26	6.39	±1.26
15	T.B.B(D.V)	2.11	±0.96	9.31	±1.69
16	T.B.B(V.V)	1.66	±0.5	9.75	±1.89

**Table 4:** *Leucauge decorata* Principle Component Analysis of 16 variables

Variable	PC 1	PC 2
LEG1	0.265	-0.080
LEG2	0.260	-0.102
LEG3	0.227	-0.276
LEG4	0.266	-0.031
PL(DV)	0.240	0.228
PB(DV)	0.221	0.099
OL(DV)	0.267	-0.017
OB(DV)	0.268	0.005
PL(VV)	0.232	-0.307
PB(VV)	0.067	0.837
OL(VV)	0.269	-0.001
OB(VV)	<b>0.271</b>	0.005
TBL(DV)	0.268	<b>0.035</b>
TBB(DV)	0.269	0.032
TBL(VV)	0.270	-0.052
TBB(VV)	0.264	0.210
Eigenvalue	13.420	1.263
% of Var.	83.873	7.891
Cum. %	83.873	91.764

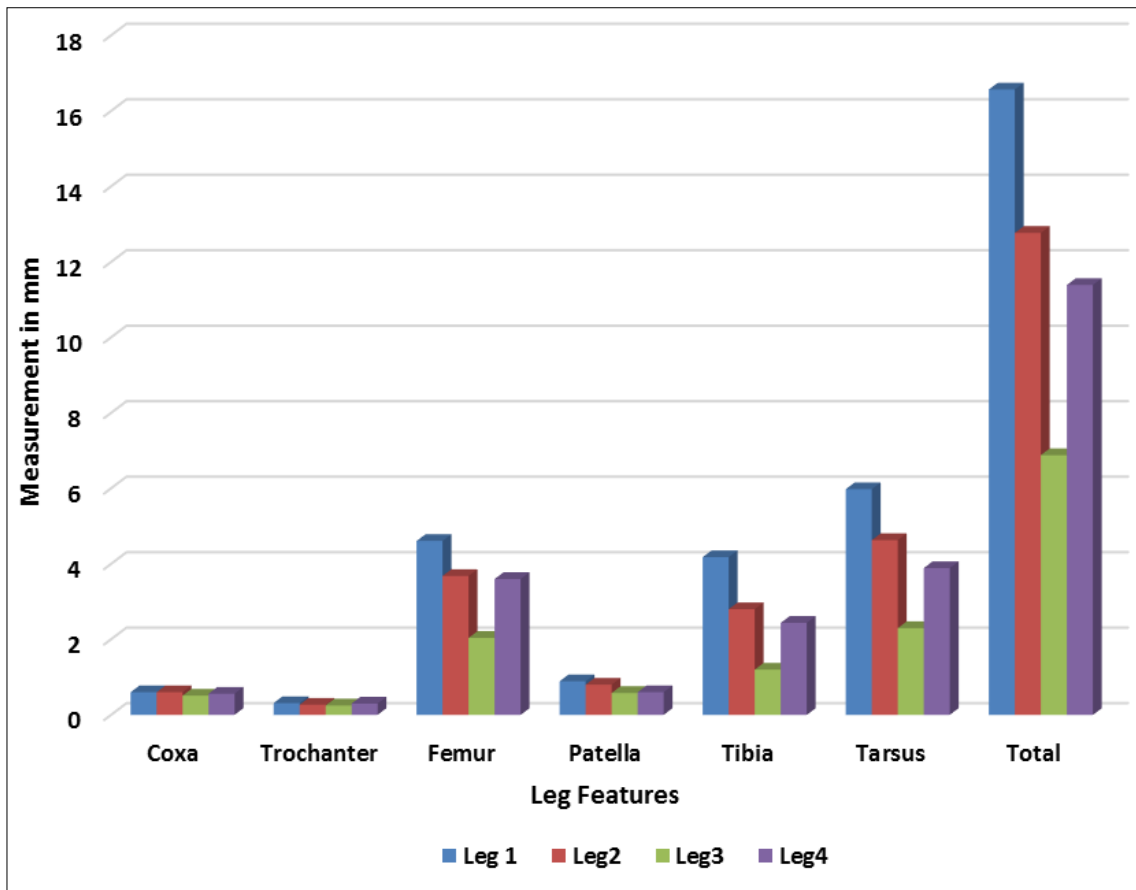


Fig 1: *Leucauge decorata* Female Leg Morphometric Measurement in mm

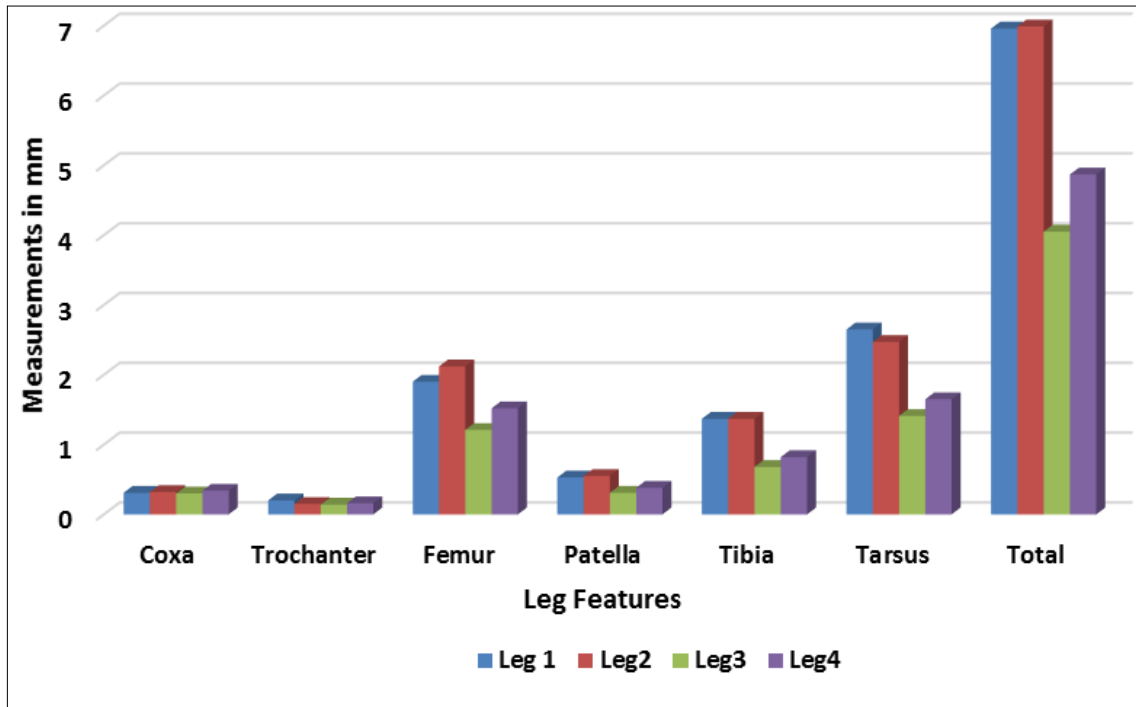


Fig 2: *Leucauge decorata* Male Leg Morphometric Measurement in mm

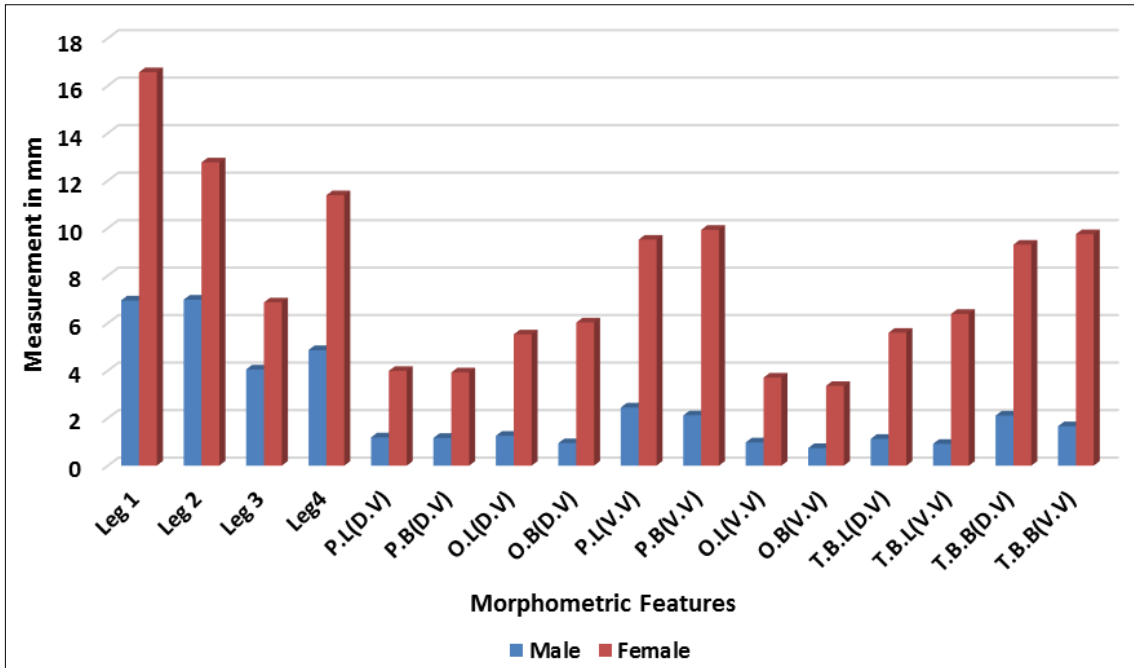


Fig 3: *Leucauge decorata* Comparison of Male and Female Morphometric Measurements in mm

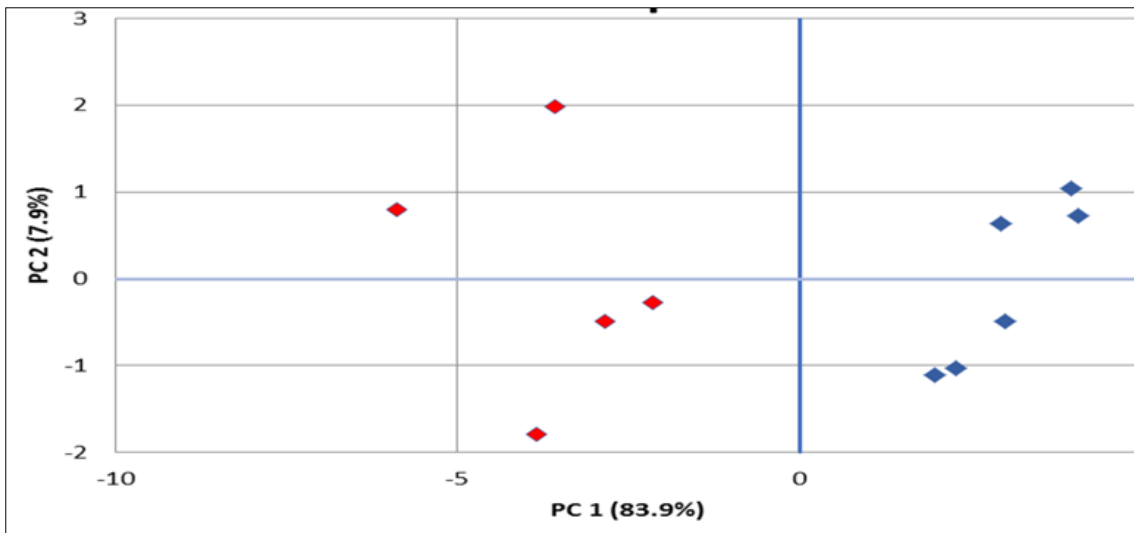
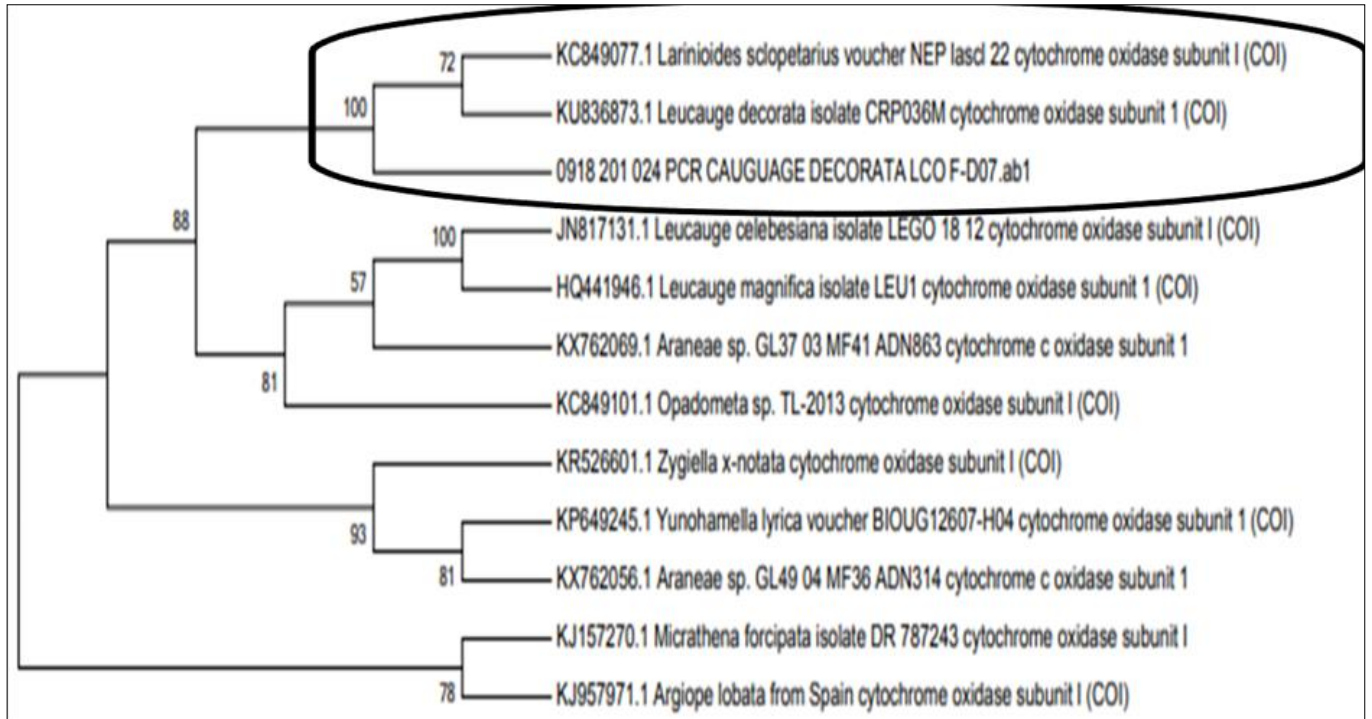
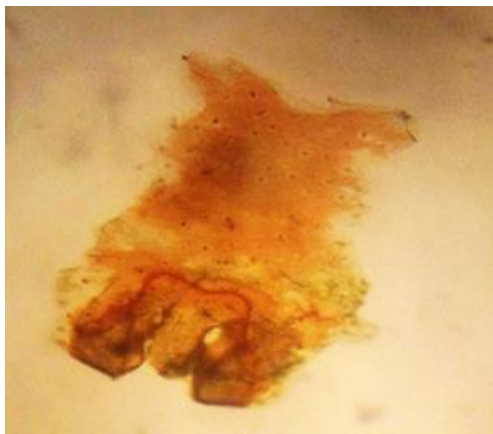


Fig 4: *Leucauge decorata* Principle Component Analysis (red indicates males and blue indicates females)



**Fig 5:** Phylogenetic Analysis of Cytochrome oxidase I Gene of *Leucauge decorata*



**Fig 06a:** Epigynum (♀)



**Fig 06b:** Pedipalp (♂)

**References**

1. Asghar U, Malik MF, Anwar F, Javed A, Raza A. DNA Extraction from Insects by Using Different Techniques: A Review. *Advances in Entomology*. 2015; 3:132-138
2. Bork Reva Ann, "Non-destructive DNA extraction

- methods that yield DNA barcodes in spiders. *Honors Program Theses*, 2015, 168.
3. Dzulhelmi Muhammad Nasir, Wong Chun Xing, Asraf Bakri, Faszly Rahim, Norma-Rashid Yusoff Short Communication: Spiders of Sabah: Fifty new records including the description of a new *Leucauge* species Biodiversity as E-ISSN: 2085-4722 Pelagia Research Library. 2016; 17(2):799-807
4. Hafiz Muhammad Tahir, Abida Butt, Rakhshanda Naheed, Muhammad Bilal, Imtiaz Alam. Active density of spiders inhabiting the citrus field in Lahore Pakistan. *Pak. J. zoo*. 2011; 43(4):683-688.
5. Jonathon Shlens A Tutorial on Principal Component Analysis, 2005.
6. Jonathan Dees, Jennifer L Momsen, Jarad Niemi, Lisa Montplaisir (Student Interpretations of Phylogenetic Trees in an Introductory Biology Course CBE—Life Sciences Education. 2014; 13:666-676,
7. Lienhard A, Schäffer S. Extracting the invisible: obtaining high quality DNA is a challenging task in small arthropods. *PeerJ*. 2019; 7:e6753 DOI 10.7717/peerj.6753
8. Macías-Hernández N, Athey K, Tonzo V, Wangenstein OS, Arnedo M, Harwood JD, *et al*. Molecular gut content analysis of different spider body parts. *PLoS ONE*. 2018; 13(5):e0196589.
9. Samira Paydar. A simplified and optimized protocol for total DNA extraction from insect species: applicable for studying genetic diversity and PCR-based specimen identification via partial amplification of cytochrome oxidase I (COI) gene *Cell Molecular Biology (Noisy le Grand)*. 2018; 64(12).
10. Thierry NN, Tang H, Xu L, Hu F. Effect of mesh size, twine material and trawl gear accessories on the bottom trawls hydrodynamic performance. *International Journal of Fisheries and Aquatic Research*. 2019;4(4):01-9.