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ROLE OF GEOGRAPHICAL SEPARATION IN MOLECULAR EVOLUTION OF THE GIANT WOOD SPIDER (*NEPHILA PILIPES*)

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Abstract:
The Giant Wood Spider (*Nephila pilipes*) is one of the commonly encounter Orb-weaver spiders in the wild. In order to define the clear phylogeny of Orb-weaving spiders, scientists have done molecular studies and behavioural studies. Even though, there is an ambiguity related to the monophyly of Orb-weaving spider. There are no evidences showing the monophyly of the two typical orb web taxa. In current study, two geographical locations known to be geographically separated for centuries are chosen. Molecular analysis of *N. pilipes*, has been done by sequencing *COI*, *28S rRNA*, and *histone H3A* genes. In addition, behavioural aspects of the spider are noticed to see the effect of geographical separation on the spider *N. pilipes*. The findings from current study may help in providing insights regarding the molecular evolution of orb-weaving spiders.

Keywords: Sanjay Gandhi National Park (SGNP), Elephanta Caves Gharapuri (ECGP), Giant Wood Spider, Evolution.

I. Introduction:

Spiders are among the largest animal groups and the dominant arthropod predators in most terrestrial ecosystems. About 44,000 spider species have been described till date [1]. The orb-weaving spiders (Orbiculariae) comprise more than 25% of the total known living spider species and produce a remarkable variety of webs. The Giant Wood Spider (*Nephila pilipes*) is one of the commonly encounter Orb-weaver spiders in the wild. The wheel-shaped orb web is primitive to this spider evolutionary clade. Orb-weavers date at least to the Jurassic [2]. With no evidence for convergence of the orb web, the monophyly of the two typical orb web taxa, the cribellate Deinopoidea and ecribellate Araneoidea, remain problematic, supported only weakly by molecular studies. Thus, the current study will help in gathering more molecular evidences regarding the molecular evolution of orb-weaving spiders.

Molecular sequence data are helping researchers answer the orbicularian monophyly question. Using 28S ribosomal sequences for eight araneomorph species Orbiculariae (represented by four species) are actually paraphyletic by having the RTA clade (two species), instead of the Deinopoidea, as the sister group of Araneoidea [3]. Using sequences of the nuclear protein-coding gene elongation factor-1 gamma, orbicularians are paraphyletic with respect to the RTA clade in their parsimony analyses but monophyletic in maximum-likelihood analyses [4]. Recently, orbicularian relationships and monophyly using DNA sequences from six genes, now conventional in spider phylogeny (*COI*, *16S rRNA*, *18S rRNA*, *28S rRNA*, *histone H3*, and *wingless*), and morphological and behavioural characters [5]. In their analyses, which included representatives from only 12 of the 22 orbicularian families, orbicularian monophyly was recovered (with Nicodamidae nested within Orbiculariae) only when the morphological and behavioral data were included.

Informative nucleotide data would be extremely useful, because a number of araneoid lineages have been difficult to place using morphological data (or the conventional markers) and their exact positions have varied across studies [6,7]. Orbicularian phylogeny is an inherently difficult problem to resolve, both for morphological and genetic data, because it involves ancient cladogenetic events compressed in a relatively narrow time span.

Current research work has been planned to study the molecular changes that orb-weaver member species Giant Wood Spider *(Nephila pilipes)* could have occurred due to geographical separation in two localities nearby Mumbai region. History of Mumbai establishment goes back in 19th Century where seven nearby islands were joined to form present Mumbai city. One of the islands which is nearby Mumbai, named Elephanta Island (EI), Gharapuri. EI has been detached and separated from Mumbai by many Centuries. The distance between Sanjay Gandhi National Park (SGNP) and EI is more than 40 Kms. In addition, this distance has a major barrier of sea water (around 10 Kms) which creates a major barrier or vicariant event for any spider species. Thus, the orb-weaver *N. pilipes* in these two localities are geographically separated for centuries and may have undergone different selection pressures throughout the period. Study of the mitochondrial gene *Cytochrome c Oxidase subunits I* (COI) and nuclear genes *Histone 3* (H3) and *28S rRNA* gene from the *N. pilipes* located at SGNP and EI, will help in understanding the role of geographic separation of this species. The study will also give emphasize on number of physiological, morphological and behavioral variations in *N. pilipes* at two localities under study. The hypothesis of this study is, due to different localities and different selection pressures on Giant Wood Spider, it could have accumulated number of nucleotide variations. The results of the study will help in adding more information related to phylogenetic evolution of orb-weaver spiders.

Spiders are exceptionally diverse and abundant in terrestrial ecosystems. In contrast to megadiverse orders of insects, evolutionary diversification of spiders is not coupled with major trophic shifts. All spiders are predators of arthropods, and spiders are dominant consumers at intermediate trophic levels [8, 9]. Spider diversification is instead linked to key innovations in silk use [10]. The araneoid orb web with stretchy capture spirals, coated by adhesive viscid silk secretions, provides access to abundant flying insects [10].

Despite similar architectures, the 2 types of orbs differ fundamentally in function. A major distinction is that viscid threads depend on water absorbed by the chemical glue coating them to maintain stickiness [11]. In contrast, cribellate threads lose stickiness when water mats together their puffy fibrils. Overall, most characters supporting orb web monophyly relate to the spinning of the orb itself and, if the orb architecture is strongly adaptive, they may easily be convergent. Inferring the evolutionary origin of orb web weaving is also necessary to understand the subsequent transformation and loss of the orb and associated web spinning behaviours.

Inferring the evolutionary origin of orb web weaving is also necessary to understand the subsequent transformation and loss of the orb and associated web spinning behaviors. Although the orb weaver (Orbiculariae) clade constitutes around 1/4 of the world's spider diversity, most do not spin orb webs. Cobweb spiders (Theridiidae) and sheet web spiders (Linyphiidae) encompass almost half of all extant species in the orb weaver clade. Moreover, many speciose families of spiders do not spin prey capture webs at all. Thus, a robust phylogenetic hypothesis is needed to determine how spider diversification relates to transformations in web architectures and silk specializations.

Morphological data has generally been successful at revealing the broad strokes of spider phylogeny, especially over the past 20 years or so [12, 13]. But there are a handful of mysterious higher-level taxa that remain to be convincingly placed, usually due to conflicting or ambiguous morphological evidence.

Higher level systematics of spiders currently relies heavily on morphological and behavioral data [13, 14]. Molecular data are used almost exclusively at the species/genus level or within families [15, 16]. However, DNA has proven useful for groups of orbicularian spiders, including the biogeography of Hawaiian tetragnathid and linyphiid species [17, 18], relationships among cobweb weaving genera, and relationships among micropholcommatids. These studies did include

more distantly related taxa as outgroups, but the relationships among them varied greatly. The few DNA-based higher level analyses of spiders focus on clades outside orb weavers, such as the infraorder Mygalomorphae (tarantulas and relatives) [16], micropholcommatids and a few ecribellate orb weavers, or the RTA clade spiders (including wolf spiders, crab spiders, and their relatives).

One of the most hotly debated aspects of the speciation process is its geographical context, and the nature and importance of isolation in the initial divergence of taxa [19]. Ecological speciation, in which reproductive isolation evolves as a consequence of divergent natural selection on traits between contrasting environments, is now recognized as an important mechanism of speciation [20]. However, the geographical context of speciation in situations of adaptive radiation, where multiple close relatives occur in sympatry, is still the subject of considerable debate [21].

II. Materials and Methods:

II.1) Sampling Collection:

The selected species of spider *N. pilipes* is under the category of least concern according to the IUCN Red data Book 2017. The samples (total six) were collected with the permission of local research committee. The samples were collected from selected two locations Sanjay Gandhi National Park (SGNP) 19°15′N 72°55′E and Elephanta caves Gharapuri (ECGP), 18° 57' 47.7108" N and 72° 55' 53.1912" E. The orb-weaver spider *N. pilipes* were identified using standard key of spider identification. The specimens were preserved in 95% alcohol till they were brought to the laboratory. No harm was done during the observational studies to the orb-weaver spider.

II.2) Behavioural and Morphological observations:

In the field, the *N. pilipes* were observed for number of behavioural aspects like web building, prey catching and body postures in different temperatures. The observations were made and photographed.

II.3) Genomic DNA extraction:

Specimen were preserved initially into 95% ethanol (in the field) and later kept at -197^{0}C before extraction of DNA (in the laboratory). Whole genomic DNA was extracted from 1–4 legs following Cetyltrimethylammonium bromide (CTAB) method.

II.4) PCR of genes under study:

Three gene fragments will be amplified in 25 µl reactions: around 540 bp region from *Cytochrome c Oxidase subunit I* (*COI*), around 330 bp region of *Histone 3* (*H3*), around 770 bp region of *28S rRNA*. The primers for these three genes were designed by Primer-3 software (table 1).

Table 1. Sequence of gene specific primes used for PCR amplification

Gene Name	Expected sequence size (bp)	Primer type	Primer sequence (5'→3)	Primer length (nucleotides)
Histone 3 Subunit A gene (H3A)	298	F	AAGCAGCTGGCTACCAAGG	19
		R	CTCTCTTGGCGTGGATAGCG	20
Cytochrome Oxidase subunit I (COI, Mitochondrial Gene)	511	F	AGGTGTAGGTGCAGGATGAAC	21
		R	GTGAGCCCACACTACAAACC	20
28S rRNA Gene (28S rRNA)	395	F	CGAGTCACTGGGTCTCGC	18
		R	CCGAAACGACCTCAACCTATT	21

Each reactions consisted of 2.5 µl of 10X Apex buffer (Bangalore Genei), 0.42 µl of 10 mM dNTP, 2.4 µl of 25mM MgCl2, 1 µl each of forward and reverse 10 mM primers, between 1.5 µl and 2.5 µl of BSA, 0.3 µl Apex Taq DNA Polymerase (Bangalore Genei), 1–4 µl of template DNA, and water to 25 µl. Reaction conditions included an initial denaturation step at 95^0C for 2 min, followed by 35 cycles of 95^0C for 30 seconds, annealing temperatures and time as the primer sets used and 72^0C for 1 min (H3 and COI) or 1 min 30 s (28S), followed by a final extension at 72^0C for 7 min, and a hold at 4^0C.

II.5) Nucleotide sequencing:

The sequencing was done in a laboratory having required facility. In order to perform 10 µl cycle sequencing reactions using 1.63 µl 5 X buffer, 0.5 µl 10 mM primer, and 0.75 µl BigDye® Terminator. Template DNA and water amounts was adjusted based on the concentration of DNA in each sample. Cycle sequencing parameters followed the protocol [22] with a variable annealing temperature, dependent on the melting temperature of the individual primer. Reaction sequences was obtained from an ABI 3130XL genetic analyser (Applied Biosystems). All sequences will be checked for contamination using a BLAST search. The generated trimmed sequences will be deposited in NCBI Genbank.

II.6) Phylogeny analysis:

Bayesian analysis was performed using MrBayes version 3.1.2 [23]. A mixed model analysis was conducted for each of the four alignments. For the two protein coding genes, each codon position will be modelled independently; the ribosomal gene will also be modelled independently for a total of eight data partitions. Gaps will be treated as missing, not as a fifth character state. Best fit models for each partition were determined independently according to the non-hierarchical Akaike information criterion as implemented in [24].

III. Results:

III. 1) Eco-spatial correlation:

To investigate the extent of eco-spatial correlation in our data, we performed a Mantel test between the ecological and geographical distance matrices. To further assess the relative contribution of environmental variables and geographical distance, matrix regression with a randomization (MMRR) method implemented.

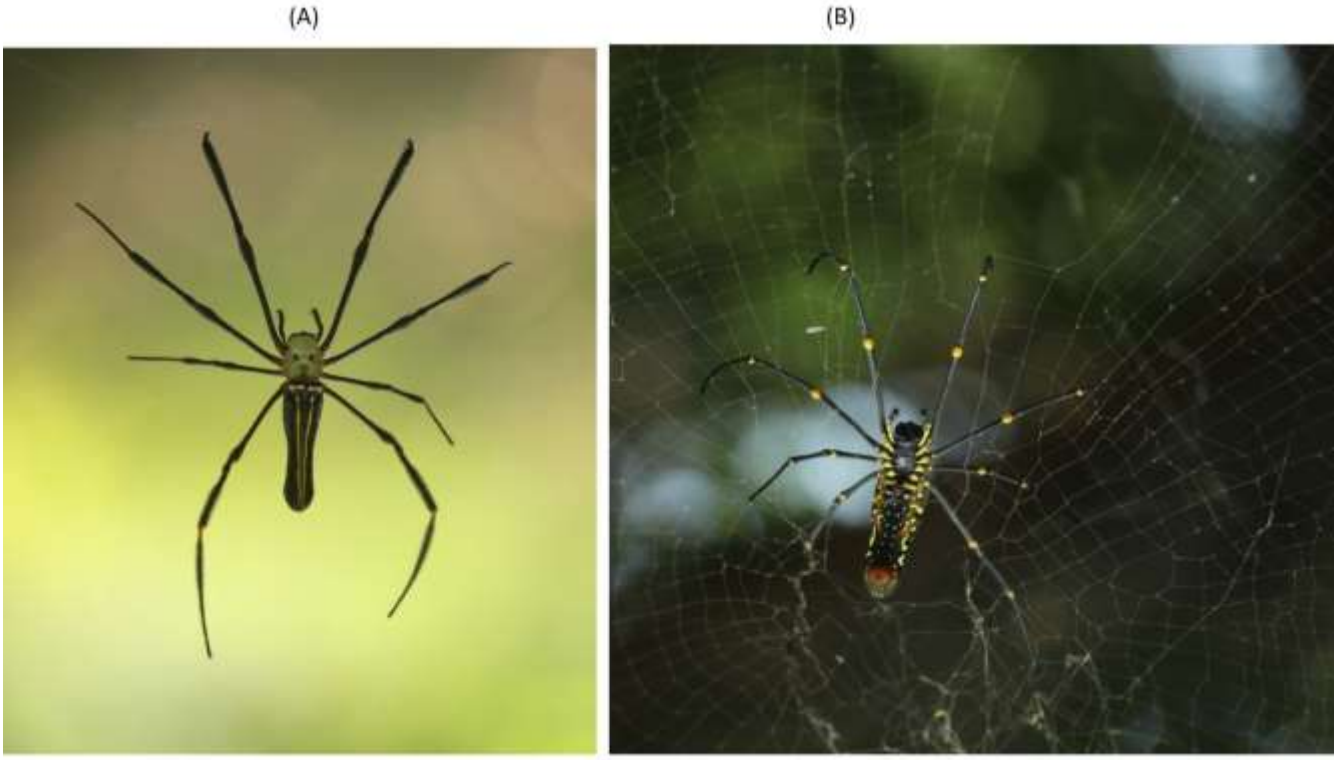

Figure 1. The Giant Wood Spider (*Nephila pilipes*). (A) Dorsal view, (B) Ventral view

III. 2) Gene sequencing:

***Nephila pilipes* (SGNP) histone 3 subunit A (H3A) gene, partial cds**

GGTGGTAGTAAAGAAACCTCATCGTTACAGGCCCGGAACGGTAGCTTTGAGAGAATCCGTCGTTACCAAAAATCTACCGA
GCTCCTCATCCGTAAATTGCCTTTCCAGAGGTTGGTGAGGGAAATCGCTCAGGATTTCAAAACCGATCTTCGATTCCAGA
GCTCTGCTGTCATGGCCCTTCAGGAAGCCAGCGAAGCTTACCTCGTCGGTTTGTTCGAAGACACTAACTTGTGCGCTATC
CACGCCCAAGAGAGAAA

***Nephila pilipes* (ECGP) histone 3 subunit A (H3) gene, partial cds**

GGTGGAGTAAAAAACCTCATCGTTACAGGCCCGGAACCGTTGCTCTGAGAGAAATCCGTCGTTACCAAAAGTCTACCGAG
CTCCTCATCCGTAAATTGCCTTTCCAGAGGTTGGTGAGGGAAATCGCTCAGGATTTCAAAACCGATCTTCGATTCCAGAG
CTCTGCTGTCATGGCCCTTCAGGAAGCCAGCGAAGCTTACCTCGTCGGTTTGTTCGAAGACACTAACTTGTGCGCTATCC
C

***Nephila pilipes* (SGNP) Cytochrome Oxidase I (COI) gene, partial cds**

GCTTCTTTAGAAGGTCATGCTGGAAGATCTGTAGATCTTGCTATTTTTTCTTTACATTTAGCGGGTGCTTCTTCAATTAT
AGGGGCTATTAACTTTATTTCAACAATTTTAAATATGCGATCATATGGAATATCTATAGAGAAAGTTCCTTTATTTGTAT
GATCTGTATTGATTACTGCTGTATTACTTTTACTTTCATTACCAGTATTAGCTGGTGCAATTACAATATTATTAACTGAT
CGAAATTTTAATACTTCTTTTTTTGACCCTTCTGGGGGTGGGGATCCTATCTTATTTCAACATTTATTTTGATTTTTTGG
TCATCCTGAAGTTTATATCTTAATTTTACCAGGATTTGGTATTGTTTCTCATATTATTAGAGCTTCTGTAGGAAAGCGAG
AACCTTTTGGAAATTTAGGAATAATTTATGCAATAGTAGGTATTGGGGGAATAGGGTTTGTAGTGTGAAATCAC

***Nephila pilipes* (ECGP) Cytochrome Oxidase I (COI) gene, partial cds**

GCTTCCTTTAGAAGTCATGCTGGGAGATCTGTAGATTTTGCTATTTTTTCTCTACATTTAGCGGGTGCTTCTTCAATTAT
AGGGGCTATTAATTTTATTTCAACAATTTTAAATATGCGATCATATGGAATATCTATAGAGAAGGTTCCTTTATTTGTAT
GATCTGTATTGATTACTGCTGTATTACTTTTACTTTCATTACCAGTATTAGCTGGTGCAATTACAATATTATTAACTGAT
CGAAATTTTAATACTTCTTTTTTTGACCCCTCTGGGGGAGGGGATCCTATCTTATTTCAACATTTATTTTGATTTTTTGG
TCATCCTGAAGTTTATATCTTAATTTTACCAGGATTTGGTATTGTTTCTCATATTATTAGAGCTTCTGTAGGAAAGCGAG
AACCTTTTGGAAATTTAGGAATAATTTATGCAATAGTAGGTATTGGAGGAATAGGGTTTGT

***Nephila pilipes* (SGNP) 28S rRNA gene, partial cds**

GGCAAAATTAATTCTACTCTTCGTAGTCGGGATCCCCCGCAGACATGCGAGGGGCGCACCGACGGCCTGCCACGCTCCTC
GGAGTCGAGGCGGAGCCTGAGCACACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGACAGGACGAAGACAGGGGA

AACCCTGTTGGAAGTCCGAAGCGGTTCTGACGTGCAAATCGATCGTCTGATCCGCGTATAGGGGCGAAAGACTAATCGAA
CCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGATCGAAATTCAGTCACGATCGGTAAAGC
GAATGATTAGAGGCCTTGGGGCCGAAACGACCTCAACCTATTAAA

***Nephila pilipes* (ECGP) 28S rRNA gene, partial cds**

GGCACAATTAATTCTACTCTTCGTAGTCGGGATCCCCCGCAGACATGCGAGGGGCGCACCGACGGCCTGCCACGCTCCTC
GGAGTCGAGGCGGAGCCCGAGCACACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGACAGGACGAAGACAGGGGA
AACCCTGTTGGAAGTCCGAAGCGGTTCTGACGTGCAAATCGATCGTCTGATCCGCGTATAGGGGCGAAAGACTAATCGAA
CCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGATCGAAATTCAGTCACGATCGGTAAAGC
GAATGATTAGAGGCCTTGGGGCCGAAACGACCTCCAACCTA

III. 3) Nucleotide sequence alignment:

Nucleotide sequence alignment of H3A gene for SGNP and ECGP specimens of *N. pilipes* shown in figure 2.

```
H3A of SGNP; Length: 257
>
Sequence ID: H3A of SGNP Length: 241
Range 1: 1 to 240

Score:405 bits(219), Expect:5e-118,
Identities:235/242(97%),  Gaps:3/242(1%), Strand: Plus/Plus

H3A of SGNP  1    GGTGGTAGTAAAGAAACCTCATCGTTACAGGCCCGGAACGGTAGCTTTGAGAG-AATCCG  59
H3A of ECGP  1    .....-......-.........................C..T...C......A......  58

H3A of SGNP  60   TCGTTACCAAAAATCTACCGAGCTCCTCATCCGTAAATTGCCTTTCCAGAGGTTGGTGAG  119
H3A of ECGP  59   ............G.............................................  118

H3A of SGNP  120  GGAAATCGCTCAGGATTTCAAAACCGATCTTCGATTCCAGAGCTCTGCTGTCATGGCCCT  179
H3A of ECGP  119  ............................................................  178

H3A of SGNP  180  TCAGGAAGCCAGCGAAGCTTACCTCGTCGGTTTGTTCGAAGACACTAACTTGTGCGCTAT  239
H3A of ECGP  179  ............................................................  238

H3A of SGNP  240  CC  241
H3A of ECGP  239  ..  240
```

Figure 2. Nucleotide sequence comparison for H3A gene for SGNP and ECGP samples.

Nucleotide sequence alignment of COI gene for SGNP and ECGP specimens of *N. pilipes* shown in figure 3.

```
COI of SGNP;  Length: 474
>
Sequence ID: COI of SGNP; Length: 461
Range 1: 1 to 461

Score:797 bits(431), Expect:0.0,
Identities:452/462(98%),  Gaps:2/462(0%), Strand: Plus/Plus

COI of SGNP  1    GCTT-CTTTAGAAGGTCATGCTGGAAGATCTGTAGATCTTGCTATTTTTTCTTTACATTT  59
COI of ECGP  1    ....C........-.........G............T.............C.......  59

COI of SGNP  60   AGCGGGTGCTTCTTCAATTATAGGGGCTATTAACTTTATTTCAACAATTTTAAATATGCG  119
COI of ECGP  60   ................................T.........................  119

COI of SGNP  120  ATCATATGGAATATCTATAGAGAAAGTTCCTTTATTTGTATGATCTGTATTGATTACTGC  179
COI of ECGP  120  .......................G.....................................  179

COI of SGNP  180  TGTATTACTTTTACTTTCATTACCAGTATTAGCTGGTGCAATTACAATATTATTAACTGA  239
COI of ECGP  180  ............................................................  239

COI of SGNP  240  TCGAAATTTTAATACTTCttttttttGACCCTTCTGGGGGTGGGGATCCTATCTTATTTCA  299
COI of ECGP  240  ..............................C........A.....................  299

COI of SGNP  300  ACATTTATTTTGATTTTTTGGTCATCCTGAAGTTTATATCTTAATTTTACCAGGATTTGG  359
COI of ECGP  300  ............................................................  359

COI of SGNP  360  TATTGTTTCTCATATTATTAGAGCTTCTGTAGGAAAGCGAGAACCTTTTGGAAATTTAGG  419
COI of ECGP  360  ............................................................  419

COI of SGNP  420  AATAATTTATGCAATAGTAGGTATTGGGGGAATAGGGTTTGT  461
COI of ECGP  420  ............................A..............  461
```

Figure 3. Nucleotide sequence comparison for COI gene for SGNP and ECGP samples.

Nucleotide sequence alignment of 28S rRNA gene for SGNP and ECGP specimens of *N. pilipes* shown in figure 4.

```
28srRNA of SGNP; Length: 365


>
Sequence ID: 28srRNA of SGNP Length: 361
Range 1: 1 to 361

Score:649 bits(351), Expect:0.0,
Identities:358/361(99%),  Gaps:1/361(0%), Strand: Plus/Plus

28srRNA of SGNP  1    GGCAAAATTAATTCTACTCTTCGTAGTCGGGATCCCCCGCAGACATGCGAGGGGCGCACC  60
28srRNA of ECGP  1    ....C.......................................................  60

28srRNA of SGNP  61   GACGGCCTGCCACGCTCCTCGGAGTCGAGGCGGAGCCTGAGCACACACGTTGGGACCCGA  120
28srRNA of ECGP  61   .........................................C.....................  120

28srRNA of SGNP  121  AAGATGGTGAACTATGCCTGGACAGGACGAAGACAGGGGAAACCCTGTTGGAAGTCCGAA  180
28srRNA of ECGP  121  ............................................................  180

28srRNA of SGNP  181  GCGGTTCTGACGTGCAAATCGATCGTCTGATCCGCGTATAGGGGCGAAAGACTAATCGAA  240
28srRNA of ECGP  181  ............................................................  240

28srRNA of SGNP  241  CCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGATCGAAAT  300
28srRNA of ECGP  241  ............................................................  300

28srRNA of SGNP  301  TCAGTCACGATCGGTAAAGCGAATGATTAGAGGCCTTGGGGCCGAAACGACCTC-AACCT  359
28srRNA of ECGP  301  ............................................................  360

28srRNA of SGNP  360  A  360
28srRNA of ECGP  361  .  361
```

Figure 4. Nucleotide sequence comparison of 28srRNA gene for SGNP and ECGP samples.

III.6) Phylogenetic tree analysis based on nucleotide sequence:

Phylogenetic tree for H3A gene sequence of SGNP specimen of *N. pilipes* shown in figure 5.

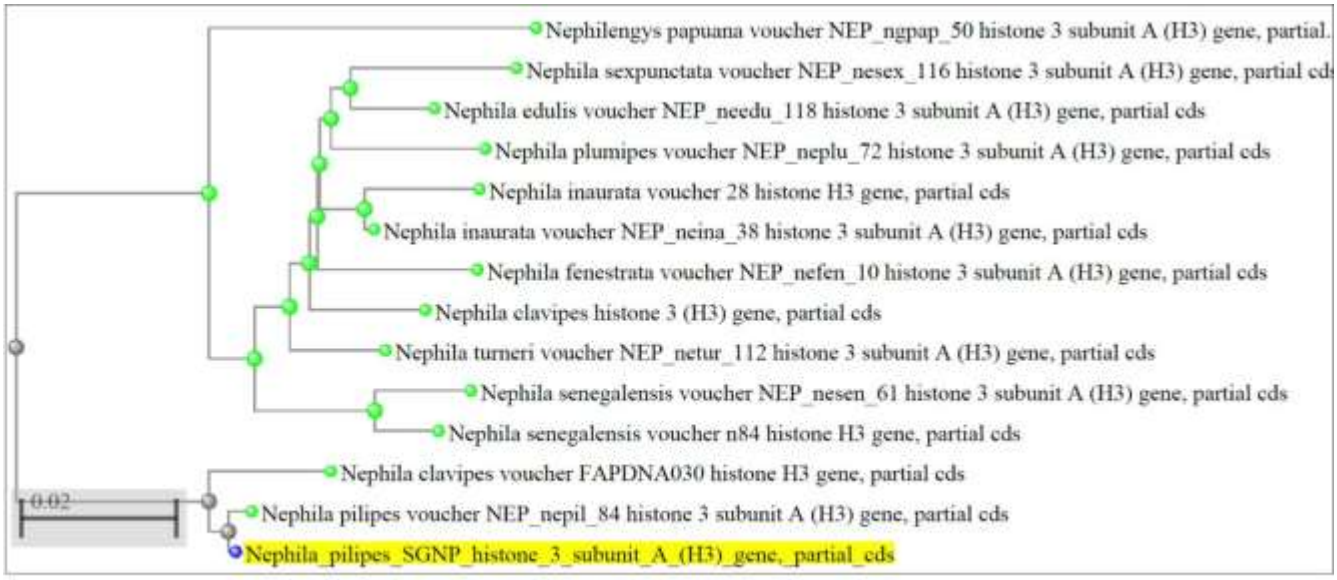

Figure 5. Phylogenetic tree based on the nucleotide sequence of H3A gene from SGNP sample of *N. pilipes*.

Phylogenetic tree for H3A gene sequence of ECGP specimen of *N. pilipes* shown in figure 6.

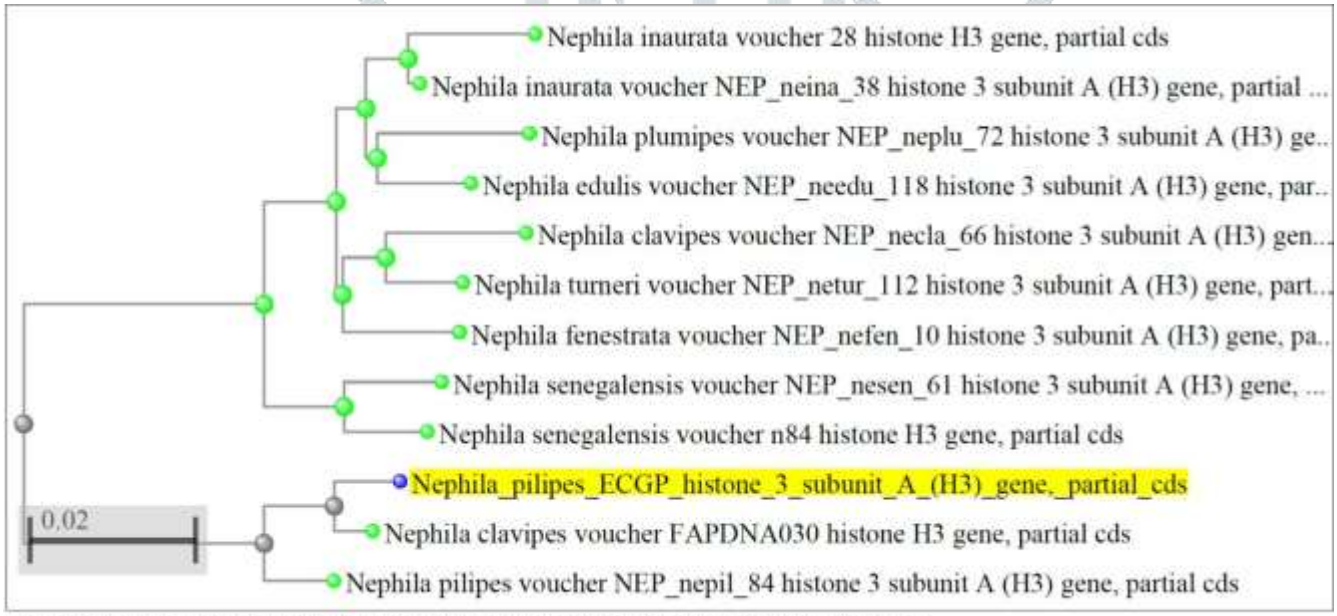

Figure 6. Phylogenetic tree based on the nucleotide sequence of H3A gene from ECGP sample of *N. pilipes*.

Phylogenetic tree for COI gene sequence of SGNP specimen of *N. pilipes* shown in figure 7.

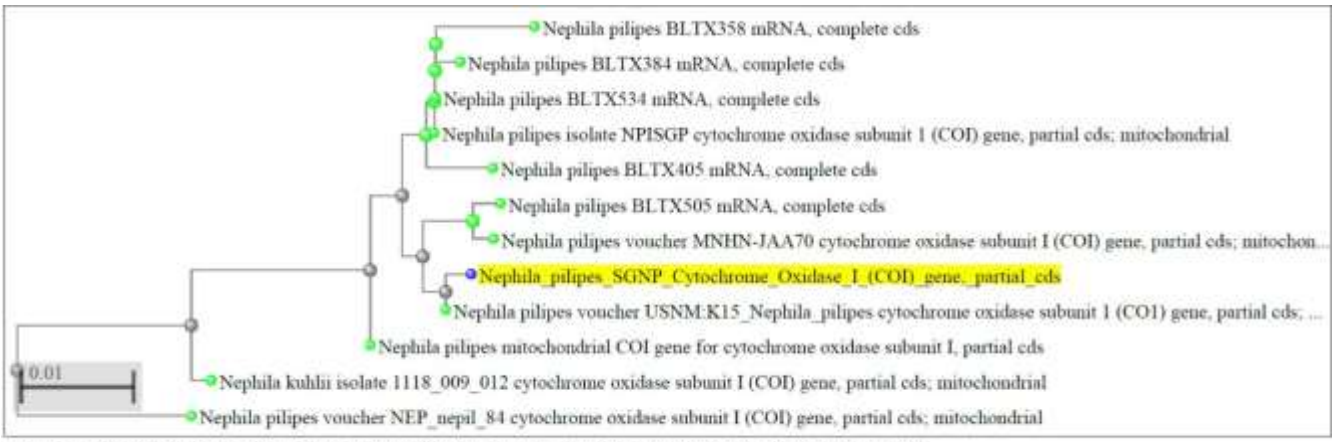

Figure 7. Phylogenetic tree based on the nucleotide sequence of COI gene from SGNP sample of *N. pilipes*.

Phylogenetic tree for COI gene sequence of ECGP specimen of *N. pilipes* shown in figure 8.

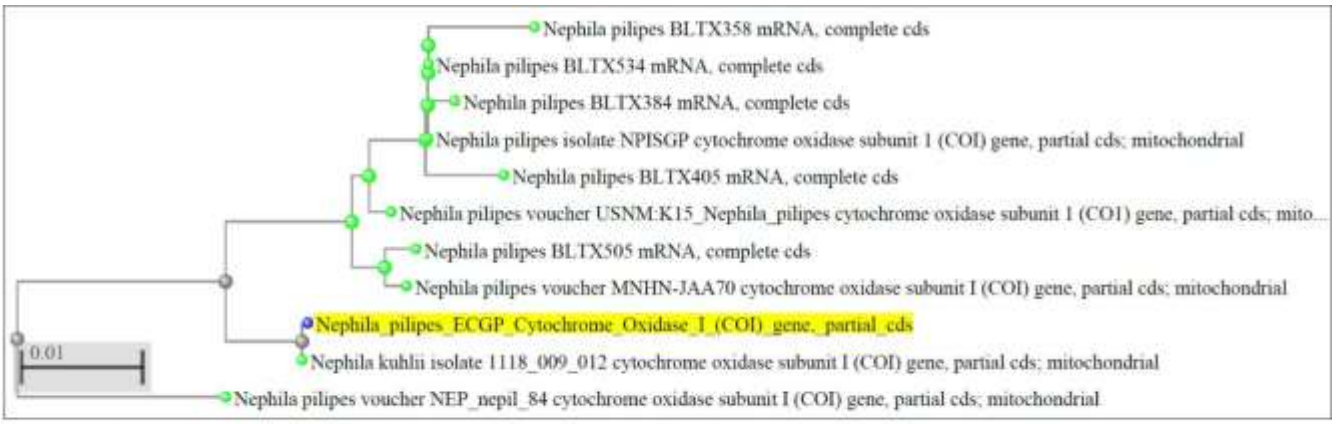

Figure 8. Phylogenetic tree based on the nucleotide sequence of COI gene from ECGP sample of *N. pilipes*.

Phylogenetic tree for 28S rRNA gene sequence of SGNP specimen of *N. pilipes* shown in figure 9.

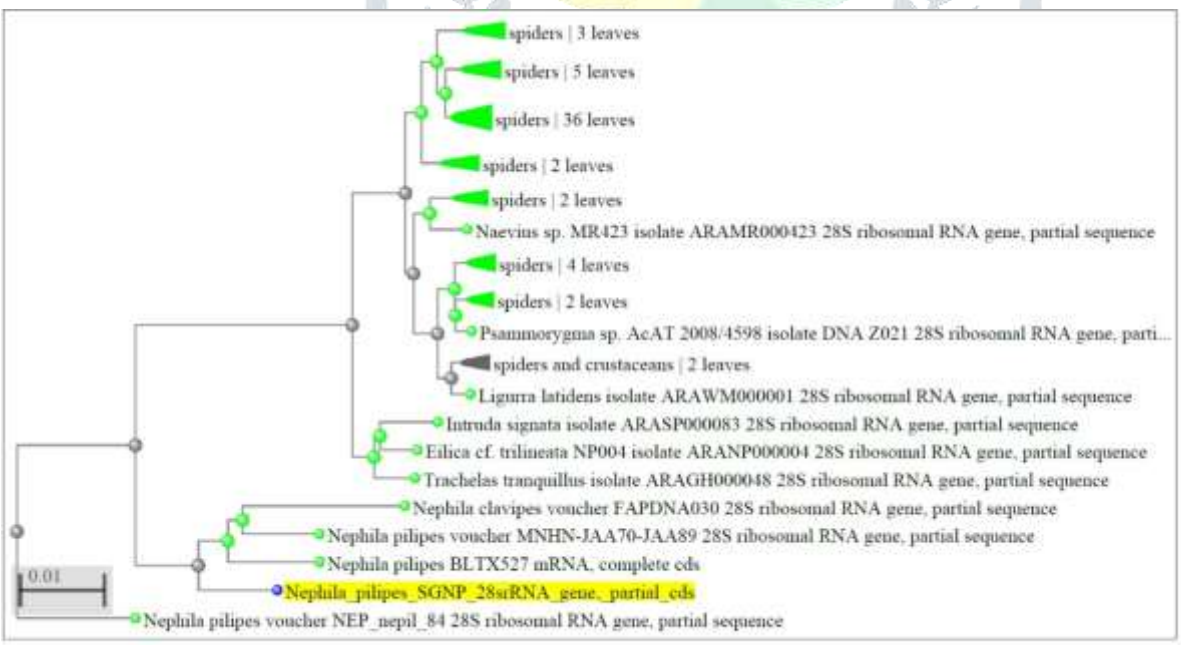

Figure 9. Phylogenetic tree based on the nucleotide sequence of 28srRNA gene from SGNP sample of *N. pilipes*.

Phylogenetic tree for H3A gene sequence of SGNP specimen of *N. pilipes* shown in figure 10.

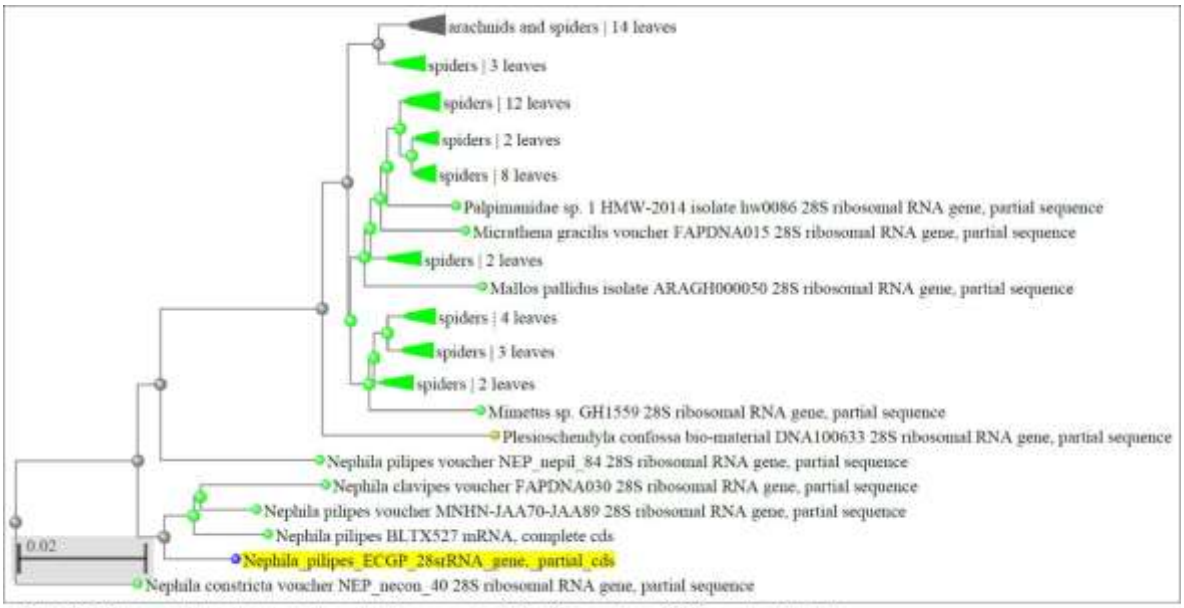

Figure 10. Phylogenetic tree based on the nucleotide sequence of 28srRNA gene from ECGP sample of *N. pilipes*.

III.7) Amino acid sequence alignment:

Amino acid sequence alignment of predicted amino acid sequence of H3A gene from SGNP and ECGP specimens of *N. Pilipes* shown in figure 6.

```
SGNP H3A Protein; Length: 85

>ECGP_H3A_Protein; Length: 79
Range 1: 2 to 79

Score:127 bits(320), Expect:1e-45,
Method:Compositional matrix adjust.,
Identities:64/78(82%), Positives:67/78(85%), Gaps:0/78(0%)

SGNP H3A Protein  3   SKETSSLQARNGSFERIRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQE  62
ECGP H3A Protein  2   ..KPHRYRPGTVALRE.............................................  61

SGNP H3A Protein  63  ASEAYLVGLFEDTNLCAI  80
ECGP H3A Protein  62  ..................  79
```

Figure 11. Amino acid sequence alignment for H3A gene for SGNP and ECGP samples of *N. pilipes*.

Amino acid sequence alignment of predicted amino acid sequence of COI gene from SGNP and ECGP specimens of *N. Pilipes* shown in figure 7.

```
SGNP COI Protein; Length: 158

>Cytochrome Oxidase I
Sequence ID: SGNP COI Protein; Length: 153
Range 1: 1 to 153

Score:291 bits(746), Expect:4e-108,
Method:Compositional matrix adjust.,
Identities:149/153(97%), Positives:149/153(97%), Gaps:0/153(0%)

SGNP COI Protein  1    ASLEGHAGSSVDLAIFSLHLAGASSIMGAINFISTILNMRSYGMSMEKVPLFVWSVLITA  60
ECGP COI Protein  1    ..FSS.......F.............................................  60

SGNP COI Protein  61   VLLLLSLPVLAGAITMLLTDRNFNTSFFDPSGGGDPILFQHLFWFFGHPEVYILILPGFG  120
ECGP COI Protein  61   ............................................................  120

SGNP COI Protein  121  IVSHIISASVGKREPFGNLGMIYAMVGIGGMGF  153
ECGP COI Protein  121  ................................  153
```

Figure 12. Amino acid sequence alignment for COI gene for SGNP and ECGP samples of *N. pilipes*.

IV. Discussion:

In the present study eco-spatial correlation data, nucleotide sequence data, molecular phylogeny data has given new insight about the role of geographical separation of spider *N. pilipes*. When we studied the behavioural aspects of the spider like, the presence of males and females per web, we almost always (95%) of times, notice one male and one

female per web. In certain few cases (5%) of time, the data shows presence of two or more males per web. We also could notice that female always prefer to occupy position in the centre of the web, whereas as males were observed to be present on the periphery of the web. We also measured the average diameter of the web, which remains the same average 42 inches. These similarities suggest involvement of instinct behaviour in web building. In a ratio of body length female: male, the average body ratio is 6:1. These observations did not differ in the two locations under study (SGNP and ECGP).

The gel images of gene specific PCR shows, the PCR amplicon length close to the desired products. The H3A gene amplicon is around 250bp, COI gene amplicon is around 500bp, 28S rRNA gene amplicon is more than 250bp and less than 500 bp for SGNP and ECGP specimens. After nucleotide sequencing, the length of the H3A gene sequences obtained from the chromatograms are 257ntds and 241ntds for SGNP and ECGP respectively. Similarly, the length of the COI mitochondrial gene sequences obtained from the chromatograms indicates 474ntds and 461ntds for SGNP and ECGP respectively. Whereas the length of the 28S rRNA nuclear gene sequences obtained from the chromatograms shows 365ntds and 361ntds for SGNP and ECGP respectively. As expected, mitochondrial sequences are relatively AT rich (COI gene), whereas 28S rRNA gene and H3A gene sequences are GC rich.

Nucleotide sequence alignment for H3A gene between SGNP and ECGP shows, 97% sequence similarity (235 of 242 bases are matching). The sequence alignment shows 3 gaps and 4 bases variation. Each H3A gene of SGNP and ECGP specimen translates into 85 and 79 length amino acid respectively. When the nucleotide sequence was translated into the amino acid sequence, it shows less sequence similarities (64 of 78 amino acids are matching). A complete stretch of 14 amino acids sequence differs in both the peptide sequence. This suggests protein variation between *N. pilipes* of two locations SGNP and ECGP, but further clarification is needed by getting the actual protein sequencing of H3A protein.

A number of nuclear as well as mitochondrial DNA markers have been proven to have profound uses [26]. Mitochondrial DNA sequences have been widely used for studies on population and molecular systematics of insects as it has a number of specific biological properties, which make mtDNA an appropriate marker for molecular biodiversity. Firstly, mtDNA is highly variable because of its high mutation rate, which can generate some signal about population history over short time period. Secondly, it is maternally inherited which means that the whole genome behaves as a single, nonrecombining locus. These unique properties allow the development of universal primers and easy recovery from small or degraded biological samples due to its high copy number in most cells with a different evolution rate in different regions of mtDNA. These structural and evolutional characteristics of mtDNA sequences make it a marker of choice for various studies.

For nucleotide sequence alignment of COI gene between SGNP and ECGP, 98% sequence similarity is observed (452 of 462 bases are matching). The variation is observed at 9 nucleotide and 1 gap of nucleotide. As COI is mitochondrial gene, the higher sequence similarity is expected. In the translation of COI gene, invertebrate mitochondrial codons were used. This virtual translation shows open reading frame making 158 and 153 amino acids chain from SGNP and ECGP specimens respectively. On comparison of these amino acid sequence, it shows 97% sequence similarity (149 of 153 amino acids are matching). The amino acid comparison shows variation at 4 positions, out of which 3 are tandem. One of the reasons for such high sequence similarity of COI protein could be the highly conserved nature of the gene.

COI nucleotide and amino acid data can be fitted to a two-dimensional structural model, which is analogous to that proposed for hexapods [25]. Considering levels of nucleotide variation in light of this model reveals differences across structural domains, particularly at 1st and 3rd codon positions.

A nucleotide sequence alignment of 28S rRNA gene shows, 99% of sequence similarity (358 of 361 bases are matching). The alignment shows 1 gap and 2 variations in the nucleotide sequences. As 28S rRNA gene does not make protein, so no amino acid sequence similarity study was carried out.

In the phylogenetic tree analysis for each nucleotide sequence, a nucleotide BLAST was carried out. The nucleotide sequences were BLAST in NCBI nucleotide database with retrieving highly similar sequences. For making phylogenetic tree, Neighbour joining method was used, maximum distance permitted was adjusted to 0.1, the trees were sorted based on the distance.

The nearest closest species for H3A gene of SGNP *N. pilipes* and ECGP *N. pilipes* is observed to be *N. clavipes*. This shows the genetic relatedness and highly conserved nucleotide sequence of *N. pilipes* in both loculations under study. We could also observe that, *N. pilipes* and *N. clavipes* forms separate clade for H3A gene.

In the phylogenetic studies of COI gene for SGNP and ECGP specimens of *N. pilipes*, the nearest closest spider species is *N. kuhlii*. With the conditions chosen for making phylogenetic tree, the COI sequence mostly shows sequences of N. pilipes from the NCBI database. This shows the highly conserved nature of COI gene.

The phylogenetic tree analysis for 28S rRNA nuclear gene of SGNP and ECGP shows, be *N. clavipes* as the most recent and closest related spider species. Though tree shows no separate clade for *28S rRNA* gene of *N. pilipes*.

V. Conclusion:

The study supports the view of highly conserved nature of *H3A*, *COI*, and *28S rRNA* genes, in *N. pilipes* spider specimens collected from geographically isolated locations, Sanjay Gandhi National Park (SGNP) and Elephanta Caves Gharapuri (ECGP). We also observed high similarities in the behavioural and physiological aspects like web diameter size, position of female in the web, number of males and females per web, and body length ratio of female to male. Thus, despite the geo-spatial separation of the spider *N. pilipes* shows nucleotide sequence conservation of genes under study and instinctive conserve behaviour of the species.

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VII. Reference:

1) **Wunderlich J.** (2004). Fossil spiders in amber and copal. *Beitrage Araneol.* 3A&B:1–1908
2) **Penney D, Selden PA.** (2011). Fossil Spiders: The Evolutionary History of a Megadiverse Order. Manchester, UK: *Siri Scientific Press*
3) **Hausdorf B.** (1999). Molecular phylogeny of araneomorph spiders. *J. Evol. Biol.* 12:980–85
4) **Ayoub NA, Garb JE, HedinM,Hayashi CY.** (2007). Utility of the nuclear protein-coding gene, elongation factor-1 gamma (*EF-1γ*), for spider systematics, emphasizing family level relationships of tarantulas and their kin (Araneae: Mygalomorphae). *Mol. Phylogenet. Evol.* 42(2):394–409
5) **Blackledge TA, Scharff N, Coddington JA, Szu¨ ts T,Wenzel JW, et al.** (2009). Reconstructing web evolution and spider diversification in the molecular era. *Proc.Natl. Acad. Sci. USA* 106(13):5229– 34
6) **Lopardo L, GiribetG, Hormiga G.** (2011). Morphology to the rescue: molecular data and the signal of morphological characters in combined phylogenetic analyses—a case study from mysmenid spiders (Araneae, Mysmenidae), with comments on the evolution of web architecture. *Cladistics* 27:278–330
7) **Lopardo L, Hormiga G.** (2008). Phylogenetic placement of the Tasmanian spider *Acrobleps hygrophilus* (Araneae, Anapidae) with comments on the evolution of the capture web in Araneoidea. *Cladistics* 24(1):1–33
8) **Wise DH** (1993) Spiders in Ecological Webs (*Cambridge Univ Press, New York*) p 328.
9) **Foelix RF** (1996) Biology of Spiders (*Oxford Univ Press, New York*) 2nd Ed p 330.
10) **Bond JE, Opell BD** (1998) Testing adaptive radiation and key innovation hypotheses in spiders. *Evolution* 52:403–414.
11) **Opell BD, Schwend HS** (2008) Persistent stickiness of viscous capture threads produced by araneoid orb-weaving spiders. *J Exp Zool A* 309A:11–16.
12) **Agnarsson, I., Avilés, L., Coddington, J.A., Maddison, W.P.,** (2006). Social theridiid spiders – repeated origins of an evolutionary dead-end. *Evolution* 60, 2342–2351.
13) **Griswold, C.E., Ramírez, M., Coddington, J.A., Platnick, N.,** (2005). Atlas of phylogenetic data for entelegyne spiders (Araneae: Araneomorphae: Entelegynae) with comments on their phylogeny. *Proc. Calif. Acad. Sci.* 56, 1–324.
14) **Lopardo L, Hormiga G** (2008) Phylogenetic placement of the Tasmanian spider Acrobleps hygrophilus (Araneae, Anapidae) with comments on the evolution of the capture web in Araneoidea. *Cladistics* 24:1– 33.
15) **Agnarsson I, Maddison WP, Avile´s L** (2007) The phylogeny of the social Anelosimus spiders (Araneae : Theridiidae) inferred from six molecular loci and morphology. *Mol Phylogenet Evol* 43:833–851.

16) **Hedin M, Bond JE** (2006) Molecular phylogenetics of the spider infraorder Mygalomorphae using nuclear rRNA genes (18S and 28S): Conflict and agreement with the current system of classification. *Mol Phylogenet Evol* 41:454–471.
17) **Hormiga G, Arnedo M, Gillespie RG** (2003) Speciation on a conveyor belt: Sequential colonization of the Hawaiian islands by Orsonwelles spiders (Araneae, Linyphiidae). *Syst Biol* 52:70–88.
18) **Gillespie RG** (2005) Geographical context of speciation in a radiation of Hawaiian Tetragnatha spiders (Araneae, Tetragnathidae). *J Arachnol* 33:313–322
19) **Coyne, J.A.** (1994). Ernst Mayr and the origin of species. *Evolution* 48:19–30.
20) **Schluter, D.** (2001). Ecology and the origin of species. *Trends in Ecology & Evolution* 16:372–380.
21) **Glor, R.E., M. E. Gifford, A. Larson, J. B. Losos, L. Rodriguez-Schettino, A. R. et.al.** (2004). Partial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles. *Proceedings of the Royal Society of London B* 271:2257–2265.
22) **Platt, A.R., Woodhall, R.W., George, A.L.J.,** (2007). Improved DNA sequencing quality and efficiency using an optimized fast cycle sequencing protocol. *BioTechniques* 43, 58–62.
23) **Huelsenbeck, J.P., Ronquist, F.,** (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
24) **Nylander, J.A.A.,** (2008). MrModeltest Version 2.3. Evolutionary Biology Centre, Uppsala University.
25) **Lunt, D. H., Zhang, D.-X., Szymura, J. M., and Hewitt, G. M.** (1996). The insect cytochrome oxidase I gene: Evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol. Biol. 5: 153–165
26) **Morin P.A., LuikartG.,WayneR.K. and SNP workshop group**. (2004). SNPs in ecology, evolution and conservation. Trends Ecol. Evol. 19, 208–216.