Diversity of aphid pests and assessment of molecular phylogenetic relationship based on mitochondrial gene sequence

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**Abstract**
An experiment was conducted to determine the aphid pests’ diversity in Jahangirnagar University Campus from January to December, 2015. Total ten aphid pests were identified viz, *Acyrthosiphon pisum, Aphis craccivora, Aphis fabae, Aphis gossypii, Aphis nerii, Brevicoryne brassicae, Hyadaphis coriandri, Lipaphis erysimi, Macrosiphum euphorbiae, Myzus persicae*.

Exploration of aphid pests showed that they caused serious damage to agricultural crops by attacking different part of plants. Seasonal abundance of aphid pests showed that they had close relation with meteorological factor. From the last of September, aphid populations were found to decrease gradually mainly due to rain and appearance of many natural enemies in the crop. Along with diversity study, phylogenetic relationship of aphid pests was constructed based on mitochondrial COI gene. In sequence alignment, out of ten aphids *Acyrthosiphon pisum* showed non conserve region than rest. Analysis of interspecific genetic divergence exposed the genetic range of aphid pests was 0.05-0.11. For confirmation of origin and evolution of aphid pests, phylogenetic tree and haplotype analysis was performed. Both Neighbor-Joining and Maximum likelihood methods for tree construction and haplotype analysis showed *My. persicae* was genetically most diverge among all other studied aphids. Perhaps the control program for one aphid species will be applicable for other aphid pests because they originated from common ancestor and they were closely related to each other. This information would be helpful in Integrated Pest Management program for successful control of aphid pests in Bangladesh.

**Keywords:** Aphid; diversity; Seasonal abundance; COI gene; Phylogenetic analysis; Haplotype

1. Introduction

Aphids (Hemiptera: Aphididae) are the most destructive and widely distributed herbivorous insects accounting more than 4,300 described species [3, 4, 15]. They attack the crops and cause harm by imbibing the sap from different parts of the plant and lessen the market price [39]. Aphids are the most common vector of plant viruses [1]. This pest account for yield losses ranging from 37 to 90% [28]. Most plants have one or more species of aphid that have been known to feed on it. Through their huge reproductive ability, aphids can cause severe damage to crops. At present, the main aspect of the aphid control program is based on the use of chemical insecticides. Continuous usage of chemicals has some disadvantages like pest resistance, environmental contaminations and sometimes outbreak of secondary pests [27, 29, 44]. Some aphids have developed resistance to at least seventy different synthetic compounds [11, 37]. These entomological backlashes have bound the scientists to be concerned with environment friendly pest management programs [19].
IPM requires an understanding of the ecology of the cropping system, including that of the pests, their natural enemies, and the nearby environment. Timely monitoring of pest populations gives information needed to decide if and when to apply a specific control measure for optimal effectiveness. There are scarcities of research on aphid management in environmentally safe manner, more specifically using seasonal information and natural enemies in Bangladesh. In Bangladesh, aphid fauna are mostly unrecorded [21]. This situation led to design a research to know some valuable information about aphid diversity, its nature of damage, seasonal abundance and relation of climate with the pest incidence.

Understanding of aphid phylogeny will eventually help to make the proper pest control strategy. Attempt to reconstruction of aphid Phylogeny has been accomplished around 20 years ago but ended with some conflict [18, 45]. The reason of conflict was insufficient phylogenetic data. Recently some efforts have been taken to understand the aphid phylogenetic relation based on Mitochondrial COI gene. COI gene have only maternal transmission and they emerge more quickly than nuclear genes and comprise much higher potential coding region than nuclear genomes [7, 36]. COI gene has been widely used because it is the standard tool of identifying the organisms and also helps in understanding phylogenetic analysis [8]. Aphid classification at various levels has been attempted using morphological characters, host-plants associations and life cycles [3, 18]. However, morphological studies very often tend to be uncertain. The phylogenetic analysis was also performed to know the relationship among aphid. This finding will help in pest control program in control measure of aphid. Thus our farmer will able to produce quality agricultural products in increased number in an environmental safety manner and thus could reach the millennium development goals.

2. Materials and Methods

2.1 Study area of aphid faunal survey and preparation of experimental plots
Aphids were collected from different vegetables, ornamental trees, shrubs, flowers, and other crops that are economically valuable from different parts of Dhaka city, capital of Bangladesh (23° 42’ N, 90° 24’ E) from January – December, 2015. Home garden, commercial nurseries, different crop fields, botanical garden and Insect Rearing and Experimental Station (IRES) was main focusing area to collect aphid. For conducting this research few small plots of different crops (bean, eggplant, cabbage, and mustard) were prepared in the Insect Rearing and Experimental Station (IRES) of Jahangirnagar University in addition to observation in other parts of the campus and its surroundings. In the small plots any chemical fertilizer or insecticide were not used to keep the insects undisturbed. Data obtained from IRES were used to determine seasonal abundance of the pest. The ecological condition supports the prey predator relationships because this area is free of chemical usage.

2.2 Collection and identification of aphid
Aphids were collected from the host plant using soft camel hair brush and preserved into a small vial for further studies in the laboratory of Molecular Entomology, Wazed Miah Science Research Center, Jahangirnagar University, Savar, Dhaka. Aphids were identified using proper keys [5, 13, 14, 26, 33, 43].

2.3 Survey on the host of aphid and observe nature of damage
Different types of plants were checked to find aphid pest. Different aphids attack different plant in different time of year. This is why all economic important plants were checked throughout the year. Plants, where aphids were found to attack as pest were considered as host plant.

2.4 Monitor seasonal abundance of the pest
Aphids were checked throughout the cropping season to check its seasonal abundance for a particular crop. Aphids were count every week. At least 5 plants were taken randomly. Then aphids of 10 randomly taken leaves of a plant were counted using a magnifying glass. Total number of aphids was then averaged to count its abundance. Both wings and wingless were taken into consideration while count. In the present experiment bean aphid (Aphis fabae) of bean plant (Lablab purpureus) was target pest.

2.5 Meteorological data
Records for mean relative humidity, mean air temperature (°c), and total rainfall (mm) during the sampling period in JU campus were collected from the Department of Geography, Jahangirnagar University.

2.6 Collection of data for phylogenetic analysis
COI gene sequence of ten (10) aphid pests was taken to understand the aphid phylogenetic relationship. This situation led to design a research to know some valuable information about aphid diversity, its nature of damage, seasonal abundance and relation of climate with the pest incidence.
Table 1: Information of Aphid pests COI gene sequences

<table>
<thead>
<tr>
<th>Name of Species</th>
<th>GenBank Accessions (COI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyrthosiphonpisum</td>
<td>KR579669.1</td>
</tr>
<tr>
<td>Aphis craccivora</td>
<td>KR084999.1</td>
</tr>
<tr>
<td>Aphis fabae</td>
<td>GU324636.1</td>
</tr>
<tr>
<td>Aphis gossypii</td>
<td>KR085028.1</td>
</tr>
<tr>
<td>Aphis nerii</td>
<td>KR044621.1</td>
</tr>
<tr>
<td>Brevicorynebrasicae</td>
<td>KJ814964.1</td>
</tr>
<tr>
<td>Hyadaphiscoriandri</td>
<td>DQ499054.1</td>
</tr>
<tr>
<td>Lipaphiserysimi</td>
<td>KJ814967.1</td>
</tr>
<tr>
<td>Macrosiphumeuphorbiae</td>
<td>KR045247.1</td>
</tr>
<tr>
<td>Myzuspersicae</td>
<td>KJ814969.1</td>
</tr>
</tbody>
</table>

2.7 Sequence alignment, Genetic distances and Phylogenetic analysis

COI gene sequences were aligned using ClustalW algorithm with the help of MEGA tools (version 6) with gap opening penalty 15, gap extensions penalty 6.66, transition weight 0.5 and delay divergent cutoff 30% [24, 38]. BioEdit Sequence Alignment Editor Version 7.1.3 help to produce multiple sequence alignment image [16].

Kimura’s two parameter (K2P) genetic distances was carried out using MEGA 6. The evolutionary history was inferred using both the Neighbor-Joining (NJ) and maximum likelihood (ML) tree method based on MEGA 6 [35, 41]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches [12]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

Haplotypes were constructed using the program Pop art 1.7 based on TCS network [10]. Haplotype identifies the mutation rate among the aphid species.

3. Result

Different species of aphids that were collected and identified following proper keys have been mentioned in table 2. Different agriculturally important hosts of the aphids were also recorded, identified and mentioned in the same table. A total of ten agricultural important aphids were identified up to species level from the study area. Their short identification procedure has been described in the table 2. All identified species were pest of different agricultural important crops. Most of the aphids were found as pest of more than one plant due to their polyphagous habit.

Table 2: Different aphids and their agricultural host plants found in the JU campus

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Short taxonomic description of the pest</th>
<th>Host plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea aphid</td>
<td><em>Acyrthosiphon pisum</em></td>
<td>Pale green with black legs; 4-5mm long, green, with a shiny appearance; antennae are pale green or brown, but always have a dark band at the joint of segments III and IV.</td>
<td>Pea</td>
</tr>
<tr>
<td>Cowpea aphid</td>
<td><em>Aphis craccivora</em></td>
<td>Velvety black, with a distinct wax cover; Abdomen: aptera - dorsum with black patch which is variable in size; Alata - dorsum with black bands on all segments.</td>
<td>Pea, bean</td>
</tr>
<tr>
<td>Bean aphid</td>
<td><em>Aphis fabae</em></td>
<td>Black or very dark brown species with a variable abdominal sclerotic pattern - confined to abdominal tergites 6-8 in smaller apterae but broken bands present in larger ones; siphunculi and caudal are dark.</td>
<td>Bean, carrot, lettuce, spinach</td>
</tr>
<tr>
<td>Cotton aphid</td>
<td><em>Aphis gossypii</em></td>
<td>Wingless are generally dark green or blue-green with dusky siphunculi; Abdomen: aptera - no dorsal markings, reticulated pattern on dorsum; alata - no black, dorsal bands on segments 1 to III and usually none on IV and V.</td>
<td>Cucumber, pumpkin, eggplant, papaya</td>
</tr>
</tbody>
</table>
### Milkweed aphid

*Aphis nerii*

- Wingless forms are yellow with black cornicles, antennae, legs, and cauda.

Oleander, milkweed

### Cabbage aphid

*Brevicoryne brassicae*

- Gray green with a powdery, waxy covering; Abdomen: aptera - broken, dark, dorsal bands on all segments; alata - broken, dark, dorsal bands on all segments.

Radish, cabbage, cauliflower, broccoli

### Coriander Aphid

*Hyadaphis coriandri*

- Yellow-green, dusted with greyish wax; short, dusky, slightly swollen, cornicles that are about twice as long as wide.

Coriander

### Mustard aphid

*Lipaphis erysimi*

- Yellowish green, gray green or olive green with a white waxy bloom covering the body.

Mustard, broccoli, cabbage, radish, tomato

### Potato aphid

*Macrosiphum euphorbiae*

- Pink or green; pear-shaped; long, dark antennae; abdomen: no dorsal markings

Sweet potato, cabbage, bean tomato, eggplant

### Green peach aphid

*Myzus persicae*

- Wingless adult aphids vary from green to pale yellow; winged adults are green with black or dark brown markings on their abdomens.

Brinjal, potato, cabbage, carrot, tomato, papaya

### 3.1 Intensity of infestation in relation to time

Large aphid populations were found to cause curling, yellowing, and distortion of leaves and stunting of shoots. They were also found to produce large quantities of a sticky substance known as honeydew, which often turns black with the growth of a sooty mold fungus.

Aphids were found to attack almost all parts of plant viz., leaf, twig, flower and fruit (Fig 1). Direct damage caused by aphids feeding caused yield losses of more than 50% in some susceptible bean or mustard varieties (data not presented in this paper). Observation revealed that direct feeding damage occurs when colonies of 30 or more aphids were developed on individual growing tips. The degree of damage depends on the varietal susceptibility, the growth stage of the crop, the percentage of plants infested, the number of aphids per growing tip, and the duration of the infestation. Different time of the year was not same for aphid infestation. Aphid infestation was observed heavily in the months January, February, and December to most crops. But some early susceptible crop variety was found to attack in the mid time of year.

![Fig 1: Damage caused by aphids: a) Aphids sucking sap of bean twig b) stunted growth of bean c) stunted growth of broccoli d) Leaf curling of egg-plant e) Aphid attack f) Egg-plant flower](image)

### 3.2 Seasonal abundance of bean aphids

Seasonal abundance of bean aphid into different parts of plants, viz., leaf, flower and bean were observed in some experimental plots of IRES. Aphids were found to attack vegetative part of bean (*Aphis fabae*) on mid-August. Mostly newly emerged leaf and twig are their choice place to infest. They were not found in mature leaves. When the flower emerged or fruit comes out, pests were found to attack those parts also in addition to leaf during September to October, 2015. Fig. 2 shows...
the seasonal abundance of this pest in bean over the cropping season. From the last of September population were found to decrease gradually mainly due to rain and appearance of many natural enemies in the crop. In month of September, aphid infestation was most and in the month of October aphid population decreased slowly.

![Graph showing seasonal abundance of bean aphids at IRES in different parts of the plant](image)

**Fig 2:** Seasonal abundance of bean aphids at IRES in different parts of the plant (values indicate mean±sem (nymphs + adults) per 10 leaves.

### 3.3 Relation of Aphid with Environmental factors

Meteorological data were recorded with a view to check its relationship with the aphid pests of JU campus. Mean relative humidity (%), mean air temperature (°C), and total rainfall (mm) of the study area throughout the year has been mentioned in the Fig. 3. It was found from the figure 1 and 2 that aphid has a relationship with weather parameters. Aphids were abundant in the month of January, February and December when temperature was low (18.92 °C, 21.54 °C, 20.15 °C respectively), coupled with light rain. Aphids were least in May to August when temperature and rain was maximum. Heavy rain was found to wash away these pests.

![Graph showing mean monthly temperature, humidity and rainfall](image)

**Fig 3:** Mean monthly temperature, humidity and rainfall recorded from Jahangirnagar University campus during January-December, 2015.
3.4 Multiple sequence alignment analysis

Multiple sequence alignment was presented by showing the non-conserved region in letter and 70% identical or conserved region by dots (Fig 4). Acyrthosiphon pisum showed large amount of non-conserved region than other aphid species. Other species showed high conserved region among them.
**Fig 4:** Multiple sequence alignment of aphid pests based on COI gene sequences performed by Bioedit. Dot denotes the conserved region.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence 400-440</th>
<th>Sequence 450-490</th>
<th>Sequence 500-540</th>
<th>Sequence 550-590</th>
<th>Sequence 600-640</th>
<th>Sequence 650-690</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrystosiphon_pisum</td>
<td>AGAATTCATCTATTTAA</td>
<td>AGAATTCCTTTTACCGA</td>
<td>AGAATTCGTTTACCTTTA</td>
<td>AGAATTCCTTTTACCGA</td>
<td>AGAATTCGTTTACCTTTA</td>
<td>AGAATTCCTTTTACCGA</td>
</tr>
<tr>
<td>Aphis_craccivora</td>
<td>T..................</td>
<td>T..................</td>
<td>T..................</td>
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<td>T..................</td>
<td>T..................</td>
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<tr>
<td>Aphis_faba</td>
<td>G..................</td>
<td>C..................</td>
<td>T..................</td>
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<td>G..................</td>
<td>C..................</td>
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<tr>
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<td>T.C..................</td>
<td>C.C..................</td>
<td>C.C..................</td>
<td>C.C..................</td>
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<tr>
<td>Brevicoryne_brassica</td>
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<td>T..................</td>
<td>T..................</td>
<td>A..................</td>
<td>T..................</td>
<td>C..................</td>
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<tr>
<td>Hyadaphis_coriandi</td>
<td>T..................</td>
<td>C..................</td>
<td>T..................</td>
<td>T..................</td>
<td>T..................</td>
<td>C..................</td>
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<tr>
<td>Macrosiphum_euphorbi</td>
<td>C..................</td>
<td>C..................</td>
<td>C..................</td>
<td>C..................</td>
<td>C..................</td>
<td>C..................</td>
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<tr>
<td>Myzus_persicae</td>
<td>T..................</td>
<td>T..................</td>
<td>T..................</td>
<td>T..................</td>
<td>T..................</td>
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</tbody>
</table>

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3.5 Genetic distance

Interspecific genetic divergence range of aphid pests was 0.05-0.11. *My. persicae* showed highest (0.11) pairwise distance than rest. *L. erysimi* and *M. euphorbiae* both showed lowest (0.05) pairwise distance among studied aphid species.

![Table 3](Table3.png)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acyrthosiphon pisum</em></td>
<td>-</td>
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<tr>
<td><em>Aphis craccivora</em></td>
<td>0.08</td>
<td>-</td>
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<tr>
<td><em>Aphis fabae</em></td>
<td>0.09</td>
<td>0.06</td>
<td>-</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Aphis gossypii</em></td>
<td>0.10</td>
<td>0.08</td>
<td>0.07</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphis nerii</em></td>
<td>0.10</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brevicoryne brassicae</em></td>
<td>0.06</td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hyadaphis coriandri</em></td>
<td>0.07</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.09</td>
<td>0.06</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lipaphis erysimi</em></td>
<td>0.08</td>
<td>0.09</td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
<td>0.05</td>
<td>0.08</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td><em>Macrostiphum euphorbiae</em></td>
<td>0.05</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
<td>0.09</td>
<td>0.06</td>
<td>0.07</td>
<td>0.08</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Myzus persicae</em></td>
<td>0.10</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.09</td>
<td>0.08</td>
<td>0.09</td>
<td>0.09</td>
<td>-</td>
</tr>
</tbody>
</table>

3.6 Phylogenetic analysis

In both phylogenetic trees (Maximum likelihood and Neighbor-Joining tree) grouped the ten aphid species into three clusters. In neighbor joining tree, *A. craccivora, A. fabae, A. gossypii* and *A. nerii* were clustered in one group. *B. brassicae* and *L. erysimi* were grouped within same cluster and showed close relationship. *Ac. pism*, *M. euphorbiae, H. coriandri* and *My. persicae* clustered into another group and showed close relationship. Same results were also shown in maximum likelihood tree. According to branch length, *My. persicae* was genetically most distant species from the common ancestor observed in both tress (Fig 5).

![Fig. 5](Fig5.png)

**Fig. 5:** Evolutionary relationship among ten aphid species was constructed using MEGA6 software. In (a): Neighbor joining tree, the optimal tree with the sum of branch length = 0.36257569 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. In (b): Maximum likelihood tree, the evolutionary history was inferred by based on the Kimura 2-parameter model. The tree with the highest log likelihood (-2213.9018) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The bar at the bottom 0.01 is a scale for the genetic change.
3.7 Haplotype
Haplotype analysis of mitochondrial COI gene of aphid showed high genetic diversity among them. *A. craccivora* and *A. fabae* separated from its immediate common ancestors by 23 and 14 mutational steps. Again these two species were separated from *A. gossypii* by 9 mutational steps. *L. erysimi, B. brassicae, A. nerii, A. pisum, M. euphorbiae, H. coriandri* and *M. persicae* were diverged from its immediate common ancestors by 16, 11, 32, 17, 15, 15 and 30 mutation number respectively. *M. persicae* showed highest amount of mutated sites among other aphid species and genetically distant from its common ancestors by 30 mutational numbers (Fig 6).

Fig 6: Mitochondrial COI gene haplotype analysis of aphid species constructed by Popart 1.7 based on TCS network. Red circles represent the haplotype and small yellow circles represent the immediate common ancestors. Mutational steps are presented by hatch marks and numbers.

4. Discussion
A total of 10 agricultural important aphid pests have been recorded from Jahangirnagar University and its surroundings during this study period (Table 2). In Bangladesh, only about 30 aphid species under 20 genera infesting 58 host plants have been listed in national encyclopedia of Bangladesh, Banglapedia. This represents less than 0.75% of the world and 2.94% of the Oriental and 4.59% of Indian aphid fauna [21]. Bean aphid and Mustard aphid was previously described for their different management aspects elsewhere in Bangladesh [9, 20, 40]. Bhagat described 31 aphid species, distributed over 20 genera, damaging 31 species of agricultural crops of 12 different plant
families in India [2]. Rafi et al. (2010) found fourteen different winter vegetables and 13 ornamental plants attacking aphid in Aligarh region, Uttar Pradesh of India [3]. Globally, more than 4,000 species of aphids of 10 families have been described, some 250 of which are pests of crops and ornamental plants [13, 43].

Present study reported abundant aphids in the month of January, February and December when temperature was relatively low coupled with foggy weather. Aphids were least in May to August when temperature and rain fall was maximum (Fig 2, 3). When we consider Bean aphid (Aphis fabae), the infestation was found mostly high in the month of September. Life of cycle Lablab purpureus needs three month (August, September, October) for its vegetation, flowering and fruiting. Present findings were somewhat related to similar research of other researchers. Hasan et al. (2009) observed that aphid populations were correlated with the variation of weather parameters in mustard ecosystem. Aphid infestation increased because of trace amount of rainfall, dew point coupled with very low sunshine hours. Mustard aphid population declined after end of January. Kumar et al., (1997) reported that average temperature of 18.06 °C (Maximum 22.81° and Minimum 13.31 °C) under the influence of high relative humidity with the range from 80.71% to 86.5% provided conductive conditions for aphid incidence. Among the environmental factors, the maximum temperature and dew point positively correlated with aphid population [28]. Aphids reproduce quickly when conditions are right. During the spring and summer females give birth to live offspring (not eggs) which are all female. At the end of the summer males are produced, and mated females lay eggs that hatch the next spring [23] (Davidson and Antonelli, 2003).

To know the aphid phylogeny relationship, we obtained the COI gene sequences of ten aphid species from NCBI(www.ncbi.nlm.nih.gov). Except Acrhyosphion pisum, all species of aphid showed high conserve region (Fig 4). Interspecific K2P genetic divergence range of aphid was 0.05-0.11. Among studied all aphid species, My. persicae showed highest (0.11) pairwise genetic distance than rest (Table 3).

Some phylogenetic experiments have been performed to solve the mysterious problem of cryptic species of aphid [8, 34]. Previous works on aphid describing three distinct groups with either viviparous, oviparous and parthenogenetic groups [43]. More recent efforts have applied DNA sequence data to the reconstruction of Aphididae phylogeny [30]. In most cases, molecular tools have proved important for species identification and delimitation in aphids [6, 14, 31, 32]. To avoid the problems associated with the presence of too many mutations or insertion–deletion events, scientists now chose to work on a well-conserved coding region of mitochondrial DNA. Thao et al., 2004 experimented upon aphid COI gene to understand the phylogenetic relationship [42]. Rebjith et al., 2013 experimented on aphid pest and Brevicoryne brassicae, Hyperomyzus carduelinus and Brachycoroid helichrysi revealed that the intraspecific and interspecific distances ranged from zero to 3.8% and 2.31 to 18.9%, respectively [34].

In our present study, A. crassivora, A. fabae, A. gossypii and A. nerii showed monophyletic clade and closely related to each other in neighbor joining tree. B. brassicae and L. erysimi belonged to the same cluster. The highest bootstrap value (88%) was observed in cluster of Ac.pisum and M. euphorbiae(Fig 5. a). In maximum likelihood tree, highest bootstrap value (86%) was observed in the cluster of A. nerii, A. gossypii, A. craccivora and A. fabae (Fig 5.b). Ac. pisum and M. euphorbiae were closely related to each other. B. brassicae and L. erysimi were in monophyletic clade showing genetic close relationship. The haplotype analysis was done by TSC network and the results revealed that My. persicae was genetically most distant from its common ancestors by highest (30) mutation number (5) (Fig 6).From all phylogenetic analysis, it can be conclude that My. persicae was genetically most diverge among all other studied aphids.

5. Conclusions
Aphids are considered one of the most injurious pests of crops and economically important plants of agriculture and horticulture. Proper pest control program needs the biology, ecology and phylogenetic data to pests. In this study, seasonal abundance of bean aphid pest over the cropping season exposed that population decreased gradually from the last of September. Aphid attacked different parts of the plants specially fruiting part. Phylogenetic data revealed that all aphid pests are closely related and arose from a common ancestor. Thus making control program of one aphid pest will be applicable for other aphid pests because they share close phylogenetic relation. Further detailed studies on different aspects of aphid IPM will help the farmers to get rid of this pest in an environmental safety manner.

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7. References


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