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E Arockia Lenin
 PHM Division, National
Institute of Plant Health
Management, Hyderabad,
Telangana, India.

Intergeneric affinity of sixty two Aphidiine parasitoids (Hymenoptera: Braconidae: Aphidiinae) from different geographical regions

E Arockia Lenin**Abstract**

The mitochondrial DNA sequences of Cytochrome Oxidase subunit – I gene of Aphidiine parasitoids (Hymenoptera: Braconidae: Aphidiinae) were subjected to phylogenetic analysis to understand the intergeneric and interspecific variations and the role of geographical isolation on speciation; using CLUSTAL W in MEGA version 5.1. This analysis includes sixty two species belonging to eleven genera from sixteen countries viz., Algeria, Canada, Chile, China, Czech Republic, Iran, France, Greece, Libya, Montenegro, Poland, Serbia, Slovenia, Spitsbergen (Svalbard), Switzerland, USA. The pair-wise genetic distances were calculated and phylograms were constructed using Maximum Likelihood, Maximum Parsimony, and Neighbor-Joining methods. This analysis revealed phylogenetic relationships and the role of geographical isolation on speciation.

Keywords: Aphidiine, parasitoid, phylogeny, speciation, geographical isolation.

1. Introduction

Aphidiinae (Braconidae: Insecta: Hymenoptera) is an important solitary endoparasitoids (Mackauer & Starý 1967^[12]; Starý 1970, 1988^[22]) and many of them are important natural enemies of aphid pests in biocontrol programs (Brewer & Elliott 2004^[4]). Moreover, monophyly of Aphidine parasitoids evidenced by not only from morphological and behavioural information but also from molecular and embryological data (Mackauer, 1961^[13]; Tremblay, 1967^[29]; Tremblay and Calvert, 1971^[28]; Chou, 1984^[6]; Gaˆrdenfors, 1986^[11]; Quicke and van Achterberg, 1992^[17]; Whitfield, 1992^[30]; Belshaw and Quicke, 1997^[3]; Smith *et al.*, 1999)^[21]. Morphological characteristics were often failed to estimate the phylogeny of parasitoids due to extensive conflict between character systems and how the analyses were conducted (Mason, 1981)^[14]. In many cases researchers are unable to resolve many relationships within the subfamily and it has become clear that molecular studies are needed to resolve the phylogeny in an unambiguous manner. The COI and 16S genes have been used in many insect phylogenetic studies (Simon, *et al.*, 1994^[24]; Ambrose, *et al.*, 2014)^[1] at both lower and higher taxonomic levels as well as in the study of diverse hymenopteran groups (Dowton and Austin, 1994^[7]). Here an attempt has been made to find out the generic relationships at the subfamily level and to study the role of geographical isolation among these highly diversified parasitic wasps. Hence, we have included more number of Aphidiine species from Asian, European, American continents (Table 1).

Material and methods**Taxon sampling**

To understand the phylogeny of sixty two Aphidiine parasitoids based on Cytochrome oxidase subunit I gene, DNA sequences of these species (Table 1) were subjected to phylogenetic analysis. The sequences were retrieved from NCBI.

Phylogenetic analysis

The DNA sequences were subjected to pairwise distance analysis and the following phylogenetic trees were constructed: Maximum Parsimony, Maximum Likelihood and Neighbor-Joining by using MEGA 5 software (Tamura *et al.*, 2011)^[26]. Pairwise distance analysis was carried out with gap opening penalty 15 and gap extension penalty 6.66 (Clustal W) (Thompson *et al.*, 1994).

Correspondence**E Arockia Lenin**
 PHM Division, National
Institute of Plant Health
Management, Hyderabad,
Telangana, India.

Maximum Parsimony

The Maximum Parsimony analyses were carried out using MEGA5 (Tamura *et al.*, 2011) [26]. Bootstrap method was used with 100 replications and gap/missing data treatment by complete selection and the search method was Subtree-Pruning-Regrafting (SPR) and substitution based on nucleotide sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) was used (Felsenstein, 1985) [9]. The Maximum parsimony tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar, 2000) using search level 1. The substitution type based nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding and all the positions containing gaps and missing data were eliminated.

Maximum Likelihood

Maximum Likelihood analyses were run in MEGA 5 (Tamura *et al.*, 2011) [26]. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993) [25]. Initial tree for the heuristic search was obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The substitution type based nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding and all the positions containing gaps and missing data were eliminated.

Neighbor-Joining

Neighbor-Joining analyses were determined using MEGA5 (Tamura *et al.*, 2011) [26]. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987) [18]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) was used (Felsenstein, 1985) [9]. The evolutionary distances were computed using the Tajima-Nei method (Tajima & Nei, 1984) [24]. Codon positions included were 1st+2nd+3rd+Noncoding and all positions containing gaps and missing data were eliminated.

Results and Discussion

The aim of this study is to evaluate the intergeneric and intrageneric relationships at subfamily level and the role of geographical isolation among aphidiine parasitoids from different geographical locations worldwide. The results clearly indicate that the COI gene could be an adequate marker to study phylogeography, intrageneric and intergeneric affinity at subfamily level. Based on Cytochrome Oxidase subunit I gene sequences, three phylograms were constructed. The results of Maximum Parsimony, Maximum Likelihood and Neighbor-

Joining trees were analyzed. Similar phylogenetic analyses have been reported among Aphidiinae (Mackauer, 1961; [13] Mackauer and Stary', 1967 [12]; Stary', 1970 [22]; Tremblay and Calvert, 1971 [28]; Ga'rdenfors, 1986 [11]; O'Donnell, 1989 [16]; Finlayson, 1990) [10]. Phylograms obtained from Neighbor-Joining and Maximum Likelihood analyses were almost similar to Maximum Parsimony analysis at generic level but the arrangement of species was found to be different. Monophyly was observed for all the species of *Praon* in ML, NJ and MP trees; *Aphidius* and *Adialytus* species in ML and NJ tree; and *Adialytus*, *Lysiphlebus*, *Euphidius* and *Pauesia* in MP tree. Polyphyly was also exhibited by *Aphidius*, *Lysiphlebus*, *Praon* and *Ephedrus* species in all trees. The Allopatric speciation was observed among *Aphidius colemani* species from France, Libya, Algeria and Greece.

Lysiphlebus species of France and Czech Republic are closer to each other despite geographical isolation. Similar observations have been made in *Adialytus salicaphis* species from France and Iran; and *Aphidius ervi* from France and USA; *Aphidius uzbekistanicus* from China and Poland; *Aphidius colemani* from France, Libya, Algeria and Greece; *Praon yomenae* from France and Serbia; *Praon dorsale* from Switzerland and Montenegro. Moreover, *Praon volucre* from Serbia, Switzerland and France are closely related in ML tree but not in NJ and MP trees due to inclusion of *Praon bicolor* from France. A deep divergence was observed among *Aphidius* and *Praon* species of various regions due to geographical barrier. The influence of speciation has been studied among *Cotesia* species (Arctander *et al.* 1999; [2] Sezonlin *et al.* 2006) [19]. On the other hand geographical barrier plays an important role in *Aphidius rhopalosiphii* from Montenegro and France; *Aphidius platensis* from Iran and Chile; and *Praon volucre* from Serbia, Switzerland and Montenegro.

The intergeneric affinity was observed among *Lysiphlebus*, *Adialytus*, *Aphidius*, *Praon* and *Ephedrus* species. In this study, *Praon* is found to be more closer to *Felciconus* and *Lipolexis* rather than being closer to *Binodoxys* as suggested by Downton *et al.* (1994) [7]. The following are observed to be the sister taxa in all the trees: *Ephedrus helleni* and *Ephedrus lacertosus*; *Binodoxys angelicae* and *Binodoxys centaurea*; *Praon barbatum*, *Praon staticobii* and *Praon gallicum*; *Aphidius aquilus*, *Aphidius salicis* and *Aphidius schimischeki*; and *Aphidius avenae* and *Aphidius rosea*.

The intergeneric affinity was observed between *Euaphidius*, *Adialytus* and *Aphidius* in ML, NJ and MP trees. *Binodoxys*, *Falcons* and *Lipolexis*; *Monoctonus*, *Pauesia* and *Ephedrus* in ML and NJ Trees; *Monoctonus* and *Ephedrus nacheri* in MP tree. In addition to this, a separate lineage exhibiting unique characteristics was observed for *Aphidius ribis* in ML and NJ trees; *Falciconus pseudoplatani* and *Lipolexis gracilis* in MP tree; *Euaphidius cingulatus* in ML and MP trees.

Table 1: Sixty two Aphidiine parasitoids subjected to phylogenetic analyses.

Species	Locality	Genbank Accession No.
<i>Adialytus ambiguus</i> Förster	Iran	KJ719613.1
<i>Adialytus salicaphis</i> Fitch	France	JN620542.1
<i>Adialytus salicaphis</i> F.	Iran	KJ719621.1
<i>Adialytus thelaxis</i> S.	Serbia	KJ719623.1
<i>Aphidius avenae</i> Haliday	France	JN620547.1
<i>Aphidius aquilus</i> Mackauer	France	JN620544.1
<i>Aphidius colemani</i> Viereck	France	JN620548.1
<i>Aphidius colemani</i> V.	Libya	KJ615373.1
<i>Aphidius colemani</i> V.	Algeria	KJ615362.1

<i>Aphidius colemani</i> V.	Greece	KJ615371.1
<i>Aphidius eadyi</i> Stary	France	JN620552.1
<i>Aphidius ericaphidis</i> Pike & Starý	Canada	EU574902.1
<i>Aphidius ervi</i> H.	France	JN620557.1
<i>Aphidius ervi</i> H.	USA	AY427884.1
<i>Aphidius funebris</i> M.	France	JN620560.1
<i>Aphidius leclanti</i> Tomanovic & Chaube	Spitsbergen, Svalbard	JQ723426.1
<i>Aphidius matricariae</i> H.	France	JN620563.1
<i>Aphidius platensis</i> Brethes	Iran	KJ615364.1
<i>Aphidius platensis</i> B.	Chile	KJ615361.1
<i>Aphidius rhopalosiphi</i> de Stefani-Perez	France	JN620572.1
<i>Aphidius rhopalosiphi</i> de S.	Montenegro	KJ615376.1
<i>Aphidius ribis</i> H.	France	JN620579.1
<i>Aphidius rosae</i> H.	France	JN620581.1
<i>Aphidius salicis</i> H.	France	JN620584.1
<i>Aphidius schimitscheki</i> S.	France	JN620586.1
<i>Aphidius sonchi</i> Marshall	France	JN620588.1
<i>Aphidius transcaspicus</i> Telenga	Greece	KJ615375.1
<i>Aphidius urticae</i> H.	France	JN620591.1
<i>Aphidius uzbekistanicus</i> Luzhetzki	China	KF597708.1
<i>Aphidius uzbekistanicus</i> L.	Poland	JN164742.1
<i>Aphidius viaticus</i> Sedlag	France	JN620596.1
<i>Binodoxys acalephae</i> M.	France	JN620601.1
<i>Binodoxys angelicae</i> H.	France	JN620604.1
<i>Binodoxys centaurea</i> H.	France	JN620610.1
<i>Ephedrus helleni</i> M.	France	JN620617.1
<i>Ephedrus lacertosus</i> H.	France	JN620620.1
<i>Ephedrus nacheri</i> Quilis Perez	France	JN620621.1
<i>Euaphidius cingulatus</i> Ruthe	France	JN620629.1
<i>Falciconus pseudoplatani</i> M.	France	JN620632.1
<i>Lipolexis gracilis</i> F.	France	JN620636.1
<i>Lysiphlebus cardui</i> M.	Czech Republic	KM408531.1
<i>Lysiphlebus fabarum</i> M.	France	JN620652.1
<i>Lysiphlebus fabarum</i> M.	Czech Republic	KM408525.1
<i>Lysiphlebus testaceipes</i> Cresson	France	JN620656.1
<i>Lysiphlebus confuses</i> Tremblay & Eady	Serbia	KM408535.1
<i>Monoctonus caricis</i> H.	France	JN620659.1
<i>Monoctonus crepidis</i> H.	France	JN620663.1
<i>Pauesia juniperorum</i> S.	France	JN620665.1
<i>Praon abjectum</i> H.	Serbia	KC128669.1
<i>Praon barbatum</i> M.	France	JN620671.1
<i>Praon bicolor</i> M.	France	JN620673.1
<i>Praon dorsale</i> H.	Switzerland	KJ698526.1
<i>Praon dorsale</i> H.	Montenegro	KJ698491.1
<i>Praon flavinode</i> H.	France	JN620677.1
<i>Praon gallicum</i> S.	France	JN620680.1
<i>Praon longicorne</i> M.	Montenegro	KJ698489.1
<i>Praon staticobii</i> Tomanovic & Petrovic	Slovenia	KJ698524.1
<i>Praon volucre</i> H.	France	JN620690.1
<i>Praon volucre</i> H.	Serbia	KJ698488.1
<i>Praon volucre</i> H.	Switzerland	KJ698515.1
<i>Praon yomenae</i> Takada	France	JN620694.1
<i>Praon yomenae</i> T.	Serbia	KJ698530.1

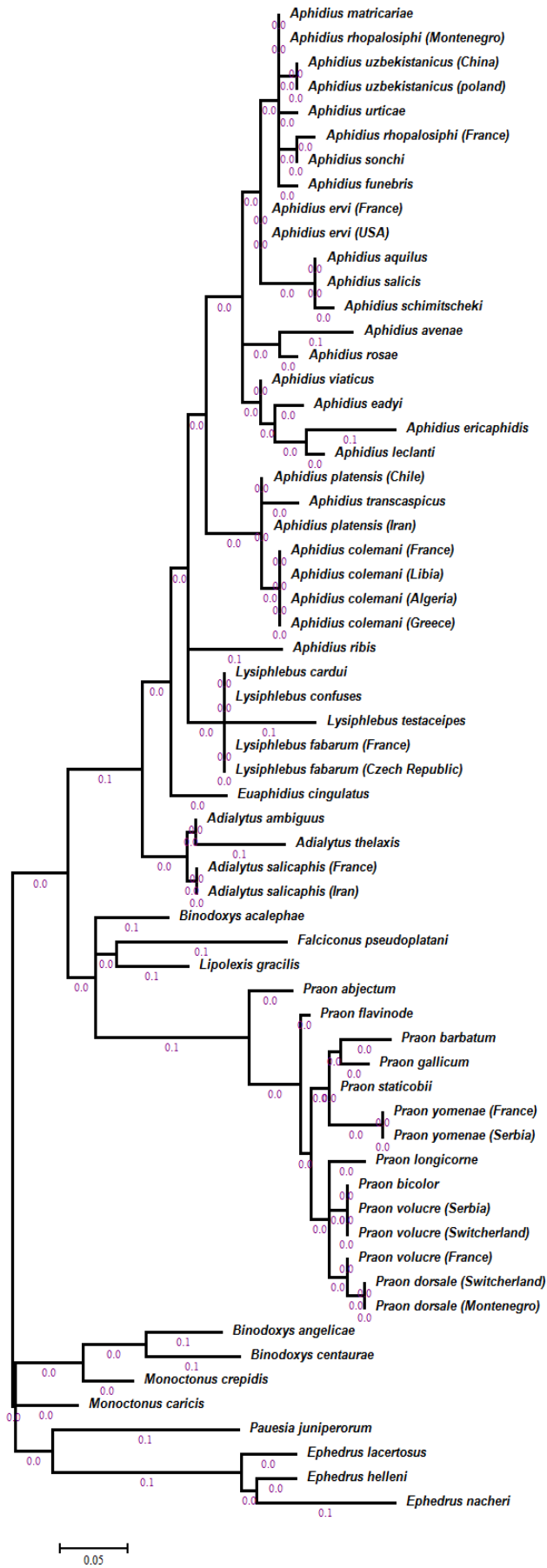


Fig 1: Maximum Likelihood tree based on COI gene variations showing the relationships among sixty two aphidiine parasitoids.

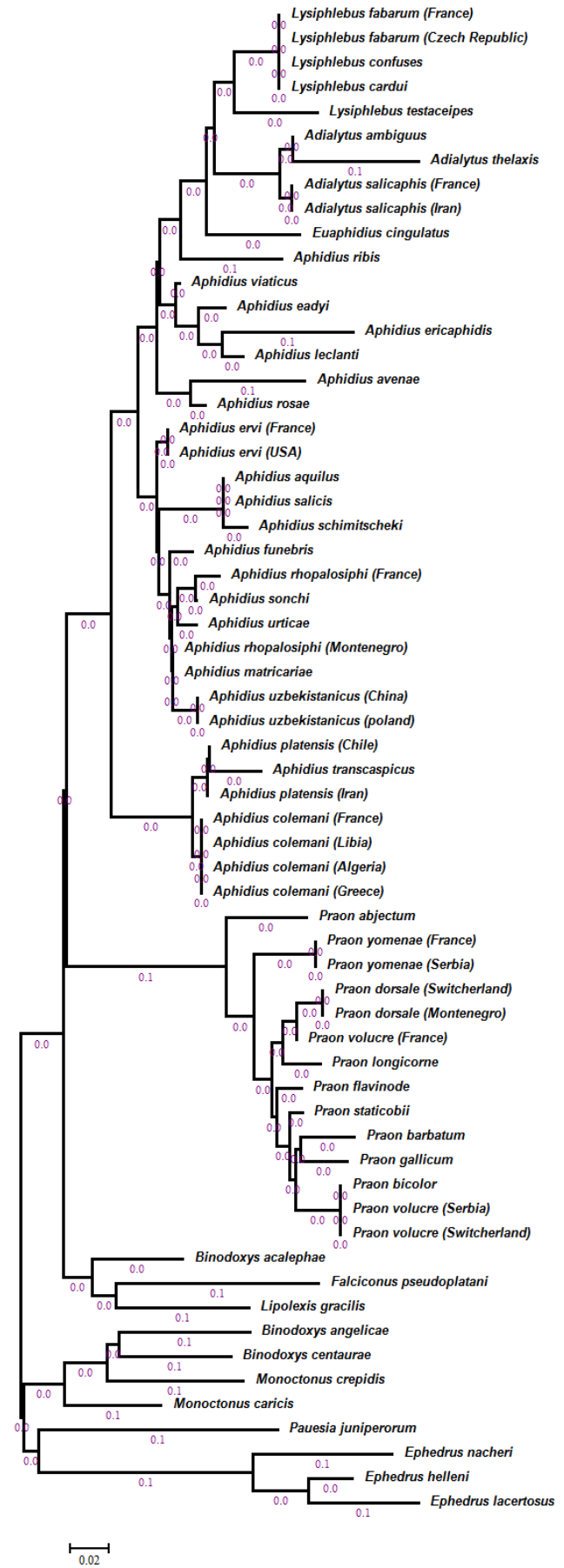


Fig 2: Neighbour -Joining tree based on COI gene variations showing the relationships among sixty two aphidiine parasitoids.

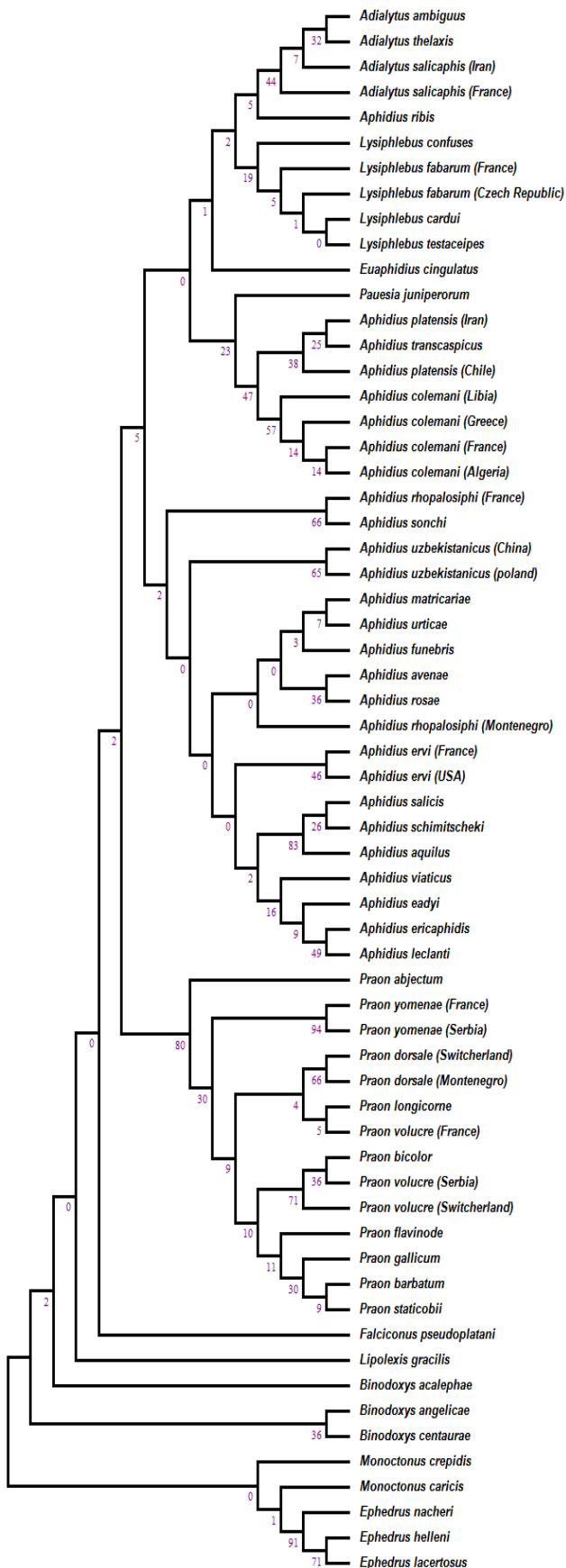


Fig 3: Maximum Parsimony tree based on COI gene variations showing the relationships among sixty two aphidiine parasitoids.

Conclusion

This study was intended to provide the geographic and generic context for understanding the relationships among Aphidiine parasitoids. The results further reveal the utility of Cytochrome Oxidase subunit I DNA sequences in phylogenetic analysis.

The findings clearly describe the intergeneric and intrageneric phylogenetic affinity and diversity of aphidiine parasitoids from different geographical locations. Further studies in this direction could lead to meaningful revision, regrouping, or replacement of species.

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