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Identification and Computational Characterization of miRNAs of *Apis Florae* and their Phylogenetic Analysis

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Abstract—*MicroRNAs(miRNAs), areendogenous, ~22-nucleotidelong RNA molecules. They bind to the complementary sites on target mRNAs and regulate protein production of the target transcript by unknown mechanisms. Since the discovery of first miRNA in Caenorhabditiselegans, different approaches have been pursued for the prediction of miRNAs and their target(s). Because of many difficulties and limitations involved in the experimental identification of spatially and temporally expressed miRNAs, many computational approaches have been successfully employed for prediction of miRNAs and their target(s). In the present study is demonstrated a genome-wide computational approach to predict miRNAs and their phylogenetic analysis in Apisflorea from known micro RNAs of Apismellifera. 67 miRNAs were predicted by genome-wide homology search against all the reported miRNAs of Apismellifera. These miRNAs were further validated by phylogenetic analysis. Over one third of the genome appears to be regulated by miRNAs which are involved in fundamental cellular processes and are negative regulators of gene expression. MicroRNAs (miRNAs) are evolutionary conserved across broad phylogenetic distances and have gained considerable attention about evolution, genetic and phylogenetic analysis. Comparing sequences of miRNA precursors within a species and between closely related species should thus help to determine patterns of molecular evolution and the timescales at which different aspects of the evolution of miRNAs can be best investigated. Prediction and phylogenetic analysis of microRNA from honey bee will serve as a purpose as it is not only valued by farmers for its ability to produce honey and pollinate crops, it also has its importance in agriculture, the honey bee serves as a model organism for studying human health issues including immunity, allergic reaction, antibiotic resistance, development, mental health, longevity and diseases of the X chromosome.*

1. INTRODUCTION

Micro-RNAs (miRNAs) are a subset of small non-coding RNAs that are 18-24 nucleotides long and have a key role in regulating gene expression in eukaryotes. They are produced from a primary full-length transcript (pri-miRNA), which is cleaved to form hairpin structures around 70 nucleotides in length [1].These are called precursor miRNAs (pre-miRNAs) and are exported to the cytoplasm to be processed further to functional mature miRNAs by the ribonuclease Dicer[2].

Finding miRNA precursors in genomes is an important task, where computational methods are required. The goal of these methods is to select potential pre-miRNAs which could be validated by
experimental methods. Experimental identification of miRNAs is difficult and cumbersome. Consequently, several computational methods have been developed and employed for reliable and rapid identification of temporally, spatially and meagrely expressed miRNA genes. Among many approaches that are being used for the prediction of miRNAs, the one based on phylogenetic conservation of sequences across multiple species is reported to provide reliable prediction of functional miRNAs [3].

The miRFinder program predicts miRNAs based on the mature miRNA sequences[4].We carried out a whole genomehomology search of *A. florea* against all the miRNAs of *A.mellifera* reported in the miRNA registry. We intentionally used the phylogenetic conservation approach considering that each predicted miRNA could be compared with its experimentally validated miRNA homologue.

Honey bees play a critical role in agriculture. The most important role honey bees play is actually not honey production, but pollination [5]. Honey Bee stings are routinely used for treatment of arthritis, multiple sclerosis and other auto-immune diseases[6].miRNAs usually have perfect or near-perfect pairing with their messenger RNA targets and induce gene repression through degradation of their target transcripts [7, 12].A miRNA may have multiple different mRNA targets, and a given target might similarly be targeted by multiple miRNAs[8].MicroRNAs are significant phylogenetic markers because of their astonishingly low rate of evolution[9].

2. MATERIALS AND METHODS

2.1 Prediction of miRNAs

A total of 218 precursors and 222 known mature miRNA sequences of A*pismellifera*were identified and downloaded

from miRBase[10].The genome sequence of target organism *Apisflorea*was available at NCBI database.

2.2 Homology search

A BLASTn [11] search of all the 222 miRNA sequences in the whole genome sequence of *A. florea* was carried out with the evalue< 0.01 and the default parameters were used, including low complexity filter. The two criteria used for screening the BLAST results were:1) More than 80% identity between each potential *A.mellifera*, 2) The length difference between each potential *T. castaneum* miRNA and the corresponding miRNA in the reference set is not more than three base.

2.3 Secondary structure validation

Pre-miRNA sequences were extracted using a sliding window of about 100 nt. in size (moving in increments of approximately 10 nt.) from the region ~80 nt upstream of the beginning of the mature miRNA to ~80 nt downstream of the miRNA. Extracted miRNA precursor sequences were then submitted to Mfold for checking of the fold-back secondary structure[13]. The three criteria used for selecting premiRNA structures were: a)Structure should have free energy change (ΔG) less than or equal to –18kcal/mole, b)The bulge size (un-pairing) should not be more than 7 bp and c)Mature miRNA hit should be on the stem region of hairpin structure.

2.4 Phylogenetic analysis

To study conserved micro RNAs as predicted in *Apisflorea* genome phylogenetic analysis was performed using MEGA 5 software [14, 15]. Blastn homology search was performed for all predicted precursor micro RNAs to find their homologues. The blast results were selected on basis of two criteri a)The e-value < 0.002, b)Sequence identity should be more than 85%.

Phylogenetic analysis of the miRNA sequences selected on basis of above criteria through UPGMA was obtained using MEGA5 software. A tree was constructed by the software showing the ancestral relationship among the sequences. The tree gives different clusters, showing their relationship with each other. The sequences which lie in the same cluster are closely related with those lying in distant clusters.

3. RESULTS AND DISCUSSIONS

A BLASTn search of all the known mature miRNAs of *A.mellifera* against the whole genome sequences of *A.florea* resulted in 149 hits. The 149 extracted precursor sequences of *A.florea*were submitted to Mfold to predict secondary structure. The secondary structure of the sequences were selected on the basis of three criteria: Delta G value less than or equal to -18kcal/mol, bulge size (un-pairing) should not be more than 7 bp,mature miRNA hit on the stem region of hairpin structure.Finally, the screening resulted in 67 potential miRNAs (Table 1) and their premiRNA sequences, which can fold back and make typical hairpin-like secondary structures. This result made us find mature potential microRNAs of *Apisflorea* that conserved as in *Apismellifera*. The genomic locations of every potential microRNA can be seen in the Table 1.

Table 1: Details of genomic locations and sequences of 67 predicted microRNAs (miRNAs) from *Apisflorea*.

TABLE **iRNA**	TABLE **inkage group**	TAB **trand**	TABL **tarting position**	TABLE **nding position**	TABLE **atch extent**	TABLE VII. **Mature miRNA sequence**
TABLE fl-miR-1	TABLE G_5	TAB	TABL 06	TABLE 87	TABLE 2/22	TABLE XIV. UGGAAUGUAAAGAAGUAUGGAG TABLE XV.
TABLE fl-miR-3719	TABLE G_3	TAB	TABL 790105 TABL	TABLE 790084 TABLE	TABLE 2/22	TABLE XXIV. UACGGAUUGCGUGACUUUUCGA TABLE XXV.
TABLE fl-miR-3759	TABLE G_3	TAB	TABL 39387	TABLE 39565	TABLE 9/19	TABLE XXXII. GACUCACGUCGACUGGGUGUCCGC TABLE XXXIII.
TABLE fl-miR-3049-3p	TABLE G_3	TAB	TABL 53947	TABLE 54128	TABLE 2/22	TABLE XL. UCCGUCCAACUCCUUUCCGUCU TABLE XLI.
TABLE fl-miR-279a	TABLE G_6	TAB	TABL 394785	TABLE 394966	TABLE 2/22	TABLE XLVIII. UGACUAGAUCCACACUCAUUAA TABLE XLIX.
TABLE fl-miR-275	TABLE G_7	TAB	TABL 929131 TABL	TABLE 929153 TABLE	TABLE 3/23	TABLE LVIII. UCAGGUACCUGAAGUAGCGCGCG TABLE LIX.
TABLE fl-miR-6000b	TABLE G_9	TAB	TABL 13385	TABLE 13567	TABLE 3/23	TABLE LXVI. UAGAGACGAGUAGUACCCACGAG TABLE LXVII.
TABLE fl-miR-92c	TABLE G_2	TAB	TABL 55419	TABLE 55602	TABLE 4/24	TABLE LXXIV. AGGUUGGGAUGUGGGCAUUAUUUG TABLE LXXV.
TABLE fl-miR-3773	TABLE G_3	TAB	TABL 34649	TABLE 34830	TABLE 2/22	TABLE LXXXII. UAAGCACAGCUCUUGUCUGUAA TABLE LXXXIII.
TABLE fl-miR-33	TABLE G_5	TAB	TABL 626100	TABLE 626278	TABLE 9/19	TABLE XC. GUGCAUUGUAGUUGCAUUG TABLE XCI.
TABLE fl-miR-927b	TABLE G_6	TAB	TABL 62387	TABLE 62569	TABLE 3/23	TABLE XCVIII. UUUUAGAAUUCCUACGCUUUACC TABLE XCIX.
TABLE fl-miR-7	TABLE G_4	TAB	TABL 440076	TABLE 440258	TABLE 3/23	TABLE CVI. UGGAAGACUAGUGAUUUUGUUGU TABLE CVII.

TABLE fl-miR-1000	TABLE G_4	TAB	TABL 00	TABLE 80	TABLE 3/23	TABLE CXIV. AUAU UGUCUUGUCACA GCAGU TABLE CXV.
TABLE fl-miR-277	TABLE G_3	TAB	TABL 7240 86	TABLE 72426 8	TABLE 3/23	TABLE CXXII. UAAA UGCACUAUCUGG UACGACA TABLE CXXIII.
TABLE fl-miR-210	TABLE G_8	TAB	TABL 2180 03	TABLE 97614 8	TABLE 1/21	TABLE CXXX. U UGUGCGUGUGAC AGCGGCUA TABLE CXXXI.
TABLE fl-miR-29b	TABLE G_6	TAB	TABL 2178 24	TABLE 21800 3	TABLE 0/20	TABLE CXXXVIII. U AGCACCAUUUGA AAUCAGU TABLE CXXXIX.
TABLE fl-miR-6067	TABLE G_5	TAB	TABL 4241 6	TABLE 42597	TABLE 2/22	TABLE CXLVI. A AACGGAUCAAGC UUUUUGUGA TABLE CXLVII.
TABLE fl-miR-8	TABLE G_2	TAB	TABL 4225 22	TABLE 42270 4	TABLE 3/23	TABLE CLIV. UAAU ACUGUCAGGUAA AGAUGUC TABLE CLV.
TABLE fl-miR-92b	TABLE G_6	TAB	TABL 5293 45	TABLE 52952 6	TABLE 2/22	TABLE CLXII. AAUU GCACCCGUCCCGG CCUGA TABLE CLXIII.
TABLE fl-miR-3723	TABLE G_6	TAB	TABL 2075 5	TABLE 20934	TABLE 0/20	TABLE CLXX. ACAG CGUUUCAAAGUU UUCGUA TABLE CLXXI.
TABLE fl-miR-278	TABLE G_6	TAB	TABL 7299 1	TABLE 73172	TABLE 2/22	TABLE CLXXVIII. U CGGUGGGACUUU CGUCCGUUU TABLE CLXXIX.
TABLE fl-miR-3776	TABLE G_6	TAB	TABL 8546 77 TABL	TABLE 85469 6 TABLE	TABLE 0/20	TABLE CLXXXVIII. GGAGGGGGGGAG AGAGAAGCG TABLE CLXXXIX.
TABLE fl-miR-iab-4	TABLE G_4	TAB	TABL 4748 07	TABLE 47498 8	TABLE 0/20	TABLE CXCVI. A CGUAUACUGAAU GUAUCCUGA TABLE CXCVII.
TABLE fl-miR-252a	TABLE G_3	TAB	TABL 4411 65	TABLE 44134 6	TABLE 2/22	TABLE CCIV. AUAA GUACUAGUGCCG CAGGAG TABLE CCV.
TABLE fl-miR-6043	TABLE G_4	TAB	TABL 9288 74	TABLE 92905 5	TABLE 2/22	TABLE CCXII. AUGG UGACCGUGAUCU AUUCCA TABLE CCXIII.
TABLE fl-miR-283	TABLE G_4	TAB	TABL 7551	TABLE 7730	TABLE 0/20	TABLE CCXX. AAAU AUCAGCUGGUAA UUCU TABLE CCXXI.
TABLE fl-miR-927b	TABLE G_7	TAB	TABL 8237 99	TABLE 82398 0	TABLE 2/22	TABLE CCXXVIII. U UUUAGAAUUUGU ACGCUCUGU TABLE CCXXIX.
TABLE fl-miR-9b	TABLE G_7	TAB	TABL 9624 3	TABLE 96425	TABLE 3/23	TABLE CCXXXVI. G CUUUGGUAAUCU AGCUUUAUGA TABLE CCXXXVII.
TABLE fl-miR-6051	TABLE G_7	TAB	TABL 6584 4	TABLE 66025	TABLE 2/22	TABLE CCXLIV. A AAAUUGCGCACG CUCUGAAUAU TABLE CCXLV.
TABLE fl-miR-6039	TABLE G_9	TAB	TABL 2290 7	TABLE 23088	TABLE 2/22	TABLE CCLII. AAUC GAACGCGUGAGU UUACGU TABLE CCLIII.
TABLE fl-miR-980	TABLE G_9	TAB	TABL 8387 80	TABLE 83896 2	TABLE 3/23	TABLE CCLX. AAGC UGCCUUUUGAAG GGCAACA TABLE CCLXI.
TABLE fl-miR-3782	TABLE G_9	TAB	TABL 6258	TABLE 6441	TABLE 4/24	TABLE CCLXVIII. C CUGCAGAGACAU CUGGCGGACAC TABLE CCLXIX.
TABLE fl-miR-2	TABLE G_4	TAB	TABL 3631 5	TABLE 36497	TABLE 3/23	TABLE CCLXXVI. U AUCACAGCCAGC UUUGAUGAGC TABLE CCLXXVII.
TABLE fl-miR-3718a	TABLE G_4	TAB	TABL 0321 86	TABLE 03236 6	TABLE 1/21	TABLE CCLXXXIV. CCCCUGUCCUGUC CCGAUAG TABLE CCLXXXV.
TABLE fl-miR-31a	TABLE G_2	TAB	TABL 1257 1	TABLE 12752	TABLE 2/22	TABLE CCXCII. G GCAAGAUGUCGG CAUAGCUGA TABLE CCXCIII.
TABLE fl-miR-263b	TABLE G_6	TAB	TABL 6382 2	TABLE 64002	TABLE 1/21	TABLE CCC. CUUG GCACUGGAAGAA UUCAC TABLE CCCI.
TABLE fl-miR-6054	TABLE G_3	TAB	TABL 2460 11	TABLE 24619 2	TABLE 2/22	TABLE CCCVIII. C ACGGUUGAACGA UGUGACGGU TABLE CCCIX.
TABLE fl-miR-11	TABLE G_3	TAB	TABL 3483 11	TABLE 34849 2	TABLE 2/22	TABLE CCCXVI. C AUCACAGGCAGA GUUCUAGUU TABLE CCCXVII.
TABLE fl-miR-193	TABLE G_3	TAB	TABL 93	TABLE 73	TABLE 1/22	TABLE CCCXXIV. U ACUGGCCUGCUA AGUCCCAA TABLE CCCXXV.
TABLE fl-miR-279c	TABLE G_6	TAB	TABL 1806 50	TABLE 18083 1	TABLE 2/22	TABLE CCCXXXII. U GACUAGAGUCAC ACUCGUCCA TABLE CCCXXXIII.
TABLE fl-miR-993	TABLE G_6	TAB	TABL 0829 19	TABLE 08310 1	TABLE 3/23	TABLE CCCXL. G AAGCUCGUCUCU ACAGGUAUCU TABLE CCCXLI.
TABLE fl-miR-315	TABLE G_7	TAB	TABL 9941 2	TABLE 99572	TABLE 2/22	TABLE CCCXLVIII. UUUGAUUGUUGC UCAGAAAGC TABLE CCCXLIX.

TABLE fl-miR-929	TABLE G_7	TAB	TABL 491686	TABLE 491866	TABLE 1/21	TABLE CCCLVI. AUUGACUCUAGUAGGGAGUCC TABLE CCCLVII.
TABLE fl-miR-12	TABLE G_5	TAB	TABL 6294	TABLE 6476	TABLE 3/23	TABLE CCCLXIV. UGAGUAUUACAUCAGGUACUGGU TABLE CCCLXV.
TABLE fl-miR-2796	TABLE G_5	TAB	TABL 806050	TABLE 806232	TABLE 3/23	TABLE CCCLXXII. GUAGGCCGGCGGAAACUACUUGC TABLE CCCLXXIII.
TABLE fl-miR-2944	TABLE G_5	TAB	TABL 734791	TABLE 734973	TABLE 3/23	TABLE CCCLXXX. UAUCACAGCAGUAGUUACCUGGU TABLE CCCLXXXI.
TABLE fl-miR-6045	TABLE G_5	TAB	TABL 96819	TABLE 97000	TABLE 2/22	TABLE CCCLXXXVIII. UUCACUGGACGGCAAUGGGCU TABLE CCCLXXXIX.
TABLE fl-miR-971	TABLE G_5	TAB	TABL 171	TABLE 353	TABLE 3/23	TABLE CCCXCVI. UUGGUGUUCUACCUUACAGUGAG TABLE CCCXCVII.
TABLE fl-miR-3783	TABLE G_4	TAB	TABL 93989	TABLE 94171	TABLE 3/23	TABLE CDIV. UACUUUCAAUUGUUUGAUGAGGU TABLE CDV.
TABLE fl-miR-87	TABLE G_5	TAB	TABL 484597	TABLE 484597	TABLE 0/20	TABLE CDXII. GUGAGCAAAGUUUCAGGUGU TABLE CDXIII.
TABLE fl-miR-279b	TABLE G_6	TAB	TABL 735259 TABL	TABLE 735236 TABLE	TABLE 4/24	TABLE CDXXII. UGACUAGAUCGAAAUACUCGUCCC TABLE CDXXIII.
TABLE fl-miR-281	TABLE G_2	TAB	TABL 03990	TABLE 04172	TABLE 3/23	TABLE CDXXX. UGUCAUGGAGUUGCUCUCUUUGU TABLE CDXXXI.
TABLE fl-miR-125	TABLE G_2	TAB	TABL 766057	TABLE 766238	TABLE 2/22	TABLE CDXXXVIII. CCCUGAGACCCUAACUUGUGA TABLE CDXXXIX.
TABLE fl-miR-996	TABLE G_8	TAB	TABL 394562	TABLE 394742	TABLE 1/21	TABLE CDXLVI. UGACUAGAUACAUACUCGUCU TABLE CDXLVII.
TABLE fl-miR-6005-3p	TABLE G_9	TAB	TABL 599763	TABLE 599944	TABLE 2/22	TABLE CDLIV. ACAUGCGUAAGAGAUUAUAUGU TABLE CDLV.
TABLE fl-miR-9a	TABLE G_2	TAB	TABL 76916	TABLE 77098	TABLE 3/23	TABLE CDLXII. CUUUGGUUAUCUAGCUGUAUGA TABLE CDLXIII.
TABLE fl-miR-981	TABLE G_3	TAB	TABL 06730	TABLE 06911	TABLE 2/22	TABLE CDLXX. UUCGUUGUCAACGAAACCUGCA TABLE CDLXXI.
TABLE fl-miR-282	TABLE G_6	TAB	TABL 67036	TABLE 67223	TABLE 8/28	TABLE CDLXXVIII. AUUUAGCCUCUCCUAGGCUUUGUCUGU TABLE CDLXXIX.
TABLE fl-miR-263a	TABLE G_6	TAB	TABL 72455	TABLE 72638	TABLE 4/24	TABLE CDLXXXVI. UAAAUGGCACUGGAAGAAUUCAC TABLE CDLXXXVII.
TABLE fl-miR-Let-7	TABLE G_8	TAB	TABL 765559	TABLE 765739	TABLE 1/21	TABLE CDXCIV. UGAGGUAGUAGGUUGUAUAGU TABLE CDXCV.
TABLE fl-miR-79	TABLE G_8	TAB	TABL 96246	TABLE 96426	TABLE 1/21	TABLE DII. UAAAGCUAGAUUACCAAAGCA TABLE DIII.
TABLE fl-miR-317	TABLE G_4	TAB	TABL 747358	TABLE 747542	TABLE 5/25	TABLE DX. UGAACACAGCUGGUGGUAUCUCAGU TABLE DXI.
TABLE fl-miR-137	TABLE G_6	TAB	TABL 078	TABLE 279	TABLE 2/22	TABLE DXVIII. UUAUUGCUUGAGAAUACACGUA TABLE DXIX.
TABLE fl-miR-932	TABLE G_2	TAB	TABL 784347	TABLE 784528	TABLE 2/22	TABLE DXXVI. UCAAUUCCGUAGUGCAUUGCAG TABLE DXXVII.
TABLE fl-miR-306	TABLE G_4	TAB	TABL 95991	TABLE 96172	TABLE 2/22	TABLE DXXXIV. UCAGGUACUGAGUGACUCUGAG TABLE DXXXV.
TABLE fl-miR-305	TABLE G_4	TAB	TABL 929218	TABLE 929400	TABLE 3/23	TABLE DXLII. AUUGUACUUCAUCAGGUGCUCUG TABLE DXLIII.
TABLE fl-miR-307	TABLE G_4	TAB	TABL 78861799	TABLE 231580	TABLE 3/23	TABLE DL. UCACAACCUUUUUGAGUGAGCGA TABLE DLI.
TABLE fl-miR-219	TABLE G_5	TAB	TABL 263	TABLE 445	TABLE 3/23	TABLE DLVIII. UGAUUGUCCAAACGCAAUUCUUG TABLE DLIX.
TABLE fl-miR-3726	TABLE G_2	TAB	TABL 431744	TABLE 431925	TABLE 2/22	TABLE DLXVI. AACGAGUGGUGGAUGCCAGCGU TABLE DLXVII.
TABLE fl-miR-3049-5p	TABLE G_3	TAB	TABL 53907	TABLE 54089	TABLE 3/23	TABLE DLXXIV. CGGGAAGGUAGUUGCGGCGGAU TABLE DLXXV.

67 predicted micro RNAs in the present study included three copies of afl-mir-279 without one or two mismatches and two copies of afl-mir-92, afl-mir-9, afl-mir-263, afl-mir-927 with one or two mismatches in mature micro RNA sequence. Micro RNAs are conserved along species,this will be further verified by using phylogenetic approach.

4. PHYLOGENETIC ANALYSIS OF PREDICTED *APISFLOREA* MIRNAS

Blast search for all the predicted precursor micro RNAs was performed. The resulted hits were selected on basis of more than 85% identity ande-value should be less than 0.002. This resulted in selection of 4 potential precursor sequences having the accession no.NW_003791204, NW_003789938, NW_003789699,NW_003790748. Each of the four *Apisflorea* miRNA sequences were studied for their similarity patterns and BLAST was therefore performed. The sequences having lowest e-value are more closely related, while the difference in e-value shows the dissimilarity among them.Phylogenetic analysis of the four *A.flore*amiRNA : microRNA 277 (MIR277), 971 (MIR971), iab-4 (MIRiab-4), 8 (MIR8),9a (MIR9a) has been done through MEGA 5.0.

5. INTERPRETATION OF PHYLOGENETIC TREES

The UPGMA rooted tree diagram of miRNA 277(mir 277) shows different clusters formation. Organism that originated from same ancestors, are placed in same clusters, whereas, those which are distant from each other are placed in separate clusters. *D.erecta*mir-210 precursor RNA, *D.sechellia* mir-210 precursor RNA, *D.yakuba* mir-210 precursor RNA, *D.willistoni* mir-210 precursor RNA,*D.mojavensis* mir-210 precursor RNA lie in the same clusters. *D.psuedoobscura*mir-210a precursor RNA and *D.persimilis* mir-210 lie in same cluster. *A .mellifera* mir-210 and *Nasoniavitripennis*also lie in same cluster and are distant from the rest of the clusters. (Fig. 1).

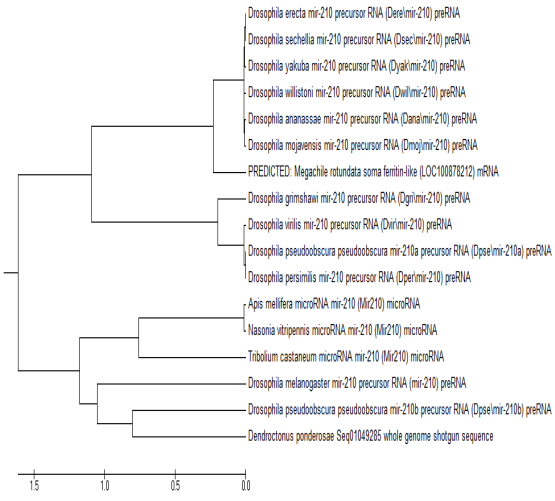

Fig. 1: Phylogenetic tree of *A.flore*amicroRNA 277 (MIR277)

6. CONCLUSIONS

We have predicted and characterized 67 miRNAs by genome-wide homology search against all the reported miRNAs. These miRNAs were further validated by statistical and phylogenetic analyses. These miRNAs in *A. florea* will serve as useful resources for initiating studies on their experimental validation and functional analyses of miRNA-regulated phenotypes in *A. florea* through gene knockdown and transgenesis.In this study, miRNAs were analyzed for their relationship with each other and pattern of variations among different organisms, and it is hoped that it will enhance our understandings on the use of miRNAs in therapeutics for the treatment of various diseases which are the main focus of modern research these days in molecular studies. Four sequences NW_003791204 , NW_003789938, NW_003789699,NW_003790748 were predicted from *A.mellifera* and phylogeneticanalysis of the miRNAs through MEGA 5 was performed which showed their relationship among each other and pattern of variations among different organisms. miRNAs are evolutionary conserved across broad phylogenetic distances. Comparing sequences of miRNA precursors within a species and between closely related species should thus help to determine patterns of molecular evolution and the timescales at which different aspects of the evolution of miRNA precursors are best investigated. The predicted miRNAs reported in the present study will serve as potential resources to initiate their experimental validation. The outcome of this experimental validation should give valuable information to carry out functional analysis of miRNA-regulated phenotypes relevant to basic and applied biology of insects in general and hymenopetrans in particular.

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