Using *de novo* transcriptome analysis to identify RNAi machinery in the spittlebug *Mahanarva spectabilis* Distant, 1909 (Hemiptera: Cercopidae)

Isabela dos Santos Begnami[1*], Gustavo Fernando Ferreira Gonçalves[2], Wilson Malagó Júnior[3], Marcos Rafael Gusmão[3], Anete Pereira de Souza[2], Bianca Baccili Zanotto Vigna[3]

[1]Universidade Estadual de Campinas, Campinas, Brazil. [2]Universidade Federal de São Carlos, São Carlos, Brazil. [3]Embrapa (Brazilian Agricultural Research Corporation) Southeastern Livestock, São Carlos, Brazil.
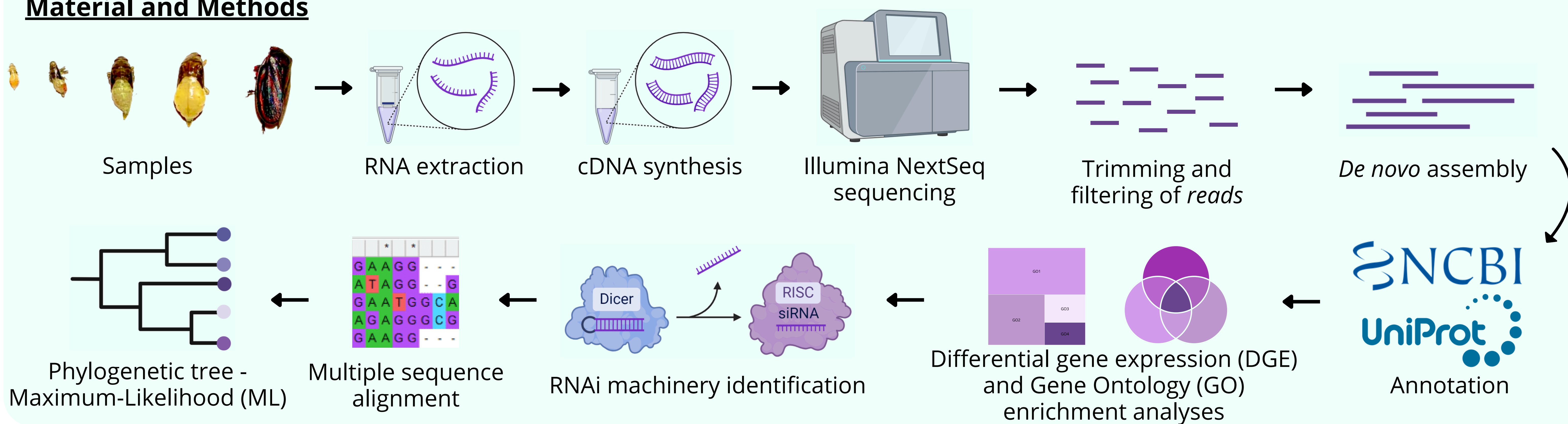*e-mail: isabegnami@gmail.com

Introduction

- *Mahanarva spectabilis* is a major pest of forage grasses in Brazil;
- 160 M ha of pasture area, 80% of which are of susceptible species;
- Biological control by *Metarhizium anisopliae* is ineffective;
- RNAi technology offers a promising approach for insect control.

Objective

To provide molecular information on *Mahanarva spectabilis* at different developmental stages, aiming to explore the RNA interference (RNAi) mechanism for potential gene silencing applications in pest control.

Material and Methods

Samples → RNA extraction → cDNA synthesis → Illumina NextSeq sequencing → Trimming and filtering of *reads* → *De novo* assembly → NCBI UniProt Annotation → Differential gene expression (DGE) and Gene Ontology (GO) enrichment analyses → RNAi machinery identification → Multiple sequence alignment → Phylogenetic tree - Maximum-Likelihood (ML)

Results and Discussion

Table 1. *M. spectabilis* transcriptome assembly metrics.

	Unigenes	cd-hit
Total transcripts predicted	309.002	197.003
GC percentual	35,69%	34,48%
N50	635	853
Contigs mean size (pb)	308	364
Bowtie2 mapping	94.80%	90.59%
BUSCO (Hemiptera_odb10)	Complete: 96,5% Duplicate: 7,8%	Complete: 96,6% Duplicate: 5,3%

Table 2. RNAi machinery genes identified in *M. spectabilis* data.

| RNAi Pathway	Gene	Description
dsRNA cleavage	Dicer-1	Endoribonuclease Dcr-1
	Dicer-2	Endoribonuclease Dcr-2
	Drosha	Ribonuclease 3
Endonucleases	Ago2	Protein argonaute 2
	Ago3	Protein argonaute 3
	Piwi	Piwi-like protein Siwi
dsRNA binding	Loqs	Protein Loquacious
	Pasha	Microprocessor complex subunit DGCR8
dsRNA transport and uptake	SID	SID1 transmembrane family member 2
	Chc	Clathrin heavy chain
	Scavenger	Scavenger receptor class B member 1
Auxiliary RISC-associated or regulatory factors	Armitage	RNA helicase armi
	Belle/DDX3	ATP-dependent RNA helicase DDX31
	Gawky	Protein Gawky
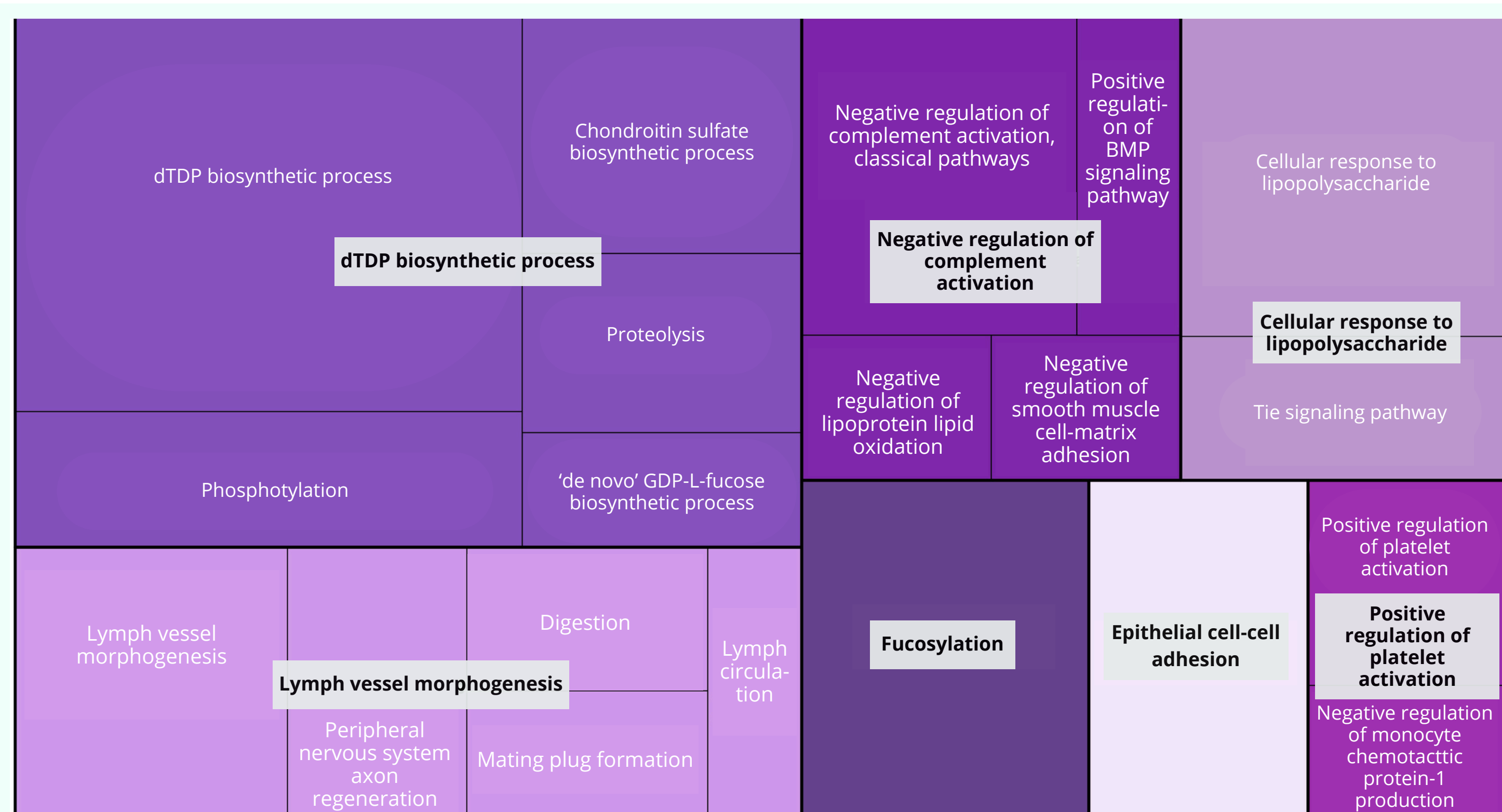
Figure 1. TreeMap of GO terms enriched in Nymphs vs. Adults DGE comparisons, summarized by ReviGO.
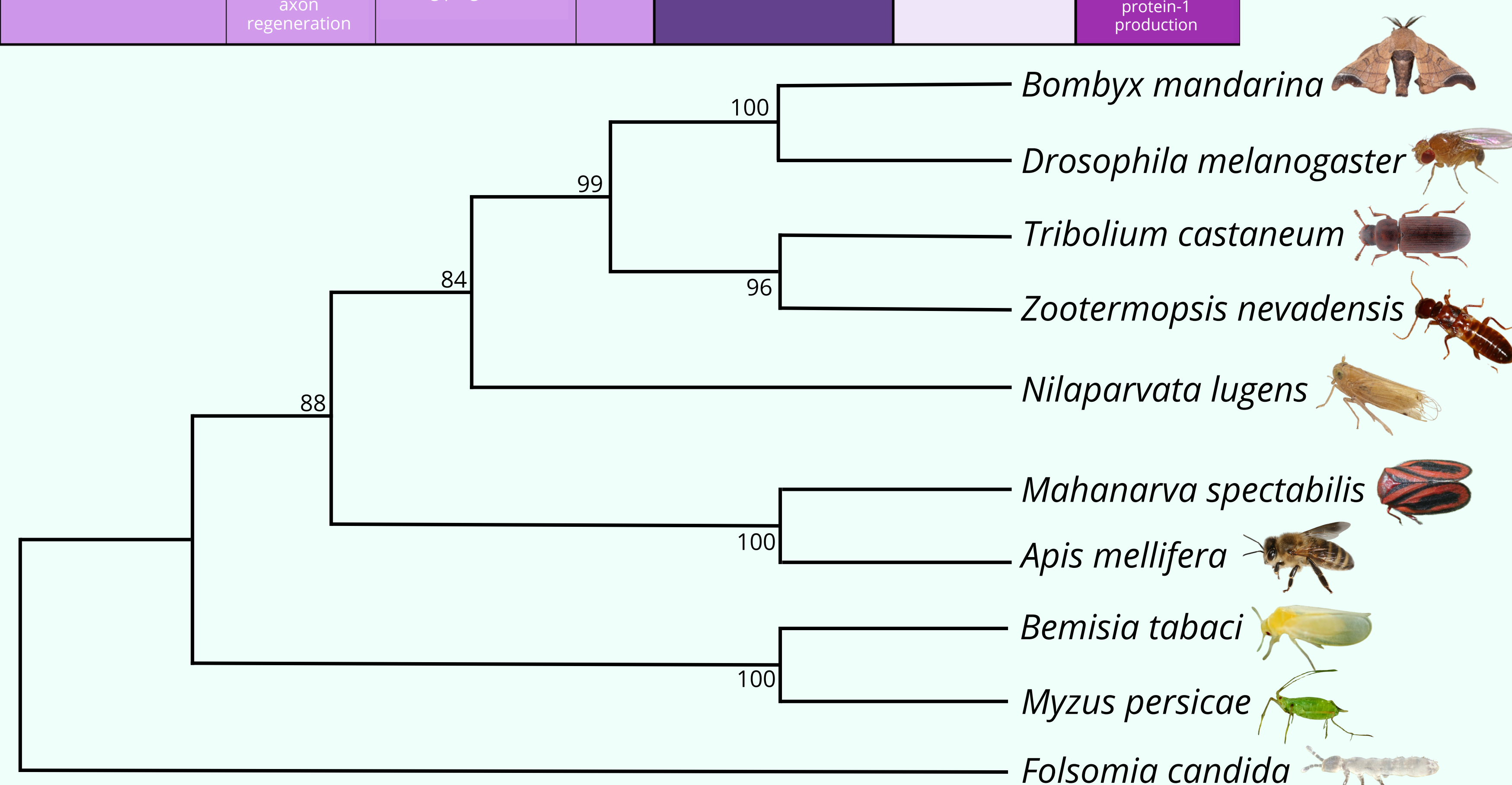

Figure 2. Phylogenetic tree for the Drosha gene constructed using the ML method with the General Time Reversible model, incorporating Gamma Distributed rates and Invariant Sites.

Conclusions and Perspectives

- *Mahanarva spectabilis* has an active RNAi mechanism, mostly in the nymphal and adult stages;
- This transcriptome will now be used to search targets for silencing.

Acknowledgements