

Evolutionary conserved microRNAs are ubiquitously expressed compared to tick-specific miRNAs in the cattle tick *Rhipicephalus (Boophilus) microplus*

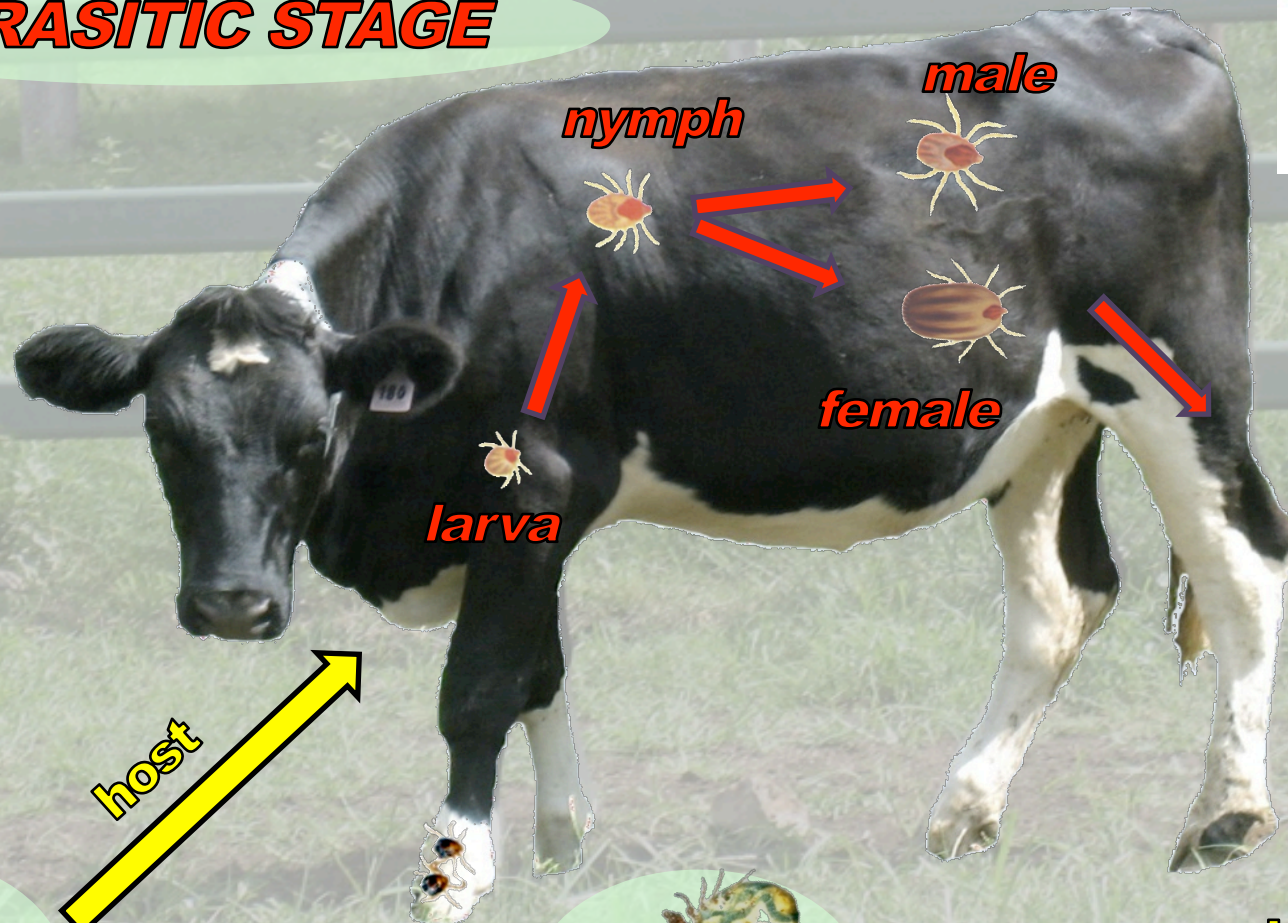
Presented by:
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Towards the control of cattle tick

- *R. microplus* causes significant economic losses in cattle production in Australia (\$150M p.a.) and worldwide.
- Current control strategies are not appropriate. Further understanding of the biology of ticks is required to identify suitable vaccine candidates.
- MicroRNAs have been shown to be key regulators of a large number of biological processes in a range of species.
- No microRNAs were known for the cattle tick (*R. microplus*)
 - 36 miRNAs were found in *Ixodes scapularis* a tick from which diverged ~241MYA.

nymph & adult stages

PARASITIC STAGE



larva

nymph

male

female

**engorged females
drop off**

larvae hatch

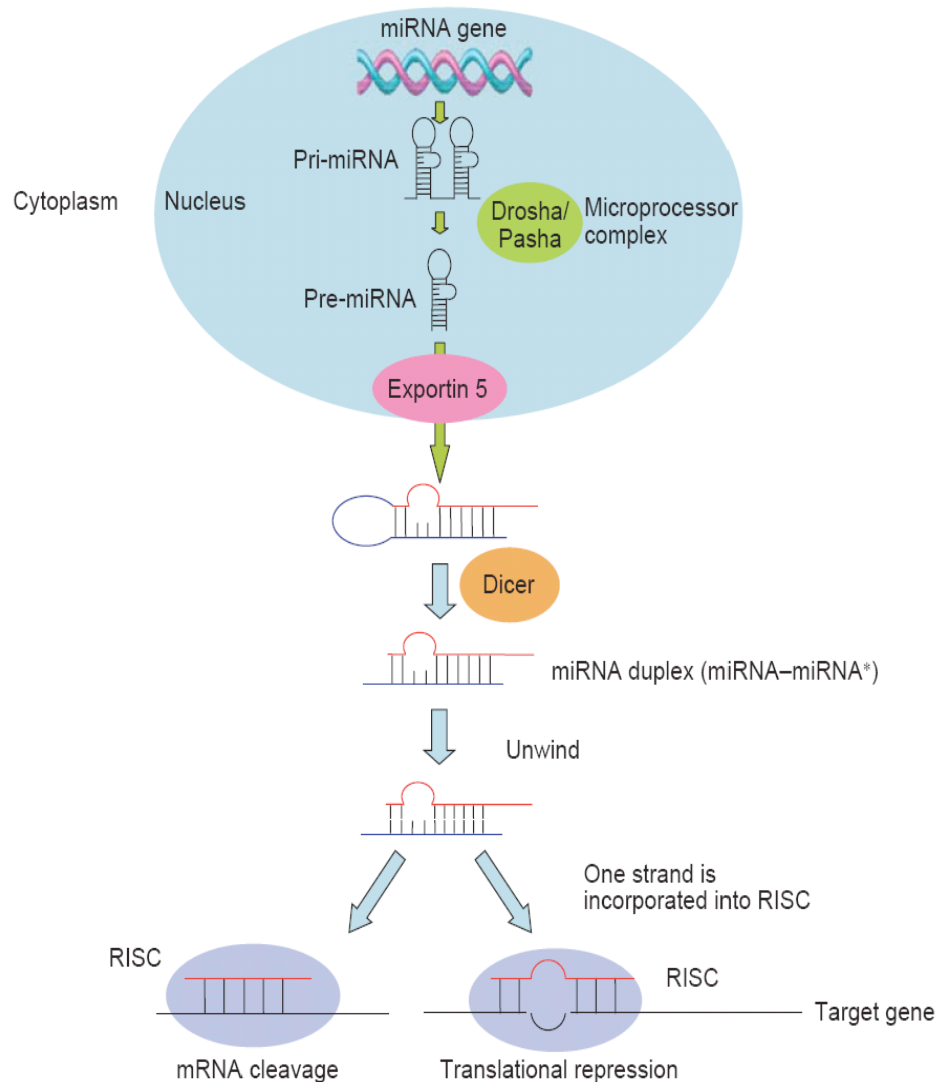
eggs are laid

NON-PARASITIC STAGE



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Department of Primary Industries and Fisheries

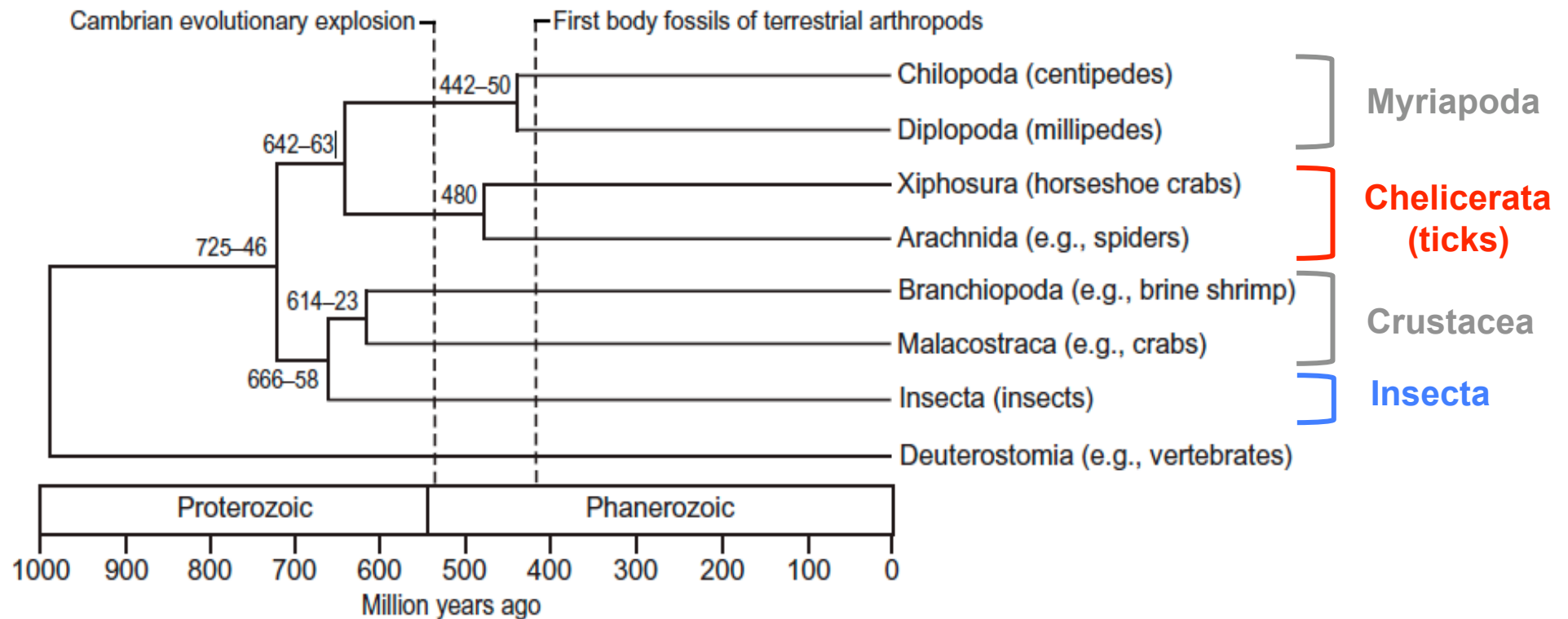
microRNA pathway



miRNA function in *Drosophila*:

- Cell proliferation/anti-apoptosis (**bantam**)
- Fat storage/anti-apoptosis (**mir-14**)
- Homeostasis/anti-apoptosis (**mir-278**)
- Anti-apoptosis (**mir-2**)
- Photoreceptor differentiation (**mir-7**)
- Neurogenesis/neurodegeneration (**mir-9**)
- Muscle differentiation (**mir-1**)
- Homeotic transformation (**iab-4**)
- Energy metabolism/fat storage (**mir-278**)
- Metamorphosis (**let-7, mir-100, 125, 34**)

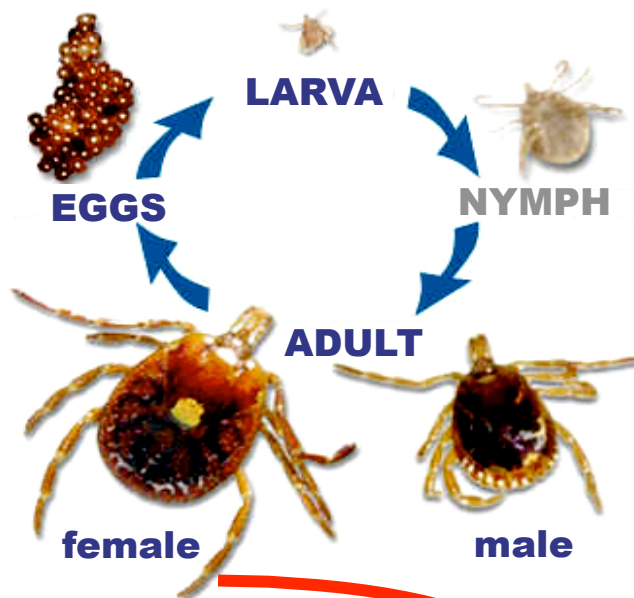
Arthropod Evolution



Ticks and flies diverged 520~725 Million Years Ago

Pasani et al. (2004) *BMC Biology*
Wheeler et al. (2009) *Evol. & Dev.*

RNA collection and next gen Illumina/Solexa sequencing



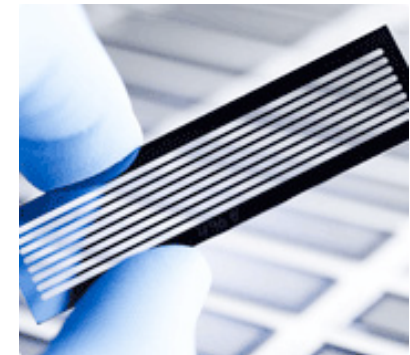
1. Collect Total RNA/small RNA fraction

- Eggs
- Larvae (larvae, frustrated larvae)
- Adult ticks (female, male)
- Key organs

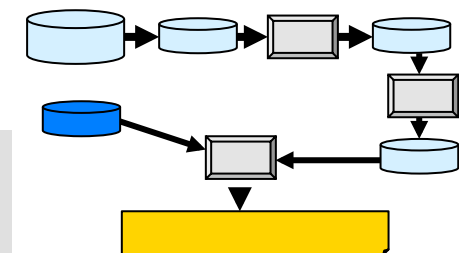
2. Construct small RNA libraries

3. Illumina/Solexa sequencing

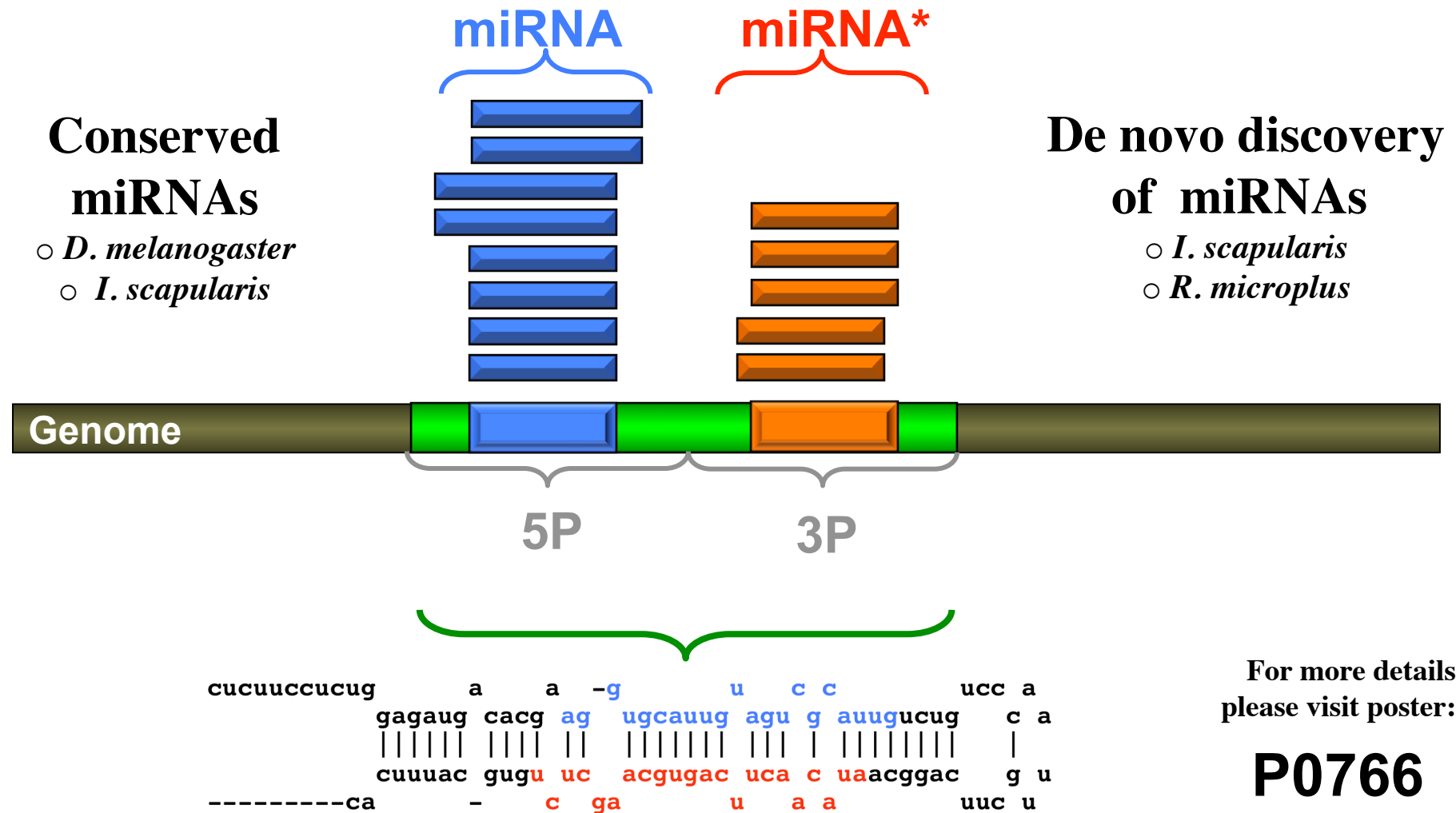
- Eggs: 4,215,404
- Larvae: 9,437,803
- Adult ticks: 8,319,734
- Gut: 3,501,156
- Sal gland: 4,579,483
- Ovaries: 5,206,221



Life stages = 21,972,942 short reads
Key organs = 13,286,860 short reads



Identification of evolutionary conserved and novel tick-specific miRNAs



For more details
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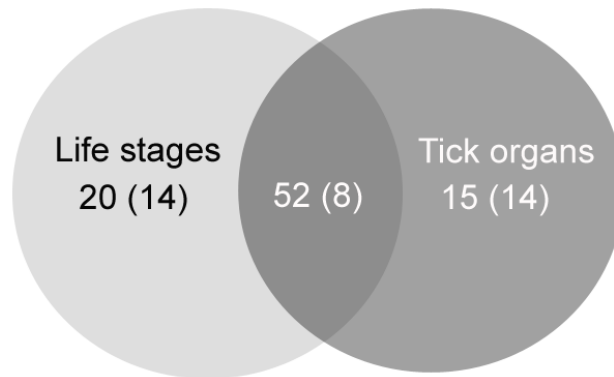
Short read statistics for evolutionary conserved & novel tick-specific miRNAs

Sample	Total reads	Evolutionary conserved miRNAs	Novel tick-specific miRNAs	Total miRNAs
A) Life cycle stages				
Eggs	4,215,404	8,775	868	9,643
Larvae	3,964,440	732,690	39,959	772,649
Frustrated Larvae	5,473,363	628,561	31,617	660,178
Female	4,248,307	996,324	1,398	997,722
Male	4,071,427	344,877	683	345,560
sub total	21,972,941	2,711,227	74,525	2,785,752
B) Female tick organs				
Gut	3,501,156	413,240	1,028	414,268
Salivary glands	4,579,483	257,096	1,898	258,994
Ovaries	5,206,221	120,485	2,833	123,318
sub total	13,286,860	790,821	5,759	796,580
Total	35,259,801	3,501,629	80,287	3,582,332

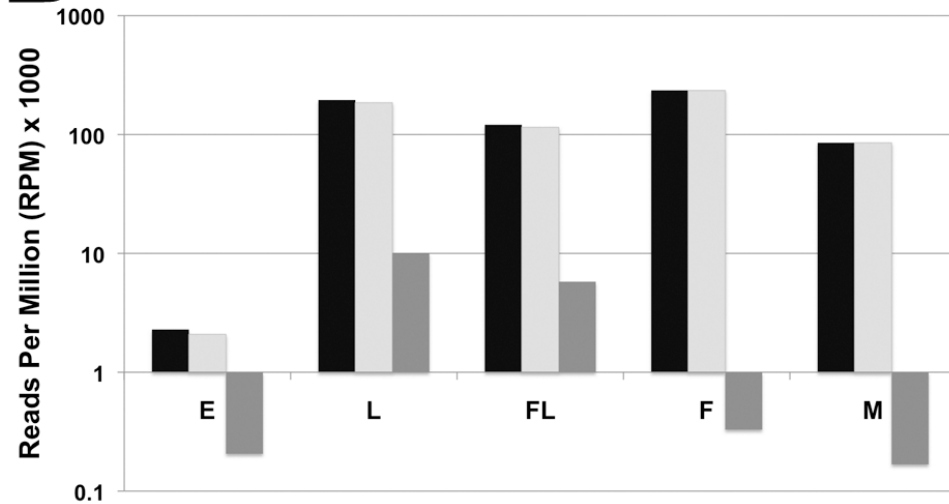
- The majority [A) 97.3%; B) 97.7%] of miRNA transcripts correspond to evolutionary conserved miRNAs
- Novel tick-specific miRNAs are only highly expressed during larval stages → Development?

Global expression and fold changes of *R. microplus* miRNAs

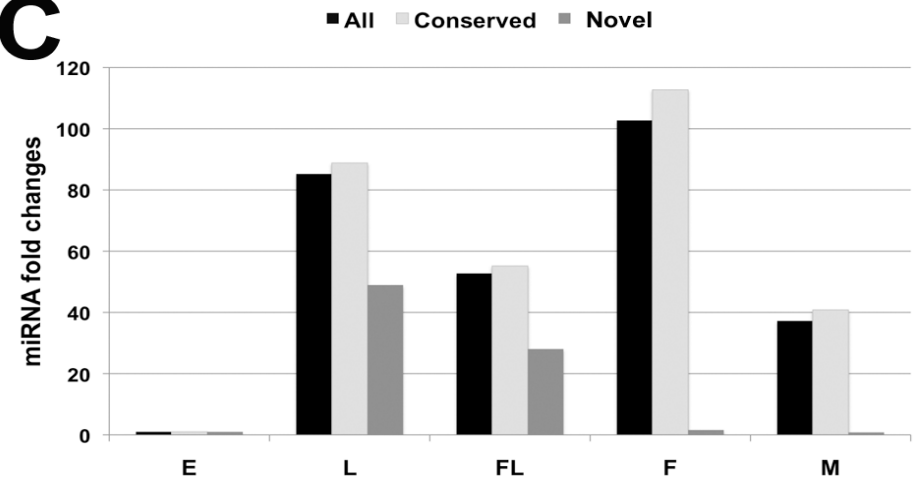
A



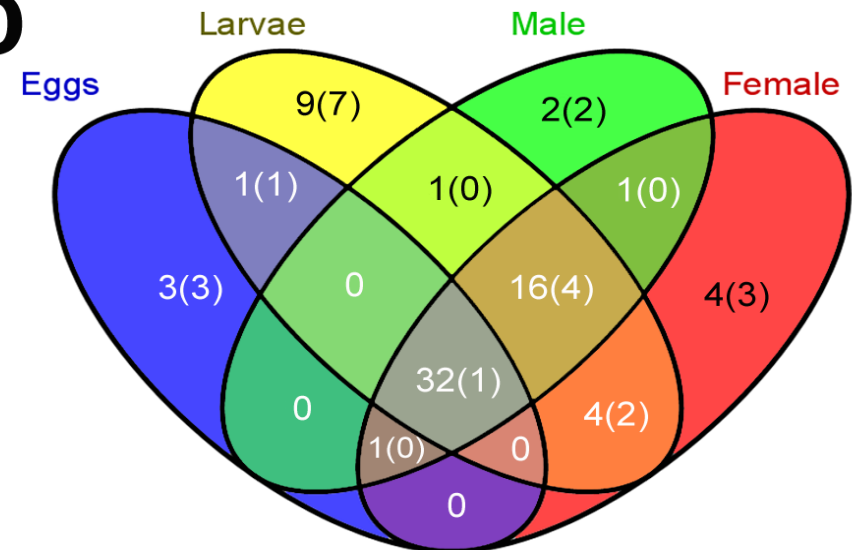
B



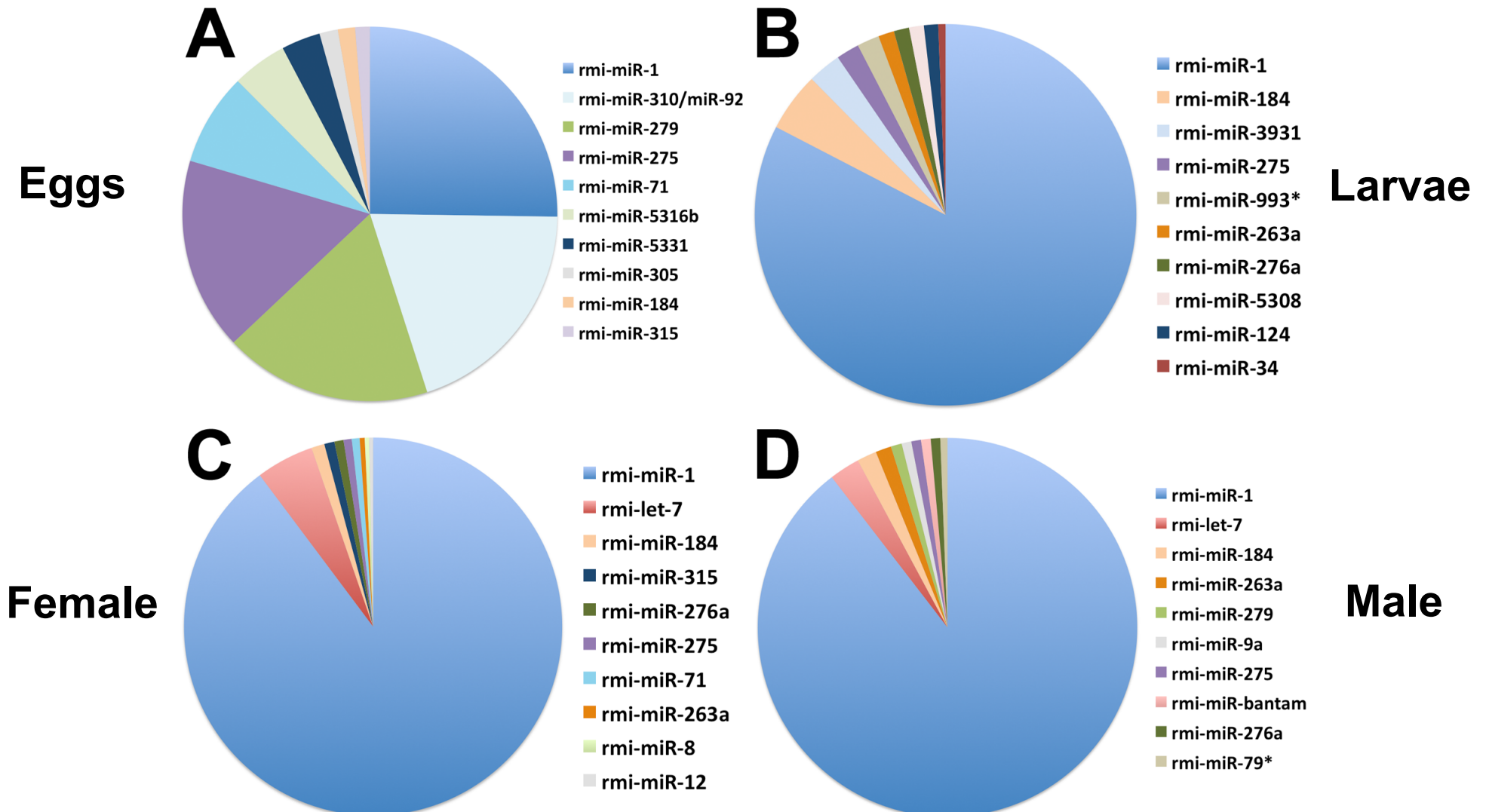
C



D



Top 10 most abundant *R. microplus* miRNAs expressed in various cattle tick life stages



Examples of novel tick-specific miRNAs

```

5' AC          C          GGUAUU
   UCUAAGUUAAUCU CAAGCCCAAU U
   AGAUUCGAUUAGA GUUCGGGUUA C
3' CC          A          ACUGCU
    
```

isc-miR-5305

```

5' CU          AC          U  AA  UAGGGA
   GGGGAGGAGC  CGCGUG  UAC  CUUCG  G
   UCCUCCUCG GUGCAC AUG GAGGC A
3' C          CA          U  A  CGGTA
    
```

isc-miR-5306

```

5' C          U  G  G  A  AG
   AAAACCCUCUC GUGCU UGGA GU AUAUAU U
   UUUUGGGGGAG CACGA ACCU CA UAUAUA A
3' A          U  A  G  CA
    
```

isc-miR-5308

```

5'          G  UU  A  A  UC  A
   CGGCUC  GG  CAUCAU AGGAUAUCAUC AUAUU  GC A
   GUUGAG  CC  GUAGUA UUCUGUAGUAG UAUAA  CG U
3'          G  UC  C  A  C-  G
    
```

isc-miR-5314

```

5' C          U  AU  UUGATA
   AGGCUCG  CGAAAA  GUUCUG  UGGCA  G
   UUCGAGC GCUUUU CGGGGC ACUGU A
3' U          A  C  C  UAAUA
    
```

rmi-miR-5316b

```

5' A          U
   AGCGCGAGAACA AACGACGACACAGAGACAAGAAG G
   UCGCGCUCUUGU UUGCUGCUGUGUCUCUGUUCUUU U
3' A          U  C
    
```

rmi-miR-5317a

```

5' C          A  AGAG
   CAAGCUUGAAGG AAACAAGGGAGACAGGAA C
   GUUCGAACUCC  UUUGUUCCUCUGUCCUU G
3'          GAC
    
```

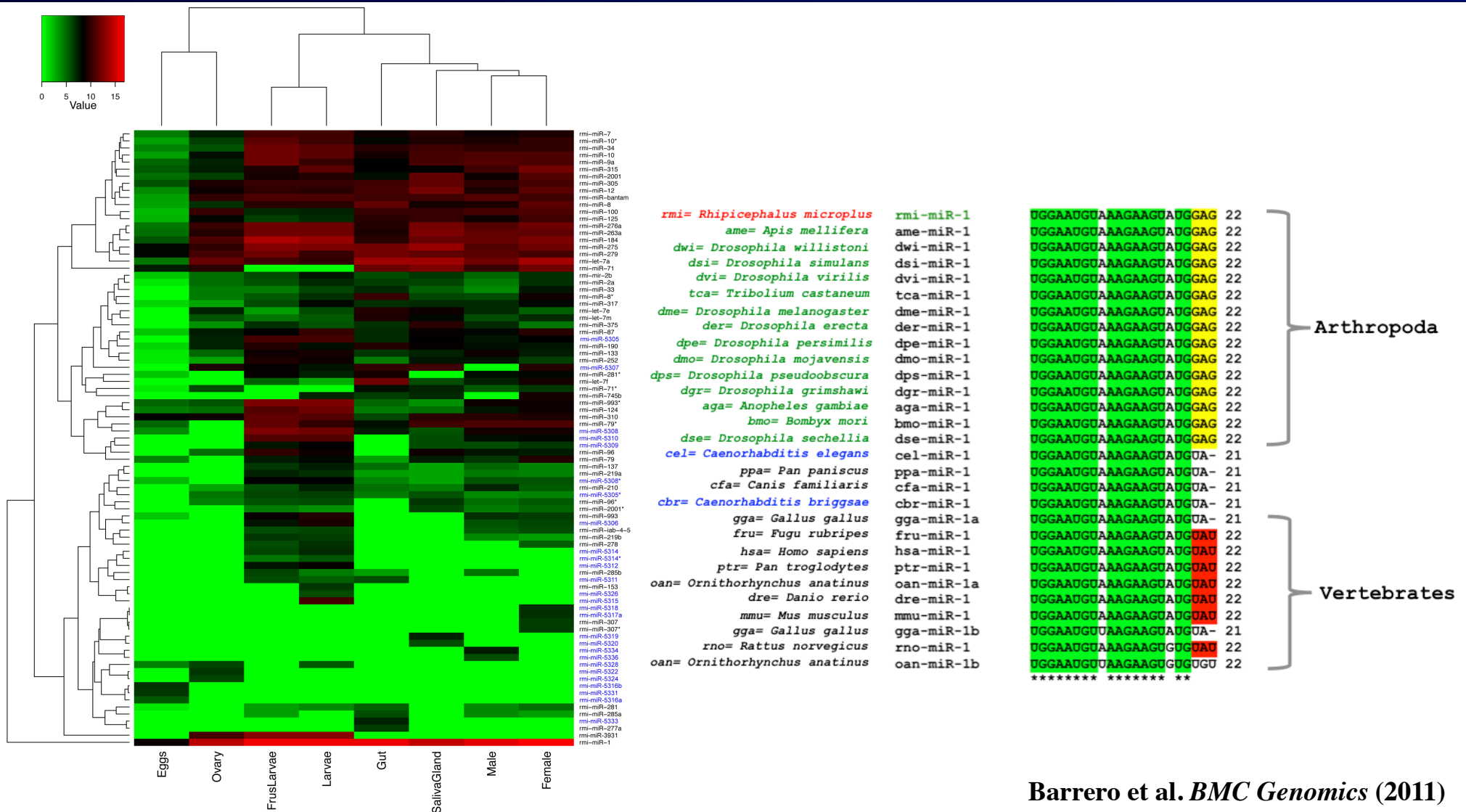
rmi-miR-5325

```

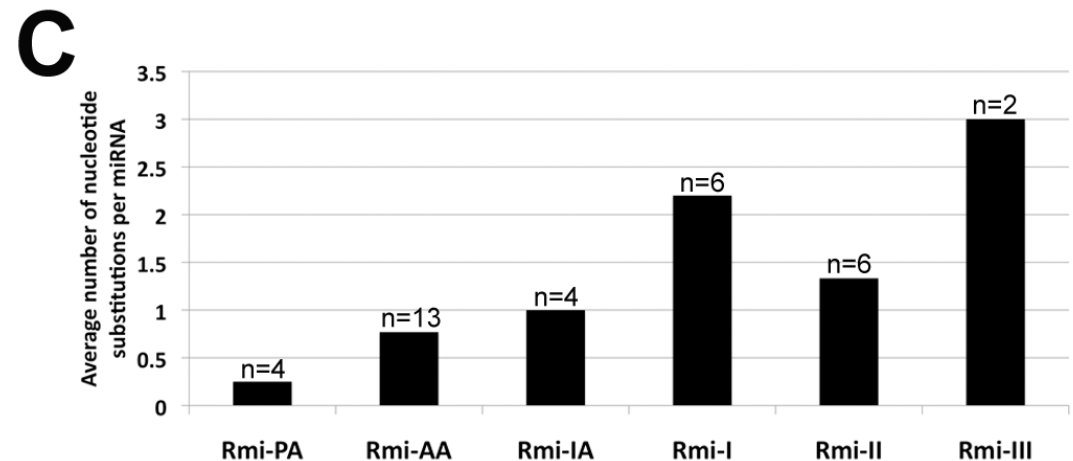
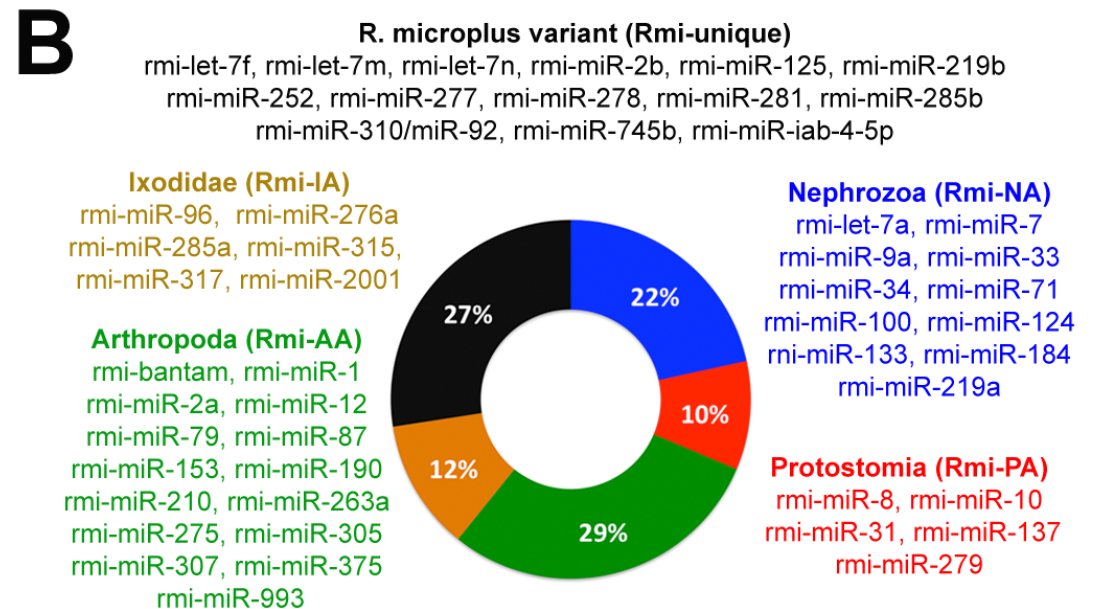
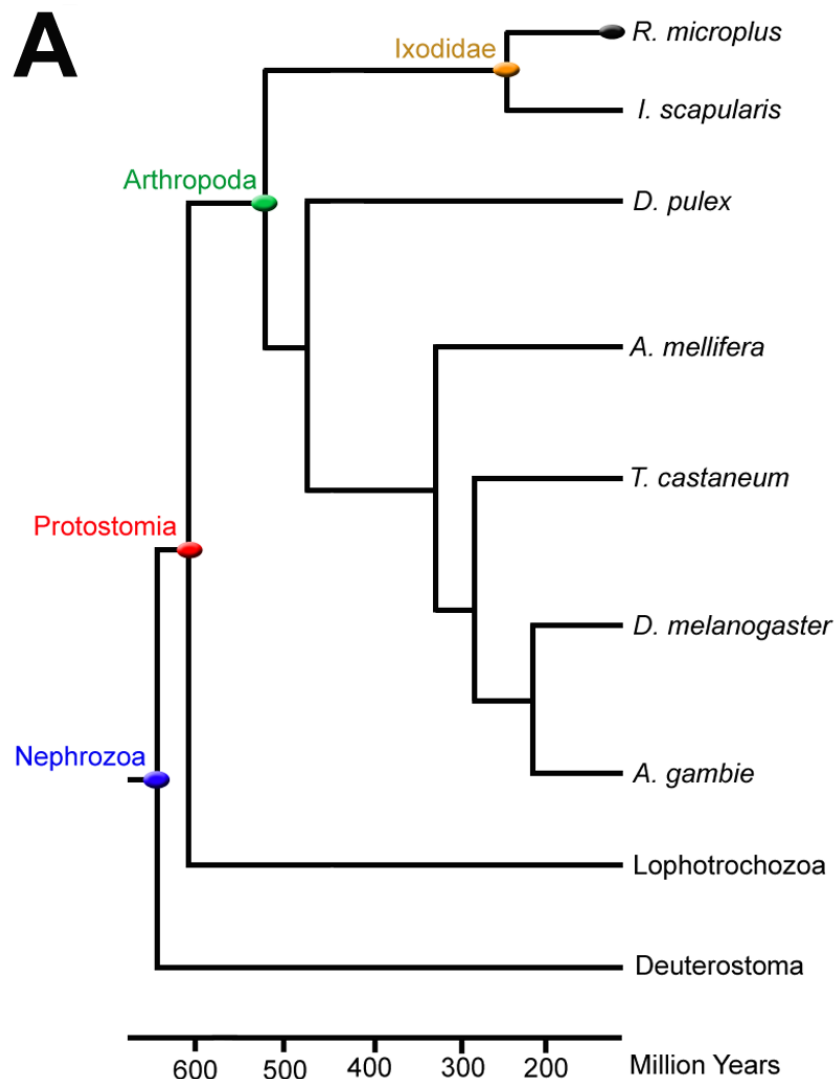
5' U  -  C  CG  CGGU  GAA  CAGAA
   CUUUG AUCU AUU GCG UCCUCGA GCA  \
   GGGAC  UAGA  UGA  CGC  GAGGAGUU  CGU  A
3' U  G  U  A-  U---  GUC  UCAAG
    
```

rmi-miR-5336

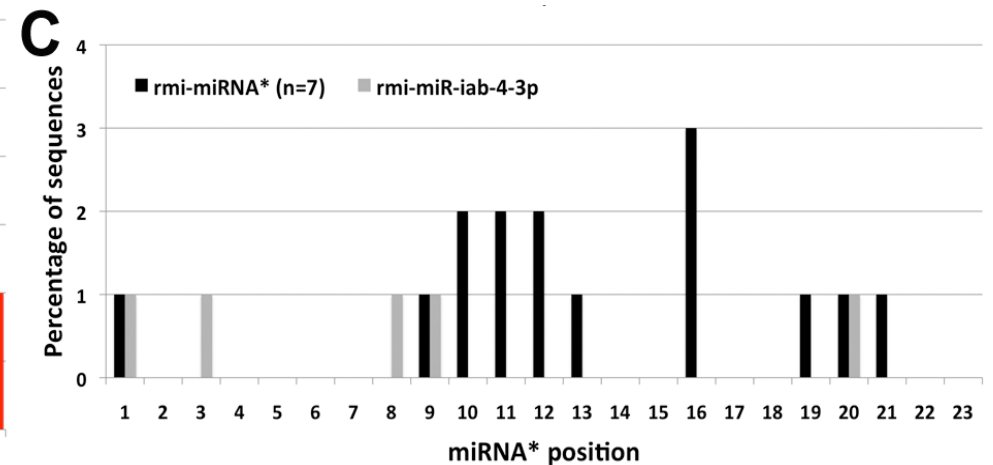
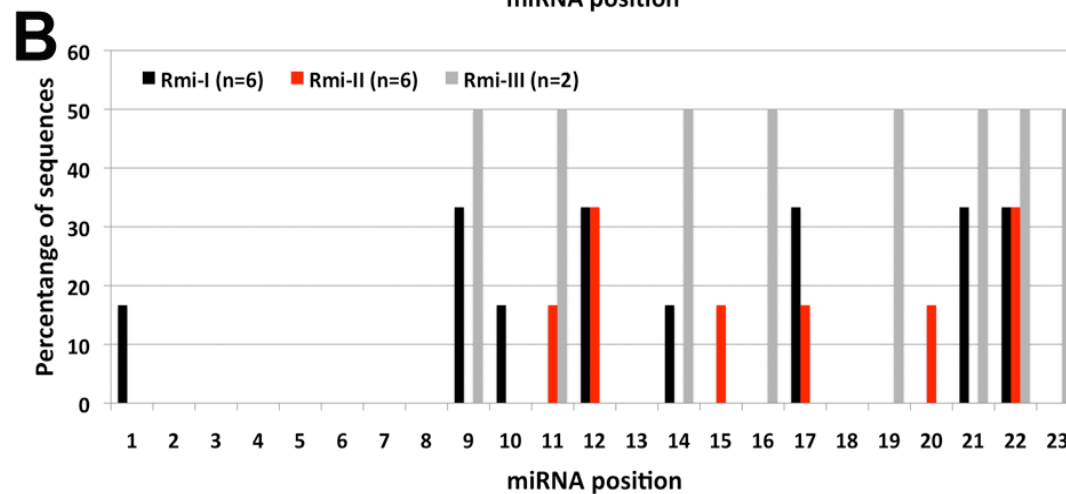
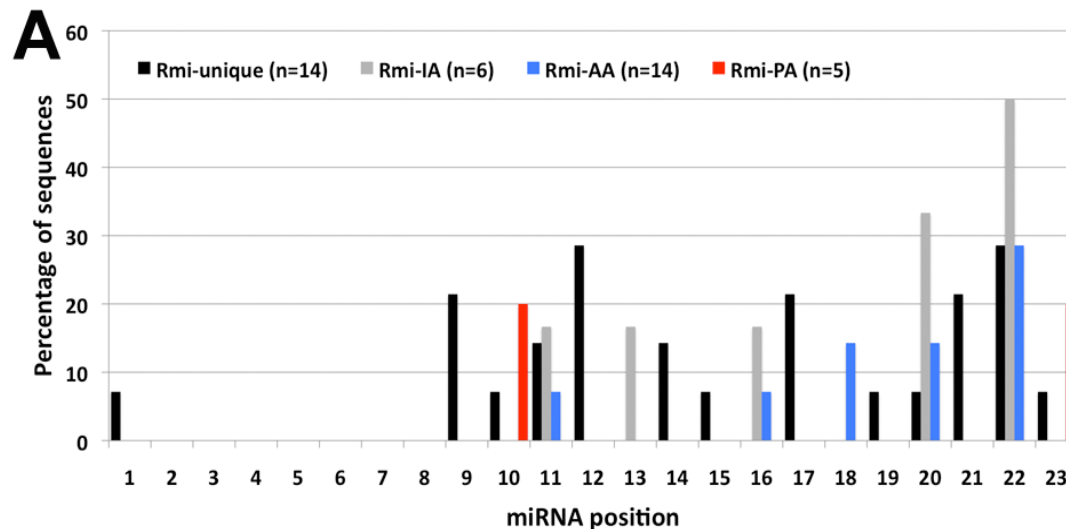
Clustering of tick samples based on their miRNA expression profiles



Evolutionary conservation and nucleotide changes of *R. microplus* miRNAs



Mutational profile of *R. microplus* miRNAs as compared to ancestral sequences

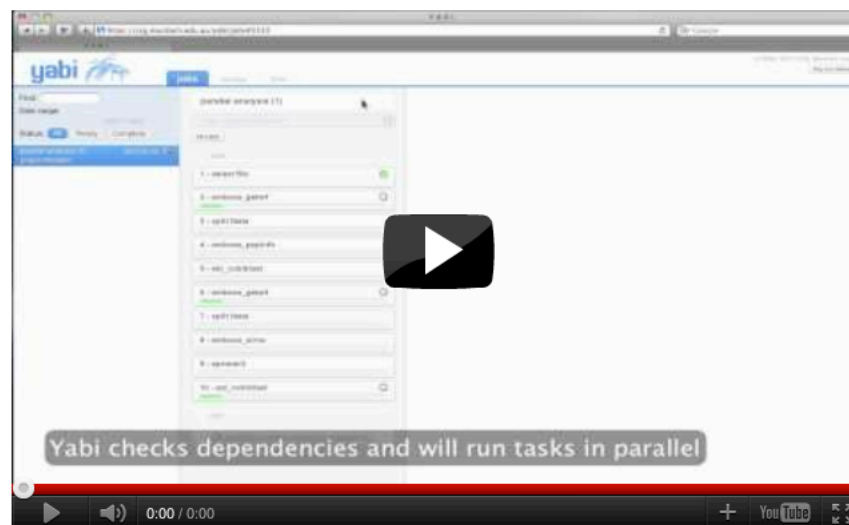


- Anciently acquired miRNAs show no nucleotide changes
- miRNAs and miRNA* sequences show similar biases in nucleotide changes

YABI: Web-based workflow environment



Workflows made easy.



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Hosted on Google Code:
<http://code.google.com/p/yabi/>



Yabi has radically transformed the way we process and analyse 2nd generation DNA sequence data. Through a user-friendly dynamic html interface we can design a simple pipeline that sorts, trims and queries sequence data against databases - we put raw data in one end and obtain meaningful outputs at the end of the pipeline. Put simply, YABI has enabled us to spend more time analysing the actual data and less time 'fighting' it.

**For more details
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<http://ccg.murdoch.edu.au/yabi/>

Summary

- We found 87 miRNAs in *R. microplus*, of these 51 are conserved in a range of species while 36 represent novel tick-specific miRNAs.
- Evolutionary conserved miRNAs are ubiquitously expressed at various levels in most life stages and organs, which contrast with the restricted expression profiles of novel tick-specific miRNAs.
- 73% of conserved *R. microplus* miRNAs are perfectly conserved throughout evolution with 11, 5 and 15 of these conserved since the Nephrozoan (640 MYA), Protostomian (620MYA) and Arthropoda (540 MYA) ancestor, respectively.
- Anciently acquired miRNAs in the *R. microplus* lineage accumulate the least amount of nucleotide substitutions as compared to more recently acquired miRNAs.
- We have implemented a web-based analytical environment to aid in the processing of next gen sequencing data for miR-seq and other genomics/transcriptomics/proteomics applications

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