RNAi pathway components and function in *Paramecium bursaria*

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Overview

Motivation

RNAi in ciliates

Experimental RNAi induction in *P. bursaria*

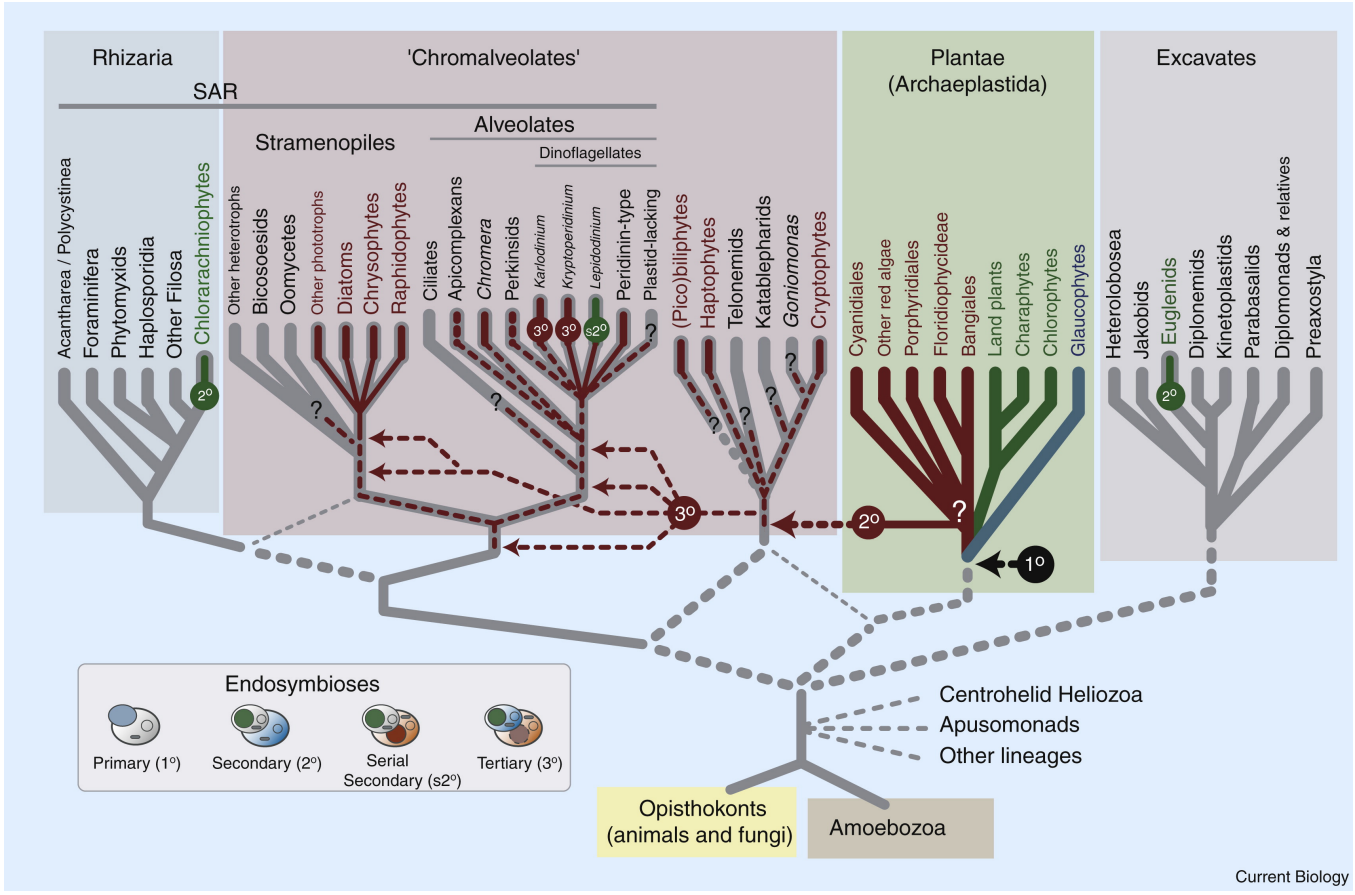
RNAi pathway components in active *P. bursaria* transcriptome

In-silico analysis of potential endosymbiont 'cross-talk'

Conclusions

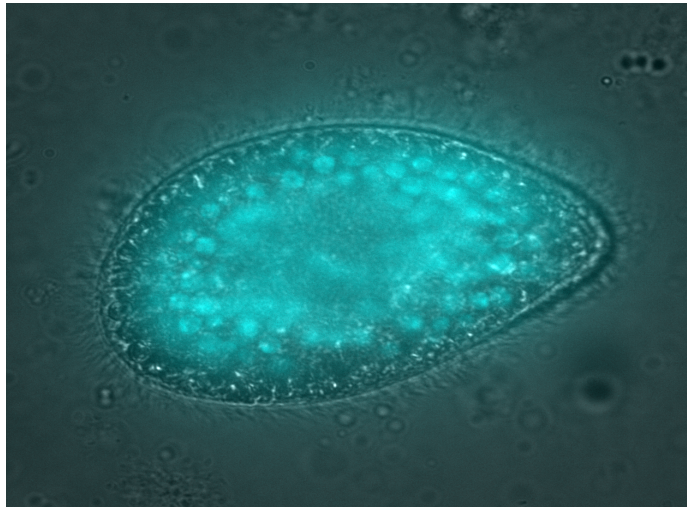
Why is *Paramecium bursaria* potentially a good model for (secondary photosynthetic) endosymbiosis?

Broad diversity of second order plastid endosymbioses



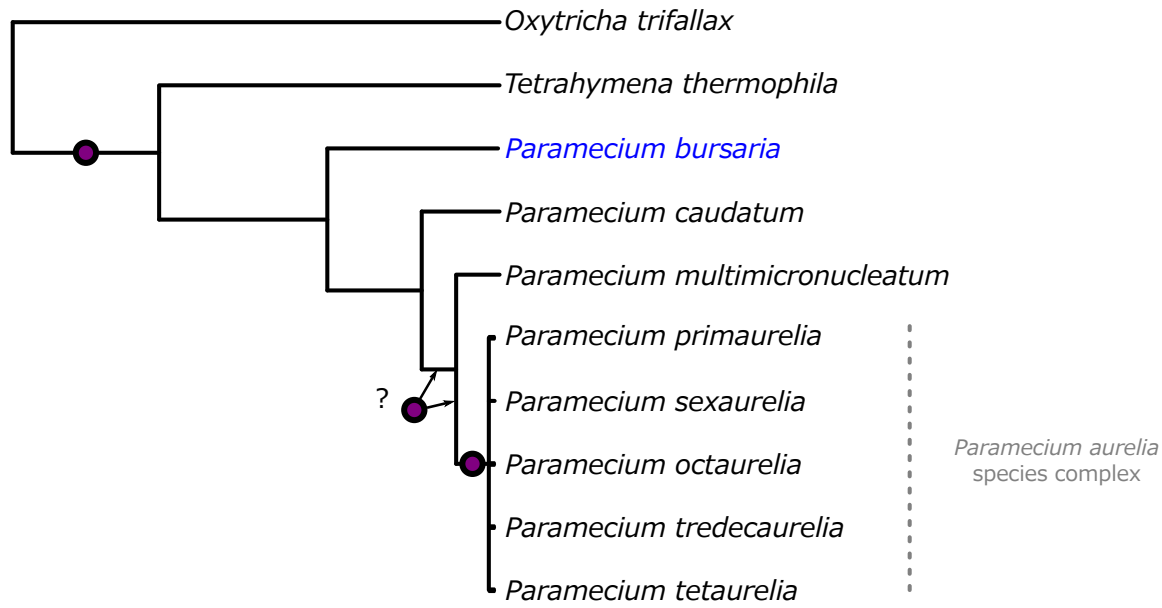
Reproduced from [Arc09]

Paramecium bursaria and its green algal endosymbionts



- 100 µm to 160 µm serial phagotrophic ciliate (nuclear dimorphism).
- $\sim$ 300 endosymbiotic algae in stable heritable facultative(?) endosymbiosis.
- Multiple independent origins of these endosymbioses.
- Single cell transcriptome and genome of *P. bursaria-Micractinium reisseri* CCAP 1660/12.
- *P. bursaria* bulk transcriptome [KSD$^+$14].

RNAi pathways in the ciliates



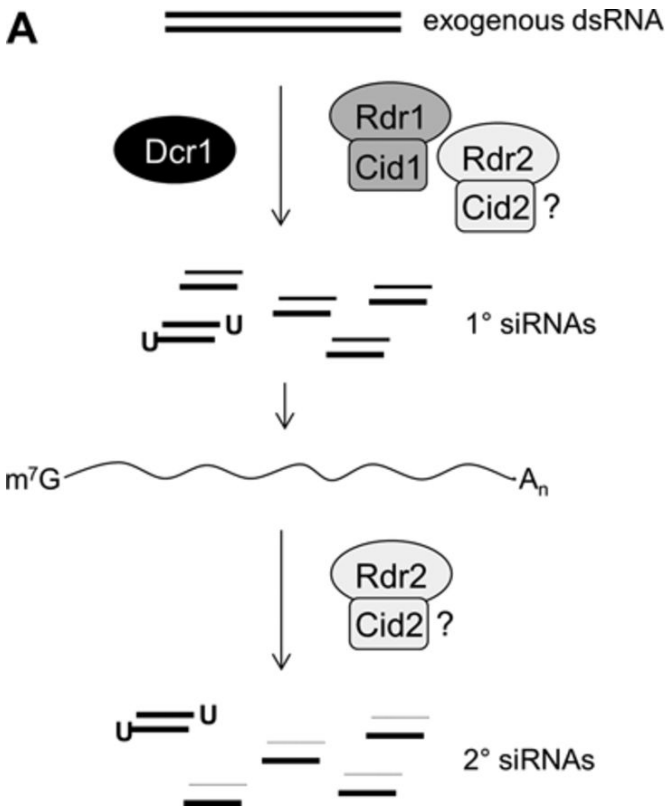
A good model needs a means to test hypotheses:

- Ciliate specific scnRNA system [MG04, CMM13].
- siRNA pathways present in *Paramecium tetaurelia* [GS01, GS02] (and *Tetrahymena thermophila* [CL06, YC05]):
  1. Transgene inducible pathway [GS01].
  2. Exogenous dsRNA inducible pathway (feeding or injection) [GS02].

Transgene pathway

- Microinjection and transformation of MAC with high-copy transgenes lacking 3' UTR [GS01]
  1. 23nt siRNA generated from transgene transcripts (Dcr1, Rdr2, Rdr) and Cid2) [LNS$^+$09, MCT$^+$14].
  2. mRNA cleavage (Ptiwi13 and Ptiwi14)

Exogenous dsRNA pathway

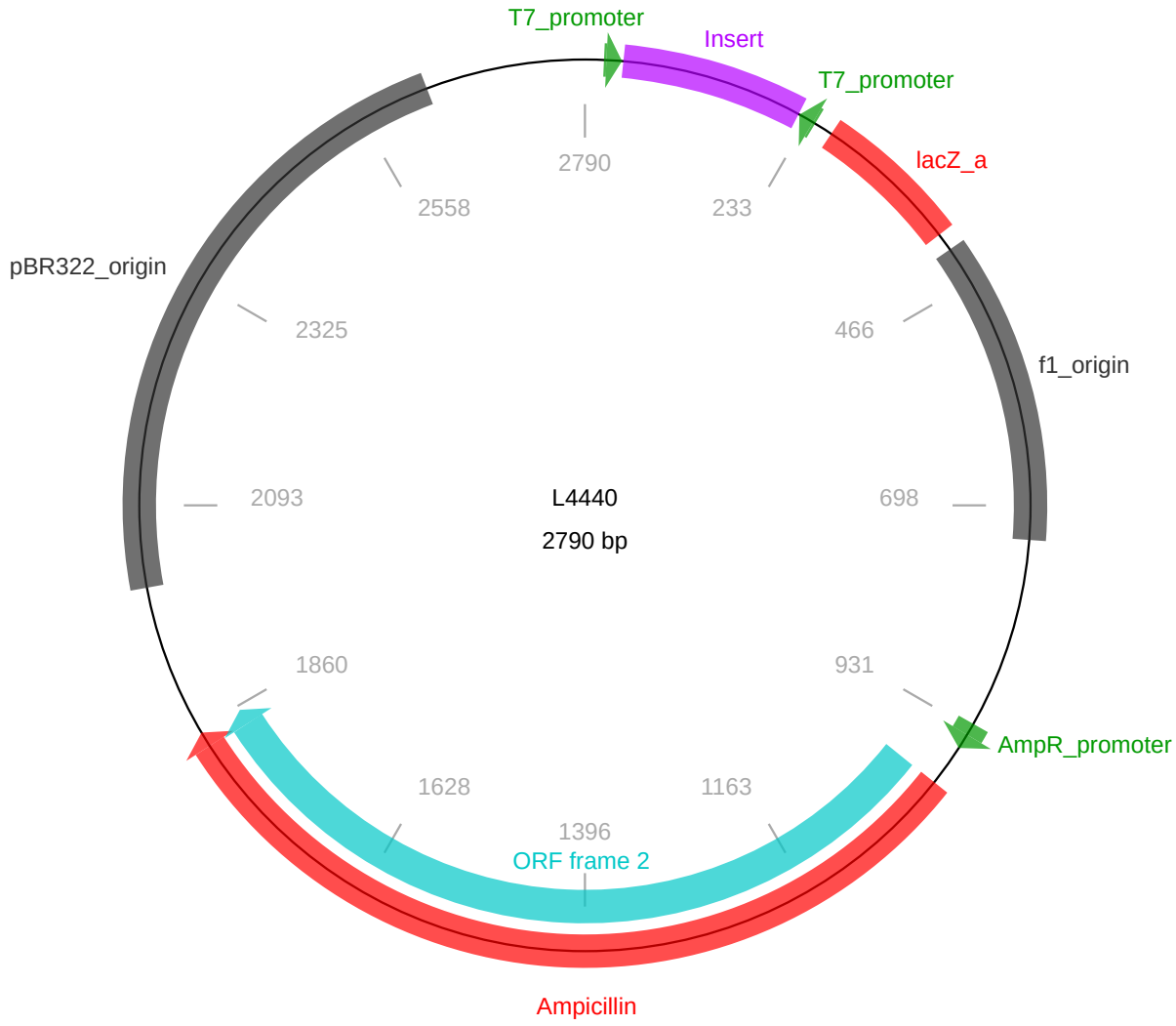


Reproduced from [CGA+15]

- Exogenous dsRNA via feeding (or microinjection) [GS02].
- 1° siRNA targeted cleavage (Ptiwi13) [BGK+11].
- Undefined role in MAC for 2° siRNA (Ptiwi12, Ptiwi15) [MCT+14, CGA+15, BGK+11]
- Pds1 involved in uptake of dsRNA from vacuole? [CGA+15].
- Activated at low levels by ssRNA from normal food bacteria [CGA+15].

So, can we experimentally induce RNAi in *P. bursaria*?

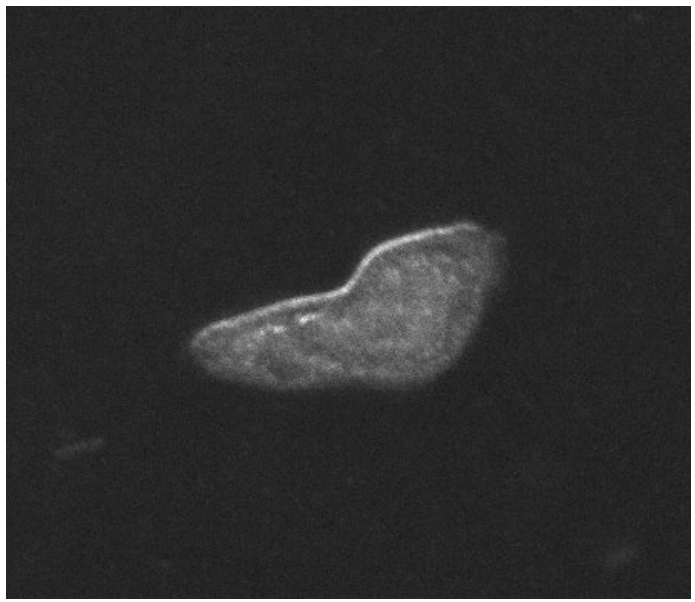
Experimental feeding vector



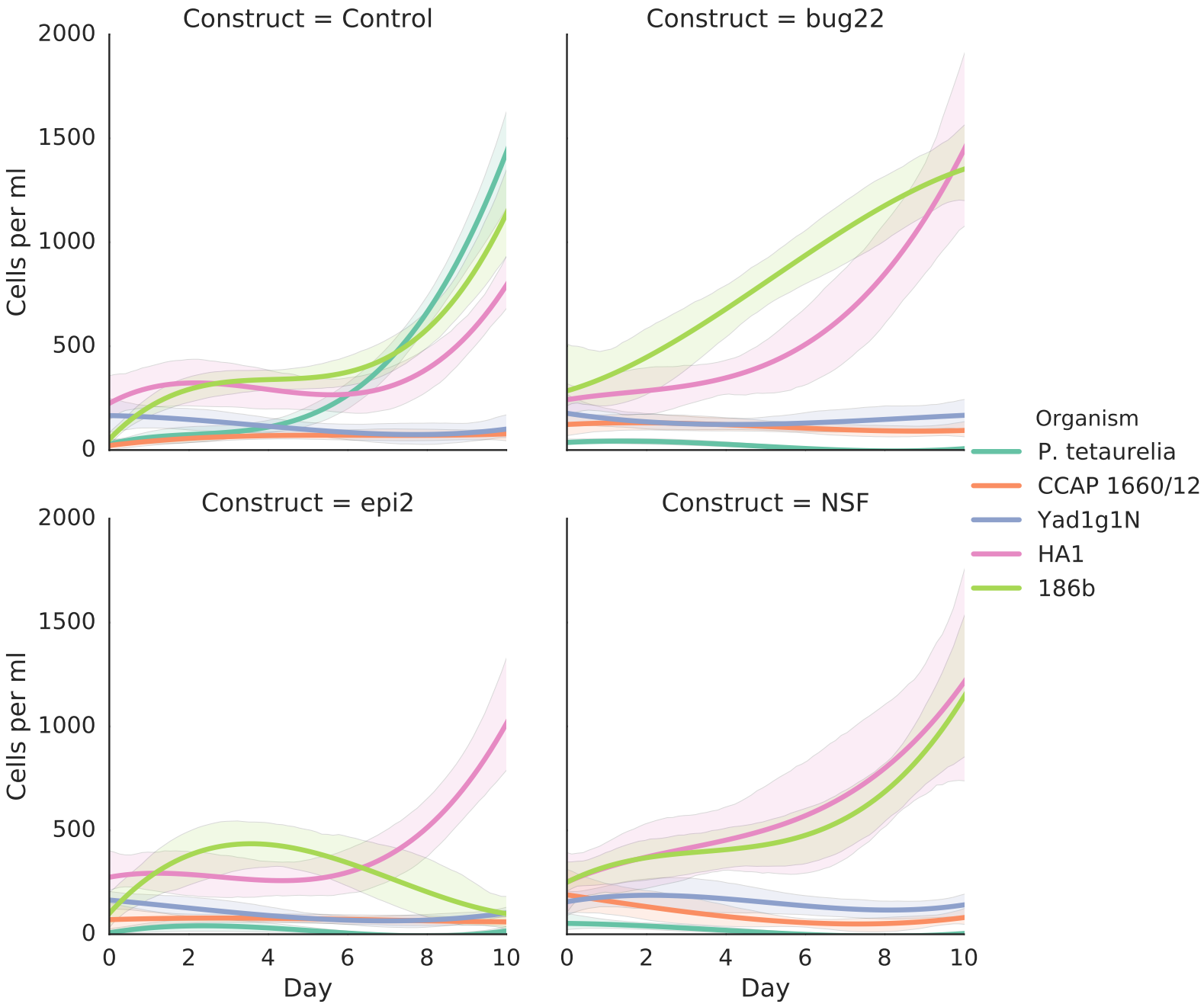
Transformed into *E. coli* with IPTG-inducible T7 polymerase and RNAse III deficiency.

Construct inserts

Gene	**Function**	**RNAi phenotype in *P. tetaurelia***
epi2	Epiplasmin	"Monstrous" cells
NSF	Membrane fusion factor	Lethal
bug22	Basal body/ciliary protein	Slow swimming and death



RNAi feeding had mixed results

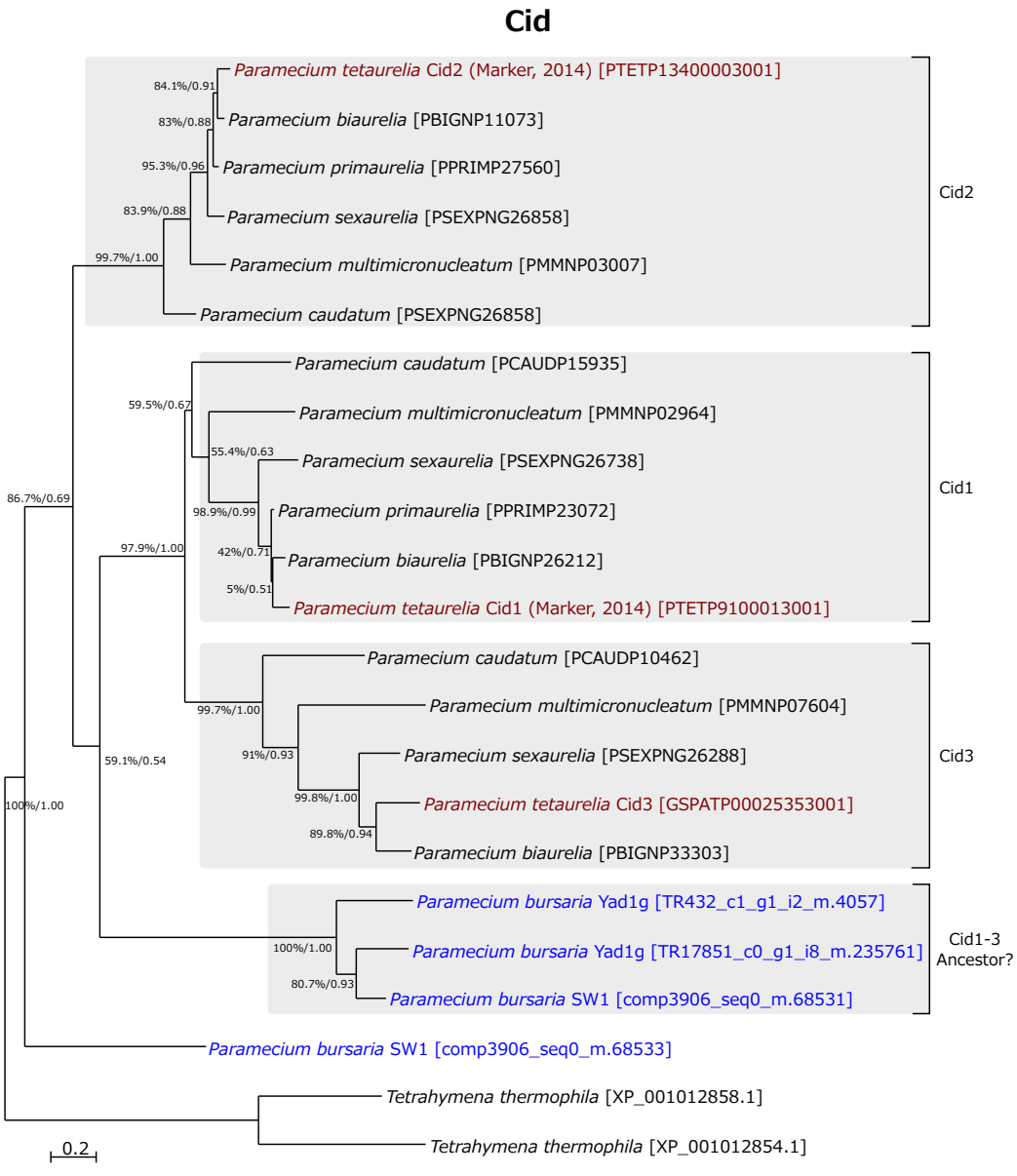


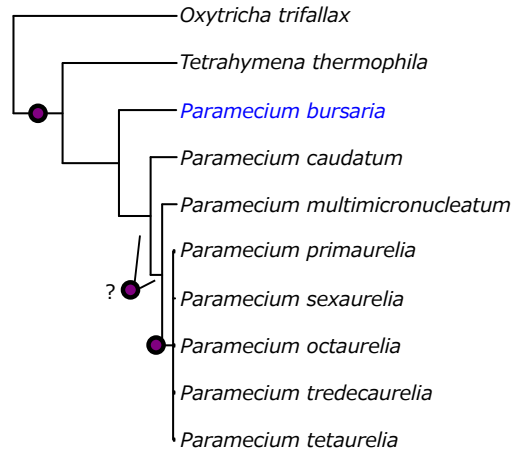
Are all the known RNAi pathway components present in the active transcriptome(s)?

Summary of known RNAi components

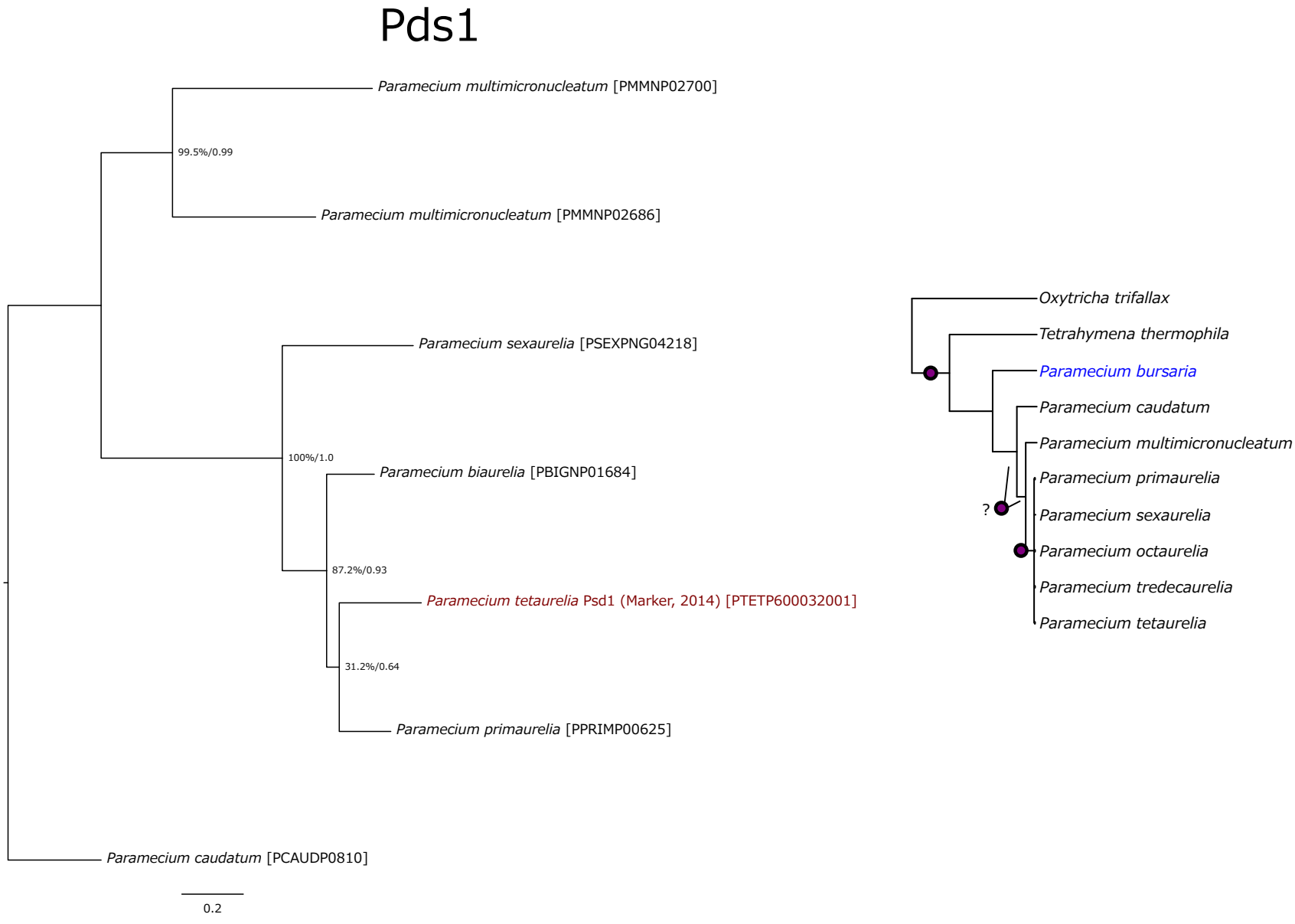
Pathway	Component	Function
transgene-induced siRNA	Rdr3	RdRP
	Ptiwi14	Piwi
both pathways	Rdr2	RdRP
	Dcr1	Dicer
	Ptiwi13	Piwi
	Cid2	Nucleotidyl transferase
exogenous dsRNA-induced siRNA	Rdr1	RdRP
	Cid1	Nucleotidyl transferase
	Ptiwi12	Piwi
	Ptiwi15	Piwi
	Pds1	Import of dsRNA?

Cid ancestor

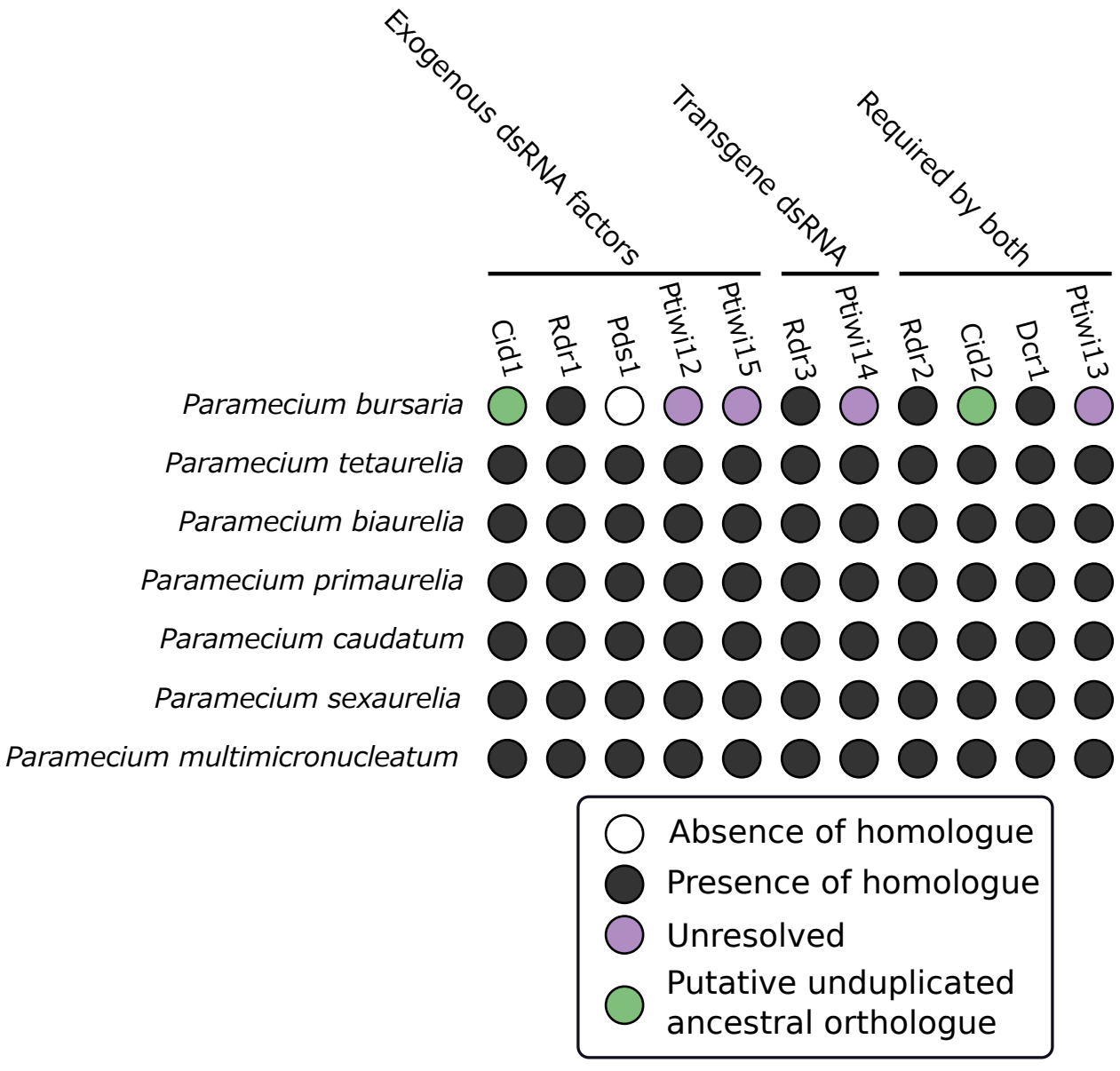




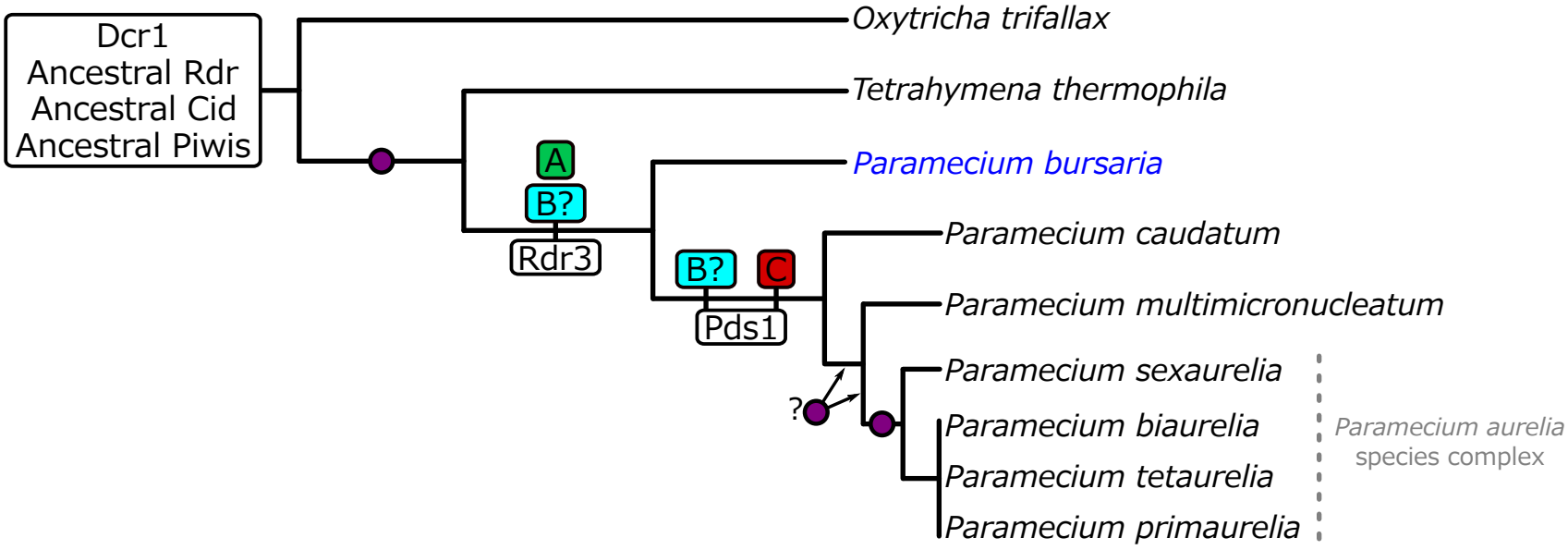
Pds1 absent


Pds1
Paramecium multimicronucleatum [PMMNP02700]
99.5%/0.99
Paramecium multimicronucleatum [PMMNP02686]
Paramecium sexaurelia [PSEXPNG04218]
100%/1.0
Paramecium biaurelia [PBIGNP01684]
87.2%/0.93
Paramecium tetaurelia Psd1 (Marker, 2014) [PTETP600032001]
31.2%/0.64
Paramecium primaurelia [PPRIMP00625]
Paramecium caudatum [PCAUDP0810]
0.2
Oxytricha trifallax
Tetrahymena thermophila
Paramecium bursaria
Paramecium caudatum
Paramecium multimicronucleatum
Paramecium primaurelia
?
Paramecium sexaurelia
Paramecium octaurelia
Paramecium tredecaurelia
Paramecium tetaurelia

Presence/absence of known pathway components



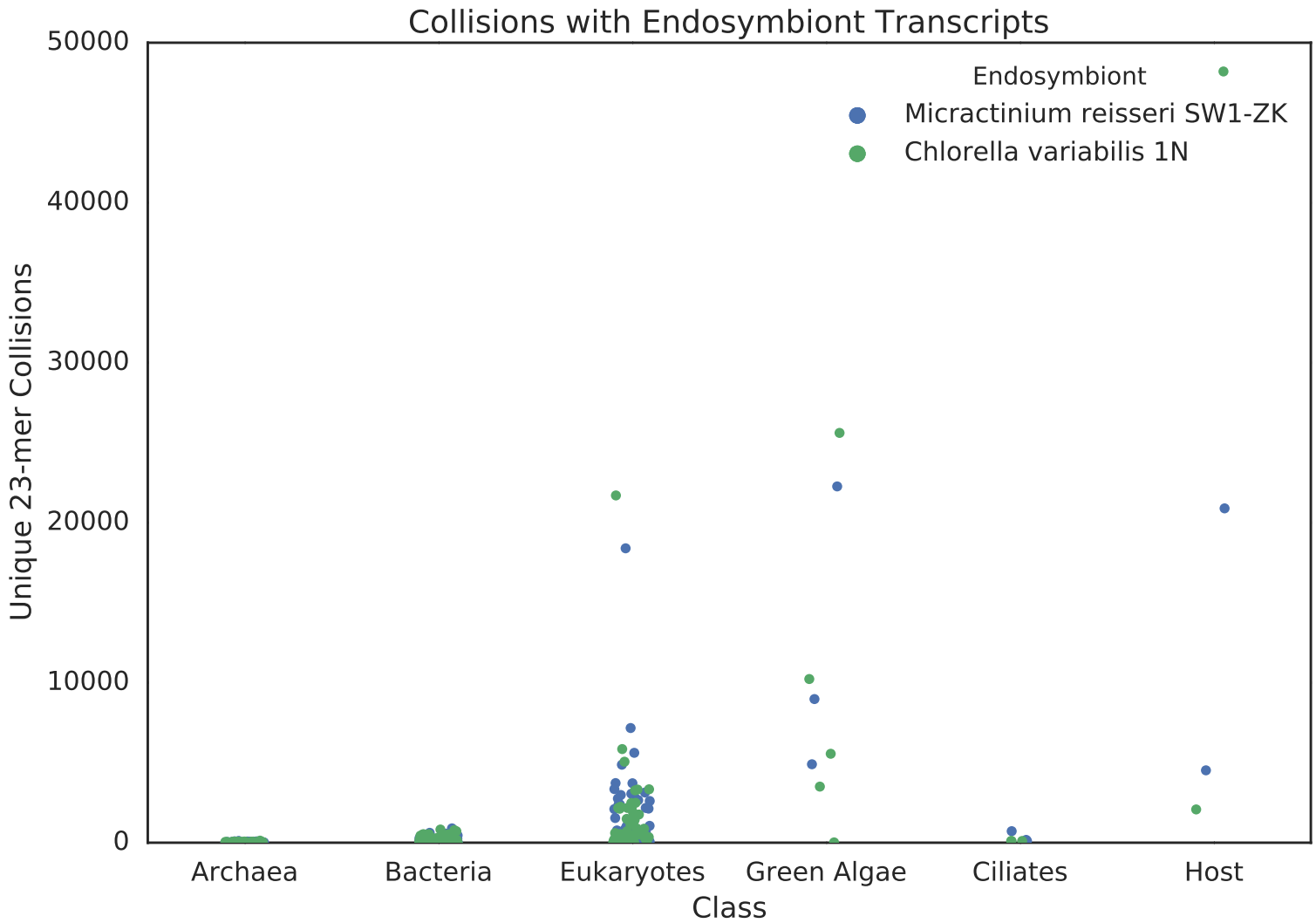
Putative RNAi component evolution scenario



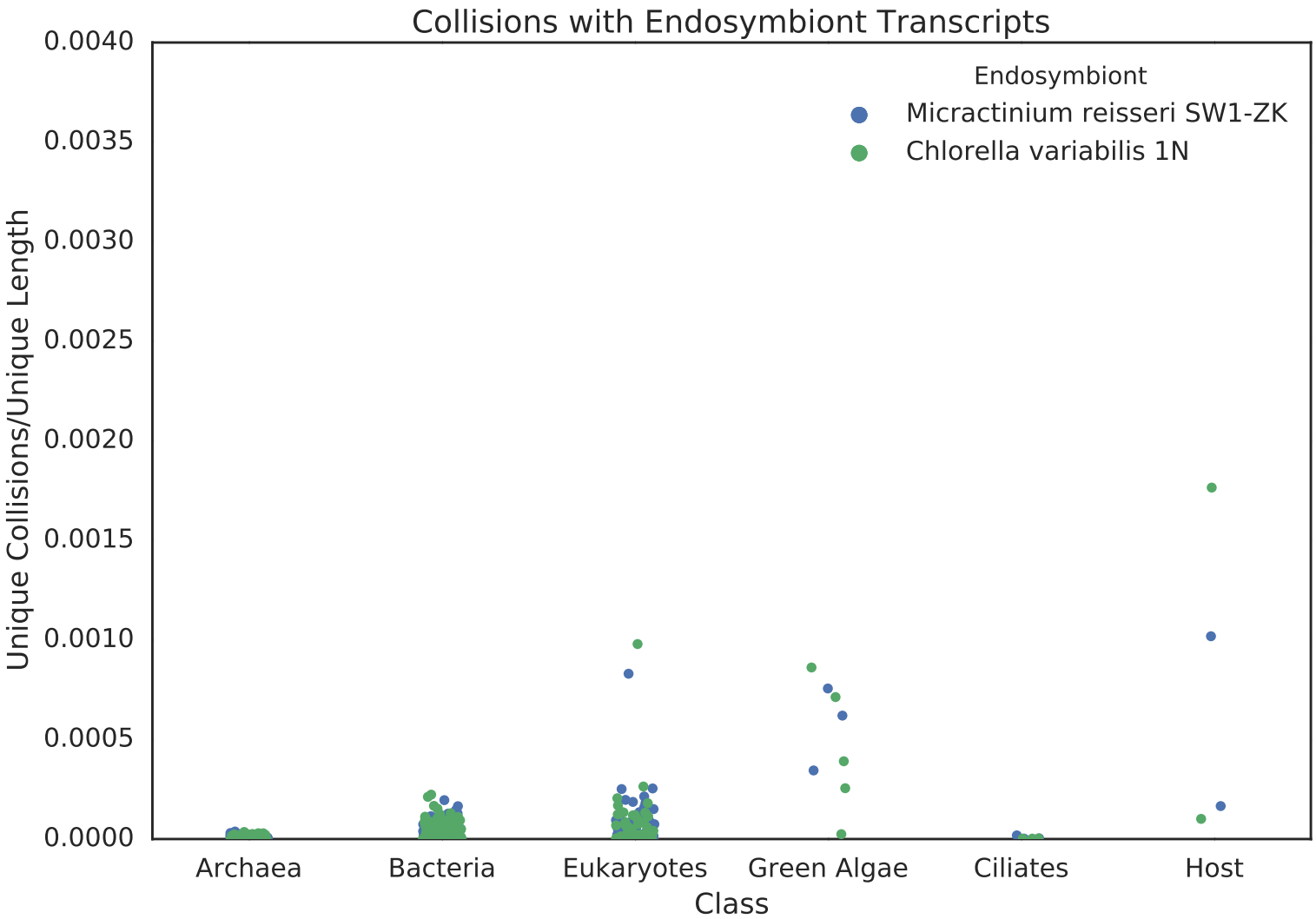
A	Duplication of ancestral Rdr into Rdr1 and Rdr2
B?	Duplication of ancestral Cid into Cid2 and Cid1-Cid3 ancestor
C	Duplication of Cid1-Cid3 ancestor into Cid1 and Cid3
●	Whole genome duplication

Could having a eukaryotic endosymbiont and RNAi activated by dsRNA in vacuoles be deleterious?

Higher level of collisions with eukaryotes



Collisions are a function of genome size



Conclusions

- RNAi phenotypes not inducible in most *P. bursaria* strains via feeding.
- *P. bursaria* lacks Pds1 thus may be unable to take up RNA from digestive vacuoles.
- High levels of 23-mer collisions between *P. bursaria* and endosymbiont transcriptomes may lead to deactivation of dsRNA uptake from vacuoles.
- Presence of other factors in active transcriptomes of multiple *P. bursaria* indicate transgene and microinjected exogenous dsRNA pathways may function although technically demanding.

Acknowledgements

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References

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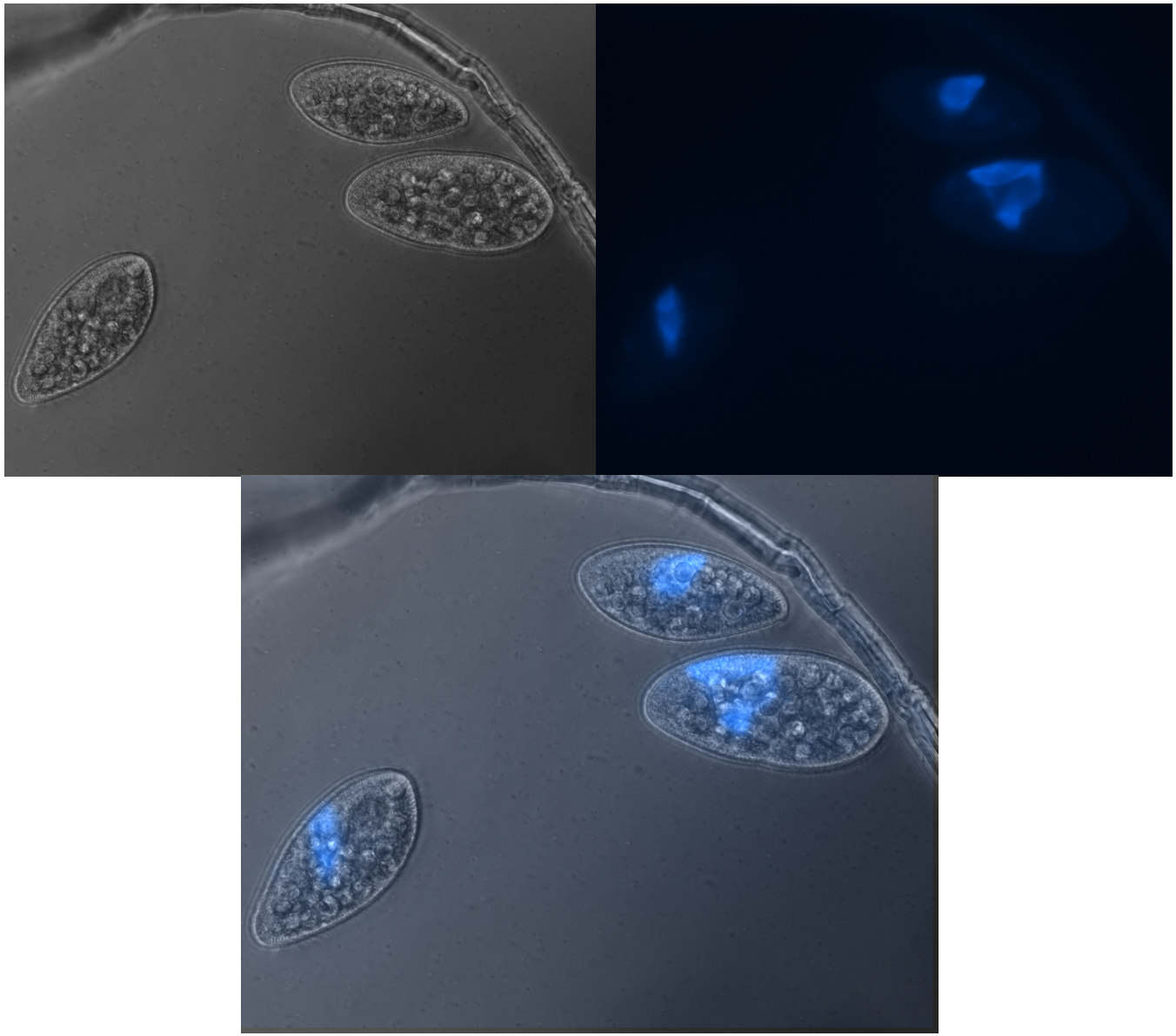
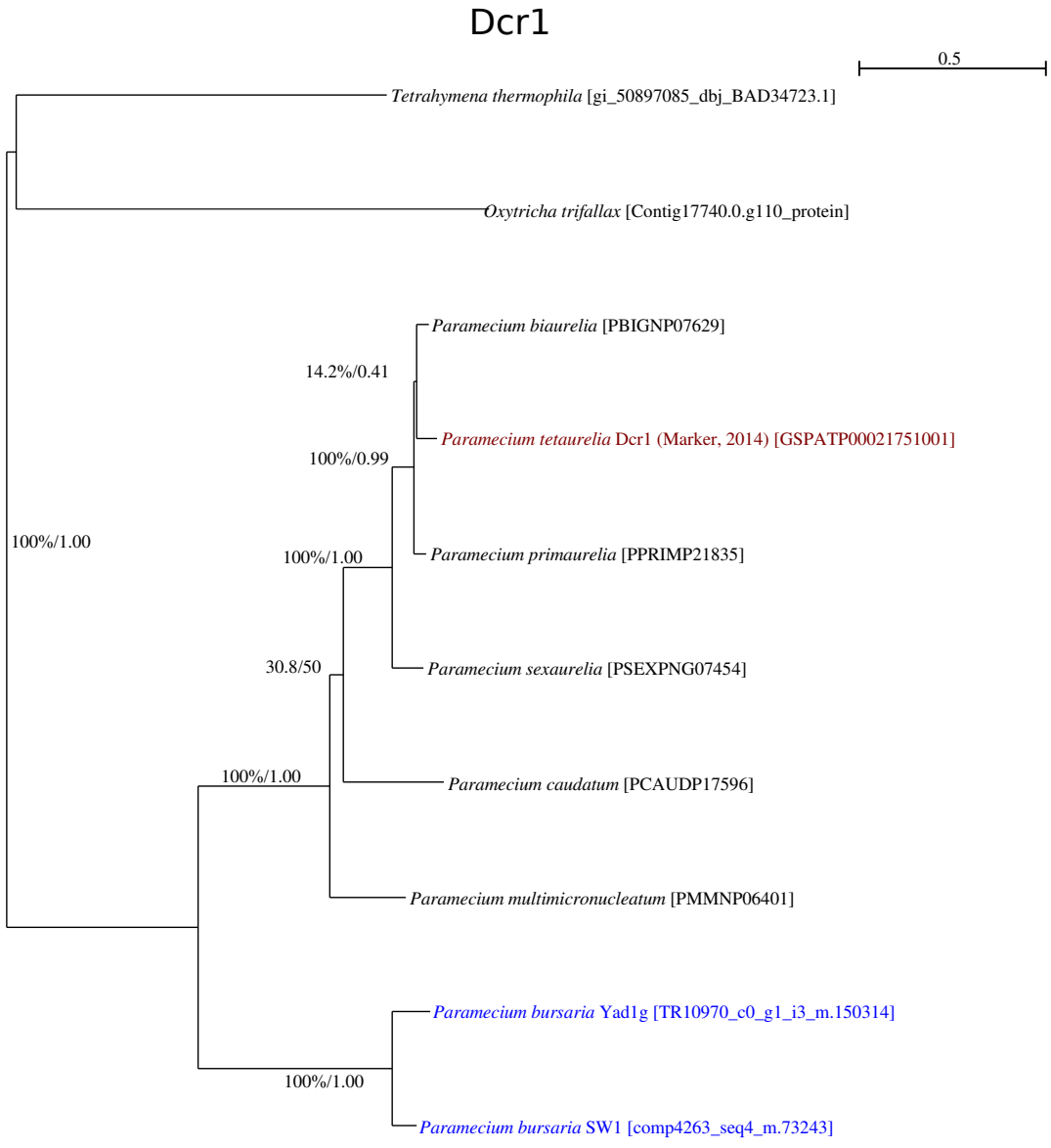
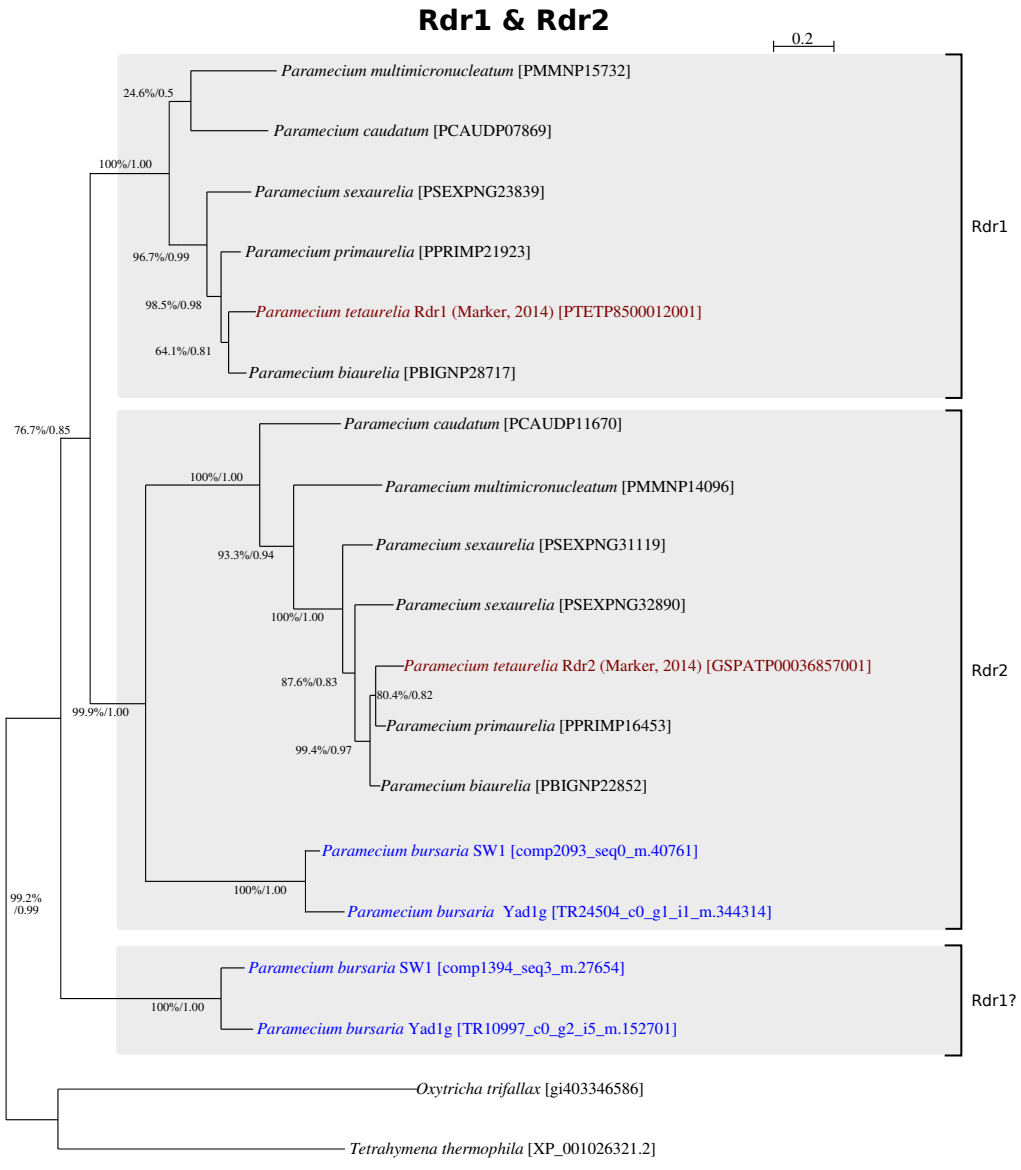
Microinjection proved difficult



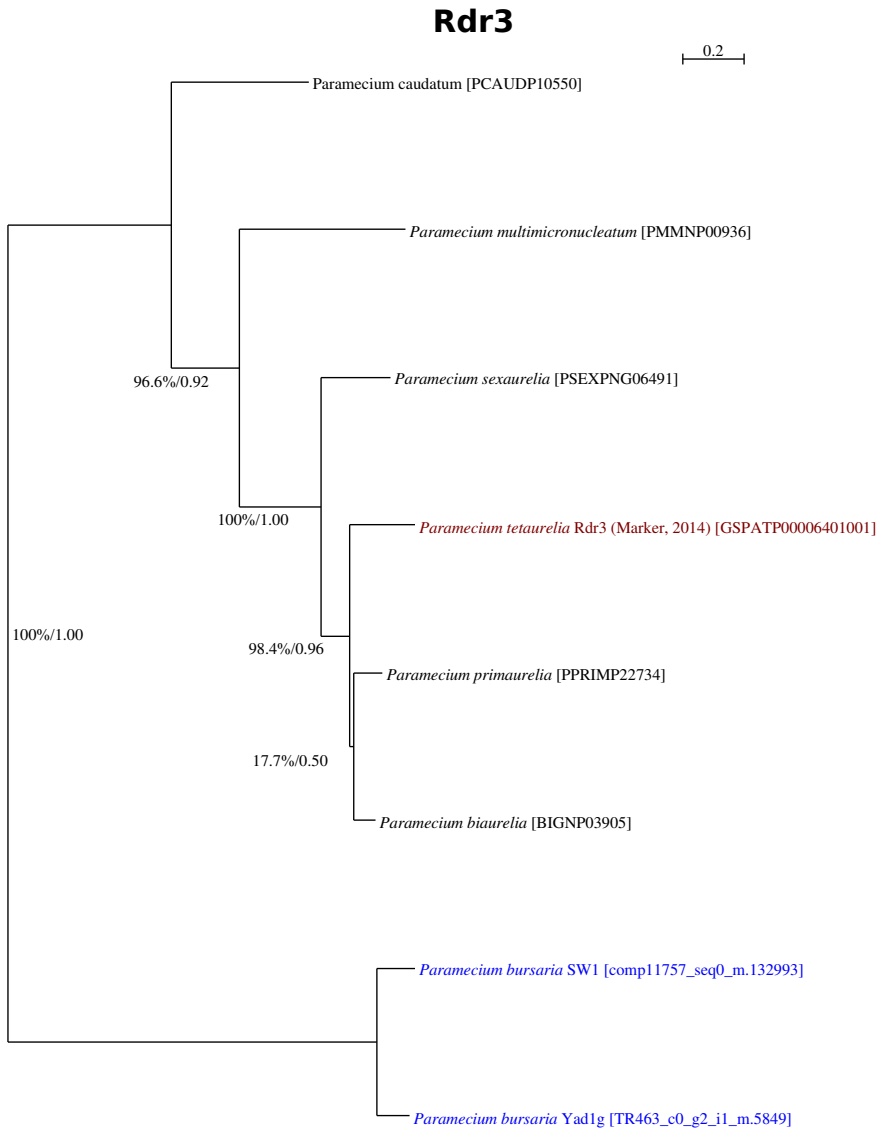
Dcr1


Dcr1
0.5
Tetrahymena thermophila [gi_50897085_dbj_BAD34723.1]
Oxytricha trifallax [Contig17740.0.g110_protein]
Paramecium biaurelia [PBIGNP07629]
14.2%/0.41
Paramecium tetaurelia Dcr1 (Marker, 2014) [GSPATP00021751001]
100%/0.99
100%/1.00
Paramecium primaurelia [PPRIMP21835]
100%/1.00
30.8/50
Paramecium sexaurelia [PSEXPNG07454]
100%/1.00
Paramecium caudatum [PCAUDP17596]
Paramecium multimicronucleatum [PMMNP06401]
Paramecium bursaria Yad1g [TR10970_c0_g1_i3_m.150314]
100%/1.00
Paramecium bursaria SW1 [comp4263_seq4_m.73243]

Rdr1 Rdr2


Rdr1 & Rdr2
0.2
Paramecium multimicronucleatum [PMMNP15732]
24.6%/0.5
Paramecium caudatum [PCAUDP07869]
100%/1.00
Paramecium sexaurelia [PSEXPNG23839]
96.7%/0.99
Paramecium primaurelia [PPRIMP21923]
98.5%/0.98
Paramecium tetaurelia Rdr1 (Marker, 2014) [PTETP8500012001]
64.1%/0.81
Paramecium biaurelia [PBIGNP28717]
Rdr1
76.7%/0.85
Paramecium caudatum [PCAUDP11670]
100%/1.00
Paramecium multimicronucleatum [PMMNP14096]
93.3%/0.94
Paramecium sexaurelia [PSEXPNG31119]
100%/1.00
Paramecium sexaurelia [PSEXPNG32890]
87.6%/0.83
Paramecium tetaurelia Rdr2 (Marker, 2014) [GSPATP00036857001]
80.4%/0.82
99.9%/1.00
Paramecium primaurelia [PPRIMP16453]
99.4%/0.97
Paramecium biaurelia [PBIGNP22852]
Paramecium bursaria SW1 [comp2093_seq0_m.40761]
100%/1.00
Paramecium bursaria Yad1g [TR24504_c0_g1_i1_m.344314]
Rdr2
99.2%
/0.99
Paramecium bursaria SW1 [comp1394_seq3_m.27654]
100%/1.00
Paramecium bursaria Yad1g [TR10997_c0_g2_i5_m.152701]
Rdr1?
Oxytricha trifallax [gi403346586]
Tetrahymena thermophila [XP_001026321.2]

Rdr3


Rdr3
0.2
Paramecium caudatum [PCAUDP10550]
Paramecium multimicronucleatum [PMMNP00936]
96.6%/0.92
Paramecium sexaurelia [PSEXPNG06491]
100%/1.00
Paramecium tetaurelia Rdr3 (Marker, 2014) [GSPATP00006401001]
100%/1.00
98.4%/0.96
Paramecium primaurelia [PPRIMP22734]
17.7%/0.50
Paramecium biaurelia [BIGNP03905]
Paramecium bursaria SW1 [comp11757_seq0_m.132993]
Paramecium bursaria Yad1g [TR463_c0_g2_i1_m.5849]

Psd1 Structure