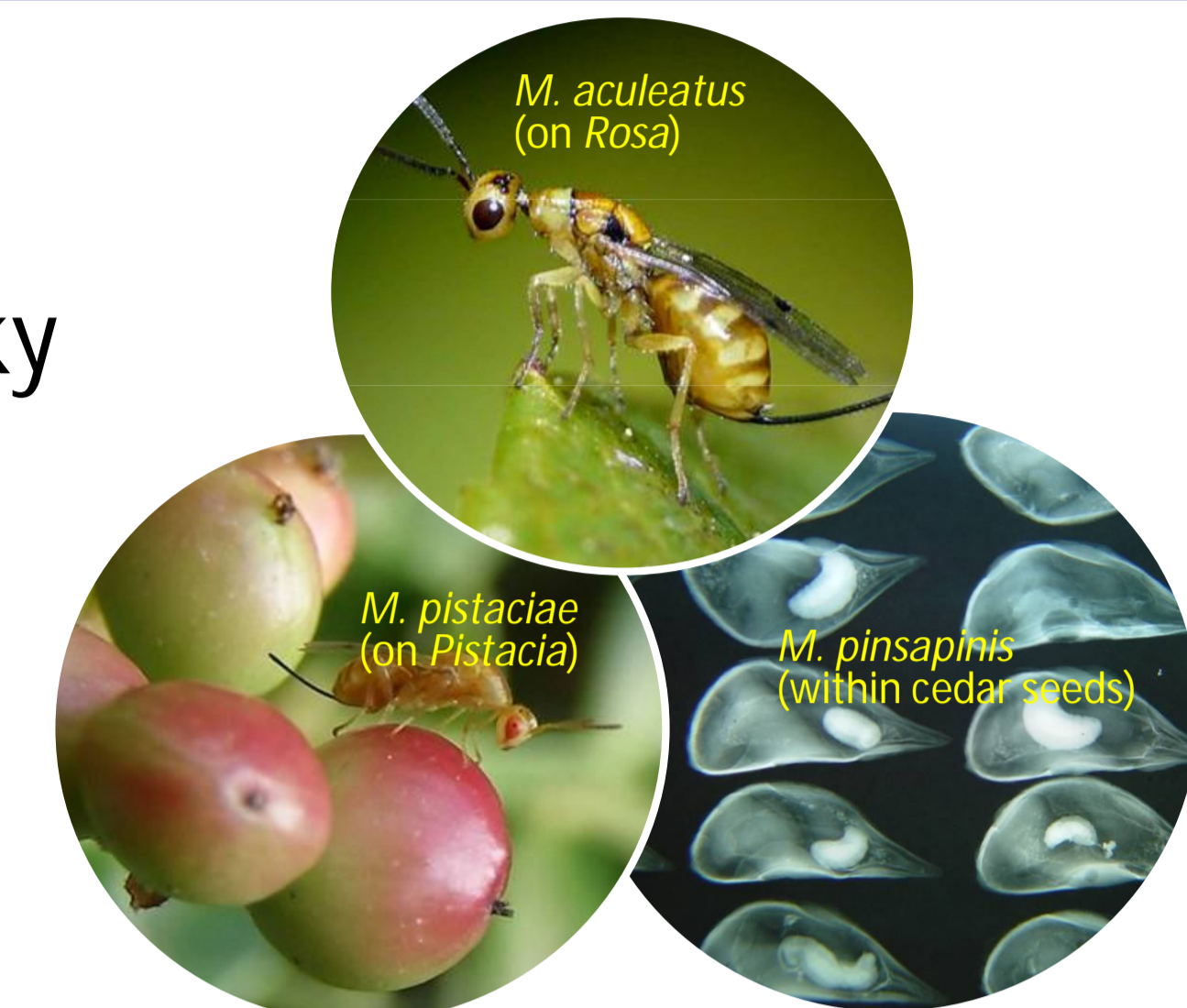


# HOST PLANT SPECIALIZATION MATTERS IN THE EPIDEMIOLOGY OF *WOLBACHIA* ACROSS PHYTOPHAGOUS WASPS (HYMENOPTERA: TORYMIDAE)

T. BOIVIN, H. HENRI, F. VAVRE, C. GIDOIN, P. VEBER, J-N. CANDAU, A. ROQUES & M-A. AUGER-ROZENBERG

Contact: thomas.boivin@paca.inra.fr

**Goal of the study** Focusing on seed wasps of the genus *Megastigmus*, we investigated whether ecological specialization is a likely mechanism of spread of thelytoky within insect communities. In these wasps, arrhenotoky is the dominant and ancestral form of parthenogenesis as thelytoky characterizes only 15 % of species, but both arrhenotokous and thelytokous species can co-occur on similar host-plant families (e.g. Rosaceae or Pinaceae). We explored the joint effects of phylogeny, ecology and endosymbionts on the spread of thelytoky within insects.



## Association between endosymbiont infection and thelytoky

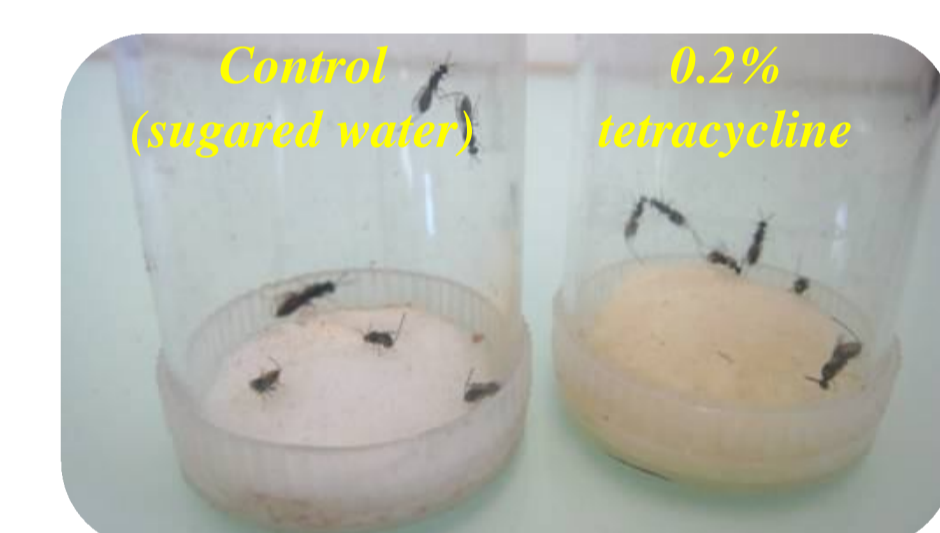
We tested for thelytoky caused by endosymbionts by screening in a large sample of arrhenotokous and thelytokous species for four endosymbiont candidates, and by performing antibiotic treatments.

- **Wolbachia infection is fixed in all thelytokous species**
- Arrhenotokous species are non-infected
- **Wolbachia curation: male production is reversible**

### PCR screening for parthenogenesis-inducing endosymbionts

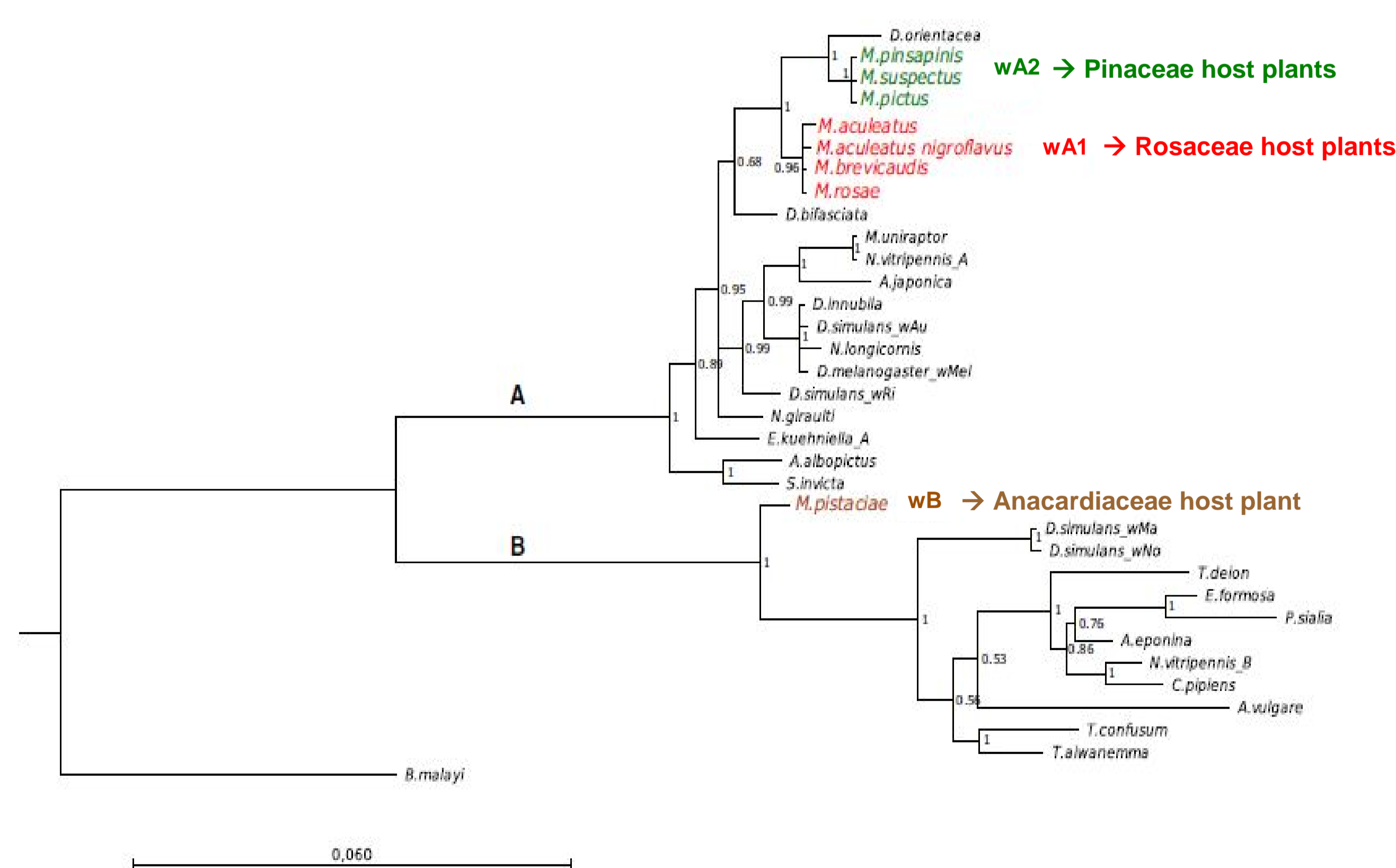
Parthenogenesis and species (N)	Wolbachia (infection %)	Cardinium	Arsenophomus	Rickettsia	Arthropod COI
<b>Thelytoky</b>					
<i>M. aculeatus</i> (43)	+ (100)	-	-	-	-
<i>M. aculeatus nigrovariegatus</i> (21)	+ (100)	-	-	-	-
<i>M. brevicaudis</i> (1)	+ (100)	NA	NA	NA	-
<i>M. boristi</i> (1)	+ (100)	NA	NA	NA	-
<i>M. pictus</i> (30)	+ (100)	-	-	-	-
<i>M. pinsapinis</i> (60)	+ (100)	-	-	-	-
<i>M. pistaciae</i> (30)	+ (100)	-	-	-	-
<i>M. rosae</i> (30)	+ (100)	-	-	-	-
<i>M. rosae alba</i> (5)	+ (100)	-	-	-	-
<i>M. suspectus</i> (65)	+ (100)	-	-	-	-
<b>Arrhenotoky</b>					
<i>M. amicornum</i> (20)	-	-	-	-	+
<i>M. atlanticus</i> (20)	-	-	-	-	+
<i>M. cryptomeriae</i> (20)	-	-	-	-	+
<i>M. pinus</i> (20)	-	-	-	-	+
<i>M. rafii</i> (30)	-	-	-	-	+
<i>M. spermotrophus</i> (30)	-	-	-	-	+
<i>M. schimitscheki</i> (30)	-	-	-	-	+
<i>M. transvaalensis</i> (20)	-	-	-	-	+

### Antibiotic treatments on the thelytokous *M. pinsapinis* (on *Cedrus*, Pinaceae)

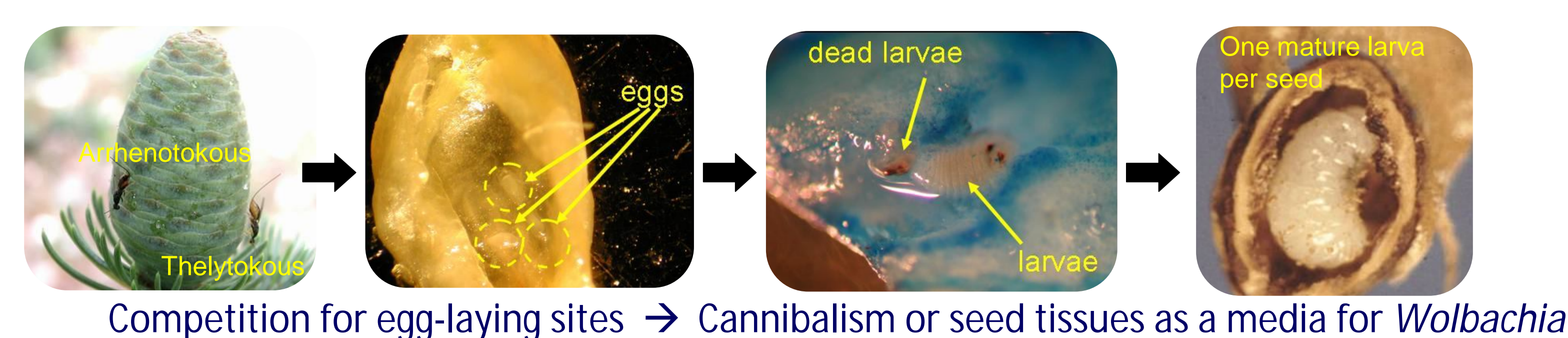


Treatment (N)	Wolbachia infection		Progeny produced by treated females		
	% of wsp positives**	Number of mothers	Mean size***	Mean % of males	
control (50)	100	44	21.4 ± 5.3 <sup>a</sup>	0	
tetracycline (50)	4.7	42	18.7 ± 7.8 <sup>a</sup>	92 ± 1.1	

Figure 1. Phylogenetic placement of the *Wolbachia* strains infecting *Megastigmus* spp. among other *Wolbachia* belonging to A and B supergroups. This Bayesian-likelihood inference phylogeny is based on concatenated sequences data set for the four MLST loci *coxA*, *gatB*, *ftsZ* and *hcpA* (34 strains, 1650 bp).



**Horizontal transfer pathways** Seeds are narrow niches favoring highly overlapping breeding sites: opportunities for intimate ecological connections between infected and non-infected species

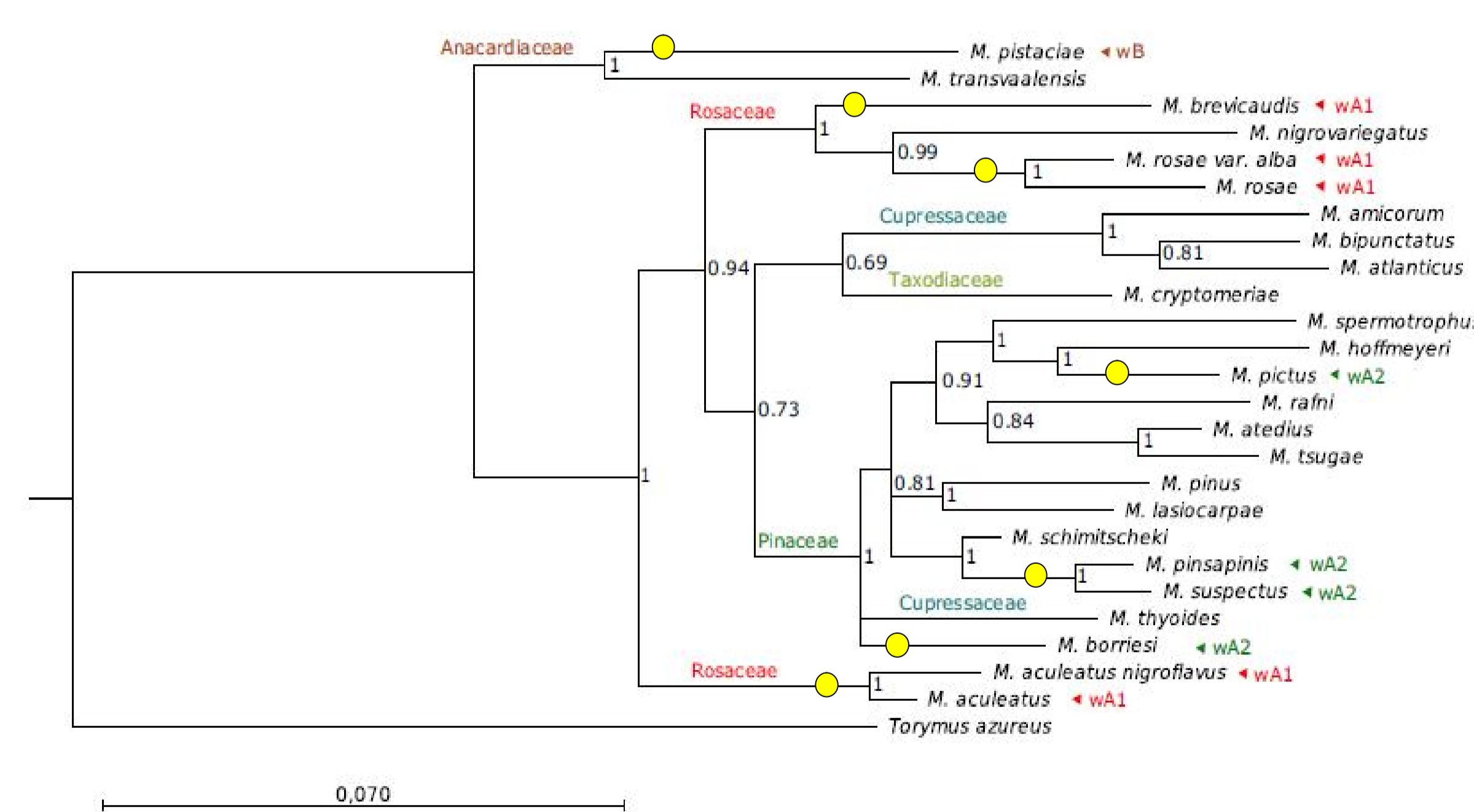


## Evolutionary *Wolbachia*-*Megastigmus*-host plant associations

We performed phylogenetic reconstructions using *Wolbachia* multilocus sequence typing (MLST) and wasp COI sequences to examine the evolution of *Wolbachia*-mediated thelytoky in *Megastigmus* and its possible connections to host plant specialization.

- We characterized **3 highly homogeneous *Wolbachia* lineages (wA1, wA2 and wB)**, with a strict association with host plant but not with *Megastigmus* (Fig.1)
- Phylogenetic signals of ecological specialization in the wasp COI tree (Fig.2): **1 basal clade on Anacardiaceae, 2 more recent clades on Rosaceae, 1 monophyletic clade on Pinaceae**
- **Thelytoky occurs sporadically** in the *Megastigmus* tree, with 7 independent *Wolbachia* acquisitions (●, Fig.2)
- **Non-random plant-specific distribution** of thelytokous species (P=0.0051)
- **Incongruence between *Wolbachia* and *Megastigmus* phylogenies (P>0.05) suggests horizontal transmission**

Figure 2. Bayesian-likelihood inference phylogeny based on cytochrome oxidase I (COI) sequences in *Megastigmus* (26 taxa, 962 bp). Host plant families are indicated at each branch forming a monophyletic group (except for the Cupressaceae). *Megastigmus* species infected by *Wolbachia* are indicated by arrowheads followed by the name of the *Wolbachia* lineage.



**Conclusion** Ecological specialization in *Wolbachia*'s hosts was probably a critical driving force for *Wolbachia* infection and spread of thelytoky, but also a constraint.

