

**The ghost sex-life of the paedogenetic beetle *Micromalthus debilis***  
M. Alejandra Perotti, Daniel K. Young, & Henk R. Braig

1 **SUPPLEMENTARY Materials**

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3 **The ghost sex-life of the paedogenetic beetle *Micromalthus debilis***

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10 **Supplementary figures** **2**

11 **Movie snapshot and legend** **4**

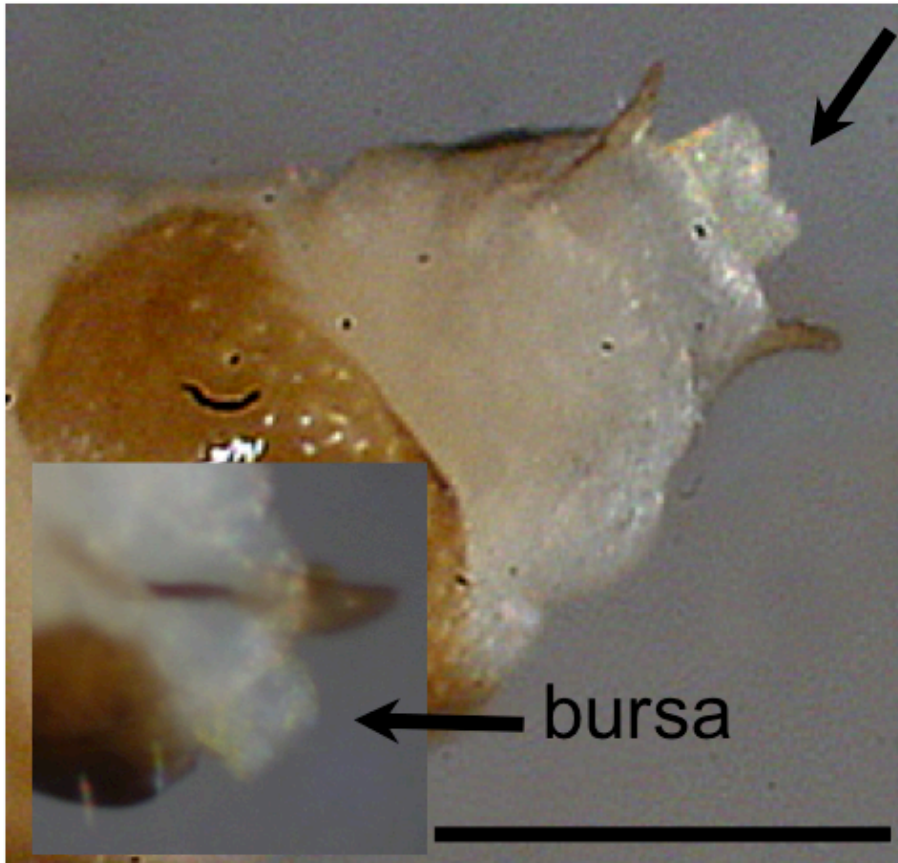
12 **Supplementary tables** **5**

13 SUPPLEMENTARY FIGURES

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15 SUPPLEMENTARY FIGURE S1

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20 Everted *bursa copulatrix*, arrows showing the position of the *bursa* in the adult  
21 female (insert shows the bursa in lateral position) (Bar 100  $\mu$ m).

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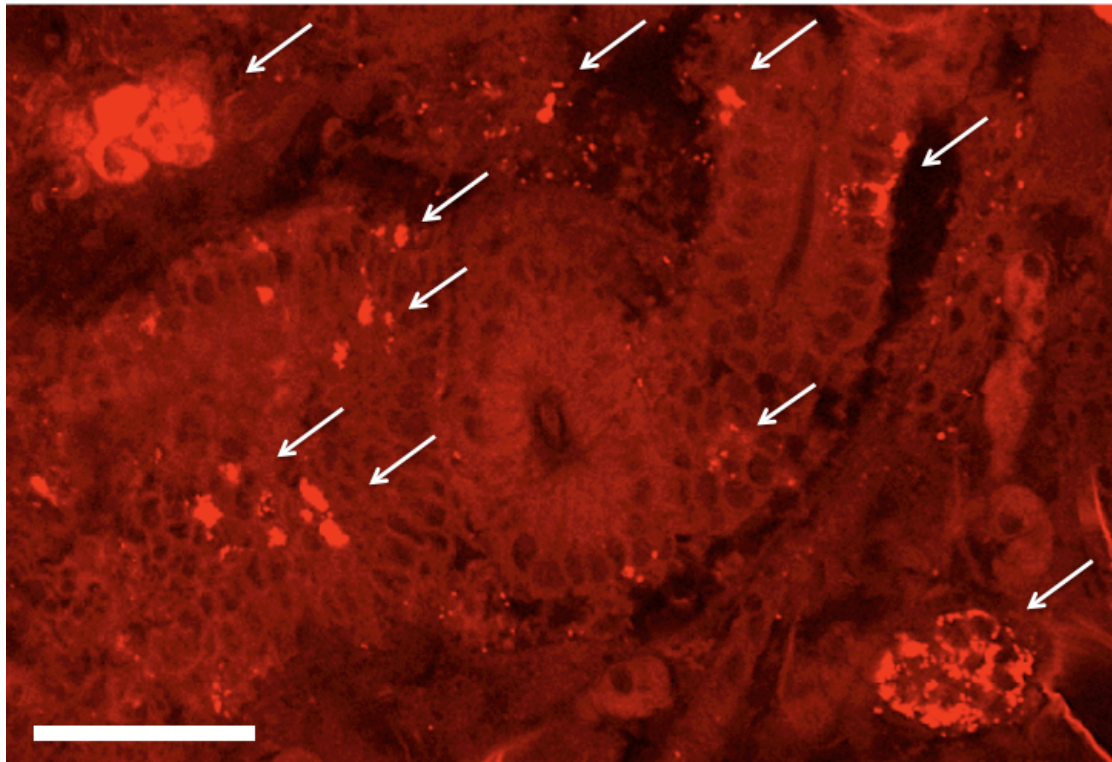
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34 **SUPPLEMENTARY FIGURE S2**

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40 *Rickettsia*-specific FISH probe labelled with Cy5 on *Micromalthus* larvae showing

41 oesophagus and midgut in the centre of the picture. Arrows point to *Rickettsia*-

42 filled mycetocytes in the epithelium of the midgut and in the upper left corner

43 and lower right corner a paired mycetomic organ (Bar 50  $\mu$ m).

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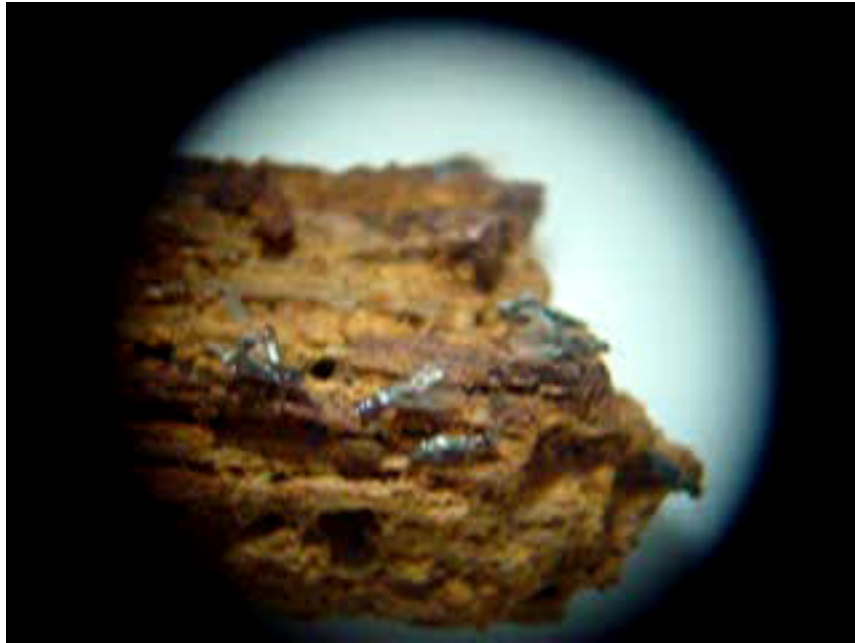
50 **SUPPLEMENTARY MOVIE (SNAPSHOT)**

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56 **Movie legend**

57 Movie S1: Female dance: Immediately after emergence from their pupae,  
58 females produce an up-down, sideways shaking of their abdomens while beating  
59 their wings.

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64 **SUPPLEMENTARY TABLES**

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66 **Table S1:** Primary sex ratios and effect of Heat (and drought) Treatment (HT) on  
 67 *Micromalthus* larvae. Forty-eight mature populations of larvae were used, of logs of  
 68 different origins (defined Locations are found in Methods). Populations were used in  
 69 2 Sets of 2 x 12 populations each, Set 1: HT applied and Set 2: Controls or never  
 70 exposed to HT. Larvae were counted before and after the HT, with Treat1 indicating  
 71 Before and Treat2 indicating After exposure (or not, like for Set2). Larvae were  
 72 extracted, sexed and counted in each of the two sets.

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Sets	Locations	Treat 1 Females	Treat 1 Males	Treat 2 Females	Treat 2 Males
1-HT applied	1	235	0	0	0
1-HT applied	1	257	1	0	0
1-HT applied	2	229	1	0	0
1-HT applied	2	258	1	0	0
1-HT applied	3	178	3	1	0
1-HT applied	3	111	0	0	0
1-HT applied	3	199	0	7	0
1-HT applied	3	127	1	0	0
1-HT applied	4	243	1	0	0
1-HT applied	4	222	2	0	0
1-HT applied	4	251	1	0	0
1-HT applied	3	192	0	0	0
2-NO HT	2	200	0	235	1
2-NO HT	2	250	0	199	0
2-NO HT	1	233	2	226	0
2-NO HT	1	271	1	249	2
2-NO HT	3	156	1	121	0
2-NO HT	3	209	0	173	0
2-NO HT	3	177	0	184	1
2-NO HT	3	134	0	129	1
2-NO HT	4	216	3	201	4
2-NO HT	4	210	1	266	0
2-NO HT	4	263	5	298	1
2-NO HT	3	186	0	232	0

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80 **Table S2:** Pairs of datasets to compare *Micromalthus* larva and adult sex proportions  
81 by locations (the only populations of larvae and adults that could be paired, according  
82 to Location).

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<b>Sex</b>	<b>Replicate</b>	<b>Location</b>	<b>Larvae Control</b>	<b>Larvae After-HT</b>	<b>ADULTS</b>
Females	1	2	235	0	72
Females	2	2	199	0	200
Male	1	2	1	0	1
Male	2	2	0	0	0
Females	1	3	121	0	132
Females	2	3	173	0	59
Male	1	3	0	0	6
Male	2	3	0	0	31

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87 **Table S3:** Results of Behavioural observations of *Micromalthus* adults. Three  
 88 Groups of females (called Experiments) were used. The origin of each Group set was  
 89 also controlled (Locality). Behaviours: A: Male discrimination; B: Female-female  
 90 mounting; C: Female dance; D: Female-male mounting; E: Female-female combat;  
 91 and F: Females injuring males.

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Experiments	Number of Females	Behaviours						Locality
		A	B	C	D	E	F	
Related	17	17	9	6	0	0	0	2
Related	18	18	8	8	0	0	0	2
Related	18	18	9	10	0	0	0	2
Related	15	14	11	8	0	0	0	3
Related	15	7	10	13	4	3	0	3
Related	20	20	14	19	0	0	0	3
Related	20	20	6	14	0	0	0	5
Related	20	18	10	17	0	0	0	3
Related	12	12	12	11	0	0	0	3
Not related	9	0	9	0	8	6	2	2
Not related	14	0	7	0	7	5	0	2
Not related	20	4	16	10	15	7	0	3
Not related	20	0	4	3	14	11	0	3
Not related	20	0	5	6	17	13	0	3
Not related	17	0	2	5	12	9	0	3
Not related	17	2	11	7	17	15	0	5
Not related	20	0	17	8	17	17	0	3
Controls	6	0	5	6	0	0	0	2
Controls	9	0	6	8	0	0	0	2
Controls	11	0	5	11	0	0	0	2
Controls	10	0	4	10	0	0	0	3
Controls	14	0	5	13	0	0	0	3
Controls	8	0	3	8	0	0	0	5

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