

Title:

Algal diversity in *Paramecium bursaria*: species identification, detection of *Choricystis parasitica*, and assessment of the interaction specificity

Authors:

Felicitas E. Flemming ¹, Alexey Potekhin ^{2,3}, Thomas Pröschold ⁴ & Martina Schrällhammer ¹

Affiliations:

¹ Microbiology, Biological Institute II, Albert Ludwigs University of Freiburg, Freiburg, Germany

² Department of Microbiology, Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia

³ Laboratory of Cellular and Molecular Protistology, Zoological Institute RAS, Saint Petersburg, Russia

⁴ University of Innsbruck, Research Department for Limnology, Mondsee, Austria

Supplementary Tables and Figures

Table S1: Primer combinations and their PCR program specifications.

Table S2: General PCR program to amplify the SSU rRNA gene.

Table S3: Model parameters calculated by PAUP for all datasets.

Table S4: Combinations of aposymbiotic *Paramecium bursaria* strains and isolated algae used in the re- and cross-infection experiments.

Figure S1. Diagnostic PCR for identification of *Micractinium conductrix*.

Table S1: Primer combinations and their PCR program specifications. Annealing temperatures (a - c) as well as annealing times (x - z) are listed for primer combinations for the general PCR program (Supplementary Table S2).

Forward	Primer combinations		PCR program
		Reverse	
Chlo_G800F		ITS055R	a = 60.0 °C, b = 58.0 °C, c = 56.0 °C, x, y, z = 1.0 min
Chori_F238		Chori_R841	a = 60.0 °C, b = 58.0 °C, c = 56.0 °C, x, y, z = 1.0 min
Penic_F82		Penic_R1280	a = 54.0 °C, b = 52.0 °C, c = 50.0 °C, x, y, z = 0.5 min
Penic_F661		28S_R457	a = 54.0 °C, b = 52.0 °C, c = 50.0 °C, x, y, z = 1.0 min

Table S2: General PCR program to amplify the SSU rRNA gene. Annealing temperatures (a - c) and times (x - z) are defined in accordance with primer combinations in Supplementary Table S1.

Temperature [°C]	Time [min]	Number of cycles
94	15	-
94	0.5	5
a	x	
72	1.5	
94	0.5	15
b	y	
72	1.5	
94	0.5	20
c	z	
72	1.5	
72	10	-
15	∞	-

Table S3: Model parameters calculated using the automated model selection tool of the software PAUP for all datasets. Substitution rates for G - T were always 1.000.

Dataset	Model	Substitution rates					Base frequencies			
		A - C	A - G	A - T	C - G	C - T	A	C	G	T
Peniculida_SSU	GTR+I+G	1.2263328	3.1510719	2.3681959	0.480006	5.80928	0.26905658	0.19029093	0.26006192	0.2806
Pbursaria_ITS	GTR+I	2.031	3.719	5.586	1.029	7.185	0.34625661	0.16416535	0.1502948	0.3393
Chlorellaceae_SSU-ITS	GTR+I+G	0.725494	1.1143	0.853439	0.58392	2.84971	0.203232	0.281986	0.271048	0.243734
<i>Choricystis</i> _SSU	TIM+I+G	2.7129043	1.5123113	1.5123113	8.5500965	1.000	0.26388291	0.2010175	0.29374726	0.2414

Table S4: Combinations of aposymbiotic *Paramecium bursaria* strains and isolated algae used in the re- and cross-infection experiments.

	Aposymbiotic receiver strains					
	<i>P. bursaria</i> JPN (natural host of <i>Chl. variabilis</i>) syngen R3		<i>P. bursaria</i> RanNy (natural host of <i>M. conductrix</i>) syngen R2		<i>P. bursaria</i> Scot (natural host of <i>M. conductrix</i>) syngen R1	
<i>Chl. variabilis</i> (freshly isolated from <i>P. bursaria</i> CBS)	nd	nd	nd	nd	x	+
<i>Chl. variabilis</i> (freshly isolated from <i>P. bursaria</i> JPN)	x	+	x	+	nd	nd
<i>Chl. variabilis</i> (freshly isolated from <i>P. bursaria</i> Tüb2015)	x	+	nd	nd	nd	nd
<i>Chl. variabilis</i> (obtained from algal culture)	x	+	nd	nd	nd	nd
<i>M. conductrix</i> (freshly isolated from <i>P. bursaria</i> Scot)	x	+	nd	nd	nd	nd
<i>M. conductrix</i> (freshly isolated from <i>P. bursaria</i> RanNy)	x	+	nd	nd	nd	nd
<i>Chor. parasitica</i> (obtained from <i>P. bursaria</i> Frieds)	x	+	nd	nd	x	+

nd: not determined; x: conducted re- / cross-infection experiment; +: successful establishment of symbiosis

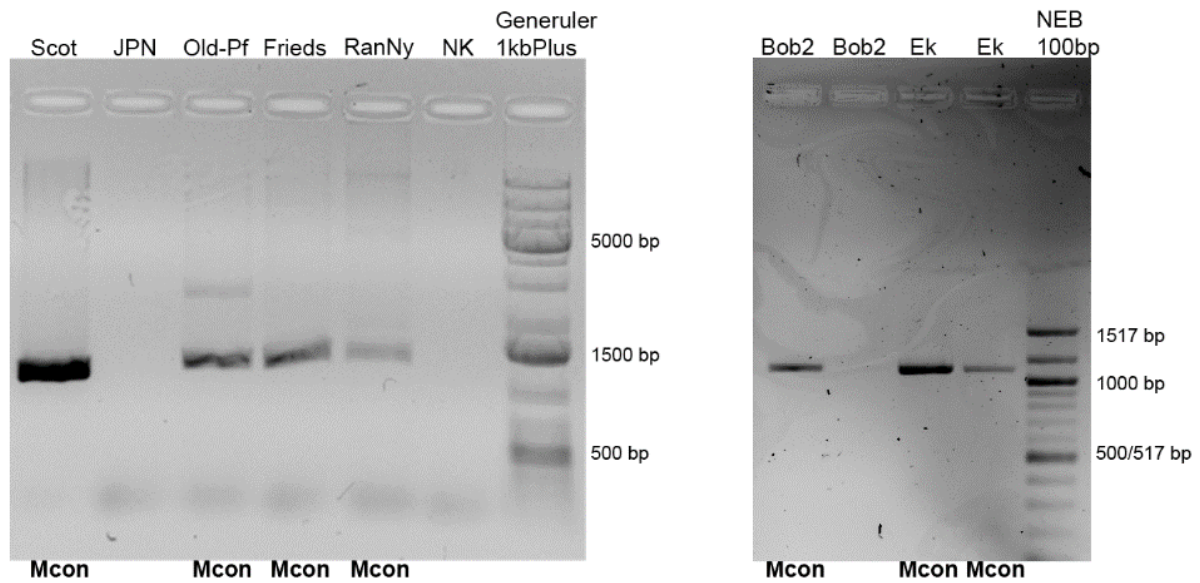


Figure S1. Diagnostic PCR for identification of *Micractinium conductrix*. The PCR was performed according to Spanner et al. [50] with the primer combination MconF & ITS055R. Positive results were obtained for strains Scot, Old-Pf, Frieds, RanNy, Bob2, and Ek, labelled with Mcon at the lower border of the agarose gel. Strain JPN carries *Chlorella variabilis*. For strains Bob2 and Ek, the PCR was carried out with either 2 µl (left) or 4 µl (right) template DNA. NK – negative control (without template DNA).