

SUPPORTING INFORMATION

High Diversity Revealed in Leaf Associated Protists (Rhizaria: Cercozoa) of Brassicaceae by Sebastian Ploch, Laura Rose, David Bass, Michael Bonkowski

Supplemental Material and Methods

Supplemental Table 1 Presence/absence of OTUs for all four species at the two locations, total number of OTUs per species by location combination, and number of unique OTUs are given.

Supplemental Materials and Methods

DNA extraction protocol based on Michiels et al. (2003) modified and adjusted for small scale extractions. The leaf samples were homogenized with metal beads in a 2-ml tube using a shaker mill (MM 200; Retsch GmbH, Germany). 800 μ l of CTAB-buffer (100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 2% Polyvinylpyrrolidone, 0.2% 2-Mercaptoethanol) was added, mixed thoroughly and incubated for 60 min at 60 °C while mixing regularly. 800 μ l of phenol/chloroform/isoamyl alcohol (25:24:1; Carl Roth, Germany) was added and mixed by inverting the tube. The mixture was centrifuged for 10 min at 8,000 g, and 700 μ l of the aqueous phase was transferred into a new 2-ml tube. 15 μ l RNase-A-solution (10 mg ml⁻¹ RNase A) was added and incubated at 37 °C for 15-30 min. 700 μ l phenol/chloroform/isoamyl alcohol (25:24:1) was added, mixed by inverting and centrifuged as above. 600 μ l of the aqueous phase was transferred to a new 2-ml tube, 600 μ l chloroform/isoamyl alcohol (24:1; Carl Roth, Germany) added, mixed by inverting and centrifuged as above. 500 μ l of the aqueous phase was transferred to a new 2-ml tube, 1,000 μ l precipitation-buffer (100% ethanol, 150 mM sodium acetate) added, and incubated in the freezer (-20°C) for at least 1 h. After centrifugation (30 min, 4 °C, 8,000 g) the supernatant was discarded, 500 μ l ice-cold washing-buffer (70% ethanol) added, and centrifuged for 5 min at 8,000 g. The washing step was repeated once, ethanol carefully removed and the pellet dried. Subsequently, the pellet was dissolved in sterile water.

Supplemental Table 1 Presence/absence of OTUs for all four species at the two locations, total number of OTUs per species by location combination, and number of unique OTUs are given.

	Düsseldorf				Frankfurt			
	<i>A. thaliana</i>	<i>C. hirsuta</i>	<i>C. pratensis</i>	<i>D. verna</i>	<i>A. thaliana</i>	<i>C. hirsuta</i>	<i>C. pratensis</i>	<i>D. verna</i>
OTU01	1	1	1	1	1	1	1	1
OTU02	1	1	1	0	1	1	0	1
OTU03	0	1	1	1	1	1	0	1
OTU04	1	1	1	1	1	1	0	0
OTU05	0	1	0	0	1	1	0	1
OTU06	0	1	0	1	1	0	1	0
OTU07	0	1	1	1	0	1	1	0
OTU08	0	0	0	1	0	1	0	0
OTU09	0	1	1	0	0	0	1	0
OTU10	0	0	0	0	1	0	0	0
OTU11	0	0	0	1	0	0	0	0
OTU12	0	1	0	0	0	0	0	0
OTU13	0	0	0	1	0	0	0	0
OTU14	1	0	0	0	0	0	0	0
OTU15	1	0	0	0	0	0	0	0
OTU16	0	0	1	0	0	0	0	0
OTU17	0	0	0	0	1	0	0	0
OTU18	1	0	0	0	0	0	0	0
OTU19	0	0	0	0	1	0	0	0
OTU20	1	0	0	0	0	0	0	0
OTU21	0	0	1	0	0	0	0	0
OTU22	0	0	0	0	0	1	0	0
OTU23	0	0	0	0	0	0	0	1
OTU24	0	0	0	0	0	1	0	0
total no.	7	9	8	8	9	9	4	5
unique	4	1	2	2	3	2	0	1