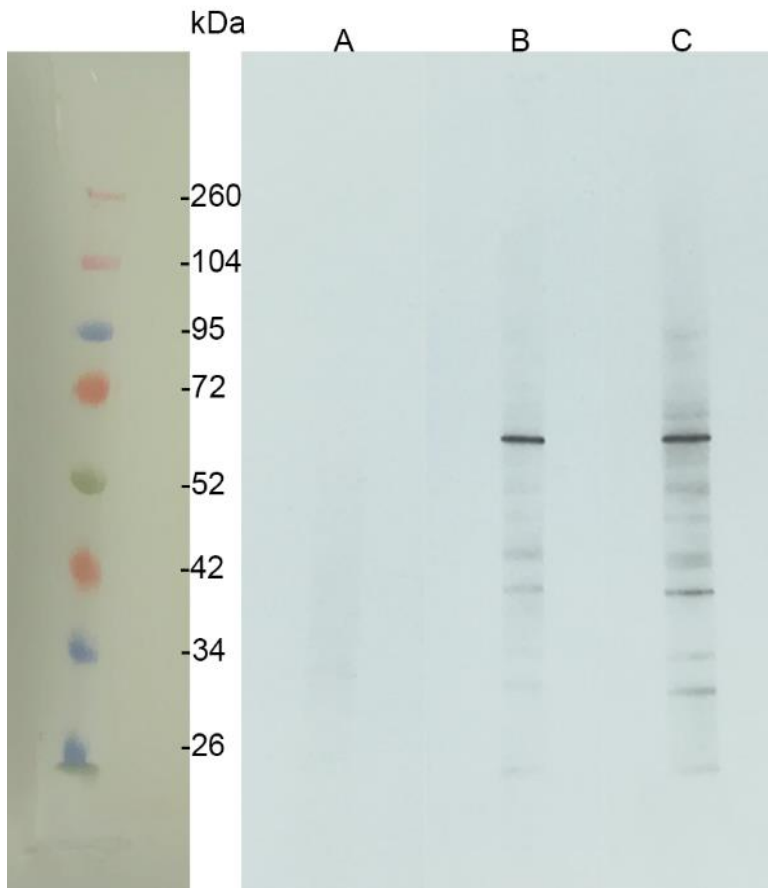
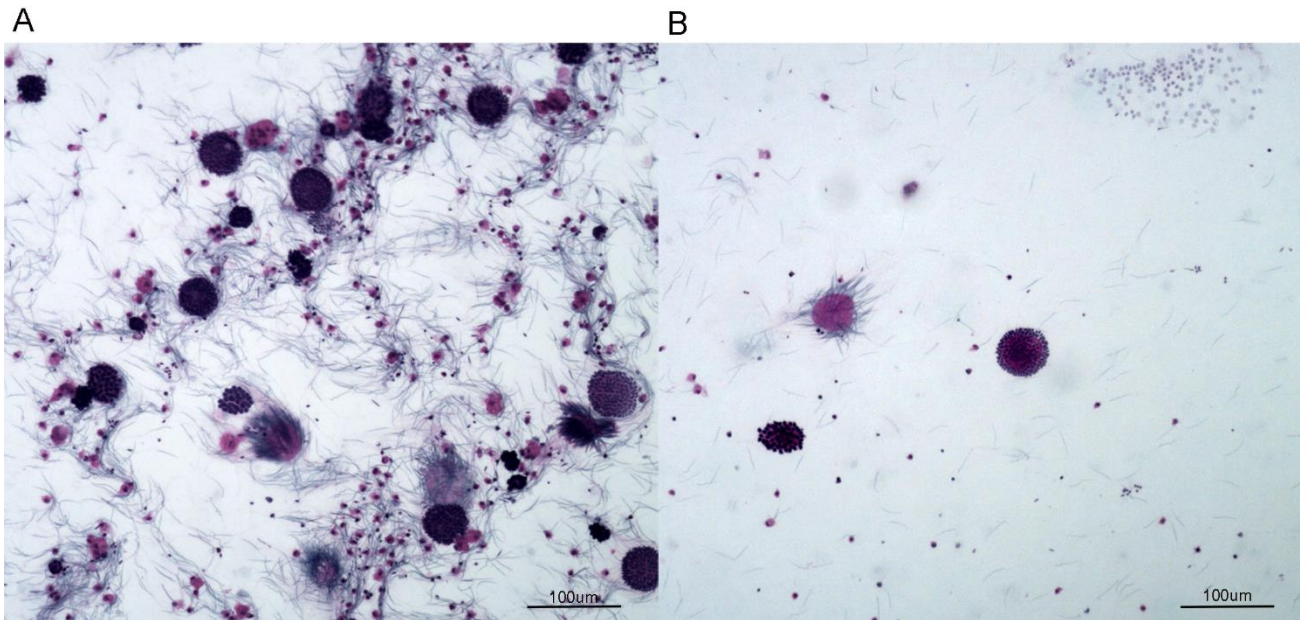


Supplementary Material

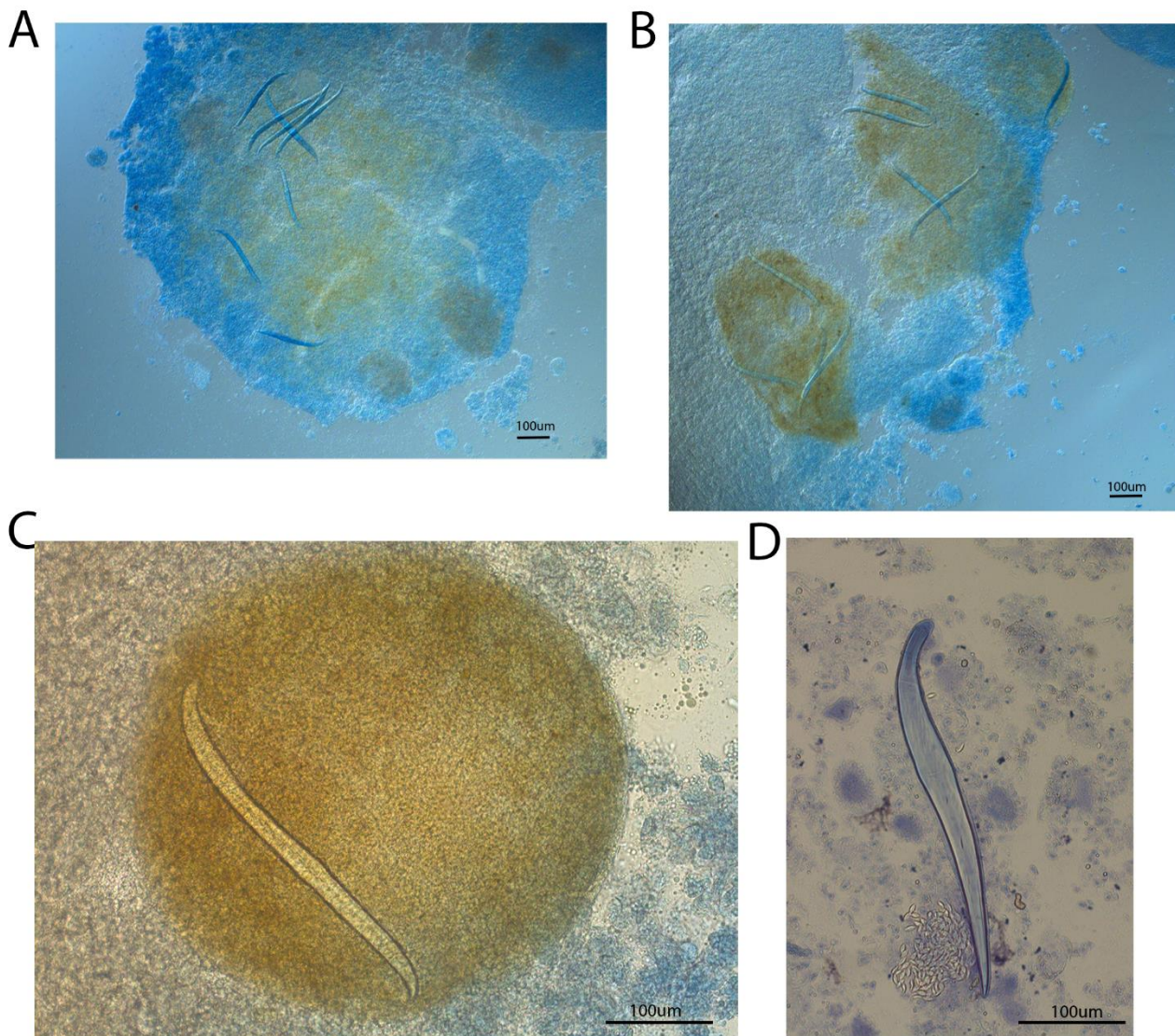
1 Supplementary Figures



Supplementary Figure 1. Western blot analysis of seminal vesicles protein lysate probed with rabbit anti- NF- κ B p65 antibody followed by detection with HRP conjugated goat anti-rabbit IgG. Seminal vesicles from 8 adult earthworms were dissected and cultivated in 60% RPMI medium with the antibiotic mixture for 6 hours with (ctrl) or without profilin antigen. Tissues were lysed in T-Per Tissue protein extraction reagent (Thermo Scientific) with proteinase inhibitor Arrest (Thermo Scientific) in combination with bead-beating (lysing matrix D, MPG). After 30 minutes incubation on ice, samples were centrifuged for 20 minutes (14000 rpm) and supernatants were separated in 12% SDS PAGE under reducing conditions. After separation, the proteins were electroblotted to nitrocellulose membrane (Hybond-C pure, Amersham). The membranes were blocked with 2% low-fat milk in PBS-T for 1h at RT and then incubated with rabbit anti- NF- κ B p65 antibody (D14E12, Cell Signaling; 1:1000 diluted in blocking solution) overnight at 4 °C. After washing with PBS-T, HRP conjugated goat anti-rabbit IgG (7074, Cell Signaling; 1:10000 diluted in blocking solution) was applied to the membranes for 1h at RT. Chemiluminescence reagents (SuperSignal West Pico kit, Thermo Scientific) and X-ray film (Carestream) were used for visualization of the binding of Ab specific for the NF- κ B. A) no primary antibody added, B) protein lysate from non-treated seminal vesicles, C) protein lysate from seminal vesicles treated with profilin antigen.



Supplementary Figure 2. Smears of earthworm seminal vesicles. (A) a typical smear of seminal vesicles before antibiotic treatment, (B) reduced the number of sperm cells as well as all forms of developing spermatocytes as a consequence of antibiotic treatment. Stained by Hematoxylin/Eosin.



Supplementary Figure 3. Earthworm bristles in seminal vesicles. (A, B, C) bristles in seminal vesicle tissues surrounded by melanization reaction, stained with Methylene Blue, (D) bristle associated with released sporocysts, stained with Trypan Blue.

Supplementary Tables

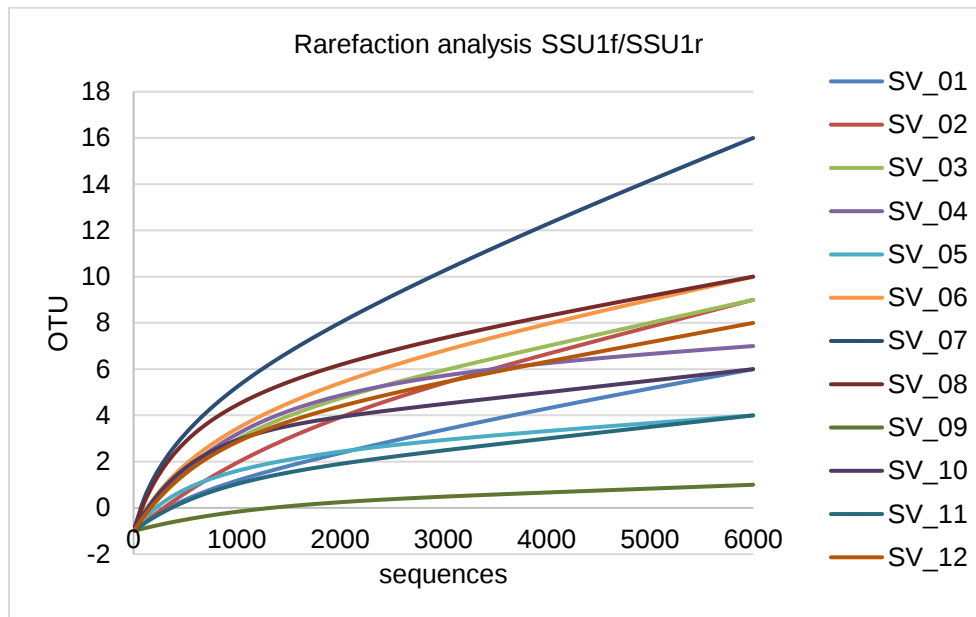
Supplementary Table S1. Primers for gregarine 18S rRNA used for HTS.

Primers for gregarine 18S rRNA		
Name	Direction	Sequence
SSU1f	forward	5'-XXXXXXXXCCGCACCATGCATGTCTAAGTATAAGTT-3'
SSU2f	forward	5'-XXXXXXXXACAGTTGTCAATCAAATGACTCTTTC-3'
SSU1r	reverse	5'-XXXXXXXXGCTGCAAGCATAGGTTGGTTCT-3'
Api1r	reverse	5'-XXXXXXXXCCTAATCTATCCCCATCACGATGC-3'
Combinations of primers		Size of amplicons (bp)
341F/806R		465
SSU1f/SSU1r		200
SSU2f/Api1r		477

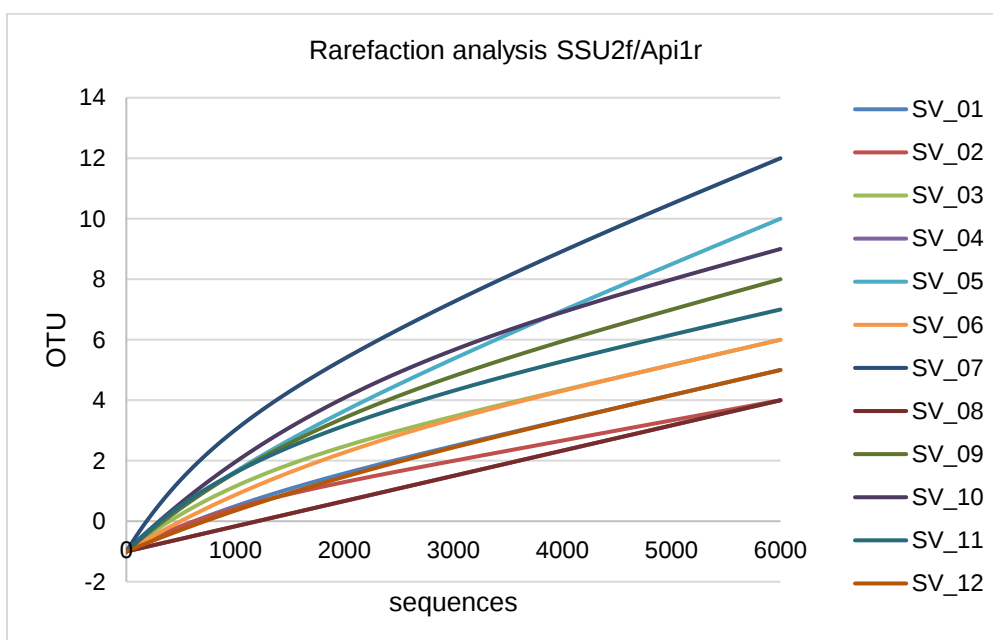
X represents a barcode base, linkers are in *italic*

Supplementary Table S2.

Combination of primers: SSU1f/SSU1r												
	SV_01	SV_02	SV_03	SV_04	SV_05	SV_06	SV_07	SV_08	SV_09	SV_10	SV_11	SV_12
Shannon-Wiener Diversity Index	0.47	0.22	0.73	0.69	0.34	0.46	0.69	0.72	0.18	0.56	0.29	0.67
Shannon Entropy	0.67	0.32	1.06	0.99	0.49	0.66	0.10	1.04	0.26	0.80	0.42	0.97
Species Richness (S)	9	12	12	10	7	13	19	13	4	9	7	11
Total Abundance	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000
Simpson Diversity Index	0.73	0.90	0.50	0.54	0.83	0.75	0.58	0.54	0.92	0.67	0.86	0.56
Evenness	0.21	0.09	0.29	0.30	0.18	0.18	0.23	0.28	0.13	0.25	0.15	0.28
Species Richness - 80% diversity	1	1	2	2	1	1	2	2	1	2	1	2
Chao-1	14	33	27	10.33	8	20.5	37.33	18	4	12	10	21
Number of reads	34381	23201	31426	22049	30157	8942	51871	30116	42017	11324	49491	8013



Combination of primers: SSU2f/Api1r												
	SV_01	SV_02	SV_03	SV_04	SV_05	SV_06	SV_07	SV_08	SV_09	SV_10	SV_11	SV_12
Shannon-Wiener Diversity Index	0.70	0.50	0.52	0.41	0.67	0.67	0.26	0.15	0.51	0.65	0.71	0.68
Shannon Entropy	1.01	0.72	0.76	0.60	0.95	0.97	0.37	0.21	0.74	0.94	1.02	0.98
Species Richness (S)	8	7	9	7	13	9	15	7	11	12	10	8
Total Abundance	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000
Simpson Diversity Index	0.50	0.69	0.67	0.76	0.55	0.53	0.88	0.94	0.69	0.56	0.51	0.52
Evenness	0.34	0.26	0.24	0.21	0.26	0.31	0.10	0.07	0.21	0.26	0.31	0.33
Species Richness - 80% diversity	2	1	2	1	2	2	1	1	1	2	2	2
Chao-1	18	13	19	17	31	19	27	17	18	17	15	18
reads	33165	27794	23506	23154	20053	19407	18919	17851	11711	11486	9506	6502



Supplementary Table S2. Alpha diversity and rarefaction analysis of samples from HTS. The alpha diversity and rarefaction analysis of OTUs based on gregarine 18S rRNA from seminal vesicles of *E. andrei* earthworms were counted from 6000 subsampled sequences for both combinations of primers. OTUs were generated at 97% identity.

Supplementary Table S3. Estimates of evolutionary divergence between sequences obtained from SSU2/Api fragments.

[illegible]

[illegible]

Supplementary Table S3. The number of base differences per site between sequences from found clusters and clusters with representative sequences of other species are shown. The analysis involved 22 (A) or 49 (B) nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were 543 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.