Supplementary data

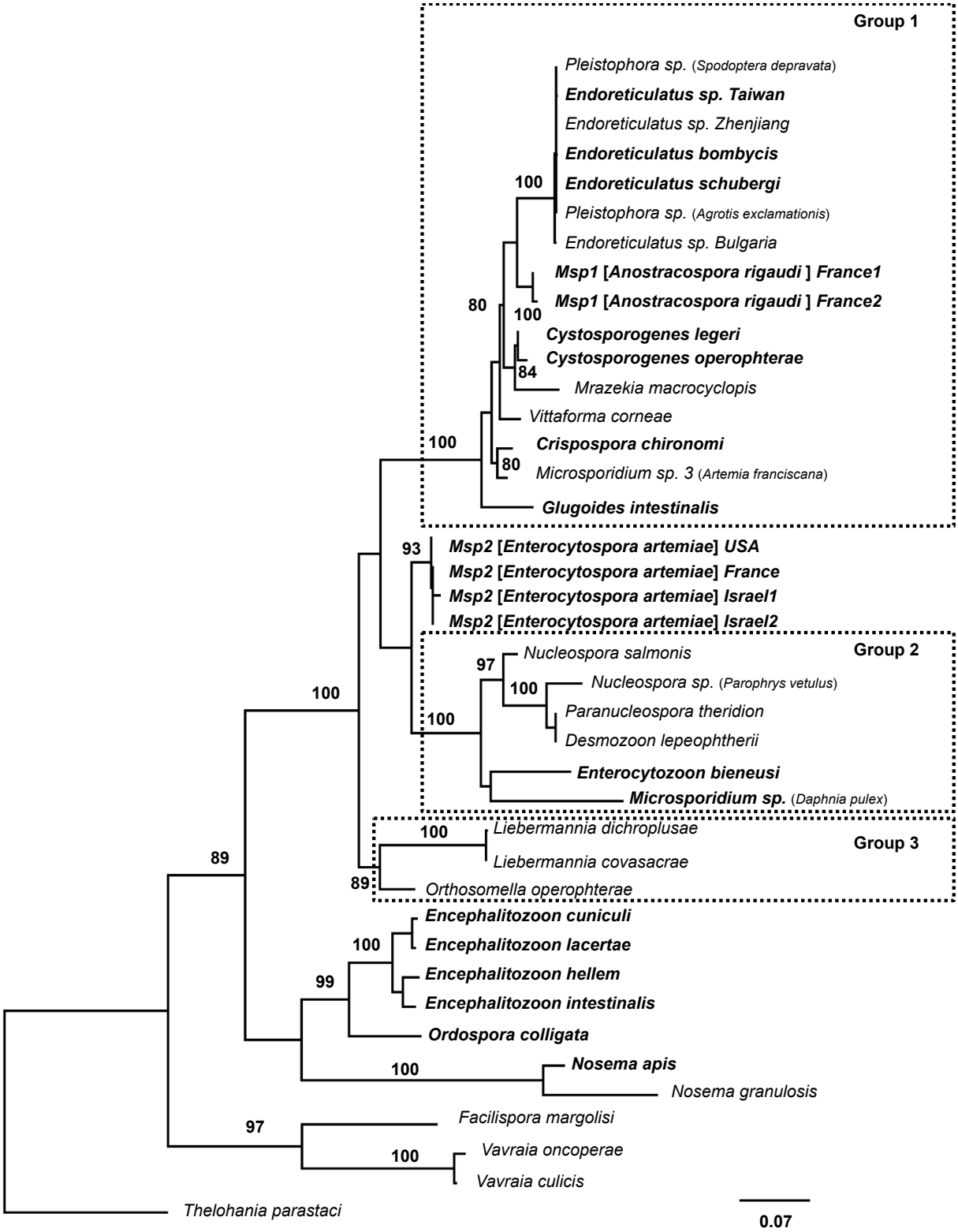

Fig. S1. Maximum likelihood tree of the SSU rDNA of *Msp1* (*Anostracospora rigaudi*) and *Msp2* (*Enterocytospora artemiae*) along with related microsporidian species infecting crustacean and non-crustacean hosts (excluding the following short sequences: *Hepatospora* spp., *Enterospora canceri, Enterocytozoon hepatopenaei* and *Myospora metanephrops*, final length ~720bp). Names of species infecting the digestive tract epithelium appear in bold. The host species of undescribed microsporidia appear between parentheses. Numbers above branches indicate bootstrap supports from 100 resamplings (only values above 80 are reported). Bootstrap support for the node showing *Msp2* as a sister species to Group 2 was very low (45%).

Table S1. List of microsporidian sequences used for the SSU rDNA phylogenetic analyses.

Organism	GenBank Acc. Number
Crispospora chironomi	GU130407
Cystosporogenes legeri	AY233131
Cystosporogenes operophterae	AJ302320
Desmozoon lepeophtherii	HM800847
Encephalitozoon cuniculi	L39107
Encephalitozoon hellem	L39108
Encephalitozoon intestinalis	L39113
Encephalitozoon lacertae	AF067144
Endoreticulatus schubergi	L39109
Endoreticulatus bombycis	AY009115
Endoreticulatus sp. Bulgaria	AY502945
Endoreticulatus sp. Taiwan	AY502944
Endoreticulatus sp. Zhenjiang	FJ772431
Enterocytozoon bieneusi	L07123
Enterocytozoon hepatopenaei	FJ496356
Enterospora canceri	HE584634
Facilispora margolisi	HM800849
Glugoides intestinalis	AF394525
Hepatospora eriocheir	HE584635
Hepatospora sp. (Cancer Pagarus)	HE584633
Liebermannia covasacrae	EU709818
Liebermannia dichroplusae	EF016249
Microsporidium sp. (Daphnia pulex)	AF394528
Microsporidium sp. 1 France1	JX915758
Microsporidium sp. 1 France2	JX915759
Microsporidium sp. 2 France	JX915760
Microsporidium sp. 2 Israel1	JX915756
Microsporidium sp. 2 Israel2	JX915757
Microsporidium sp. 2 USA	JX839889
Microsporidium sp. 3 (Artemia franciscana)	JX839890
Mrazekia macrocyclopis	FJ914315
Myospora metanephrops	HM140497
Nosema apis	X73894
Nosema granulosis	FN434088
Nucleospora sp. (Parophrys vetulus)	AF186007
Nucleospora salmonis	U78176
Ordospora colligata	AF394529
Orthosomella operophterae	AJ302317
Paranucleospora theridion	FJ594990
Pleistophora sp. (Agrotis exclamationis)	U10342
Pleistophora sp. (Spodoptera depravata)	D85500
Thelohania parastaci	AF294781
Vavraia culicis	AJ252961
Vavraia oncoperae	X74112
Vittaforma corneae	L39112

Table S2. Mean genetic distances between *Msp1, Endoreticulatus* spp.*, Cystosporogenes* spp.*,* the remaining species from Group 1 ("Others in Group 1") and *Msp2*.

	Msp1	***Endoreticulatus***	***Cystosporogenes***	**Others in Group1**
Msp1				
Endoreticulatus	0.035			
Cystosporogenes	0.066	0.048		
Others in Group1	0.060	0.047	0.060	
Msp2	0.134	0.103	0.143	0.125

Table S3. Mean genetic distances between *Msp2* and all major clades from the phylogeny in Fig. S1.

	Group1	***Msp2***	***Group2***	***Group3***	***Encephalitozoon/ Ordospora***	***Nosema***
Group1						
Msp2	0.117					
Group2	0.225	0.163				
Group3	0.185	0.145	0.219			
Encephalitozoon/ Ordospora	0.248	0.190	0.284	0.237		
Nosema	0.328	0.303	0.399	0.378	0.329	
Vavraia	0.418	0.392	0.439	0.402	0.414	0.505

Table S4. Comparison of the different gut microsporidia related to *Msp1* (*Anacostracospora rigaudi*) and *Msp2* (*Enterocytospora artemiae*)

Microsporidian species	Host	Distribution	Site of infection	Parasitophorous vacuole	Meront nuclei
Anacostracospora rigaudi	Anostraca: Artemia	Southern France, Ukraine	Intestinal epithelium	Appears during sporogony	Monokaryotic
Enterocytospora artemiae	Anostraca: Artemia	Southern France, Israel, USA	Intestinal epithelium	Appears during merogony	Monokaryotic
Ordospora duforti	Anostraca: Artemia	Spain	Intestinal epithelium	Appears during merogony	Monokaryotic
Vavria anostraca	Anostraca: Artemia	Brazil	Intestinal epithelium, muscle	Appears during sporogony	Monokaryotic
Glugoides intestinalis	Cladocera: Daphnia	Europe	Intestinal epithelium	Appears during merogony	Monokaryotic
Cystosporogenes operophterae	Lepidoptera: Operophtera	UK	Salivary glands, intestinal epithelium	Appears during merogony	Monokaryotic
Endoreticulatus schubergi	Lepidoptera: Choristoneura	USA	Intestinal epithelium	Appears during merogony	Monokaryotic
Crispospora chironomi	Diptera: Chironoma	Russia	Intestinal epithelium	Appears during merogony	Diplokaryotic
Orthosomella operophterae	Lepidoptera: Operophtera	UK, USA	Silk gland, intestinal epithelium and other tissues	None	Monokaryotic
Desmozoon lepeophtherii	Copepod: Lepeophtheirus	UK	Desmocyte	None	Diplokaryotic
Enterocytozoon bieneusi	Primates: Homo	worldwide	Intestinal epithelium	None	Unikaryotic
Enterocytozoon hepatopenaei	Decapoda: Penaeus	Thailand	Hepatopancreas epithelium	None	unknown
Enterospora canceri	Decapoda: Cancer	UK	Hepatopancreatocytes nucleoplasm	Appears during merogony	unknown
Hepatospora eriocheir	Decapoda: Eriocheir	UK, China	Hepatopancreas epithelium	Appears during merogony	Monokaryotic
Liebermannia covasacrae	Orthoptera: Covasacris	Argentina	Salivary gland	Appears during merogony	Diplokaryotic

Table S4. (continued)

Microsporidian species	Merogonial division	Sporont nuclei	Sporogonial division	Number of sporoblasts/spores	Spore shape
Anacostracospora rigaudi	unknown	Monokaryotic	Plasmotomy	32-64*	Oval
Enterocytospora artemiae	Binary fission	Monokaryotic	Plasmotomy	64-128*	Sub-spherical
Ordospora duforti	Plasmotomy from rosette	Monokaryotic	Plasmotomy	4 and 8	pyriform
Vavria anostraca	Plasmotomy	Monokaryotic	Plasmotomy	unknown	Oval
Glugoides intestinalis	Plasmotomy	Monokaryotic	Plasmotomy	16	Oblong to lightly reniform
Cystosporogenes operophterae	Binary fission	Monokaryotic	Plasmotomy	>128	Oval
Endoreticulatus schubergi	binary/multiple division	Monokaryotic	Plasmotomy	32	Oval
Crispospora chironomi	Plasmotomy by rosette	Monokaryotic	Plasmotomy	unknown	1/ sub-spherical; 2/oval
Orthosomella operophterae	Plasmotomy	Monokaryotic	Plasmotomy from sausage-shaped sporogonial plasmodium	12	Oblong to lightly reniform
Desmozoon lepeophtherii	Binary fission followed by plasmotomy	Monokaryotic	Plasmotomy	unknown	sub-spherical
Enterocytozoon bieneusi	Plasmotomy	Monokaryotic	Plasmotomy	unknown	sub-spherical to oval
Enterocytozoon hepatopenaei	unknown	Monokaryotic	Binary fission	unknown	Oval
Enterospora canceri	Plasmotomy	Monokaryotic	Plasmotomy	>20	Oval
Hepatospora eriocheir	Rosette-like division	Monokaryotic	Plasmotomy/Rosette-like division	unknown	Oval
Liebermannia covasacrae	Binary fission	Monokaryotic	Binary fission	unknown	Ovocylindrical

* Estimation from repeated observations of parasitophorous vacuoles on semi-thin sections.

Table S4. (continued)

Microsporidian species	Spore size (µm)	Spore nuclei	Spore wall thickness (nm)	Polar tube	Polar tube coils	Polar tube rows	Polar tube diameter (nm)
Anacostracospora rigaudi	1.3x0.7	Monokaryotic	55	Isofilar	5 to 6	1 to 2	60
Enterocytospora artemiae	1.2x0.9	Monokaryotic	80	Isofilar	4	1	100
Ordospora duforti	1.70x0.98	Monokaryotic	140-150	Isofilar	8 to 11	2	97
Vavria anostraca	mi:2.8-3.5x 1.5-2.0; ma: 3.5-5.0x2.0-3.0**	Monokaryotic	94x97	Anisofilar	mi: 11-12; ma: 15-18**	mi: 1; ma:2**	mi: 70-90; ma: 92-120**
Glugoides intestinalis	2.4-2.7x1.1-1.7	Monokaryotic	80-85	Isofilar	5 to 8	1	77-85
Cystosporogenes operophterae	2.7x1.6	Monokaryotic	125	Isofilar	10 to 12	1	120
Endoreticulatus schubergi	2.5x1.5	Monokaryotic	50	Isofilar	7 to 9	1	100
Crispospora chironomi	1/ 1.5-2; 2/2.5x1.5	1/ monokaryotic; 2/diplokaryotic	60	Isofilar	4	1	85
Orthosomella operophterae	3.5x1.3	Monokaryotic	50	Isofilar	6 to 7	1	75
Desmozoon lepeophtherii	2.34x1.83	Monokaryotic	75	Isofilar	5 to 8	2	65-85
Enterocytozoon bieneusi	1.5x0.8	Monokaryotic	30-35	Isofilar	4 to 7	1	75
Enterocytozoon hepatopenaei	1.1x0.7	Monokaryotic	55	Isofilar	5 to 6	2	666
Enterospora canceri	1.3x0.7	Monokaryotic	32	Isofilar	4 to 5	2	85
Hepatospora eriocheir	1.8x0.9	Monokaryotic	55	Isofilar	7 to 8	1	120
Liebermannia covasacrae	2.2-3.4x1.1-1.7	Monokaryotic	120	Isofilar	3 to 5	1	120

** mi: microspore, ma: macrospore