Table S1. Identification of *Prototheca* isolates (*n*=70) from environmental samples and mastitis milk by *cytB* PCR-RFLP analysis.

Source	**Strain ID**	**No. of isolates by species/genotype**		
		P. zopfii gen. 1	*P. zopfii* gen. 2	*P. blaschkeae*
Environmental (*n*=18)	EA10, EB1	2	-	-
	EA2, EA3, EB5, EB9, ED2-ED4, ED6, EE2, EE5, EE7, EE8, EH9, EH10	-	14	-
	ED7, ED8	-	-	2
Mastitis milk (*n*=52)	D3-D9, E1-E9, F1-F9, G1-G9, H1, 2E7, 2E9, 2E10, 2C6-2C9, 2D8, 2F1-2F3, 2F5, 2F7, 2F8, 2G5, 2G6	-	51	-
	H3	-	-	1
TOTAL		**2 (2.9%)**	**65 (92.8%)**	**3 (4.3%)**

Table S2. Primers used for PCR amplification and sequencing of the mitochondrial *cytb* gene and rDNA loci.

Locus	Primer[a]		Product size (bp)	Reference
	designation	nucleotide sequence (5'→3')		
cytb	cytB_F1	GyGTwGAACAyATTATGAGAG	644	This study
	cytB_R2	wACCCATAArAArTACCATTCWGG		
SSU	SSU-F1*	AACCTGGTTGATCCTGCCAGTAGTC	1803 - 3637	(1)
	SSU-R1*	TGATCCTTCTGCAGGTTCACCTACG		
	21M13F**	TGTAAAACGACGGCCAGT		-
	M13Rev**	CAGGAAACAGCTATGACC		
	SSU3**	GCCTGAGAAACGGCTACCAC		(2)
	SSU5**	GTGGTAGCCGTTTCTCAGGC		
	SSU_F2**	TGTCAGAGGTGAAATTCTTGG		This study
ITS	ITS4*	TCCTCCGCTTATTGATATGC	742 - 2806	(3)
	ITS5*	GGAAGTAAAAGTCGTAACAAGG		
	21M13F**	TGTAAAACGACGGCCAGT		-
	M13Rev**	CAGGAAACAGCTATGACC		
	ITS2**	GCTGCGTTCTTCATCGATGC		(3)
	ITS3**	GCATCGATGAAGAACGCAGC		
	PWITS_F2**	CCAACTACGCGGTCAGGTCAC		This study
	PWITS_R2**	GCCCGAATGTrCrTCCTCAGTC		
	ITS_F1**	GCACGTCVYGTGTTTCCACTYTG		
	ITS_F2**	GTTAGGGTGGMTAGATGTTAKG		
	ITS_R1**	CGCTCACACCTACCAAGCCA		
	ITS_R2**	CMTAACATCTAKCCACCCTAAC		
D1/D2 LSU	NL1*	GCATATCAATAAGCGGAGGAAAAG	625 - 738	(4)
	NL4*	GGTCCGTGTTTCAAGACGG		

[a] Primers were used for both amplification and sequencing (*) or for sequencing only (**); Degenerate nucleotides: y, C or T; h, A, C, or T; r, A or G; w, A or T.

Table S3. Pairwise similarity matrix of *cytB* sequences for *Prototheca* species (genotypes) and outgroup species.

Species (genotype) ***cytB***	***Prototheca zopfii* gen. 1**	***Prototheca zopfii* gen. 2**	***Prototheca blaschkeae***	***Prototheca cutis***	***Prototheca miyajii***	***Prototheca wickerhamii***	***Prototheca stagnora***	***Prototheca ulmea***	***Prototheca tumulicola***	***Auxenochlorella* sp.**	***Chlorella* sp.**	***Chlamydomonas* sp.**	***Helicosporidium* sp.**	***Prototheca* spp.**
***Prototheca zopfii* gen. 1**	**97.66 - 100**	93.98 - 94.48	89.13 - 90.97	78.93 - 79.60	78.60 - 78.76	80.60 - 81.27	85.28 - 86.29	83.28 - 83.95	86.62 - 87.79	79.93 - 80.27	76.42 - 78.09	64.72 - 67.39	78.60 - 79.60	78.60 - 94.48
***Prototheca zopfii* gen. 2**	93.98 - 94.48	**99.83 - 100**	89.13 - 89.46	80.77 - 80.94	79.77 - 79.93	80.77 - 80.94	85.45 - 85.62	83.78 - 83.95	86.62 - 86.79	80.27 - 80.43	77.26 - 77.93	63.55 - 66.05	78.76 - 78.93	79.77 - 94.48
Prototheca blaschkeae	89.13 - 90.97	89.13 - 89.46	**99.50 - 100**	78.60	78.76 - 79.26	80.77 - 81.10	84.28 - 84.62	83.11 - 83.44	86.29 - 86.79	79.77 - 80.10	76.59 - 78.76	63.88 - 67.56	80.27 - 80.77	78.60 - 90.97
Prototheca cutis	78.93 - 79.60	80.77 - 80.94	78.60	**100**	88.29	85.79 - 85.95	78.93	79.43	80.43	86.12	78.76 - 81.10	62.71 - 66.72	81.94	78.93 - 88.29
Prototheca miyajii	78.60 - 78.76	79.77 - 79.93	78.76 - 79.26	88.29	**100**	85.12 - 85.28	81.61	80.27	82.27	85.45	78.93 - 80.94	62.37 - 65.55	82.11	78.60 - 88.29
Prototheca wickerhamii	80.60 - 81.27	80.77 - 80.94	80.77 - 81.10	85.79 - 85.95	85.12 - 85.28	**99.50 - 100**	80.60 - 80.77	78.60 - 78.76	82.27 - 82.44	84.45 - 84.95	78.93 - 79.93	63.71 - 67.39	81.10 - 81.27	78.60 - 85.95
Prototheca stagnora	85.28 - 86.29	85.45 - 85.62	84.28 - 84.62	78.93	81.61	80.60 - 80.77	**100**	86.45	90.80	81.94	77.93 - 79.26	63.88 - 67.56	78.93	78.93 - 90.80
Prototheca ulmea	83.28 - 83.95	83.78 - 83.95	83.11 - 83.44	79.43	80.27	78.60 - 78.76	86.45	**100**	87.79	79.60	78.26 - 79.43	63.21 - 65.22	78.09	78.60 - 87.79
Prototheca tumulicola	86.62 - 87.79	86.62 - 86.79	86.29 - 86.79	80.43	82.27	82.27 - 82.44	90.80	87.79	**100**	82.78	80.43 - 82.44	63.38 - 66.72	79.77	80.43 - 90.80
***Auxenochlorella* sp.**	79.93 - 80.27	80.27 - 80.43	79.77 - 80.10	86.12	85.45	84.45 - 84.95	81.94	79.60	82.78	**100**	80.43 - 82.44	61.04 - 66.22	82.61	79.60 - 86.12
***Chlorella* sp.**	76.42 - 78.09	77.26 - 77.93	76.59 - 78.76	78.76 - 81.10	78.93 - 80.94	78.93 - 79.93	77.93 - 79.26	78.26 - 79.43	80.43 - 82.44	80.43 - 82.44	**86.12 - 100**	61.71 - 64.21	77.93 - 78.43	76.42 - 82.44
***Chlamydomonas* sp.**	64.72 - 67.39	63.55 - 66.05	63.88 - 67.56	62.71 - 66.72	62.37 - 65.55	63.71 - 67.39	63.88 - 67.56	63.21 - 65.22	63.38 - 66.72	61.04 - 66.22	61.71 - 64.21	**76.09 - 100**	62.71 - 65.72	62.37 - 67.56
***Helicosporidium* sp.**	78.60 - 79.60	78.76 - 78.93	80.27 - 80.77	81.94	82.11	81.10 - 81.27	78.93	78.09	79.77	82.61	77.93 - 78.43	62.71 - 65.72	**100**	78.09 - 82.11

Table S4. Pairwise similarity matrix of SSU sequences for *Prototheca* species (genotypes) and outgroup species.

Species (genotype) **SSU**	***Prototheca zopfii* gen. 1**	***Prototheca zopfii* gen. 2**	***Prototheca blaschkeae***	***Prototheca cutis***	***Prototheca miyajii***	***Prototheca wickerhamii***	***Prototheca stagnora***	***Prototheca ulmea***	***Prototheca tumulicola***	***Auxenochlorella* sp.**	***Chlorella* sp.**	***Chlamydomonas* sp.**	***Helicosporidium* sp.**	***Prototheca* spp.**
***Prototheca zopfii* gen. 1**	**99.79 - 100**	99.48 - 99.79	97.60 - 97.91	86.42 - 86.62	86.21 - 86.52	77.95 - 78.37	93.73 - 94.04	94.25 - 94.46	93.31 - 93.52	84.22 - 84.43	85.06 - 85.48	83.59 - 84.22	77.43 - 77.64	77.95 - 99.79
***Prototheca zopfii* gen. 2**	99.48 - 99.79	**99.90 – 100**	97.91 - 98.12	86.52 - 86.62	86.42 - 86.62	78.16 - 78.47	94.04 - 94.25	94.36 - 94.46	93.63 - 93.73	84.54 - 84.64	85.16 - 85.48	83.70 - 84.22	77.74 - 77.85	78.16 - 99.79
Prototheca blaschkeae	97.60 - 97.91	97.91 - 98.12	**99.90 – 100**	87.36	87.46 - 87.57	77.85 - 78.16	95.51 - 95.72	95.72 - 95.82	95.09 - 95.19	85.27	85.89 - 86.10	84.54 - 84.95	77.64 - 77.74	77.85 - 97.91
Prototheca cutis	86.42 - 86.62	86.52 - 86.62	87.36	**100**	98.33 - 98.64	79.73 - 79.83	86.94 - 87.04	87.15	86.42	91.43	92.69 - 92.89	90.70 - 91.85	79.83	79.73 - 98.64
Prototheca miyajii	86.21 - 86.52	86.42 - 86.62	87.46 - 87.57	98.33 - 98.64	**99.69 - 100**	78.79 - 79.31	86.83 - 87.04	86.83 - 86.94	86.42 - 86.52	91.22 - 91.54	91.75 - 92.27	89.66 - 91.12	79.41 - 79.73	78.79 - 98.64
Prototheca wickerhamii	77.95 - 78.37	78.16 - 78.47	77.85 - 78.16	79.73 - 79.83	78.79 - 79.31	**95.61 - 100**	78.06 - 78.27	78.16 - 78.37	77.85 - 78.06	78.27 - 78.37	79.31 - 80.15	78.47 - 79.52	74.82 - 75.34	77.85 - 79.83
Prototheca stagnora	93.73 - 94.04	94.04 - 94.25	95.51 - 95.72	86.94 - 87.04	86.83 - 87.04	78.06 - 78.27	**99.90 - 100**	95.82 - 95.92	96.55 - 96.66	84.74 - 84.85	86.00 - 86.31	84.74 - 85.37	78.06 - 78.16	78.06 - 96.66
Prototheca ulmea	94.25 - 94.46	94.36 - 94.46	95.72 - 95.82	87.15	86.83 - 86.94	78.16 - 78.37	95.82 - 95.92	**100**	96.03	85.06	86.52 - 86.73	84.85 - 85.89	78.27	78.16 - 96.03
Prototheca tumulicola	93.31 - 93.52	93.63 - 93.73	95.09 - 95.19	86.42	86.42 - 86.52	77.85 - 78.06	96.55 - 96.66	96.03	**100**	84.12	85.58 - 85.68	84.54 - 85.27	78.06	77.85 - 96.66
***Auxenochlorella* sp.**	84.22 - 84.43	84.54 - 84.64	85.27	91.43	91.22 - 91.54	78.27 - 78.37	84.74 - 84.85	85.06	84.12	**100**	93.10 - 93.42	90.39 - 90.80	79.94	78.27 - 91.54
***Chlorella* sp.**	85.06 - 85.48	85.16 - 85.48	85.89 - 86.10	92.69 - 92.89	91.75 - 92.27	79.31 - 80.15	86.00 - 86.31	86.52 - 86.73	85.58 - 85.68	93.10 - 93.42	**99.58 - 100**	94.15 - 94.98	80.46 - 80.56	79.31 - 92.89
***Chlamydomonas* sp.**	83.59 - 84.22	83.70 - 84.22	84.54 - 84.95	90.70 - 91.85	89.66 - 91.12	78.47 - 79.52	84.74 - 85.37	84.85 - 85.89	84.54 - 85.27	90.39 - 90.80	94.15 - 94.98	**94.15 - 100**	79.21 - 80.36	78.47 - 91.85
***Helicosporidium* sp.**	77.43 - 77.64	77.74 - 77.85	77.64 - 77.74	79.83	79.41 - 79.73	74.82 - 75.34	78.06 - 78.16	78.27	78.06	79.94	80.46 - 80.56	79.21 - 80.36	**100**	74.82 - 79.83

Table S5. Pairwise similarity matrix of ITS sequences for *Prototheca* species (genotypes) and outgroup species.

Species (genotype) **ITS**	***Prototheca zopfii* gen. 1**	***Prototheca zopfii* gen. 2**	***Prototheca blaschkeae***	***Prototheca cutis***	***Prototheca miyajii***	***Prototheca wickerhamii***	***Prototheca stagnora***	***Prototheca ulmea***	***Prototheca tumulicola***	***Auxenochlorella* sp.**	***Chlorella* sp.**	***Chlamydomonas* sp.**	***Helicosporidium* sp.**	***Prototheca* spp.**
***Prototheca zopfii* gen. 1**	**100**	96.77	91.71 - 92.17	74.65 - 75.12	73.73 - 76.50	72.81 - 74.19	85.71	82.03	82.49	76.04	78.34 - 80.18	74.65 - 77.88	70.51	72.81 - 96.77
***Prototheca zopfii* gen. 2**	96.77	**100**	91.71 - 92.17	73.73 - 74.19	72.35 - 76.96	71.89 - 73.73	87.56	83.87	83.87	75.58	78.80 - 81.11	76.04 - 78.80	70.97	71.89 - 96.77
Prototheca blaschkeae	91.71 - 92.17	91.71 - 92.17	**99.54 - 100**	76.04 - 76.96	73.73 - 78.80	71.43 - 74.19	85.25 - 85.71	83.87 - 84.33	81.57 - 82.03	76.96 - 77.42	78.34 - 80.65	75.58 - 78.34	70.05 - 70.51	71.43 - 92.17
Prototheca cutis	74.65 - 75.12	73.73 - 74.19	76.04 - 76.96	**99.08 - 100**	76.96 - 94.01	75.12 - 77.88	75.12 - 75.58	78.34 - 78.80	76.50 - 76.96	88.48 - 88.84	85.71 - 88.02	79.26 - 85.25	76.04 - 76.50	73.73 - 94.01
Prototheca miyajii	76.50	76.96	78.34 - 78.80	93.55 - 94.01	**100**	74.19 – 76.96	78.80	79.72	77.88	88.02	87.10 - 89.40	79.72 - 87.10	78.34	74.19 - 94.01
Prototheca wickerhamii	72.81 - 74.19	71.89 - 73.73	71.43 - 74.19	75.12 - 77.88	74.19 – 76.96	**87.56 - 100**	69.59 - 72.81	71.89 - 74.65	72.35 - 74.19	75.12 - 76.96	77.42 - 80.18	73.27 - 76.04	70.97 - 73.27	69.59 – 77.88
Prototheca stagnora	85.71	87.56	85.25 - 85.71	75.12 - 75.58	72.81 - 78.80	69.59 - 72.81	**100**	85.71	81.11	76.50	78.34 - 80.18	75.58 - 77.42	72.81	69.59 - 87.56
Prototheca ulmea	82.03	83.87	83.87 - 84.33	78.34 - 78.80	74.65 - 79.72	71.89 - 74.65	85.71	**100**	87.10	77.88	79.26 - 80.65	77.42 - 80.18	74.65	71.89 - 87.10
Prototheca tumulicola	82.49	83.87	81.57 - 82.03	76.50 - 76.96	74.19 - 77.88	72.35 - 74.19	81.11	87.10	**100**	78.34	80.18 - 82.49	77.42 - 80.18	70.97	72.35 - 87.10
***Auxenochlorella* sp.**	76.04	75.58	76.96 - 77.42	88.48 - 88.84	76.96 - 88.02	75.12 - 76.96	76.50	77.88	78.34	**100**	88.02 - 90.32	81.11 - 84.33	80.65	75.12 - 88.84
***Chlorella* sp.**	78.34 - 80.18	78.80 - 81.11	78.34 - 80.65	85.71 - 88.02	78.80 - 89.40	77.42 - 80.18	78.34 - 80.18	79.26 - 80.65	80.18 - 82.49	88.02 - 90.32	**90.32 - 100**	79.26 - 87.10	78.80 - 80.65	77.42 - 89.40
***Chlamydomonas* sp.**	74.65 - 77.88	76.04 - 78.80	75.58 - 78.34	79.26 - 85.25	73.73 - 87.10	73.27 - 76.04	75.58 - 77.42	77.42 - 80.18	77.42 - 80.18	81.11 - 84.33	79.26 - 87.10	**88.02 - 100**	75.12 - 76.04	73.73 - 87.10
***Helicosporidium* sp.**	70.51	70.97	70.05 - 70.51	76.04 - 76.50	72.81 - 78.34	70.97 - 73.27	72.81	74.65	70.97	80.65	78.80 - 80.65	75.12 - 76.04	**100**	70.51 - 78.34

Table S6. Pairwise similarity matrix of D1/D2 LSU sequences for *Prototheca* species (genotypes) and outgroup species.

Species (genotype) **LSU**	***Prototheca zopfii* gen. 1**	***Prototheca zopfii* gen. 2**	***Prototheca blaschkeae***	***Prototheca cutis***	***Prototheca miyajii***	***Prototheca wickerhamii***	***Prototheca stagnora***	***Prototheca ulmea***	***Prototheca tumulicola***	***Auxenochlorella* sp.**	***Chlorella* sp.**	***Chlamydomonas* sp.**	***Helicosporidium* sp.**	***Prototheca* spp.**
***Prototheca zopfii* gen. 1**	**99.58 - 100**	98.60 - 99.16	96.09 - 96.37	84.22	83.52 - 83.66	80.31 - 80.59	91.20 - 91.34	91.48	91.62 - 91.76	84.22	81.84 - 82.68	80.03 - 81.42	79.47	80.31 - 99.16
***Prototheca zopfii* gen. 2**	98.60 - 99.16	**99.72 - 100**	95.95 - 96.23	83.66 - 83.80	82.96 - 83.10	79.89 - 80.17	90.92	91.06 - 91.20	90.92 - 91.06	83.66 - 83.80	81.56 - 82.40	79.75 - 81.01	79.33	79.89 – 99.16
Prototheca blaschkeae	96.09 - 96.37	95.95 - 96.23	**100**	83.80	83.52	81.42 - 81.56	91.62	91.48	92.32	84.08	82.12 - 82.40	80.45 - 81.42	78.77	81.42 - 96.37
Prototheca cutis	84.22	83.66 - 83.80	83.80	**100**	97.35	81.98 - 82.40	83.10	84.64	82.68	92.04	87.99 - 88.27	83.38 - 85.89	81.98	81.98 - 97.35
Prototheca miyajii	83.52 - 83.66	82.96 - 83.10	83.52	97.35	**100**	82.40 - 82.54	82.54	84.36	82.12	91.48	87.57 - 87.85	83.80 - 85.75	80.59	82.12 - 97.35
Prototheca wickerhamii	80.31 - 80.59	79.89 - 80.17	81.42- 81.56	81.98 - 82.40	82.40 - 82.54	**97.49 - 100**	80.17 - 80.45	79.75 - 79.89	80.59 - 81.01	81.70 - 82.26	79.75 - 81.15	79.75 - 81.98	81.28 - 81.84	79.75 - 82.54
Prototheca stagnora	91.20 - 91.34	90.92	91.62	83.10	82.54	80.17 - 80.45	**100**	90.92	93.58	82.82	82.26 - 82.40	80.31 - 82.40	79.89	80.17 - 93.58
Prototheca ulmea	91.48	91.06 - 91.20	91.48	84.64	84.36	79.75 - 79.89	90.92	**100**	92.32	85.61	83.66 - 83.94	80.59 - 82.26	79.61	79.75 - 92.32
Prototheca tumulicola	91.62 - 91.76	90.92 - 91.06	92.32	82.68	82.12	80.59 - 81.01	93.58	92.32	**100**	83.52	81.56 - 81.98	80.87 - 82.54	80.17	80.59 - 93.58
***Auxenochlorella* sp.**	84.22	83.66 - 83.80	84.08	92.04	91.48	81.70 - 82.26	82.82	85.61	83.52	**100**	88.13	82.54 - 84.92	81.42	81.70 - 92.04
***Chlorella* sp.**	81.84 - 82.68	81.56 - 82.40	82.12 - 82.40	87.99 - 88.27	87.57 - 87.85	79.75 - 81.15	82.26 - 82.40	83.66 - 83.94	81.56 - 81.98	88.13	**96.37 - 100**	83.10 - 87.71	79.61 - 80.17	79.75 - 88.27
***Chlamydomonas* sp.**	80.03 - 81.42	79.75 - 81.01	80.45 - 81.42	83.38 - 85.89	83.80 - 85.75	79.75 - 81.98	80.31 - 82.40	80.59 - 82.26	80.87 - 82.54	82.54 - 84.92	83.10 - 87.71	**87.43 - 100**	77.65 - 80.17	79.57 - 85.89
***Helicosporidium* sp.**	79.47	79.33	78.77	81.98	80.59	81.28 - 81.84	79.89	79.61	80.17	81.42	79.61 - 80.17	77.65 - 80.17	**100**	78.77 - 81.98

Table S7. Primers used for amplification of the SSU and ITS loci of *Prototheca* spp. – a compilation from the published literature.

No.[a]	Primer[b]		Locus	Reference
	designation	nucleotide sequence (5'→3')		
1.	SSU-F1	AACCTGGTTGATCCTGCCAGTAGTC	SSU	(5,6)
2.	SSU-R1	TGATCCTTCTGCAGGTTCACCTACG		
3.	18 PZF1	ACAATACGTAGCGATGCCGAACT		(7-9)
4.	18 PZR1	GCCAGCCAGAGGACGCCGAA		
5.	proto18S-4f[*]	GACATGGCGAGGATTGACAGA		(6,7,10,11)
7.	proto18S-4r[**]	AGCACACCCAATCGGTAGGA		(6,10)
6.	PZGT 2/r[**]	GTCGGCGGGGCAAAAGC		(7)
8.	proto18S-4R-1[**]	ATCACAGACCTGTTATC		(11)
9.	O18SF1	TACCACATCCAAGAAAGGCA		(9)
10.	O18SR1	CCTTGGCAAATGCTTTCGCA		
11.	PP1[+]	CCTTGTCAGGTTGATTCC		(12)
12.	SP1[++]	CAGCAGTCCCTCTAAGAAGCG		
13.	PP2[++]	AGCAGTCCCTCTAAGAAG		
14.	Fw_1	TGCTGCAGTTAAAAAGCTCGT		(13)
15.	Rw_1	TCCAAGAATTTCACCTCTGACA		
16.	Fw_2	AGCCTGCGGCTTAATTTGAC		
17.	Rw_2	CGGCCCAGAACATCTAAGG		
18.	ITS-1[#]	TCCGTAGGTGAACCTGCGG	ITS	(14)
19.	ITS-2[##, ‡‡]	GCTGCGTTCTTCATCGATGC		(5,14)
20.	ITS-3[†]	GCATCGATGAAGAACGCAGC		
21.	ITS-4[††, ‡‡]	TCCTCCGCTTATTGATATGC		(5,6,14)
22.	ITS-5[#, ‡]	GGAAGTAAAAGTCGTAACAAGG		(5,6)
23.	Pb-ITS-F	AAGGCCCTGCGTTCTTCGCA		(15)
24.	Pb-ITS-R	GCGTGTTCCCGACCGAGAGA		
25.	Pz-ITS-F	TTCGACCGAACGAAACGA		
26.	Pz-ITS-R	AATTCCTGGCATTGGCGACA		
27.	28S-F1	AAGCATATCAATAAGCGGAGG	D1/D2 LSU	(5)
28.	653/NL4	GGTCCGTGTTTCAAGACGG		
29.	NL1	GCATATCAATAAGCGGAGGAAAAG		(4)

[a] The numbers correspond with those provided in brackets in Fig. 9-11.

[b] As the same forward primers were sometimes used with different reverse primers, these primer combinations were indicated with the superscripts: [*], [+], [#], [†], [‡], single for forward primers, and double for reverse primers.

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