

Supplementary Tables

Gallo Vaulet, L.; Vanstreels, R.E.T.; Gallo, L.; Entrocassi, A.C.; Peker, L.; Blanco, G.S.; Rago, M.V.; Rodriguez Fermepin, M.; Uhart, M.M. 2022. *Chlamydiaceae*-Like Bacterium in Wild Magellanic Penguins (*Spheniscus magellanicus*). Diversity. Correspondence to: Ralph E. T. Vanstreels (ralph_vanstreels@yahoo.com.br).

Table S1. Diagnostic results for cloacal swabs of Magellanic penguins (*Spheniscus magellanicus*) tested using a real-time PCR test targeting the 23S rRNA gene of *Chlamydiaceae*.

Colony	Stage and season	Age class	Swab storage ¹	Samples tested	Samples positive	%	Quantification cycle, mean \pm SD (range)
Punta Norte/San Lorenzo	Post-guard 2013/2014	Chick	D	3	0	0	Not applicable
		Adult	D	13	8	61.5	35.3 \pm 2.3 (32 – 38)
	Guard 2017/2018	Chick	D	6	1	16.7	38.6
			CM	5	3	60.0	39.4 \pm 0.5 (39 – 39.9)
		Adult	D	15	5	33.3	35.9 \pm 2.7 (31.6 – 39.1)
			CM	13	5	38.5	39.1 \pm 0.6 (38.2 – 39.6)
Punta Tombo	Post-guard 2013/2014	Adult	D	22	10	45.5	36.1 \pm 3.3 (31 – 40)
Cabo Dos Bahías	Post-guard 2013/2014	Adult	D	20	10	50.0	37.3 \pm 2.5 (33 – 42)
			D	10	2	20.0	30.8 \pm 9.2 (24.3 – 37.3)
	Incubation 2015/2016	Adult	CM	18	7	38.9	36.5 \pm 2.1 (32.5 – 38.3)
			CM	26	7	26.9	35.7 \pm 3.0 (31 – 38)
Isla Vernacci Fondo	Post-guard 2013/2014	Adult	D	16	3	18.8	37.7 \pm 1.5 (36 – 39)
Total				167	61	36.5	36.5 \pm 3.0 (24.3 – 42)

Note: ¹ Swabs were placed in either dry tubes (D) or in tubes containing culture medium (CM) before freezing.

Table S2. Sequence of primers and probes employed.

Primer or Probe	Sequence (5' - 3')
Ch23S-F	CTG AAA CCA GTA GCT TAT AAG CGG T
Ch23S-R	ACC TCG CCG TTT AAC TTA ACT CC
Ch23S-p	FAM-CTC ATC ATG CAA AAG GCA CGC CG-TAMRA
Cpps-F	CAC TAT GTG GGA AGG TGC TTC A
Cpps-R	CTG CGC GGA TGC TAA TGG
Cpps-S	FAM-CGC TAC TTG GTG TGA C-BHQ1
16SIGF	CGG CGT GGT TGA GGA AT
16SIGR	TCA GTC CCA GTG TTG GC
16SF2	CCG CCC GTC ACA TCA TGG
23SIGR	TGG CTC ATC ATG CAA AAG GCA

Table S3. GenBank accession codes of the DNA sequences evaluated in this study (16S rRNA gene, 16S/23S intergenic spacer, and 23 S rRNA gene).

Organism	Accession codes
<i>Chlamydiales</i> sp. SM1420 (this study)	OL870320.1 and OL873322.1
<i>Chlamydiales</i> sp. 12-1761_J072	LN810447.1 and LN810461.1
<i>Chlamydiales</i> sp. 12-3998_AO053	LN810449.1 and LN810463.1
<i>Chlamydiales</i> sp. 136621	KP211380.1
<i>Chlamydiales</i> sp. C122	GU068510.1
<i>Chlamydia abortus</i> S26/3	CR848038.1:992074-996789
<i>Chlamydia avium</i> 10DC88	CP006571.1:718307-723013
<i>Chlamydia buteonis</i> RSHA	NZ_CAAAFM010000001.1:300013-304729
<i>Chlamydia caviae</i> GPIC	AE015925.1:1020808-1025525
<i>Chlamydia felis</i> Fe/C-56	AP006861.1:147704-152421
<i>Chlamydia gallinacea</i> 08-1274/3	CP015840.1:281447-286166
<i>Chlamydia ibidis</i> 10-1398/6	NZ_APJW010000003.1:241606-246333
<i>Chlamydia muridarum</i> Nigg	CP027211.1:133832-138572
<i>Chlamydia pecorum</i> E58	CP002608.1:159116-163827
<i>Chlamydia pneumoniae</i> CWL029	AE001363.1:1000564-1005278
<i>Chlamydia poikilothermis</i> S15-834K	LS992154.1:1002686-1007399
<i>Chlamydia psittaci</i> 6BC	CP002586.1:1019219-1023929
<i>Chlamydia serpentis</i> H15-1957-10C	LT993738.1:972227-976940
<i>Chlamydia suis</i> SWA-2	NZ_LT821323.1:887563-892290
<i>Chlamydia trachomatis</i> D/UW-3/CX	AE001273.1:854128-858862
<i>Candidatus Chlamydia coralli</i> G3/2742-324	NZ_NWQK010000001.1:446079-450797
<i>Candidatus Chlamydia sanziniae</i> 2742-308	CP014639.1:554775-559459
<i>Candidatus Chlamydia testudinis</i> 17-3921_L77	NZ_CACSG010000003.1:75444-80165
<i>Chlamydiifrater phoenicopteri</i> 14-2711_R47	NZ_LR777658.1:1150415-1155131
<i>Chlamydiifrater volucris</i> 15-2067_O50	NZ_LR777654.1:57265-61981
<i>Criblamydia sequanensis</i> CRIB-18	DQ124300.1 and JN201882.1
<i>Parachlamydia acanthamoebae</i> UV-7	FR872580.1:768411-773250
<i>Simkania negevensis</i> Z	FR872582.1:370012-374799
<i>Waddlia chondrophila</i> WSU_86-1044	CP001928.1:1836312-1840994
<i>Candidatus Amphibiichlamydia ranarum</i> AMCS11/3	JN402380.1
<i>Candidatus Amphibiichlamydia salamandrae</i> AMCS11/1	JN392919.1
<i>Candidatus Clavichlamydia salmonicola</i> ET	NZ_WTCQ010000001.1:165185-169940
<i>Candidatus Similichlamydia latridicola</i> Hat2	QQBG01000012.1:206-4857
<i>Candidatus Rhabdochlamydia porcellionis</i> 15C	CP075585.1:1017984-1022723

Table S4. Estimates of evolutionary divergence (number of base substitutions per site in the 16S–23S rRNA region) of *Chlamydiaceae*-like strains from aquatic birds.

Organism	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Unidentified <i>Chlamydiales</i> from seabirds							
(1) SM1420 from Magellanic penguin	–	0.0148	0.0295	0.0354	0.0390	0.0613	0.0655
(2) 136621 from chinstrap penguin	0.0148	–	0.0421	0.0469	0.0426	0.0595	0.0686
(3) C122 from glaucous-winged gull	0.0295	0.0421	–	0.0044	0.0015	0.0296	0.0304
(4) 12-3998_AO053 from European herring gull	0.0354	0.0469	0.0044	–	0.0062	0.0323	0.0362
(5) 12-1761_J072 from European herring gull	0.0390	0.0426	0.0015	0.0062	–	0.0299	0.0351
<i>Chlamydiifrater</i>							
(6) <i>Chlamydiifrater volucris</i> 15-2067_O50	0.0613	0.0595	0.0296	0.0323	0.0299	–	0.0071
(7) <i>Chlamydiifrater phoenicopteri</i> 14-2711_R47	0.0655	0.0686	0.0304	0.0362	0.0351	0.0071	–
<i>Chlamydia</i>							
<i>Chlamydia ibidis</i> 10-1398/6	0.1448	0.1737	0.0695	0.0769	0.0762	0.0600	0.0614
<i>Chlamydia pneumoniae</i> CWL029	0.1496	0.2036	0.0903	0.0853	0.0847	0.0669	0.0668
<i>Chlamydia serpentis</i> H15-1957-10C	0.1528	0.1891	0.0865	0.0819	0.0825	0.0623	0.0624
<i>Ca. Chlamydia coralli</i> G3_2742-324	0.1559	0.2020	0.0900	0.0872	0.0865	0.0625	0.0632
<i>Chlamydia buteonis</i> RSHA	0.1560	0.1844	0.0815	0.0786	0.0798	0.0631	0.0626
<i>Chlamydia psittaci</i> 6BC	0.1569	0.1788	0.0790	0.0818	0.0817	0.0651	0.0638
<i>Ca. Chlamydia sanziniae</i> 2742-308	0.1587	0.2049	0.0853	0.0868	0.0836	0.0706	0.0731
<i>Chlamydia abortus</i> S26/3	0.1601	0.1895	0.0814	0.0836	0.0835	0.0674	0.0670
<i>Chlamydia gallinacea</i> 08-1274/3	0.1659	0.1646	0.0689	0.0829	0.0822	0.0666	0.0672
<i>Chlamydia pecorum</i> E58	0.1677	0.2066	0.0833	0.0813	0.0813	0.0667	0.0666
<i>Chlamydia avium</i> 10DC88	0.1714	0.1861	0.0762	0.0830	0.0846	0.0692	0.0680
<i>Ca. Chlamydia testudinis</i> 17-3921_L77	0.1733	0.2189	0.0839	0.0885	0.0898	0.0667	0.0677
<i>Chlamydia felis</i> Fe/C-56	0.1737	0.1740	0.0791	0.0858	0.0857	0.0694	0.0693
<i>Chlamydia poikilothermis</i> S15-834K	0.1749	0.1779	0.0797	0.0881	0.0866	0.0697	0.0703
<i>Chlamydia caviae</i> GPIC	0.1795	0.1732	0.0781	0.0887	0.0885	0.0702	0.0703
<i>Chlamydia muridarum</i> Nigg	0.2386	0.1912	0.0875	0.1139	0.1189	0.0939	0.0940
<i>Chlamydia trachomatis</i> D/UW-3/CX	0.2471	0.2037	0.0928	0.1217	0.1252	0.1003	0.1003
<i>Chlamydia suis</i> SWA-2	0.2488	0.1792	0.0906	0.1184	0.1228	0.0975	0.0970
Other <i>Chlamydiales</i>							
<i>Ca. Amphibiichlamydia salamandrae</i> AMCS11/1	0.1578	0.1854	0.1126	0.1120	0.1144	0.1029	0.1036
<i>Ca. Amphibiichlamydia ranarum</i> AMCS11/3	0.1773	0.1991	0.1083	0.1055	0.1061	0.0950	0.0984
<i>Ca. Clavichlamydia salmonicola</i> ET	0.2644	0.1876	0.1002	0.1397	0.1387	0.1290	0.1307
<i>Criblamydia sequanensis</i> CRIB-18	0.2857	0.2506	0.1667	0.2046	0.2060	0.1940	0.1946
<i>Parachlamydia acanthamoebae</i> UV-7	0.3406	0.2651	0.1709	0.2067	0.2029	0.1954	0.1946
<i>Waddlia chondrophila</i> WSU_86-1044	0.3649	0.2854	0.1929	0.2301	0.2301	0.2208	0.2181
<i>Simkania negevensis</i> Z	0.4060	0.3654	0.2117	0.2385	0.2426	0.3822	0.3810
<i>Ca. Rhabdochlamydia porcellionis</i> 15C	0.4405	0.3579	0.2010	0.2526	0.2534	0.2475	0.2457
<i>Ca. Similichlamydia latridicola</i> Hat2	0.4626	0.5073	0.2563	0.2939	0.2917	0.2619	0.2604