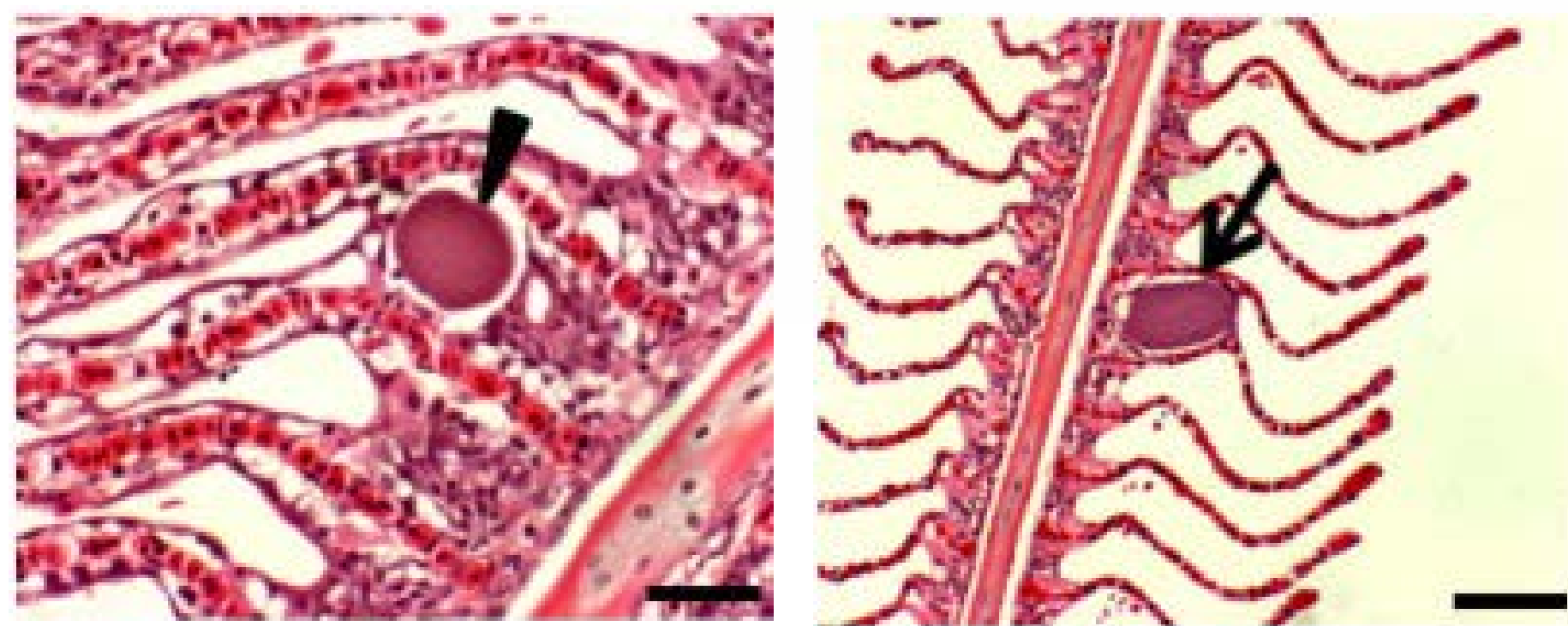


Culture-independent genome sequencing and analysis of the chlamydial agent of epitheliocystis in Yellowtail Kingfish

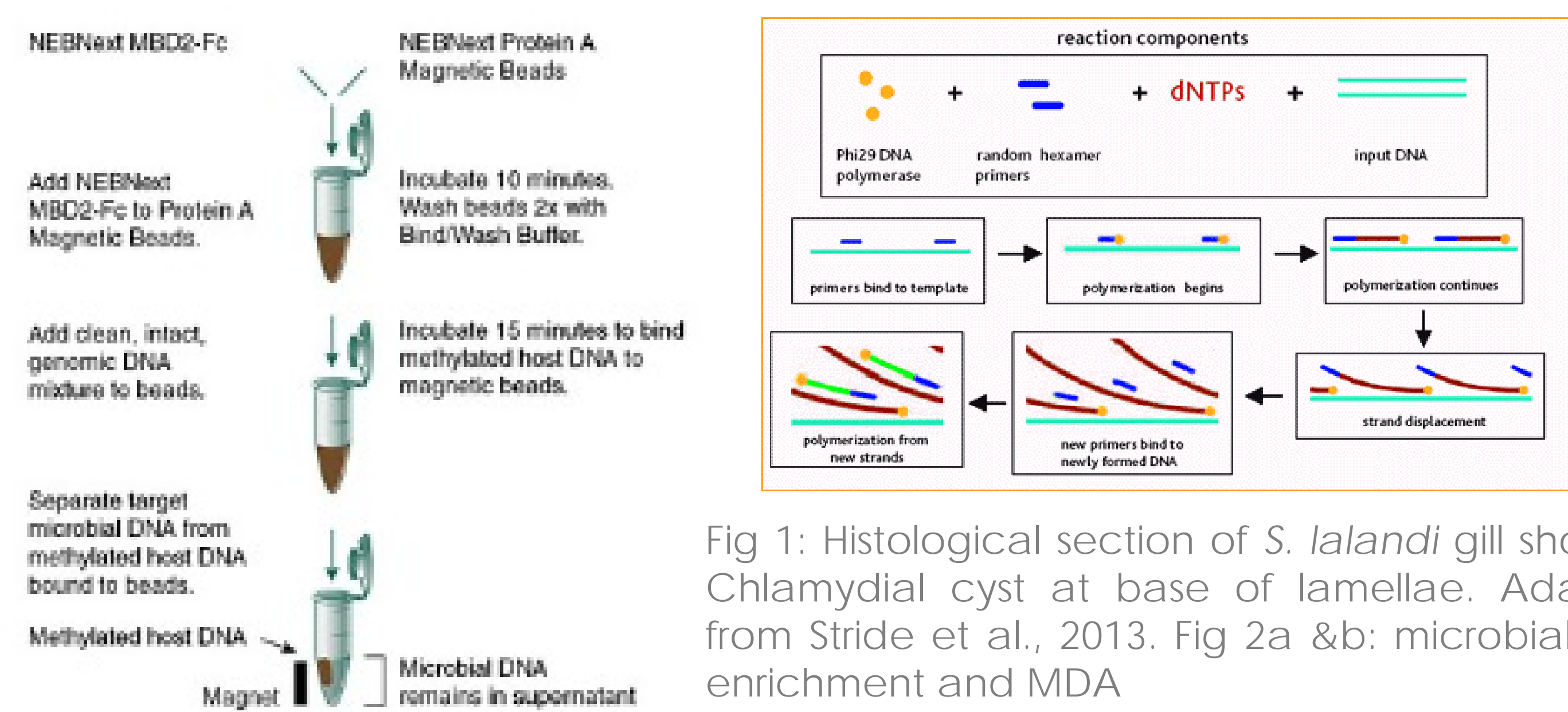
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Background: Several recently described *Chlamydiales* families are associated with epitheliocystis in fish. Due to the lack of culture systems, little is known about the biology of these *Chlamydia*-like organisms (CLOs).



Aim: We aimed to characterise and investigate the genome of the novel pathogen, *Ca. Parilichlamydia carangidicola*, the epitheliocystis agent of Yellowtail Kingfish (*Seriola lalandi*) to better understand the biology, pathogenicity, and evolutionary relationships among the *Chlamydiae*.



Methods: DNA from gills of YTK with epitheliocystis

→ Microbial DNA enrichment

→ depletion of methylated DNA (Fig 2)

→ multiple displacement amplification (MDA) (Fig 2)

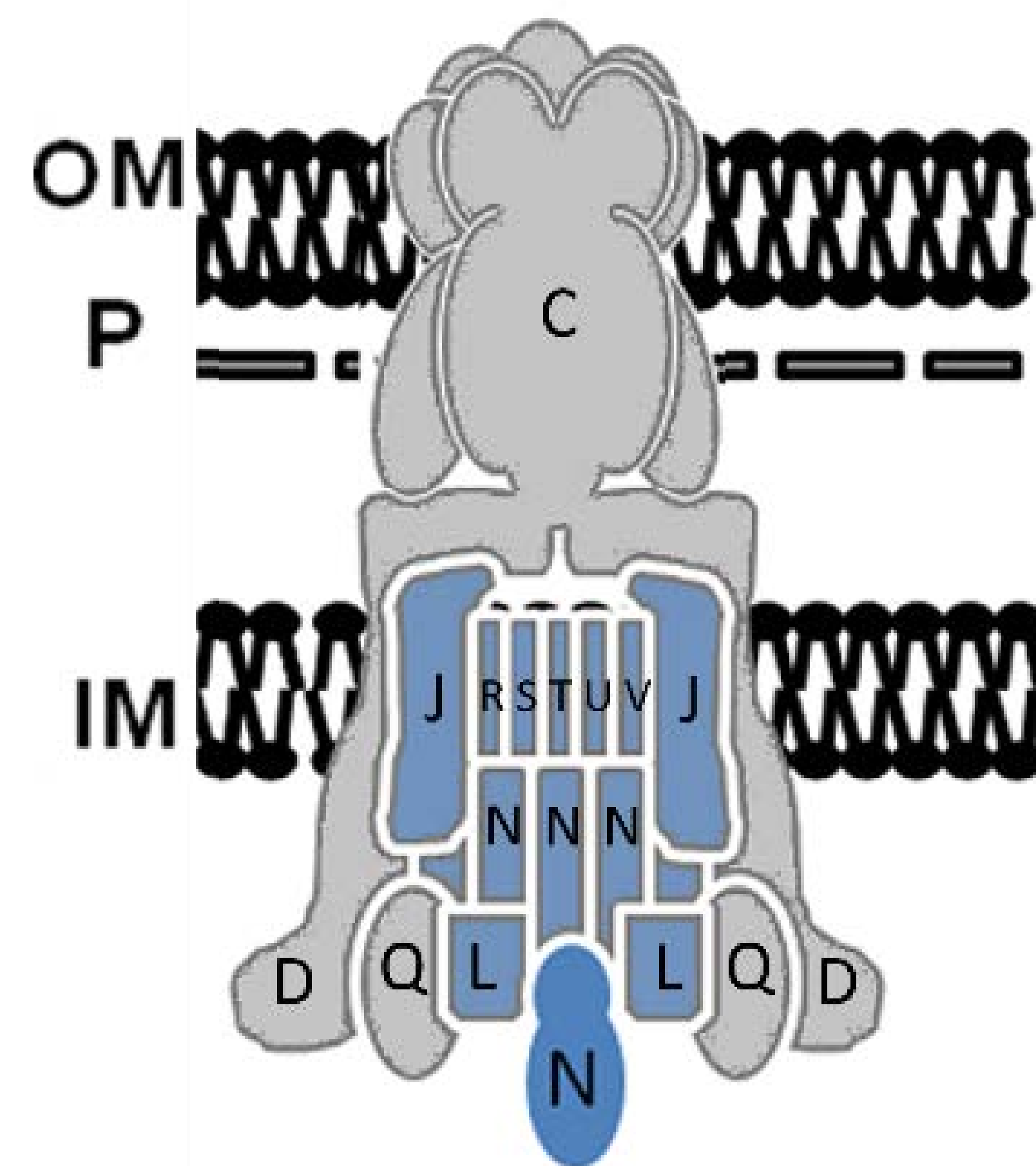
→ Genome sequencing on an Illumina MiSeq

→ Bioinformatics analysis

→ SPADEs, RAST, BLAST, Geneious and DNAPlotter

Results: *Ca. P. carangidicola* genome sequence

Species, strain	Genome characteristics					Reference(s)
	Size (Mbp)	Plasmid size (Kbp)	Predicted CDSs	Hypothetical proteins	GC Content (%)	
<i>Ca. Parilichlamydia carangidicola</i> 11YTK14	2.1	Np	1933	599	39	This study
<i>C. trachomatis</i> D/UW-3/CX	1.0	7.5	894	266	41	(Stephens, et al., 1998)
<i>Simkania negevensis</i> "Z"	2.5	132	2519	450	38	(Collingro, et al., 2011)
<i>Waddlia chondrophila</i> WSU 86-1044	2.1	15.5	1934	764	43	(Bertelli, et al., 2010)
<i>Parachlamydia acanthamoebae</i> UV-7	3.1	Np	2788	368	39	(Collingro, et al., 2011)
<i>Criblamydia sequanensis</i> CRIB-18	2.9	89.5	2426	641	38	(Bertelli, et al., 2014)



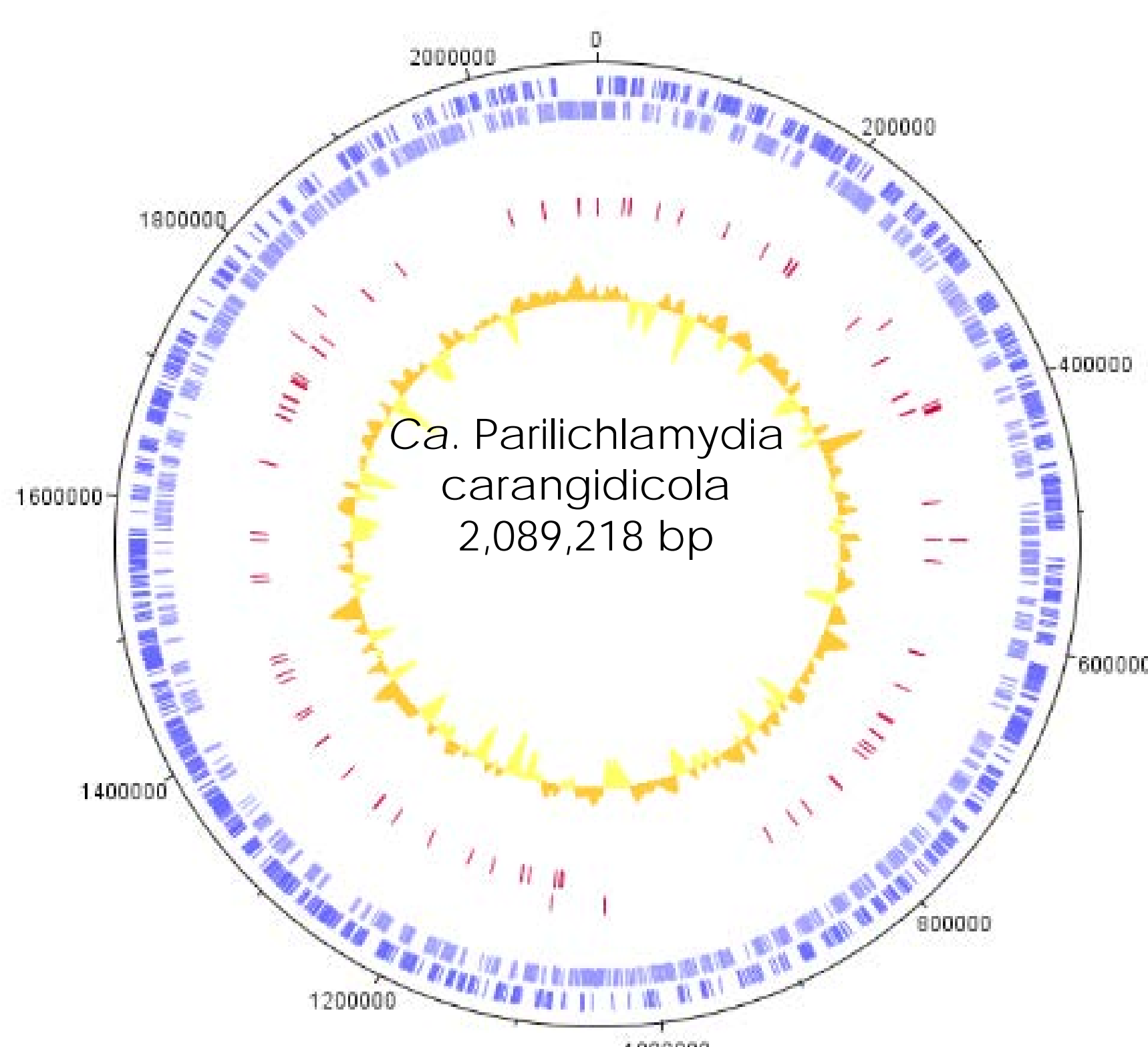
Left (Fig 4): Structural components of the T3SS (eg. inner membrane ring) are conserved (Fig 4; blue), while outer membrane ring components (grey) & needle formation proteins (not shown) appear absent from this assembly. One T3SS chaperone is predicted (not shown)

Fig 4: *Ca. P. carangidicola* T3SS components. Adapted from Betts-Hampikian and Fields, Frontiers in Microbiology (2010).

Below: structural components appear to be most closely related to those of *P. acanthamoebae*

Amino acid similarity (%) between *Ca. P. carangidicola* and *Chlamydiae* T3SS components

	<i>C. trachomatis</i> DUW3CX	<i>C. sequanensis</i> CRIB-18	<i>P. acanthamoebae</i> UV-7	<i>W. chondrophila</i> WSU 86-1044	<i>S. negevensis</i> Z
SctJ	34	32.5	33.8	34.4	33.9
SctR	38.1	43.5	41.5	43.3	41.9
SctL	24	26	35	30	31.1
SctT	28.5	30.2	29.1	27.8	29.4
SctU	45.3	49.7	51.4	49.2	43.9
SctV	62.4	65.5	68.5	67	62.9
SctS	50	56.2	59.6	65.2	61.8



Above: characteristics of representative Chlamydial genomes. Left (Fig 3): Circular representation of the *Ca. P. carangidicola* genome. Blue rings; CDSs in the forward and reverse directions, red rings; RNAs, orange & yellow ring; GC content.

Summary: We used a culture-independent sequencing technique to determine the first draft genome of *Candidatus Parilichlamydia carangidicola*; an epitheliocystis agent from Yellowtail kingfish. The draft genome is 2,089,218 bp long, and contains 1933 predicted coding sequences. Preliminary analysis reveals the presence of many conserved proteins, such as structural components of the T3SS. The availability of the *Ca. P. carangidicola* genome provides us with new insights into the evolution of members of the order *Chlamydiales*, and can be used to inform future characterisation of novel CLOs.