

Supplementary Materials

Table S1 - Primers used to isolate 584 bp length of jellyfish mt 16S rRNA gene.

Assay	Label	Sequence (5'-3')	Target Sequence Length (bp)
Universal Jellyfish Assay	16SL_F Aa_H16S_15141H	GACTGTTTACCAAAAACATA AGATTTTAATGGTCGAACAGAC	584

Table S2 - List of marine fish species NCBI accession numbers and country of origin for all existing mt 16S rRNA/complete genome sequences used for *in silico* testing of the endogenous control assay.

Species	Accession Number	Country of Origin	<i>In silico</i> Amplification
<i>Lates calcarifer</i>	KR349919	Australia	Yes
<i>Platycephalus fuscus</i>	KT862661	Australia	Yes
<i>Acanthopagrus butcheri</i>	KX234643	Australia	Yes
<i>Acanthopagrus australis</i>	JN688792	China	Yes
<i>Acanthopagrus pacificus</i>	MK919144	Vietnam	Yes
<i>Rhabdosargus sarba</i>	KM272585	China	Yes
<i>Scartelaos histophorus</i>	NC_017888	China	Yes
<i>Eleutheronema tetradactylum</i>	KT593869	China	Yes
<i>Lutjanus johnii</i>	NC_024572	Australia	Yes
<i>Sillaginidae punctatus</i>	MF572031	China	Yes
<i>Triacanthus blaculeatus</i>	AP009174	Japan	Yes
<i>Monodactylus argenteus</i>	NC_009858	Japan	Yes
<i>Hephaestus fuliginosus</i>	MH606192	China	Yes
<i>Gnathanodon speciosus</i>	NC_054367	China	Yes

Table S3 - Primer selection characteristics.

Selection Characteristics	Sense Primer	Anti-Sense Primer	MGB Probe
bp length	24	22	20
T _m (Nearest Neighbour)	64.98°C	65.23°C	N/A
GC%	45.8	54.5	45
Hairpins	34.4 (weak)	None	None

GC Clamps	2	3	N/A
Self-Dimer	None	None	None
Hetero-Dimer	None	None	None

Table S4 - Base pair (bp) differences between *Chironex fleckeri* identifiers and sympatric jellyfish species.

Species	Total bp Differences	Primer bp Differences (Sense & Anti-Sense)	Probe bp Differences
<i>Alatina alata</i>	17	6 & 5	6
<i>Carukia barnesi</i>	28	8 & 8	12
<i>Carybdea xaymacana</i>	32	11 & 10	11
<i>Copula sivickisi</i>	22	8 & 5	9
<i>Morbakka fenneri</i>	31	10 & 7	14
<i>Tamoya ohboya</i>	33	9 & 8	16

Table S5 - Efficiencies of the *Chironex fleckeri* specific detection assay when independent and multiplexed with the endogenous control assay.

	Target Assay Only	Multiplexed Assay
Efficiency (%)	93.47	92.68
R ²	0.999	0.999
Slope	-3.48	-3.51

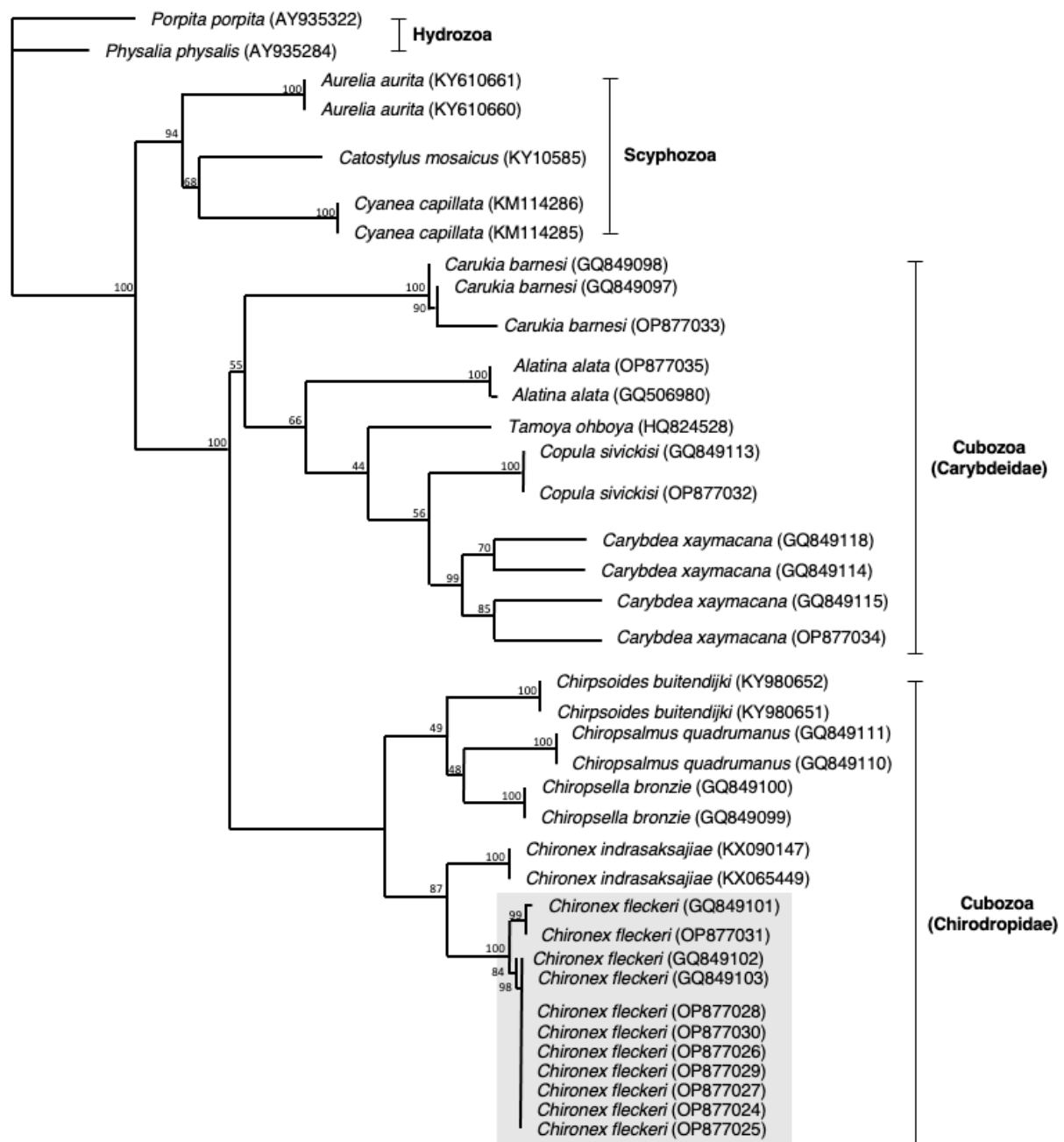


Figure S1 - Intraspecific similarity of *Chironex fleckeri* sequences and interspecific divergence between *C. fleckeri* and sympatric species for the mt 16S rRNA gene. Bootstrap values greater than 50% are displayed above the branch nodes. The tree was maximum likelihood generated utilising the substitution models GTR+F+I+G4 with sequence regions ranging from ≤418 - ≥763 bp

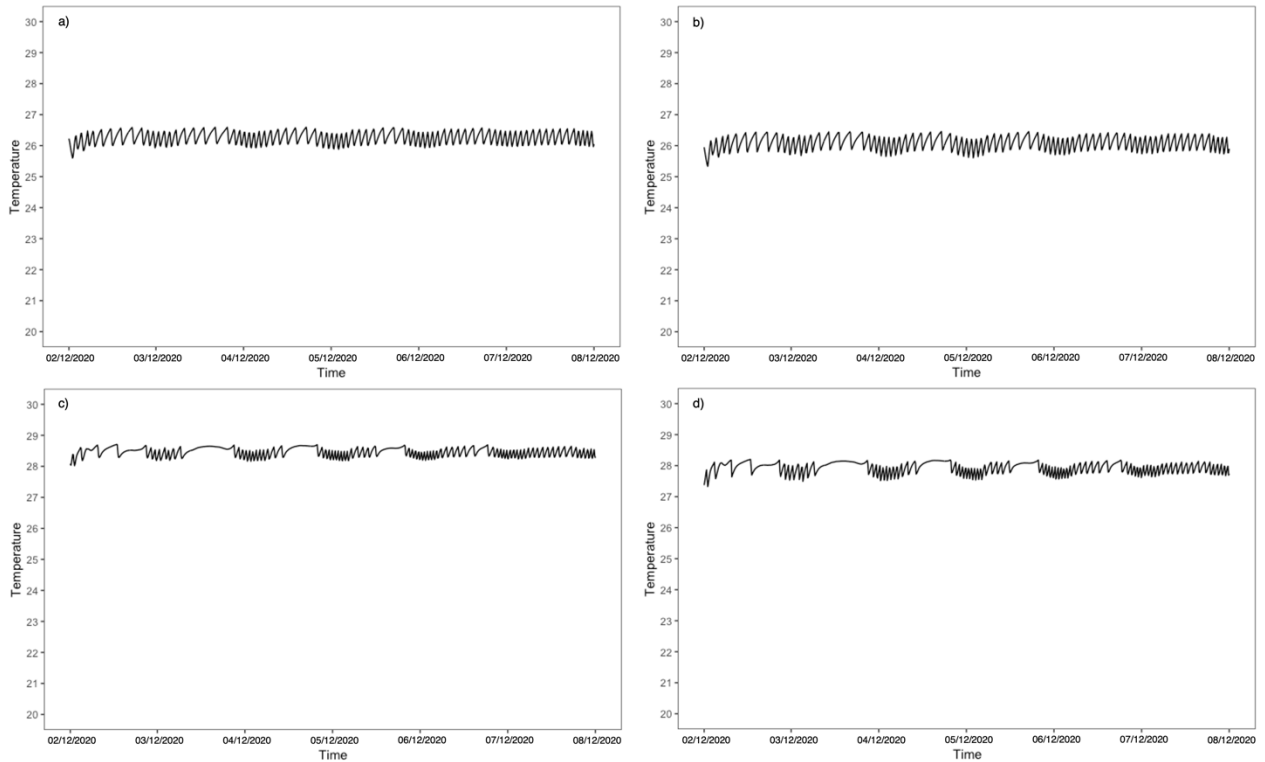


Figure S2 – Tinytag TG-3100 data logger temperature data for duration of the *C. fleckeri* eDNA decay experiment. a) Logger 1 – 26 °C bath, b) Logger 2 – 26 °C bath, c) Logger 3 – 28 °C bath, d) Logger 4 – 28 °C bath.

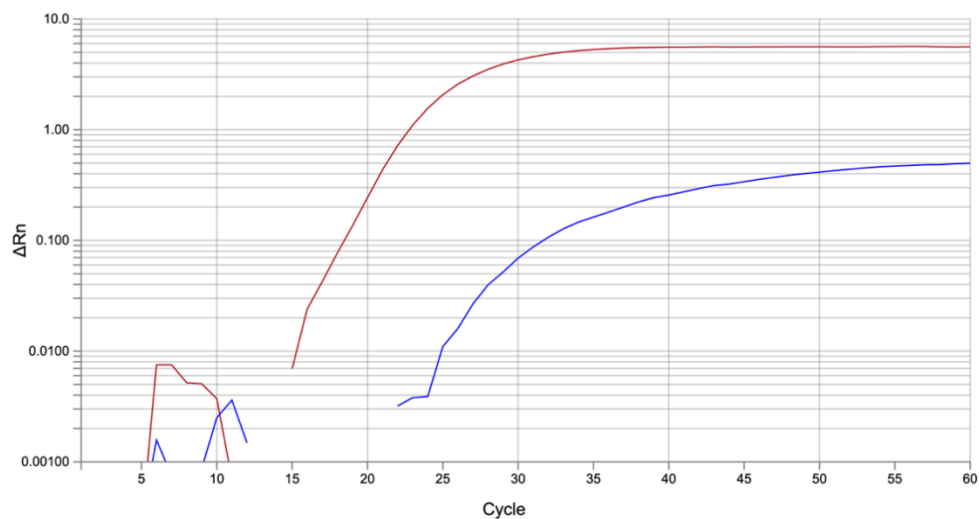


Figure S3 - Amplification Plot of Multiplexed Assay showing amplification of FAM dye-labelled *Chironex*-specific assay (red) and VIC dye-labelled endogenous control assay (blue).