SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Map and photographs of shark strandings occurring in SF Bay in spring of 2017. A) Map of SF Bay showing locations of stranded sharks and bat rays. (MSI) Marine Science Institute. (AOTB) Aquarium of the Bay. (**B**-**D**) Representative photographs of stranded leopard sharks around SF Bay taken between March and August 2017.

Figure S2: Presence of *M. avidus* in CSF from additional shark species in SF Bay. DNA samples from SF Bay sharks (S1, S2, and S3) and Southern California sharks (S4, S5) were tested by PCR using universal primers for the ciliate *cox1* gene (expected size 700bp), and nested primers specific to the *cox1* gene of *M. avidus* (expected size 422bp). Presence of shark DNA was assessed with universal shark rDNA primers (expected 1.3-1.6kb). (NTC) indicates no-template control. M: 100bp (Universal ciliate and *M. avidus*), or 1kb+ (Universal shark) ladder.

Figure S3: Absence of non-*M. avidus* pathogenic ciliates in CSF from leopard sharks in SF Bay. DNA samples from SF Bay leopard sharks (LS1-12) and negative control animals (bat ray (BR) and S4) were tested by PCR using universal primers for the ciliate *cox1* gene (expected size 700bp, arrow), and nested primers specific to the *cox1* gene of *Pseudocohnilembus longsietus* (expected size 341bp), *Pseudocohnilembus persalinus* (expected size 229bp), and *Uronema marinum* (expected size 285bp). Positive controls for *P. longsietus*, *P. persalinus*, and *U. marinum* were unavailable. Presence of shark DNA was assessed with universal shark rDNA primers (expected 1.3-1.6kb). (+) marks samples where amplification with primers specific to the *cox1* gene of *M. avidus* produced a band of the expected size (see Figure 2A). M: 100bp (Universal ciliate), 25bp (species-specific), or 1kb+ (Universal shark) ladder.

Figure S4: Absence of *M. avidus* in CSF from leopard sharks in Southern California. DNA samples from leopard sharks from Southern California (LS13-16) and a positive control SF Bay leopard shark (LS11) were tested by nested PCR using primers specific to the *cox1* gene of *M. avidus* (expected size 422bp). (NTC) indicates no-template control. Presence of shark DNA was assessed with universal shark rDNA primers (expected 1.3-1.6kb). M: 100bp (*M. avidus*) or 1kb+ (Universal shark) ladder.

Figure S5: Presence of ciliate SSU sequence in SF Bay sharks. DNA samples from leopard sharks (A) and other shark species (B) was amplified using universal ciliate ribosomal SSU primers (expected size 500bp, arrows). Bands above 1000bp and below 400bp likely represent non-specific amplification and did not reveal ciliate sequences by Sanger sequencing. (NTC) indicates no-template control reaction. M: 100bp ladder.

Figure S6: Neighbor-joining phylogenetic trees from ribosomal RNA genes. Trees were constructed from SSU rDNA (A) or LSU rDNA (B) nucleotide sequences of Philasterid species, using *Tetrahymena pyriformis* as the outgroup. New sequences in this study are in bold. See Tables S5 and S6 for reference sequence accession numbers. Nodes are labeled with bootstrap values based on 1,000 resamplings. Scale bar, nucleotide substitutions per site.

Figure S7: Precipitation in regions draining to SF Bay. Six-month cumulative precipitation (solid lines) ending in March of given year, in California Climate District 2 (Sacramento Drainage, black) and Climate District 5 (San Joaquin Drainage, grey). Mean October-March precipitation values for 1901-2000 plotted as baseline (dashed lines). Map of California (right) shows climate districts, respective to their outflow into SF Bay (star). Red highlights years with abnormal shark deaths in the spring. Precipitation data from NOAA National Centers for Environmental Information.

Fig. S1

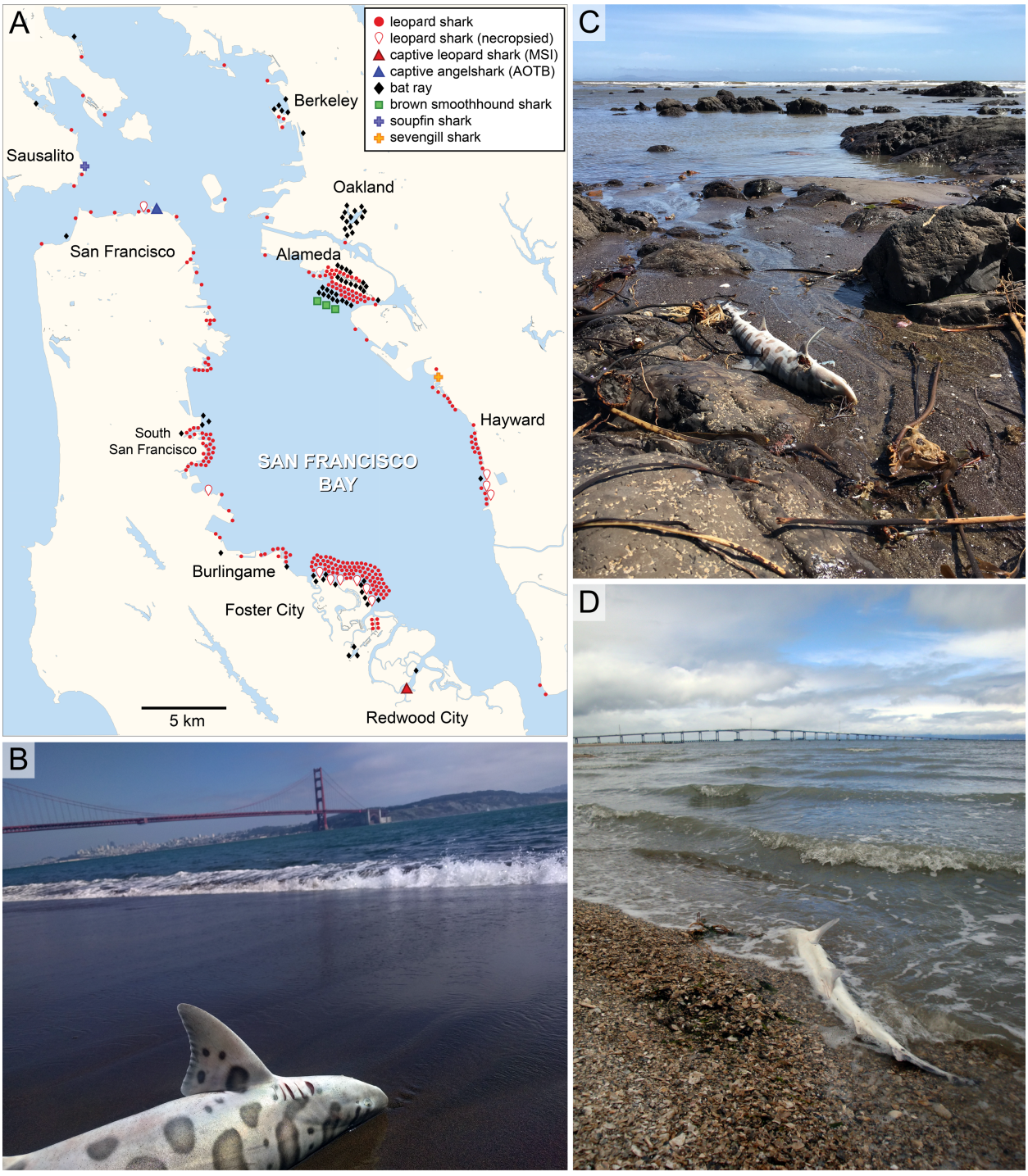
A
leopard shark
leopard shark (necropsied)
captive leopard shark (MSI)
captive angelshark (AOTB)
bat ray
brown smoothhound shark
soupfin shark
sevengill shark
Berkeley
Sausalito
Oakland
San Francisco
Alameda
Hayward
South
San Francisco
SAN FRANCISCO
BAY
Burlingame
Foster City
Redwood City
5 km
B
C
D

Fig. S2

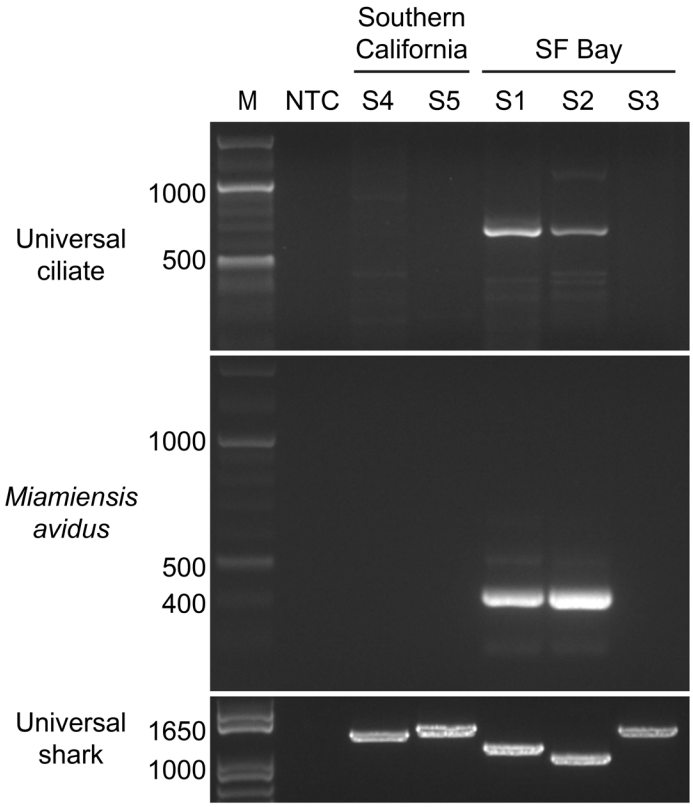
Southern California
SF Bay
M
NTC
S4
S5
S1
S2
S3
Universal ciliate
1000
500
Miamiensis avidus
1000
500
400
Universal shark
1650
1000

Fig. S3

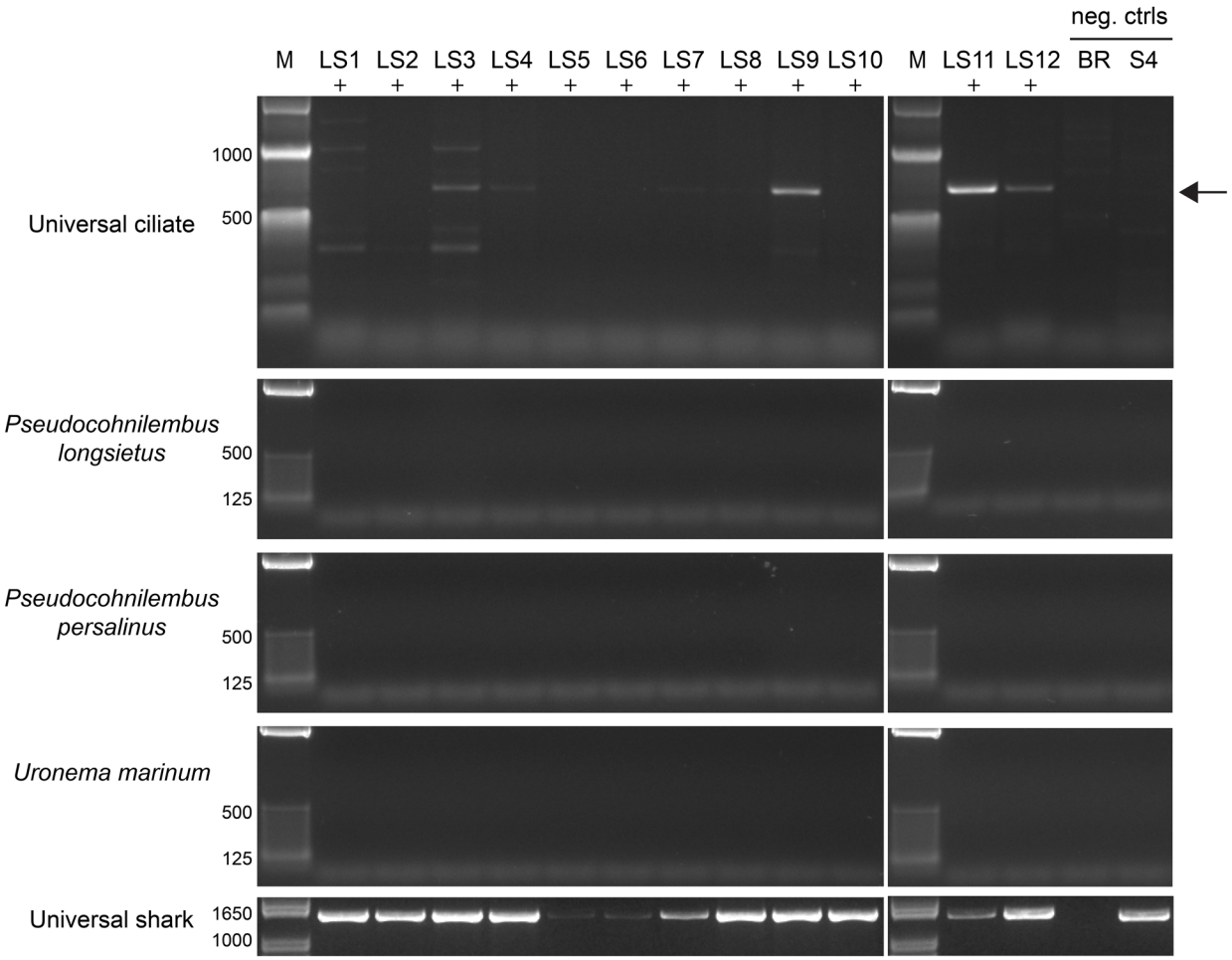
neg. ctrls
M
LS1
LS2
LS3
LS4
LS5
LS6
LS7
LS8
LS9
LS10
M
LS11
LS12
BR
S4
+
Universal ciliate
1000
500
Pseudocohnilembus longsietus
500
125
Pseudocohnilembus persalinus
500
125
Uronema marinum
500
125
Universal shark
1650
1000

Fig. S4

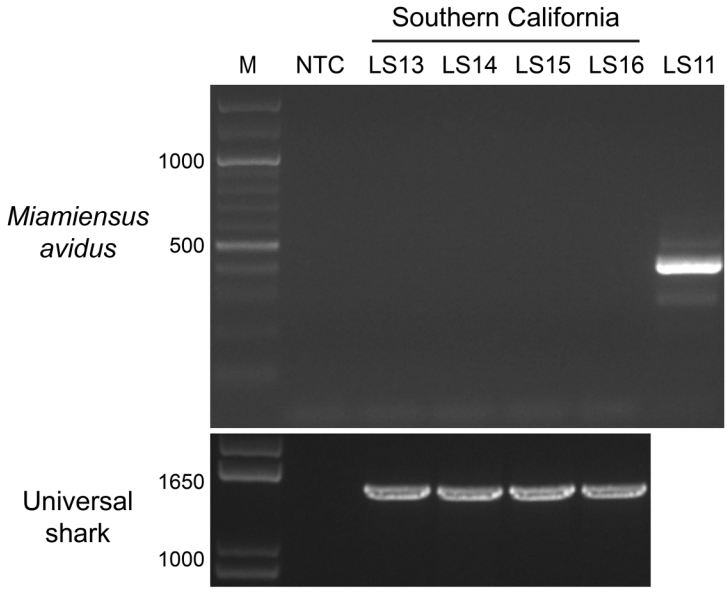
Southern California
M
NTC
LS13
LS14
LS15
LS16
LS11
1000
500
Miamiensus avidus
1650
1000
Universal shark

Fig. S5

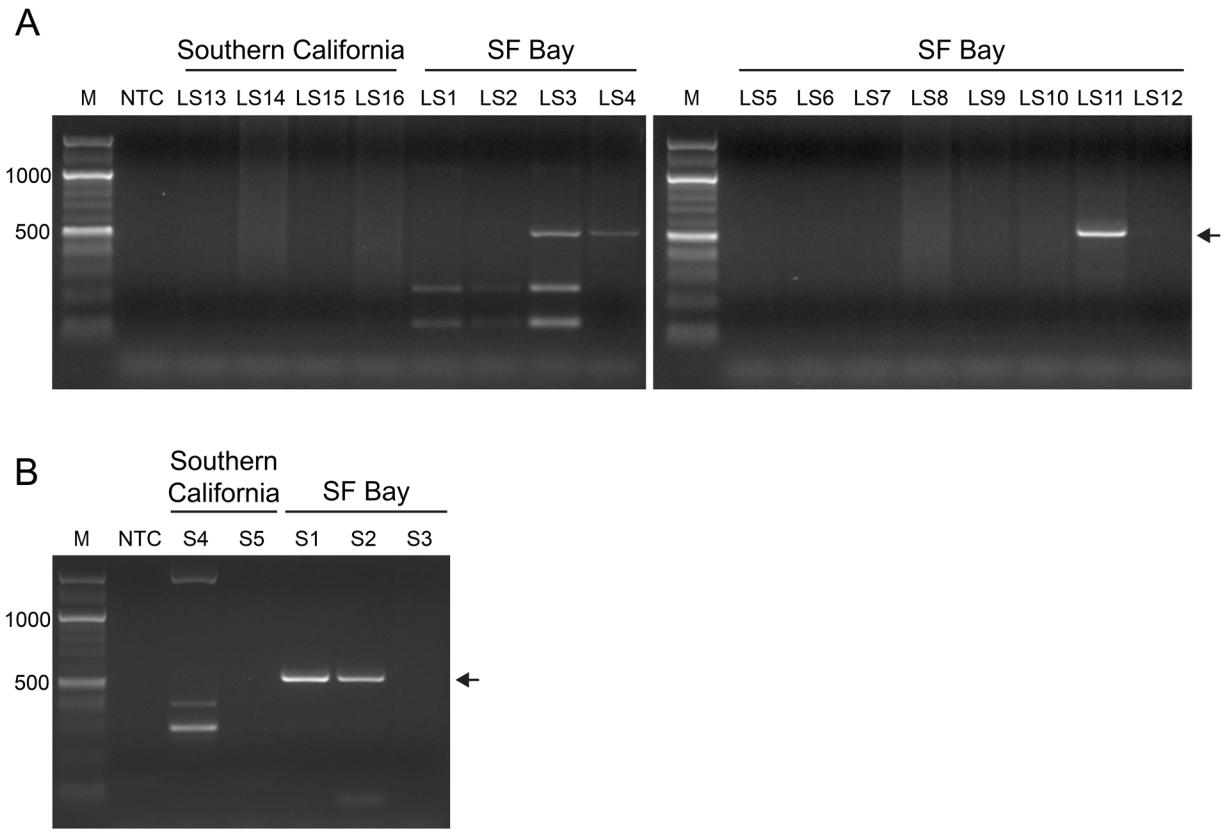
A
Southern California
SF Bay
SF Bay
M
NTC
LS13
LS14
LS15
LS16
LS1
LS2
LS3
LS4
M
LS5
LS6
LS7
LS8
LS9
LS10
LS11
LS12
1000
500
B
Southern California
SF Bay
M
NTC
S4
S5
S1
S2
S3
1000
500

Fig. S6

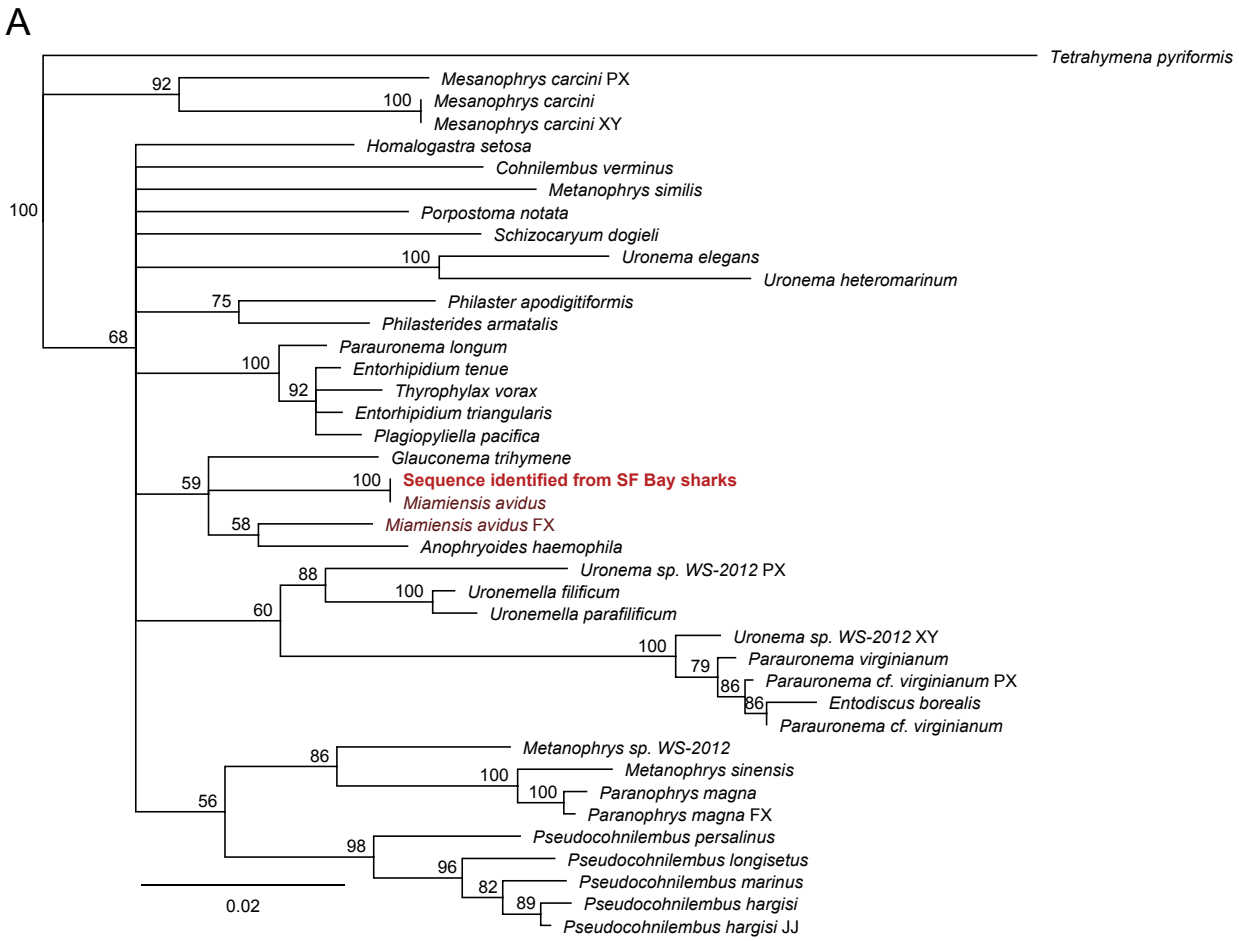
A
Tetrahymena pyriformis
Mesanophrys carcini PX
Mesanophrys carcini
Mesanophrys carcini XY
Homalogastra setosa
Cohnilembus verminus
Metanophrys similis
Porpostoma notata
Schizocaryum dogieli
Uronema elegans
Uronema heteromarinum
Philaster apodigitiformis
Philasterides armatalis
Parauronema longum
Entorhipidium tenue
Thyrophylax vorax
Entorhipidium triangularis
Plagiopyliella pacifica
Glauconema trihymene
Sequence identified from SF Bay sharks
Miamiensis avidus
Miamiensis avidus FX
Anophryoides haemophila
Uronema sp. WS-2012 PX
Uronemella filificum
Uronemella parafilificum
Uronema sp. WS-2012 XY
Parauronema virginianum
Parauronema cf. virginianum PX
Entodiscus borealis
Parauronema cf. virginianum
Metanophrys sp. WS-2012
Metanophrys sinensis
Paranophrys magna
Paranophrys magna FX
Pseudocohnilembus persalinus
Pseudocohnilembus longisetus
Pseudocohnilembus marinus
Pseudocohnilembus hargisi
Pseudocohnilembus hargisi JJ
0.02
B
Tetrahymena pyriformis
Cohnilembus verminus
Sequence identified from SF Bay sharks
Miamiensis avidus
Pseudocohnilembus hargisi
Mesanophrys carcini PX
Mesanophrys carcini XY
Paranophrys magna
Metanophrys sinensis
Metanophrys sp. WS-2012
Uronema heteromarinum
Uronema sp. WS-2012 XY
Parauronema virginianum
Parauronema cf. virginianum PX
Uronema marinum
Uronema sp. WS-2012 PX
Uronemella parafilificum
Parauronema longum
Porpostoma notata
Philasterides armatalis
Philaster apodigitiformis
0.02

Fig. S7

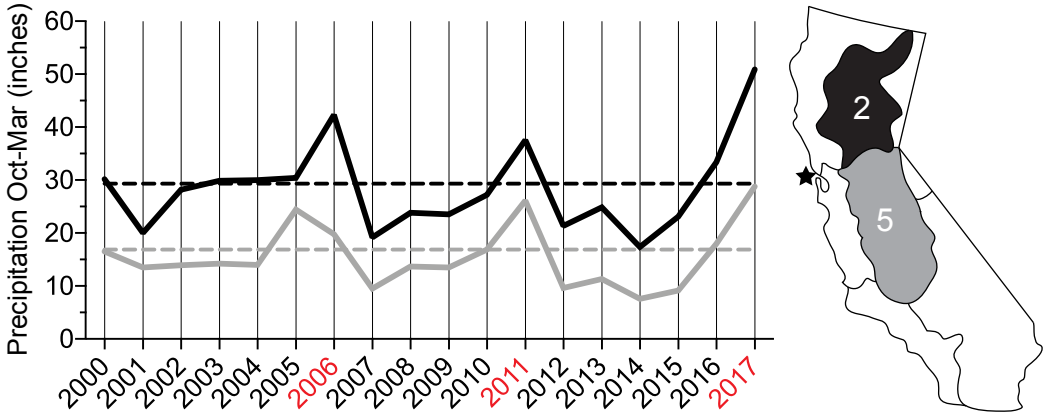
Precipitation Oct-Mar (inches)
60
50
40
30
20
10
0
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2
5

Table S1. Specimen details

* captive shark on display in aquaria. Est = estimated. ND = not determined. M = male, F = female.

	Fish ID	Shark Species	Stranding / Collection Date	Necropsy Date	Total Length (cm)	Fork Length (cm)	Body Weight (kg)	Sex
Stranded sharks from SF Bay	LS01	*Triakis semifasciata*	4/9/17	4/12/17	125.1	109.9	8.85	M
	LS02	*Triakis semifasciata*	4/15/17	4/17/17	122.5	106.3	7.94	M
	LS03	*Triakis semifasciata*	4/25/17	4/25/17	113.7	95.9	5.25	M
	LS04	*Triakis semifasciata*	4/25/17	4/25/17	49.5	42.5	0.25	M
	LS05	*Triakis semifasciata*	4/25/17	4/25/17	111.8	96.5	5.25	M
	LS06	*Triakis semifasciata*	4/25/17	4/25/17	73.0	62.9	1.50	M
	LS07	*Triakis semifasciata*	4/25/17	4/25/17	48.3	41.3	0.25	M
	LS08	*Triakis semifasciata*	4/26/17	4/27/17	96.7	85.2	3.52	M
	LS09	*Triakis semifasciata*	4/26/17	4/28/17	69.9	59.7	1.50	M
	LS10	*Triakis semifasciata*	5/2/17	5/3/17	100.5	88.3	3.88	M
	LS11	*Triakis semifasciata*	5/13/17	5/16/17	96.0	82.5	3.12	F
	LS12	*Triakis semifasciata* *	5/23/17	5/24/17	66.4	58.4	1.05	F
	S1	*Squatina californica* *	5/17/17	5/18/17	ND	ND	ND	ND
	S2	*Notorynchus cepedianus*	5/17/17	5/20/17	293.4	ND	100 (est.)	M
	S3	*Galeorhinus galeus*	7/5/17	7/11/17	177.0	ND	22.50	M
Controls from Central/South CA	LS13	*Triakis semifasciata*	7/18/17	7/20/17	128.7	113.2	10.20	M
	LS14	*Triakis semifasciata*	7/18/17	7/20/17	85.2	75.0	2.72	F
	LS15	*Triakis semifasciata*	7/18/17	7/20/17	73.2	65.0	1.81	M
	LS16	*Triakis semifasciata*	7/18/17	7/20/17	82.9	72.7	2.45	F
	S4	*Carcharodon carcharias*	4/8/17	4/9/17	299.7	279.4	310.00	M
	S5	*Galeorhinus galeus*	5/24/17	5/25/17	185.0	160.0	ND	F

Table S2: mNGS findings

Details on metagenomic Next-Generation Sequencing (mNGS) performed using RNA extracted from CSF of sharks from SF Bay or from Southern /Central California, or using no-template controls (NTC). [1] Number of unprocessed read pairs. [2] Credible shark pathogens identified in metagenomic analysis. [3] Number of read pairs assigned to Ciliophora (TaxID 5878). [4] Ciliophora reads as in [3], expressed as reads per million raw reads (rpm). * *Mortierella alpina* was identified, but pathogenicity is uncertain.

	Fish ID	Shark Species	Raw reads [1]	Credible pathogen [2]	Ciliophora reads [3]	Ciliophora rpm [4]
Stranded sharks from SF Bay	LS08	*Triakis semifasciata*	676,814	Scuticociliate	979	1446
	LS10	*Triakis semifasciata*	974,483	Scuticociliate	1477	1516
	LS12	*Triakis semifasciata*	1,555,887	Scuticociliate	349	224
	S1	*Squatina californica*	760,593	Scuticociliate	773	1016
	S2	*Notorynchus cepedianus*	2,393,172	Scuticociliate	154	64
Southern/ Central CA	S4	*Carcharodon carcharias*	558,798	None*	0	0
	S5	*Galeorhinus galeus*	145,710	None	0	0
NTC	n/a	n/a	3,826	None	0	0

Table S3: Primers used in this study
Degenerate nucleotides (D: A/G/T, H: A/C/T, M: A/C, R: A/G, W: A/T and Y: C/T) are underlined

Primer name	Target	Sequence (5' - 3')	Amplicon size	Source
OX09-26	Universal ciliate *cox1*	GDTAYTTACAAGTWATTACYGCWCATGG	716-719 bp	Whang 2013
OX09-27	Universal ciliate *cox1*	TAAAACYCTYCTATGYCTCATACC		
OX09-142	*Miamiensis avidus cox1*	AGTAATAATAGAACATTTAACGAATTTAATAACAC	422 bp	Whang 2013
OX09-143	*Miamiensis avidus cox1*	CGTCTTGTAATTAATAAATTTGTAAACGATAC		
OX09-144	*Uronema marinum cox1*	AACATAGAGCATATAGAGAGTACTCTAA	285 bp	Whang 2013
OX09-145	*Uronema marinum cox1*	TTCATCCAGCTGTTGTTAATGT		
OX09-146	*Pseudocohnilembus persalinus cox1*	TAAATCTAATCATCGTAATAATAGAGAATTGTTAG	229 bp	Whang 2013
OX09-147	*Pseudocohnilembus persalinus cox1*	CTTATCGATACGACTAACTGCAT		
OX09-148	*Pseudocohnilembus longisetus cox1*	AAATCAAATCATAGAAATAATAGAGAATTTTTAAATG	341 bp	Whang 2013
OX09-149	*Pseudocohnilembus longisetus cox1*	GCTCCAACACCAGTATATTTAATG		
Cil3	Universal ciliate ribosomal SSU	GTAGGCTCTTTACCTTGA	~500 bp	Jung 2005
Cil4	Universal ciliate ribosomal SSU	CAAATCACTCCACCAACT		
FISH5.8SF	Universal shark ribosomal ITS2	TTAGCGGTGGATCACTCGGCTCGT	1.3-1.6 kb	Pank 2001
FISH28SR	Universal shark ribosomal ITS2	TCCTCCGCTTAGTAATATGCTTAAATTCAGC		

Table S4: Mitochondrial cytochrome oxidase I (mt *cox1*) gene sequences used in this study.

Taxonomic name	Name in phylogenetic tree	GenBank Accession No.
Miamiensis avidus	*Miamiensis avidus*	GQ855300
	Miamiensis avidus strain A3	EU831214
	Miamiensis avidus strain Mie0301	EU831233
	Miamiensis avidus strain Nakajima	EU831226
	Miamiensis avidus strain YK1	EU831228
	Miamiensis avidus strain YS3	EU831218
Pseudocohnilembus longisetus	*Pseudocohnilembus longisetus*	GQ500580
Pseudocohnilembus persalinus	*Pseudocohnilembus persalinus* GQ	GQ500579
	Pseudocohnilembus persalinus GU	GU584095
Tetrahymena pyriformis	*Tetrahymena pyriformis*	EF070300
Uronema marinum	*Uronema marinum*	GQ500578

Table S5. SSU-rDNA gene sequences used in this study

Taxonomic name	Name in phylogenetic tree	GenBank Accession No.
Anophryoides haemophila	*Anophryoides haemophila*	U51554
Cohnilembus verminus	*Cohnilembus verminus*	HM236339
Entodiscus borealis	*Entodiscus borealis*	AY541687
Entorhipidium tenue	*Entorhipidium tenue*	AY541688
Entorhipidium triangularis	*Entorhipidium triangularis*	AY541690
Glauconema trihymene	*Glauconema trihymene*	GQ214552
Homalogastra setosa	*Homalogastra setosa*	EF158848
Mesanophrys carcini	*Mesanophrys carcini*	AY103189
	Mesanophrys carcini PX	JN885085
	Mesanophrys carcini XY	JN885086
Metanophrys similis	*Metanophrys similis*	AY314803
Metanophrys sinensis	*Metanophrys sinensis*	HM236336
Metanophrys sp. WS-2012	*Metanophrys sp. WS-2012*	JN885084
Miamiensis avidus	*Miamiensis avidus*	AY550080
	Miamiensis avidus FX	JN885091
Paranophrys magna	*Paranophrys magna*	AY103191
	Paranophrys magna FX	JN885089
Parauronema virginianum	*Parauronema cf. virginianum*	FJ595488
	Parauronema cf. virginianum PX	JN885082
	Parauronema virginianum	JN885087
Parauronema longum	*Parauronema longum*	HM236338
Philaster apodigitiformis	*Philaster apodigitiformis*	FJ648350
Philasterides armatalis	*Philasterides armatalis*	FJ848877
Plagiopyliella pacifica	*Plagiopyliella pacifica*	AY541685
Porpostoma notata	*Porpostoma notata*	HM236335
Pseudocohnilembus hargisi	*Pseudocohnilembus hargisi*	AY212806
	Pseudocohnilembus hargisi JJ	JN885090
Pseudocohnilembus longisetus	*Pseudocohnilembus longisetus*	FJ899594
Pseudocohnilembus marinus	*Pseudocohnilembus marinus*	Z22880
Pseudocohnilembus persalinus	*Pseudocohnilembus persalinus*	AY551906
Schizocaryum dogieli	*Schizocaryum dogieli*	AF527756
Tetrahymena pyriformis	*Tetrahymena pyriformis*	EF070254
Thyrophylax vorax	*Thyrophylax vorax*	AY541686
Uronema elegans	*Uronema elegans*	AY103190
Uronema heteromarinum	*Uronema heteromarinum*	FJ870100
Uronema sp. WS-2012	*Uronema sp. WS-2012* PX	JN885083
	Uronema sp. WS-2012 XY	JN885088
Uronemella filificum	*Uronemella filificum*	EF486866
Uronemella parafilificum	*Uronemella parafilificum*	HM236337

Table S6. LSU-rDNA gene sequences used in this study

Taxonomic name	Name in phylogenetic tree	GenBank Accession No.
Cohnilembus verminus	*Cohnilembus verminus*	JN885111
Mesanophrys carcini	*Mesanophrys carcini* PX	JN885112
	Mesanophrys carcini XY	JN885113
Metanophrys sinensis	*Metanophrys sinensis*	JN885114
Metanophrys sp. WS-2012	*Metanophrys sp. WS-2012*	JN885129
Miamiensis avidus	*Miamiensis avidus* FX	JN885115
Paranophrys magna	*Paranophrys magna* FX	JN885116
Parauronema virginianum	*Parauronema cf. virginianum* PX	JN885117
	Parauronema virginianum	JN885128
Parauronema longum	*Parauronema longum*	JN885118
Philaster apodigitiformis	*Philaster apodigitiformis*	JN885119
Philasterides armatalis	*Philasterides armatalis*	JN885120
Porpostoma notata	*Porpostoma notata*	JN885121
Pseudocohnilembus hargisi	*Pseudocohnilembus hargisi* JJ	JN885122
Tetrahymena pyriformis	*Tetrahymena pyriformis*	X54004
Uronema heteromarinum	*Uronema heteromarinum*	JN885123
Uronema marinum	*Uronema marinum*	JN885124
Uronema sp. WS-2012	*Uronema sp. WS-2012* XY	JN885125
	Uronema sp. WS-2012 PX	JN885126
Uronemella parafilificum	*Uronemella parafilificum*	JN885127

SUPPLEMENTAL METHODS

Details on mNGS analysis

Due to the lack of complete shark genome sequences, subtraction of sequences aligning to the host organism was performed in two steps. First, paired end reads were aligned using the rapid aligner Spliced Transcripts Alignment to a Reference (STAR) (v2.5.1b) (Dobin et al. 2013) against a custom database containing the whole genome assembly of the elephant shark (*Callorhinchus milii*, RefSeq Assembly Accession: GCF_000165045.1) and the mitochondrial genome of the great white shark (*Carcharodon carcharias*, NCBI Reference Sequence: NC_022415.1); and secondly using the more stringent aligner Bowtie2 (v2.2.4) (Langmead and Salzberg 2012) in "very-sensitive-local" mode to align against a database built from the great white shark heart transcriptome (Sequence Read Archive at NCBI under the study accession number SRP016555 (Richards et al. 2013)), the elephant shark whole genome assembly (as above), and all nucleotide records in NCBI under taxonomy ID 7777 (class Chondrichthyes, aka cartilaginous fishes). Before the Bowtie2 alignment read pairs were quality filtered using PriceSeqFilter (v1.2) (Ruby, Bellare, and DeRisi 2013) with settings "-rqf 85 0.98 -rnf 90", collapsed on a similarity of 95% using cd-hit-dup (v4.6.4) to remove PCR duplicates (Fu et al. 2012), and filtered for reads with <0.45 lzw compression ratio to remove reads with low complexity. After these steps, read pairs in which both mates remained unmapped were then passed on to GSNAPL (v2015-12-31) (Wu and Nacu 2010), which identified reads aligning to the NCBI nucleotide database (downloaded July 2015, indexed with k = 16mers, and preprocessed with RepeatMasker (vOpen-4.0; www.repeatmasker.org) to remove repetitive sequences). Finally, the same reads were aligned to the NCBI protein (non-redundant) database (July 2015) using the RapSearch2 algorithm (Zhao, Tang, and Ye 2012).

The interpretation of alignment results depended on identifying organisms in the samples from the epizootic that were dissimilar from the host, not seen in the water control, and unlikely to be commensal or a contaminant. Credible pathogens were also expected to be absent from samples taken from asymptomatic sharks outside the geographic area of the main leopard shark mortality event in SF Bay, and present in epizootic sharks at a conservative level of abundance (>20 non-redundant, mapped read pairs per million raw read pairs (rM) at the genus level based on nucleotide alignment).

To add confidence in the species determination of a putative eukaryotic pathogen, we assembled genomic regions commonly used in species identification, including the 18S small subunit (SSU) and 28S large subunit (LSU) of the nuclear ribosomal RNA locus (rDNA) as follows: unique, non-host read pairs were pooled from all samples containing reads that aligned to ciliates by the initial pipeline. These reads were then aligned against a custom database of all nucleotide records under taxonomy ID 5878 (phylum Ciliophora) using Bowtie (v2.2.4) (Langmead and Salzberg 2012). The aligning reads were *de novo* assembled using Geneious (Biomatters Ltd, v9.1.8), and contigs that aligned to the ciliate ribosomal DNA (rDNA) locus by nucleotide BLAST (BLASTn) (Altschul et al. 1990) were manually inspected for accuracy and read coverage (>4 unique read pairs coverage, >85% nucleotide identity). Final, high-confidence contigs for the ciliate SSU and LSU sequences were deposited in GenBank (Accession numbers MH062876, MH064355). Species-specific alignment for the SSU and LSU contigs was determined using BLASTn (Altschul et al. 1990).

Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman. 1990. “Basic Local Alignment Search Tool.” *Journal of Molecular Biology* 215 (3): 403–10. doi:10.1016/S0022-2836(05)80360-2.

Dobin, Alexander, Carrie A Davis, Felix Schlesinger, Jorg Drenkow, Chris Zaleski, Sonali Jha, Philippe Batut, Mark Chaisson, and Thomas R Gingeras. 2013. “STAR: Ultrafast Universal RNA-Seq Aligner.” *Bioinformatics (Oxford, England)* 29 (1): 15–21. doi:10.1093/bioinformatics/bts635.

Fu, Limin, Beifang Niu, Zhengwei Zhu, Sitao Wu, and Weizhong Li. 2012. “CD-HIT: Accelerated for Clustering the next-Generation Sequencing Data.” *Bioinformatics (Oxford, England)* 28 (23): 3150–52. doi:10.1093/bioinformatics/bts565.

Langmead, Ben, and Steven L Salzberg. 2012. “Fast Gapped-Read Alignment with Bowtie 2.” *Nature Methods* 9 (4): 357–59. doi:10.1038/nmeth.1923.

Richards, Vincent P, Haruo Suzuki, Michael J Stanhope, and Mahmood S Shivji. 2013. “Characterization of the Heart Transcriptome of the White Shark (Carcharodon Carcharias).” *BMC Genomics* 14 (1): 697. doi:10.1186/1471-2164-14-697.

Ruby, J. Graham, Priya Bellare, and Joseph L. DeRisi. 2013. “PRICE: Software for the Targeted Assembly of Components of (Meta) Genomic Sequence Data.” *G3* 3 (5): 865–80. doi:10.1534/g3.113.005967.

Wu, Thomas D, and Serban Nacu. 2010. “Fast and SNP-Tolerant Detection of Complex Variants and Splicing in Short Reads.” *Bioinformatics (Oxford, England)* 26 (7): 873–81. doi:10.1093/bioinformatics/btq057.

Zhao, Yongan, Haixu Tang, and Yuzhen Ye. 2012. “RAPSearch2: A Fast and Memory-Efficient Protein Similarity Search Tool for next-Generation Sequencing Data.” *Bioinformatics (Oxford, England)* 28 (1): 125–26. doi:10.1093/bioinformatics/btr595.