Science Advances

Supplementary Materials for

Marine heatwaves threaten cryptic coral diversity and erode associations among coevolving partners

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The PDF file includes:

Figs. S1 to S23
Tables S1 to S9
Legends for files S1 and S2

Other Supplementary Materials for this manuscript includes the following:
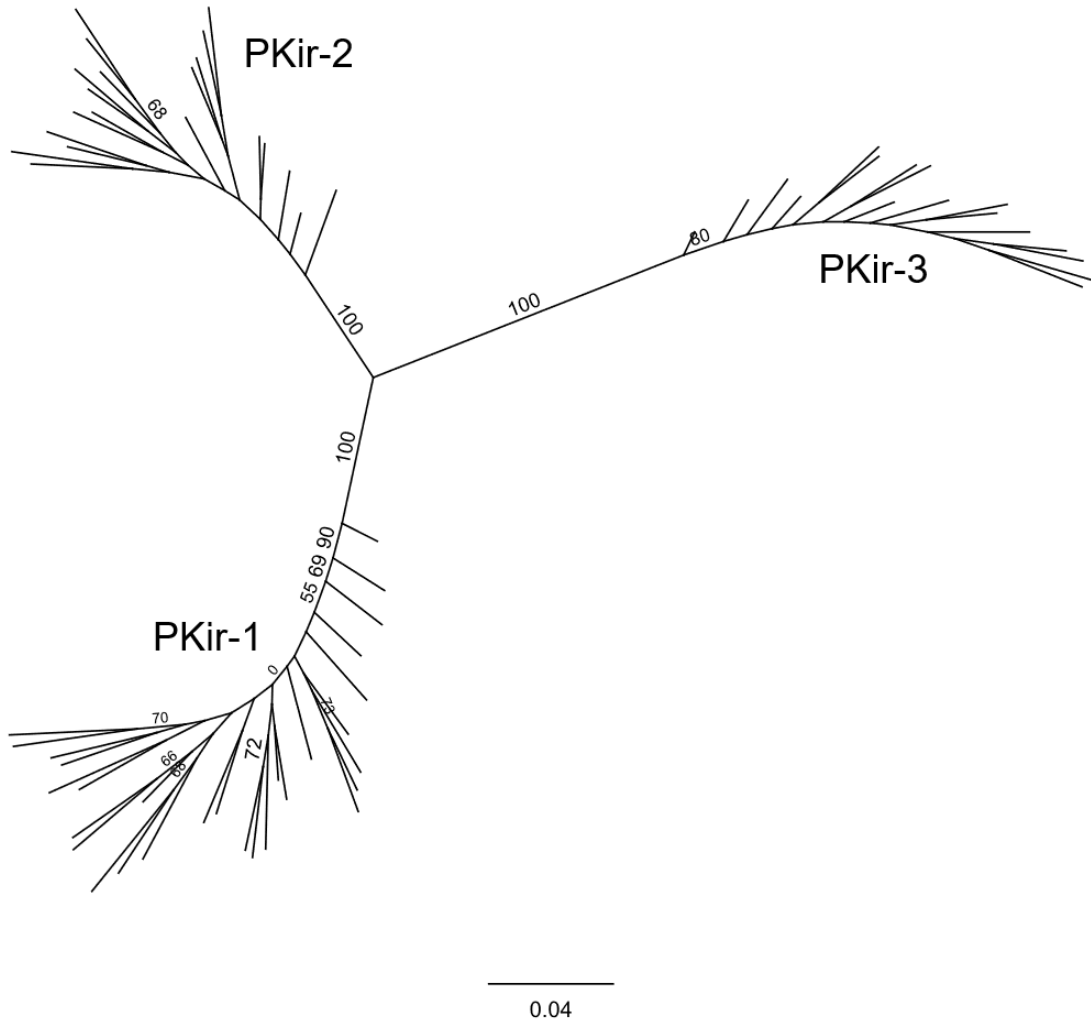
Files S1 and S2

Fig S1. Maximum likelihood phylogenetic tree showing relationships between colonies across and within lineages based on 2bRAD data. Numbers indicate bootstrap support values; only those > 50 are shown. Tip (sample) labels are not shown.
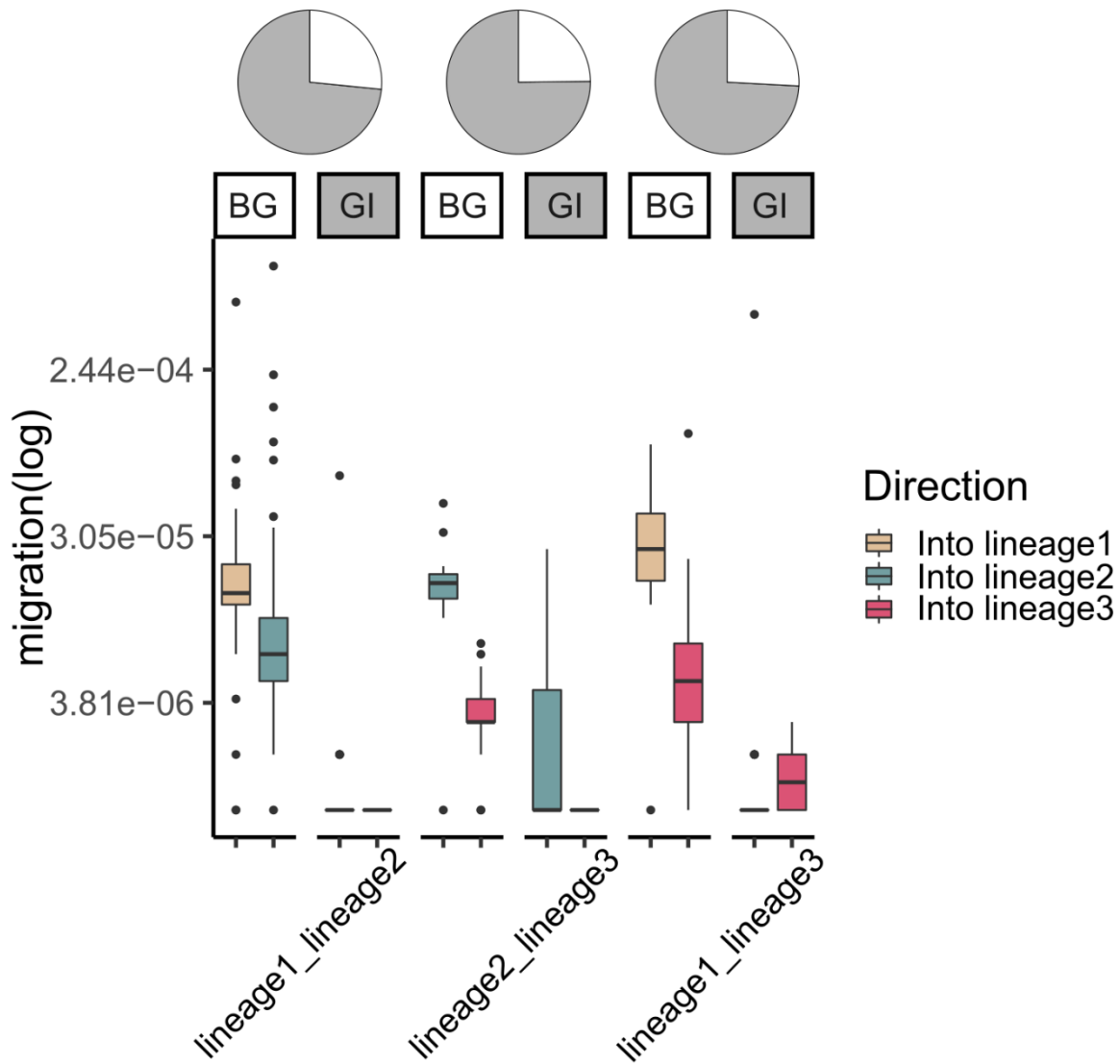
Fig S2. Bootstrapped rates of gene flow between lineage pairs. Values shown are the Moments migration parameter output. GI represents genomic islands of decreased gene flow relative to BG, the remainder of the genome. Note these GIs make up a majority of the genome (percentage shown in pie-chart).
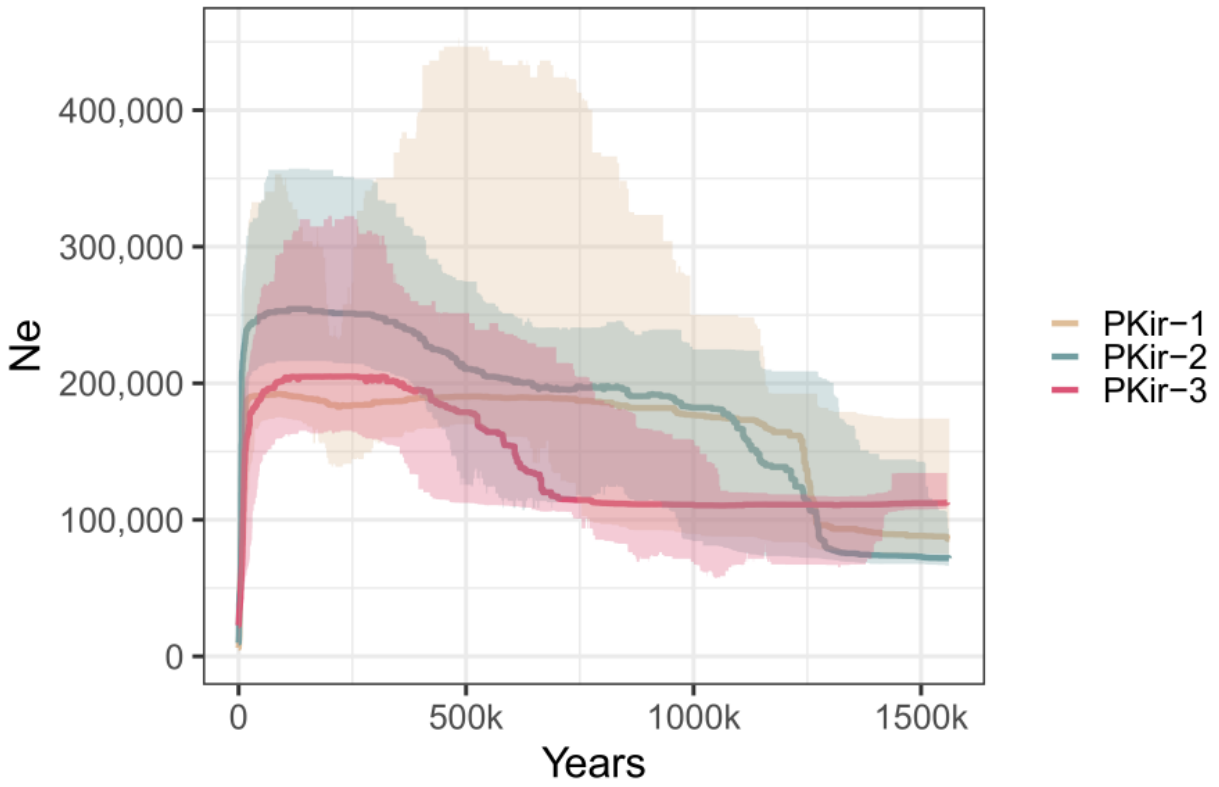
Fig S3. Reconstruction of effective population size (N$_e$) over the past 1.5 million years in all three lineages of *Porites*.
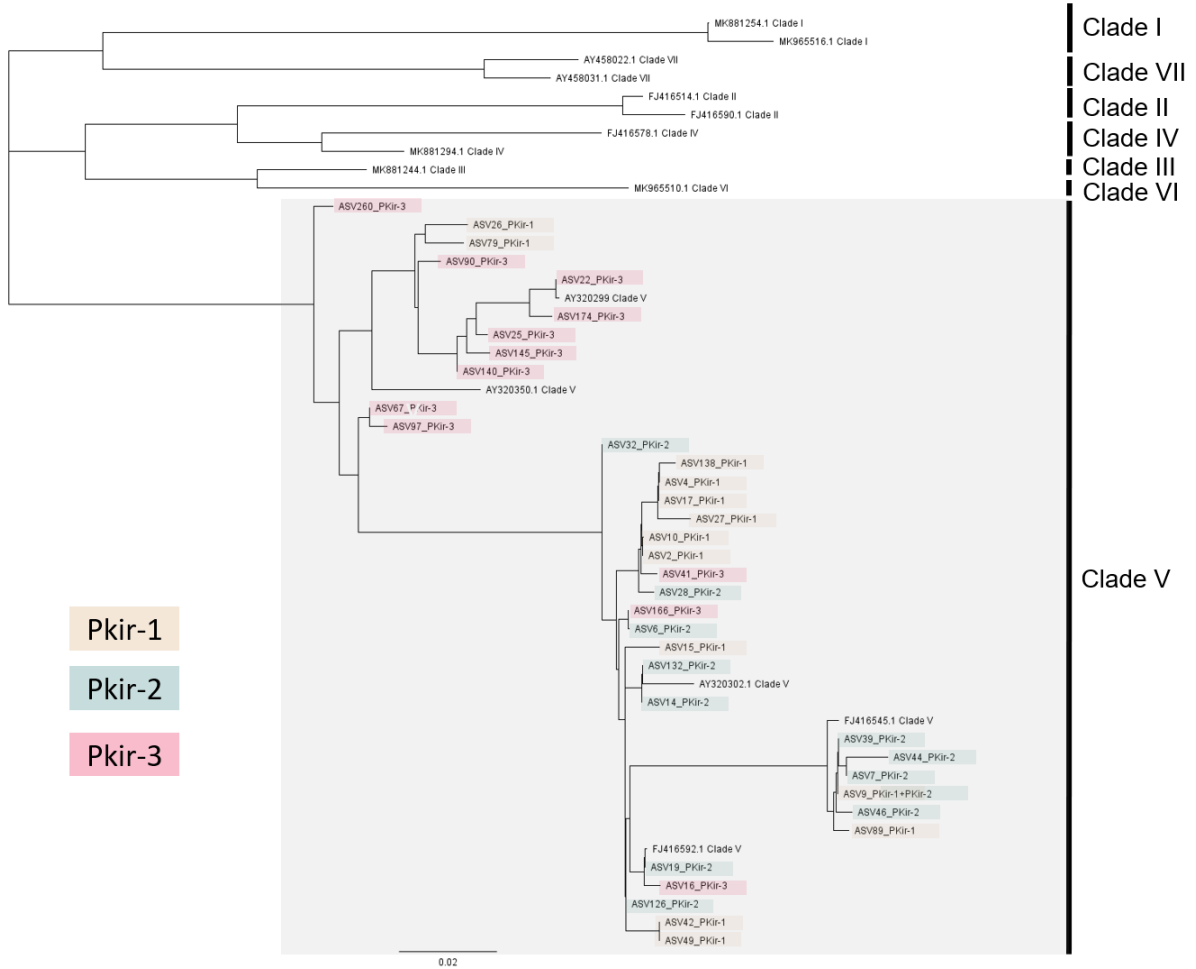
Fig S4. Dominant ITS2 sequence variants from colonies in this study, compared to reference sequences from *Porites* clades I – VII. Sequences from this study are highlighted by the lineage with which they are associated. Clade V which includes all dominant ITS2 variants from this study is highlighted in grey. The phylogeny is a Neighbor-joining tree.
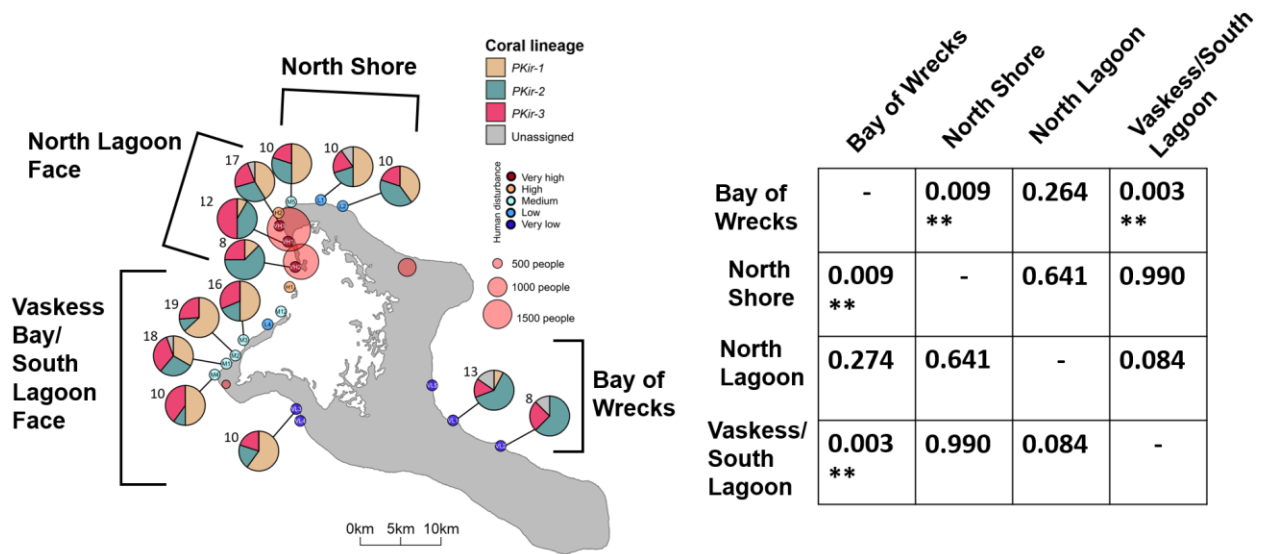
	Bay of Wrecks	North Shore	North Lagoon	Vaskess/South Lagoon
Bay of Wrecks	-	0.009 **	0.264	0.003 **
North Shore	0.009 **	-	0.641	0.990
North Lagoon	0.274	0.641	-	0.084
Vaskess/ South Lagoon	0.003 **	0.990	0.084	-

Fig S5. Results of fisher's exact test comparing the distribution of lineages before the heatwave across regions. There was an overall significant effect of region on lineage (P < 0.001). Shown is the scheme used to group sites into regions (left) and the results of pairwise fisher's exact tests among regions (right). Numbers in each cell indicate P-values and asterisks indicate significance (* = P < 0.05, ** = P < 0.01, *** = P < 0.001).
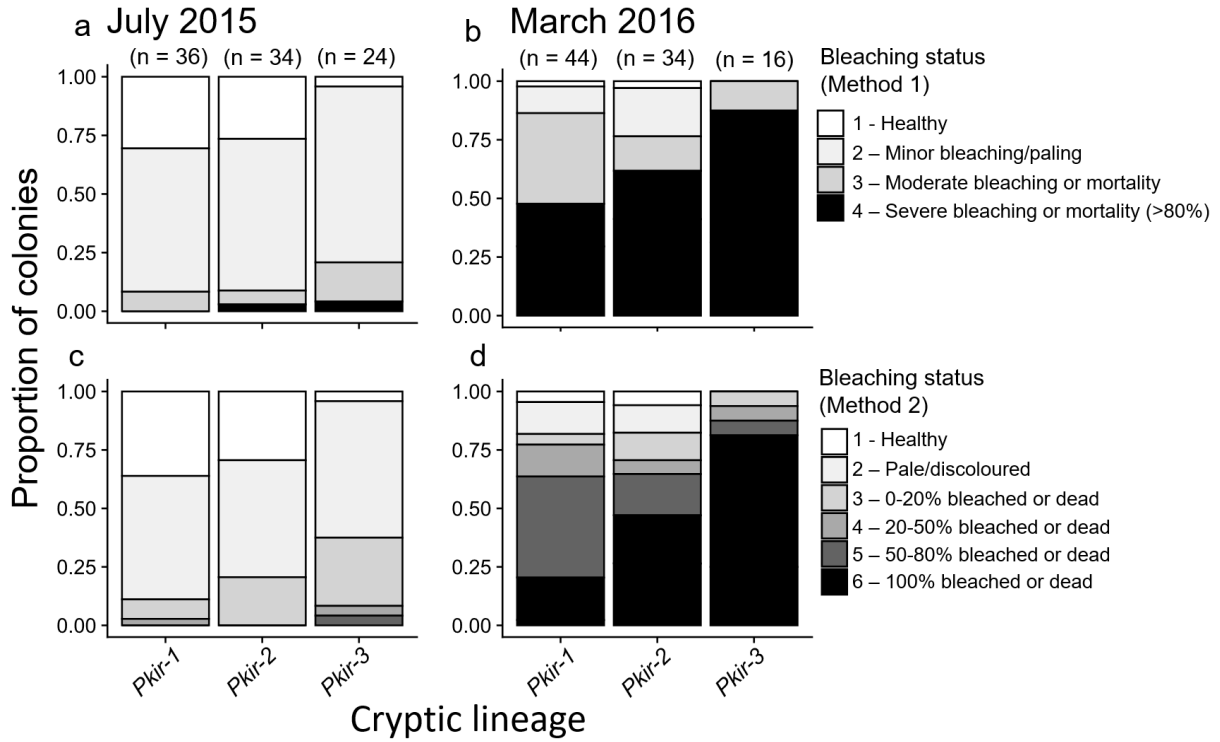
Fig S6. Bleaching status of colonies early (July 2015) and late (March 2016) in the heatwave. Shading indicates the degree of bleaching experienced by a particular colony at each timepoint based on two different methods of discretely categorizing bleaching (see Methods).
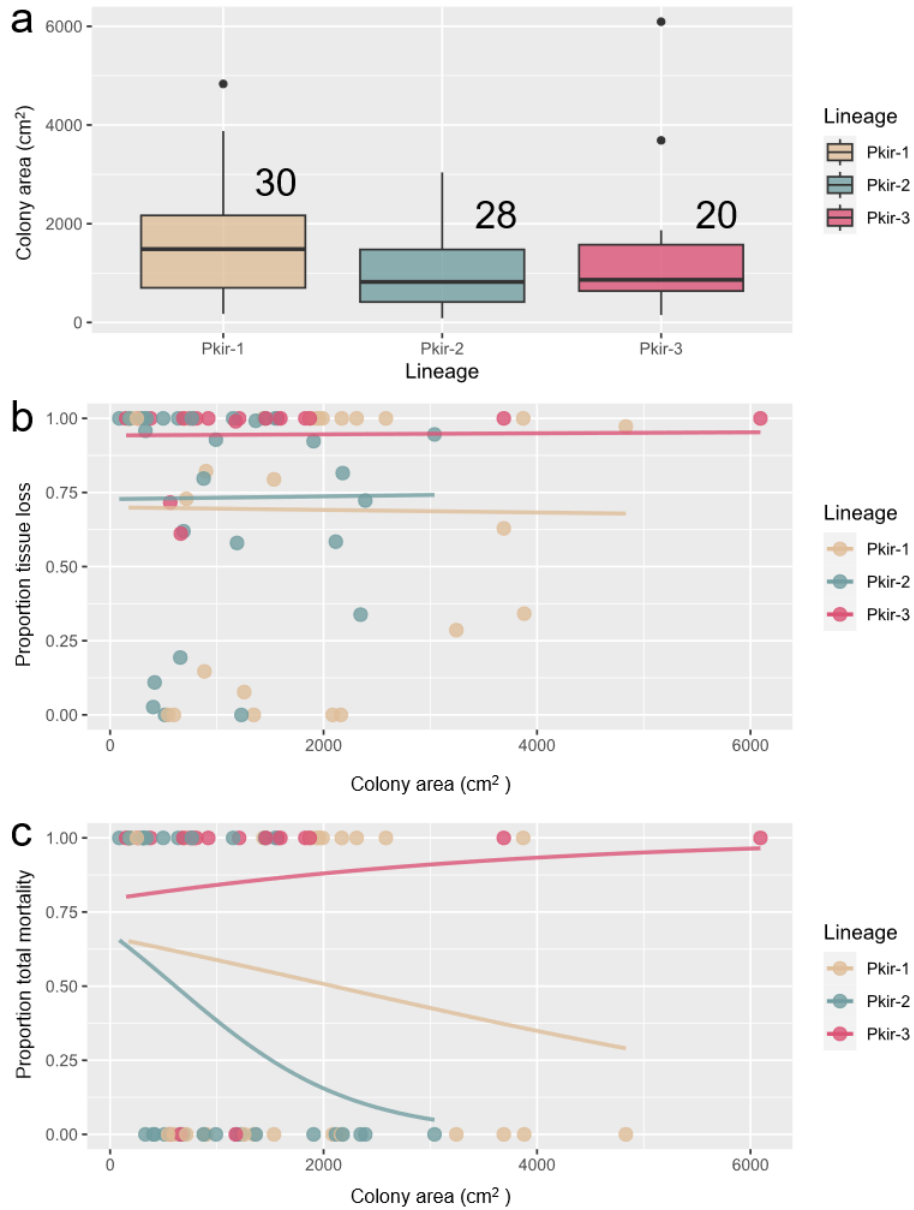
Fig S7. Comparison of colony size (i.e., surface area) between the three *Porites* lineages and testing for effects of size on survival. Only samples tracked through the heatwave and assigned to a lineage are shown here (n = 78). Shown in (a) is a comparison of colony surface area across the three lineages. There is no significant relationship between coral lineage and colony size before the heatwave (ANOVA: F = 2.0652, P = 0.1339). Shown also are tissue death (b) and total mortality (c) for each lineage. Regression lines are those best fit with GLM models (b: quasibinomial; c: binomial). However, neither relationship is significant (tissue loss – GLM – size: X^2 = 0.0127, P = 0.9103; lineage: X^2 = 11.3919, P = 0.0034; size*lineage: X^2 = 0.6204, P = 0.7333; total mortality – GLM: size: X^2 = 2.3777, P = 0.1231; lineage: X^2 = 11.8851, P = 0.0026; size*lineage: X^2 = 5.8273, P = 0.0543).
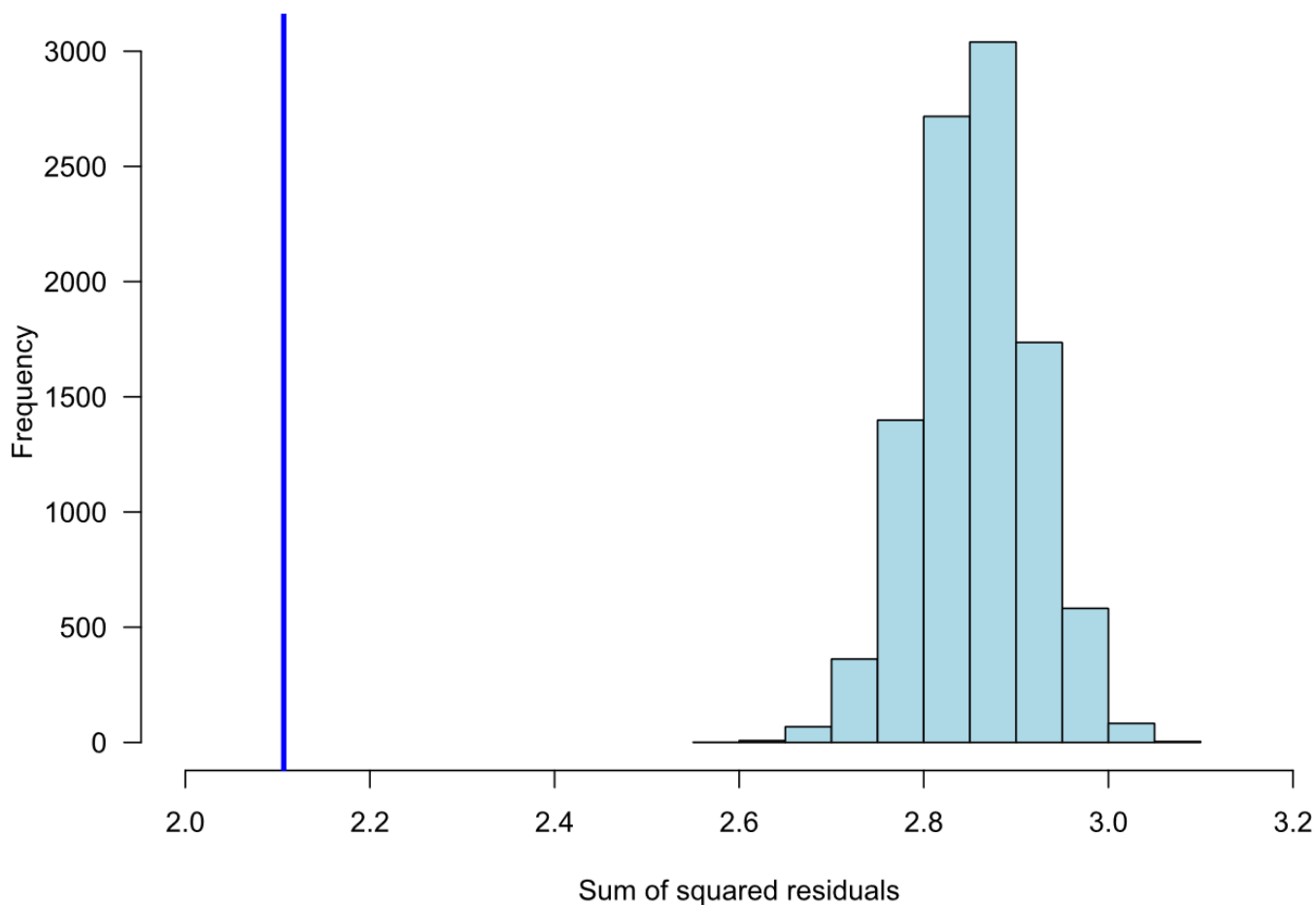
Fig S8. Comparison of sum of squared residuals (m^2_{XY}) from observed data and null model simulations for PACo analysis. Shown is a histogram of sum of squared residuals generated from running 10,000 simulations of the null model (r0). The observed value is shown as a blue line.
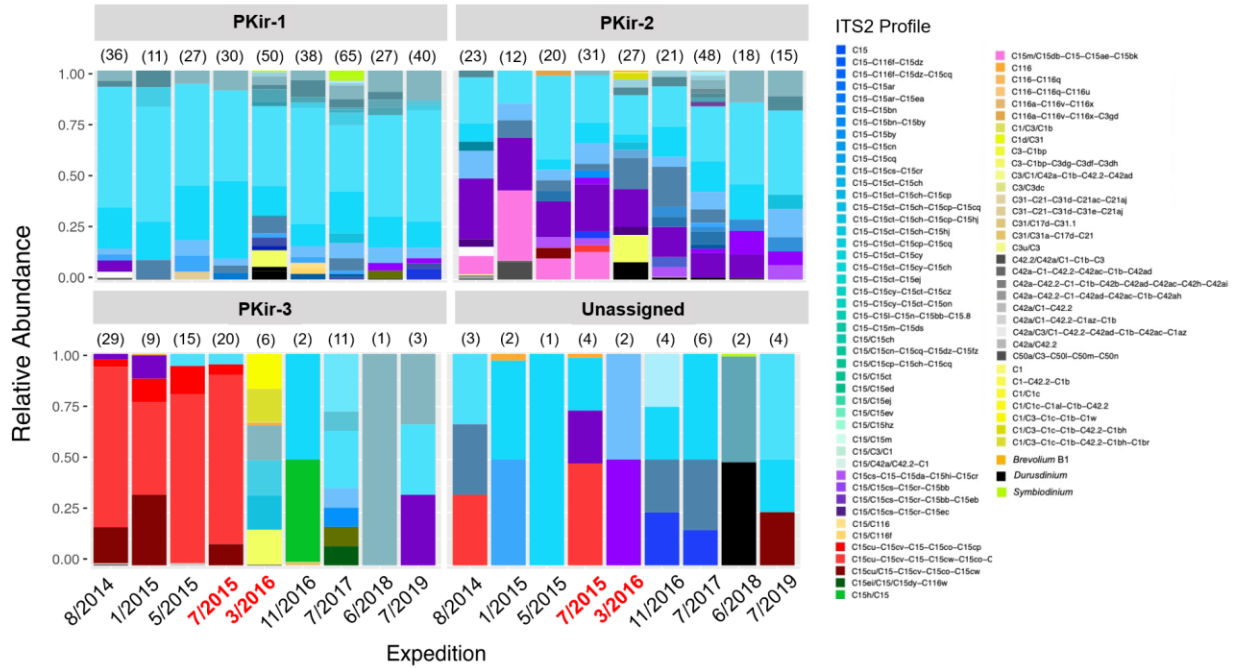
Fig S9. Symbiodiniaceae assemblages associated with each *Porites* lineage during each expedition. Numbers above each bar indicate sample size. 110 profiles were found across all samples but only *Cladocopium* profiles are shown below the level of genus. See Table S9 for full list of ITS2 profiles.

Fig S10. Barplot showing the relative abundance of Symbiodiniaceae ITS2 profiles associated with PKir -1 colonies sampled before and after the heatwave at each site. Values above each bar indicate the number of colonies sampled at each timepoint.

Fig S11. Barplot showing the relative abundance of Symbiodiniaceae ITS2 profiles associated with PKir -2 colonies sampled before and after the heatwave at each site. Values above each bar indicate the number of colonies sampled at each timepoint.

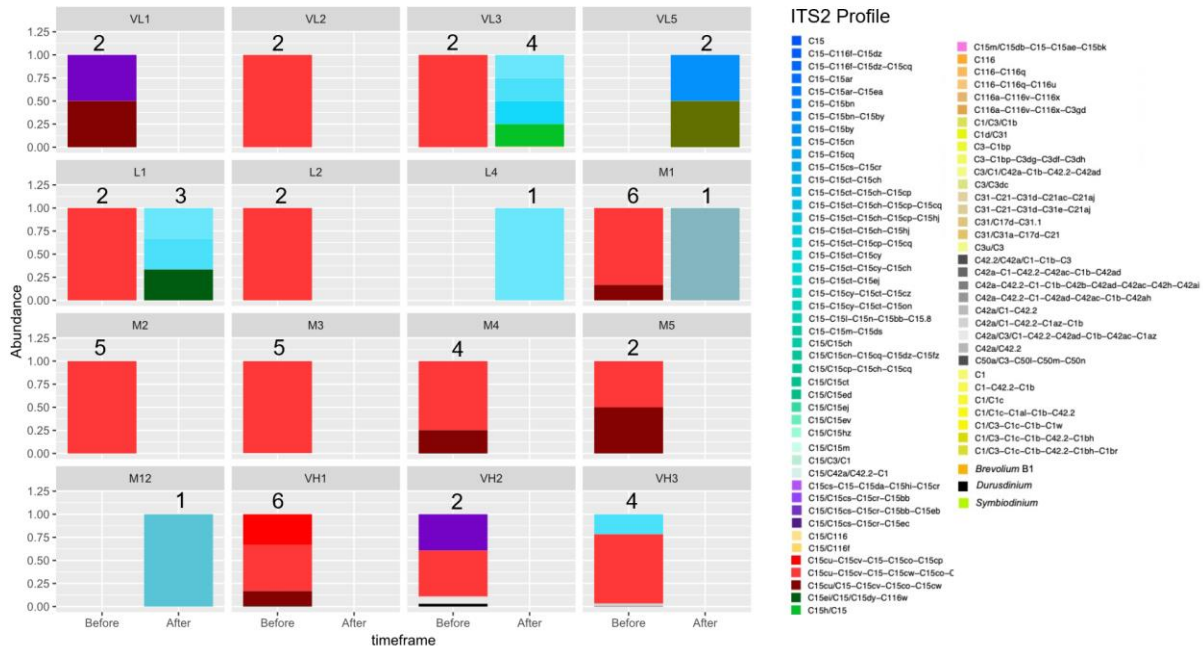**Fig S12. Barplot showing the relative abundance of Symbiodiniaceae ITS2 profiles associated with PKir -3 colonies sampled before and after the heatwave at each site.** Values above each bar indicate sample the number of colonies sampled at each timepoint.
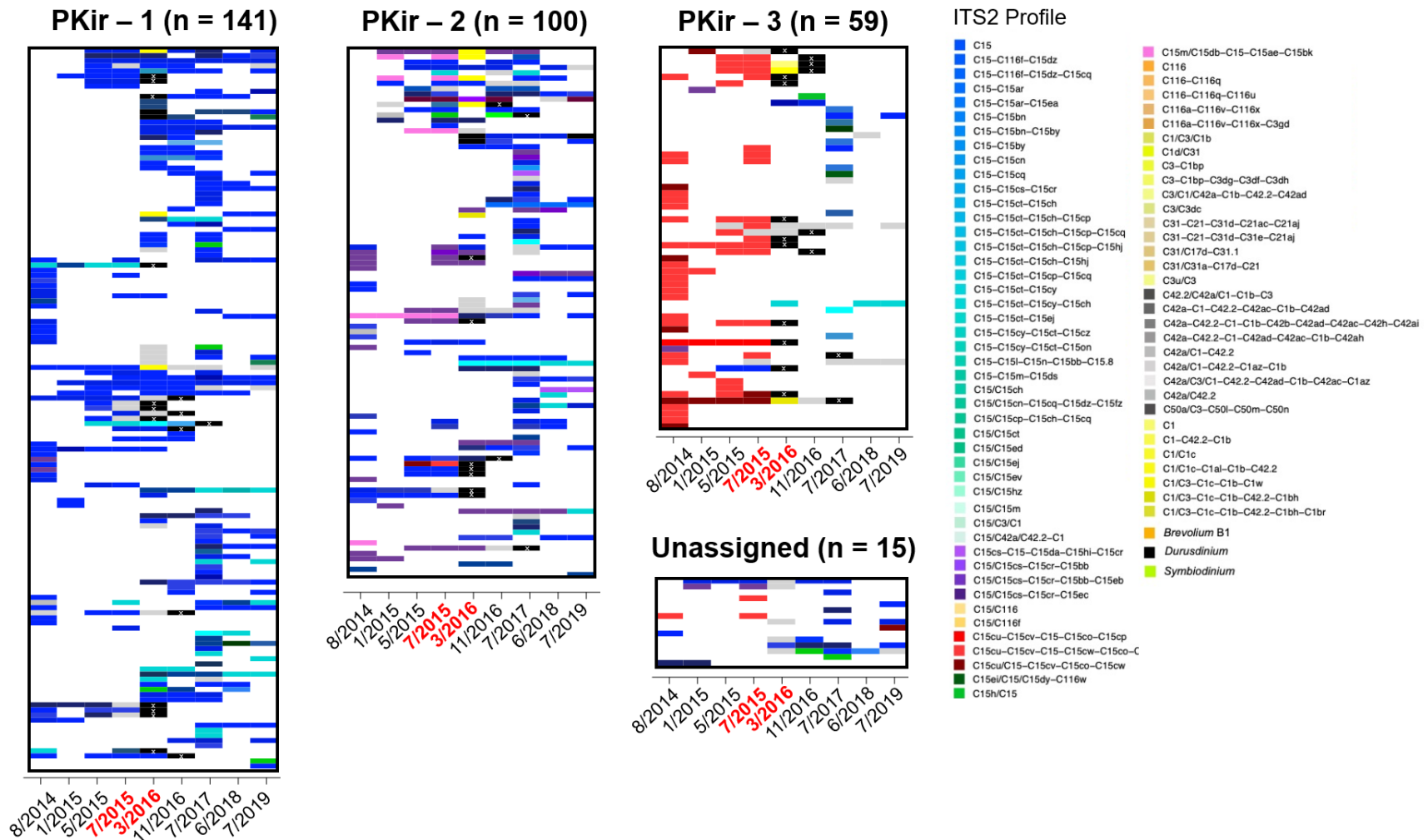
Fig S13. Time series of Symbiodiniaceae associated with tracked colonies of each cryptic *Porites* lineage. Each row represents an individual colony, with colour at each time point indicating the dominant symbiont profile. Colonies within each lineage are arranged in order of human disturbance (highest on top, lowest on bottom). Note that colonies that survived to 2017 were considered alive for survivorship analyses. All samples shown with >200 sequence reads (despite cut-off of >500 in main text analyses). Expeditions during the heatwave are shown in red text.
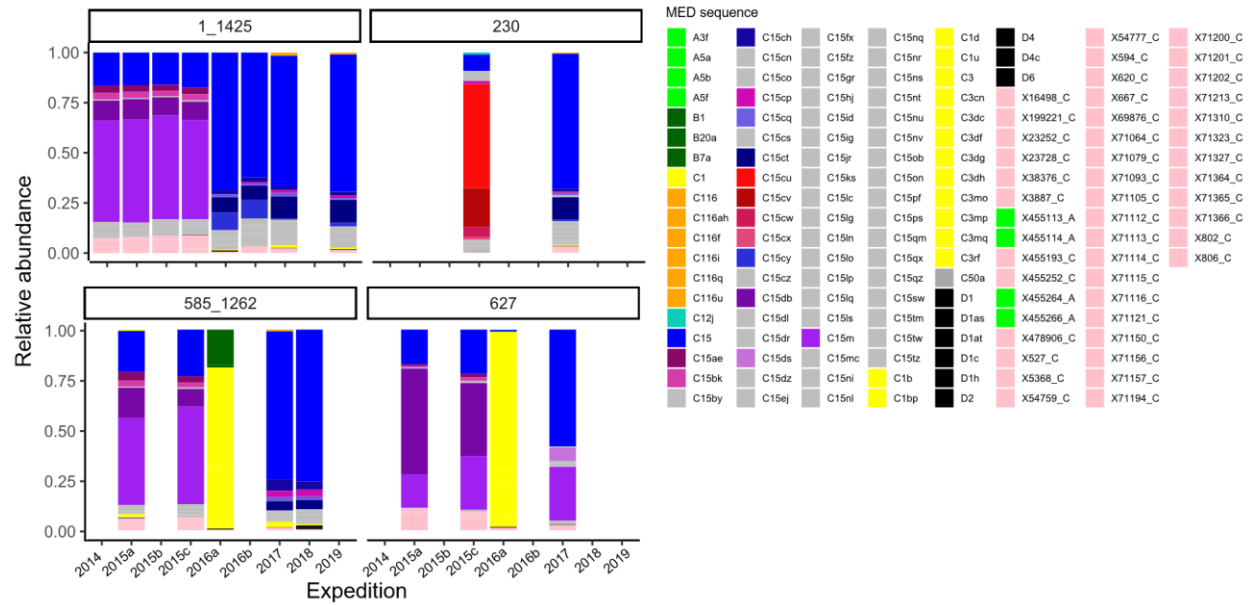
Fig S14. Colonies considered to have shifted (switched or shuffled) symbionts during or after the 2015-2016 marine heatwave. Only the most common DIVs are coloured to draw attention to the loss, acquisition or change in relative abundance of common DIVs.
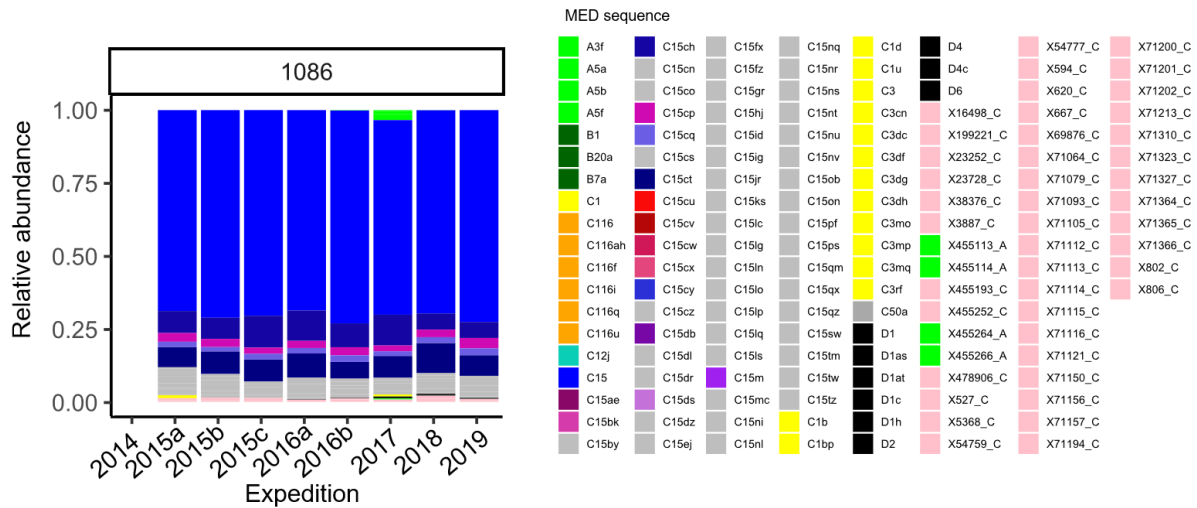
Fig S15. Example of colonies that had different ITS2 profiles through time but were not considered to have shifted symbionts due to high similarities in the abundances of DIVs. Only the most common DIVs are coloured to draw attention to the stability of common DIVs. Dominant ITS2 profile in 2015a, 2015b, 2015c, 2017, 2018 was C15-C15ct-C15ch-C15cp-C15hj; in 2016a, 2016b and 2019 the ITS2 profile was C15-C15ct-C15ch-C15cp-C15cq.
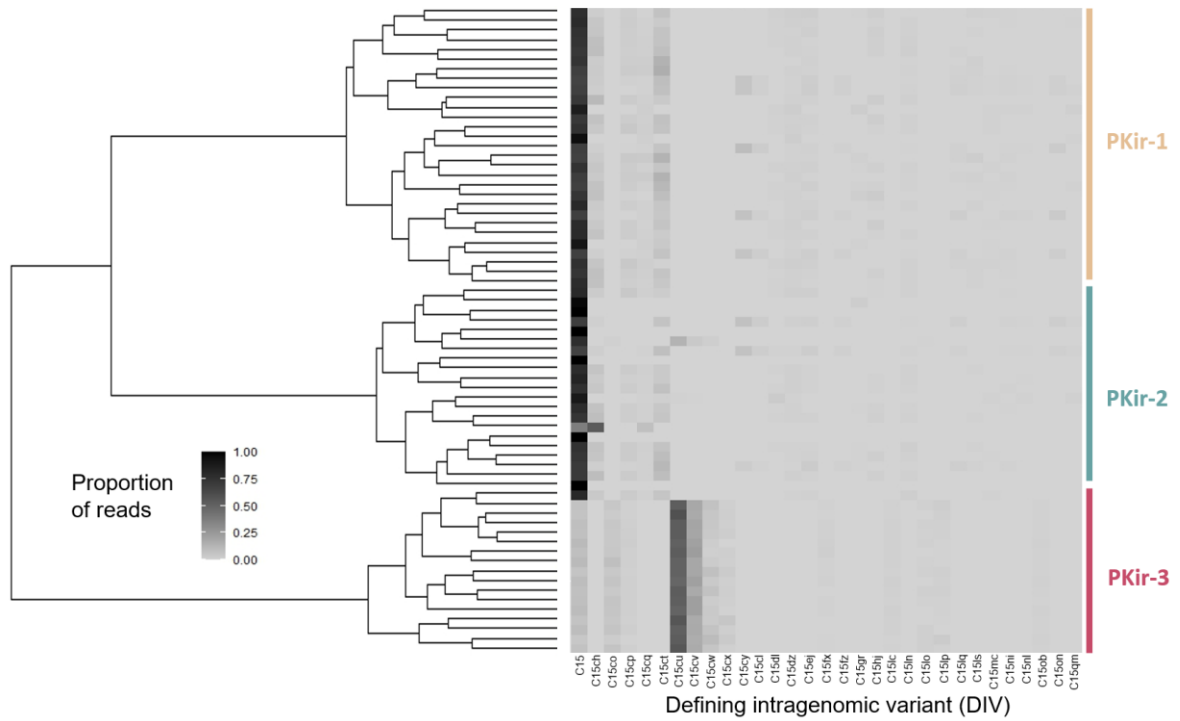
Fig S16. Heatmap showing the relative abundance of common symbiont DIVs associated with the 67 colonies also sequenced with 2bRAD. Phylogenetic tree is based on maximum likelihood of 2bRAD SNP dataset.
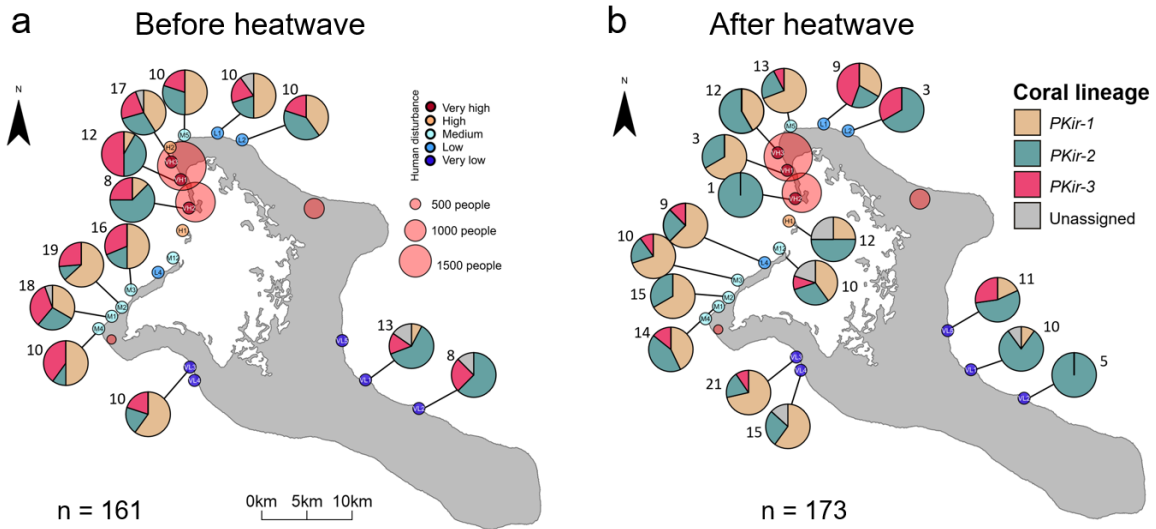
Fig S17. Maps of Kiritimati with pie charts showing the relative abundance of each lineage at each site before and after the heatwave. Numbers indicate the number of colonies sampled and sequenced with either 2b-RAD or ITS2 metabarcoding. Circles indicate sites, colored by level of human disturbance and scaled by human population size. Note that panel b includes colonies that were tagged and tracked, known to survive but never successfully sampled and/or sequenced for ITS2 after the heatwave.
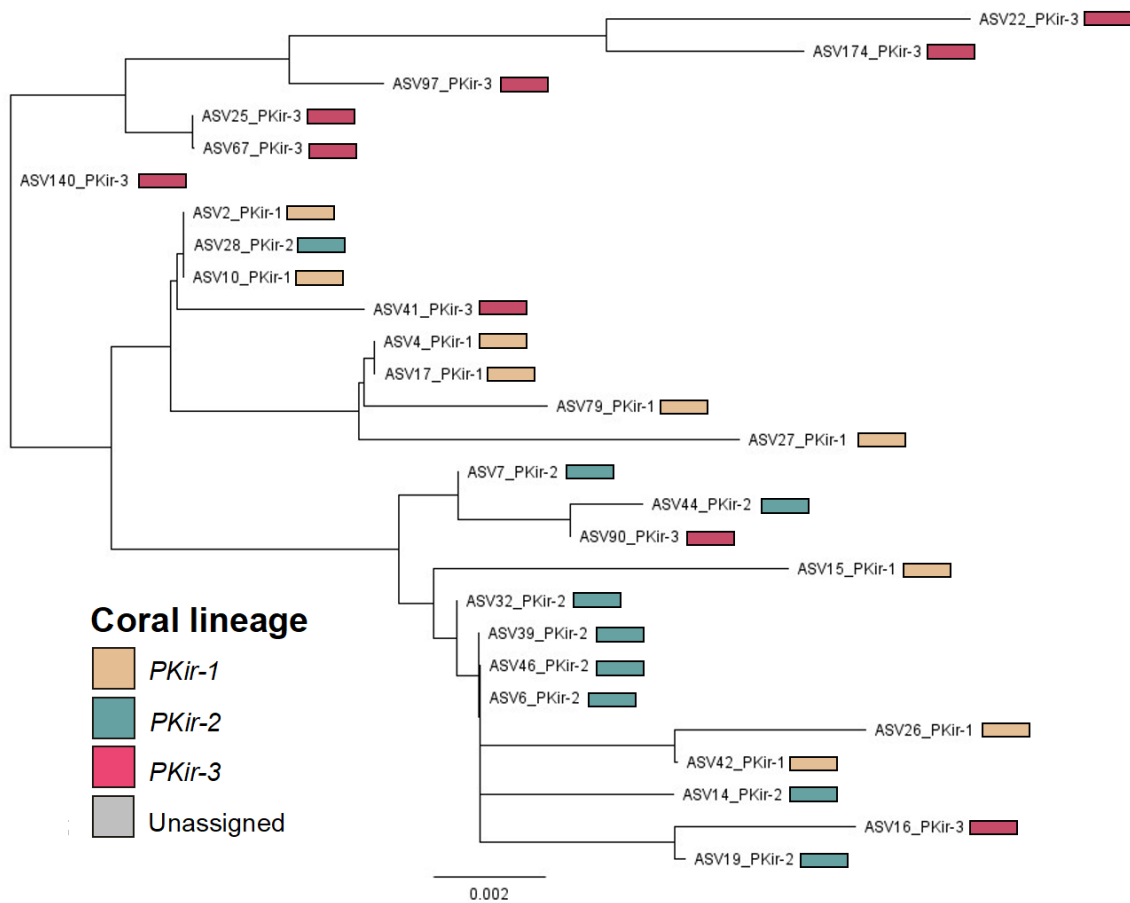
Fig S18. Neighbour-joining tree of the dominant ITS2 sequence variants used to assign colonies to lineage (n = 28). Each tip represents a sequence variant and the colour indicates the lineage(s) found to be associated with that particular variant. The tree was produced in Geneious Prime using a Tamura-Nei distance model. Sequence variants are from all colonies that were sequenced with both RADSeq and ITS2 barcoding (n = 67 colonies).

Fig S19. Neighbour-joining tree of the dominant ITS2 sequence variants (n = 36) in each colony sequenced with both 2bRAD and ITS2 barcoding. Each tip represents a sequence variant and the colour indicates the lineage(s) found to be associated with that particular variant. The tree was produced in Geneious Prime using a Tamura-Nei distance model.
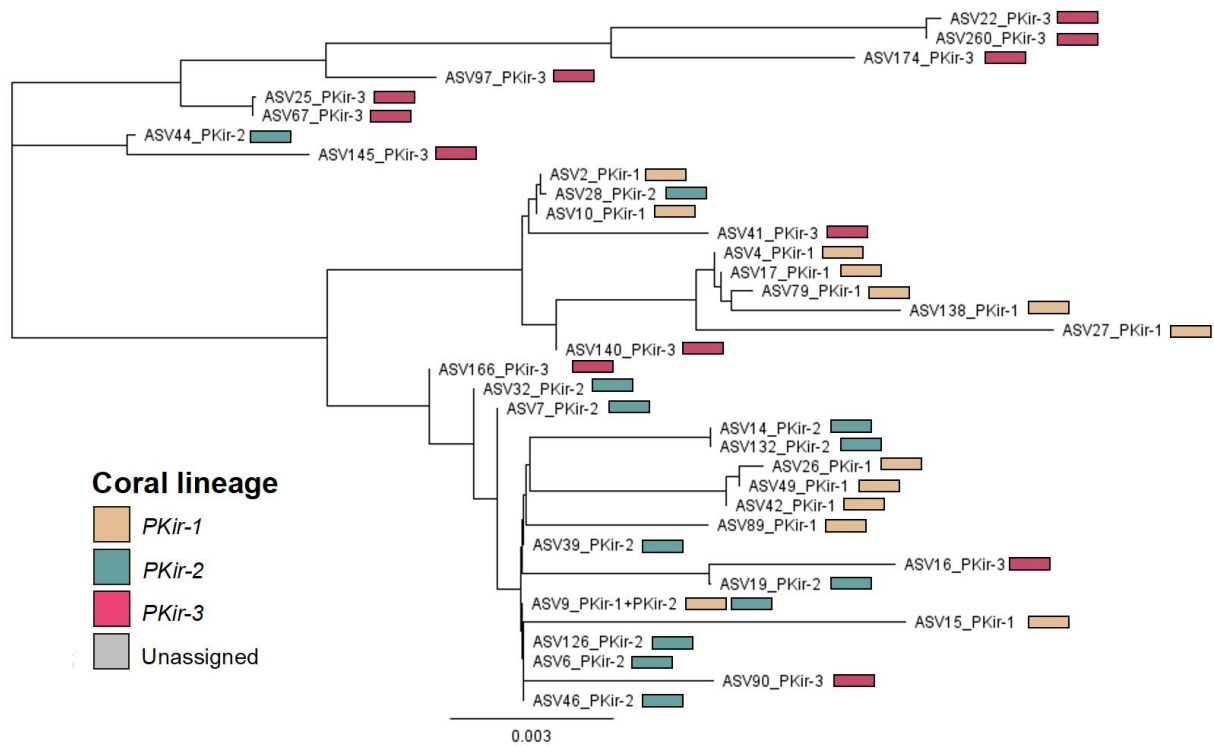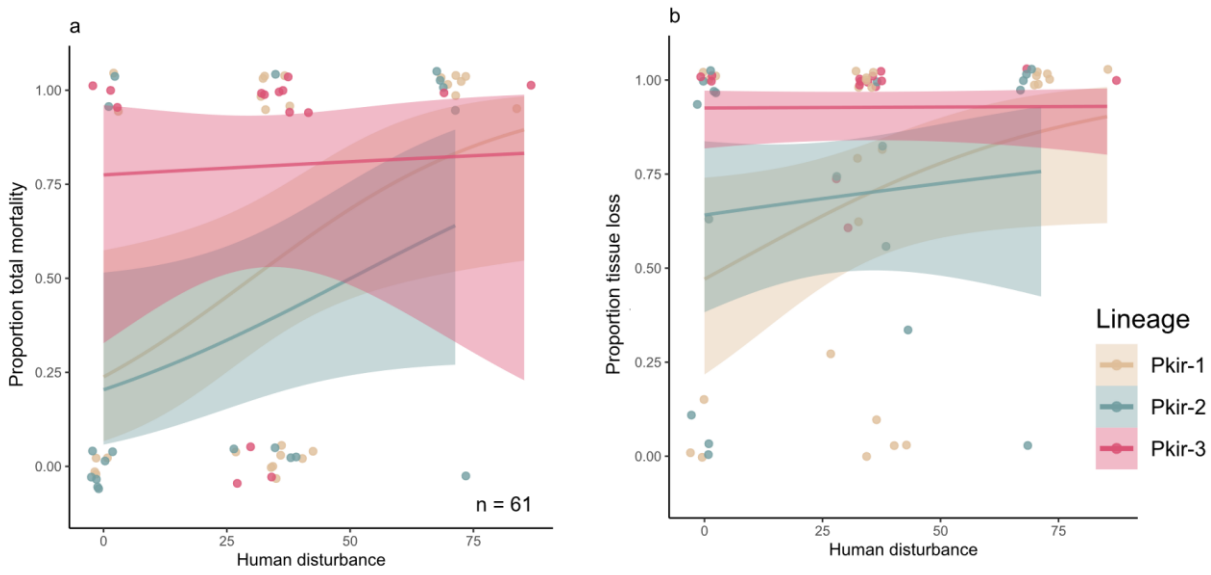
Fig S20. Survivorship by coral cryptic lineage and chronic human disturbance for samples sequenced with 2bRAD (n = 61). Shown are both the probability that coral colonies experienced complete mortality between 2015 and 2017 (a) and the average percentage of partial or total mortality that each colony experienced (b) both in relation to human disturbance and *Porites* lineage. In (a), each point represents a colony that either survived (0) or died (1). The proportion of colonies that died at each value is estimated by the logistic regression line. In (b), proportion mortality indicates the amount of colony death that was experienced while each line shows the model fit with human disturbance for each lineage. Note that human disturbance is a relative metric based on fishing pressure and distance to Kiritimati's villages (see ref. , *84*). Note that data points are jittered for visualization. For both mortality metrics, there was significant effect of lineage and disturbance on mortality but no interaction (Total mortality - binomial GLM: Disturbance: $X^2 = 9.4720$, P = 0.00209, Lineage: $X^2 = 6.4462$, P = 0.03983, Disturbance*Lineage: $X^2 = 1.1549$, P = 0.56078; Proportion tissue death - quasibinomial GLM: Disturbance: $X^2 = 4.7173$, P = 0.02986, Lineage: $X^2 = 8.0775$, P = 0.01762, Disturbance*Lineage: $X^2 = 1.2764$, P = 0.52823).
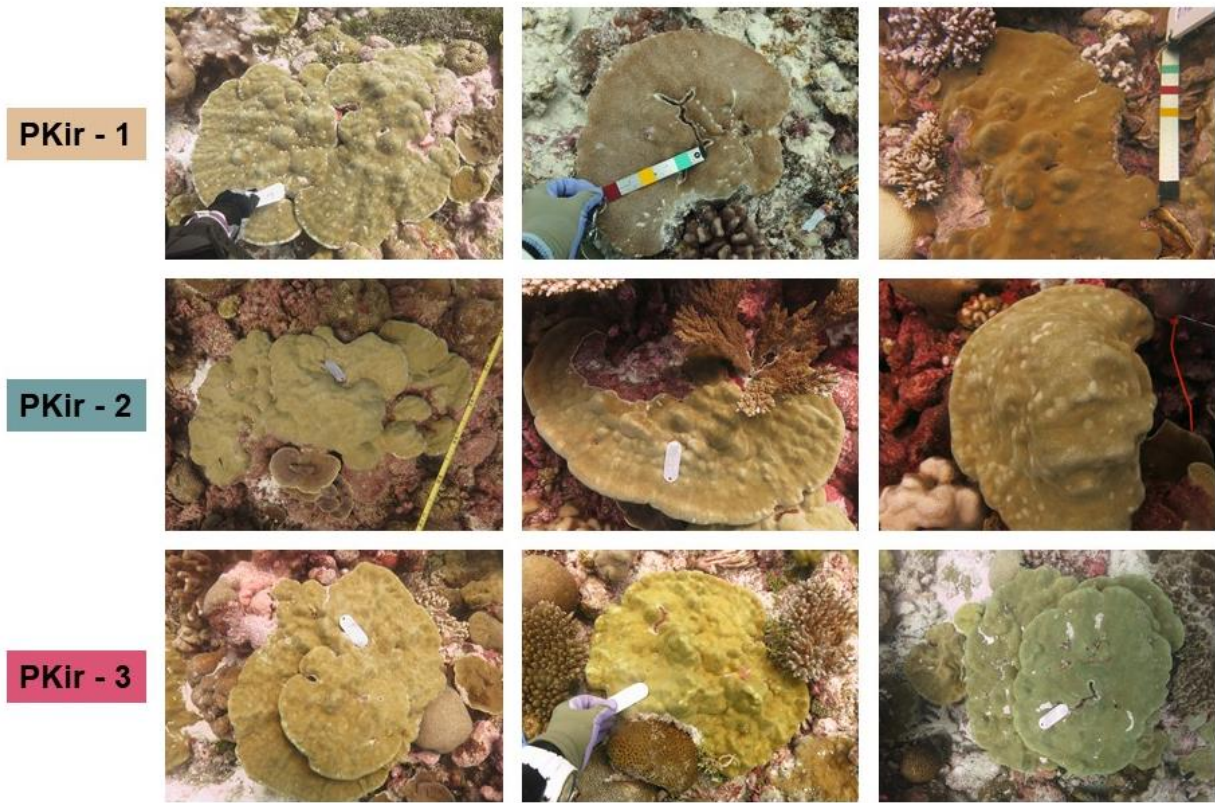
Fig S21. Examples of colonies from each cryptic lineage of Porites. Shown are three example images of each cryptic lineage (arranged in rows) taken before the heatwave. Photos were taken by the Baum lab coral field team (Julia K. Baum, Danielle C. Claar, Kristina Tietjen).
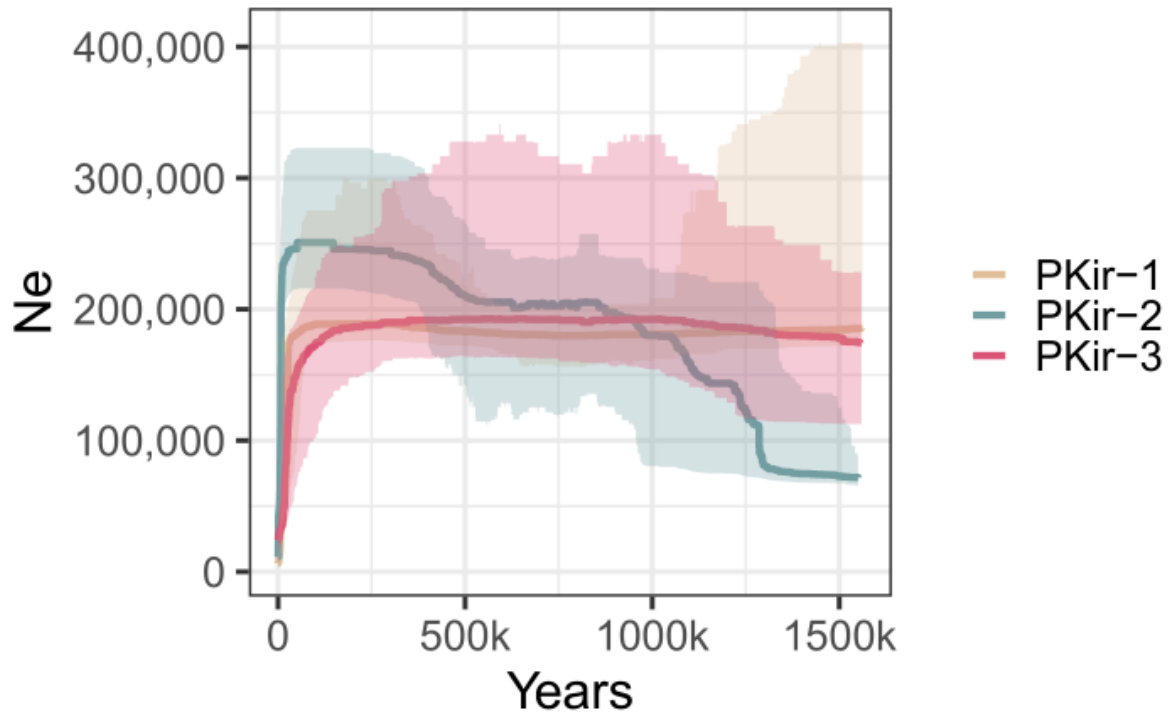
Fig S22. Reconstruction of effective population size (N$_e$) over the past 1.5 million years in all three lineages of *Porites* using a generation time of 18 years.
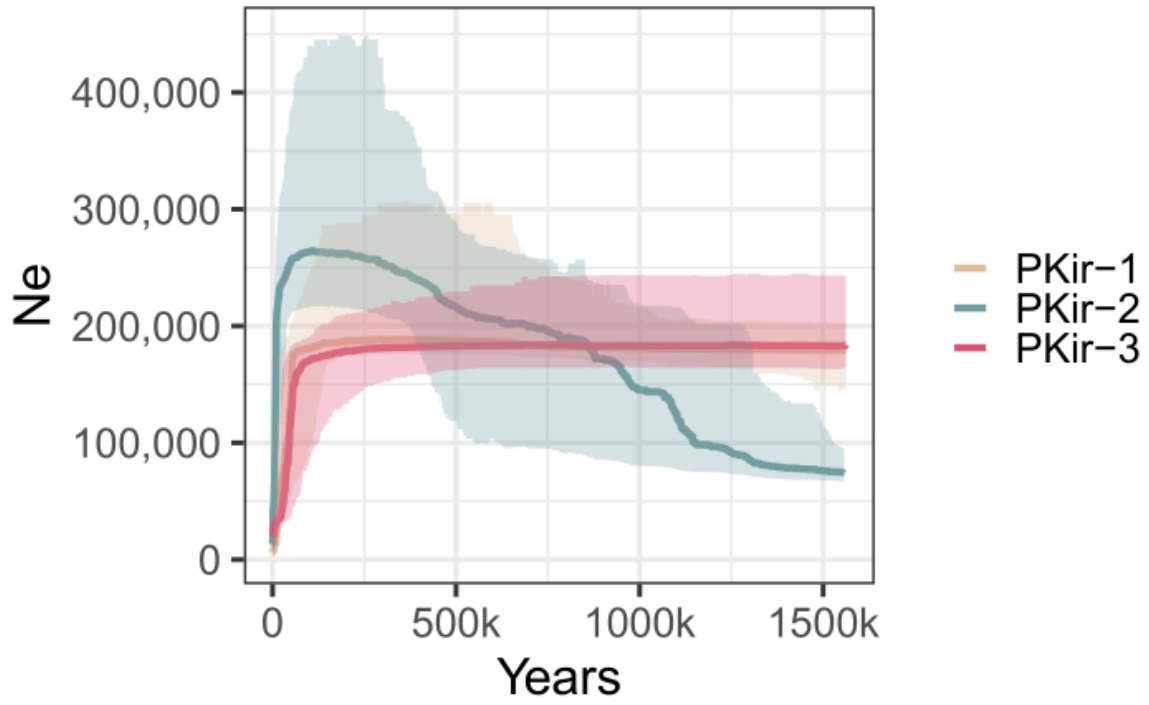
Fig S23. Reconstruction of effective population size (N$_e$) over the past 1.5 million years in all three lineages of *Porites* using a generation time of 35 years.

Supp. File 1. Results of outlier analysis using colonies samples with 2bRAD. See README sheet for more information. [Excel spreadsheet]

Supp File 2. Additional information on genomic analyses. See README sheet for more information [Excel spreadsheet]

Table S1. Global F_{st} between lineage pairs.

	PKir - 1	PKir - 2	PKir - 3
PKir - 1	-	0.263	0.361
PKir - 2	0.263	-	0.236
PKir - 3	0.361	0.326	-

Table S2. Summary of sample sizes by technique used to characterize host or symbiont genotypes. Symbionts only considered sequenced if >500 reads. All colonies sequenced with 2bRAD also had host ITS2 sequences. Note that colonies with "no host data" were included alongside those with ambiguous ITS2 sequences (n = 11) to form the "unassigned" (to lineage) group (n = 15 colonies).

Symbiont	Host			Total
	2bRAD + ITS2	ITS2 only	No host data	
ITS2 before heatwave only	44	86	2	132
ITS2 after heatwave only	0	132	2	134
ITS2 both before and after heatwave	23	6	0	29
ITS2 during heatwave only	0	10	0	10
No symbiont data	0	10	0	10
Total	67	244	4	315

Table S3. Results of phylogenetic signal testing on DIVs (found in > 10% of samples) using a phylogeny inferred from 2bRAD data and symbiont DIVs based on ITS2 metabarcoding. Shown are the metrics for each test and their associated P-values. Note that DIVs are MED-sequences identified by SymPortal as defining of a particular symbiont profile (see Materials & Methods)

DIV name	Moran's I	P-value	Blomberg's K	P-value
C15hj	0.06	< 0.001*	0.25	0.281
C15ct	0.15	< 0.001*	0.35	0.050*
C15ni	0.04	< 0.001*	0.22	0.389
C15dl	0.03	< 0.001*	0.20	0.389
C15ln	0.05	< 0.001*	0.21	0.483
C15ch	0.02	<0 .001*	0.39	0.389
C15lp	0.22	< 0.001*	0.55	0.122
C15cw	0.30	< 0.001*	0.75	0.009*
C15lo	0.20	< 0.001*	0.36	0.062
C15cx	0.25	< 0.001*	0.56	0.031
C15cu	0.37	< 0.001*	2.13	0.005*
C15cv	0.37	< 0.001*	1.95	0.005*
C15co	0.34	< 0.001*	1.39	0.005*
C15cq	0.08	< 0.001*	0.25	0.281
C15cy	-0.01	0.533	0.22	0.389
C15cz	-0.01	0.550	0.21	0.389
C15nl	-0.01	0.546	0.20	0.459
C15cp	0	0.294	0.19	0.624
C15qm	-0.01	0.547	0.22	0.389
C15lc	0.34	< 0.001*	1.31	0.005*
C15dz	0.18	< 0.001*	0.34	0.062
C15gr	0	0.382	0.22	0.389
C15fx	0.23	< 0.001*	0.50	0.050*
C15lq	0.01	0.062	0.21	0.389
C15on	0	0.383	0.22	0.389
C15fz	-0.01	0.546	0.21	0.434
C15ej	0.09	< 0.001*	0.23	0.389
C15	0.33	< 0.001*	1.48	0.005*
C15mc	0.04	< 0.001*	0.24	0.343
C15ls	0.04	< 0.001*	0.20	0.480
C15ob	0.34	< 0.001*	1.12	0.005*

Table S4. Results of phylogenetic signal testing on ITS2 profiles (found in > 10% of samples) using a phylogeny inferred from 2bRAD data and symbiont profiles (output from SymPortal) based on ITS2 metabarcoding. Shown are the metrics for each test and their associated P-values.

ITS2 Profile name	Moran's I	P-value	Blomberg's K	P-value
C15-C15ct-C15ch-C15cp-C15cq	0.02	0.0190	0.25	0.1512
C15-C15ct-C15ch-C15cp-C15hj	0.03	<0.001	0.24	0.1511
C15cu-C15cv-C15-C15cw-C15o-C15cx	0.34	<0.001	1.05	0.003*

Table S5. Summary of sample sizes for all colonies that were sampled before the heatwave, highlighting the number of colonies that were tracked versus the total number of colonies sampled during broader population-level sampling

Lineage	All samples	Tracked colonies only
PKir-1	60	30
PKir-2	50	28
PKir-3	44	20
Unassigned	7	1
Total	161	79

Table S6. Summary of sample sizes for tracked colonies of known survival status (as of 2017).

Site	PKir-1 Survived	Died	PKir-2 Survived	Died	PKir-3 Survived	Died	Unassigned Survived	Died	Total
VH1	0	1	0	2	0	2	0	0	5
VH2	0	1	0	4	0	1	0	0	6
VH3	0	6	1	1	0	1	0	0	9
M1	2	1	1	0	0	3	0	0	7
M2	2	4	1	0	0	2	0	0	9
M3	3	1	0	1	1	2	0	0	8
M5	2	0	2	0	0	1	0	0	5
L1	1	0	0	0	2	0	0	0	3
L2	0	0	2	1	0	0	0	0	3
VL1	0	1	4	2	0	1	1	0	9
VL2	0	0	4	0	0	2	0	0	6
VL3	4	1	2	0	0	2	0	0	9
Total	14	16	17	11	3	17	1	0	79

Table S7. Summary of sample sizes by site for samples taken late during in the heatwave (2016 timepoint). Note that these include colonies for which either symbiont or host sequences were recovered (not necessarily both).

Site	PKir-1	PKir-2	PKir-3	Unassigned
VH1	1	3	2	0
VH2	1	4	1	0
VH3	3	2	1	0
M1	5	2	4	0
M2	10	1	1	0
M3	5	3	3	0
M12	4	3	0	1
L2	1	4	0	0
L3	9	3	3	0
L4	4	1	0	0
VL1	1	8	1	2

Table S8. NCBI accession numbers for *Porites* sequences used to assign lineage.

ID IN THIS STUDY	SEQUENCE ID	GENBANK ACCESSION	LINEAGE
ASV2	PKir1-1	OQ825068	PKir-1
ASV4	PKir1-2	OQ825069	PKir-1
ASV10	PKir1-3	OQ825073	PKir-1
ASV15	PKir1-4	OQ825075	PKir-1
ASV17	PKir1-5	OQ825077	PKir-1
ASV26	PKir1-6	OQ825081	PKir-1
ASV27	PKir1-7	OQ825082	PKir-1
ASV42	PKir1-8	OQ825086	PKir-1
ASV79	PKir1-9	OQ825090	PKir-1
ASV9	PKir12-1	OQ825072	PKir-1, PKir-2
ASV6	PKir2-1	OQ825070	PKir-2
ASV7	PKir2-2	OQ825071	PKir-2
ASV14	PKir2-3	OQ825074	PKir-2
ASV19	PKir2-4	OQ825078	PKir-3
ASV28	PKir2-5	OQ825083	PKir-3
ASV32	PKir2-6	OQ825084	PKir-3
ASV39	PKir2-7	OQ825085	PKir-3
ASV44	PKir2-8	OQ825087	PKir-3
ASV46	PKir2-9	OQ825088	PKir-3
ASV16	PKir3-1	OQ825076	PKir-3
ASV22	PKir3-2	OQ825079	PKir-3
ASV25	PKir3-3	OQ825080	PKir-3
ASV67	PKir3-4	OQ825089	PKir-3
ASV90	PKir3-5	OQ825091	PKir-3
ASV97	PKir3-6	OQ825092	PKir-3
ASV140	PKir3-7	OQ825093	PKir-3
ASV174	PKir3-8	OQ825094	PKir-3

Table S9. List of ITS2 profiles found across all samples sequenced in this study.

Profile		
A3	C15-C15ct-C15ej	C42a/C1-C42.2-C1az-C1b
A5b	C15-C15cy-C15ct-C15cz	C42a/C3/C1-C42.2-C42ad-
A5b-A5a-A5c-A5e-A5g	C15-C15cy-C15ct-C15on	C1b-C42ac-C1az
A5b-A5a-A5c-A5g	C15-C15l-C15n-C15bb-C15.8	C42a/C42.2
A5b-A5a-A5g	C15-C15m-C15ds	C50a/C3-C50l-C50m-C50n
A5b-A5j-A5k	C15/C116	D1-D4-D17d-D4c-D1r-
A5b-A5j-A5k-A5l	C15/C116f	D17e-D17c-D17j
A5b/A5a	C15/C15ch	D1-D4-D1al
A5b/A5f-A5a	C15/C15cn-C15cq-C15dz-C15fz	D1-D4-D1c-D1al-D2
A6b-A3g-A3	C15/C15cp-C15ch-C15cq	D1-D4-D4c-D1h
A6b/A3-A3g-A3ai	C15/C15cs-C15cr-C15bb	D1-D4-D4c-D6-D1c
B1	C15/C15cs-C15cr-C15bb-C15eb	D1-D4-D6-D1as-D1bb-
C1	C15/C15cs-C15cr-C15ec	D1ba-D1ax
C1-C42.2-C1b	C15/C15ct	D1-D4c-D4-D6c
C1/C1c	C15/C15ed	D1/D1as-D1ax-D1at-D4-D6-
C1/C1c-C1al-C1b-C42.2	C15/C15ej	D1au-D1av-D1aw
C1/C3-C1c-C1b-C1w	C15/C15ev	D1/D1d-D4
C1/C3-C1c-C1b-C42.2-C1bh	C15/C15hz	D1/D4
C1/C3-C1c-C1b-C42.2-C1bh-C1br	C15/C15m	D1/D4-D1c
C1/C3/C1b	C15/C3/C1	D1/D4-D2a
C116	C15/C42a/C42.2-C1	D1/D4-D4c-D1c
C116-C116q	C15cs-C15-C15da-C15hi-C15cr	D1/D4-D4c-D5-D2
C116-C116q-C116u	C15cu-C15cv-C15-C15co-C15cp	D1/D4-D4c-D6
C116a-C116v-C116x	C15cu-C15cv-C15-C15cw-C15co-	D1/D4/D4c
C116a-C116v-C116x-C3gd	C15cx	D1/D4/D4c-D2
C15	C15cu/C15-C15cv-C15co-C15cw	D1/D6
C15-C116f-C15dz	C15ei/C15/C15dy-C116w	D1/D6/D4
C15-C116f-C15dz-C15cq	C15h/C15	D4-D5m
C15-C15ar	C15m/C15db-C15-C15ae-C15bk	
C15-C15ar-C15ea	C1d/C31	
C15-C15bn	C3-C1bp	
C15-C15bn-C15by	C3-C1bp-C3dg-C3df-C3dh	
C15-C15by	C3/C1/C42a-C1b-C42.2-C42ad	
C15-C15cn	C3/C3dc	
C15-C15cq	C31-C21-C31d-C21ac-C21aj	
C15-C15cs-C15cr	C31-C21-C31d-C31e-C21aj	
C15-C15ct-C15ch	C31/C17d-C31.1	
C15-C15ct-C15ch-C15cp	C31/C31a-C17d-C21	
C15-C15ct-C15ch-C15cp-C15cq	C3u/C3	
C15-C15ct-C15ch-C15cp-C15hj	C42.2/C42a/C1-C1b-C3	
C15-C15ct-C15ch-C15hj	C42a-C1-C42.2-C42ac-C1b-C42ad	
C15-C15ct-C15cp-C15cq	C42a-C42.2-C1-C42ad-C42ac-C1b-	
C15-C15ct-C15cy	C42ah	
C15-C15ct-C15cy-C15ch	C42a/C1-C42.2	