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A root for massive crown-of-thorns starfish outbreaks in the Pacific Ocean

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47 **Abstract**

48

49 Recurring outbreaks of crown-of-thorns starfish (COTS) severely damage
50 healthy corals in the Western Pacific Ocean. To determine the source of
51 outbreaking COTS larvae and their dispersal routes across the Western Pacific,
52 complete mitochondrial genomes were sequenced from 243 individuals
53 collected in 11 reef regions. Our results indicate that Pacific COTS comprise two
54 major clades, an East-Central Pacific clade (ECP-C) and a Pan-Pacific clade
55 (PP-C). The ECP-C consists of COTS from French Polynesia (FP), Fiji, Vanuatu
56 and the Great Barrier Reef (GBR), and does not appear prone to outbreaks. In
57 contrast, the PP-C, which repeatedly spawns outbreaks, is a large clade
58 comprising COTS from FP, Fiji, Vanuatu, GBR, Papua New Guinea, Vietnam,
59 the Philippines, Japan, Micronesia, and the Marshall Islands. Given the nature of
60 Pacific Ocean currents, the vast area encompassing FP, Fiji, Vanuatu, and the
61 GBR likely supplies larvae for repeated outbreaks, exacerbated by
62 anthropogenic environmental changes, such as eutrophication.

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66 **Introduction**

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68 Coral reefs are the most biodiverse marine ecosystems and because they
69 nurture edible marine species, furnish biochemicals and novel pharmaceutical
70 leads, provide coastal protection and employment, and contribute to regional
71 cultures, marine managers, communities and governments are calling for their
72 preservation (De'ath et al. 2012). However, many coral reefs are currently
73 experiencing severe, cumulative disturbances, including coral bleaching
74 (Hughes et al. 2017), cyclones/typhoons (Harmelin-Vivien 1994), and massive
75 outbreaks of crown-of-thorns starfish (COTS), *Acanthaster cf. solaris* (previously,
76 *Acanthaster planci*) (Birkeland and Lucas 1990; Yasuda et al. 2009; Timmers et
77 al. 2012; Hughes et al. 2014; Yasuda 2018).

78 COTS are considered the major and most destructive predators of
79 reef-building corals in the Indo-Pacific (Birkeland 1990). Although they are highly
80 fecund (Birkeland and Lucas 1990), under normal, undisturbed conditions COTS
81 populations remain relatively constant and their impacts on coral communities
82 are minimal (Fabricius et al. 2010). On the other hand, recent anthropogenic
83 activities have adversely affected the marine environment resulting in an
84 increased discharge of nutrients (Fabricius et al. 2010) and climate change
85 (Uthicke et al. 2013), both of which are linked to increased COTS pelagic larval
86 duration (PLD) (Yamaguch 1973). This relatively long PLD, which can last
87 several weeks, greatly increases the overall survival rate and may assist
88 expansion of COTS into new habitats with comparatively homogeneous
89 populations in widespread localities (Birkeland and Lucas 1990; Vogler et al.
90 2013). This extended PLD, in association with strong ocean currents, is
91 hypothesized to cause successive secondary population outbreaks of COTS,
92 especially in the Great Barrier Reef (GBR) of Australia, and Japan (Birkeland
93 and Lucas 1990; Benzie and Stoddart 1992; Kenchington 1997; Yasuda, 2018),
94 with substantial loss of coral cover, thereby diminishing the integrity and
95 resilience of reef ecosystems (Timmers et al. 2012; Hughes et al. 2014). In the
96 GBR, one-third of coral reef damage is attributed to COTS predation (Timmers

97 et al. 2012). Similarly, in the Ryukyu Archipelago (RA) and temperate regions of
98 Japan, at least two waves of chronic and successive outbreaks spanning 60
99 years have decimated corals. Since 2000, over 980,000 COTS have been
100 removed from reefs of Amami Island and the Ryukyus (Nakamura et al. 2014;
101 Yasuda 2018) (website <http://www.churaumi.net/onihitode/onihitode1.html>), and
102 from 2011 well over 300,000 COTS have been collected in the GBR (website
103 <http://www.environment.gov.au/marine/gbr/case-studies/crown-of-thorns>),
104 highlighting the protracted nature and high cost of programs to maintain healthy
105 coral reefs.

106 Extensive studies of COTS biology, including population genetics, have
107 been conducted (Benzie 1992; Yasuda et al. 2009; Yasuda et al. 2015; Harrison
108 et al. 2017; Pratchett et al. 2017). For example, genetic studies based on partial
109 mitochondrial gene sequences revealed the geographic distributions of four
110 COTS lineages, two in the Indian Ocean, one in the Red Sea, and one in the
111 Pacific Ocean (Vogler et al. 2008). Studies using either genes of mitochondrial
112 cytochrome oxidase subunit I, II and III or microsatellite locus heterozygosity, or
113 both, have generally demonstrated a genetically homogenous pattern of *A. cf.*
114 *solaris* in the Western Pacific (Vogler et al. 2013; Tusso et al. 2016), as well as in
115 regions associated with western boundary currents (Yasuda et al. 2009), the
116 Hawaiian Islands (Timmers et al. 2011), French Polynesia (Yasuda et al. 2015),
117 and the GBR (Harrison et al. 2017). However, no study has addressed which
118 lineage of extant Pacific COTS is the oldest, what mechanisms supported their
119 expansion across the entire Pacific Ocean, and does COTS genetic connectivity
120 facilitate outbreaks, especially in the Western Pacific. To answer these questions,
121 we sequenced entire mitochondrial genomes (Inoue et al. 2020) of 243 COTS
122 specimens collected from 11 representative localities of the Pacific and
123 conducted molecular phylogenetic analyses.

124

125 **Methods**

126

127 ***Acanthaster cf. solaris***

128 A total of 243 adult crown-of-thorns starfish were collected from 2006~2018 at
129 reefs in the Pacific Ocean (Fig. 1). Fifty-three specimens were collected in
130 French Polynesia, including 13 specimens from Bora-Bora, 16 from Moorea, 9
131 from Raiatea, and 15 from Tahiti (Supplementary Table S1). Ten specimens
132 were collected from Fiji, 31 from Vanuatu, and 20 from the GBR (10 each from
133 Clack and Shell Reefs). We collected 9 specimens from Papua New Guinea, 4
134 from the Philippines, 10 from Vietnam, 48 from the Ryukyu Archipelago of Japan
135 and 29 from the Kagoshima Islands of Japan (Table S1). In addition, 9
136 specimens from Micronesia, 8 from the Marshall Islands, and 12 from the USA (9
137 from Hawaii and 3 from California) were also collected. Collection sites and
138 sample numbers are reported in Supplementary Table 1.

139

140 **DNA sequencing and assembly of mitochondria genomes**

141 Tube feet of adult COTS were dissected with scissors and fixed in 99.5% ethanol.
142 Specimens were kept at 4°C until use for DNA sequencing. Genomic and
143 mitochondrial DNA were extracted using the automated Nextractor® NX-48S
144 system. Extraction was performed following the manufacturer's protocol using an
145 NX-48 Tissue DNA kit (Genolution Inc., Seoul, Korea). Tube foot tissue was
146 incubated in lysis buffer overnight and extracted DNA was purified with
147 Agencourt AMPure XP magnetic beads immediately before library preparation.
148 DNA concentration was determined with Qubit dsDNA broad range (Thermo
149 Scientific Inc., USA), and the quality of high molecular-weight DNA was checked
150 using an Agilent 4150 TapeStation (Agilent, USA). PCR-free shotgun libraries
151 were constructed using NEBNext® Ultra™ II FS DNA Library Prep Kits for
152 Illumina (New England BioLabs Inc, UK), following the manufacturer's protocols.
153 Sequencing was performed using an Illumina NovaSeq 6000 sequencer
154 (Illumina Inc., USA).

155

156 Sequencing was performed using Illumina HiSeq 2500 and Novaseq
sequencers. Approximately 10X coverage of nuclear genome DNA sequences

157 was obtained. After removing low-quality reads, under default parameters,
158 paired-end reads were assembled using GS *De novo* Assembler version 2.3
159 (Newbler, Roche) and NOVOPlasty 2.6.3 (Dierckxsens et al. 2017) with the
160 published *A. planci* sequence I (Yasuda et al. 2006) as seed input. Usually, the
161 largest scaffolds contained mitochondrial DNA sequences. Analysis of the
162 genomes using MitoAnnotator (Iwasaki et al. 2013) resulted in the circular
163 structure of the genome. That is, the genome consists of a gene set of
164 cytochrome oxidase subunits I, II and III (COI, COII and COIII), cytochrome b
165 (Cyt b), NADH dehydrogenase subunits 1-6 and 4L (ND1-6 and 4L), ATPase
166 subunits 6 and 8 (ATPase6 and 8), two rRNAs, and 22 tRNAs (see Fig. 1 of 26).

167 As mentioned above, we collected 243 individuals representing 11 coral
168 reef regions of the Pacific Ocean (Fig. 1) and determined the complete
169 mitochondrial genome sequences (16,210~16,246 bp, depending on the
170 individual) of all specimens. Genome sequencing coverage per individual was
171 1,827X on average, ranging from 34X to 136,220X, indicating the data
172 robustness from each specimen. We unambiguously aligned 16,218 bp of
173 sequences, including 1,822 variable sites, which were used for unrooted tree
174 analyses (Fig. 2). On the other hand, 16,219 bp of unambiguously aligned sites,
175 including 3,159 variable sites, were used for rooted tree analyses, with
176 mitochondrial sequences of *A. brevispinus* as an out group (Fig. 3).

177

178 **Phylogenetic analysis**

179 Whole mitochondrial genome sequences were aligned using MAFFT (Katoh et al.
180 2005). Multiple sequence alignments were trimmed by removing poorly aligned
181 regions using TRIMAL 1.2 (Capella-Gutiérrez et al. 2009) with the option
182 “gappyout.” To examine population structures, maximum likelihood (ML) trees
183 were created using RAxML 8.2.6 (Stamatakis 2014). Trees were estimated with
184 the “-f a” option, which invokes rapid bootstrap analysis with 100 replicates and
185 searches for the best-scoring ML tree, using the GTRCAT model (Stamatakis
186 2006).

187

188 **Principal component analysis (PCA)**

189 Population structures were analyzed using model-free approaches. Based on
190 mitochondrial genome sequences, principal component analysis (PCA) was
191 performed on all individuals, using PLINK 1.9 (Purcell and Chang 2015).
192 Pairwise genetic distances among localities were estimated with Weir and
193 Cockerham's F_{ST} (Weir and Cockerham 1984) and Nei's genetic distance (Nei
194 1972) using StAMPP (Pembleton et al. 2013).

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198 **Results and Discussion**

199

200 A total of 243 adult COTS were collected from 11 representative coral reef
201 regions (14 countries) throughout the Pacific Ocean (Fig. 1; Supplementary
202 Table S1), including Bora Bora, Moorea, Raiatea, and Tahiti in French Polynesia,
203 Fiji, Vanuatu, the GBR (Clack and Shell Reefs) of Australia, Papua New Guinea,
204 the Philippines, Vietnam, Japan (the Ryukyu Archipelago and islands of
205 Kagoshima), Micronesia, the Marshall Islands, and Hawaii and California, USA.

206 Complete mitochondrial genome sequences (a circular genome
207 consisting of 16,221 bp, on average) (Inoue et al. 2010) were determined for all
208 specimens (Supplementary Fig. S1). The mean read coverage was 1,827X,
209 ranging from 34 to 136,220X, indicating that data were robust and suitable for
210 establishing the complete sequence of each individual and for subsequent
211 molecular phylogenetic analyses and principal component analysis (PCA).

212 An unrooted molecular phylogenetic tree was constructed for all
213 specimens, based on 16,218 unambiguously aligned bases, including 1,822
214 variable sites (Fig. 2). A rooted tree using the corresponding mitochondrial
215 sequence of *Acanthaster brevispinus* (Yasuda et al. 2006), a closely related, and
216 possibly ancestral species of *A. planci*, *sensu lato* (Lucas and Jones 1976) was
217 used as an outgroup (Fig. 3). The rooted tree was based on 16,219
218 unambiguously aligned bp, including 3,159 variable sites. Both trees yielded
219 similar profiles of COTS population diversification.

220 Both trees indicated that COTS populations in the Pacific represent two

221 major clades, tentatively called the East-Central Pacific clade (ECP-C) and
222 Pan-Pacific clade (PP-C). Diversification of the two clades was evident in a long
223 branch distance between the two in the unrooted tree. That is, the two clades are
224 separated by 0.004 mitochondrial DNA sequence substitutions per site (Fig. 2),
225 and there is discrete branching of the two groups in the rooted tree (Fig. 3).

226 The ECP-C consists of four major lineages, tentatively called the
227 Eastern Pacific lineage (EP-L), the East-Central Pacific lineages, ECP-L1 and
228 L2, and the Hawaiian lineage (ECP-H) (Figs. 2 and 3). The EP-L consists of
229 COTS from French Polynesia (Tahiti, Bora Bora, Moorea, and Raiatea) and
230 California (Fig. 3). ECP-L1, contains COTS from French Polynesia, plus
231 populations from California and Fiji. ECP-L2 comprises two subgroups, but both
232 include COTS of French Polynesia, Fiji, Vanuatu, and GBR (Clack and Shell
233 Reefs). The genetic homogeneity of COTS among these French Polynesian
234 populations was noted in a previous study (Yasuda et al. 2015). The two
235 California COTS pertain to EPC-C, one belonging to EP-L and the other to
236 EC1-L (Fig. 3). The external morphology of the California COTS is significantly
237 different from counterparts in other areas of the Pacific. Specifically, they tend to
238 have shorter arms, and were initially classified as a separate species,
239 *Acanthaster elichii* (Timmers et al. 2012). However, allozyme analysis revealed
240 them to have stronger affinity to COTS of the Western Pacific than to their
241 closest geographical neighbors, the Hawaiian COTS (discussed later), and were
242 therefore renamed *Acanthaster planci* (Nishida and Lucas 1988). This suggests
243 a common ancestry for Eastern Pacific COTS and California COTS. Accordingly,
244 all COTS are now classified as *Acanthaster cf solaris* (Haszprunar and Spies,
245 2014)

246 Near the root position, as viewed from the ECP-C/PP-C boundary of the
247 unrooted tree (Fig. 2) and in the third branch of the rooted tree (Fig. 3), ten
248 Hawaiian COTS formed a discrete group, without individuals from any other
249 Pacific reefs (ECP-H). This genetic isolation was exceptional but had 100%
250 bootstrap support (Fig. 3). This result agrees well with previous studies,
251 suggesting that North Central Pacific COTS, including Hawaii, form a distinct

252 clade among Pacific COTS (Timmers et al. 2012; Vogler et al. 2013). ECP-H is
253 likely independent of other Pacific COTS or of cryptic COTS species. Future
254 nuclear genomic studies should be able to confirm this possibility.

255 In contrast to the four lineages of ECP-C, all of which are comparatively
256 well separated or isolated, eight lineages or subgroups of PP-C, PP-L1, PP-L2
257 and PP-L3A-L3F, appeared more genetically similar (Figs. 2 and 3). PP-L1,
258 which includes COTS from Fiji, the Philippines and Japan, and PP-L2, which
259 comprises starfish from Fiji, Vanuatu, and Japan, branched earlier and are
260 separated from the other PP lineages (Fig. 2, Fig. 3). PP-L3 is a very large group,
261 including not only Western Pacific COTS, but also Eastern Pacific populations
262 from French Polynesia, Fiji, Vanuatu, GBR, Papua New Guinea, the Philippines,
263 Japan, Micronesia, and the Marshall Islands. It consists of six lineages (PP-L3A
264 to PP-L3F) that are not strictly geographically defined, in that each subgroup
265 comprises individuals from several of these areas. Of special interest is PP-L3B,
266 which has the largest geographic, including COTS from all locations of French
267 Polynesia, Fiji, Vanuatu, GBR, Papua New Guinea, the Philippines, Vietnam,
268 Japan, Micronesia, and the Marshall Islands. PP-L3C also includes COTS from
269 various locations including Vanuatu, GBR, Papua New Guinea, Japan,
270 Micronesia, and the Marshall Islands. PP-L3D includes COTS not only from
271 Japan, Micronesia and the Marshall Islands, but also Fiji. On the other hand,
272 PP-L3F appears to be a lineage more specific to East Asia, comprising COTS
273 populations in the Philippines, Vietnam, and Japan.

274 Principle component analysis (PCA) of specimens from all sampling
275 locations (Supplementary Table S1) supported the results of molecular
276 phylogenetic analyses (Fig. 4). PCA resulted in five independent groups,
277 corresponding to EP-L, ECP-L1, EPC-L2, ECP-H, and PP-L, respectively.
278 Notably, a mixture of COTS from all locations across the Pacific was evident in
279 PP-L (Fig. 4, upper right corner). When compared to molecular phylogeny
280 results (Figs. 2 and 3), grouping of EP-L, ECP-L1 and EPC-H was more strongly
281 demonstrated in PCA (Fig. 4). In addition, PCA suggested an affinity of ECP-L2
282 with PP-L, although this was not as strong (Fig. 4).

283 The present results provide several clues regarding the evolutionary
284 history of COTS in the Pacific Ocean. First, based on comparisons of complete
285 mitochondrial DNA sequences, COTS in the Pacific are genetically subdivided
286 into two major clades, ECP-C and PP-C. We speculate that because ECP-C
287 COTS are confined to the Eastern and Central Pacific and are less affected by
288 anthropogenic factors, they are not prone to major outbreaks, even though they
289 show local outbreaks (Birkeland 1990). In contrast, PP-C which occurs across
290 the entire Pacific, including more highly populated regions, spawns massive
291 outbreaks.

292 ECP-C was divided into four sub-groups, EP-L, ECP-L1, ECP-L2 and
293 ECP-H. The former three are distinguishable by their geographic distributions.
294 EP-L is confined to four countries of French Polynesia + California, ECP-L1
295 encompasses French Polynesia + California + Fiji, and ECP-L2 is confined to
296 French Polynesia, Vanuatu, and GBR. This sub-grouping suggests two possible
297 scenarios relative to their distributional history in the Eastern and Central Pacific.
298 One is the EP-L ancestry hypothesis, in which COTS originated in French
299 Polynesia, experienced a bottleneck-like founder effect (Yasuda et al. 2015), and
300 then expanded into the central and western regions, ultimately reaching the
301 GBR. In contrast, in the ECP-L2 ancestry hypothesis, a comparatively broad
302 region encompassing GBR, Vanuatu, Fiji and French Polynesia is the original
303 source of COTS, from which EP-L and ECP-L1 became established as separate,
304 independent lineages long ago. The latter scenario is the more plausible and is
305 discussed further below.

306 The inclusion of Californian COTS in EP-L and ECP-L1, as well as the
307 grouping of the independent Hawaiian lineage within EP-L, suggests that COTS
308 larval migration in the Eastern Pacific has played an important role in their
309 expansion across the wider Pacific. Another interesting observation is that
310 COTS of Micronesia and the Marshall Islands may not be members of EP-L but
311 may belong in PP-L. This suggests that the westward flow of the South
312 Equatorial Current into the Coral Sea may become disrupted by complex
313 topography, carrying larvae to the intersection of the Equatorial Counter Current,

314 which is an eastward flowing, wind-driven current, thereby transporting
315 Eastern-Central COTS larvae toward California (Wyrcki 1967) (Fig. 1). While this
316 partially supports the ECP-L2 ancestry scenario, at present, there is no evidence
317 to explain the origin of the Hawaiian COTS population, which arrived by
318 unknown means and has is completely isolated. Given that Hawaiian COTS are
319 independent of current outbreaks in the Pacific (Timmers et al. 2012), their origin
320 remains a key question in future genomic studies.

321 On the other hand, PP-L contains COTS from almost all regions of the
322 Pacific, including French Polynesia, Fiji, Vanuatu, GBR, Papua New Guinea,
323 Vietnam, the Philippines, Japan, Micronesia, and the Marshall Islands. The two
324 PP-L subgroups, PP-L3B and PP-L3C, both contain COTS from all these
325 localities. It is highly likely that this type of population genetic profile reflects the
326 trajectory of repeated outbreaks across the entire Pacific Ocean, with the
327 exception of the U.S. population. One possible explanation is that dispersal of
328 long-lived COTS larvae spawned in the central Pacific is facilitated by the South
329 Equatorial Current, which flows at an average velocity of 20 nautical miles per
330 day from Fiji and Vanuatu toward the GBR, where it bifurcates into the New
331 Guinea Coastal Undercurrent (Trembl et al. 2008; Sokolov et al. 2000). In
332 combination with the North Equatorial Current, which originates from the
333 Californian Current, it bifurcates into the strong Kuroshio Current that flows from
334 the northeastern Philippines toward Japan (Qi and Lukas 1996) (Fig. 1). An
335 earlier divergence of PP-L1 and L2, both including COTS from Fiji and Vanuatu,
336 suggests a contribution of these COTS with western Pacific populations via the
337 southernmost branches of the South Equatorial Current.

338 Further support linking repeated outbreaks to the PP-L population
339 comes from comparisons of the entire ~384-Mb genome sequences of the two
340 COTS, one from the GBR and the other from Okinawa (OKI), separated by over
341 5,000 km (Hall et al. 2017). An unexpected result of this study was the
342 exceptionally low heterozygosity of the genomes, 0.88% and 0.92% for the GBR
343 and OKI populations, respectively. In addition, reciprocal BLAST analysis of
344 scaffolds longer than 10 kb showed 98.8% nucleotide identity between the GBR

345 and OKI genomes, evidence of the great similarity of their nuclear DNA
346 sequences. Inclusion of these two specimens in a rooted tree (Fig. 2 and Fig. 3,
347 arrows) revealed that GBR COTS belong to PP-L3A and Oki COTS to PP-L3F.
348 Intriguingly, our results suggest a very strong resemblance of the nuclear
349 genomes of these two COTS lineages.

350 These results raise yet another possibility with respect to the
351 geographical extent of the distribution of various COTS lineages. Most of the
352 COTS that belong to ECP-L1 are from Vanuatu. However, other Vanuatu COTS
353 belong to PP-L3B, PP-L3B, or PP-L3D. Both lineages of COTS coexist in
354 Vanuatu, one with the capacity for large outbreaks and the other without. An
355 objective of future population genomics studies will be to sequence and compare
356 complete genomes of both ECP-L and PP-L COTS to try to discover the genetic
357 and genomic features that encode the capacity for outbreaks.

358 Based on the combined results of molecular phylogeny and PCA, it is
359 likely that the oldest lineages of extant Pacific COTS originated from a broad
360 Pacific region, including Fiji, Vanuatu, GBR, and French Polynesia (Fig. 5).
361 Some populations have lived harmoniously in these regions, with some lineages
362 moving eastward toward California. On the other hand, some COTS populations
363 have extended their range to cover nearly all of the Pacific, and they are
364 especially prolific in the Western Pacific (Fig. 5). The shorter branch length with
365 highly diverse haplotypes in the admixed PP-3C implies a founder effect during
366 their westward migration, followed by population expansion. These populations
367 have developed a capacity for greatly enhanced larval survival, possibly
368 triggered by anthropogenic environmental changes, such as eutrophication. Our
369 results therefore shed light on an important issue in which regulation of future
370 COTS outbreaks depends on a better management of this pest in the central
371 Pacific, and better human waste management.

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376 **Data accessibility.** All the sequence data are accessible under

377 <https://www.ncbi.nlm.nih.gov/bioproject/PRJDB10499>.

378 **Authors' contribution.** N.Y., J.I., M.R.H., C.A.M. and N.S. designed the
379 research. N.Y., M.R.H., M.R.N., M.A., M.D.F., M.N., N.T., R.R-C. and S.H.F.
380 collected samples. T.B.H.S. and R.K. sequenced COTS mitochondrial DNA.
381 N.Y., J.I., K.H., C.A.M. and N.S. analyzed data. N.Y., J.I., C.A.M. and N.S. wrote
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384

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401

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545 **Figure Legends**

546

547 **Figure 1. a**, A single adult of crown-of-thorns starfish on reef-building corals. **b**,
548 An outbreak of crown-of-thorns starfish (COTS) covering and eating
549 scleractinian corals, causing severe damage to the reef. **c**, Collection sites of
550 COTS in the Pacific Ocean. 243 COTS were collected at 23 locations in 14
551 countries, representing 11 reef regions: French Polynesia (Bora Bora, Moorea,
552 Raiatea and Tahiti), Fiji, Vanuatu, Great Barrier Reef, Australia (GBR; Clack and
553 Shell Reefs), Papua New Guinea (PNG), the Philippines, Vietnam, Japan,
554 Micronesia, the Marshall Islands, and USA (Hawaii and California). Locations in
555 Japan and French Polynesia are enlarged in (A) and (B). Red arrows show the
556 main currents in the Pacific Ocean. KC = Kuroshio Current, CC = California
557 Current, NEC = North Equatorial Current, ECC = Equatorial Countercurrent,
558 SEC = South Equatorial Current, EAC = East Australian Current and NGCU =
559 New Guinea Coastal Undercurrent.

560 **Figure 2.** An unrooted phylogenetic tree of individual *Acanthaster cf. solaris*
561 using the maximum-likelihood (ML) method, based on mitochondrial genome
562 sequences (16,218 bp, including 1,822 variable sites). Red arrowheads indicate
563 sequences (OKI and GBR) decoded in the genome paper (Hall et al. 2017).

564 **Figure 3.** A rooted phylogenetic tree of *Acanthaster cf. solaris* using the
565 maximum-likelihood (ML) method, based on mitochondrial genome sequences
566 (16,219 bp, including 3,159 variable sites). Numbers at some nodes indicate
567 bootstrap values (>50%) based on 100 replicates for internal branch support.
568 Arrowheads at the right indicate sequences (OKI and GBR) decoded in the
569 genome paper (Hall et al. 2017). The *A. brevispinus* sequence (NC_007789.1)
570 was selected for rooting. The color relationship to sampling locations is shown in
571 the insert (left, bottom).

572 **Figure 4.** Principle Component Analysis identifies five COTS populations, EP-L,
573 ECP-L1, ECP-L2, ECP-H, and WP-CL1/2. WP-L3 contains COTS collected from

574 all countries, suggesting that this population is the source of repeated outbreaks
575 in the Pacific. Color codes are shown at the right side.

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577 **Figure 5.** A summary diagram to show a possible root for crown-of-thorns
578 starfish outbreaks in the Western Pacific Ocean. The Pacific hosts two major
579 groups of COTS. The East-Central Pacific group comprises COTS from French
580 Polynesia, Fiji, Vanuatu, and the GBR (blue and yellow). The Whole Pacific
581 group contains COTS from the entire Western Pacific (purple). The latter has
582 experienced repeated outbreaks, while the former has experienced local
583 outbreaks. This suggests an importance of better management of this pest in the
584 central Pacific region, including Fiji, Vanuatu, and the GBR.

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588 **Figure S1.** An alignment of the complete mitochondrial DNA sequences of
589 *Acanthaster planci* (NC_007788.1 and specimen name, M2), All sequence data
590 are accessible at: <https://www.ncbi.nlm.nih.gov/bioproject/PRJDB10499>.

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Figure 1.

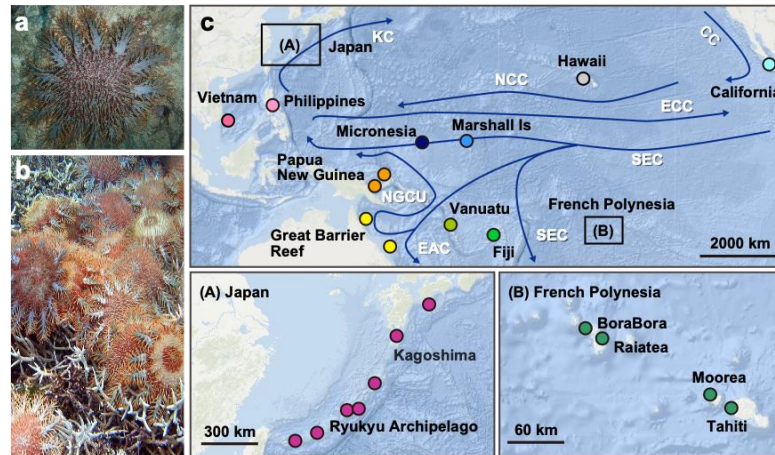


Figure 1. a, A single adult of crown-of-thorns starfish on reef-building corals. **b,** An outbreak of crown-of-thorns starfish (COTS) covering and eating scleractinian corals, causing severe damage to the reef. **c,** Collection sites of COTS in the Pacific Ocean. 243 COTS were collected at 23 locations in 14 countries, representing 11 reef regions: French Polynesia (Bora Bora, Moorea, Raiatea and Tahiti), Fiji, Vanuatu, Great Barrier Reef, Australia (GBR; Clack and Shell Reefs), Papua New Guinea (PNG), the Philippines, Vietnam, Japan, Micronesia, the Marshall Islands, and USA (Hawaii and California). Locations in Japan and French Polynesia are enlarged in (A) and (B). Blue arrows show the main currents in the Pacific Ocean. KC = Kuroshio Current, CC = California Current, NEC = North Equatorial Current, ECC = Equatorial Countercurrent, SEC = South Equatorial Current, EAC = East Australian Current and NGCU = New Guinea Coastal Undercurrent.

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Figure 2.

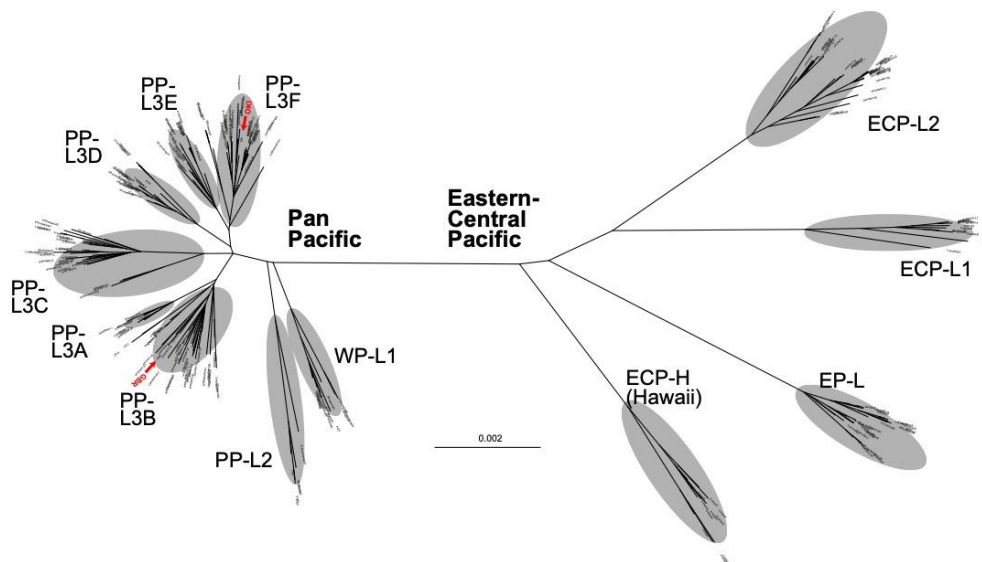


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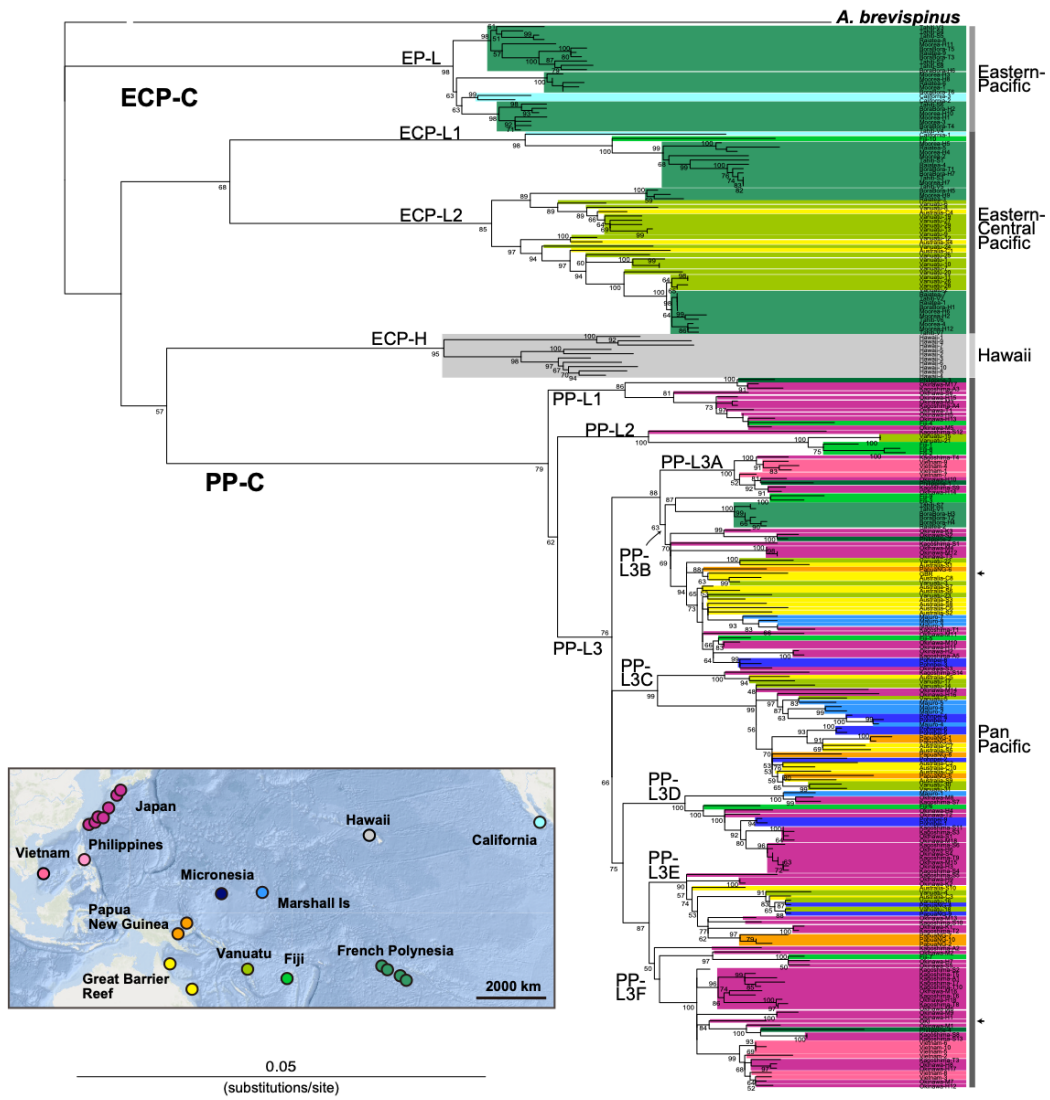


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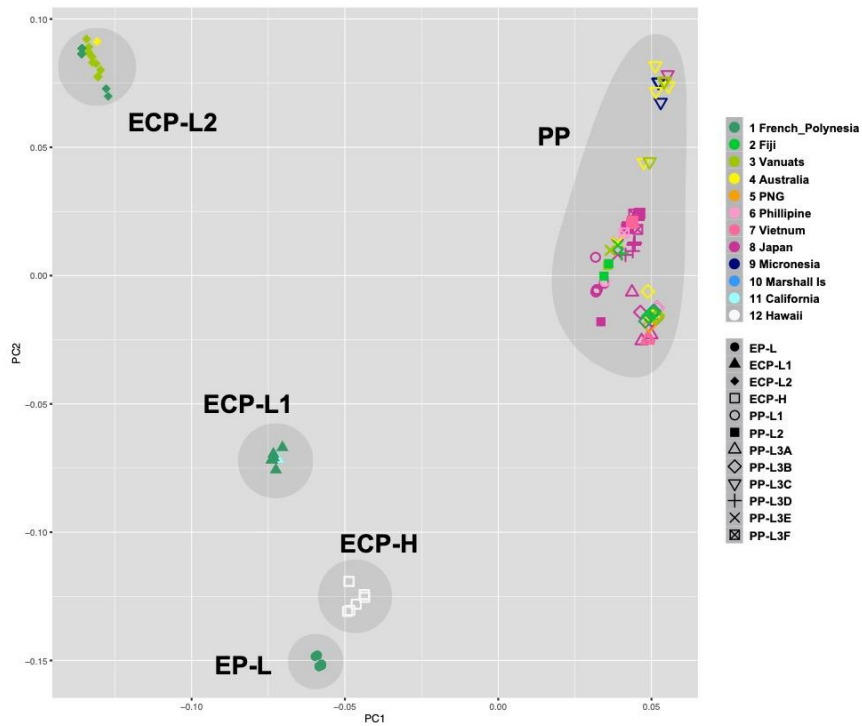


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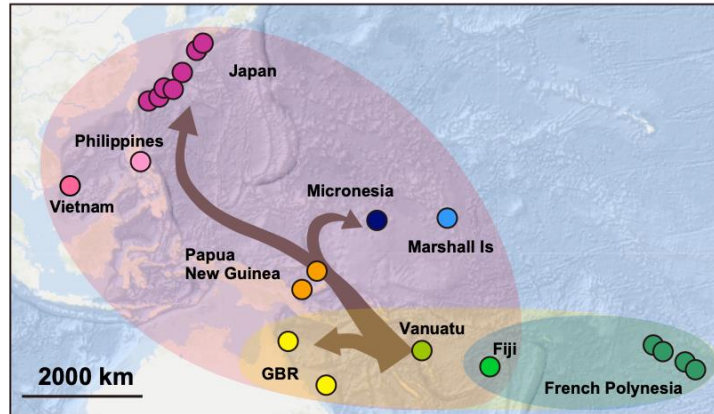


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