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RESEARCH

Speciation of coral-associated barnacles: generalists *versus* specialists in the Indo-West Pacific

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Abstract Coral-associated invertebrates possess a continuum of host specificity in their host usage, with generalists inhabiting a wide selection of host corals and specialists inhabiting a narrower range of hosts. The population genetics of three coral-associated barnacle species with different degrees of host specificity were examined using cytochrome c oxidase I gene sequences. The relative generalist *Cantellius euspinulosum* showed deep and host-related intraspecific divergence in two sympatric groups in the Indo-West Pacific (K2P = 4.43%). There are host coral species exclusive to each group, suggesting the possibility of intraspecific host-driven divergence. The relative specialists *C. septimus* and *Darwiniella angularis* only showed genetic divergence associated with geographical distribution, not with the host coral species. Historical demography analyses indicated that all species underwent expansion around the mid to late Pleistocene. Higher contemporary population size was observed in the *C. euspinulosum* groups, albeit not significantly different from that of *C. septimus*, implying that neither generalists' nor specialists' propensities guarantee higher fitness advantages regarding expansion. Generalists appeared to have greater gene flow at geographical scales, but their population structures were driven by host specializations. In contrast, the relatively specialist species have fewer stepping stones for dispersal and often have genetic differentiation correlated with geographic scales.

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Introduction

Coral reefs are among the most diverse marine ecosystems in the world, housing obligate symbiotic invertebrates that account for more than 56% of their apparent biodiversity (Stella et al. 2011). Understanding the patterns of gene flow and population structure of coral-associated organisms is essential for comprehending the mechanisms generating such great biodiversity. Population genetic studies of free-living marine invertebrates in the Indo-Pacific region are extensive, examining the interplaying dynamics of larval dispersal periods, oceanographic currents, and historical geological events, such as Pleistocene glaciations, which configure genetic differentiation patterns (Chan et al. 2020; Kim et al. 2020; Tsang et al. 2012). In contrast to free-living marine species, the influence of host coral distribution, their degree of host specificity, and the gene flow among populations of coral-associated organisms is poorly understood.

Coral-associated barnacles are a group of the obligate coral macrosymbionts that thrive inside coral skeletons for habitat and protection (Fig. 1) (Chan et al. 2013; Ross & Newman 1969, 1973), and commence their symbiotic life cycle during the planktonic larvae settlement period (Dreyer et al. 2022). Coral barnacles are suspension feeders, adaptively feeding through a protruded cirri and capturing nearby zooplankton (Anderson 1978). Previous studies revealed the importance of carbon and ammonium exchange between the barnacle and its host coral, suggesting a mutualistic relationship (Achituv et al. 1997; Achituv & Mizrahi 1996). Among the variety of coral-associated fauna,

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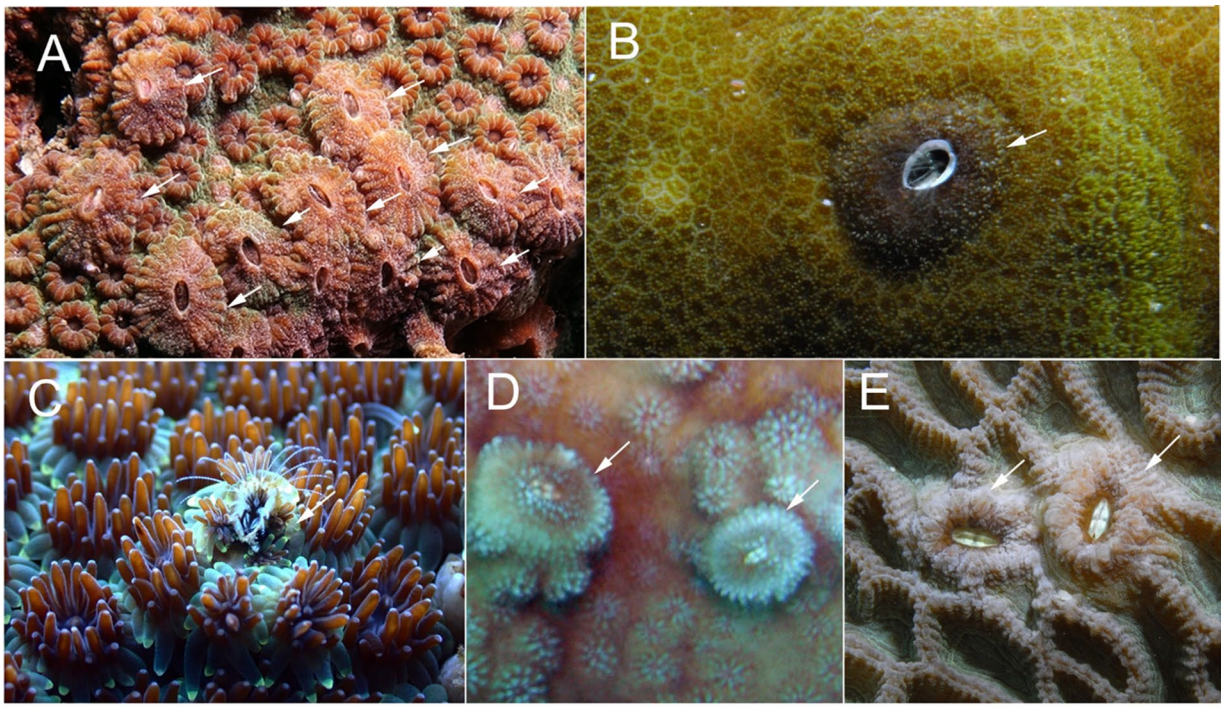
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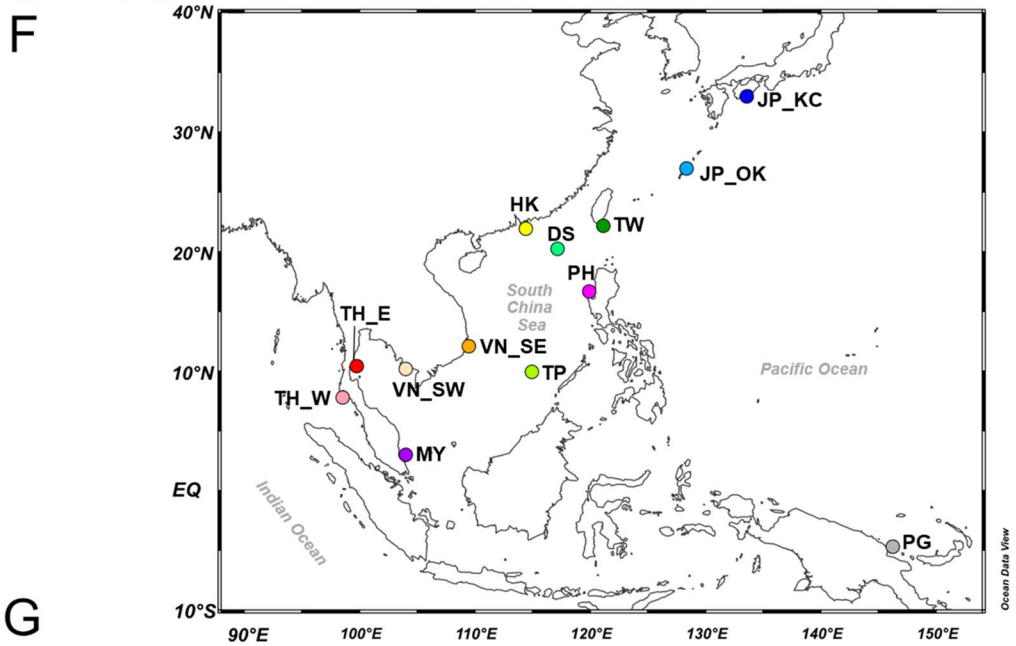
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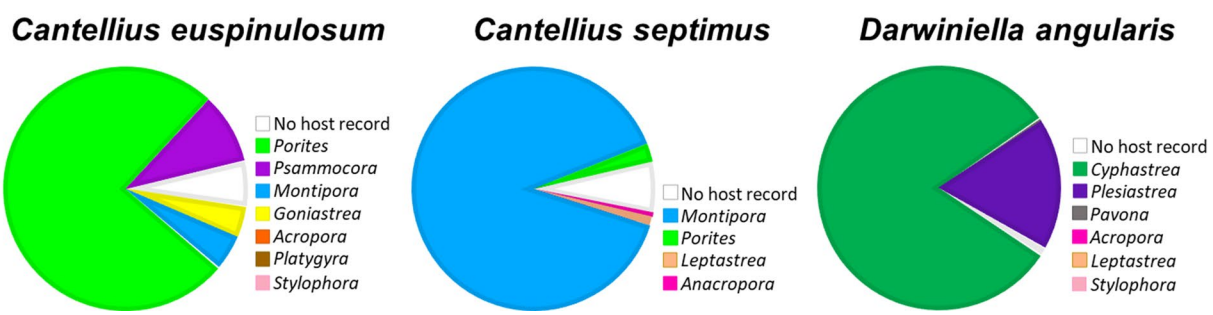
G
Cantellius euspinulosum
No host record
Porites
Psammocora
Montipora
Goniastrea
Acropora
Platygyra
Stylophora
Cantellius septimus
No host record
Montipora
Porites
Leptastrea
Anacropora
Darwiniella angularis
No host record
Cyphastrea
Plesiastrea
Pavona
Acropora
Leptastrea
Stylophora

◂**Fig. 1** Coral barnacles have an obligate symbiotic relationship with host corals and exhibit a continuum of host specificity. (A) *Darwiniella angularis* on *Cyphastrea* coral. (B) *Cantellius cf. euspinulosum* on *Porites* coral. (C) *Nobia grandis* on *Galaxea* coral. (D) *Cantellius septimus* on *Montipora* coral. (E) *Trevathana sp*. on *Platygyra* coral. (F) Sampling map of *Cantellius euspinulosum*, *C. septimus,* and *Darwiniella angularis* for this population genetics and host specificity study in the Indo-West Pacific. Note that all the sites are in the West Pacific except TH_W, which lies on the Indian Ocean border. Site colors correspond to the haplotype diagrams in Fig. 2. (G) Host specificity (percentage occurrence of host corals) of *Cantellius euspinulosum*, *C. septimus,* and *Darwiniella angularis* recorded in the present study. *C. euspinulosum* is a relative generalist compared to *C. septimus* and *D. angularis*. All the coral barnacles are highlighted with white arrows. For site abbreviations, please see Table 2.

coral-associated barnacles have greater variations in host specificity than coral-associated bivalves, annelids, gall crabs, and tube worms (van der Schoot & Hoeksema 2024). There is a continuum of host specificity in coral-associated barnacles—with *generalists* inhabiting a wider selection of host corals, and *specialists*, which limitedly thrive within fewer host choices (Achituv & Hoeksema 2003; Achituv & Newman 2002; Tsang et al. 2014). *Armatobalanus allium* (Darwin 1854) is a known generalist that can inhabit more than ten host coral species (Tsang et al. 2014), while the genus *Cantellius* (Ross & Newman 1973) covers both generalist and specialist propensities. On the other hand, genera including *Trevathana* (Anderson 1992), *Savignium* (Leach 1826), *Darwiniella* (Anderson 1992), and *Nobia* (Sowerby 1839) are relatively specialists, living in a smaller array of host corals compared to that of *Armatobalanus* and *Cantellius* (Fig. 1) (Tsang et al. 2014). Molecular studies of pyrgomatinid barnacles revealed that there are two sister clades—one comprised of the generalist *Armatobalanus* (Hoek 1913) and *Cantellius* and the other clade composed of the rest of the other genera within the subfamily. Phylogenetically, generalist species of Pyrgomatinae are more basally located, whereas specialists are positioned closer to the clade tips (Tsang et al. 2014). This signified an evolutionary trend of increasing host specificity among coral-associated barnacles.

A recent study showed that the host specificity of coral-associated barnacles can be driven by the actively selective behavior of the cypris larvae (Yap et al. 2023), therefore accentuating the dire importance of larval recruitment and supply toward their preferred hosts (Liu et al. 2016). The host specificity of coral barnacles can consequently influence their population structure and subsequent genetic divergence (Mokady & Brickner 2001; Tsang et al. 2009, 2014). Species with broader ranges of host usage may have a greater degree of gene flow among geographical populations because the higher availability of hosts can provide stepping stones for larval dispersal. Conversely, species with more restricted host preferences may experience reduced gene flow. However, this hypothesis still needs to be further tested using a spectrum of coral-associated barnacles that inhabit different host corals and exhibit varying ranges of host usage. The present study aims to test the hypothesis of greater gene flow in generalists and specialists by examining three coral-associated barnacle species: *Cantellius euspinulosum* (Broch 1931), *C. septimus* (Hiro 1938), and *Darwiniella angularis* (Chen et al. 2012), each with different degrees of host specificity in the Indo-West Pacific region. *Cantellius euspinulosum* is a generalist, primarily living on *Porites* (Link 1807) corals but also found on other genera including *Montipora* (Blainville 1830), *Goniastrea* (Milne Edwards & Haime 1848a), *Acropora* (Oken 1815), *Platygyra* (Ehrenberg 1834), and *Stylophora* (Schweigger 1820). In contrast, *C. septimus* and *D. angularis* are more specialized, with *C. septimus* primarily inhabiting *Montipora,* whereas *D. angularis* primarily thrives on *Cyphastrea* (Milne Edwards & Haime 1848a) corals.

Materials and methods

Target species and DNA sequencing

The coral-associated barnacles *Cantellius euspinulosum*, *C. septimus,* and *Darwiniella angularis* were sampled throughout the West Pacific region and Phuket, Thailand, in the Indian Ocean (Fig. 1F). Kim et al. (2021) suggested *C. euspinulosum* is a cryptic species complex. However, before any further taxonomic revision on this species, we still used the name *C. euspinulosum* to represent the specimens collected in the present study. All three barnacle species were collected by SCUBA diving with a hammer and chisel and then fixed in 95% ethanol (methods following Chan et al. 2016). A photograph of the host coral was taken in situ upon the collection of barnacles for identification of the host corals. The host corals were identified following Veron (2000). Updated scientific name of corals followed by the World List of Scleractinia (https://www.marinespecies.org/scleractinia) (Hoeksema & Cairns 2025). Total genomic DNA was extracted from the soft tissue of each specimen using Qiagen (Chatsworth, CA) QIAquick Tissue Kit following the manufacturer's instructions. A partial DNA sequence of mitochondrial gene cytochrome c oxidase subunit I (COI) was amplified by polymerase chain reaction (PCR) with barnacle-specific primers (Chan et al. 2013; Tsang et al. 2014).

The COI region was preferred due to its efficiency as a DNA barcode marker. It also possessed more polymorphic sites within species compared to another popular mitochondrial marker 12S (data not shown) and bore the advantage of having robust reference data, i.e., mutation rate, therefore favorable for further population parameter estimations. The PCR solution contained 40 ng template DNA, 5 µl Taq DNA Polymerase Master Mix (1.5 mM $MgCl_2$; Ampliqon,

Denmark), 1 µM of each primer, and ddH_2O to a final volume of 10 µl. The reaction was conducted under the following conditions: 2 min at 95 °C for initial denaturing, 35 cycles of 30 s at 95 °C, 1 min at 48 °C, 1 min at 72 °C, with a final extension for 5 min at 72 °C. The products were then purified using the DNA Gel purification kit (Tri-I Biotech, Taipei, Taiwan). Sequencing of the purified PCR products was performed on an ABI 3730XL Genetic Analyzer with BigDye terminator cycle sequencing reagents (Applied Biosystems, Foster City, California, USA). Sequences were then aligned with CLUSTAL X (Thompson et al. 1997) using default settings and visually adjusted in Geneious v8.1.8 (https://www.geneious.com).

Genetic diversity, connectivity, and demographic history

Genetic diversity, connectivity, and demographic history of each species were explored. Standard genetic diversity indices, including the number of haplotypes (Na), haplotype diversity (h), and nucleotide diversity (π), were estimated using Arlequin v3.5 (Excoffier & Schneider 2005). Genetic connectivity and potential differentiation patterns were estimated with haplotype network analyses implemented in PopART (Leigh & Bryant 2015) using TCS (Clement et al. 2000) as the network inference method. Neutrality statistics, including Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997), and the mismatch distribution analysis (MMA) (Rogers & Harpending 1992) were applied to test departures from the neutrality of mutations using Arlequin (Fay & Wu 1999; Hein et al. 2004; Nielsen 2001; Simonsen et al. 1995; Tajima 1989). Genetic distances were calculated in MEGA X (Kumar et al. 2018).

The demographic history of each target species was reconstructed with both MMA and the Bayesian skyline plots (BSP) (Drummond et al. 2005). MMA was conducted for 1,000 bootstrap replicates to search for signatures of rapid expansion and estimate the approximate time for each species (Rogers & Harpending 1992; Schneider & Excoffier 1999). Significance (P value) was calculated as the proportion of simulations producing a larger sum of square deviation (SSD) than the observed SSD. When a rapid expansion was detected, the parameter τ was computed, and the time since expansion in years before present was converted with mutation rate. The mutation rate per nucleotide (µ) was set as 1.55% per million years per generation, converted from 3.1% divergence per million years (Wares 2001). The raggedness index of the observed mismatch distribution was also calculated (Harpending 1994; Harpending et al. 1993). BSP was conducted to estimate the genealogy and demographic history using BEAST v1.10.4 (Barido-Sottani et al. 2017; Drummond & Rambaut 2007; Suchard et al. 2018). The configuration files were first generated with BEAUti v1.10.4 (Drummond & Rambaut 2007). Datasets were run for 100 (*C. euspinulosum* group 1 and 2) and 50 (*C. septimus* Pacific population) million generations under the strict clock model. For all analyses, each codon of COI was partitioned and unlinked for substitution rate parameters and base frequencies. A coalescent Bayesian skyline tree model with 10 groups and uniform priors was applied. The chain was sampled every 1,000 generations, and the first 10–20% generations were discarded as burn-in before reaching the stationary status. Trace plots were inspected to assess convergence, mixing, and stationary during the MCMC process, and the effective sample sizes (ESS) were also checked and confirmed to be more than 200 in Tracer v1.7.1 (Rambaut et al. 2018). The analysis of each dataset was run three times to ensure consistency, and the results of the three replicate runs were combined with LogCombiner v1.10.4 (Drummond & Rambaut 2007). The historical population dynamics were then analyzed in Tracer. On the other hand, preliminary BSP calculations for *D. angularis* failed to produce substantial ESS values despite multiple trials and parameter recombination and were henceforth discontinued.

Results

Host specificity of the coral-associated barnacles

The three target barnacle species had different degrees and genera of host coral usage through the Indo-West Pacific region (Fig. 1G). *Cantellius euspinulosum* lives mainly on *Porites* (~75% occurrence), but also can be found on other genera, including *Psammocora* (Dana 1848), *Montipora*, *Goniastrea* (4–9% occurrence), and with a few individuals found on *Acropora*, *Platygyra,* and *Stylophora* (< 1% occurrence). *Cantellius septimus* mainly lives on *Montipora* (89% occurrence) and is occasionally found on *Leptastrea* (Milne Edwards & Haime 1848b) (< 1% occurrence) and *Porites* (< 2.5%). *Darwiniella angularis* mainly lives on *Cyphastrea* (80% occurrence) and *Plesiastrea* (Milne Edwards & Haime 1848a) (17% occurrence), and with very few occurrences on *Leptastrea*, *Pavona* (Lamarck 1801)*, Stylophora,* and *Acropora* (< 1% occurrence) (Table 1).

Genetic pattern based on COI

A total of 516, 165, and 828 sequences were collected for the three target species, *C. euspinulosum*, *C. septimus*, and *D. angularis*, respectively (Table 2). The final aligned data matrix comprised 542, 543, and 420 nucleotide sites, including the shorter sequences from GenBank. High haplotype diversity (0.97, 0.99, 0.96) and nucleotide diversity (0.030, 0.018, 0.014) were observed in all species (Table 3). Different genetic connectivity patterns were observed across

Table 1 Host coral records of the three coral barnacle species (Chan et al. 2013, this study)

Species name	Host coral
Cantellius euspinulosum	*Acropora* sp.
	Goniastrea favulus
	Goniastrea minuta
	Goniastrea retiformis
	Montipora sp.
	Montipora venosa
	Pavona varians
	Platygyra sp.
	Porites cylindrica
	Porites lichen
	Porites lobata
	Porites lutea
	Porites rus
	Porites sp.
	Psammocora contigua
	Psammocora nierstraszi
	Psammocora profundacella
	Stylophora pistillata
Cantellius septimus	*Anacropora* sp.
	Galaxea astreata
	Leptastrea purpurea
	Montipora angulata
	Montipora danae
	Montipora efflorescens
	Montipora foliosa
	Montipora sp.
	Montipora undata
	Porites sp.
Darwiniella angularis	*Acropora solitaryensis*
	Asteopora sp.
	Cyphastrea chalcidium
	Cyphastrea microphthalma
	Cyphastrea serailia
	Cyphastrea sp.
	Goniastrea pectinata
	Leptastrea purpurea
	Leptoria phrygia
	Plesiastrea versipora
	Stylophora pistillata

the three coral barnacle species (Fig. 2). In *C. euspinulosum*, three groups (Groups 1–3) were detected, each being separated by several mutational changes. Two groups (1 and 2) were separated by 13 mutational changes yet considerably appeared to be geographically overlapped (Fig. 2A). A third group (Group 3), only collected from Papua New Guinea (PG) and southeastern Vietnam (VN_SE), was at least 16 mutational changes apart from Group 2. The most widespread haplotype was observed across 10 sites, namely: Taiwan (TW); Kochi (JP_KC) and Okinawa, Japan (JP_OK); Malaysia (MY); southeastern (VN_SE) and southwestern Vietnam (VN_SW); the Philippines (PH); Dongsha Atoll (DS); and Taiping Island (TP). *Porites* spp., *Montipora* sp., *Goniastrea minuta* (Veron 2000) were the predominant host corals for Groups 1 and 2, having *Porites* sp. as most common (Fig. 3A). On the other hand, *Montipora venosa* (Ehrenberg 1834), *Platygyra* sp., *Porites rus* (Forskål, 1775), *Psammocora* spp., and *Stylophora pistillata* (Esper 1792) only occurred at Group 1; *Acropora* sp., *Goniastrea favulus* (Dana 1848), *Porites cylindrica* (Dana 1848), *P. lutea* (Milne Edwards & Haime 1848b), and *Stylophora pistillata* exclusively occurred at Group 2. Group 3 was only found on *Porites* sp. The % K2P genetic distances across each group were: 4.43% (Group 1 *vs* 2), 4.57% (Group 2 *vs* 3), and 5.36% (Group 1 *vs* 3), whereas within-group K2P distances were all below 0.79%.

In *C. septimus*, the Phuket, Thailand, samples (TH_W) from the Indian Ocean appeared genetically distant from the rest of the Pacific samples, being apart by at least 16 mutational changes. Additionally, samples from Papua New Guinea also exhibited unique haplotypes (Fig. 2B). The most widespread and prevalent haplotype was observed in Taiwan, Kochi, and Okinawa, Japan, Malaysia, the Philippines, and eastern Thailand (TH_E). Pacific samples were predominantly found on *Montipora* spp. and with a few from *Anacropora* sp. (Oken 1815), *Leptastrea purpurea* (Dana 1848), and *Porites* sp. Phuket, Thailand, samples were exclusively found on *M. danae* (Fig. 3B). In *D. angularis*, all the Phuket, Thailand, samples alongside a few from Malaysia, Hong Kong (HK), southwestern Vietnam, and Oman, Red Sea (OM) formed a segregated group. Contrarily, the rest of the Pacific samples were highly mixed and closely interconnected. This main grouping was separated by 13 mutational changes. Samples from Papua New Guinea (PG) exhibited unique haplotypes and were separated only by at least 7 mutational changes from its closest Pacific relatives (Fig. 2C). The most widespread haplotype was detected across 7 sites, namely: Kochi, Japan; Malaysia; eastern Thailand; southeastern Vietnam; Hong Kong; and Taiwan. Regardless of the sampling locations and population structuring, *D. angularis* exhibited varied host coral usage, spanning mostly from *Cyphastrea serailia* (Forskål, 1775), *C. chalcidium* (Forskål, 1775), *Cyphastrea sp*., and *Plesiastrea versipora* (Lamarck 1816) (Fig. 3C).

For all three species and subgroups, significantly negative Tajima's D and Fu's Fs values suggested population expansion except Tajima's D in *C. euspinulosum* and *D. angularis* Indian Ocean population (Table 3). Based on the mismatch distribution test, both the sum of square deviation (SSD) and the Raggedness index were not significantly different from a sudden expansion model except *C. euspinulosum* Group 1 and *D. angularis* (Table 3). The expansion time was the oldest in *C. euspinulosum* and the youngest in *C. euspinulosum* Group 1 based on the τ values. The sample size after expansion (θ_1) was about the same, except infinite for *C. euspinulosum* Group 1. The expansion time was estimated as *C. euspinulosum* Group 1: 0.005 (0.001–0.005 for 95% confidence interval); *C. euspinulosum* Group 2: 0.361 (0.225–0.458);

Table 2 Sampling location and number of individuals of each of the three coral barnacle species studied herein

Abb	Location	*Cantellius euspinulosum*	*Cantellius septimus*	*Darwiniella angularis*
JP_KC	Kochi, Japan	22	14	436
JP_OK	Okinawa, Japan	25	12	0
TW	Taiwan	129	47	71
HK	Hong Kong	56	2	197
DS	Dongsha Atoll	11	4	0
PH	Bolinao, Philippines	2	1	0
TP	Taiping Island	4	2	0
VN_SE	Nha Trang, Vietnam	53	22	4
VN_SW	Phú Quốc, Vietnam	6	0	32
TH_E	Chumphon, Thailand	6	7	9
TH_W	Phuket, Thailand	6	3	28
MY	Johor, Malaysia	91	15	38
PG	Madang, Papua New Guinea	105	36	12
OM	Oman	0	0	1
	Total	**516**	**165**	**828**

Table 3 Sample size (n), genetic diversity of COI for the three coral barnacle species, *Cantellius euspinulosum* (Ceu), *C. septimus* (Cse) and *Darwiniella angularis* (Dan), including the number of haplotypes (Nh), haplotype diversity (h), nucleotide diversity (π), neutrality tests including Tajima's D and Fu's Fs, mismatch distribution test including τ, θ_0, θ_1, Harpending's raggedness index, and the sum of squared differences (SSD). The mismatch distribution test was only conducted when the sample size was higher than 100. Ceu_1, 2 and 3 correspond to the three haplotypes in Fig. 2A. Cse_P and Dan_P: Pacific population of *C. septimus* and *D. angularis*. Dan_I: Indian Ocean population of *D. angularis*. Note no genetic diversity and neutrality test can be conducted on Indian population of *C. septimus* due to low sample size (see Fig. 2B). CI: confidence interval. *P < 0.05 (0.02 in Fs), **P < 0.01, ***P < 0.001

		Diversity			Neutrality test	
	n	Nh	h	π	D	Fs
Ceu	516	247	0.970	0.02985	−0.88950	−23.48410*
Ceu_1	221	114	0.911	0.01499	−1.88535***	−24.39797***
Ceu_2	259	108	0.945	0.00974	−1.98465*	−24.97531**
Ceu_3	36	25	0.973	0.00662	−1.55753*	−20.68724***
Cse	165	121	0.988	0.01811	−1.84320**	−24.29281***
Cse_P	162	119	0.987	0.01715	−1.91086**	−24.36327***
Dan	828	229	0.959	0.01368	−1.95493**	−24.31293***
Dan_P	776	199	0.953	0.00969	−2.13268***	−24.93870**
Dan_I	52	30	0.952	0.01081	−1.49870	−19.78899***

	Mismatch distribution test				
	τ (95% CI)	θ_0	θ_1	SSD	Raggedness index
Ceu	23.992 (13.697–29.158)	0.000	32.309	0.006	0.003
Ceu_1	0.08789 (0.016–0.086)	0.000	9999.000	0.726***	0.003
Ceu_2	6.063 (3.787–7.697)	0.000	28.242	0.005	0.014
Ceu_3	N/A	N/A	N/A	N/A	N/A
Cse	11.348 (7.438–14.238)	0.031	31.202	0.001	0.002
Cse_P	11.295 (8.127–13.252)	0.012	32.574	0.001	0.003
Dan	4.561 (3.426–6.246)	0.009	29.033	0.005***	0.014
Dan_P	4.410 (2.645–4.801)	0.000	49.141	0.003	0.017
Dan_I	N/A	N/A	N/A	N/A	N/A

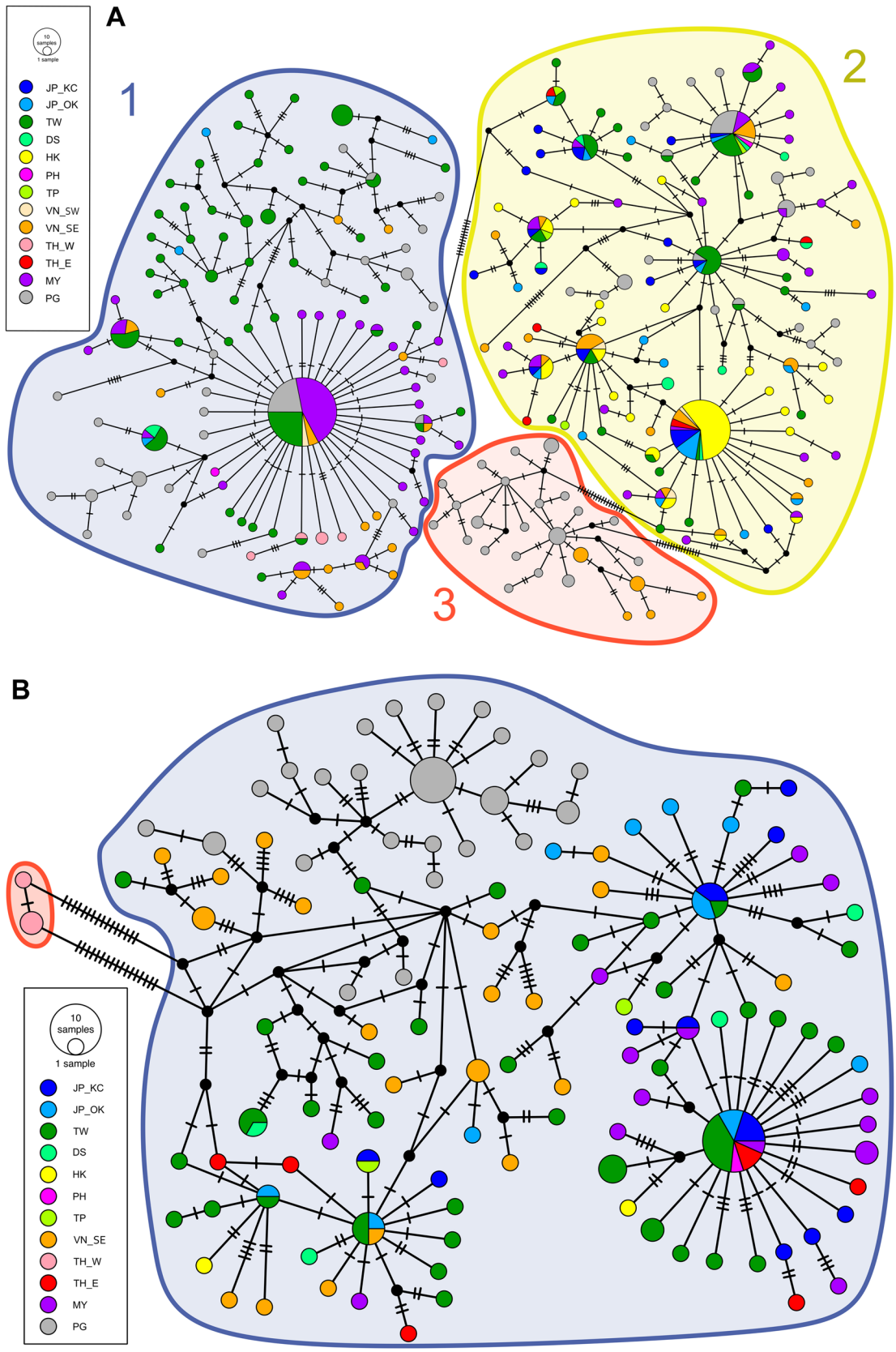

Fig. 2 Haplotype networks of (A) 516 *Cantellius euspinulosum*, (B) 165 *C. septimus,* and (C) 828 *Darwiniella angularis* based on 542, 543, and 420 bp of mitochondrial COI sequences, respectively. The haplotypes are color-coded according to sampling locations. Groups with distinct genetic differences were identified and circled

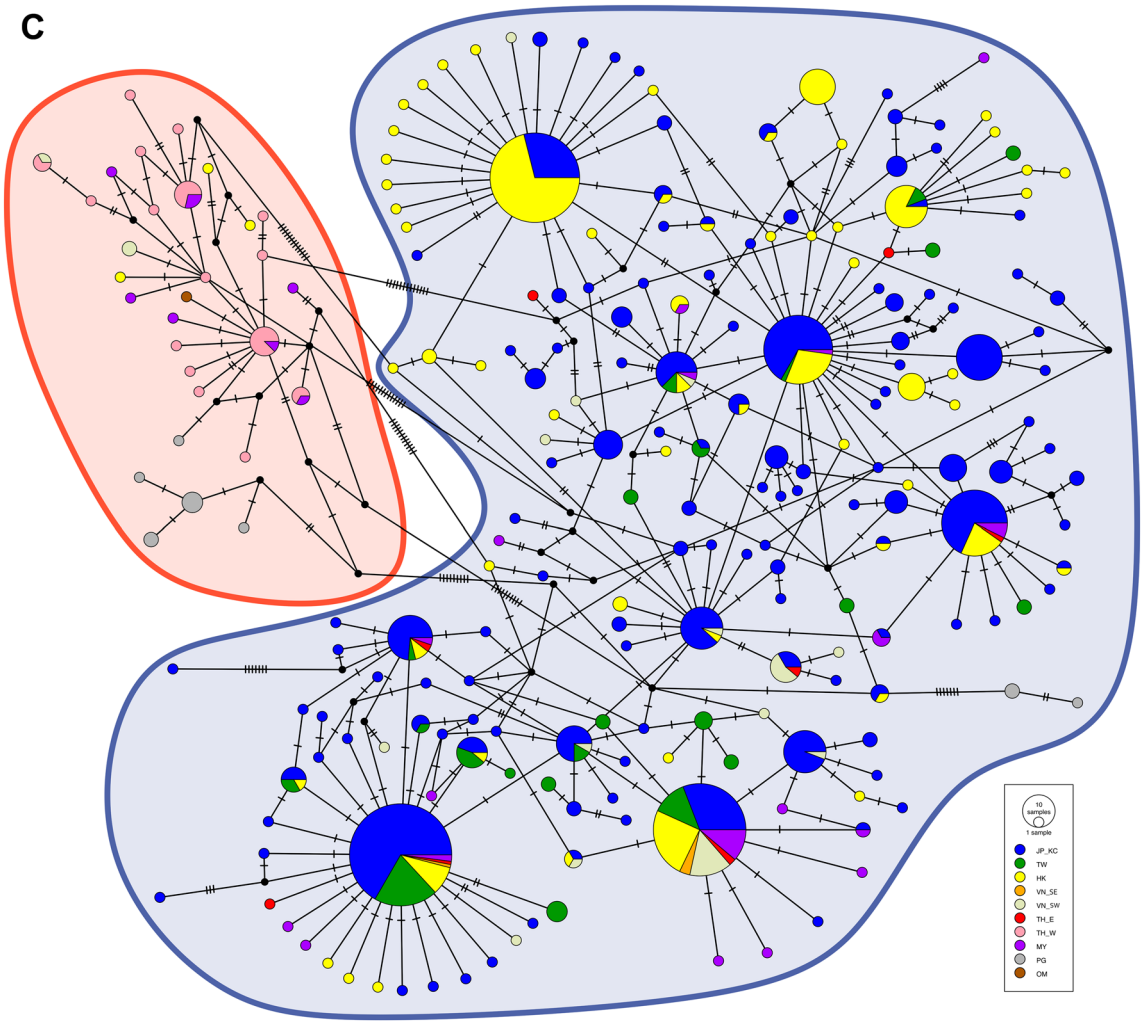

Fig. 2 (continued)

C. septimus Pacific population: 0.671 (0.483–0.787); and *D. angularis* Pacific population: 0.339 (0.203–0.369) million years before present based on the τ values of 0.08789 (0.016–0.086), 6.063 (3.787–7.697), 11.295 (8.127–13.252), 4.410 (2.645–4.801) and u value of 8.401, 8.401, 8.417, and 6.510 per million years per generation for the 542, 542, 543, 420 nucleotide-long COI, respectively (Table 3). The expansion timing estimated with Bayesian Skyline Plot for *C. euspinulosum* Group 1: 0.131; *C. euspinulosum* Group 2: 0.106; and *C. septimus* Pacific population: 0.247 million years ago with an effective population size change from the magnitude of 10^6 to 10^7–10^8 (Fig. 4).

Discussion

Cantellius euspinulosum, *C. septimus,* and *D. angularis* are widely distributed in the Indian and Western Pacific Oceans, and we observed variations in population genetic patterns among these three species with different degrees of host specificity. The relatively generalist *C. euspinulosum* has high gene flow between the Indian and Pacific populations, which is similar to other generalist coral-associated barnacles such as *Cantellius arcuatus* (Hiro 1938), *Galkinius tabulatus* (Chan et al. 2013), and *G. equus* (Chan et al. 2013) (Yu et al. 2023). However, sympatric host-driven genetic differentiation was observed in the Pacific populations of *C. euspinulosum* (Figs. 2 and 3), with two sympatric groups displaying significant mutational changes (Groups 1 and 2), which mostly inhabited *Porites* spp. There were several host corals of *C. euspinulosum* which were exclusively utilized by Groups 1 and 2, respectively. For instance, *Montipora venosa*, *Platygyra* sp., *Porites rus,* and *Psammocora* were only inhabited by Group 1, whereas *Acropora* sp., *Goniastrea favulus*, *Porites cylindrica*, *P. lutea*, and *Stylophora pistillata* were exclusively inhabited by Group 2. The

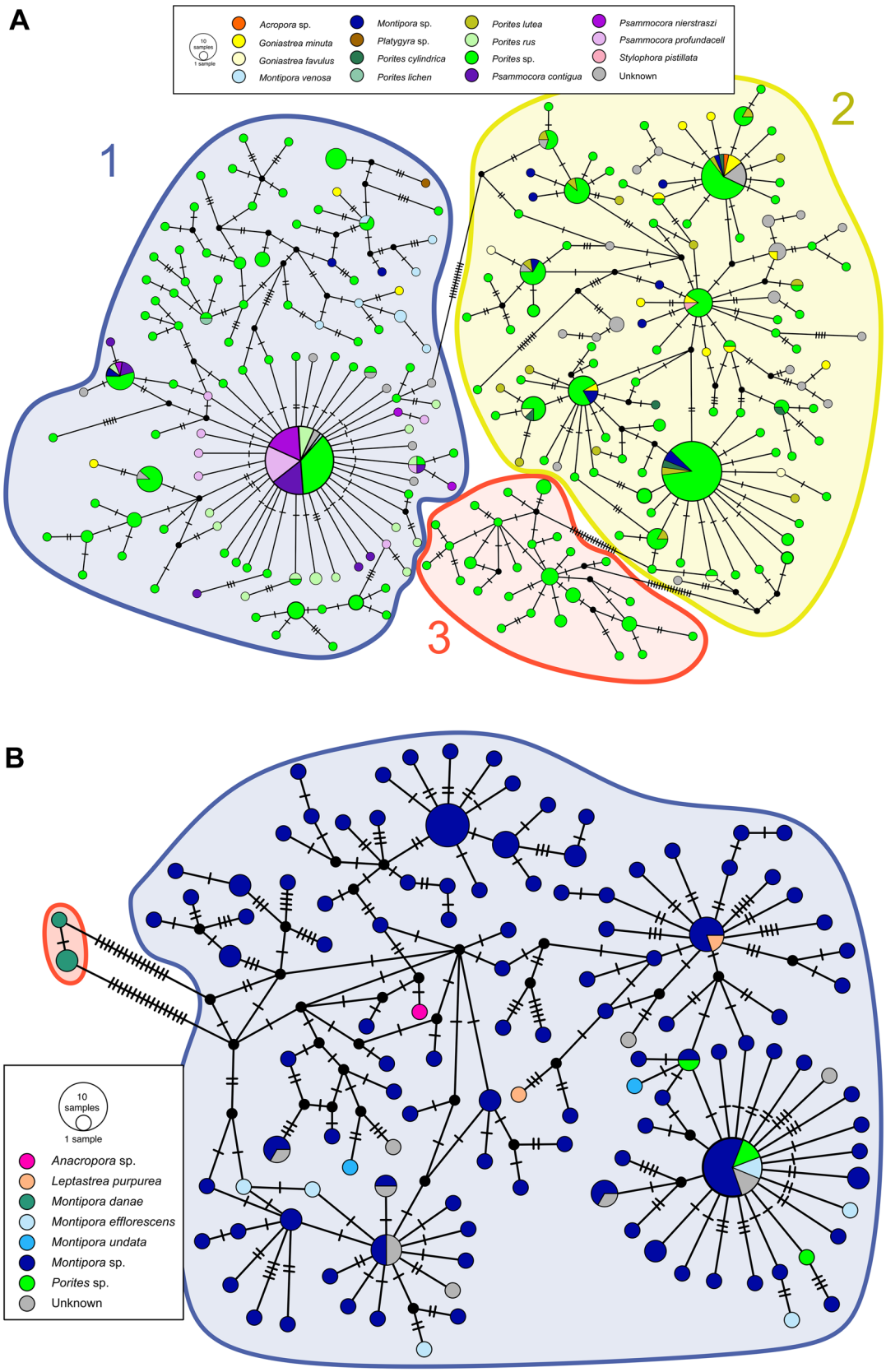

Fig. 3 Haplotype network of (A) *Cantellius euspinulosum*, (B) *C. septimus,* and (C) *Darwiniella angularis* color-coded according to host coral

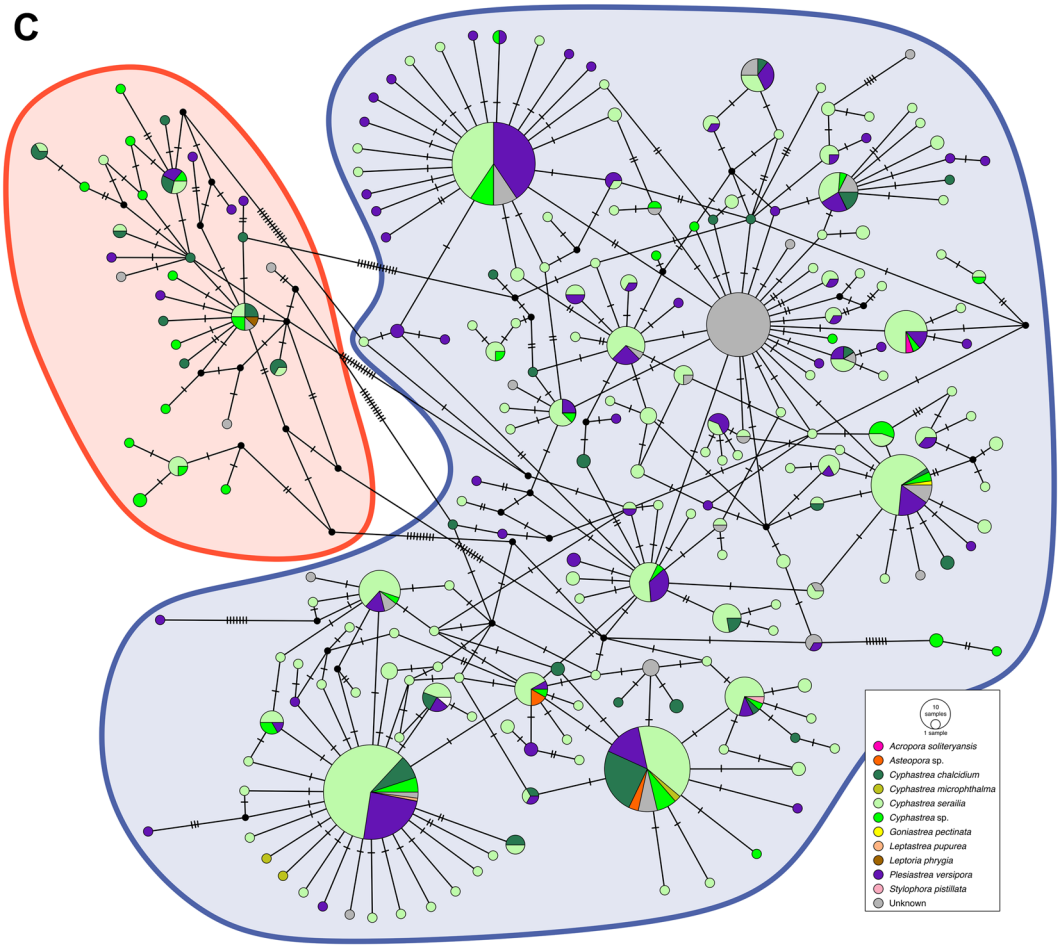

Fig. 3 (continued)

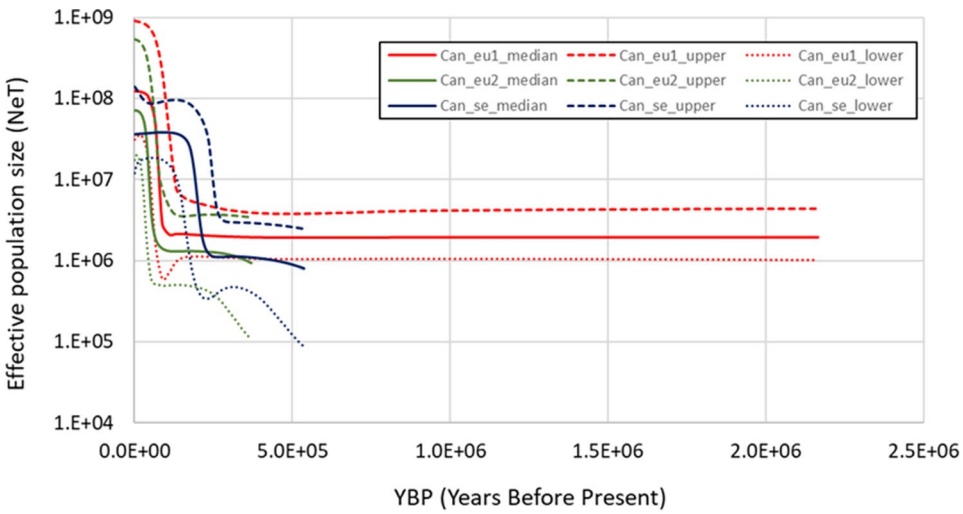

Fig. 4 Bayesian Coalescent Skyline Plot of *Cantellius euspinulosum* Group 1 (Can_eu1; red) and 2 (Can_eu2; green) and *C. septimus* Pacific population (Can_se; blue). The lines represent the median, upper, and lower 95% confidence interval of effective population size through time, respectively

assemblage of *C. euspinulosum* suggested the possibility of host-driven intraspecific differentiation, especially for the exclusive host corals for Groups 1 and 2. The occurrence of barnacles on non-*Porites* host corals was a minority (< 10%), indicating that these are probably rare hosts. Host specialization, as an adaptive strategy, is regarded as advantageous, especially during resource competition and microhabitat partitioning of the settling coral barnacle. Generalist species

can have spatial variation in host specificity, which is correlated with the latitudinal diversity of coral communities. In high latitude marginal coral communities in Jeju Island, South Korea, which has a lower diversity of corals, *C. arcuatus* only inhabits *Montipora millepora* (Crossland 1952) (Chan et al. 2018), but it inhabits greater number of hosts in Taiwan (Chan et al. 2018), indicating that generalist species can have host-shift depending on the availability of host corals. Similar cases on host shifts, prompted by the host coral's availability, have also been observed across various taxa, such as fishes (Munday et al. 2004), nudibranchs (Faucci et al. 2006; Hoeksema et al. 2018), and the coral's algal symbionts (LaJeunesse et al. 2004). Developing host specialization strategies provides a competitive advantage over generalists by having greater efficiency in utilizing a specific coral as their host, hence a crucial factor for their overall survivability (Caley & Munday 2003). Furthermore, this is also known to develop the barnacle's physiological actions against their host's defensive response (Liu et al. 2016; Yap et al. 2023). Although more evidence is required to confirm full reproductive isolation across the genetic groups, it can be presumed that continued host specialization and assortative mating will likely lead to eventual speciation. Host-associated genetic differentiation has also been reported in *Wanella milleporae* (Darwin 1854), particularly inhabiting *Millepora* corals (Tsang et al. 2009, 2014). In *W. milleporae*, corresponding differences were also observed in the barnacle's morphology and its preference toward the type of coral growth forms (e.g., propensity to either an encrusting or branching-type *Millepora*). These morphological features are functionally suited to their respective hosts and are highly useful, particularly during the settlement period. This warranted a thorough examination of possible morphological differentiations across *C. euspinulosum* genetic groups and their plausible preferentiality toward a particular host coral morphotype.

The high intraspecific % K2P divergence of the mitochondrial COI gene observed in *C. euspinulosum* is comparable to that of other interspecific % K2P distance on barnacles, such as *Tetraclita* (Schumacher 1817) 3.4% ~ 4.2% (Chan et al. 2007), *Galkinius* (Perreault 2014) 7.0% ~ 13.2% (Chan et al. 2013), and across other species of *Cantellius* 4.1% ~ 5.0% (Mokady et al. 1999). This high genetic distance indicates that *C. euspinulosum* forms a species complex in the Indo-Pacific, and these genetic groupings may be putatively sorted as distinct cryptic species. The sympatric existence of the two *C. euspinulosum* groups in the Pacific implied a disruptive selection of the species, thus signifying the absence of geographical barriers. This disruptive selection in *C. euspinulosum* might have started when individuals developed differential preferences in host coral species (Coyne 2007). Additionally, with their sessile mode of adult life, lack of asexual reproduction, clumped distribution, and exclusive undertaking of internal fertilization, *C. euspinulosum*'s mode of reproduction can only rely on proximity to find potential mates. In the long run, this might have created a sort of reproductive isolation among their kind. The high diversity of coral reefs in the Indo-Pacific also suggests the abundance of novel or potentially untapped habitats for *C. euspinulosum*. This can promote future opportunities to evolve with more habitat specialization techniques, ultimately leading to species divergence.

In contrast to *C. euspinulosum, C. septimus* and *D. angularis* showed genetic differentiations between the Indian and Pacific Oceans, which correspond to the well-recognized historic Indo-Pacific Barrier (Fig. 2) (Fleminger 1986; Rocha et al. 2007). Similar Indo-Pacific differentiations were also reported in the relatively specialist coral-associated barnacles *Hiroa stubbingsi* (Ross & Newman 1973), *Galkinius maculosus* (Chan & Liu 2017), and *Savignium* spp. (Yu et al. 2023). The historic separation of these two ocean basins has resulted in the genetic separation of a variety of marine populations ranging from corals (Richards et al. 2016) to fishes (Chenoweth et al. 1998; Gaither et al. 2011; Ishikawa et al. 2004) and many invertebrates (Benzie 1999; Crandall et al. 2008; Duda Jr & Palumbi, 1999; Lal et al. 2017; Williams & Benzie 1997). For *C. septimus* and *D. angularis*, it is noteworthy to consider that the representatives of the Indian Ocean population were limited to only the Phuket region in the Andaman Sea. Differences in the intrinsic coral compositions of the Pacific and Indian Oceans might be a factor in the structuring of the two coral barnacle species. However, host matching and literature reviews corroborated that coral species found in the Andaman Sea area were also widely present in the Pacific region (Brown 2007; Hongo & Montaggioni 2015; Majumdar et al. 2018; Phongsuwan & Chansang 2012). Additionally, Obura (2012) proposed that the Andaman region should be regarded more as part of the Pacific marine realm due to the high affinity and relatedness of its fauna with that of the South China Sea and Western Pacific, than that of its adjacent Indian Ocean neighbors. Nevertheless, more representation of barnacle species from the greater Indian Ocean region will be relevant in understanding their genetic structuring from the wider inter-oceanic perspective. Despite the proximity, the contemporary disconnect of the Andaman Sea samples from the rest of the Western Pacific can perhaps be attributed to the biological limitation of the barnacle species and the oceanographic features of the area. Larval development of *D. angularis* is about 10 to 16 days (Liu et al. 2016; Yap et al. 2023). Monsoons and other oceanographic influences can act as key factors in these barnacles' population structuring based on the 2-week period of larval dispersal. Brown (2007) highlighted that the surface waters of the Andaman Sea circulate as a double gyre—anticlockwise during northeast monsoons and clockwise during southwesterly monsoons (Brown 2007).

This kind of circulation within the Andaman Sea and its adjacency with the narrow Malacca Strait—the gateway to the Pacific, could be a key driver in the genetic structuring of the species. The widespread connectivity of the Pacific group implied a lack of a biogeographic barrier for the species in the area and complementary distribution of present-day oceanographic conditions that assist successful larval transport throughout the region (Kool et al. 2011).

There are occurrences of distinct geographic haplotypes of *C. euspinulosum* (Group 3), *C. septimus,* and *D. angularis*. Separation of the Papua New Guinea group in the southern Pacific possibly entailed historical and present-day influences. Historically, periodic fluctuations in sea level have resulted in land bridges and genetic disjunction of marine populations from Papua New Guinea and Australia versus the rest of the Indo-Pacific (Ludt & Rocha 2015; Voris 2000). Presently, the isolation impacts of adjacent oceanic currents (e.g., Indonesian throughflow and Leeuwin current) and the presence of the Palau, Yap, and Mariana trenches appear to separate the oceanographic connections between these northern and southern parts of the Pacific and limit larval transport across these two regions. Similar genetic disjunctions between the north and south of the Pacific were also found in other sessile marine invertebrates including the coastal isopod *Alloniscus oahuensis* (Budde-Lund 1885), the deep-water barnacle *Scalpellum stearnsii* (Pilsbry 1890), and the deep-sea hydrothermal vent barnacle *Neoverruca* (Newman & Hessler 1989) (Huelsken et al. 2013; Lind et al. 2007; Macaranas et al. 1992; Santamaria & Koch 2023; Vogler et al. 2013). Although we recognize the lack of samples from the greater Indonesian Archipelago, representatives from this area might bridge the connection of the Papua New Guinea haplotypes with the rest of the southern Pacific.

There are sudden population size expansions observed for all of the three study species and subgroups based on the results of neutrality tests, mismatch distribution test, and the BSP. For *C. euspinulosum* Groups 1 and 2 and *C. septimus* Pacific population, the estimated timing according to the coalescence-based method BSP ranged from 0.11 to 0.24 MYA. This timing is quite close to *Semibalanus cariousus* (Pallas 1788), a barnacle species inhabiting rocky shores (Marko et al. 2010), yet much older than the deep-sea barnacle *Scalpellum stearnsii* (Lin et al. 2020), suggesting that similar historic events affected the population growth in shallow water. The current population size ranges from 36 to 105 million, and the two groups of the generalist *C. euspinulosum* are 2–3 times higher than the specialist *C. septimus*. This might signify higher fitness as a generalist, in having a more diverse number of hosts, considering the comparable life history traits of these two congeneric species. In other coral-related commensalisms, generalist attributes such as the flexibility of coral and algal relationships likewise displayed positive correlations with geographic distribution, dispersal ability, and demographic history (Zarate et al. 2024). For *C. euspinulosum* and other generalist coral-associated barnacles, this can also reflect that their strategies maximize exploitation advantages on resource availability, environmental and habitat accustomation, and perhaps resilience against disturbances, which in turn lead them to higher present-day effective population sizes.

Historically, during the Pleistocene, which spanned from 2.58 to 0.01 MYA (Head et al. 2021), the planet experienced repeated cycles of glaciations and deglaciations, which shaped climate and oceanographic dynamics, including in the Indo-Pacific region (Bates et al. 2014; Windler et al. 2021). Specifically, the two lattermost subdivisions of the Pleistocene—mid (~0.77 to ~0.13 MYA) and late subperiods (~0.13 to 0.01 MYA), contained the expansion times of our coral barnacle species and their intraspecific groups. We acknowledge that the mismatch distribution and BSP analyses suggested different expansion times, probably due to the differing aspects of their calculational methods, thus posing caveats on our interpretation (Grant 2015). All of the genetic groups, except for *C. euspinulosum* Group 1 (~0.005 MYA on mismatch distribution) and *C. euspinulosum* Group 2 (~0.106 MYA on Bayesian skyline), depicted population expansions during the mid to late Pleistocene and Holocene (~0.01 MYA to present). These Pleistocene and Holocene geological periods are separated and marked by distinct climatic patterns, which in turn affected population histories of a number of marine species (Cabanne et al. 2016). A noteworthy event during the mid-Pleistocene is the shift of the glacial cycle from 41,000 years to longer 100,000 year cycle patterns (Bates et al. 2014). The Indo-Pacific experienced a dramatic decrease in sea surface temperature, cooler winters, changes in thermocline depths, intensified monsoons, and faunal–floral turnovers (Li et al. 2008). Additionally, the South China Sea region was also a semi-enclosed basin with water passages closed during glacial periods, yet sediment deposition from shelves was occurring (Li et al. 2008). On the other hand, glacial periods were more amplified with pronounced precipitations and oceanographic upwelling, thus causing high marine productivity during the late Pleistocene (Dauner et al. 2022; Glasser et al. 2004). During one of its interglacial episodes, the Sangamonian interval (Otvos 2015), polar ice caps completely melted and the sea level rose to approximately 8 m higher than today—the highest record in recent times. In our context, this type of climatic and geological variation might have been a favorable opportunity for coral barnacles to diversify and thrive in the newly opened habitats. The oldest fossils of *Cantellius* and *Darwiniella* were found in Holocene terraces in Japan (Otvos 2015). The fossil records of pyrgomatinid barnacles matched with the expansion period recorded for *Cantellius* and *Darwiniella* in the present study. Currently, fossil records of coral-associated barnacles are scarce, and it is difficult to

deduce how diversification occurred during the Pleistocene to Holocene periods.

This study revealed that the genetic population structuring of three Indo-Pacific coral-associated barnacle species is a result of the interaction of host coral utilization, oceanographic currents, and historical events. The relatively generalist barnacle *C. euspinulosum* lacks Indo-Pacific genetic differentiation but has genetic differentiation in different hosts, indicating that host-driven genetic differentiations can happen in generalists when species invade new or a minority of novel host populations. The relative specialist species, *C. septimus* and *D. angularis*, often have differentiation between the Pacific and the Indian Oceans, suggesting that a shorter larval development period with less host usage may have fewer stepping stones to cross the Indian and Pacific Ocean current boundaries. The distinct genotypes in the Papua New Guinea waters observed in all three studied species suggest oceanographic separations between the northern and southern Pacific.

Availability Data And Materials

All haplotype sequences generated in this study have been deposited in GenBank under the accession numbers PQ482814 to PQ483060 (*Cantellius euspinulosum*), PQ482689 to PQ482809 (*C. septimus*), and PQ487815 to PQ488043 (*Darwiniella angularis*).

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Author contributions H.C.L. and B.K.K.C. conceptualized the study, collected and analyzed the data, wrote the manuscript, and secured funding. L.C.H. contributed to the analyses, results interpretation, drafts, and facilitated the manuscript preparation and submission. Y.N. rendered comments and supported the coral identification and interpretation. B.K.K.C. provided the overall supervision. All authors performed research and approved the final version of the manuscript.

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Declarations

Conflict of Interests The authors declare no competing interests.

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