

Patterns of population structure and historical demography of *Conus* species in the tropical Pacific*

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Abstract. We compared patterns of genetic population structure and historical demography of several tropical Pacific *Conus* species that have similar though not completely overlapping distributions. Although these species possess similar life histories with planktonic larval durations of at least three weeks and exhibit genetic homogeneity across broad expanses of their distributions, they show distinct patterns of genetic population structure that are largely associated with genetic differentiation of geographically isolated populations. While previous analyses of *C. ebraeus* (Linnaeus, 1758) and *C. miliaris* (Hwass in Bruguière, 1792) detected high levels of genetic differentiation among isolated locations in the eastern Pacific and at Easter Island for each species, respectively, *C. chaldaeus* (Röding, 1798) does not show robust evidence of population differentiation across the East Pacific Barrier, albeit the sample size from the eastern Pacific is small. In addition, unlike *C. chaldaeus* and *C. ebraeus*, *C. sanguinolentus* (Quoy and Gaimard, 1834) exhibits a strong genetic break at Hawaii. Analyses of mismatch distributions suggest recent population expansion of *C. chaldaeus* and *C. sanguinolentus* as well as the Hawaiian endemic species *C. abbreviatus* (Reeve, 1843) during the past one million years as was observed previously for tropical Pacific populations of *C. ebraeus* and *C. miliaris*. Taken together, these results show that although high dispersal rates appear to genetically homogenize broadly distributed species in the tropical Pacific, stochasticity in long-distance dispersal likely instigates genetic differentiation of geographically isolated and peripheral populations and results in discordant phylogeographic patterns among even closely related species. Thus, population divergence and speciation in the tropical Pacific likely occur among populations at isolated locations though gene flow tends to prevent differentiation at broad geographic scales in species with high potentials for dispersal.

Key words: Conidae, phylogeography, genetic differentiation, population expansion, cytochrome oxidase subunit I

Phylogeographic investigations can illuminate the factors responsible for current and historical distributional patterns of species and the origins and maintenance of earth's biotic diversity. Indeed, comparative phylogeography is a powerful approach for identifying processes related to the origins of contemporary patterns of genetic population structure among sets of species inhabiting the same biogeographic region and can reveal historical vicariant events that have similarly influenced these species (Avice 2000). For example, similar patterns of genetic subdivision among Atlantic Ocean and Gulf of Mexico populations of multiple phylogenetically disparate species suggest that populations in these regions were vicariantly separated in the past (Reeb and Avice 1990). In addition, concordant patterns of genetic differentiation among populations of tropical marine species in the Indian and Pacific Oceans support a previous vicariant separation of these populations associated with past low sea level stands

(Benzie and Stoddart 1992, McMillan and Palumbi 1995, Lavery *et al.* 1996, Miya and Nishida 1997, Williams and Benzie 1997, 1998, Duke *et al.* 1998, Benzie 1999, Duda and Palumbi 1999, Lessios *et al.* 1999, 2001, Williams *et al.* 2002, Bay *et al.* 2004, Teske *et al.* 2005, Reid *et al.* 2006, Crandall *et al.* 2008).

Many works have explored patterns of population genetic structure of a variety of species in the tropical Pacific, from Winans' (1980) allozyme studies of the milkfish *Chanos chanos* (Forsskål, 1775) to Crandall and co-authors' (2008) recent and impressively sample-rich phylogeographic studies of two neritid gastropod species based on analyses of mitochondrial gene sequences. The main conclusions from these investigations are that (1) patterns of population genetic structure differ considerably among species in the tropical Pacific and (2) dispersal ability and oceanic current patterns are subtly or in some cases strongly related to the degree of genetic subdivision among samples from different locations

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(e.g., McMillan *et al.* 1992, also Palumbi 1994). Nonetheless, differences in dispersal ability alone are usually insufficient to account for different phylogeographic patterns among species in this region (e.g., Palumbi *et al.* 1997, Crandall *et al.* 2008). Factors such as stochasticity in dispersal events (e.g., Palumbi *et al.* 1997, Lessios and Robertson 2006) and physiological or ecological phenomena associated with persistence and successful recruitment to particular locations (e.g., Hilbish and Koehn 1985, Lee and Ó Foighil 2004, Marko 2004, Kelly and Palumbi 2010) also affect genetic population structures of marine taxa.

Conus chaldaeus (Röding, 1798) and *C. ebraeus* (Linnaeus, 1758) are sister species (Duda and Kohn 2005) that show nearly identical distributions and are two of just three transpacific *Conus* species that occur in both the Indo-West Pacific and eastern Pacific (Röckel *et al.* 1995) (Figs. 1A, 1B). These species also have similar estimated minimum planktonic larval periods of 25–27 days (Kohn and Perron 1994). Based on phylogenetic reconstructions, *C. miliaris* (Hwass in Bruguière, 1792) shared a most recent common ancestor with *C. chaldaeus* and *C. ebraeus* approximately 10 million years ago (mya) (Duda and Kohn 2005). The range of *C. miliaris* overlaps largely with distributions of both *C. chaldaeus* and *C. ebraeus* though it is the only of these three species that is established at Easter Island [note: although *C. ebraeus* is reported to occur at Easter Island (Röckel *et al.* 1995), it is only rarely observed at this location and does not appear to be established (Duda, pers. obs.)] and in extreme northwestern parts of the Indian Ocean, including the Red Sea; unlike *C. chaldaeus* and *C. ebraeus*, it is absent from Hawaii and the Marquesas (Röckel *et al.* 1995) (Fig. 1C). *Conus miliaris* has an estimated minimum pelagic larval period of 23–27 days (Kohn and Perron 1994). Röckel *et al.* (1995) report that *C. sanguinolentus* (Quoy and Gaimard, 1834) occurs throughout much of Indo-West Pacific, is absent at Easter Island and purportedly does not occur in the central Indian Ocean and at Hawaii (Fig. 1D). Nonetheless, as described below and as discussed by Walls (1979) and Röckel *et al.* (1995), this species is likely confused with *C. lividus* (Hwass in Bruguière, 1792) which occurs in parts of the central Indian Ocean and within the Hawaiian Archipelago (Fig. 1D). *Conus lividus* has an estimated minimum pelagic larval period of at least four weeks (Kohn and Perron 1994), but the life history of *C. sanguinolentus* has not been described. In light of the possibility that descriptions of the life history of *C. lividus* may have actually considered *C. sanguinolentus*, we suspect that *C. sanguinolentus* also has a planktonic larval phase of at least four weeks. *Conus sanguinolentus* and *C. lividus* are closely related, but are not sister species (the eastern Pacific species *C. diadema* (Sowerby, 1834) is the sister species of *C. sanguinolentus*), and shared a most recent common ancestor with *C. chaldaeus*, *C. ebraeus* and *C. miliaris* about 25–30 mya (Duda and Kohn 2005). Based on

the similar life histories and present distributions of these species, we predict that they exhibit similar phylogeographic patterns and demographic histories as those determined previously for *C. ebraeus* and *C. miliaris*.

Previous analyses of phylogeographic patterns and demographic histories of two widespread *Conus* species in the tropical Pacific, *C. ebraeus* and *C. miliaris*, revealed similar patterns of population differentiation at geographically isolated locations (eastern Pacific and Easter Island, respectively), genetic homogeneity across large parts of the distributions of these species, and recent population expansion (Duda and Lee 2009, Duda and Lessios 2009). To compare patterns of population genetic structure and demographic histories of additional members of the marine gastropod genus *Conus*, including a sister species of *C. ebraeus* that exhibits an almost identical distribution in the Pacific and Indian Oceans, we examined sequences of a region of the mitochondrial cytochrome oxidase c subunit I (*COI*) gene of individuals of three widespread species, *C. chaldaeus*, *C. lividus* and *C. sanguinolentus*, from multiple geographic locations, and a species that is restricted to the Hawaiian Archipelago, *C. abbreviatus* (Reeve, 1843) that has a similar life history as the other species presented above (*i.e.*, a minimum planktonic larval phase duration of 26–32 days) (Kohn and Perron 1994), to address the following questions. Do *Conus* species with similar distributions as well as similar life history modes and anticipated high levels of gene flow as *C. ebraeus* and *C. miliaris* lack genetic population structure throughout large parts of their ranges in the tropical Pacific? Does *C. chaldaeus* exhibit genetic subdivision of Indo-West Pacific and eastern Pacific populations as observed for its sister species, *C. ebraeus* (Duda and Lessios 2009)? We also augmented the sample size of *C. ebraeus* from the eastern Pacific and obtained mitochondrial gene sequences from these specimens to more rigorously examine the putative genetic disjunction between the Indo-West Pacific and eastern Pacific that Duda and Lessios (2009) determined based on a relatively small sample size ($N = 10$) from the eastern Pacific. Although *C. abbreviatus* is largely restricted to the Hawaiian Archipelago (Röckel *et al.* 1995), we utilized information from this species to compare demographic histories of species with different range sizes.

MATERIALS AND METHODS

Specimens

We obtained specimens of *Conus abbreviatus*, *C. chaldaeus*, *C. lividus* and *C. sanguinolentus* from the field or from museum collections that came from many locations in the Indo-West Pacific, including Reunion Island (*C. sanguinolentus*), Papua New Guinea (*C. lividus* and *C. sanguinolentus*),

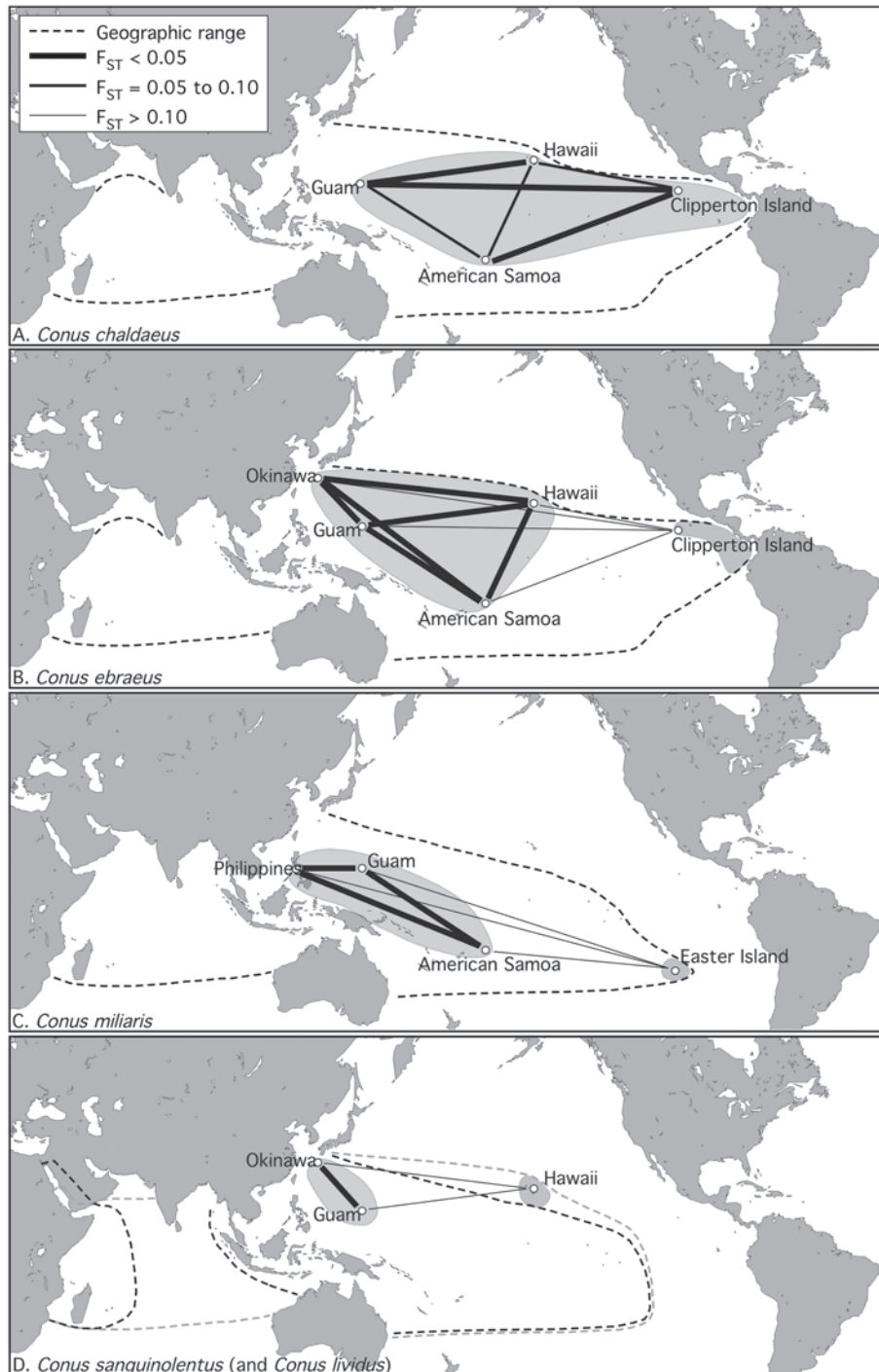


Figure 1. Geographic distributions (from Röckel *et al.* 1995) and patterns of genetic subdivision of tropical Pacific *Conus* species; sample locations where $N > 8$ and pairwise estimates of Φ_{ST} were obtained are indicated in figure. Dashed lines indicate geographic distributions of species. Solid lines indicate estimated values of Φ_{ST} ; line thickness corresponding to arbitrary cut-offs of Φ_{ST} values (less than 0.05, 0.05–0.1, and greater than 0.1) illustrate inferred levels of genetic differentiation among locations with sample sizes greater than eight individuals as indicated in the figure inset. Locations are grouped by shading to show genetically subdivided groups (i.e., with Φ_{ST} values greater than 0.1). **A.** *Conus chaldaeus*; **B.** *Conus ebraeus*; **C.** *C. miliaris* (based on Duda and Lee 2009); **D.** *C. sanguinolentus*, geographic distribution of *C. lividus* shown with dashed grey lines (see text).

Okinawa (*C. lividus* and *C. sanguinolentus*), Guam (*C. chaldaeus*, *C. lividus* and *C. sanguinolentus*), American Samoa (*C. chaldaeus*, *C. lividus* and *C. sanguinolentus*), French Polynesia (*C. sanguinolentus*), Hawaii (all four species) and Clipperton Island (*C. chaldaeus*). We 'preidentified' specimens of *C. lividus* and *C. sanguinolentus* based on the presence or absence of a pale central band on the last whorl of the shell, respectively. Ultimately, we confirmed or re-evaluated identifications of these two species based on the sequences obtained from these individuals (as described below). The sample size of *C. lividus* was too small to permit phylogeographic investigation of this species and so all analyses proceeded with the other species. We also obtained additional specimens from Clipperton Island from the Invertebrate Zoology collections at the Santa Barbara Museum of Natural History to increase the sample size of *C. ebraeus* from the eastern Pacific that was used in a previous study (*i.e.*, Duda and Lessios 2009).

DNA sequences

We extracted DNA from specimens using the Omega-Biotek EZNA Mollusc DNA kit (Doraville, Georgia, USA). We amplified a region of approximately 650 basepairs (bp) (excluding primers) of the mitochondrial *COI* gene with LCO1490 and HCO2198 primers (Folmer *et al.* 1994) at an annealing temperature of 45°C. We sequenced the amplification products directly in both directions with their original amplification primers at the University of Michigan DNA Sequencing Core facility. We evaluated chromatograms and sequence calls with Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and aligned sequences by eye with Se-Al v.2.0a11 (Rambaut 2002). We also included in our analyses *COI* sequences from three individuals that were preidentified as either *Conus lividus* or *C. sanguinolentus* from American Samoa, French Polynesia and Réunion Island that were gratefully provided by Christopher P. Meyer (National Museum of Natural History) and a previously published sequence from an individual of *C. lividus* from American Samoa (GenBank accession number AY588192, Table 1).

Sequence analyses

We constructed haplotype networks based on analyses of *COI* sequences of *Conus chaldaeus*, *C. lividus* and *C. sanguinolentus* using TCS 1.21 (Clement *et al.* 2000). *COI* sequences from specimens of both *C. lividus* and *C. sanguinolentus* were initially combined; positions of specimens within the resultant network were used to confirm or re-evaluate the identities of these specimens (described in *Results*). Numbers of haplotypes and haplotype diversity of each species and each location were determined with Arlequin version 2.0 (Schneider *et al.* 2000). Modeltest v.3.7 (Posada and Crandall 1998) was

Table 1. Genbank accession numbers of *COI* sequences of *Conus* species.

Species	GenBank accession numbers
<i>C. abbreviatus</i>	HQ852701–HQ852725
<i>C. chaldaeus</i>	HQ852592–HQ852682
<i>C. ebraeus</i>	AY588175 ¹ , EF547559–EF547649 ² , HQ852683–HQ852700
<i>C. lividus</i>	AY588192 ¹ , HQ852563–HQ852591
<i>C. miliaris</i>	AY588203 ¹ , FJ392914–FJ393023 ³ , FJ411486–FJ411516 ³
<i>C. sanguinolentus</i>	HQ852472–HQ852562

¹from Duda and Rolán 2005

²from Duda and Lee 2009

³from Duda and Lessios 2009

used to determine the best model of nucleotide substitution for each species.

To examine patterns of phylogeography, we estimated Φ_{ST} values for all pairwise comparisons of samples of locations with sample sizes greater than eight individuals with Arlequin. We also used Arlequin to determine if values of Φ_{ST} deviated significantly from the null hypothesis of no differences between locations based on the proportion of 10,100 permutations of haplotypes between locations that gave Φ_{ST} values greater to or equal the observed Φ_{ST} values. Moreover, we conducted an analysis of molecular variance (AMOVA) using Arlequin to examine the partitioning of genetic variance within and among groups of populations (*i.e.*, samples from each location) for each species; group memberships contained samples from locations that exhibited Φ_{ST} values that were less than 0.01 and not significant. For *Conus chaldaeus* this included a combined sample from Guam and Hawaii ('Guam+Hawaii'); for *C. sanguinolentus* it included a combined sample from Okinawa and Guam ('Okinawa+Guam').

We examined the historical demography of populations with analyses of mismatch distributions (Rogers and Harpending 1992, Rogers 1995) of *COI* sequences also with Arlequin. As above, population sets were comprised of samples from different locations that showed no evidence of population structure (*i.e.*, with Φ_{ST} values < 0.01). We compared the mismatch distribution of these populations to expectations for demographic population expansion by examining the sum of squared deviations and Harpending's raggedness index of observed and expected distributions under a model of demographic population expansion with Arlequin. Estimates of times since population expansion (τ , the number of generations scaled by the mutation rate) and population sizes before (θ_0) and after (θ_1) expansion ($\theta = N_e \mu$, where N_e is the effective population size and μ is the mutation rate) and their 95% confidence intervals were also determined with Arlequin. Estimates of τ (*i.e.*, $\tau = 2\mu t$) were converted to

absolute time using a *COI* divergence rate of 3.7 substitutions per million year as utilized by Duda and Lessios (2009). We also estimated Tajima's *D* (Tajima 1989) and Fu's *F_s* (Fu 1997) statistics to evaluate demographic histories of populations of these species as well as *Conus miliaris* (these statistics were not originally determined by Duda and Lee 2009) with Arlequin. Significantly negative values of these statistics usually indicate recent population expansion though they can also be evidence of recent selective sweeps (Tajima 1989, Fu 1997).

We also conducted analyses using IMA (Hey and Nielsen 2007) to estimate scaled times of separation of populations, effective population sizes, and directional migration rates where appropriate. Initial runs were started with population samples (as defined above) of *Conus abbreviatus*, *C. chaldaeus*, *C. ebraeus* and *C. sanguinolentus* using initial parameter values suggested in the program manual and the Hasegawa, Kishino and Yano (1985) (HKY) model of nucleotide substitution. For *C. chaldaeus* we ran three independent analyses that included two sets of populations: Clipperton Island and American Samoa, Clipperton Island and Guam+Hawaii, and American Samoa and Guam+Hawaii. Final runs included input parameters that bounded the range of parameter values observed in test runs, a geometric heating scheme with 20 chains, a burn-in of 10^5 steps and at least 10^5 saved genealogies (*i.e.*, at least 10^6 additional steps after burn-in). In addition, we also employed msBayes (Hickerson *et al.* 2007) to test for simultaneous divergence of Indo-West Pacific and eastern Pacific populations of *C. chaldaeus* and *C. ebraeus*. We utilized default bounds for prior distributions of all parameters as generated by the program and evaluated the posterior probabilities of models with one and two divergence times for the population-pairs of these species.

RESULTS

DNA sequences

We recovered *COI* sequences from 91 individuals of *Conus chaldaeus* from Guam ($N = 24$), American Samoa ($N = 29$), Hawaii ($N = 29$) and Clipperton Island ($N = 9$) (Table 2, Fig. 2A); an additional 18 individuals of *C. ebraeus* from Clipperton Island (Table 2, Fig. 2B); 114 individuals that were preidentified as *C. lividus* or *C. sanguinolentus* (Table 2, Fig. 2C) (location information is provided below) (the total number also includes sequences provided by C.P. Meyer and one previously published sequence); and 25 individuals of *C. abbreviatus* from Hawaii (Table 2, Fig. 1E).

The sequences from *Conus lividus* and *C. sanguinolentus* occurred in two main groups in the network; sequences from these clades differed at a minimum of 57 sites (Fig. 2C). Based on the segregation patterns of individuals identified as *C. lividus* and *C. sanguinolentus* among these clades, the majority

Table 2. Sample sizes and information on *COI* haplotype diversity of *Conus* species from locations in the tropical Pacific. *N*, sample size.

Sample	<i>N</i>	Number of haplotypes	Haplotype diversity (SE)
<i>Conus abbreviatus</i>			
Hawaii	25	22	0.990 (0.014)
<i>Conus chaldaeus</i>			
Guam	24	15	0.964 (0.019)
American Samoa	29	17	0.899 (0.047)
Hawaii	29	16	0.923 (0.034)
Clipperton Island	9	6	0.833 (0.127)
<i>Conus ebraeus</i>			
Clipperton Island	28	16	0.947 (0.022)
Indo-West Pacific ¹	80	41	0.942 (0.018)
<i>Conus miliaris</i> ²			
Easter Island	61	40	0.965 (0.014)
Non-Easter Island	80	74	0.997 (0.003)
<i>Conus sanguinolentus</i>			
Okinawa	14	5	0.791 (0.067)
Guam	48	11	0.764 (0.042)
Hawaii	25	8	0.543 (0.119)

¹ from Duda and Lessios 2009

² from Duda and Lee 2009.

of the individuals assayed were *C. sanguinolentus* and approximately half of the individuals that were identified as *C. lividus* were actually *C. sanguinolentus*. In total, *COI* sequences were determined from 88 individuals of *C. sanguinolentus* from Réunion Island ($N = 1$) (sequence from C.P. Meyer), Papua New Guinea ($N = 1$), Okinawa ($N = 14$), Guam ($N = 48$), American Samoa ($N = 1$) (sequence from C.P. Meyer), French Polynesia ($N = 1$) (sequence from C.P. Meyer) and Hawaii ($N = 25$) (including one previously published sequence). All locations except French Polynesia contained at least one specimen that was incorrectly identified as *C. lividus*. Because of the small sample size of *C. lividus* ($N = 27$), further analyses were not conducted on samples of this species, but *C. lividus* occurred at all locations examined except for Réunion Island and French Polynesia where only one specimen from each location was available for study. All new sequences (including the three provided by C.P. Meyer) were deposited in GenBank (Table 1).

Sequence analyses

We detected 37 unique *COI* haplotypes from 91 individuals of *Conus chaldaeus* (Table 2, Fig. 2A). We obtained *COI* sequences from an additional 18 individuals of *C. ebraeus* from Clipperton Island, including several haplotypes that Duda and Lessios (2009) did not previously observe at this location (Table 2, Fig. 2B). We identified 23 unique *COI* haplotypes from 88 specimens of *C. sanguinolentus* (Table 2, Fig. 2C). Haplotype diversity was high for all samples; *C.*

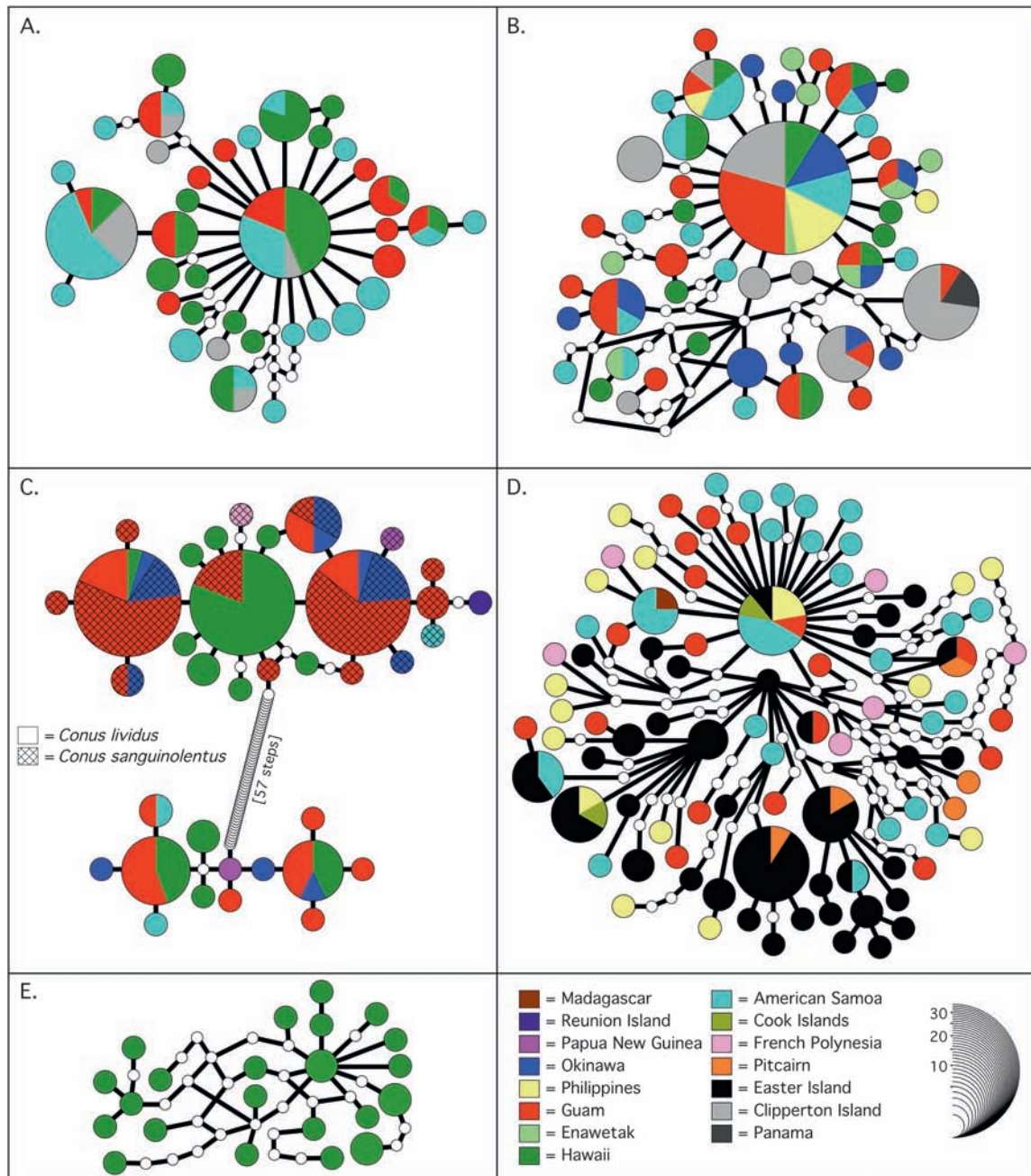


Figure 2. Haplotype networks of *COI* sequences of tropical Pacific *Conus* species. Each branch represents a single step/substitution between haplotypes; hypothetical haplotypes are indicated with small empty circles. Areas of other circles representing observed haplotypes are proportional to the observed frequency of each haplotype; scale given in lower right. Locations of haplotypes are color-coded and shown as pie diagram within haplotype circles based on legend in lower right. **A**, *Conus chaldaeus*; **B**, *C. ebraeus*; **C**, *C. lividus* and *C. sanguinolentus*; **D**, *C. miliaris* (figure adapted from Fig. 2 in Duda and Lee 2009); **E**, *C. abbreviatus*.

sanguinolentus, particularly at Hawaii, showed the lowest levels of diversity (Table 2). We also observed 22 unique *COI* haplotypes from 25 individuals of *C. abbreviatus* (Table 2, Fig. 2E). For comparison, this information is also presented

for the Indo-West Pacific populations of *C. ebraeus* and *C. miliaris* (Table 2, Figs. 2B, 2D).

As observed previously for *Conus ebraeus* and *C. miliaris* (Duda and Lee 2009, Duda and Lessios 2009), Modeltest

revealed that the HKY model (Hasegawa *et al.* 1985) was the most appropriate model of nucleotide substitution for the new datasets examined. Nonetheless, the HKY model is unavailable in Arlequin and so we used Tamura-Nei (Tamura and Nei 1993) distances. As discussed previously by Duda and Lessios (2009), Tamura-Nei and HKY distances differ at most at the fifth decimal place and so use of Tamura-Nei distances in Arlequin are unlikely to have noticeably affected our results.

We detected signals of genetic population differentiation for all species that were examined. For *Conus chaldaeus*, significant Φ_{ST} values were detected for comparisons that included samples from (1) American Samoa and Guam and (2) American Samoa and Hawaii (Table 3). Nonetheless, these values (0.063 and 0.065) were much lower than estimates of Φ_{ST} for samples of the other species. Moreover, the Φ_{ST} values from pairwise comparisons that included samples from Clipperton Island, the location with the smallest sample size ($N = 9$), ranged from -0.030 (compared to sample set from American Samoa) to 0.039 (compared to Guam) and 0.058 (compared to Hawaii), but none were significantly different from null expectations. Based on analyses of a larger sample size of *C. ebraeus* from Clipperton Island, we confirmed the previous finding of Duda and Lessios (2009) that this species exhibits a genetic break between populations in the Indo-West Pacific and eastern Pacific. Although our estimates of Φ_{ST} (0.098–0.119) are lower than those estimated previously [0.112 – 0.210; Duda and Lessios (2009)], they still differed significantly from null expectations. Pairwise estimates of Φ_{ST} values of *C. sanguinolentus* were restricted to samples from Okinawa, Guam and Hawaii due to the small sample sizes at other locations. While the estimate of Φ_{ST} between samples from Okinawa and Guam was low (-0.011) and not significantly different from zero, pairwise comparisons between samples from these locations and Hawaii yielded quite large Φ_{ST} values (0.322 and 0.235, respectively) that were significantly greater than null expectations (Table 4).

AMOVAS were conducted for two groups of *Conus chaldaeus* that contained samples from American Samoa and samples from Guam and Hawaii, two groups of *C. sanguinolentus* that contained samples from Hawaii and samples from Okinawa and Guam, and two groups of *C. ebraeus* that included samples from Clipperton Island and

Table 4. Pairwise Φ_{ST} values for samples of *Conus sanguinolentus* from different locations in the tropical Pacific.

	Guam	Hawaii
Okinawa	-0.011 ^{NS}	0.322*
Guam		0.235*

NS, not significant; *, $P < 0.001$

samples from Okinawa, Philippines, Guam, American Samoa, Enawetak and Hawaii (data for these latter samples from Duda and Lessios 2009). In all cases, most of the genetic variance was partitioned within populations (*i.e.*, samples from each location) with values of 93.6%, 76.8% and 87.8% recorded for *C. chaldaeus*, *C. sanguinolentus* and *C. ebraeus*, respectively; only minor fractions of the variance were partitioned among populations within groups (0.4%, -0.3% and -0.4%, respectively). AMOVA results showed that 6.0%, 23.5% and 12.6% of the genetic variance was partitioned among groups for *C. chaldaeus*, *C. sanguinolentus* and *C. ebraeus*, respectively.

Observed mismatch distributions of *COI* haplotypes corresponded to expectations of a model of recent population expansion for all populations examined, including *C. abbreviatus* from Hawaii, *C. chaldaeus* from American Samoa and combined samples from Hawaii and Guam, *C. ebraeus* from Clipperton Island and *C. sanguinolentus* from Hawaii and combined samples from Okinawa and Guam (Table 5). The 95% confidence intervals of estimates of the time since expansion scaled by mutation rate are broadly overlapping for all populations. Conversion of the values to absolute time provides estimates of times since expansion within the past one million years for most populations (Table 5). The only outlier is the population of *C. ebraeus* at Clipperton Island with an estimated time since expansion that ranged from 400 thousand to two million years ago; the population of this species from the Indo-West Pacific appears to have undergone expansion more recently (20,000–70,000 years ago). In most cases, analyses of Tajima’s D and Fu’s F_s confirmed results from analyses of mismatch distributions (*i.e.*, significantly negative values of these statistics that suggest recent population expansion were observed) (Table 5). Exceptions included the Clipperton Island population of *C. ebraeus* based on Tajima’s D estimate and the combined samples of *C. sanguinolentus* from Okinawa and Guam for both Tajima’s D and Fu’s F_s .

Parameter estimates from the IMA runs are presented in Table 6. In all cases results from multiple runs gave similar results. As indicated, a few of the estimated parameters exhibited incomplete posterior distributions in the analyses, presumably due to lack of sufficient information in the data. Estimates of θ ($\theta = 2N_e\mu$, where N_e = effective population size, μ = mutation rate) are largely comparable to values

Table 3. Pairwise Φ_{ST} values for samples of *Conus chaldaeus* from different locations in the tropical Pacific.

	American Samoa	Hawaii	Clipperton
Guam	0.063*	0.004 ^{NS}	0.039 ^{NS}
American Samoa		0.064**	-0.030 ^{NS}
Hawaii			0.058 ^{NS}

NS, not significant; *, $P < 0.01$; **, $P < 0.005$

Table 5. Mismatch distribution statistics from analyses of patterns of variation of *COI* haplotypes of populations of *Conus* species from the tropical Pacific. *N*, sample size; SSD, sum of squared deviations of observed and expected mismatch under a model of recent population expansion; HRI, Harpending's Raggedness index; τ , time since expansion scaled by the mutation rate (μ) (i.e., $\tau = 2\mu t$); *t*, absolute time in millions of years (my) since expansion based on estimated mutation rate (i.e., converted values of τ); θ_0 and θ_1 , initial and current estimates of theta ($\theta = 2N_e\mu$, N_e = effective population size); *D*, Tajima's (1989) *D* statistic; *F_s*, Fu's (1997) *F_s* statistic.

Sample	<i>N</i>	SSD	HRI	Mismatch distribution statistics				<i>D</i>	<i>F_s</i>
				τ (95% CI)	<i>t</i> (my) (95% CI)	θ_0 (95% CI)	θ_1 (95% CI)		
<i>Conus abbreviatus</i>									
Hawaii	25	0.003 ^{NS}	0.016 ^{NS}	5.6 (3.0–7.4)	0.8 (0.4–1.0)	0.1 (0.0–2.4)	78 (23–7250)	-1.8*	–∞***
<i>Conus chaldaeus</i>									
Guam+Hawaii	53	0.001 ^{NS}	0.032 ^{NS}	2.9 (1.5–3.5)	0.4 (0.2–0.5)	0.0 (0.0–1.5)	4081 (37–10067)	-2.0**	-22.4***
American Samoa	29	0.012 ^{NS}	0.047 ^{NS}	3.2 (1.4–5.6)	0.4 (0.2–0.8)	0.0 (0.0–1.3)	14.1 (5.1–6720)	-1.7*	-10.5***
Clipperton	9	0.143 ^{NS}	0.549*	4.7 (1.9–8.6)	0.6 (0.3–1.2)	0.0 (0.0–4.2)	12.9 (3.9–6609)	-0.9 ^{NS}	-1.0 ^{NS}
<i>Conus ebraeus</i>									
Clipperton	28	0.010 ^{NS}	0.020 ^{NS}	8.2 (3.1–13.9)	1.1 (0.4–1.9)	0.0 (0.0–4.4)	11.4 (5.0–173)	0.5 ^{NS}	-4.5*
Indo-West Pacific ¹	80	0.010 ^{NS}	0.020 ^{NS}	1.4 (0.4–5.5)	0.2 (0.1–0.7)	2.8 (0.0–8.4)	1062 (20.9–7586)	-2.3***	-11.2**
<i>Conus miliaris</i> ²									
Indo-West Pacific (non-Easter Island locations)		0.002 ^{NS}	0.008 ^{NS}	5.1 (3.1–10.1)	0.7 (0.4–1.4)	1.5 (0–2.5)	36.9 (15.8–4924)	-2.2***	-12.4***
Easter Island only		0.006*	0.021 ^{NS}	n/a	n/a	n/a	n/a	-1.8*	-13.1****
<i>Conus sanguinolentus</i>									
Okinawa+Guam	62	0.023 ^{NS}	0.063 ^{NS}	3.3 (0.9–7.1)	0.4 (0.1–1.0)	0.0 (0.0–1.8)	4.1 (1.4–2972)	-0.6 ^{NS}	-3.1 ^{NS}
Hawaii	25	0.000 ^{NS}	0.062 ^{NS}	0.9 (0.0–2.6)	0.1 (0.0–0.4)	0.0 (0.0–0.8)	3.4 (0.4–4779)	-2.1**	-5.4***

NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$

¹ from Duda and Lessios 2009, except for Tajima's *D* and Fu's *F_s* which were calculated in the present study

² from Duda and Lee 2009, except for Tajima's *D* and Fu's *F_s* which were calculated in the present study

estimated based on mismatch distributions and in most cases suggest that the populations have undergone recent expansion. In addition, except for a few cases, estimates of times of population separation from IMA do not conflict with estimates of times of population expansion based on mismatch distributions (assuming that the separation preceded the expansion). Based on these results, Indo-West Pacific and eastern Pacific populations of *Conus chaldaeus* separated earlier than those of *C. ebraeus*, but the posterior density intervals for these parameters are broadly overlapping (Table 6). Evaluation of the divergence times of the Indo-West Pacific and eastern Pacific populations of *C. chaldaeus* and *C. ebraeus* with msBayes revealed higher posterior probabilities for a model of a single timing of separation for these populations based on categorical regression (0.965) and

simple rejection (0.786) than for a model of two divergence times (0.035 and 0.214, respectively).

DISCUSSION

Phylogeographic analyses of mitochondrial sequence data revealed both concordant and discordant patterns among four species of *Conus* in the tropical Pacific (Fig. 1). First, as reported previously for *C. ebraeus* and *C. miliaris* (Duda and Lessios, 2009, Duda and Lee 2009), *C. chaldaeus* and *C. sanguinolentus* showed no genetic population structure throughout large parts of their ranges spanning several thousand kilometers (Figs. 1A, 1D). But despite rather extensive genetic homogeneity across wide geographic areas, at least three of the four species with

Table 6. Estimates of population parameters of *Conus* species based on analyses with IMA. N , number of genealogies examined; θ_i , $2N_e\mu$ (N_e = effective population size, μ = mutation rate; subscripts used for estimates of the populations compared (1 = first listed, 2 = second listed, A = ancestral population)); HPD, 95% highest posterior density interval, except where indicated; t_μ , time of population separation scaled by mutation rate; t , time of separation based on rate calibration; m , migration rate from population scaled by mutation rate where $2N_e m_i = \theta m/2$ (m_i = female migration rate; subscripts used for estimates of the populations compared (1 = first listed, 2 = second listed)).

Population	N	θ_1	θ_2	θ_A	t_μ	t (my)	m_1	m_2
comparison	(x10 ⁵)	(HPD)	(HPD)	(HPD)	(HPD)	(HPD)	(HPD)	(HPD)
<i>C. abbreviatus</i>	3.0	96.5 (55.5–171)	n/a	n/a	n/a	n/a	n/a	n/a
<i>C. chaldaeus</i>								
Hawaii+Guam and American Samoa	17.4	93.8 (27.3–324)	20.3 (4.3–88.8)	0.3 (0.0–292)	1.3 (0.4–9.9) ¹	0.35 (0.11–2.7)	1.6 (0.0–4.9)	0.1 (0.0–5.2)
Hawaii+Guam and Clipperton	2.9	68.3 (16.8–343)	9.3 (0.8–286)	0.3 (0.0–406)	1.3 (0.6–10.0) ¹	0.35 (0.16–2.7)	2.7 (0.8–6.0) ¹	0.1 ² n/a
American Samoa and Clipperton	3.0	24.3 (7.8–461) ¹	6.8 (0.0–458) ¹	8.3 (0.0–441) ¹	0.5 (0.1–10.0) ¹	0.1 (0.0–2.7)	0.0 ² n/a	4.7 (0.4–6.0) ³
<i>C. ebraeus</i>								
Indo-West Pacific and eastern Pacific	1.4	9995 ³ (1555–9995)	5.0 (0.0–85.0)	45.0 (0.0–135.0)	0.3 (0.1–0.5)	0.08 (0.03–0.14)	0.1 (0.0–4.50)	14.9 (0.0–129.9) ¹
<i>C. miliaris</i> ⁴								
Easter Island and non-Easter Island	50.0	72.6 (31.1–159.3) ⁵	6128 (3677–171,502) ⁵	31.8 (10.6–64.3) ⁵	1.7 (1.2–2.5) ⁵	0.45 (0.32–0.67) ⁵	0.6 (0.0–2.4) ⁵	1.3 (0.7–2.1) ⁵
<i>C. sanguinolentus</i>								
Hawaii and Okinawa+Guam	30.0	10.7 (4.3–46.7) ¹	9.2 (3.2–22.5)	0.0 (0.0–44.0) ¹	0.7 (0.2–10.0) ¹	0.19 (0.05–2.7)	0.2 (0.0–2.2)	0.0 (0.0–1.6)

¹ distinct peak but posterior density does not reach zero at upper limit of the prior

² multiple peaks, HPD not reported

³ increasing posterior distribution to a plateau

⁴ from Duda and Lee (2009)

⁵ 90% HPD presented as reported by Duda and Lee (2009)

adequate sample sizes (i.e., not including *C. lividus*) exhibited a uniquely located strong genetic break that occurred at peripheral and isolated locations (Figs. 1B, 1D).

In addition, most populations exhibited recent demographic population expansion that is estimated to have occurred during the past one million years (Table 5). Previous analyses of mismatch distributions of the eastern Pacific population of *Conus ebraeus* rejected the hypothesis of recent population expansion for this population (Duda and Lessios 2009). As shown here, analysis of a larger sample size from Clipperton Island failed to reject this hypothesis and so, as mentioned by Duda and Lessios, interpretations of results from mismatch distributions based on small sample sizes should be considered with caution.

Phylogeographic breaks across the East Pacific Barrier

Analysis of a larger sample size of *Conus ebraeus* confirmed previous interpretations based on the smaller sample size used by Duda and Lessios (2009) that the East Pacific Barrier limits gene flow among populations of *C. ebraeus* in the Indo-West Pacific and eastern Pacific and constitutes a strong

phylogeographic break for this species (Fig. 1B). Nonetheless, although the sample size from the eastern Pacific (i.e., Clipperton Island) was small, *C. chaldaeus* did not show this pattern very strongly: estimated Φ_{ST} values among the Clipperton Island sample and Hawaii, Guam and American Samoa samples of this species were all not significantly different from null expectations of a random distribution of haplotypes among locations and the Φ_{ST} value calculated between Clipperton Island and American Samoa samples is negligible (Table 3). Under the assumption that the small sample size was not responsible for failure to reject our null hypothesis of genetic homogeneity across the East Pacific Barrier, the discordant phylogeographic patterns of *C. chaldaeus* and *C. ebraeus*, two sister species that likely share a number of traits aside from similar dispersal abilities, was similar to the disparate patterns observed by Lessios and Robertson (2006) for 20 transpacific fish species that also span the East Pacific Barrier. Because of the small sample size of *C. chaldaeus* from the eastern Pacific, however, these interpretations should be considered with caution until a larger sample size from the eastern Pacific is obtained and examined.

The lack of strong evidence of a coincident genetic break across the East Pacific Barrier for both *Conus chaldaeus* and *C. ebraeus* may have resulted from the following factors: (1) fine scale differences in life history attributes as suggested by Crandall *et al.* (2008) as the possible causes of the discordant phylogeographic patterns of *Nerita albicilla* Linnaeus, 1758 and *Nerita plicata* Linnaeus, 1758 in the Indo-West Pacific, (2) differences in ecological features as suggested by Marko (2004) for *Nucella lamellosa* (Gmelin, 1791) and *Nucella ostrina* (Gould, 1852) in the northeastern Pacific and by Crandall *et al.* (2008) for the two nerite species, or (3) stochasticity in long-distance dispersal events or local extinctions as suggested for discordant patterns of four closely related Indo-West Pacific sea urchin species (*Echinometra*) by Palumbi *et al.* (1997) and 20 transpacific fish species by Lessios and Robertson (2006). *C. chaldaeus* and *C. ebraeus* exhibit similar life histories (Kohn and Perron 1994). In addition, although *C. chaldaeus* and *C. ebraeus* show slight differences in feeding ecologies (Kohn 1959, Kohn and Orians 1962), how these differences would have contributed to the discordant phylogeographic patterns in light of the nearly identical distributions of these species is difficult to interpret. We favor the explanation that stochasticity in long-distance dispersal or population extinction is responsible for the observed patterns. Under this scenario, *C. ebraeus* has had a longer history in the eastern Pacific than *C. chaldaeus* (due to an earlier colonization or massive dispersal event in *C. ebraeus* or a more recent extinction of *C. chaldaeus*) or only *C. chaldaeus* has experienced recent gene flow across the East Pacific Barrier. Although the 95% highest posterior density intervals are wide and some analyses failed to provide appropriate estimates of parameters, results from IMA suggest that the separation of Indo-West Pacific and eastern Pacific populations of *C. ebraeus* is actually more recent (80,000 years ago) than *C. chaldaeus* (350,000 years ago) and that migration rates are higher for populations of *C. ebraeus* (Table 6). Moreover, our data support a simultaneous divergence of Indo-West Pacific and eastern Pacific populations of these species based on model-testing with msBayes. Thus, neither of these hypotheses is supported by our data and we suspect that the sample size of *C. chaldaeus* from the eastern Pacific is perhaps too small to appropriately evaluate its history and degree of differentiation from populations in the Indo-West Pacific.

Phylogeographic patterns in the western and central Pacific

Similar to results observed for these four *Conus* species, many other demersal species with high dispersal potential do not exhibit genetic population structure within large parts of their ranges in the tropical Pacific. These include other gastropods (*Nerita albicilla* and *Nerita plicata*, Crandall *et al.* 2008), and various echinoderms (*Diadema paucispinum* A. Agassiz, 1863 and *Diadema savignyi* Michelin, 1845 Lessios *et al.* 2001; *Linckia laevigata* Linnaeus, 1758, Williams *et al.*

2002; *Tripneustes* species, Lessios *et al.* 2003). The concordance of these results suggests that possession of a relatively long duration planktonic larval phase enhances gene flow or that these species all underwent recent expansion throughout this region and there has not been enough time or local population sizes have been too large for genetic drift to cause differentiation at local scales. On the other hand, a number of tropical Pacific species with high dispersal potential exhibit genetic differentiation in this region, including several molluscs (three *Tridacna* species, Benzie and Williams 1997), several sea urchins (four *Echinometra* species, Palumbi *et al.* 1997; *Eucidaris metularia* Lamarck, 1816, Lessios *et al.* 1999), a lancelet (*Asymmetron lucayanum* clade B Andrews, 1893, Kon *et al.* 2006), and multiple fish species (*Chanos chanos*, Winans 1980 and Ravago-Gotanco and Juinio-Meñez 2004; *Dascyllus trimaculatus* Rüppell, 1829, Bernardi *et al.* 2001; *Acanthurus triostegus* Linnaeus, 1758, Planes and Fauvelot 2002; *Chlorurus sordidus* Forsskål, 1775, Bay *et al.* 2004). Although these phylogenetically disparate taxa likely exhibit differences in their potential for dispersal in terms of the lengths of their planktonic larval periods, the contrasting patterns of genetic population structure may be due to the stochasticity in long-distance dispersal or different ecological attributes as discussed above for transpacific species.

Among the four *Conus* species that have been examined to date, the greatest signal of a genetic break occurs for *C. sanguinolentus* at Hawaii. The density of our sampling of *C. sanguinolentus* in the tropical Pacific is poor and we did not include any samples from locations between Guam and Hawaii (e.g., from the Marshall Islands) or from regions in the South Pacific (e.g., American Samoa). Thus, the location of the break at Hawaii itself may be inaccurate and the range of this genetically differentiated population may actually encompass a larger geographic area. Nonetheless, the geographic isolation of the Hawaiian Archipelago in the tropical Pacific at least associates a mechanism (i.e., reduced gene flow at an isolated location) with the hypothesis that the break occurs uniquely at Hawaii. Also, the pattern observed for *C. sanguinolentus* contrasts quite strongly with the lack of genetic differentiation of samples of both *C. chaldaeus* and *C. ebraeus* at this location. Moreover, the Φ_{ST} values that are associated with pairwise comparisons of samples of *C. sanguinolentus* at Hawaii and other locations (0.235–0.322; Table 3) are at least 1.7 times greater than values associated with samples of *C. miliaris* at Easter Island and other locations in the Indo-West Pacific (0.072–0.137; Duda and Lee 2009) and samples of *C. ebraeus* in the eastern Pacific and Indo-West Pacific (0.098–0.119). In addition, these samples also exhibit the lowest level of haplotype diversity for all samples that were examined (Table 2) and results from mismatch distribution analyses suggest that this population expanded more recently than other populations of *Conus* in the tropical Pacific, including the Hawaiian endemic *C. abbreviatus* (Table 5). Together these

results suggest that *C. sanguinolentus* at Hawaii is isolated from other populations in the tropical Pacific and that it may have undergone a recent founder event with subsequent expansion at this location. Clearly, a much greater density of samples from the tropical Pacific and analyses of additional loci would help to more precisely identify the location of the genetic break as well as the history of its formation.

Inferences from the demographic histories of populations

All populations examined exhibited patterns of genetic variation that suggest recent demographic expansion within the past one million years, including the Hawaiian endemic *Conus abbreviatus* (Table 5). The nerite species examined by Crandall and coworkers (2008) show this same timing for their expansion as well. The coincidence of these timing suggests a common explanation for the demographic expansion such as expansion of suitable habitats caused by episodes of low sea-level stands during the Pleistocene (Paulay 1990).

If population sizes are large enough to limit the effects of genetic drift, the lack of genetic population structure over large parts of the ranges of tropical Pacific species may have stemmed largely from recent spatial expansion throughout this region that was coupled with the demographic expansion and may not reflect high rates of contemporary gene flow in these species. In addition, although the potential for extensive larval transport could certainly have facilitated expansion throughout large regions of the tropical Pacific in the past, it may only have had minor homogenizing effects on geographically discrete populations after the spatial expansion took place. Population differentiation at particular geographic locations in the tropical Pacific, as observed for these *Conus* species, might simply reflect the lack of recruitment to these areas during past broad geographic expansions of these species. Because of the isolated and peripheral nature of the locations where genetic differentiation was observed, the absence of recruitment to these areas is likely to have resulted from the failure of sufficient numbers of larvae to reach these locations. Nonetheless, if populations at these locations are subject to distinct local selection pressures, invading larvae from elsewhere may not have been able to successfully recruit.

CONCLUSIONS

The phylogeographic patterns of four *Conus* species in the tropical Pacific essentially reiterate the patterns observed for a number of phylogenetically disparate species in this region, including other species of molluscs, echinoderms and fishes. In particular, while some sets of species exhibit similar genetic population structures and patterns of population differentiation, few consistent trends are apparent. Nonetheless, dispersal potential clearly plays an important role in population

differentiation at broad scales, especially along the continuum from low to high dispersal species. Like other species in the tropical Pacific, four broadly-distributed *Conus* species with high potential for dispersal via planktonic larvae are genetically homogeneous over large parts of their distributions and in one case (*i.e.*, *C. chaldaeus*) also perhaps across a strong barrier to dispersal. This genetic homogeneity either resulted from a recent spatial expansion of these species in large regions of the tropical Pacific or high levels of contemporaneous gene flow among broadly separated locations. The differences in the locations of the genetic breaks in these species, however, suggest a strongly stochastic nature of dispersal. Although sporadic long-distance dispersal may eventually maintain genetic homogeneity across large parts of the ranges of species in the tropical Pacific, it may too allow for populations to undergo genetic differentiation at isolated locations that could ultimately lead to species formation.

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