

Geneological approaches to the temporal origins of the Central American gap: Speciation and divergence in Pacific *Chthamalus* (Sessilia: Chthamalidae)

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Abstract: A large section of the tropical Eastern Pacific coastline is nearly devoid of reef or consolidated habitat, and is known as the Central American Gap as it is associated with a biogeographic transition in fish and invertebrate species. We analyze phylogeographic data for intertidal barnacles (*Chthamalus*) to identify relevant temporal patterns that describe the origins of this biogeographic transition (the Mexican-Panamic Transition Zone). These contrasts of populations on either side of the transition zone include two pairs of closely related species (*C. panamensis* and *C. hedgecocki*; *C. southwardorum* and a Southern form of *C. southwardorum*), as well as gene flow data within one species (*C. panamensis*) that currently is found on both sides of the boundary between provinces. Using sequence data from a prior phylogenetic study, we used traditional (net nucleotide divergence) measures as well as coalescent analyses that incorporate the isolation-migration model to identify the likely time of separation between Northern and Southern taxa in two species pairs. A total of 67 individuals were sequenced at two mitochondrial (cytochrome c oxidase I, 16S) and one nuclear (elongation factor 1-alpha) gene regions. Our analyses indicate that the regional isolation of these intertidal barnacles occurred approximately 315-400kya, with subsequent expansion of *C. panamensis* from the Southern region into the North much more recently. There are insufficient survey data to conclusively document the absence of species from this group within the Central American Gap region near the Gulf of Tehuantepec. However, appropriate habitat is quite sparse in this region and other environmental factors, including upwelling and water temperature, are likely to be associated with isolation of many species in the Mexican and Panamic provinces *sensu stricto*. Some taxa may maintain gene flow across this region, but very few genetic studies have been completed on such taxa. Until further work is done, distinguishing between prior hypotheses of a faunal gap, or a faunal transition zone, is somewhat speculative. Additional taxonomic revision will be necessary in *Chthamalus* but is beyond the scope of this paper. Rev. Biol. Trop. 61 (1): 75-88. Epub 2013 March 01.

Key words: Pacific, intertidal, barnacle, phylogeography, transition zone, Central American Gap, *Chthamalus*.

The conundrum of marine speciation has become a common subject of investigation in recent years. The long dispersal potential of many marine organisms and the paucity of absolute physical barriers would suggest the existence of a small number of widespread species, and yet in actuality marine systems are incredibly diverse, with high levels of endemism (Palumbi 1994). What we have come to recognize is the dynamic nature of habitat and

species distributions through time. Histories of allopatry, expansion, and habitat loss are not always readily apparent from contemporary species distributions (McGovern *et al.* 2010). It is of interest to characterize these historical events when they appear to have influenced large numbers of taxa. In particular, what ancient and contemporary forces separate particular marine provinces, and ecoregions within provinces (Spalding *et al.* 2007)? The origins

of biogeographic transition zones may be environmental in nature (associated with habitat) or may be caused by ancient events that are no longer concordant with environmental patterns.

One such transition zone is found along the Mexican coast of the Tropical Eastern Pacific (TEP) ocean between roughly 13-15°N latitude. Called the “Central American Gap” (CAG) by some (Hastings 2000, Pitombo & Burton 2007) and the Mexican-Panamic transition zone (MPTZ) in other sources (Laguna 1990), this region separates the Mexican and Panamic *sensu stricto* marine provinces with a “stretch of coastline over 1 200km in length dominated by sandy/muddy shores and mangrove-lined lagoons” (Hastings 2000). This region is known to influence the distribution of rocky shore and reef fish, as well as invertebrates, and is a likely factor in the separation of cryptic species in the region.

The widespread barnacle genus *Chthamalus* has been the subject of several biogeographic studies in the TEP (Laguna 1990, Wares 2001, Pitombo & Burton 2007, Wares *et al.* 2009). Very low levels of morphological variation have made determining species distributions difficult, and until recently many species in the ‘*fissus* group’ (defined by two minute but verified synapomorphies) were considered a single species or species complex (Dando 1987). Recent phylogenetic studies have indicated that cryptic diversity is yet to be fully explored in this group, with fine-scale morphological variation and DNA barcoding being used to fully describe two new species, *C. hedgecocki* and *C. southwardorum* (Pitombo & Burton 2007). Based on morphology alone, the latter is reported to be distributed from Bahio Kino, Mexico, to Puerto Chicama, Perú, but molecular data were previously only available from the Northernmost samples. Pitombo & Burton (2007) predicted that a sibling species group may still exist in this widespread species range, which crosses the CAG; this prediction was founded in part on allozyme data presented by Dando & Southward (1980), but no formal revision or analysis has been completed to date.

To evaluate both the potential for further cryptic diversity and the insights this diversity can provide for understanding the temporal habitat dynamics of the CAG, we have analyzed previously sampled populations of *C. southwardorum* from sites North and South of this faunal transition and use mitochondrial and nuclear sequence-based markers to measure genetic differentiation among these sites. The same methods are applied to a closely related species pair, *C. hedgecocki* and *C. panamensis*, that are found in more wave-exposed coastal habitats. We use analytical approaches based on coalescent theory that allow the co-estimation of divergence times among populations and the migration rates among them (the isolation-migration model; Hey & Nielsen 2004). This approach enables us to separate the effects of ancestral polymorphism and gene flow at loci where alleles are shared between the two regions. Using substitution rates generated from a phylogenetic analysis of the genus (Wares *et al.* 2009), we have also evaluated the likely time frame at which these regional populations could have become isolated from one another.

MATERIALS AND METHODS

Study system: The taxonomy of this species complex has been confused in the literature for nearly 30 years. This has been in part because although data were available to indicate there were two pairs of *Chthamalus* along this coast (Northern and Southern pairs of wave-exposed habitat and wave-sheltered habitat species), the taxonomy was only partially updated by Pitombo & Burton (2007), after a series of intervening papers that applied unofficial and inconsistent nomenclature. The four species evaluated here, with an attempt at taxonomic history for each, are: *C. panamensis* Pilsbry – though its exact distribution has been in question given the difficulty in field identification of this species, *panamensis* has been consistently used for this taxon. Found in wave-exposed coastal habitats, this species resides primarily in Southern locations in the

TEP. Pitombo & Burton (2007) indicated that it may range as far North as 20°N.

C. hedgecocki Pitombo & Burton – originally part of the *C. fissus* (Darwin) species complex (Hedgecock 1979), this species was distinguished from the rest of the complex using phenotypic and electrophoretic data by Dando & Southward (1980) and unofficially called *C. "mexicanus"*. The same name was also used by Laguna (1990), but this species was officially described as *C. hedgecocki* in Pitombo & Burton (2007). It is found in wave-exposed coastal habitats, primarily at Northern locations in this region.

C. southwardorum Pitombo & Burton – as with *C. hedgecocki*, this was originally distinguished as a separate taxon by Dando & Southward (1980) and unofficially called *C. "cortesianus"* (Laguna 1990). Formally described as *C. southwardorum* in Pitombo & Burton (2007), it can be found in wave-sheltered coastal habitats, solely at Northern locations in this region.

C. southwardorum "Farfan" form – named for the putative type locality in Panama (at

which 100% of individuals were of this form in 1978, P. Dando *pers. comm.*), populations of this form were identified as distinct by Dando & Southward (1980) but with no formal description. It has been confused in the literature with *C. southwardorum sensu stricto*, which may influence our understanding of regional biogeography. The "Farfan" form is found in wave-sheltered coastal habitats, solely at Southern locations in this region. Recent data further suggest a difference between the forms (Wares *et al.* 2009), though the nomenclature of the *C. southwardorum* A and B clades is inconsistent in that source.

Note that other congeneric species, including *C. fissus* and *C. anisopoma*, may be sympatric with these taxa in the Gulf of California or along the Baja California peninsula, but are more readily distinguished from this complex.

Chthamalus individuals were collected in 2005-2006 from seven primary locations in the TEP (Fig. 1, Table 1) as part of a global phylogeny of the group (Wares *et al.* 2009). Species in this complex are largely indistinguishable in the field based on external morphology

TABLE 1

Collection information for phylogenetic analysis of *Chthamalus southwardorum* in the Tropical Eastern Pacific. Numbers under each locus name indicate the sample size available for that locus from each location

Site	Taxon	COI	16S	EF1
Bahia Kino (Mex); 28°48' N-111°54' W	<i>C. southwardorum</i>	8	9	1
La Paz (Mex); 24°12' N-110°00' W	<i>C. southwardorum</i>	6	4	9
Mazatlán (Mex); 23°12' N-105°30' W	<i>C. hedgecocki</i>	0	0	1
Mazatlán (Mex); 23°12' N-105°30' W	<i>C. panamensis</i>	2	1	0
Matanchen (Mex); 21°30' N-105°12' W	<i>C. southwardorum</i>	6	6	5
Matanchen (Mex); 21°30' N-105°12' W	<i>C. hedgecocki</i>	1	1	1
Matanchen (Mex); 21°30' N-105°12' W	<i>C. panamensis</i>	1	0	1
Punta Perula (Mex); 19°36' N-105°06' W	<i>C. hedgecocki</i>	4	2	3
Punta Perula (Mex); 19°36' N-105°06' W	<i>C. panamensis</i>	2	0	0
Tenacatita (Mex); 19°18' N-104°48' W	<i>C. hedgecocki</i>	1	1	1
Tenacatita (Mex); 19°18' N-104°48' W	<i>C. panamensis</i>	4	0	1
El Ancote (Mex); 20°48' N-105°30' W	<i>C. hedgecocki</i>	3	3	3
El Ancote (Mex); 20°48' N-105°30' W	<i>C. panamensis</i>	4	0	0
Puntarenas (CR); 9°54' N-84°54' W	<i>C. southwardorum</i>	2	4	2
Puntarenas (CR); 9°54' N-84°54' W	<i>C. panamensis</i>	5	4	4
Punta Culebra (Pan); 8°54' N-79°30' W	<i>C. southwardorum</i>	7	7	7
Punta Culebra (Pan); 8°54' N-79°30' W	<i>C. panamensis</i>	14	3	13

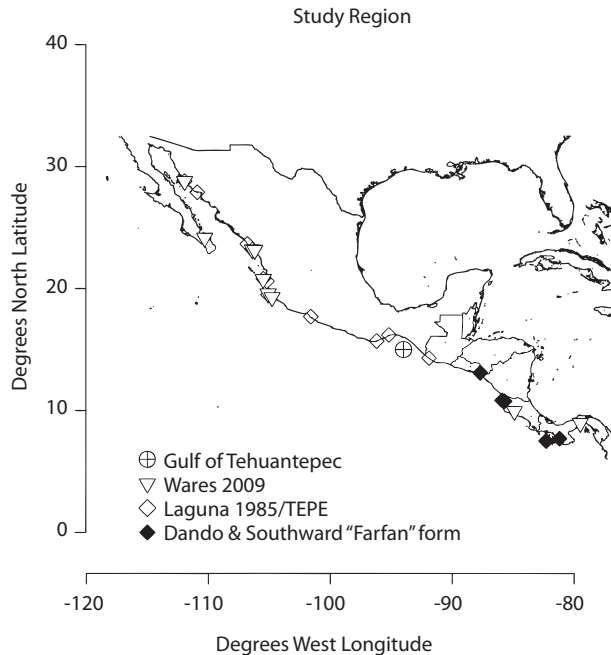


Fig. 1. Sampled locations for genetic data in this study, and sample locations from the TEPE 1978 survey. Sites at which the "Farfan" form of *Chthamalus southwardorum* were identified using phenotypic and allozyme data are indicated. The Gulf of Tehuantepec is the approximate boundary between "Southern" and "Northern" populations in this study, and the demarcation of the Mexican-Panamic transition zone as well as the "Central American Gap" discussed in the text.

(Pitombo & Burton 2007; P. Dando, *pers. comm.*). Thus, it was not possible to sample directly for particular species, but rather the available specimens were defined using standard DNA barcoding approaches with the mitochondrial cytochrome oxidase I (COI) locus (Wares *et al.* 2009). Specimens of *C. southwardorum* were found in five locations (Table 1, Fig. 1), with three locations North of the CAG and two locations to the South. Specimens of *C. hedgecocki* and *C. panamensis* were identified from three additional locations (Table 1). Molecular PCR and sequencing techniques are described in Wares *et al.* (2009). Two sequences at the 16S locus from the previous study, one each from *C. panamensis* and *C. southwardorum*, were excluded from analysis after being identified as either erroneously labeled or product of contamination. Removal of these two sequences results in the reciprocal monophyly of *C. southwardorum* and the clade

containing *C. hedgecocki* and *C. panamensis* (Wares *et al.* 2009).

Phylogenetic analysis: Here we use previously reported (Wares *et al.* 2009) aligned sequence data for two mitochondrial gene regions (COI and 16S rDNA) and one nuclear locus (elongation factor 1-alpha, EF1) from the locations indicated in table 1 and figure 1. Additional nuclear sequence data are available for these specimens, but provide little phylogenetic signal at this level and considerable homoplasy (Wares *et al.* 2009). The separation of locations by the CAG is used for all *a priori* structuring of data in subsequent analysis. Due to difficulty in amplifying some loci from some individuals, not all locations/individuals/loci are equally represented (Table 1).

Sequence data at the EF1 nuclear locus were directly sequenced and polymorphism within sequences was recoded as ambiguity,

either unknown “N” in mitochondrial data or appropriate IUPAC ambiguity codes for nuclear data. Phylogenetic reconstructions were performed on the concatenated data using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) with model estimation unlinked across loci. Bayesian estimation followed 5×10^6 generations of four MCMC chains, with a burn-in fraction of 25%. Data from the EF1 locus were analyzed with ambiguity represented as heterozygous sites for phylogenetic analysis.

Coalescent analysis: We analyzed the data as multilocus observations from the Northern and Southern sites using the program IMA2 (Hey & Nielsen 2004), which applies the isolation-migration model for estimation of the relative importance of migration and time since divergence. The IMA2 program uses a Markov Chain Monte Carlo method for assessing posterior probability densities of six model parameters: time of divergence, migration from North to South, migration from South to North, and effective population sizes for the two regional populations as well as their ancestral source population. Substitution rate estimates for each locus (Wares *et al.* 2009) were included to allow the time since divergence to be estimated from the coalescent model. These rates, in units of substitutions per locus/year, are: mtCOI (1.83×10^{-5}); mt16S (7.65×10^{-6}); nEF1 (1.86×10^{-6}). The nEF1 data were separated into haplotypes for coalescent analysis using the PHASE algorithm (Stephens *et al.* 2001) as implemented in DNAsp (Librado & Rozas 2009). This analysis was repeated for the taxon pair *C. hedgecocki* – *C. panamensis*, and for Northern and Southern populations of *C. panamensis*. Priors allowed for population mutation rates ranging from 10-100 (most likely estimate based on π of 15 was used for final calculations with a maximal range of 30, see below), migration parameter from 0-5, and separation time of four coalescent units. A burn-in period of 10 000 steps was used, followed by a run duration of 10^8 steps with 20 geometrically heated chains.

Given the potential for spatial and temporal concordance of separation between the Northern and Southern species pairs, temporal association was tested using the msBayes pipeline (Hickerson *et al.* 2006). This approach uses approximate Bayesian computation (ABC) simulation approaches to evaluate the most likely isolation scenarios associated with empirical data; in our case we used 10^6 simulated data sets and an upper prior on ancestral effective size equal to the upper prior on current effective size (otherwise default parameters for analysis).

RESULTS

Phylogenetic analysis: Data from *C. southwardorum*, *C. panamensis*, and *C. hedgecocki* used in this study are as in Wares *et al.* (2009) but with more focused analysis of genealogical relationships. Genbank voucher numbers for all sequence data are provided in Wares *et al.* (2009). Bayesian phylogenies for each taxon pair are shown in figures 2 and 3; similar results were obtained from likelihood bootstrap analysis and parsimony analysis (results not shown). Net nucleotide divergence (Nei & Li 1979) between the Northern and Southern populations of *C. southwardorum* was 0.0288 ± 0.0043 for COI, 0.0047 ± 0.0019 for 16S, and 0.0019 ± 0.0013 for EF1 (0.0044 ± 0.0016 after haplotype phase calculated). Given the substitution rates indicated above, these d_A values would suggest divergence times of approximately 5.11×10^5 , 1.69×10^5 , and 5.32×10^5 years, respectively (mean 4.04×10^5).

For comparison, the divergence of the *C. panamensis* – *C. hedgecocki* species pair involves net nucleotide divergences of 0.0210 ± 0.0040 for COI, 0.0091 ± 0.0051 for 16S, and 0.0034 ± 0.0025 for EF1. These values suggest divergence times at each locus of approximately 3.73×10^5 , 3.27×10^5 , 4.81×10^5 (mean 3.98×10^5) years.

Coalescent analysis: Homogeneity of populations in the Northern and Southern

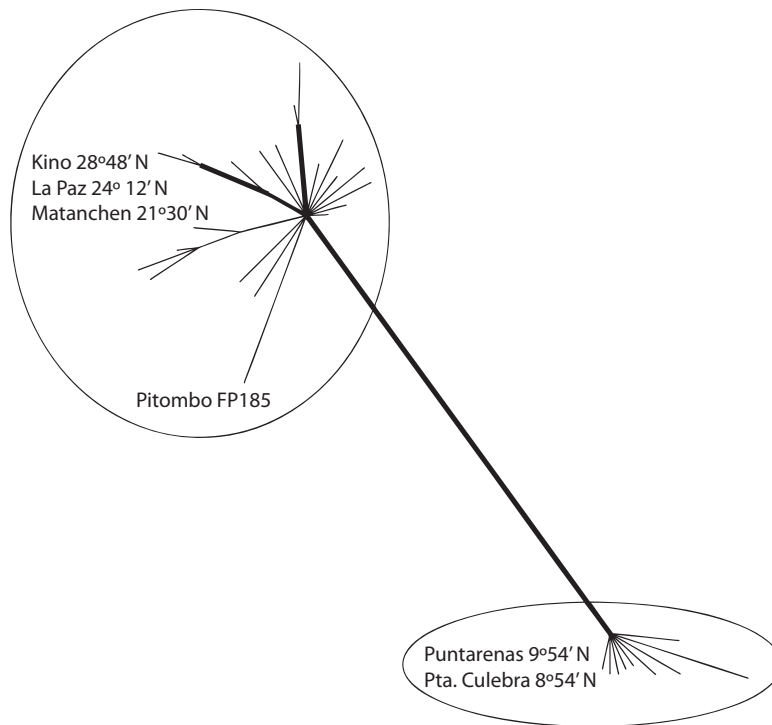


Fig. 2. Bayesian multilocus gene tree for the data from *C. southwardorum*. The two clades are separated with a posterior probability of 1.0. The type sequence for mitochondrial COI is included and labeled Pitombo FP185; the other clade represents the “Farfan” form of this species. Width of line indicates posterior probability of branch.

regions for each taxon was indicated from Hudson’s (2000) Snn statistic ($p > 0.05$ in all cases). Coalescent analysis of the nEF1 locus produced similar results for *C. southwardorum* whether using phased or unphased sequences (results not shown); each supported negligible migration between populations of *C. southwardorum*, though the age estimation based on the nEF1 locus alone is relatively uninformative as there is considerably less polymorphism. Overall, the IMA2 analyses ran sufficiently long to reach good convergence as determined by effective sample size (ESS) values and very similar likelihood distributions (Fig. 4). Effective size estimates were consistent between runs, with the ancestral effective population size $9.27\text{--}9.54 \times 10^4$, and current effective population sizes of $1.45\text{--}1.47 \times 10^5$ (Northern) and 2.36×10^5 (Southern). Migration rate estimates

between the two regional populations were consistently negligible (≤ 0.0001 migrants per 1 000 generations in either direction). Posterior probability distributions of divergence time estimates peaked approximately 340kya in all runs, with minimum 95% limits at around 220kya (Fig. 4). High 95% limits are not reported because the distributions have fat tails and thus these maximum (and mean) values are conditioned on the prior. Our time distribution range was set to correspond with the time since the rise of the Isthmus of Panama (~three million years ago).

An identical analysis performed on the *C. panamensis* – *C. hedgecocki* species pair was consistent with the above results. Migration rate estimates between the two taxa were consistently negligible (≤ 0.001 migrants per 1 000 generations in either direction). However, the

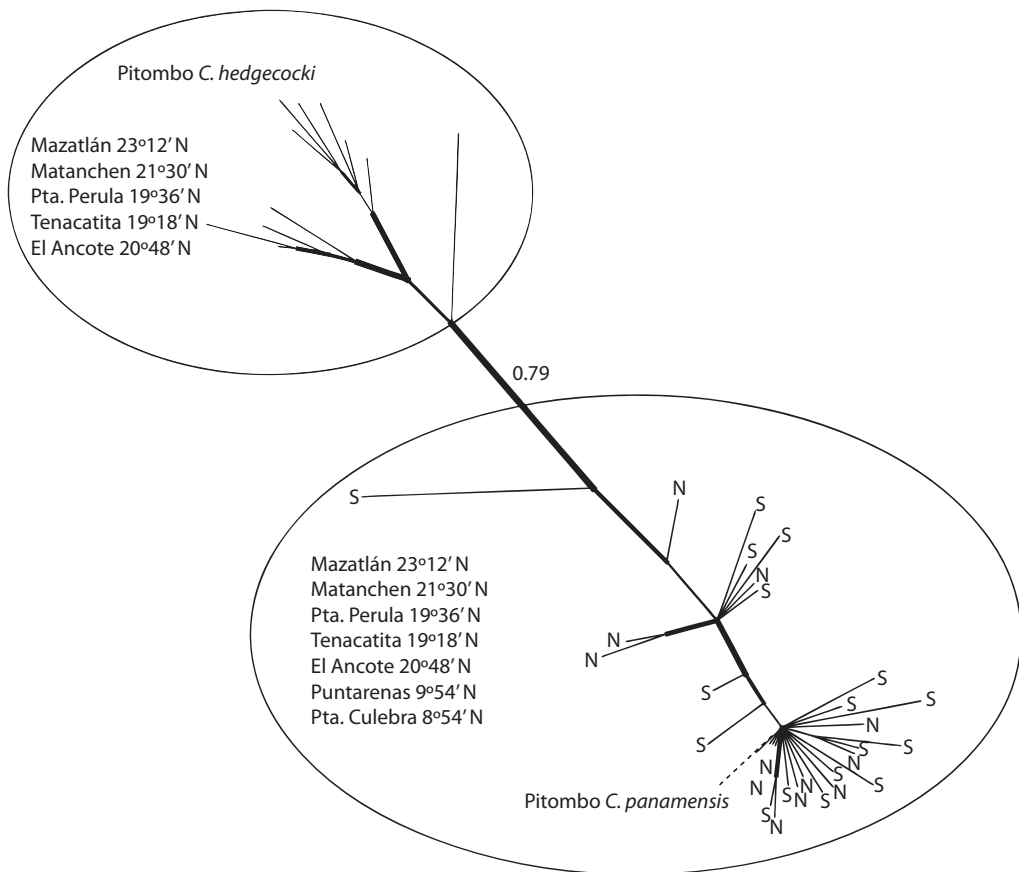


Fig. 3. Bayesian multilocus gene tree for the data from *C. hedgecocki* and *C. panamensis*. The two clades are separated with a posterior probability of 0.79. The type sequence for mitochondrial COI (from Pitombo & Burton 2007) is included and labeled for each species. Width of line indicates posterior probability of branch. 'N' and 'S' in the *C. panamensis* clade indicate individuals sampled from North and South of the CAG, respectively.

posterior probability distribution of divergence time estimates exhibited an increasing function with time, meaning no useful estimate of divergence is possible with these data.

Full analysis of the *C. panamensis* data in IMA2 indicated maximal population sizes in current Northern and Southern regions (e.g. limited only by the prior, as discussed here prior maximum on theta is 30; see Discussion). The ancestral population substitution rate estimate was approximately what would be predicted from π (13.9; 95% range 8.26-24.38). Migration rate $2Nm$ from the South to the North was estimated at (mean) 2.46 (0.16, 4.84) and from North to South at 1.48 (0.03,

4.58). Plots of migration rates between the two regions are substantially different, with a maximal posterior probability from South to North of 2.36 and from North to South of 0.003 (Fig. 5). The time of separation of the Northern and Southern populations, coestimated with migration, is approximately 166 830 years given our substitution rate estimates (95% range of 47 400-922 000).

Analysis in msBayes of the coalescent-based divergence times of the two species pairs indicated high probability that separation of the two lineages of *C. southwardorum*, and *C. panamensis* and *C. hedgecocki*, was temporally concordant. With local multinomial logit

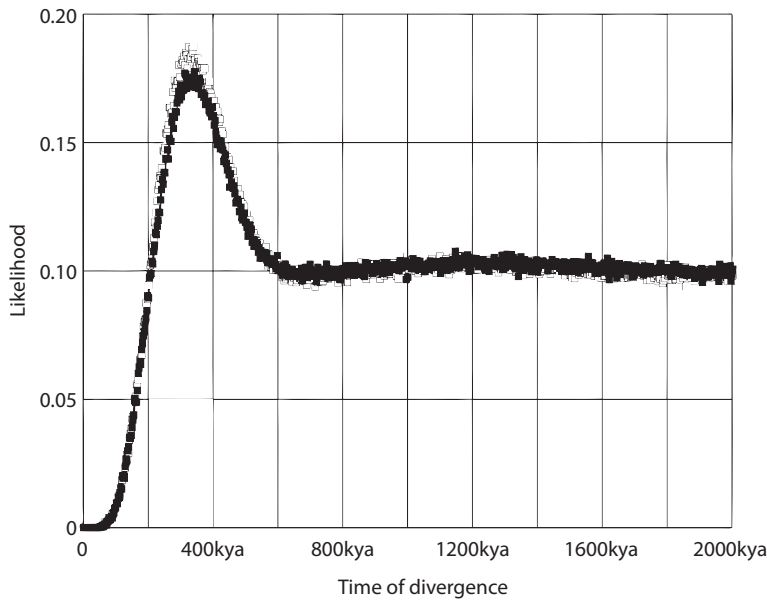


Fig. 4. Posterior distributions of divergence time estimates (horizontal axis) for Northern and Southern populations of *C. southwardorum* using IMA for the mtCOI, mt16S, and nEF1 gene regions combined. Analyses were run two times with identical inputs except random number seeds. Distributions peaked (probability distribution indicated by vertical axis) at 342 400 years ago in run 1 and 339 700 years ago in run 2, with the low 95% credibility intervals at 222 500 and 219 800 years ago respectively. (High 95% credibility intervals are not reported because distributions never reach zero and thus values are conditioned on the prior; only first 2mya are shown, distribution remains level through remainder). Migration estimates were ≤ 0.01 in both directions.

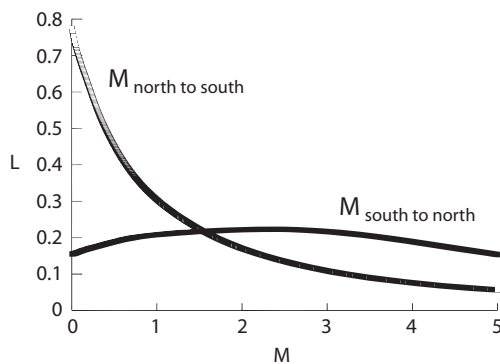


Fig. 5. Posterior distributions of likelihood (L, vertical axis) for migration rate parameters (M) in *C. panamensis*. Although the means of these distributions do not differ significantly, the shapes of these distributions and their maximal values are substantially different, with negligible North-to-South migration recovered in this analysis.

regression, the posterior probability for a single divergence time between the two pairs was 0.929, with remaining probability allowed for two distinct divergence times. The estimated time of simultaneous divergence using this approach, with the calculations of Huang *et al.* (2011), is 315 000 years (assuming a generation time of one year and the substitution rates noted above).

DISCUSSION

Our results suggest a temporal pattern of increasing isolation among TEP chthamalid barnacles; the deepest separation of the four species evaluated suggests that the sheltered-exposed species pairs were separated approximately one million years ago, based on net

nucleotide divergences ranging from 7.5-8% at mitochondrial COI. The focus of this study, the potential for geographic isolation of species across the CAG, allows us to evaluate the potential for a single environmental change promoting separation of Northern and Southern lineages of both the wave-sheltered and wave-exposed forms. Based on classical approaches such as net nucleotide divergence, as well as two different coalescent estimators, our results are surprisingly consistent in estimating a separation time between *C. hedgecocki* and *C. panamensis*, and the type and “Farfan” forms of *C. southwardorum*, of approximately 400kya, corresponding with the timing of one of the mid-Pleistocene high sea level stands (Muhs *et al.* 2004). These results also indicate strongly (92.9% posterior probability) that isolation of *C. southwardorum* Northern and Southern populations was temporally concordant with the isolation of *C. hedgecocki* and *C. panamensis*. It remains to be determined if this apparent vicariant event is associated with a biogeographic transition zone between the Mexican and Panamic provinces (requiring further analysis of species that do cross the region) or a true habitat and faunal gap (Audzijonyte & Vrijenhoek 2010).

Estimation of gene flow between these two taxon pairs (treating the Southern populations of *C. southwardorum* as a distinct taxon, or at least separate population) indicates negligible gene flow between the Northern and Southern forms of both the wave-sheltered (*C. southwardorum*) and wave-exposed (*C. hedgecocki*, *C. panamensis*) taxa. However, additional analysis of the one taxon for which we have samples spanning the region, *C. panamensis*, indicates that a more recent (~160kya) expansion from the Southern populations to the North is likely to be the cause of the current distribution, both geographic and in terms of genetic diversity. All of these results are also supported by the inclusion of data from one additional nuclear locus, though sample sizes were too small to provide sufficient additional information (results not shown).

Conclusions from our analyses must be tempered by the sparse samples available for this study. Most of the sequence data are from a single mitochondrial locus, sequenced at two distinct regions (mtCOI and mt16S). It should be emphasized that the data obtained were initially part of a separate phylogenetic analysis in which cryptic species were distinguished, without reference to external morphology in field collections. Samples of *C. southwardorum* were not recovered from sites immediately North of the CAG, nor were any chthamalids identified within the CAG during the NSF-funded Tropical Eastern Pacific Expedition of Newman in 1978 (W. Newman, P. Dando, E. Southward, *pers. comm.*). Thus, the opportunistic sample size reflects both the sampling effort and PCR success in these samples. Considering the reciprocal monophyly of regional samples at mitochondrial gene regions and extensive lineage sorting at nuclear gene regions, the results appear to be robust to sample size (Rosenberg 2007). One problematic result of the coalescent analyses was the estimate of N_e in populations of *C. panamensis* being predicated on the maximum of the prior distribution. This is often the case when the data are limiting, and may be associated with the signal of expansion noted above.

Briggs (1974) and Brusca & Wallerstein (1979) first indicated that a zoogeographic break in the marine fauna existed at approximately 15° N (roughly the Gulf of Tehuantepec). There are a number of strong environmental shifts associated with this coastal region (Udvardy 1975, Hayden & Dolan 1976). There are significant sea surface temperature and wind stresses associated with the Gulf of Tehuantepec (Sun & Yu 2006), generating strong upwelling in the Gulf and offshore movement of surface waters (Kessler 2006, Barton *et al.* 2009). Upwelling patterns alone have previously been associated with divergence of regional barnacle populations (Dando & Southward 1981). The coast in this region is predominantly sandy (Robertson & Cramer 2009, W. A. Newman, *pers. comm.*), with little consolidated habitat for barnacles or reef organisms to persist. A remaining concern

is whether this region represents a true faunal 'gap' or transition zone, a question that requires integration across more taxa (Audzijonyte & Vrijenhoek 2010).

While certain groups of species that require consolidated habitat have reduced diversity or abundance in this region, indicating a gap, the patterns of distribution in most taxa may still be consistent with an overlap zone between two biogeographic provinces. Reid (2002) provides such data for littorinid snails, and Laguna (1990) illustrates this in the distribution of barnacles along the tropical Eastern Pacific coast. Notably, there is a clear demarcation between the Mexican and Panamic provinces *sensu stricto*, but there are a number of barnacle species defined by Laguna (1990) as being of the Panamic province *sensu lato* that do broadly overlap this transition zone and the CAG.

The present study makes it apparent that at least one of these species (called *C. "mexicanus"* at the time, but presently *C. southwardorum*) does not readily cross this region. Though the samples in the present study are sparse, it is consistent with information from the TEPE survey, with no representatives of *C. southwardorum* (or the "Farfan" form) collected between 9°54' N and 21°30' N. Based on allozyme data from the earlier survey, what is now *C. southwardorum* was found in high abundance in the Northern Gulf of California (and 100% of our *Chthamalus* from Bahia Kino are *C. southwardorum*), but collections between Bahia Chamela (19°30' N), Salina Cruz (16° N) and at Isla Sacrificios (15°42' N) recovered only *C. hedgecocki* (P. Dando, W. Newman, *pers. comm.*). In the Southern range, as far North as Murciélagos, Costa Rica (10°48' N) only *C. panamensis* were recovered at the time. Certainly, even when multiple species are present, one may be extremely rare or microhabitat specific. It is an open question whether genetic analysis of other species in

this region (group IV in Laguna 1990) would indicate a population genetic pattern consistent with the divergence of *C. southwardorum*.

The generality of results presented here to the broader faunal distribution patterns of the Tropical Eastern Pacific remains ambiguous. It is clear that for some taxa that require (or form) consolidated substrate or reef habitat, the lack of such habitat in this region (the Central American Gap) is a significant demarcation in diversity along this coast (Hastings 2000, Diaz-Jaimes *et al.* 2006, Vargas *et al.* 2008). Studies that have focused only on the apparent distribution of species suggest, however, that the effect of these habitat gaps on marine diversity is minimal and the association of transition zones with gaps like the CAG is possibly stochastic (Robertson & Cramer 2009). Robertson & Cramer (2009) note that because the appearance of the CAG as a "distinctive entity" in their analysis is in part because of an absence of data, rather than information on the potential for migration or gene flow across the region, additional range-wide genetic analysis of fish and other taxa is warranted. The small number of population genetic studies that span this region suggest there are many cryptic populations along the TEP coast (Hellberg 1998, Arnau *et al.* 2000, Diaz-Jaimes *et al.* 2006, Wares *et al.* 2009, Saarman *et al.* 2010), and in many cases the break in diversity could be spatially associated with the CAG (Diaz-Jaimes *et al.* 2006, Hurtado *et al.* 2007, Pitombo & Burton 2007). In the intertidal snail *Nerita*, two species are known to span this region; one exhibits a strong break while the other does not (Hurtado *et al.* 2007), suggesting that knowledge of both habitat and interactions between life history, behavior, and physical oceanography may be necessary to predict the effect of this habitat break on coastal diversity.

Robertson & Cramer (2009) noted that the CAG may have formed recently relative

to the origins of many taxa in their study, and that more work must be done to assess intraspecific genetic variation among populations of TEP fish taxa on either side of the CAG. Although they argue that the distribution of species suggests gene flow has the potential to be quite high even across this substantial habitat gap – placing the emphasis on other forms of environmental variation for driving the diversity patterns of this coast – we show that for species with long (2-3 weeks; Miller *et al.* 1989) effective larval dispersal, gene flow is negligible. On recent time scales, the CAG may be promoting intraspecific diversification. It is important to note that the CAG may have distinct effects on different groups of organisms; the delimitation based on absence of reef fishes (Robertson & Cramer 2009) is quite narrow (15-16° N). It may be that more vagile organisms like pelagic fish are less sensitive to such barriers (Lessios & Robertson 2006), while benthic invertebrates or demersal fish have less opportunity to complete such a journey and successfully recruit (Waters *et al.* 2007). For intertidal invertebrates, and in particular the Cirripedia, this region is certainly an area of faunal transition (Laguna 1990), separating the Mexican and Panamic provinces. The mechanism for diversification may be more than simply habitat availability, as Laguna (1990; also W. A. Newman, P. Dando, *pers. comm.*) points to earlier work that suggests local currents and upwelling could act as dispersal barriers in this depositional shoreline region. Eventually, with addition of more locational information and microhabitat environmental data, there may be more explicit ways to evaluate the mechanism by which these species have been separated (Glor & Warren 2011). A more thorough spatial survey will be necessary to fully generate models that are useful for microhabitat identification (Lozier *et al.* 2009).

Assuming an average age of the isolating event between these Northern and Southern lineages of around 400kya (Fig. 4), it is not clear what model of isolation best fits these data. The analytical approaches used here are necessarily indirect (Slatkin 1985) and can have their limitations (Wares & Cunningham 2005), but the concordant results between two distinct estimators suggests our temporal estimate is robust. Hickerson & Meyer (2008) developed a method for comparing the likelihood of “soft” vicariance (in which an effectively panmictic species then becomes isolated across some stretch of its geographic range because of habitat or climate change) versus peripatric events in which a colonization from one side of the boundary establishes a new lineage on the other side. However, these methods require data from a larger number of taxa. Separating the influences of temporal isolation and recurrent gene flow is often critical in assessing alternate historical scenarios that could produce patterns of extant diversity (McGovern *et al.* 2010).

The question of whether the two subgroups, Northern and Southern, of *C. southwardorum* merit taxonomic revision is a question that has been addressed but only informally. The regional populations were considered likely to represent separate taxa based on the relative mobilities of *Ald* and *Pgk* allozymes, and some coloration differences between exposed-shore populations and sheltered populations in the Mexican (now *C. hedgecocki* and *C. southwardorum*) and Panamic (now *C. panamensis* and the Southern group of *C. southwardorum*) provinces (Dando & Southward 1980), and tergal groove morphology separating the two Panamic species. The exposed coast forms have orange tergal-scutal tissue flaps (with two pale spots on the flap in *C. hedgecocki*), and the sheltered coast forms are brown or “orange-brown” for the same tissues (P. Dando, *pers. comm.*). Pitombo & Burton (2007) also

note that *C. southwardorum* could consist of two sibling species, but this was untested in that study. At present it is simply referred to as the “Farfan” form to avoid any further taxonomic confusion in this group; full taxonomic designation will require integration of phenotypic and genetic identifiers, and will be handled separately. What may be of most general interest about these results – particularly in designing future studies of taxa that apparently cross this coastal region – is that for *Chthamalus*, the speciation of inshore (sheltered) and outer coast (exposed) forms clearly happened first (Wares *et al.* 2009), followed by the latitudinal divergence associated here with the CAG. The generality of this result should be examined further.

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RESUMEN

La taxonomía del complejo de especies de cirripedios (*Chthamalus*) se ha confundido en la literatura desde hace casi 30 años, por lo tanto analizamos datos de su filogeografía para identificar modelos temporales relevantes que describan los orígenes de la zona de transición entre las provincias Mexicana y Panameña. Estos contrastes de poblaciones a ambos lados de la zona de transición incluyen a dos pares de especies estrechamente relacionadas, así como datos de flujo de genes dentro de una especie que actualmente es encontrada en ambos lados del límite entre provincias. Usando datos de secuencia de un estudio previo de filogenética, usamos medidas tradicionales, así como análisis de coalescencia que incorporan el modelo de migración y aislamiento para identificar el tiempo probable de la separación entre los taxa del norte y del sur en dos pares de especies. Nuestros análisis indican que el aislamiento regional de estos cirripedios ocurrió aproximadamente hace 315-400 mil años, con una extensión subsecuente de *Chthamalus panamensis* de la región del sur hacia el norte mucho más reciente. No hay datos suficientes para documentar conclusivamente la ausencia de especies de este grupo dentro de la región de Centro América cerca del Golfo de Tehuantepec. Sin embargo, el hábitat apropiado es bastante escaso en esta región y otros factores ambientales, incluyendo corrientes y temperatura acuática, probablemente están relacionados con el aislamiento de muchas especies en estas provincias. Algunos taxa pueden mantener el flujo de genes a través de esta región, pero muy pocos estudios genéticos han sido realizados en tales taxa. Hasta que no se desarrollen trabajos adicionales, la distinción entre hipótesis previas de un “gap” faunal o de una zona de transición faunal es algo especulativo.

Palabras clave: océano Pacífico, zona intermareal, cirripedios, filogeografía, zona de transición, Centro América.

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