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Cover Page Footnote
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Range Expansion or Range Shift? Population Genetics and Historic Range Data Analyses of the Predatory Benthic Sea Slug *Phidiana hiltoni* (Mollusca, Gastropoda, Nudibranchia)

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Abstract.—*Phidiana hiltoni* is a conspicuous nudibranch sea slug native to the northeastern Pacific Ocean. Over the past thirty years the range of *P. hiltoni* has expanded about 200 km northward, but the mechanism that facilitated this expansion is poorly understood. In this study, we use mtDNA and microsatellite data to investigate the population structure of *P. hiltoni* in its historical range as well as in recently colonized localities. Microsatellite analyses reveal little to no genetic structure and thus high gene flow throughout the range of *P. hiltoni*. This is consistent with mtDNA analysis results, which revealed shared haplotypes between Southern, Central and Northern populations. However, AMOVA of mtDNA data did recover some genetic structure among geographic regions. This, along with same group memberships in the microsatellite data of individuals from sites like Cave Landing, suggest a certain degree of local recruitment and reduced vagility. Recently established populations in Northern California contain two unique mtDNA haplotypes that are not present elsewhere, but microsatellite data do not differentiate these from other populations. The mismatch between mtDNA and microsatellite data could be explained by the mating system of this aggressive, hermaphroditic species as well as the sporadic nature of the northward dispersal. Analyses of historical abundance data of *P. hiltoni* suggest a population decline in Southern California. Together, these results suggest a northward population shift, rather than a range expansion, possibly related to ongoing changes in nearshore oceanographic conditions in the region.

Rising ocean temperatures driven by global climate change are having dramatic impacts on coastal ecosystems around the world (McGowan et al. 1998; Sorte et al. 2011). One of the most noticeable effects is the poleward range expansion of certain species (Dawson et al. 2010; Sorte et al. 2011; Sunday et al. 2012; Canning-Clode and Carlton 2017). Particularly problematic are range expansions of predatory species, which can have significant impacts on the trophic structure of newly colonized ecosystems (Zeidberg and Robinson 2007; Gallardo et al. 2016). However, not all of these range expansions are permanent;

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some result from regular oscillations in ocean temperatures (e.g., El Niño events). In these cases, populations often return to their original range following ephemeral warming events, making it difficult to attribute in the short term any particular range shift to longer term climate change (Schultz et al. 2011). Poleward range expansions can also be accompanied by extirpation at lower latitudes, resulting in shifts at both ends of species ranges (Parmesan et al. 1999; Bates et al. 2014). Range shifts may constitute a more pervasive indication of permanent changes in the ecological structure of biotas as they can be more difficult to reverse (Parmesan et al. 1999; Schultz et al. 2011). However, range shifts are difficult to detect and precisely quantify, particularly in marine species with low abundance and/or population densities (Bates et al. 2015).

Phidiana hiltoni is a relatively large and conspicuous aeolid nudibranch native to the northeastern Pacific Ocean. Like most nudibranchs, *P. hiltoni* is a simultaneous hermaphrodite, but the mating behavior of this species is poorly understood. The diet of *P. hiltoni* consists mostly of hydroids and other cnidarians; however, individuals of this species are known to attack and consume other sea slugs, particularly small, soft-bodied aeolids and dendronotaceans, including conspecifics (Goddard et al. 2011). *Phidiana hiltoni* has relatively large eggs and lecithotrophic larval development (Goddard 2004); its larvae do not need to feed in the water column and are capable of settlement and metamorphosis within a day or two of hatching. Thus, compared to planktotrophic species, dispersal by the larvae of *P. hiltoni* is greatly reduced. Historically, *P. hiltoni* was found as far south as Isla Cedros off the coast of Baja California, Mexico and as far north as Pacific Grove, California (Goddard et al. 2011). In 1977, *Phidiana hiltoni* was discovered north of Monterey Bay (Goddard et al. 2011). Once across Monterey Bay, *P. hiltoni* rapidly made its way up the coast; it was found just north of San Francisco Bay (Duxbury Reef, Marin County) in 1992 and now is present as far north as Bodega Bay, California, representing a 200-km northward range expansion in 40 years (Goddard et al. 2011; Goddard et al. 2018). At Duxbury Reef, *P. hiltoni* quickly became the dominant sea slug, with apparent negative impacts on other nudibranch species, likely through a combination of direct predation and competition for shared hydroid prey (Goddard et al. 2011).

The mechanism behind the range expansion of *P. hiltoni* is not well understood, but has been potentially linked to warming coastal waters and shifts in ocean currents along the California coast (Schultz et al. 2011). Changes in ocean circulation, which drives larval transport, can potentially increase the risk of species introductions and/or dispersals (Harley et al. 2006; Sorte et al. 2011; Wilson et al. 2016). Whereas the dispersal potential of species with planktonic feeding larvae is relatively well understood (Scheltema 1986), less is known about how species with lecithotrophic development may respond to oceanographic changes. A majority of lecithotrophic sea slug species are found in warmer, nutrient-poor waters, where having a short-lived, non-feeding larval stage can lead to reductions in larval mortality at the cost of reduced fecundity and vagility (Goddard 2004; Goddard and Hermosillo 2008). *Phidiana hiltoni* is one of the few lecithotrophically developing nudibranchs found in temperate waters in the Northeast Pacific Ocean (Goddard 2004), and its recent range expansion may reflect long-term changes in regional nearshore circulation regimes and productivity (Rebstock 2003). These factors make *P. hiltoni* a particularly interesting system for studying the complex interactions between climate change, range shifts, and marine invasion biology.

If the dispersal of *P. hiltoni* has been facilitated by changes of oceanographic regimes and warming waters at the northern edge of its range, the most likely source for the new populations north of Monterey Bay are Central California populations. However, it is also

possible that individuals from father south were introduced into northern California either by larval dispersal or human activities. The lecithotrophic larval development of *P. hiltoni* makes it an ideal candidate for ballast water dispersal, and less likely to disperse long distances naturally in response to environmental changes. Two of the busiest commercial ports in North America are located in California, with the Los Angeles-Long Beach Harbor well within the historic range of *P. hiltoni*, and the Oakland-San Francisco Harbor (San Francisco Bay) in the center of the extended portion of the range. However, the absence of *P. hiltoni* from San Francisco Bay along with its prevalence in open-coast rocky reefs (Goddard et al. 2011) contradicts the ballast water introduction hypothesis. Another possible vector for the spread of *P. hiltoni* could be small vessel traffic between regional ports and harbors (Wasson et al. 2001), but the mobile hunting behavior of this species makes it an unlikely fouling organism. Regardless of the mechanism of dispersal, Schultz et al. (2011) noted that *P. hiltoni* has persisted at higher latitudes despite ocean temperature fluctuations from El Niño/La Niña cycles, suggesting this species may be an indicator of faunal range shifts due to climate change.

Although the range expansion and some of the associated ecological effects of *P. hiltoni* are well documented (Goddard et al. 2011), many questions remain. No genetic studies have been conducted on *P. hiltoni*, thus the population structure of the species is unknown, hampering our ability to understand the mechanisms of dispersal. Also, very little attention has been paid to the southern range limit of *P. hiltoni*, leaving unanswered the question as to whether recent observations indicate a northern range expansion or overall range shift. In the present study, we examine population structure in *P. hiltoni*, explore the genetic signature of its range expansion, and hypothesize possible dispersal mechanisms into Northern California. Additionally, we reviewed historical collection data near the southern range of *P. hiltoni* in order to document population density changes that may help to understand the population dynamics of this species.

Materials and Methods

Whole specimens and tissue samples of *Phidiana hiltoni* (Table S1) were obtained from different sources and various locations along the California coast (Fig. 1). Some individuals were collected at the shoreline during low tide, other specimens and tissue samples were provided by colleagues or obtained from collections of the Natural History Museum of Los Angeles County (LACM), the Santa Barbara Museum of Natural History (SBMNH) and the California Academy of Sciences (CASIZ). Fieldwork was conducted under the California Department of Fish and Wildlife permit #13256. Specimens collected in the field were preserved in 95% ethanol and deposited at the California State Polytechnic University Invertebrate Collection (CPIC).

DNA was extracted from sixty specimens (Table 1) using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) using standard protocols provided by the manufacturer. A fragment of the cytochrome c oxidase subunit I (COI) mitochondrial gene was amplified and sequenced using universal primers (LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′, HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). PCR reaction conditions were as follows: denaturation at 95°C for 3 min, followed by 35 cycles of 94°C for 45 sec, 50°C for 45 sec, and 72°C for 2 min with final elongation at 72°C for 10 min. Successful DNA amplification was confirmed using an agarose gel electrophoresis and ethidium bromide. The PCR products were purified with a

Table 1. Number and collection sites of specimens for which the COI sequence data (mtDNA) and microsatellite fragment genotype data (nDNA) were analyzed. For the isolates, a plus sign (+) indicates only the COI gene was analyzed, asterisk (*) indicates only microsatellite data were analyzed.

	Sampling site	mtDNA	nDNA	Collection years	Isolates
Northern California	Pillar Point	16	15	2015	CK60+, CK61, CK62, CK63, CK64, CK65, CK66, CK67, CK68, CK69, CK70, CK71, CK73, CK74, CK75, CK76
	Scott Creek	0	1	2015	CK114*
Central California	Carmel Point	2	3	2011, 2016	CK111*, CK117, CK118
	Cayucos	2	0	2017	CK130+, CK131+
	Cave Landing	18	26	2016	CK29, CK30, CK31, CK32, CK33, CK34+, CK77, CK78, CK79, CK80*, CK81, CK82, CK83, CK84*, CK86, CK87, CK88, CK89, CK90*, CK91*, CK92*, CK93, CK94, CK95*, CK96*, CK97*, CK98
	Jalama Beach	8	8	2009	CK46, CK47, CK48, CK49, CK50, CK51, CK52, CK53
Southern California	Naples	1	1	2009	CK34+, CK36*
	San Clemente Is.	1	1	1961	CK11

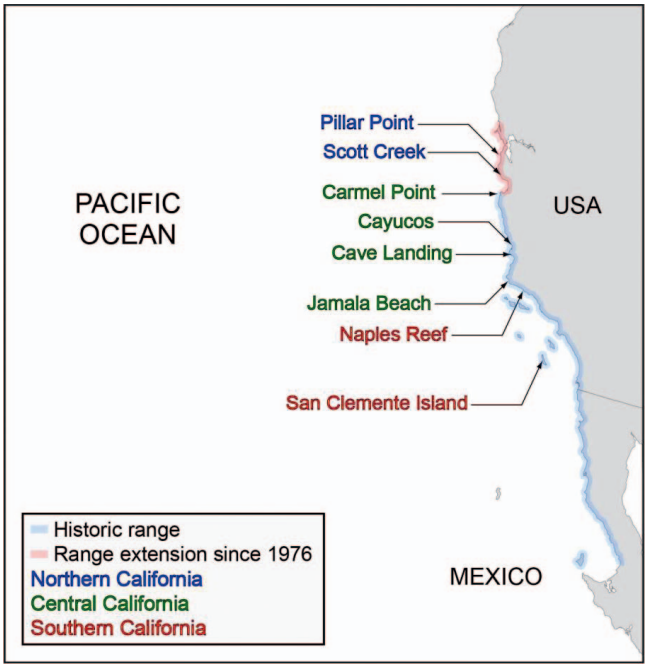

Fig. 1. Map of the historic and expanded range of *Phidiana hiltoni* along the western coast of North America. Sampling localities are indicated by arrows and coded with different grey tones by geographic regions.

GeneJET PCR Purification Kit (Fermentas, Waltham, MA) using standard protocols and were sent for sequencing to Source Bioscience Inc. (Santa Fe Springs, CA).

Sequences were assembled and aligned using Geneious v8.1.8 (Kearse et al. 2012). The geographic distribution of mtDNA haplotypes was visualized by producing a haplotype network using the program PopArt v1.7 (Leigh and Bryant 2015) using the TCS option. Haplotypes were pattern-coded by locality. Genetic structure within and among populations and among groups was examined using analysis of molecular variance (AMOVA) as implemented in Arlequin v3.5 (Excoffier and Lischer 2010). Three different AMOVA analyses were run to test for the effects of arranging populations into different groups based on the distribution of genetic variation. In the first AMOVA test, populations established after 1977 were included in the Northern California group while historic range populations were divided into two groups: Central California (populations north of Point Conception) and Southern California (populations south of Point Conception); this is the hypothesized biogeographic structure if Point Conception acts as a biogeographic barrier (Blanchette et al. 2008). To examine possible genetic similarities between newly formed populations in Northern California and those from southern Monterey Bay, two additional AMOVAs were run with different group arrangements, to examine whether this resulted in different distributions of genetic diversity among groups and among populations within groups. In the second AMOVA, the groups were kept the same except central California populations were split into two groups, Northern Central California (southern Monterey Bay) and Southern Central California (populations further south), see Blanchette et al. (2008). In the third AMOVA populations from southern Monterey Bay were pooled with Northern

California. Significance of the AMOVAs was tested using 16,000 permutations of individuals between groups. Arlequin v3.5 was also used to calculate pairwise Φ_{ST} between populations (1,000 permutations). Because the Southern California populations (Naples and San Clemente) were represented by one sequence each, populations were pooled together.

Microsatellite loci were identified through sequencing by synthesis with a MiSeq platform (Illumina, Inc., San Diego, CA). DNA was extracted from a single specimen collected from Pillar Point, California (CASIZ 190249), tagged with a unique barcode during library preparation, and pooled with other samples for Illumina sequencing. Sequencing was conducted at the UCLA Genotyping and Sequencing Core facility. Automated screening of sequences for tetranucleotide repeats and primer design were performed simultaneously in MSATCOMMANDER v1.0.8 (Faircloth 2008). Twenty-two primer pairs were purchased from Eurofins (Louisville, KY) with a M13 tail added to the 3′ end of each forward primer sequence.

Five of the twenty-two primer pairs were tested with ten specimens that consistently amplified for mtDNA to determine the PCR protocol. PCR protocol settings for the primers were optimized from a standard protocol by adjusting the annealing temperatures and elongation times until amplification was achieved. The PCR Master Mix for each locus in these tests included the forward primer with a M13 tail, reverse primer, and BSA (bovine serum albumin) and used Thermo Fisher Platinum Hot Start PCR Master Mix. The final PCR reaction conditions were as follows: denaturation at 95°C for 15 min, followed by 30 cycles of 94°C for 30 sec, 60–65°C for 30 sec, and 68°C for 30 sec with final elongation at 60°C for 10 min. All twenty-two primer pairs were tested with ten specimens that consistently amplified for mtDNA using the above conditions. Of the twenty-two primer pairs tested, ten polymorphic loci amplified reliably. Using the ten reliable primer pairs (Table S2) and the above amplification conditions, PCR was carried out with fifty-seven specimens. The PCR Master Mix for each locus in this final round now included a fluorescent M13 tag (5′-[6-FAM] AGGGTTTTCCCAGTCACGACGTT-3′) along with the original components. Genotyping was outsourced to Laragen Incorporated (Culver City, CA). Genotypes were scored using the Microsatellite Analysis External Plugin v1.4.4 implemented in Geneious v8.1.8 using the Two Surrounding Peaks setting (Kearse et al. 2012).

In total, fifty-five individual specimens were genotyped for all 10 microsatellite loci. This is a small sample size for this type of study, but specimens were difficult to obtain in the field. Collecting sea slugs is serendipitous in nature and after two years of fieldwork only a small number of specimens was obtained. Additionally, most museum specimens examined were unsuitable for molecular work. Population subdivision in the nuclear genome was inferred using STRUCTURE v2.3.4 (Pritchard et al. 2000) with the default parameters; 5 replicates for each value of *K* were run for 1,000,000 MCMC iterations following a burn-in period of 100,000. To detect the true number of clusters (*K*) using the Evanno Method (Evanno et al. 2005) the result file from STRUCTURE was processed with STRUCTURE Harvester v0.6.9.84 (Earl and vonHoldt 2012). Using the selected *K* value (3) the resulting files were processed with CLUMPP v1.1.2 (Rosenberg et al. 2002) and Distruct v1.1 (Rosenberg, 2004) to generate a graphic display of the population structure. AMOVA and F_{ST} pairwise genetic differentiation comparisons between populations were conducted following the same methodology as in the mtDNA analyses. Microsatellite data were also analyzed via Discriminant Analysis of Principal Components (DAPC) using the *adegent* package in R (Jombart et al. 2010).

To determine whether the abundance of *P. hiltoni* in Southern California has changed since the mid-20th century, counts of nudibranchs by James R. Lance dating from 1953

to 2001[1] at six rocky intertidal sites in San Diego County (Point Loma, Hill Street, False Point, Bird Rock, Windansea, and South Casa Reef) were examined and analyzed. The data for *P. hiltoni* were extracted and grouped by site, decade, and before and after 1963, the year when, excepting one brief trip to Bird Rock in 1956, Lance started sampling outer coast sites in San Diego County other than Point Loma. Counts made on consecutive or near-consecutive dates at any given site were excluded from analysis in order to reduce autocorrelation in the data; the count retained was the one with the highest number of *P. hiltoni*. Twenty-one additional counts conducted by either JG or CK from 2000 to 2016 at 4 of the same sites (Point Loma, Hill Street, Bird Rock, and South Casa Reef) were also included in the analysis. A Wilcoxon sign-rank test was implemented in JMP v13, SAS Institute Inc. (Cary, NC) and used to compare the number of *P. hiltoni* found at Point Loma before and after 1963. Additional information on the recent occurrence of *P. hiltoni* in San Diego County was obtained from the website iNaturalist (https://www.inaturalist.org/taxa/48724-Phidiana-hiltoni) and the species database on Divebums, a San Diego dive website (http://species.divebums.com/index.php?l=sciname&n=Phidiana%20hiltoni), and confirmed by the authors.

Results

The haplotype network of the mitochondrial COI gene recovered five distinct haplotypes (Fig. 2). Thirty-three individuals spanning all seven populations share the most common haplotype. Three haplotypes diverge from the most common haplotype by only two nucleotides. The most common of these three haplotypes was found exclusively in fourteen individuals from Pillar Point, Northern California. The other two haplotypes are only found in specimens originating from Cayucos and Cave Landing, Central California. An additional specimen from Cave Landing possessed a haplotype diverging from the most common haplotype by three nucleotides.

In the first AMOVA test (Northern California: Pillar Point; Central California: Cave Landing, Jalama Beach, Cayucos, and Carmel Point) most of the genetic variation is recovered among groups (70.05%) and within populations (30.59%), with virtually no variation among populations within groups (-0.63%) (Table 2). In the second AMOVA test (Northern California: Pillar Point; Northern Central California: Carmel Point; Southern Central California: Jalama Beach, Cave Landing, Cayucos) most of the genetic variation is again among groups (65.94%) and within populations (32.82%) and very little variation among populations within groups (1.24%) (Table 2). In the third AMOVA test (Northern California: Pillar Point, Carmel Point; Central California: Jalama Beach, Cave Landing, Cayucos) most of the genetic variation is found again among groups (50.47%) and within populations (33.5%) however genetic variation is found among populations within groups (16.04%) (Table 2).

A pairwise Φ_{ST} test was run on all populations and resulted in relatively high values between Pillar Point and all other populations and also between Carmel Point and Cayucos. The only significant difference in genetic variation found was between Pillar Point, Northern California and each of the four Central California populations: Jalama Beach, Cave

[1] Goddard, J.H.R. 2013. Opisthobranch gastropods observed on the outer coast of San Diego County, California by James R. Lance, 1953–2001. knb.298.2. [online] California Academy of Sciences. Available https://knb.ecoinformatics.org/knb/metacat/knb.298.2/knb [2017 Jun 15].

Table 2. AMOVA test results for mitochondrial haplotype data of three separate population groupings obtained with Arlequin v3.5, significant values ($p \leq 0.05$) in bold. **Grouping 1**: Northern California (Pillar Point), Central California (Jalama Beach, Cave Landing, Cayucos, Carmel Point), and Southern California (San Clemente, Naples). **Grouping 2**: Northern California (Pillar Point), Northern Central California (Carmel Point), Southern Central California (Jalama Beach, Cave Landing, Cayucos) and Southern California (San Clemente, Naples). **Grouping 3**: Northern California (Pillar Point, Carmel Point), Central California (Jalama Beach, Cave Landing, Cayucos), and Southern California (San Clemente, Naples).

Source of variation	d.f.	Sum of squares	Variance components	% of variation	Fixation indices	*p* value
Grouping 1						
Among Groups	2	8.258	0.33629	70.05	-0.02112	**0.01019**
Among Populations within Groups	4	0.533	-0.00304	-0.63	0.69413	**0**
Within Populations	41	6.021	0.14685	30.59	0.70046	**0.02775**
Total	47	14.812	0.48011			
Grouping 2						
Among Groups	3	8.268	0.29502	65.94	0.03642	0.20297
Among Populations within Groups	3	0.524	0.00555	1.24	0.67179	**0**
Within Populations	41	6.021	0.14685	32.82	0.65938	0.09205
Total	47	14.812	0.44742			
Grouping 3						
Among Groups	2	6.900	0.22123	50.47	0.32374	**0.01969**
Among Populations within Groups	4	1.892	0.0703	16.04	0.66502	**0**
Within Populations	41	6.021	0.14685	33.5	0.50466	0.11511
Total	47	14.812	0.43838			

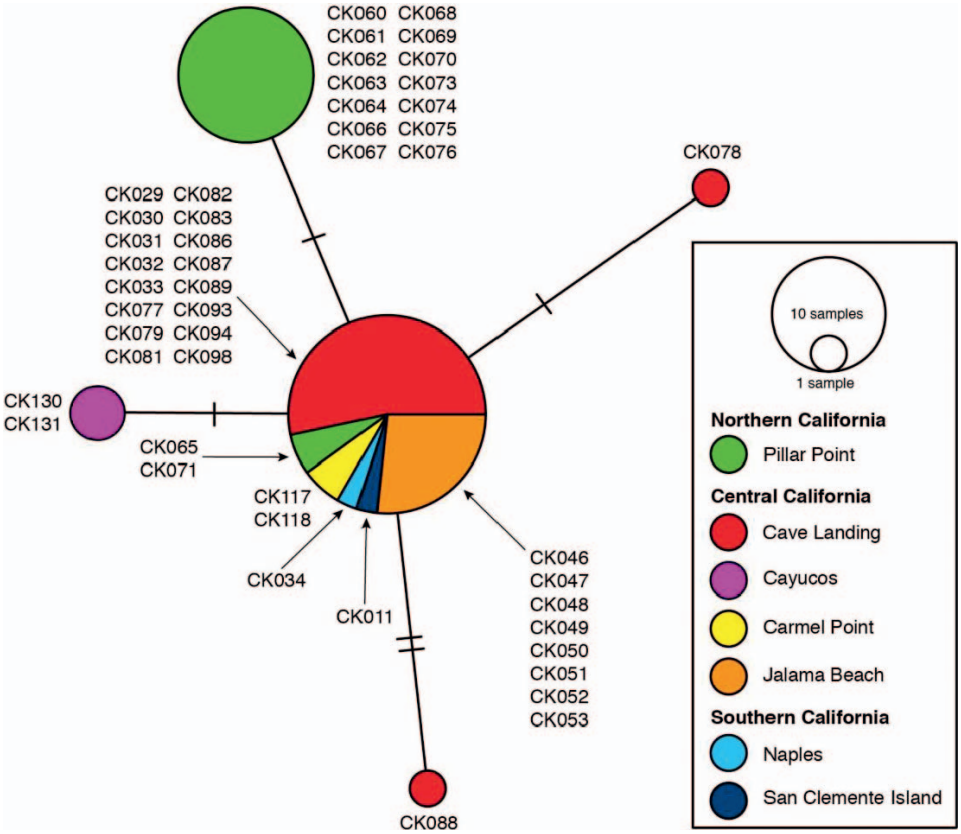

Fig. 2. Haplotype network of COI mitochondrial sequences generated with PopArt v1.7. Each circle represents a unique haplotype and its area is proportional to the number of specimens sequenced with that haplotype. Each pattern represents the geographic origin of the individual specimens, as indicated in the legend. Isolate codes are indicated next to each haplotype.

Landing, Cayucos and Carmel Point ($\Phi_{ST} = 0.69$, $p = 0.03$; $\Phi_{ST} = 0.75$, $p = 0.00$; $\Phi_{ST} = 0.69$, $p = 0.00$; $\Phi_{ST} = 0.67$, $p = 0.04$ respectively) (Table 3). This suggests genetic differentiation between Pillar Point and the other populations. However, this result, as well as the lack of significant differentiation among other pairwise comparisons, must be interpreted with caution due to the limited number of samples from all collection sites except for Pillar Point and Cave Landing.

Analysis of microsatellite data with Structure Harvester using Evanno's method (Evanno et al. 2005), a maximum value of the rate of change (ΔK) in the log probability of data was obtained at $K = 3$ (Fig. 3B). These three recovered clusters are unevenly distributed among geographic regions with no obvious geographic subdivision (cluster 1: red, cluster 2: blue, cluster 3: yellow, Fig. 3A). Moreover, all individuals exhibit a non-zero probability of belonging to any one of the three clusters.

All AMOVA tests with different groupings produced very similar results; the overwhelming majority of the genetic variation was recovered within populations (92.32–93.61%) and some among populations within groups (6.51–9.98%), with virtually no variation among groups (-3.59–1.17%) (Table 4). Pairwise F_{ST} comparisons produced very low values

Table 3. Φ_{ST} pairwise comparison values for mitochondrial haplotype data obtained with Arlequin v3.5 (lower triangular) and associated *p* values (upper triangular). Significant values ($p \leq 0.05$) in bold.

	Pillar Point	Carmel Point	Cayucos	Cave Landing	Jalama Beach	Naples	San Clemente
Pillar Point	–	**0.03062**	**0.00019**	**0.0000**	**0.03537**	0.09994	0.99994
Carmel Point	0.69490	–	0.21358	0.32000	0.99994	0.99994	0.99994
Cayucos	0.75345	0.62791	–	0.99994	0.99994	0.99994	0.99994
Cave Landing	0.68730	0.29687	-0.05381	–	0.99994	0.99994	0.99994
Jalama Beach	0.66771	0.0000	0.0000	-0.33043	–	0.99994	0.99994
Naples	0.61778	0.0000	0.0000	0.0000	0.0000	–	0.99994
San Clemente	0.61778	0.0000	0.0000	0.0000	0.0000	0.0000	–

across the entire range suggesting little to no genetic differentiation between populations (Table 5). DAPC, which attempts to group individuals using a k-means clustering algorithm, suggests that the entire metapopulation of *P. hiltoni* cannot be divided into more than one group based on microsatellite data (Fig. 4).

From 1953 to 1962, Jim Lance sampled for nudibranchs on the outer coast of San Diego County only at Point Loma, where he found at least one *Phidiana hiltoni* during half of his 28 trips during that period (Fig. 5A). In 1964 he began to sample additional outer coast sites. Since then, only two more *P. hiltoni* were found at Point Loma (Figure 5A & B), one by Lance in July 1968 and one by JG in June 2001. Similarly, *P. hiltoni* was observed on only about 10% of the trips to each of the other five outer coast sites (Fig. 5A), and was found in lower numbers per trip than had been seen in the earlier period at Point Loma (Fig. 5B). Significantly fewer *P. hiltoni* were found at Point Loma after 1963 than before (Wilcoxon rank sum test, P = 0.002) (Figs. 5B, C). Except for the 1990s, when only 10 total trips were made to two sites, *P. hiltoni* has been found in San Diego County in low numbers in each of the decades since the 1960s (Fig. 5C). Finally, *P. hiltoni* has been photographed subtidally in San Diego County at least 10 times since 2005 (http://species.divebums.com/index.php?l = sciname&n = Phidiana%20hiltoni; https://www.inaturalist.org/taxa/48724-Phidiana-hiltoni).

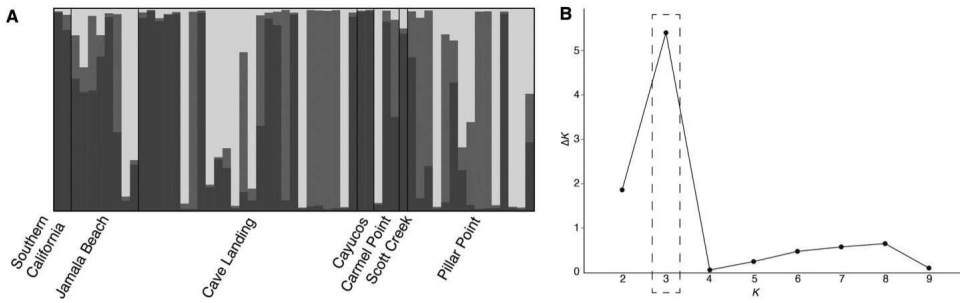

Fig. 3. Genetic clustering analysis for the entire data set of 57 individuals and 10 microsatellite regions as estimated by STRUCTURE v2.3.4. **A.** Genetic clustering plot for $K = 3$ clusters, generated with CLUMPP v1.1.2. Each grey tone represents a different genetic cluster. Bar graphs show average posterior probability of membership (*y*-axis) of each individual. Populations are delimited by dark vertical lines. **B.** Graph of ΔK = mean (|L"(*K*)|) / sd(L(*K*)) as a function of *K* (potential number of genetic clusters) generated by STRUCTURE Harvester v0.6.9.84. The most likely number of clusters is indicated by the modal value, in this case $K = 3$.

Table 4. AMOVA test results for microsatellite genotype data with three separate population groupings obtained with Arlequin v3.5, significant values ($p \leq 0.05$) in bold. **Grouping 1**: Northern California (Pillar Point), Central California (Jalama Beach, Cave Landing, Cayucos, Carmel Point), and Southern California (San Clemente, Naples). **Grouping 2**: Northern California (Pillar Point), Northern Central California (Carmel Point), Southern Central California (Jalama Beach, Cave Landing, Cayucos) and Southern California (San Clemente, Naples). **Grouping 3**: Northern California (Pillar Point, Carmel Point), Central California (Jalama Beach, Cave Landing, Cayucos), and Southern California (San Clemente, Naples).

Source of variation	d.f.	Sum of squares	Variance components	% of variation	Fixation indices	*p* value
Grouping 1						
Among Groups	2	16.091	-0.15376	-3.59	0.06387	**0.01075**
Among Populations within Groups	3	27.962	0.42745	9.98	0.09630	**0.00719**
Within Populations	104	417.174	4.01129	93.61	-0.03588	0.49775
Total	109	461.227	4.28498			
Grouping 2						
Among Groups	3	28.051	0.05088	1.17	0.07679	**0.01106**
Among Populations within Groups	3	16.002	0.28276	6.51	0.06585	0.27272
Within Populations	104	417.174	4.01129	92.32	0.01171	0.15679
Total	109	461.227	4.34492			
Grouping 3						
Among Groups	2	17.137	-0.09775	-2.27	0.06886	**0.01106**
Among Populations within Groups	3	26.916	0.3944	9.16	0.08952	**0.0245**
Within Populations	104	417.174	4.01129	93.11	-0.02269	0.37895
Total	109	461.227	4.30793			

Table 5. F_{ST} pairwise comparison values for microsatellite genotype data, obtained with Arlequin v3.5 (lower triangular) and associated p values (upper triangular), significant values ($p \leq 0.05$) in bold. The two southern California populations of Naples and San Clemente were combined into one due to low sampling numbers.

	Pillar Point	Scott Creek	Carmel Point	Cave Landing	Jalama Beach	S. California
Pillar Point	–	0.93776	**0.01968**	0.64388	0.39049	0.35181
Scott Creek	0.13279	–	0.99994	0.99994	0.99994	0.99994
Carmel Point	0.14426	0.35183	–	**0.00306**	**0.00444**	0.10229
Cave Landing	0.01779	0.17272	0.15117	–	0.06255	0.05986
Jalama Beach	0.04219	0.23112	0.19336	0.05260	–	0.19821
S. California	0.12656	0.36283	0.30272	0.15087	0.15965	–

Discussion

The California coast is a prime example of a region where climate change is impacting native marine ecosystems through changes in ocean temperatures, seawater chemistry, and coastal current regimes (Barry et al. 1995; Sagarin et al. 1999; Harley et al. 2006). With ocean temperatures increasing, species are predicted to shift their ranges poleward, a trend that has already been observed across a wide range of taxonomic groups, mostly at temperate latitudes (Dawson et al. 2010; VanDerWal et al. 2012). It is difficult to predict how environmental change, including biotic exchanges resulting from species range shifts and introductions, will affect ecological systems. Hellman et al. (2008) emphasized that

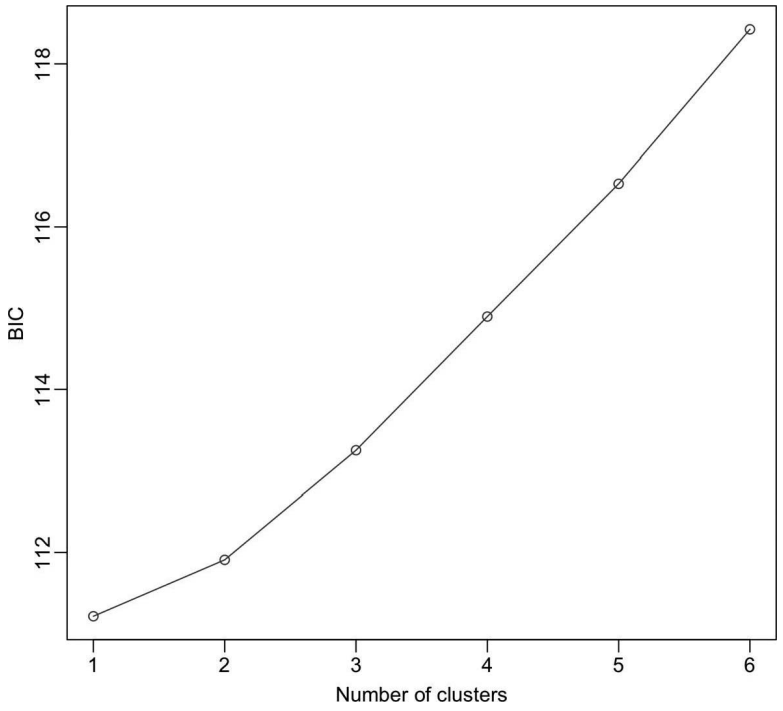

Fig. 4. Results of the DAPC analysis indicating that the data, when analyzed as principal components, cannot be divided into more than one group.

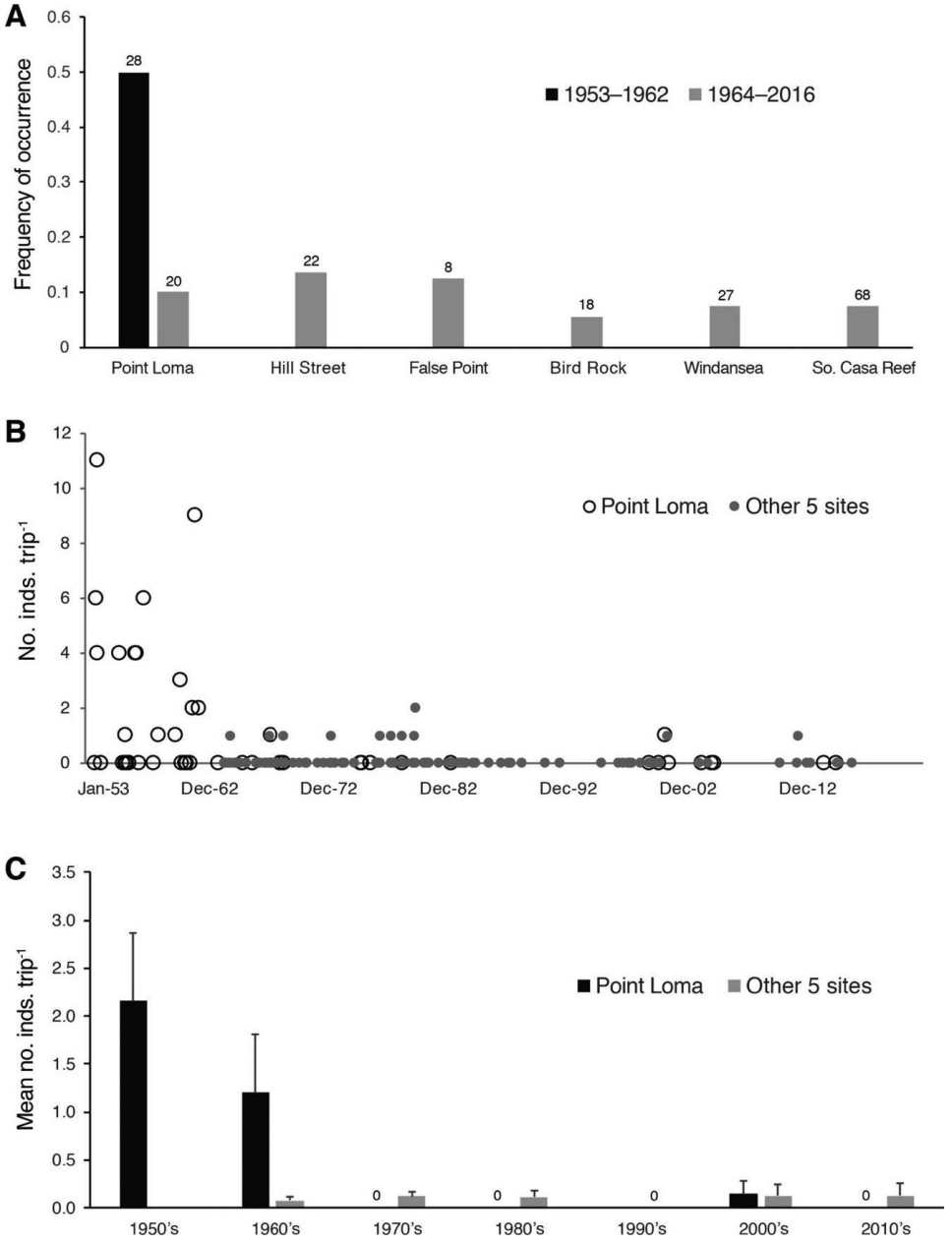

Fig. 5. Occurrence of *Phidiana hiltoni* at six rocky intertidal sites in San Diego County, 1953–2016. **A**. Frequency of occurrence by site, 1953–1962 and 1964–2016. Number of trips to each site shown above bars. **B**. Number of *P. hiltoni* found on each trip, 1953–2016. **C**. Mean number (± SE) of *P. hiltoni* found per trip at Point Loma and other sites combined, by decade.

global climate change compounds this difficulty because it transforms transport and introduction mechanisms, impacts the distribution of existing invasive species, and alters the effectiveness of control strategies. Efforts to manage and conserve marine ecosystems in the face of climate change will require improvements to the existing predictive framework to aid in preventing future introductions (Harley et al. 2006). In this context, understanding the mechanisms behind range expansions (or shifts) of individual species will contribute to a larger body of evidence, critically important for predicting the biological effects of climate change.

The data presented in this paper provide insight into the processes underlying the range expansion in *P. hiltoni*. The analysis of mitochondrial DNA (mtDNA) sequence data of *Phidiana hiltoni* across both its historical range and extended range in Northern California revealed limited genetic structure. The COI haplotype network shows very little polymorphism with only five haplotypes in total being recovered (Fig. 2). The most common of these haplotypes is shared among individuals from all seven populations, including the newly formed populations in Northern California. However, there are a few haplotypes only detected in certain populations. For example, one of the two haplotypes found at Pillar Point, north of Monterey Bay, was found nowhere else. Because populations north of Monterey Bay did not exist prior to 1977 (Goddard et al. 2011), it is likely that this unique haplotype is also present south of Monterey Bay but at such low frequency that it has not been yet detected, and may have become more common in the recently colonized populations due to founder effects. To better understand the geographic structure of *P. hiltoni* based on mtDNA, three AMOVA analyses were run, each with different population groupings. In each of the three groupings, the highest percent variation was consistently found among groups (Table 2). However, there is a decrease in this percent variation as the groupings structure are altered (70.05%, 65.94%, 50.47% respectively), suggesting that the first grouping arrangement (in which the Northern California group includes only populations found in the extended range) best represents population genetic structure according to mtDNA data. Pairwise Φ_{ST} comparisons agree with the geographic structure recovered in the AMOVAs (Table 3). Relatively high Φ_{ST} values indicating genetic differentiation were found between Pillar Point and the Central and Southern California populations, and between Carmel Point and the two populations of Cayucos and Cave Landing. This is consistent with the haplotype network results, showing that Pillar Point, Cayucos and Cave Landing possess divergent haplotypes from the most common haplotype found across the range of *P. hiltoni*.

While analyses of mtDNA sequences suggest population structure and genetic differentiation among groups, no genetic pattern corresponding to geography was detected using microsatellite data. Across the range of *P. hiltoni*, Structure analyses showed several individuals have nearly identical probabilities of cluster membership, a pattern that is particularly apparent at Cave Landing. This genetic uniformity could be an indication of self-recruitment at Cave Landing, where the concavity in the coastline (accentuated by the 750-meter long rock jetty on Point San Luis) may encourage larval retention. Goddard et al. (2011) suggested that population structure in *P. hiltoni* should be affected by upwelling shadows, resulting in local retention of short-lived larvae, particularly at the northern end of bights along the coastline (Graham and Largier 1997; Roughan et al. 2005). AMOVAs on microsatellite data included the same three distinct groupings as mitochondrial analyses except for the addition of Scott Creek to the Northern California group and the removal of Cayucos (where microsatellite data were not successfully recovered). In all AMOVA tests, most genetic variance was found within populations (93.61%, 92.32% and

93. 11%, respectively) (Table 4). Consistently low variation among groups is indicative of high levels of gene flow across the range, as seen in the mtDNA data. Pairwise comparisons of F_{ST} values from microsatellite data are also consistent with high gene flow across the range, with generally low F_{ST} values between populations (Table 5). The slightly higher F_{ST} values found between Scott Creek and Carmel Point, as well as between Carmel Point and Southern California are unreliable due to the low sample sizes from Scott Creek and Southern California.

AMOVA and pairwise Φ_{ST} comparisons using mtDNA data suggest Northern California populations are genetically distinct, and consequently the origin of the recently founded populations remains unclear. On the contrary, microsatellite data indicate very high levels of gene flow in *P. hiltoni,* with Northern California populations genetically indistinguishable from those in Central California. This discrepancy of results from mtDNA vs. nuclear data might be explained by the reproductive behavior of *P. hiltoni*. Rutowksi (1983) found that species with high rates of cannibalism require several couplings, usually with different mates, in order for all the eggs in the egg mass to be fertilized. It is very likely that *P. hiltoni* (considering the pugnacious and cannibalistic nature of this species) exhibits abbreviated coupling times, requiring several mates to fully fertilize egg masses. Additionally, *P. hiltoni* exhibits locally high population densities in Central California, potentially facilitating promiscuity and thus intense sperm competition. The main consequence of this mating system would be that egg masses produced by a single individual (functional female) will be sired by multiple partners, generating offspring with genetically identical mitochondria but different nuclear alleles. If recently established populations in Northern California are the result of sporadic events involving dispersal of larvae (or rafting of egg masses) produced by a small number of females, it is likely that founder effects facilitated retention of these rare mitochondrial haplotypes. While the diversity of nuclear alleles should also decrease due to drift and founder effects, the polyandrous mating system of *P. hiltoni* has the potential to mitigate these effects. Under this scenario, Northern California populations could harbor mitochondrial haplotypes that were previously very rare in the historic range, while nuclear alleles from the source population are more broadly represented. If this hypothesis is correct, additional sampling from Central California should detect all or most Northern California haplotypes. An alternative explanation is that the lack of genetic structure in microsatellite data is an artifact of the limited sample size. If this is the case, additional sampling across the range of *P. hiltoni* would improve the reliability of the results of this study.

Another outstanding question is what mechanism(s) allowed *P. hiltoni* to cross Monterey Bay starting in the late 1970s, or what physical or biological barriers restricted the prior range of this species. If recently established populations in Northern California are indeed the result of sporadic dispersal by a limited number of individuals, this would suggest that this dispersal was not triggered by a gradual process, such as increasing ocean temperatures, but instead by the temporary or intermittent opening of a corridor. One distinct possibility is that a weakening of the upwelling shadow in Monterey Bay (Graham and Largier 1997) due to climate change may have facilitated this process. Pennington et al. (2000) documented decadal-scale changes in the oceanographic conditions near the center of Monterey Bay region consistent with those described for the 1976–77 climate shift in the North Pacific Ocean. These include increased stratification of surface waters, warmer, less productive waters during non-upwelling seasons and a later onset of upwelling. These changes may have reduced larval retention in northern Monterey Bay, allowing *P. hiltoni* to disperse into Northern California.

Few studies have examined the population genetics of other marine invertebrate taxa that have experienced recent range expansions or range shifts. In California, a similar study by Dawson et al. (2010) examined three hypotheses/scenarios that explain the causes of range limits of species, and concluded that in the volcano barnacle, *Tetraclita rubescens*, the northern range boundary is maintained by migration load arising from flow of maladapted alleles into peripheral locations. Dawson et al. (2010) proposed that in this species (with planktonic-dispersing larvae), environmental amelioration, likely due to climate change, resulted in a reduction of the strength of selection against immigrant phenotypes in the northern range boundary, allowing the species to expand northward. The case of *P. hiltoni* is very different in several respects, but the main difference is that whereas *T. rubescens* was declining near its northern range limit, *P. hiltoni* has been and remains common. Framing of our data in the three scenarios/hypotheses proposed by Dawson et al. (2010) suggest physical barriers to dispersal is the most likely mechanism that historically restricted migration in *P. hiltoni*.

This study included a sample of 57 individual specimens collected across the range of *P. hiltoni*, but the sample size from Southern California, south of Point Conception, is small. Only two individuals were collected despite a substantial collecting effort by the senior author in this region during two consecutive years. This suggests that *P. hiltoni* could have become rare in the southern portion of its range. However, this assumption should be interpreted with caution. Bates et al. (2015) shown that abundance-related species detectability, particularly important in uncommon, difficult-to-detect marine species such as *P. hiltoni*, has the potential to confound our understanding of the true location of range edges. Bates et al. (2015) emphasized the importance of simulation and modeling, but also long-term monitoring with consistent sampling effort through time. In this case, we analyzed high-quality, long-term observational data collected at fairly regular intervals from the same region. These data suggest that the historical abundance of *P. hiltoni* in San Diego County, especially Point Loma (the type locality of *P. pugnax* Lance 1961 [= *P. hiltoni*]) has declined (Figs. 4A–C). Reasons for this decline remain unknown and warrant further investigation, although rising ocean temperatures appears to be a viable hypothesis. Notably, the abundance of another species of sea slug, *Felimare californiensis*, was once-common in Southern California, but became extinct there in the 1980's (Goddard et al. 2013). Although individuals of *F. californiensis* reappeared in 2003 and the species has since been found in a few isolated localities in Southern California (Goddard et al. 2013; Hoover 2015), its populations have not completely recovered. It is unclear whether there is a link between the decline of these two ecologically distinct species, but if there is, it may be a symptom of larger and more pervasive environmental change. The apparent decline of *P. hiltoni* in Southern California along with its dispersal northward needs to be substantiated with further monitoring and additional data analyses (Bates et al. 2015), but if confirmed, would suggest this is a true poleward range expansion rather than a temporary shift (Parmesan et al. 1999). Understanding the process by which *P. hiltoni* migrated northward may provide insight as to how other benthic organisms will respond to rising ocean temperatures and changes in ocean current systems (McGowan et al. 1998).

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Appendix

Table S1. Complete list of specimens sequenced for this study, including isolate number, locality, collection date, and GenBank accession numbers.

Isolate	Locality	Collection date	GenBank accession #
CK11	San Clemente Island, CA	1961	MK333330
CK29	Cave Landing, CA	10/8/2014	MK333291
CK30	Cave Landing, CA	10/8/2014	MK333292
CK31	Cave Landing, CA	10/8/2014	MK333293
CK32	Cave Landing, CA	10/8/2014	MK333294
CK33	Cave Landing, CA	10/8/2014	MK333295
CK34	Naples, CA	12/2009	MK333313
CK46	Tarantula Reef, Jalama Beach, CA	12/15/2009	MK333331
CK47	Tarantula Reef, Jalama Beach, CA	12/15/2009	MK333332
CK48	Tarantula Reef, Jalama Beach, CA	12/15/2009	MK333333
CK49	Tarantula Reef, Jalama Beach, CA	12/15/2009	MK333334
CK50	Tarantula Reef, Jalama Beach, CA	12/15/2009	MK333335
CK51	Tarantula Reef, Jalama Beach, CA	12/15/2009	MK333336
CK52	Tarantula Reef, Jalama Beach, CA	12/15/2009	MK333337
CK53	Tarantula Reef, Jalama Beach, CA	12/15/2009	MK333338
CK60	Pillar Point, CA	6/18/2015	MK333314
CK61	Pillar Point, CA	6/18/2015	MK333315
CK62	Pillar Point, CA	6/18/2015	MK333316
CK63	Pillar Point, CA	6/18/2015	MK333317
CK64	Pillar Point, CA	6/22/2015	MK333318
CK65	Pillar Point, CA	6/22/2015	MK333319
CK66	Pillar Point, CA	6/22/2015	MK333320
CK67	Pillar Point, CA	6/22/2015	MK333321
CK68	Pillar Point, CA	6/22/2015	MK333322
CK69	Pillar Point, CA	6/22/2015	MK333323
CK70	Pillar Point, CA	6/22/2015	MK333324
CK71	Pillar Point, CA	6/22/2015	MK333325
CK73	Pillar Point, CA	6/22/2015	MK333326
CK74	Pillar Point, CA	6/22/2015	MK333327
CK75	Pillar Point, CA	6/22/2015	MK333328
CK76	Pillar Point, CA	6/22/2015	MK333329
CK77	Cave Landing, CA	1/16/2016	MK333296
CK78	Cave Landing, CA	1/16/2016	MK333297
CK79	Cave Landing, CA	1/16/2016	MK333298
CK80	Cave Landing, CA	1/16/2016	MK333299
CK81	Cave Landing, CA	1/16/2016	MK333300
CK82	Cave Landing, CA	1/16/2016	MK333301
CK83	Cave Landing, CA	1/16/2016	MK333302
CK84	Cave Landing, CA	1/16/2016	MK333303
CK86	Cave Landing, CA	1/16/2016	MK333304
CK87	Cave Landing, CA	1/16/2016	MK333305
CK88	Cave Landing, CA	1/16/2016	MK333306
CK89	Cave Landing, CA	2/5/2016	MK333307
CK93	Cave Landing, CA	2/5/2016	MK333308
CK94	Cave Landing, CA	2/5/2016	MK333309
CK98	Cave Landing, CA	2/5/2016	MK333310
CK117	Carmel Pt, Monterey Bay CA	–	MK333289
CK118	Carmel Pt, Monterey Bay CA	–	MK333290
CK130	Cayucos, CA	–	MK333311
CK131	Cayucos, CA	–	MK333312

Table S2. List of primer pairs (and their sequences) used to amplify polymorphic microsatellite loci in *P. hiltoni*. Bolded portion of forward primers indicate M13 tail.

Primer	Sequence
Phil760625F	**AGGGTTTTCCCAGTCACGACGTT**AACGTCGTCATGGAATTCACAG
Phil760625R	GTTTATTAATGGCGGCGATGTGAC
Phil792112F	**AGGGTTTTCCCAGTCACGACGTT**AACCAATCGACGACAAGCTAAC
Phil792112R	GTTTGTCTCCGTGTTAAGTGTTGC
Phil820905F	**AGGGTTTTCCCAGTCACGACGTT**ACATTACTCCACTCGACTCAGG
Phil820905R	GTTTAGTCTCGGTCCATGAATCAGG
Phil928092F	**AGGGTTTTCCCAGTCACGACGTT**GATTCTATGCCACACACCTTGG
Phil928092R	GTTTAATGTATCTGCTTCATCCGTGC
Phil98151F	**AGGGTTTTCCCAGTCACGACGTT**AGAGGAATAGTCGCGGAACTAC
Phil98151R	GTTTCATCATTGCGTCAGATGTCC
Phil109255F	**AGGGTTTTCCCAGTCACGACGTT**CACACGTTCATACACTCACCTG
Phil109255R	GTTTAACACCGAGACAAGACATGC
Phil585958F	**AGGGTTTTCCCAGTCACGACGTT**ACTCTCTCACACCTGTCAAGTC
Phil585958R	GTTTCACCTCAGTACAGTCTCGTG
Phil918696F	**AGGGTTTTCCCAGTCACGACGTT**ACTCTCTCACACCTGTCAAGTC
Phil918696R	GTTTCACCTCAGTACAGTCTCGTG
Phil121774F	**AGGGTTTTCCCAGTCACGACGTT**GTCAAGTGAATAAGACGGCGAG
Phil121774R	GTTTCTGCCTGCTATACATCCATCC
Phil315595F	**AGGGTTTTCCCAGTCACGACGTT**GTAACACAGTGTCCGTATGTGG
Phil315595R	GTTTATCATTCTACGTGCATGCTGTC