

# Isolation and population divergence of a widespread Indo-West Pacific marine gastropod at Easter Island

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**Abstract** Oceanic islands represent excellent systems for studying the link between geographic isolation and population divergence. Easter Island is the world's most isolated island and exhibits a high level of endemism in the near-shore marine environment. Yet few studies have examined the effect of such extreme isolation on the divergence of populations of widespread species that occur at Easter Island. *Conus miliaris*, a marine gastropod distributed throughout much of the Indo-West Pacific, occurs at Easter Island where the population is ecologically and morphologically distinct from other populations of the species. To determine whether these phenotypic differences are associated with genetic isolation of the Easter Island population, we investigated the phylogeography of this species by examining mitochondrial COI sequences obtained from 141 individuals from eight localities occurring predominantly in the western, central and southeastern Pacific. Results from our analyses show that *C. miliaris* at Easter Island differs genetically from other populations. We estimate that *C. miliaris* colonized Easter Island shortly after the origin of the island  $\leq 0.7$  million years ago and that since population founding, gene flow has occurred predominantly from Easter Island to the west and that little migration has occurred into Easter Island.

## Introduction

The unique biotas of oceanic islands inspired Darwin (1859) to formulate his theory of evolution by natural selection and motivated Mayr (1942) to consider that isolation and small population size speed up the speciation process. Oceanic islands, as well as analogous systems that are island-like (e.g., isolated mountain ranges or so called 'sky-islands', lakes and other pockets of unique habitats), can be sufficiently isolated from other suitable habitats such that the remoteness promotes differentiation of organisms that happen to colonize them. Such environments thus provide natural laboratories for studying the processes involved with the formation of new species and origins of biodiversity (Emerson 2002).

Easter Island is the most isolated island in the world. Aside from Sala y Gómez Islands that occur approximately 400 km to the east of Easter Island, the next closest emergent landmass is Ducie Island, part of the Pitcairn Island group, approximately 1,600 km to the west. The Galapagos Islands are the closest landmasses to the north (approximately 3,800 km NNE from Easter Island) and the Juan Fernández Islands are the closest to the east (about 3,100 km ESE). As might be expected from such extreme isolation, levels of endemism at Easter Island of marine taxa are quite high; approximately 42% of marine molluscs (Rehder 1980), 67% of ostracods (Whatley and Jones 1999), 39% of sponges (Boyko 2001) and 22% of shore fishes (Robertson 2001) are unique to Easter Island.

Easter Island and the Sala y Gómez Islands are the only currently emergent segments of the Easter Seamount Chain (or Sala y Gómez Ridge) (Haase et al. 1997). The geographic features that comprise the Easter Seamount Chain tend to increase in age from west to east (O'Connor et al. 1995). Although early estimates of volcanic flows on Easter

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Island suggested that the island arose about 2.5–3 million years ago (mya) (Baker et al. 1974; Bonatti et al. 1977; Clark and Dymond 1977), more recent analyses show that the island originated  $\leq 0.7$  mya (Kaneoka and Katsui 1985; Haase et al. 1997). The discrepancy of these estimates is purportedly due to contamination of atmospheric argon in the earlier potassium–argon age estimates (Haase et al. 1997). Sala y Gómez Islands originated approximately 1.3–1.9 mya (O'Connor et al. 1995).

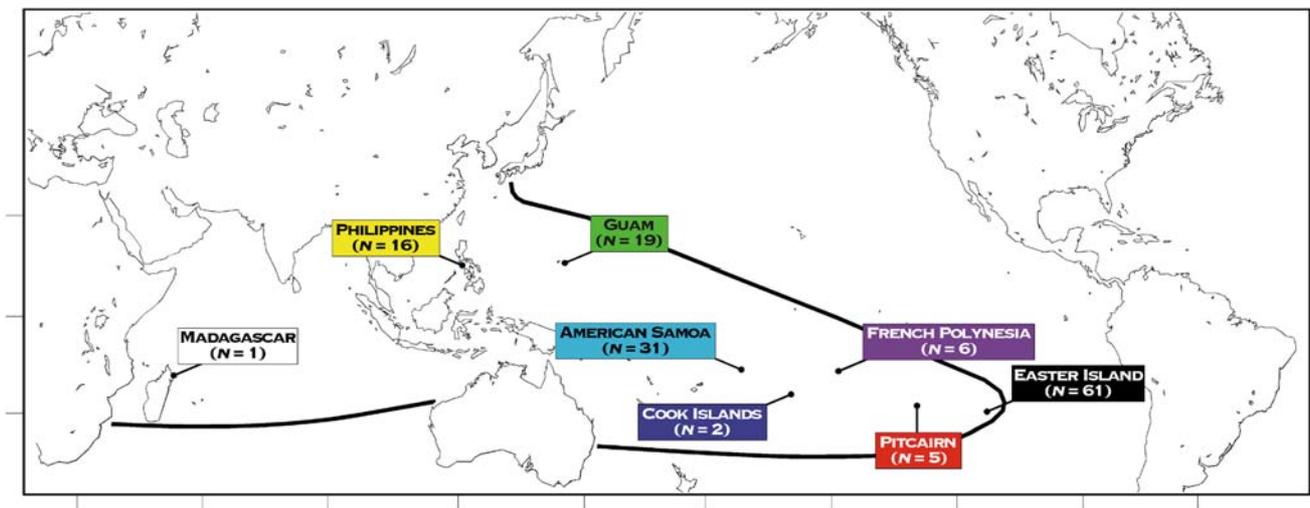
Based on the relatively young ages of Easter Island and other nearby features of the Easter Seamount Chain to the east, many of the Easter Island endemics arose recently, presumably within the last 700 thousand years if they directly colonized Easter Island. The Easter Island marine fauna shares affinity with other Indo-West Pacific localities (Rehder 1980; Whatley and Jones 1999) and so most endemics presumably arose via colonization and differentiation from source populations in the west. The extreme isolation of Easter Island has likely facilitated the divergence of populations that became established at Easter Island through limited gene flow from original source populations. Nonetheless, only a few studies have examined the phylogeography of widespread species or endemics that occur at Easter Island (Bowen et al. 2001; Lessios et al. 2001, 2003; Meyer and Paulay 2005; Lessios and Robertson 2006). In most cases, populations of widespread species at Easter Island have not diverged genetically from populations elsewhere, a pattern that is either attributed to high rates of gene flow (Bowen et al. 2001; Lessios et al. 2001, 2003) or recent colonization of Easter Island (Lessios and Robertson 2006). However, some Easter Island endemics are genetically distinct from related species elsewhere (Meyer and Paulay 2005).

The predatory marine gastropod genus *Conus* is a hyperdiverse group that includes more than 300 species in the

Indo-West Pacific (Rehder 1980). Regional diversity of *Conus* at localities throughout the Indo-West Pacific is generally quite high with a maximum of 218 in the central Indo-West Pacific and as few as 16 species in southern Australia (Williams and Duda 2008). Although a biogeographic region consisting of the Pitcairn Island group and Easter Island contains 22 *Conus* species (Williams and Duda 2008), only one of these species, *Conus miliaris*, maintains a population at Easter Island; populations of other Indo-West Pacific *Conus* species though appear to be ephemeral and individuals of these species are only rarely observed at Easter Island (Kohn 1978; Rehder 1980; DiSalvo et al. 1988).

*Conus miliaris* is broadly distributed and occurs throughout most tropical and subtropical parts of the Indo-West Pacific: from the Red Sea and eastern shores of Africa to Easter Island and Sala y Gómez islands in the southeastern Pacific (it is absent though from the Marquesas and Hawaiian Islands in the central Pacific) (Fig. 1) (Röckel et al. 1995). At most localities in the Indo-West Pacific, including the Seychelles and Maldives Islands in the western Indian Ocean and the Marianas, Marshall and Caroline Islands in the Pacific, *C. miliaris* preys almost exclusively on three species of eunicid polychaetes (Kohn 1978). The diet of *C. miliaris* at Easter Island is considerably broader, including additional species of eunicids, as well as several species of nereids, an onuphid, a capitellid and members of six other polychaete families (Kohn 1978). Based on the relatively broad diet and higher population density of *C. miliaris* at Easter Island compared to other localities as well as the nearly complete absence of congeners at this location, Kohn (1978) suggests that *C. miliaris* underwent ecological release at Easter Island.

Easter Island *C. miliaris* is also morphologically distinct: shells of specimens from Easter Island are significantly



**Fig. 1** Map of the Indian and Pacific Oceans with the range of *C. miliaris* (delimited by thick lines), collection locations and sample sizes (n)

different at eight of ten morphometric characters and darker in color than shells of *C. miliaris* from elsewhere (Kohn and Riggs 1975). Also, radular teeth of Easter Island *C. miliaris* are significantly larger (relative to shell length) than radular teeth of *C. miliaris* from other localities in the Indo-West Pacific (Kohn 1978). Based on the unique morphology of *C. miliaris* at Easter Island and the relative consistency of these characteristics at other localities in the Indo-West Pacific, Rehder (1980) recommends subspecific status for the population from Easter Island as *C. miliaris pascuensis*. Although Rehder's proposal is a potential topic for debate (that we do not address here) and it is not known if the morphological disparity has a genetic basis, the importance of this designation is that the population at Easter Island is sufficiently distinct to suggest separation from other Indo-West Pacific populations of *C. miliaris*. We assume that these populations represent allospecies sensu Mayr and Diamond (2001).

Based on studies of the development of *C. miliaris* from the Maldive Islands and Thailand, it, like many other species of *Conus*, possesses a planktotrophic larval phase with a pelagic period estimated to last about 3–4 weeks (Kohn and Perron 1994) that could permit extensive gene flow throughout its range. Based on observations of veliger larvae of Easter Island *C. miliaris* (TFD, personal observation), larval development appears similar to that of *C. miliaris* from elsewhere. Nonetheless, the exceptional isolation of Easter Island and its position at the extreme southeasterly limit of the distribution of this species (Fig. 1) presumably limit gene flow to this location and the population may be self-recruiting.

The ecological and morphological distinctiveness of Easter Island *C. miliaris* and the extreme isolation of Easter Island suggest that this population is genetically differentiated from other populations of *C. miliaris* in the Indo-West Pacific and perhaps is undergoing incipient speciation. To test this hypothesis and to examine the impact of geographic isolation on the differentiation of marine taxa, we investigated the phylogeography of *C. miliaris* in the Pacific with analyses of mitochondrial cytochrome oxidase subunit I (COI) sequences to address the following questions: is *C. miliaris* from Easter Island genetically distinct from other populations in the Pacific? When did the population at Easter Island become isolated from other populations in the Indo-West Pacific? What is the level and directionality of gene flow between populations from Easter Island and elsewhere? We used traditional measures of  $\Phi_{ST}$  and analyses of molecular variance (AMOVA) and mismatch distributions as well as recently developed Bayesian methods to assess the population genetic structure, demographic history, migration rates and timing of separation of populations of *C. miliaris* in the Indo-West Pacific.

## Methods

### Specimens

We collected specimens of *C. miliaris* at American Samoa, Guam and Easter Island and preserved them in 95% ethanol. Some of the specimens from Guam, the Philippines, Cook Islands, Moorea, Tuamotu and Pitcairn Island group were obtained from collections at the Field Museum (Chicago, IL, USA) and the Florida Museum of Natural History (University of Florida, Gainesville, FL, USA). While reasonable sample sizes (i.e.,  $n > 12$ ) were available from localities in Guam, the Philippines, American Samoa and Easter Island, we had limited numbers of specimens or sequences from other locations, including Madagascar ( $n = 1$ ), Aitutaki ( $n = 1$ ) and Rarotonga ( $n = 1$ ) in the Cook Islands, Moorea ( $n = 1$ ) and Rangiroa ( $n = 5$ ) in French Polynesia, and Henderson Island ( $n = 2$ ), Oeno Island ( $n = 2$ ) and Pitcairn Island ( $n = 1$ ) in the Pitcairn Island group ( $n = 5$ ) (Fig. 1).

### Sequence data

We extracted DNA using the E.Z.N.A.<sup>TM</sup> Mollusc DNA Kit (Omega Bio-Tek, Doraville, GA, USA) or the DNeasy Kit (Qiagen Inc., Valencia, CA, USA) from approximately 25 mg of foot tissue. We amplified a region of approximately 644 bases of the mitochondrial COI gene with Folmer primers LCO1490 and HCO2198 (Folmer et al. 1994). Amplification products were prepared for cycle sequencing by diluting 1:5 in sterile water. Sequencing was performed in both directions at the University of Michigan DNA Sequencing Core on an Applied Biosystems Model 3730 XL DNA sequencer. We analyzed chromatograms and aligned sequences with Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). Additional unpublished COI sequences from single individuals of *C. miliaris* from Easter Island and Madagascar (data provided by C.P. Meyer, National Museum of Natural History; GenBank accession numbers FJ392950 and FJ392976) as well as a published COI sequence from an individual of *C. miliaris* from American Samoa (Duda and Rolán 2005) (GenBank accession number AY588203) were included with sequences we recovered.

### Analyses

We used Modeltest 3.7 (Posada and Crandall 1998) to determine the most appropriate model of nucleotide substitution. We constructed a statistical parsimony network (Templeton et al. 1992) with TCS version 1.21 (Clement et al. 2000). We calculated genetic diversity statistics, pairwise  $\Phi_{ST}$  values and migration ( $M = 2Nm$ ) estimates, and

mismatch distributions and conducted an Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) with Arlequin version 2.0 (Schneider et al. 2000). We were ultimately only able to obtain sequences from a few specimens from three of our sites (the Cook Islands, French Polynesia and Pitcairn). Because these locations are not disconnected by any large expanse (>250 km) of deep ocean in this region (Fig. 1), we pooled data from these localities into a single sample (referred to as ‘southeastern Pacific’) and utilized this group in estimates of pairwise  $\Phi_{ST}$  values and mismatch distributions and for AMOVA as discussed above. Nonetheless, we also considered alternative grouping schemes that combined Pitcairn and Easter Island samples and that excluded Pitcairn samples entirely.

To estimate demographic parameters, including timing of separation of populations, directional rates of gene flow and effective population sizes of daughter and ancestral populations, we used the Isolation with Migration [analytic] (IMa) analysis program (Schneider et al. 2000; Nielsen and Wakeley 2001; Hey and Nielsen 2007). IMa utilizes a Bayesian method based on coalescence models to estimate the time of separation of populations  $t$  (in number of generations and scaled by mutation rate,  $\mu$ ), the demographic parameter  $\theta$  ( $\theta = N_e\mu$ , where  $N_e$  is the effective population size) of the ancestral and two daughter populations, and the scaled migration rate  $m = m/\mu$  in each direction. We initially set upper bounds for each parameter based on recommendations in the IMa manual (i.e.,  $q1 = q2 = qA = 10$ ,  $m1 = m2 = 10$ ,  $t = 10$ ) and performed multiple runs with IMa using different random number seeds to determine appropriate starting values for these bounds. Additional runs were then performed with more appropriate upper bounds to identify trends in parameter estimation. All initial runs consisted of a burn-in stage of  $10^5$  steps and an additional run of at least  $10^6$  steps with a single chain. We then performed a final run using a geometric heating scheme with 20 chains, burn-in stage of  $10^5$  steps and a run duration of at least  $10^6$  steps. Again here we considered alternative grouping schemes that combined Pitcairn and Easter Island samples and that excluded Pitcairn samples entirely.

Based on the divergence of COI sequences of a geminate pair of transisthmian *Conus*, *C. gladiator* and *C. mus*, given by Duda and Rolán (Duda and Rolán 2005) and an estimated time of separation of these species at 6.9 my (Duda and Kohn 2005), we estimate a mutation rate of 3.7 substitutions per my for the amplified fragment of COI.

## Results

We obtained sequences of a region of the mitochondrial COI gene from 141 individuals of *C. miliaris* from Madagascar, the Philippines, Guam, American Samoa, Cook

Islands, French Polynesia, Pitcairn Island and Easter Island (GenBank accession numbers AY588203, FJ392914–FJ393023 and FJ411486–FJ411516, including one previously published sequence and two sequences provided by C.P. Meyer, National Museum of Natural History). Of these sequences, 96 were unique and only 14 haplotypes were observed in more than one individual (Fig. 2). Five haplotypes that were shared by more than one individual were detected solely in individuals from Easter Island (Fig. 2). One of the two most common haplotypes was observed in ten individuals from Easter Island and one individual from Pitcairn Island and the other was observed in two individuals from the Philippines, four from American Samoa, and one individual each from the Cook Islands and Easter Island (Fig. 2). The haplotype of the individual from Madagascar was also found in three individuals from American Samoa (Fig. 2).

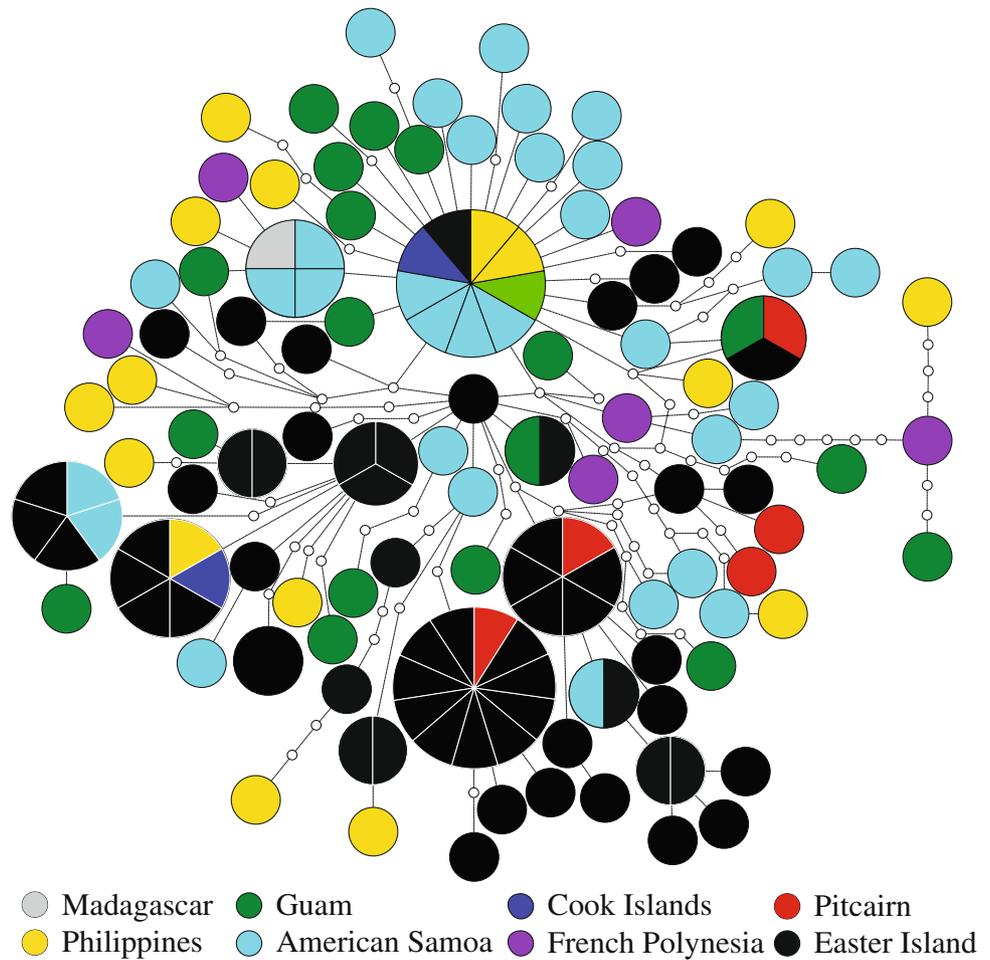
All localities showed high haplotype diversity, with values ranging from 0.965 to 1.000 (Table 1). The population from Easter Island exhibiting the lowest haplotype diversity; all others had haplotype diversities greater than or equal to 0.991 (Table 1).

$\Phi_{ST}$  values were low and not significantly different than zero for comparisons between most pairs of populations (Table 2). Comparisons of Easter Island to other localities, however, gave  $\Phi_{ST}$  values that were consistently large and significant (Table 2). The lowest  $\Phi_{ST}$  value for a comparison including Easter Island (0.072) occurred between Easter Island and the combined samples from the southeastern Pacific, the closest locations to Easter Island (Table 2).

Results from the AMOVA show that 10.3% of the genetic variance is partitioned among Easter Island and elsewhere (Table 3). Nearly 90% of the variation occurs within populations and only a fraction of the variation (0.8%) occurs among non-Easter Island localities (Table 3). We obtained similar results from alternative grouping schemes that combined Pitcairn and Easter Island samples and that excluded Pitcairn samples entirely. This implies that the sample size at Pitcairn is too small to make strong interpretations about samples from this location.

Mismatch distributions of haplotypes for all localities except Easter Island and pooled haplotypes from localities other than Easter Island do not differ significantly from a distribution simulated for recent population expansion (Table 1; Fig. 3). For the combined data, initial and current estimates of theta are 1.49 (95% confidence interval 0–3.52) and 36.9 (15.8–4,924) respectively ( $\theta = 2N_e\mu$ ,  $N_e$  is the effective population size and  $\mu$  is the mutation rate). The time since expansion scaled by mutation rate ( $\tau$ ) is 5.06 (3.09–10.1). The mutation rate estimated for COI of *Conus* at 3.7 substitutions per million years (my) provides a time since expansion for the combined non-Easter Island samples of 0.68 my (0.42–1.36 my).

**Fig. 2** Haplotype network joining COI haplotypes of 141 individuals of *C. miliaris* at the 95% confidence level. The ancestral haplotype according to ‘out-group weight’ (Castelloe and Templeton 1994) is the haplotype shared by nine individuals from the Philippines, Guam, American Samoa, Cook Islands and Easter Island. The area of each shape is proportional to the frequency of haplotypes. Hypothetical haplotypes are indicated with *small ovals*. Localities are shown in the legend



**Table 1** Sample size, haplotype diversity (Tamura-Nei) and sum of squared deviation (SSD) of mismatch distributions of populations of *C. miliaris*

	Number of individuals	Number of haplotypes	Haplotype diversity (SE)	Mismatch distribution, SSD
Philippines	16	15	0.992 (0.025)	0.017NS
Guam	19	19	1.000 (0.017)	0.006NS
American Samoa	31	28	0.991 (0.012)	0.004NS
Pitcairn	5	5	1.000 (0.127)	0.077NS
French Polynesia	6	6	1.000 (0.096)	0.025NS
Southeastern Pacific	13	13	1.000 (0.030)	0.007NS
All combined, not including Easter Island	80	74	0.997 (0.003)	0.002NS
Easter Island	61	40	0.965 (0.014)	0.006*

Because sample sizes at some localities in the southeastern Pacific that are in relatively close geographic proximity were low (e.g., Cook Islands, French Polynesia and Pitcairn), we combined haplotype data from these localities into a single population (‘southeastern Pacific’). We also combined haplotype data from all localities other than Easter Island into a single population (‘all combined, not including Easter Island’). Standard errors (SE) are indicated in parentheses. Probabilities that the least squares fit of mismatch distributions equals a simulated recent expansion are provided

NS not significant

\*  $P < 0.05$

The final IMA analysis consisted of approximately  $5.0 \times 10^7$  additional steps following a burn-in of 100,000 steps; the effective sample size estimate of time, an estimate

of the mixing properties of the run for the parameter with the slowest rate of mixing, was 633. The probability density curves for all but one parameter converged. The exception

**Table 2** Pairwise  $\Phi_{ST}$  values (below diagonal) and estimates of migration ( $M = 2Nm$ ) (above diagonal) for populations of *C. miliaris* in the tropical Pacific with  $n > 12$ 

	Philippines	Guam	American Samoa	Southeastern Pacific	Easter Island
Philippines		Inf.	18.4	Inf.	1.8
Guam	-0.013NS		Inf.	Inf.	1.8
American Samoa	0.013NS	-0.012NS		14.3	1.5
Southeastern Pacific	-0.011NS	-0.018NS	0.018NS		3.3
Easter Island	0.120**	0.121**	0.137**	0.072*	

As in Table 1, we pooled data from the Cook Islands, French Polynesia and Pitcairn into a single population ('southeastern Pacific'). Probabilities that observed  $\Phi_{ST}$  values deviate from a null hypothesis of no difference between populations were determined from the proportion of 10,100 permutations of haplotypes between populations that gave  $\Phi_{ST}$  values greater than or equal to the observed  $\Phi_{ST}$

NS not significant

\*  $P < 0.01$ ; \*\*  $P < 0.001$

**Table 3** Analysis of molecular variance (AMOVA) and  $\Phi$ -statistics of populations of *C. miliaris* grouped hierarchically into two regions

Category	Observed partition				
	Variance	% explained	$\Phi$ -statistics	$P$	$df$
Among regions	0.385	10.27	$\Phi_{CT} = 0.103$	0.206	1
Among populations within regions	0.031	0.83	$\Phi_{SC} = 0.009$	0.377	3
Within populations	3.331	88.90	$\Phi_{ST} = 0.111$	<0.00001	133

Region one included samples from the Philippines, Guam, American Samoa and the southeastern Pacific (e.g., Cook Islands, French Polynesia and Pitcairn); region two included the population from Easter Island

was the curve of theta for the set of haplotypes from Madagascar, Philippines, Guam, American Samoa, Cook Islands, French Polynesia and Pitcairn ( $\theta_{\text{non-EI}}$ ) which attained a peak at 6,128.4, slowly decreased and then stabilized at a plateau without any apparent approach of a marginal posterior probability of zero; the 90% highest posterior density (HPD90) interval was 3,677.1–171,501.6. Presumably the COI data did not contain sufficient information to accurately estimate this parameter. Nonetheless, the peak at 6,128.4 at least provides a minimum estimate of  $\theta_{\text{non-EI}}$  (Hey 2005). The theta value of the Easter Island population ( $\theta_{\text{EI}} = 72.6$ ; HPD90 interval 31.1–159.3) was considerably lower than that of the other population set and theta of the ancestral population was lower than both of these values ( $\theta_A = 31.8$ ; HPD90 interval 10.6–64.3). The scaled migration rate to Easter Island ( $m_{\text{EI}} = 0.56$ ; HPD90 interval 0.00–2.37) was less than the rate to non-Easter Island populations ( $m_{\text{non-EI}} = 1.26$ ; HPD90 interval 0.70–2.08), but the HPD90 intervals overlap considerably. Nonetheless, if the estimate of  $\theta_{\text{non-EI}}$  is reasonable, the number of female propagules ( $M = 2N_e m = \theta m/2$ ) that Easter Island has received ( $M_{\text{EI}} = 20.3$ ; range based on HPD90 intervals of  $\theta_{\text{EI}}$  and  $m_{\text{EI}}$  0.03–187.9) is much less than the number that other populations have received from Easter Island ( $M_{\text{non-EI}} = 3,860.9$ ; range based on HPD90 intervals of  $\theta_{\text{non-EI}}$  and  $m_{\text{non-EI}}$  1,287.0–178,361.7). The scaled time of divergence of the population at Easter Island from populations elsewhere in

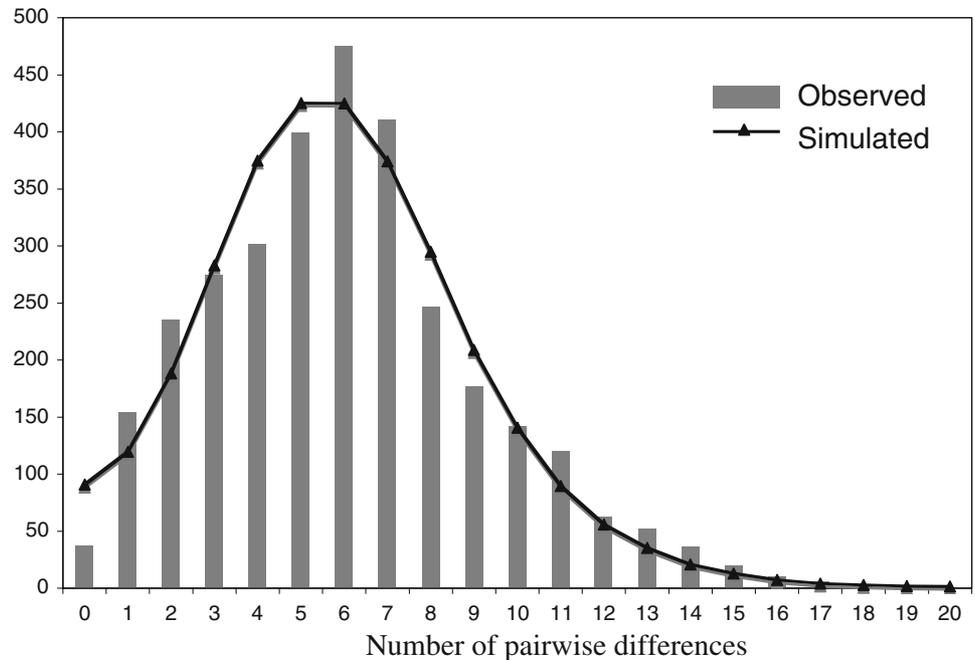
the Indo-West Pacific ( $t$ ) (i.e., an estimate of the most recent major colonization event at Easter Island) was 1.67 (HPD interval 1.18–2.49). Using our mutation rate of 3.7 substitutions per my gives a time of separation of the Easter Island population at 0.45 mya (range based on the HPD90 interval of  $t$  0.32–0.67 mya). As above, the use of alternative grouping schemes that combined Pitcairn and Easter Island samples and that excluded Pitcairn samples entirely did not significantly impact the results from the IMA analyses.

## Discussion

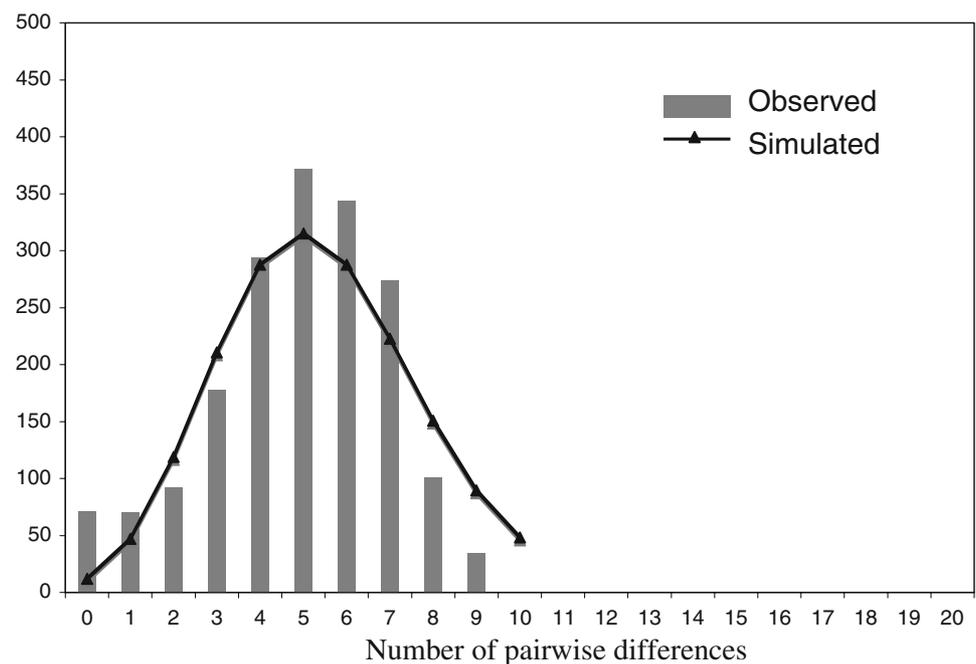
Analyses of mitochondrial COI sequences show that the predominant demographic and phylogeographic patterns in *C. miliaris* are recent population expansion and lack of genetic structure throughout most parts of the Pacific with some genetic discontinuity at Easter Island. Remarkably, but on par with results from other marine taxa that possess larval stages capable of long distance dispersal (see below), samples collected from localities separated by about 10,000 km (Philippines and combined samples from the southeastern Pacific) show no evidence of genetic differentiation (Table 2). In accordance with the previously observed distinct ecology and shell and radular tooth morphology of *C. miliaris* from Easter Island (Kohn and Riggs

**Fig. 3** Mismatch distributions of COI haplotypes of populations of *C. miliaris* from **a** combined samples from all localities except Easter Island and **b** Easter Island. Curves depicting frequency distributions of pairwise sequence differences simulated under a model of population expansion are illustrated for each set of haplotypes

### A Combined samples not including Easter Island



### B Easter Island



1975; Kohn 1978; Rehder 1980), the population at Easter Island though is genetically differentiated from other populations in the Indo-West Pacific. Based on the estimates of unidirectional migration rates determined with IMA, the genetic divergence of the Easter Island population has occurred despite rather high migration from Easter Island into the rest of the Indo-West Pacific; the migration presumably has had little impact due to the large effective

population size in this region. Pairwise  $\Phi_{ST}$  values (Table 2) and numbers of shared haplotypes among regions (Fig. 2) suggest that migration into the Indo-West Pacific occurs predominantly from Easter Island to other nearby localities in the southeastern Pacific (e.g., Pitcairn), but larger sample sizes are needed to more thoroughly address this point. Also, our results and interpretations are based on sequences from a single mitochondrial gene; although they

may reliable, our interpretations should be considered with some caution until data from nuclear markers are available.

#### Distinctiveness of the Easter Island population of *C. miliaris*

In contrast to the structure exhibited by *C. miliaris* at Easter Island, many other widespread marine species show no evidence of genetic differentiation of populations at Easter Island. These include the Chinese trumpetfish *Aulostomus chinensis* (Bowen et al. 2001), the long-spined porcupinefish *Diodon holocanthus* (Lessios and Robertson 2006) and the sea urchin *Diadema paucispinum* (Lessios et al. 2001). Also, although some pairwise  $\Phi_{ST}$  values calculated between populations of the sea urchin *Tripneustes* at Easter Island and elsewhere in the Indo-West Pacific are relatively large and significant, many others are not, a pattern attributed to stochastic haplotype distributions in short-lived populations of a large metapopulation (Lessios et al. 2003). The lack of significant structuring of these four species is attributed to high rates of gene flow among populations or recent colonization from the eastern Pacific in the case of the porcupinefish (Lessios and Robertson 2006). While *C. miliaris* exhibits considerable migration from Easter Island to other localities in the Indo-West Pacific, migration to Easter Island from these localities is much less.

Why is the pattern of population structure exhibited by *C. miliaris* at Easter Island different from these four other species? One explanation is that although the extreme isolation of Easter Island and the nature of current patterns in the southeastern Pacific presumably affects migration of all of these species in similar ways, slight differences in dispersal abilities or other issues related to larval dispersal and survival (e.g., timing of release of gametes or larvae, physiological tolerances of larvae, etc.) limits dispersal of *C. miliaris* to Easter Island. Second, migration from localities in the Indo-West Pacific to Easter Island occurs as a form of ‘sweepstakes dispersal’ (Simpson 1953) and these four other species were simply more ‘fortunate’ than *C. miliaris*. Third, larvae of *C. miliaris* are more prone to localized retention mechanisms at Easter Island and so recruitment from within is more common for *C. miliaris* at Easter Island than it is for these other species. Fourth, *C. miliaris* colonized Easter Island much earlier than these other species and has thus had more time to differentiate genetically. Although we estimated that *C. miliaris* colonized Easter Island about 450 thousand years ago, similar estimates are not available from these four other species. Finally, larvae of *C. miliaris* from localities outside Easter Island may be less fit than recruits from the resident population and so migrants ultimately contribute few if any genes to subsequent generations. The unique ecology and morphology of *C. miliaris* at Easter Island suggests that the population is

adapted to local conditions, but this hypothesis would be difficult to test.

A large percentage of marine species at Easter Island are endemic, a pattern that is echoed in the genetic divergence of the Easter Island population of *C. miliaris*. Nonetheless, we are aware of only a few studies that have examined these endemics genetically. In a study of the molecular systematics of cowries, Meyer (2003) found that the dragon-head cowrie *Monetaria caputdraconis*, a cowrie that is endemic at Easter Island, is closely allied with *M. caputserpentis caputserpentis*, a species that occurs throughout most of the Indo-West Pacific except for the Hawaiian Archipelago, and *M. c. caputophidii*, a species restricted to the Hawaiian Archipelago. Similar to the situation involving *C. miliaris*, the cowrie at Easter Island is morphologically distinct, a pattern attributed to paedomorphosis by Tissot (1988). Nonetheless, unlike *C. miliaris* at Easter Island, *M. caputdraconis* exhibits reciprocal monophyly in phylogenies constructed from analyses of mitochondrial gene sequences (Meyer and Paulay 2005). Another Easter Island cypraeid endemic, *Erosaria engleri*, shows a similar pattern of reciprocal monophyly (Meyer and Paulay 2005). These two cypraeids have achieved a greater degree of genetic separation at Easter Island than has *C. miliaris*, presumably as a result of lower levels of gene flow or longer periods of isolation at Easter Island than *C. miliaris*. Nonetheless, preliminary data from a third Easter Island endemic cypraeid, *Cribrarula garciai*, imply that this species, like the population of *C. miliaris* at Easter Island, does not exhibit reciprocal monophyly with a species outside of Easter Island (C.P. Meyer, personal communication). Clearly additional phylogeographic studies that include estimates of timings of population separation and gene flow of endemic Easter Island forms and widespread species that occur at Easter Island will be instrumental in advancing our understanding of the role of isolation in the differentiation of tropical marine taxa and the origins of the high levels of endemism at Easter Island.

Based on our results, colonization of *C. miliaris* from the Indo-West Pacific to Easter Island occurred approximately 450 thousand years ago (range 320–670 thousand years ago), a time that coincides with the timing of population expansion of *C. miliaris* in the Pacific (see below). Alternatively, an already established population at Easter Island may have received a massive influx of new recruits at this time, a phenomenon that could have eliminated any genetic signal of the original colonization event. Based on the estimated age of Easter Island of 700 thousand years (Kaneoka and Katsui 1985), the initial colonization may have taken place shortly after Easter Island arose, and so the population of *C. miliaris* at Easter Island presumably did not have to ‘island hop’ to Easter Island from older islands associated with the Easter Seamount Chain (e.g., Sala y Gómez

Islands). Nonetheless, the location of initial colonization remains ambiguous without fossil data and without COI data from *C. miliaris* from Sala y Gómez.

#### History of non-Easter Island populations of *C. miliaris*

The apparent lack of genetic structure of *C. miliaris* in most parts of the tropical Pacific is comparable to that observed for other marine species in this region, including a closely related *Conus*, *C. ebraeus* (Duda and Lessios 2009), as well as two species of neritid gastropods *Nerita albicilla* and *N. plicata* (Crandall et al. 2008) and the sea urchins *Diadema savignyi* (Lessios et al. 2001) and *Tripneustes* (Lessios et al. 2003). All of these species possess a planktonic larval phase that provides the potential for high rates of gene flow. Thus, the lack of structure within these species is not necessarily unexpected. Similar to *C. ebraeus* (Duda and Lessios 2009), *T. gratilla* (Lessios et al. 2003) and the coconut crab *Birgus latro* (Lavery et al. 1996), the mismatch distribution of *C. miliaris* from locations other than Easter Island is unimodal and suggests a recent population expansion in these regions (Fig. 3). Nonetheless, patterns of panmixia and recent expansion are hardly universal among all tropical marine organisms in the Pacific. For example turbinid gastropods (Meyer et al. 2005), giant clams (Benzie and Williams 1997), pearl oysters (Benzie and Ballment 1994; Arnaud-Haond et al. 2003), show significant structuring or patterns of isolation-by-distance in this region despite all species having a dispersing larval stage.

*Conus miliaris* exhibits a strikingly similar demographic history in the Pacific as its close relative, *C. ebraeus* and the nerites *N. albicilla* and *N. plicata* (Crandall et al. 2008). Based on results from the mismatch analysis, *C. miliaris* underwent population expansion in the Pacific during the past 680 thousand years, with a 95% confidence interval of 420–1,360 thousand years. This period overlaps considerably with the 95% confidence interval given for the time since expansion of *C. ebraeus* in the Pacific (50–740 thousand years ago) (Duda and Lessios 2009) and is also very similar to the estimated times of expansion for the *N. albicilla* and *N. plicata* (Crandall et al. 2008). *Tripneustes*, on the other hand, began to expand in the Pacific much more recently than these species, with a 95% confidence interval of the time of expansion of 51–151 thousand years ago (Lessios et al. 2003). Thus although the timing of these events are not entirely congruent and the pattern is not consistent for all marine taxa in the tropical Pacific, the late Pleistocene represents a time of population expansion in the Pacific for at least some Indo-West Pacific species.

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