

# Phylogeography of the coronulid barnacle, *Chelonibia testudinaria*, from loggerhead sea turtles, *Caretta caretta*

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## Abstract

The barnacle, *Chelonibia testudinaria*, is a common inhabitant of the marine turtle epibiont community and plays a key role in the development of this community. Phylogeographic analysis of 79 cytochrome *c* oxidase I (COX1) sequences for barnacles collected from five populations found contrasting patterns of divergence for populations in the Atlantic vs. the Pacific Ocean. Our analysis indicates that the two Pacific populations, Senri Beach, Japan and Bahia Magdalena, Mexico, were not only highly divergent from the Atlantic populations but are highly divergent from one another. We suggest that barnacles from these populations may represent cryptic species. In contrast, sequence divergence was greatly reduced among barnacles collected from Wassaw Island, GA, USA, Keewaydin, FL, USA, and Kyparissia, Pèloponnésus Island, Greece. A reduction in sequence diversity at the latter site was attributed to a recent range expansion into the Mediterranean Sea. We examined historical patterns of migration among the three Atlantic and Mediterranean populations using the program MIGRATE. This analysis indicates a high rate of migration from Keewaydin to Wassaw Island, contrasted with a much lower rate of migration in the opposite direction. The estimated migration rate from Kyparissia to Keewaydin was also non-negligible. We suggest that the association between *C. testudinaria* and loggerhead turtles and the patterns of turtle migration have played key roles in the expansion of the range of *C. testudinaria* into the Mediterranean Sea and the subsequent patterns of barnacle migration. We further propose that the difference between ocean basins, with respect to the impact of host migration on barnacle gene flow, probably stems from the fact that host-mediated dispersal in the Atlantic depends on advanced stage juveniles and adults while any host-mediated dispersal in the Pacific would have to involve early 'pelagic' stage juvenile loggerheads.

**Keywords:** barnacle, *Chelonibia testudinaria*, cytochrome *c* oxidase I, loggerhead turtles, phylogeography, population genetics

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## Introduction

The coronulid barnacle, *Chelonibia testudinaria*, is a prominent and conspicuous member of the marine turtle fouling or epibiont community. Although *C. testudinaria* is one of six to seven commonly observed members of the epibiotic community associated with turtles, world-wide, loggerhead turtles (*Caretta caretta*) host *C. testudinaria* more frequently and in higher densities than any other species (Matsuura & Nakamura 1993; Bugoni *et al.* 2001; Frick & Ross 2001;

Meischner 2001). Upwards of 94% of nesting loggerheads host at least one and as many as several dozen barnacle species at any time (Frick *et al.* 1998). Turtle barnacles are typically found attached to the carapace and plastron of host turtles (Matsuura & Nakamura 1993; Meischner 2001) but are also known to occur on the head, flippers and skin (Frick & Ross 2001). Detailed surveys have shown that adult *C. testudinaria* occur on loggerheads throughout the year in Georgia and northeast Florida (Frick & Slay 2000) and a recent study by Frick *et al.* (2002) suggests that *C. testudinaria* is the presumptive pioneer species whose recruitment facilitates the development of the loggerhead epibiotic community.

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Unfortunately, little else is known about the life history of *C. testudinaria*. Other well-studied barnacles, such as the intertidal species *Semibalanus balanoides*, have a fairly long-lived planktonic larval phase with a high capacity for dispersal (Lucas *et al.* 1979). In turn, the level of genetic differentiation among subpopulations tends to be low for these species except over very large geographical distances or when there is habitat-specific selection (Hedgecock 1986; Schmidt *et al.* 2000). In contrast to intertidal barnacles, *C. testudinaria* is an obligate commensal that spends all of its adult life attached to highly mobile hosts. The life history of one host, loggerhead turtles, includes several which undertake large migrations (e.g. Musick & Limpus 1997; Rankin-Baransky *et al.* 2001; Williams & Frick 2001). For example, evidence suggests that juvenile loggerhead turtles in the Atlantic, Indian and Pacific oceans may spend 6 years or more in oceanic nursery grounds, migrating vast distances within ocean gyres (Bjorndal *et al.* 2000). Older juvenile loggerheads which have left the oceanic nursery phase and entered the demersal-neritic phase will undertake large seasonal migrations between foraging grounds along coastal margins (Musick & Limpus 1997). Even breeding adult turtles apparently migrate between geographically distinct foraging grounds, courtship areas and nesting beaches (Rankin-Baransky *et al.* 2001). Thus, hosts such as loggerhead turtles may also represent a significant dispersal vector for *C. testudinaria*.

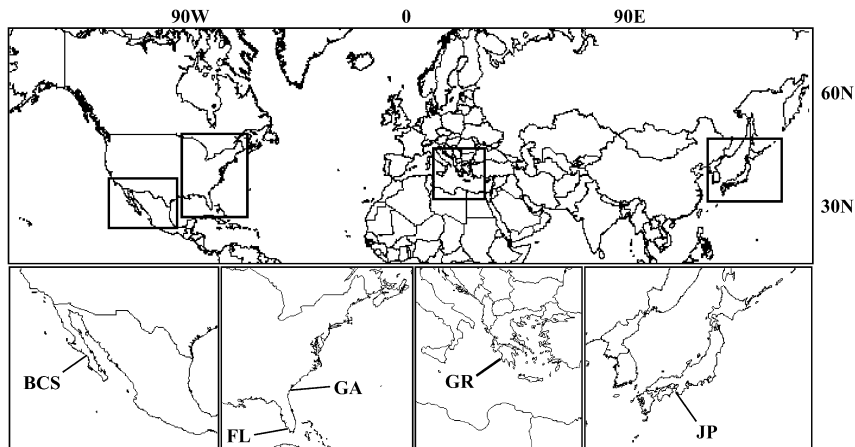
The potential for both large-scale larval and host-mediated dispersal suggests that there should be limited genetic divergence among populations of *C. testudinaria*. We have examined this hypothesis by analysing DNA sequence variation for a 588 base pair portion of the mitochondrial-encoded cytochrome *c* oxidase I gene (COX1) sampled from 79 *C. testudinaria* obtained from five locations world-wide. Based on this analysis, we have asked (i) whether there is evidence of genetic differentiation among populations of *C. testudinaria*; (ii) if differentiation is detected, to what degree the variation correlates with known patterns of

migration for *Caretta caretta*; and (iii) given that fossil evidence suggests that the association between *C. testudinaria* and *Caretta caretta* extends back to as early as the Miocene epoch (Ross 1963; Ross & Newman 1967), to what degree have changes in the range of turtles influenced genetic variation in *C. testudinaria*?

## Methods and materials

### Sample collection

Barnacles identified as *Chelonibia testudinaria*, based on shell morphology, were sampled from the carapaces of loggerhead turtles, *Caretta caretta*. Initially, barnacles were removed from female loggerheads nesting on Wassaw Island, Georgia, USA (GA; Fig. 1) between May and August of 2000 and preserved in a solution of 6 M NaCl–dimethylsulphoxide (DMSO). DNA was isolated from the visceral tissue of each barnacle using a QIAamp DNA Mini Kit (Qiagen) and used as template in a polymerase chain reaction (PCR) assay amplifying a ~680 base pair portion of the mitochondrial cytochrome *c* oxidase subunit I gene (COX1). For each reaction 1 µL (~50–100 ng) of template DNA was added to a 25-µL reaction consisting of 1× PCR buffer, 1.5 mM MgCl<sub>2</sub>, 1.25 nmol dNTPs, 0.5 U *Taq* polymerase (Invitrogen) and 30 pmol each of the oligonucleotide primers LCO (GCT-CAACAAATCATAAAGATATTGG) and HCO (TAAACCTCAGGGTGACCAAAAAATCA; Folmer *et al.* 1994). The reactions were incubated at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 35 °C for 45 s, and 72 °C for 2 min with a final extension of 72 °C for 5 min. Quadruplicate PCR were performed for each barnacle; all four PCR were subsequently pooled and gel-purified using a QIAquick Gel Extraction kit (Qiagen). Gel-purified products were directly sequenced in one direction with the LCO primer, and an ABI Prism sequencing kit (Applied Biosystems) and the sequences were read on an ABI 373A automated sequencer (Applied Biosystems).



**Fig. 1** Partial map of the northern hemisphere (upper panel) showing the relative locations of the five sampling sites in this study. Each rectangle corresponds to an enlarged view in the lower panel with labels indicating sampling locations as follows; Bahia Magdalena, Baja California, Mexico (BCS); Keewaydin, Florida, USA (FL); Wassaw Island, Georgia, USA (GA); Kyparissia, Pèloponnèsus Island, Greece (GR); Senri Beach, Wakayama Prefecture, Japan (JP). Images were generated using ARCVIEW 3.3 and the ESRI World Map database.

**Table 1** Site of collection and GenBank accession numbers for the sequences analysed in the present study

Symbol	Location	Year	Latitude	Longitude	Accession nos.
GA00	Wassaw Isl, GA	2000	31°54' N	80°58' W	AY174289 to AY174302
GA01	Wassaw Isl, GA	2001			AY174303 to AY174323
FL	Keewaydin, FL	2001	26°02' N	81°46' W	AY174324 to AY174333
GR	Kyparissia, Greece	2001	36°58' N	22°59' E	AY174354 to AY174367
JP	Senri Beach, Japan	2001	33°46' N	135°19' E	AY174334 to AY174341
BCS	Bahia Magdalena, Mexico	2001	24°38' N	112°09' W	AY174342 to AY174353

A second set of barnacles were sampled from loggerhead turtles visiting nesting beaches at Wassaw Island, as well as Keewaydin, Florida, USA (FL), Kyparissia Island, Pèloponnésus Island, Greece (GR), and Senri Beach, Wakayame Prefecture, Japan (JP), between May and August 2001 (Fig. 1, Table 1). At the same time, a fifth sample of barnacles was obtained from immature loggerhead turtles caught offshore of Bahia Magdalena, Baja California Sur, Mexico (BCS). Because the recovery of DNA from the barnacles preserved in NaCl–DMSO in 2000 was low, all barnacles sampled in 2001 were preserved in 70–90% ethanol. DNA, isolated from eight to 21 individuals at each site, was used in PCR amplifying the COX1 gene. These reactions were essentially those described above, except that the *Chelonibia*-specific primers CTFOR (GAGCATGGTCTGCTATAGTAGG) and CTREV (CTTGGTAAAGAATTGGATCTCC) replaced LCO and HCO, respectively, and the annealing temperature was increased to 50 °C. The PCR were run in quadruplicate for each barnacle, pooled, gel-purified, and sequenced in one direction using the CTFOR primer.

During the 2001 loggerhead nesting season, a high frequency of the congener, *Chelonibia caretta*, was observed on the turtles visiting Wassaw Island (Frick, personal observation). Because of the morphological similarity between *C. testudinaria* and *C. caretta*, we obtained a COX1 sequence from the latter species for comparison with *C. testudinaria* COX1 and to serve as an outgroup sequence for phylogenetic analysis. DNA was isolated from two *C. caretta* individuals from Wassaw Island and a portion of the COX1 gene was amplified using the primers CTFOR and CTREV and the methods described above. The resulting PCR products were cloned into the TA cloning vector pCR2.1 following the manufacturer's protocol (Invitrogen) and one clone for each barnacle was sequenced using the vector primers M13FOR (GTTTCCAGTCACGAC) and M13REV (CAGGAAACAGCTATGAC; Invitrogen). Analysis indicated that the nucleotide and inferred amino acid sequence of one of these sequences had high similarity to COX1 from *Chthamalus angustitergum* (GenBank no AF234799) and *Semibalanus balanoides* (GenBank no AF242699), while the second clone had little similarity to barnacle COX1 sequences and several insertion–deletions that disrupted the coding sequence. We suggest that this latter sequence may

**Table 2** Location-specific estimates of haplotypic diversity ( $h \pm$  SD) and nucleotide diversity ( $\pi \pm$  SD) for *Chelonibia testudinaria* COX1 sequences sampled from five sites

Location	$n$	Hosts	$h$	$\pi$
GA00	14	10	0.978 (0.035)	0.006 (0.001)
GA01	21	16	0.957 (0.032)	0.007 (0.001)
FL	10	8	1.000 (0.045)	0.008 (0.001)
GR	14	9	0.802 (0.094)	0.002 (0.001)
JP	8	8	1.000 (0.004)	0.007 (0.001)
BCS	12	5	0.898 (0.104)	0.008 (0.002)
Total	79	56	0.977 (0.008)	0.048 (0.006)

$n$ , number of barnacles from which sequences were obtained; Hosts, number of loggerhead turtle hosts from which barnacles were sampled.

represent either contamination or perhaps a COX1 pseudogene in *C. caretta*. Further comparison of the putative *C. testudinaria* and *C. caretta* COX1 sequences indicated that base changes have accumulated between these species at several restriction enzyme recognition sites. Because *C. testudinaria* and *C. caretta* are sympatric in much of the Atlantic, we prescreened PCR products obtained from Wassaw Island and Keewaydin barnacles using a *FokI* restriction digest. This enzyme cuts *C. testudinaria* COX1 products but not those from *C. caretta*. Thus, only PCR products cut by *FokI* were subsequently sequenced and the resulting sample size from each location is indicated in Table 2. Sequences were proofread and aligned using GENETOOL 1.0 (Biotools Inc.) and the *C. testudinaria* COX1 sequences have been deposited in GenBank (accession nos AY174289 to AY174368, Table 1). Estimates of nucleotide and haplotype diversity were obtained for each sampling location using ARLEQUIN 2.0 (Schneider *et al.* 2000).

#### Phylogenetic analyses

The phylogenetic relationships among the *C. testudinaria* COX1 sequences were estimated by the neighbour-joining method as implemented in the program PAUP\* 4.0b10 (Swofford 2000). Based on hierarchical likelihood ratio tests that were performed with the MODELTEST 3.06 program

(Posada & Crandall 1998), the neighbour-joining analysis used a general time reversible model of DNA substitution (substitution rate matrix = 3.851, 68.906, 25.314, 0, 68.906, 1.000) with a gamma distribution of rate heterogeneity across variable sites ( $\Gamma = 0.1295$ ). We employed a bootstrap analysis with 1000 repetitions to evaluate the support for the phylogenetic relationships estimated by PAUP\* with sequences from *C. caretta*, *S. balanoides* and *C. angustitergum* serving as outgroups.

A minimum spanning network was constructed using the MINSPNET algorithm in ARLEQUIN to further delineate the relationships among the 59 COX1 sequences sampled from Wassaw Island, Keewaydin, and Kyparissia. For this subset of sequences, we also applied coalescence/maximum likelihood-based approaches to estimate effective population size, population growth, and migration rates. To test whether there was evidence of population expansion, we used the program FLUCTUATE 1.4 (Kuhner *et al.* 1998). FLUCTUATE uses a Markov chain Monte Carlo approach to search through genealogies and obtain estimates of effective population size ( $\Theta = 2\mu N_e$ ) and exponential growth rate ( $g$ ) at the maximum likelihood. For this analysis, we considered the Wassaw Island, Keewaydin and Kyparissia sequences to have come from a single population. We set the transition–transversion ratio in all FLUCTUATE runs to 3.46 and used the empirical base frequencies. These values were obtained from likelihood ratio tests performed with the MODELTEST 3.06 on the reduced dataset (i.e. excluding sequences from Bahia Magdalena, Mexico and Senri Beach, Japan). FLUCTUATE was run several times with different numbers of short and long chains to check for consistency among the estimates of  $\Theta$  and  $g$ . Final estimates were obtained from a run with five short chains of 1000 steps each and three long chains of 15 000 steps each and a sampling increment of 20. A likelihood ratio test to determine the significance of the exponential growth rate ( $g$ ) was performed by comparing the log-likelihood of a model in which  $g$  was set to zero (stable population) and one in which  $g$  was allowed to vary. The test statistic was calculated as twice the difference in the log-likelihood of each model and compared to a  $\chi^2$  distribution with one degree of freedom.

We used the program Migrate 1.6.9 (Beerli & Felsenstein 2001) to estimate simultaneously the effective population size ( $\Theta = 2\mu N_e$ ) and the migration rates ( $M = 2mN_e$ ). MIGRATE also uses a Markov chain Monte Carlo approach to search through genealogies and a likelihood ratio test to obtain estimates of  $\Theta$  and  $M$ . The program assumes that  $\Theta$  is constant for each population but allows  $\Theta$  to vary between populations. For this analysis, Wassaw Island, Keewaydin and Kyparissia were treated as distinct populations and MIGRATE was used to estimate population-specific values of  $\Theta$  and pairwise migration rates. As with the FLUCTUATE analyses described above, a model of DNA substitution with a transition–transversion ratio of 3.46 was used with

the empirical base frequencies. Five initial runs of MIGRATE included  $F_{ST}$ -based estimates of  $\Theta$  and  $M$  for starting values and used 10 short chains with 5000 sampled genealogies and three long chains with 50 000 sampled genealogies each and a sampling increment of 20. The heating option was in effect, as well, with four temperatures of 1.0, 1.2, 1.5 and 3.0. After checking for consistency among the estimates obtained from these initial runs, five additional runs were performed with the estimates of  $\Theta$  and  $M$  from the previous run used as the starting values for these parameters.

## Results

### DNA sequence analysis

We obtained a total of 79 mtCOX1 sequences from barnacles (*Chelonibia testudinaria*), sampled from 56 different loggerhead turtles visiting nesting beaches or foraging grounds at five geographically separate locations. Our analysis of *C. testudinaria* COX1 was restricted to the 588 base pairs common to sequences generated with both the LCO–HCO and CTFOR–CTREV primer pairs. Nucleotide substitutions were observed at 116 of the 588 base pairs analysed (~20%); all 116 were synonymous substitutions. A total of 54 unique haplotypes were observed among the 79 *C. testudinaria* COX1 sequences. Site-specific haplotype diversity ranged from a low of 0.802 among the sequences from Kyparissia to a maximum of 1.000 for the Senri Beach and Keewaydin sequences (Table 2). Similarly, estimates of nucleotide diversity ( $\pi$ ) were fairly constant across populations, with the exception of Kyparissia where  $\pi$  was some three- to four-fold lower (Table 2). Pairwise *t*-tests indicated that this difference was significant at an overall error rate of 0.05.

### Phylogeographic analysis

Neighbour-joining analysis grouped the *C. testudinaria* haplotypes into three major clades which were well supported by bootstrap analysis (> 99%; Fig. 2). All of the haplotypes from Senri Beach, Japan in the western Pacific formed a distinct clade as did those from Bahia Magdalena, Mexico (eastern Pacific) while the haplotypes from Wassaw Island, Keewaydin and Kyparissia comprised the third major clade (western Atlantic/Mediterranean). Haplotypes from Wassaw Island and Keewaydin, whether sampled in 2000 or 2001, were dispersed throughout the western Atlantic/Mediterranean clade while those from Kyparissia were clustered within a distinct subclade that had weak bootstrap support (68%). In general, the western Atlantic/Mediterranean clade contained a few common and relatively widespread haplotypes with numerous localized haplotypes connected to the tree by short branches of roughly similar length, a pattern indicative of a recent demographic expansion.

**Table 3** Results of the coalescence analysis of effective population size ( $\Theta = 2\mu N_f$ ) and exponential growth rate ( $g$ ) for the western Atlantic and Mediterranean sequences

Model	$\Theta = 2\mu N_f$	$g$	ln L
No growth	0.036	—	0.098
Exp. growth	0.376 (0.036)	1436.1 (48.1)	3.553

$\chi^2 = 6.91$   
 $P = 0.013$

In L, log likelihood of the specified model. Population parameters are maximum likelihood estimates ( $\pm$  SD).

A minimum spanning network further highlighted the relationships among the haplotypes in the western Atlantic/Mediterranean clade (Fig. 3). Within the network there were three equally common haplotypes, GA00–8A, GR2 and GA00–3C. Haplotypes clustered around GA00–8A were separated from those associated with haplotypes GR2 and GA00–3C by four mutations. In contrast, the latter two haplotype clusters were separated by only a single mutation. All of the haplotypes sampled from Kyparissia clustered around haplotype GR2. Each of these three clusters exhibited a star-like topology, a pattern consistent with a recent population expansion (Avice 2000). Results from our coalescence-based analysis, using the program FLUCTUATE, provided further support for a population expansion. In this analysis, the null hypothesis of a stable population was rejected and the estimated population growth rate was highly positive (Table 3).

Results of the MIGRATE analysis indicated highly asymmetrical patterns of historical migration among the two western Atlantic populations and Kyparissia (Table 4). Migration from Keewaydin to Wassaw Island was strong, roughly two orders of magnitude higher than the estimated migration rate in the opposite direction. In addition, an estimate of the migration rate from Kyparissia to Keewaydin was similar in magnitude to that from Wassaw Island to Keewaydin. In contrast, the estimate rates of migration between Wassaw Island and Kyparissia (both directions) and from Keewaydin to Kyparissia were negligible.

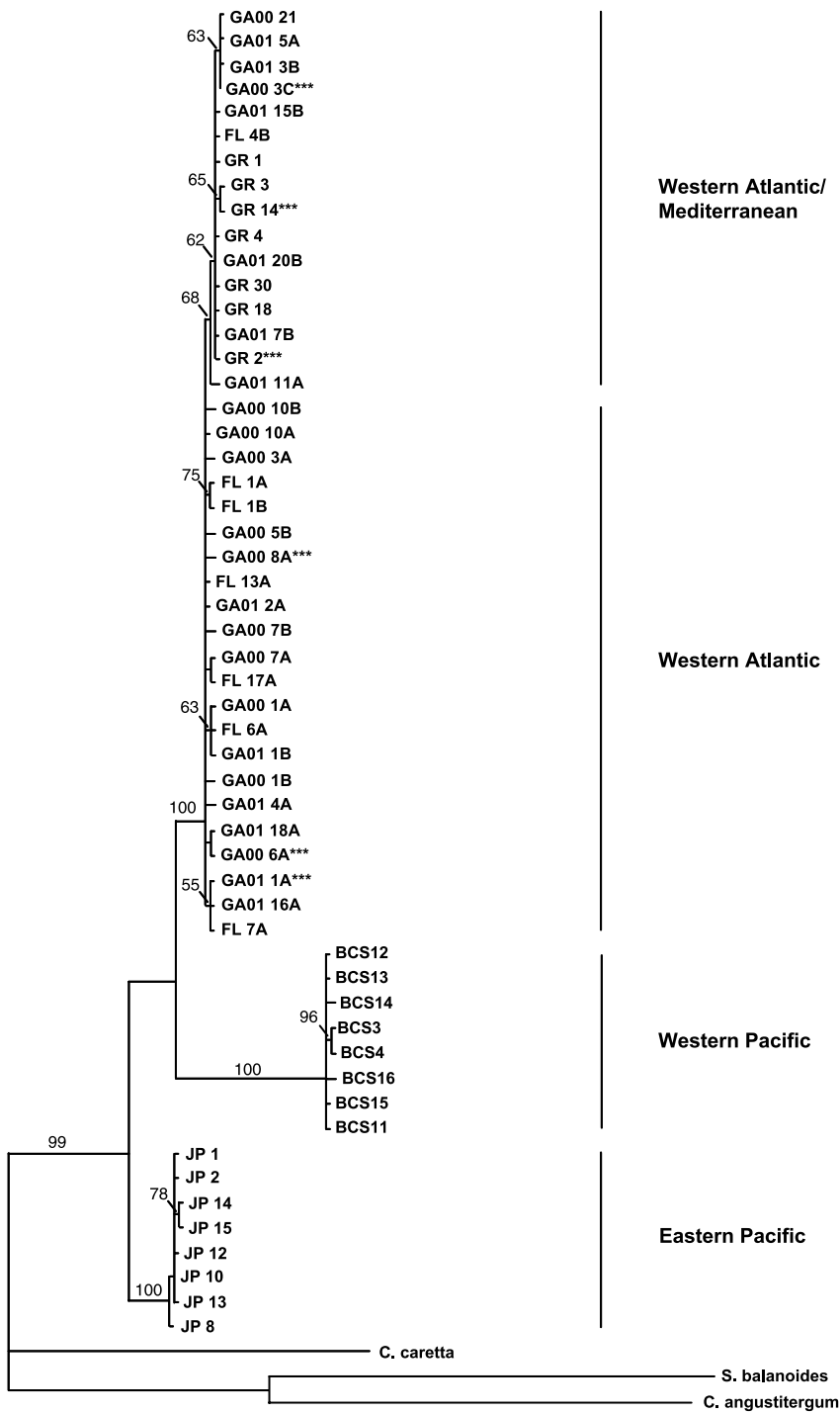
**Discussion**

Phylogenetic analysis of COX1 variation in *Chelonibia testudinaria* demonstrates that there is significant inter-population sequence divergence for this barnacle, particularly between the two Pacific Ocean populations sampled in this study. The eastern (Baja California) and western (Japan) Pacific *C. testudinaria* populations are not only highly divergent from the Atlantic and Mediterranean populations but are genetically distinct from one another. The clustering of sequences from Senri Beach and Bahia Magdalena into separate and well-differentiated clades is indicative of a complete lack of gene flow between these sites. On the one hand this observation is not too surprising. Although many related balanomorph barnacles, such as the intertidal barnacle *Semibalanus balanoides*, have a planktonic and highly vagile larval phase that may last as long as 5 weeks (Lucas *et al.* 1979), Senri Beach and Bahia Magdalena are separated by approximately 9400 km, making larval dispersal between these locations unlikely.

Dispersal in *C. testudinaria*, however, is also probably affected by the association of this species with its hosts. *Chelonibia* is an obligate commensal of marine turtles; most host turtles, especially the loggerhead *Caretta caretta*, have highly cosmopolitan distributions and undergo extensive migrations. For example, Bowen *et al.* (1995) have demonstrated that the vast majority of loggerheads off the coast of Baja California and those captured in the mid-ocean drift-net fishery carry mitochondrial DNA haplotypes identical to those found in turtles from Japanese nesting grounds. Given the lack of loggerhead nesting sites in the eastern Pacific, they concluded that there is appreciable migration between Japanese nesting sites and foraging grounds off Baja California. Moreover, Nichols *et al.* (2000) have used satellite telemetry to show directly that juvenile loggerheads migrate from Baja California to the waters of the western Pacific. Currently, we lack information on the longevity of *C. testudinaria* and the availability of alternative hosts in the Pacific. The results from our analysis, however, suggest that barnacles either do not recruit to pelagic-phase juvenile turtles, or do not survive transit across the Pacific. They also indicate that the length of

**Table 4** Estimates of effective population size and migration rates for the western Atlantic and Mediterranean populations

Population	$n$	Effective population size	Migration rate ( $2mN_f$ )		
		$\Theta = 2\mu N_f$	GA	FL	GR
Wassaw Isl (GA)	35	0.0998 (0.0187, 0.7749)	—	125.25 (83.28, 291.20)	$1.61 \times 10^{-7}$ ( $1.21 \times 10^{-7}$ , $4.02 \times 10^{-4}$ )
Keewaydin (FL)	10	0.0104 (0.0059, 0.0150)	0.69 (0.52, 2.21)	—	2.32 (0.04, 5.22)
Kyparissia (GR)	14	0.0063 (0.0027, 0.0226)	$5.86 \times 10^{-16}$ ( $4.55 \times 10^{-16}$ , 0.45)	$5.43 \times 10^{-8}$ ( $4.07 \times 10^{-8}$ , $1.36 \times 10^{-4}$ )	—

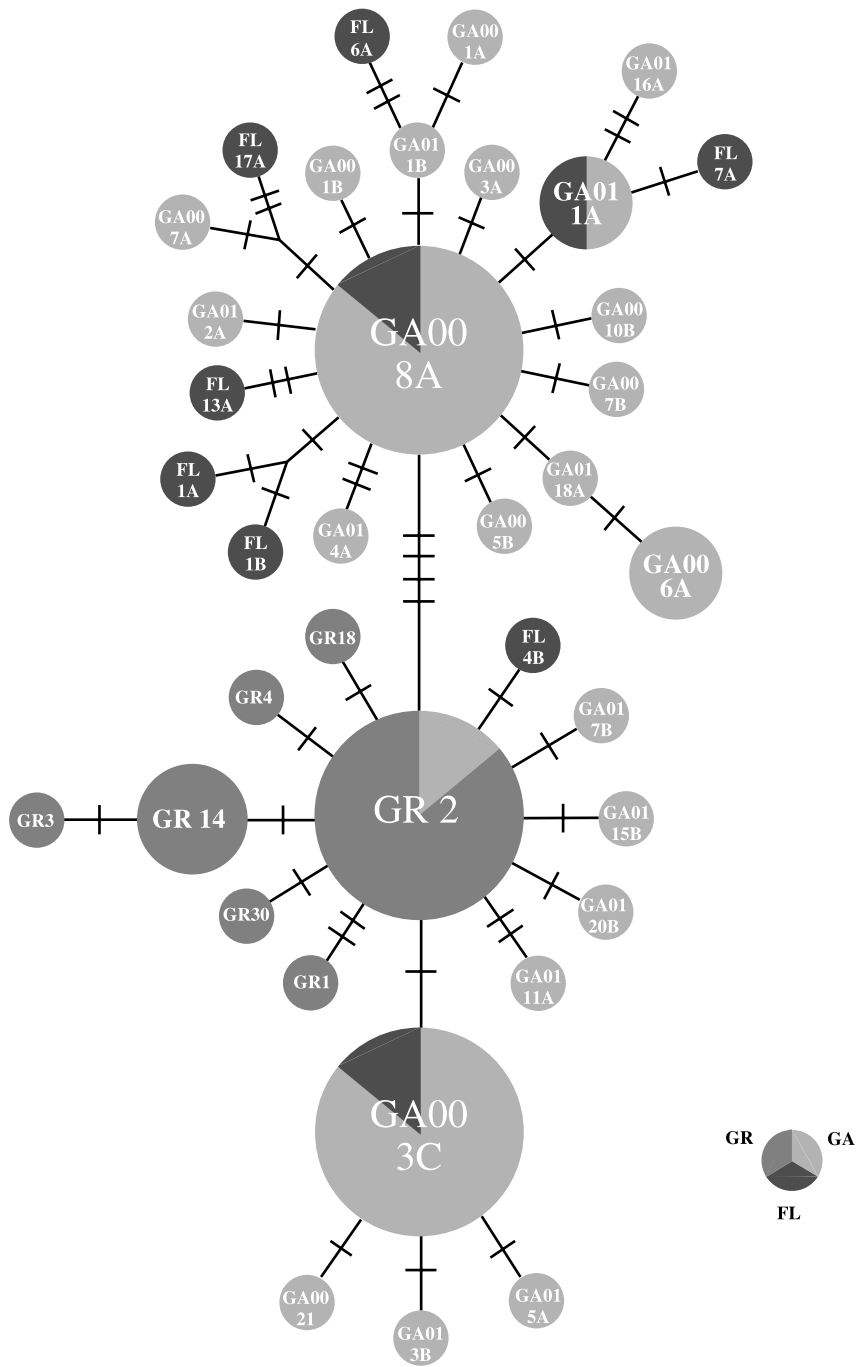


**Fig. 2** Neighbour-joining phylogram for the 54 unique *Chelonibia testudinaria* haplotypes. Sequences from *Chelonibia caretta*, *Semibalanus balanoides* and *Chthamalus angustitergum* are included as outgroups. Haplotype labels indicate sampling location, as per Fig. 1, as well as the year in which they were sampled (00 = 2000; 01 = 2001) for Wassaw Island haplotypes. Asterisks indicate haplotypes represented by more than one sequence. The number above a given branch indicates the bootstrap support for that branch (1000 replicate samplings).

time that turtles are resident in the eastern Pacific is long enough for *C. testudinaria* barnacles to complete their life cycle.

The high degree of genetic differentiation between Pacific *C. testudinaria* populations indicates the long-term separation of these populations. Although there is an extensive fossil record for barnacles, in general there are few good landmarks for calibrating a molecular clock (Spears *et al.*

1994). Even so, COX1 sequence data from barnacles in the genus *Chthamalus* (Wares 2001) and from other crustaceans (Knowlton & Weigt 1998) yield clock estimates between 2.2 and 3.1% sequence divergence per million years. Given an estimated sequence divergence of 12.7% between Senri Beach and Bahia Magdalena (data not shown), these populations appear to have been evolving independently for perhaps 3.4–5.7 million years. In fact, this level of divergence



**Fig. 3** Minimum spanning network showing the phylogenetic relationship and relative frequency of the 37 haplotypes sampled from Atlantic and Mediterranean sites. The number of nucleotide changes between adjoining haplotypes are indicated by dashes while the size of each circle represents the frequency of a given haplotype. In addition, the source population(s) for each haplotype are indicated by shading with light grey, grey and black corresponding to haplotypes from Wassaw Island, Kyparissia, and Keewaydin, respectively.

is similar to the degree of interspecific divergence in COX1 within the genus *Chthamalus*, noted by Wares (2001). In our study, specimens of *C. testudinaria* were collected primarily on the basis of shell morphology. From his analysis, Wares (2001) pointed out that speciation in the genus *Chthamalus* has occurred despite an apparent lack of significant morphological variation. Our data suggest that a similar lack of congruity between molecular and morphological diversification may characterize barnacle populations nominally recognized as *C. testudinaria*, and that the taxonomic

status of the Pacific *C. testudinaria* populations should be reconsidered.

In contrast, much lower levels of sequence divergence were evident among the Wassaw Island, Keewaydin and Kyparissia populations. Our analysis suggests that the distribution of genetic variation at these sites has been impacted by recent demographic changes. We obtained a significantly positive estimate for the population growth rate parameter (*g*) from our coalescence-based analysis employing the program FLUCTUATE. FLUCTUATE considers

only one potential model of population growth (exponential) and is known to generate estimates of both  $\Theta$  and  $g$  that are upwardly biased as a result of correlation between the parameters and because the program assumes a panmictic population (Kuhner *et al.* 1998). Even so, the large value we consistently obtained for  $g$  from the FLUCTUATE analysis, along with the phylogenetic patterns observed in both the neighbour-joining analysis and the minimum spanning network all indicate a rapid demographic expansion within the western Atlantic/Mediterranean sequence clade.

The patterns of genetic diversity in this clade suggest that the demographic changes in this clade include a significant range expansion. Estimates of nucleotide and haplotypic diversity for the Kyparissia sequences were significantly lower than for any other population we sampled. A significant decrease in genetic diversity is expected when there has been a recent expansion in the range of a species (Grosberg & Cunningham 2000). Whether range expansion is the result of gradual expansion or of long-distance dispersal, individuals in the newly colonized area will typically carry only a subset of the alleles or haplotypes found in the source population(s). A second expectation associated with a recent range expansion is that haplotypes in the new population should be phylogenetically nested within those of the source population (Grosberg & Cunningham 2000); such nesting is evident in both the neighbour-joining phylogeny and the minimum spanning network, wherein the sequences from Kyparissia form a distinct subset of those found in the western Atlantic. These observations are consistent with the historical demography of loggerhead turtles, the primary host of *C. testudinaria*, which are believed to have colonized habitats in the Mediterranean Sea after the Wisconsin glaciation, approximately 12 000 years ago (Bowen *et al.* 1993).

Low interpopulation divergence among the Wassaw Island, Keewaydin and Kyparissia populations is indicative of high levels of gene flow. Unfortunately, the lack of strongly supported clusters of sequences in the western Atlantic/Mediterranean portion of the neighbour-joining phylogeny preclude the use of a conventional phylogeographic approach (*sensu* Avise 2000) to estimate the patterns of migration between these locations. As an alternative, we used the program MIGRATE to estimate the historical patterns of movement among the Wassaw Island, Keewaydin and Kyparissia populations. Although the search strategy employed in MIGRATE runs can influence the accuracy of parameter estimates, use of the 'heating' option (as per MIGRATE documentation) greatly improved the consistency of the estimates we obtained. MIGRATE assumes a constant  $\Theta$  within each population, an assumption that may not be valid in our data set (see FLUCTUATE analysis, above). Zheng *et al.* (2003) have discussed the bias that may be imparted on estimates of migration because of the assumption of constant effective population size. They suggest

that estimates of  $\Theta$  obtained from MIGRATE represent historical averages that are likely to be smaller than present-day  $\Theta$ . However, Zheng *et al.* (2003) suggested that this bias may not affect the major patterns or asymmetries in the estimated migration matrix.

If it is assumed that the bias caused by a population expansion is negligible, then our analysis suggests strong but asymmetric migration in the western Atlantic. The estimated rate of migration from Keewaydin to Wassaw Island was 100-fold greater than migration in the opposite direction. High rates of migration between the Gulf of Mexico (Keewaydin) and western Atlantic (Wassaw Island) *C. testudinaria* populations have likely been effected by larval dispersal associated with large-scale surface circulation patterns. Changes in sea level associated with patterns of glaciation during the Pleistocene resulted in an enlarged Florida peninsula that may have repeatedly isolated populations in the Gulf of Mexico from those on the Atlantic coast (Avise 1992). Present-day surface circulation patterns, as depicted in Avise (1992), include swift currents moving out of the Gulf of Mexico which then join up with the Gulf Stream as it hugs the coast of southeast Florida. These current patterns may facilitate the movement of larvae, spawned in the Gulf of Mexico, northward along the Atlantic coast of North America while restricting the southward dispersal of barnacle larvae. Our coalescence-based results from the MIGRATE runs are consistent with such a dispersal scenario.

Genetic homogeneity for Wassaw Island and Keewaydin may also be influenced by the association between *C. testudinaria* and loggerhead turtles. Although Bowen *et al.* (1993) have observed significant levels of genetic differentiation between female loggerheads nesting at Keywahdin, FL, and sites in the southern Atlantic, these differences are generated by the natal homing behaviour of female loggerheads. Additional genetic and tagging studies have shown that advanced stage juvenile and adult loggerheads, particularly males, frequently migrate between feeding and nesting grounds along the Atlantic coast and between the Atlantic and the Gulf of Mexico (e.g. Bell & Richardson 1978; Rankin-Baransky *et al.* 2001; Williams & Frick 2001; Casale *et al.* 2002). Any barnacles recruiting to these migrating turtles provide an opportunity for the host-mediated dispersal of adult barnacles between loggerhead nesting and feeding grounds.

A small but non-negligible migration rate was also estimated by MIGRATE for movement between Kyparissia and Keewaydin. The effect of emigration from Kyparissia can also be seen in the minimum spanning network. Several haplotypes, each represented by a single sequence from Wassaw Island and Keewaydin, clustered with GR2 a haplotype which occurred at high frequency in Kyparissia. On the one hand, this result may seem surprising given the distance between the western Atlantic and the Mediterranean.

However, there are two potential hypotheses which can explain this observation. First, haplotypes that we observed only in Kyparissia, such as GR2, may be quite common in populations throughout the Mediterranean and eastern Atlantic. Under this scenario, gene flow would be high throughout the Mediterranean and eastern Atlantic region with occasional migration occurring from the eastern to the western Atlantic. Bolten *et al.* (1998) have observed extensive migration of juvenile sea turtles between nesting beaches in the western Atlantic and foraging grounds in the eastern Atlantic, which may promote transatlantic barnacle migration. Alternatively, an appreciable frequency of male juvenile loggerheads with mtDNA haplotypes typically associated with western Atlantic populations has been observed on foraging grounds in the Mediterranean Sea (Casale *et al.* 2002). Barnacles recruiting to these turtles could potentially be transported to the western Atlantic when the turtles return to natal nesting beaches or nearby foraging or courtship grounds. Our lack of sampling from the eastern Atlantic and other locations in the Mediterranean make it impossible to determine which of these hypotheses, or perhaps a combination of the two, is most plausible.

In summary, the association between the barnacle *C. testudinaria* and its host the loggerhead turtle, *Caretta caretta* is quite old, extending as far back as the Miocene epoch (Ross 1963; Ross & Newman 1967). Given that *C. testudinaria* is an obligate commensal commonly associated with marine turtles, we have suggested that host-mediated dispersal may have a significant impact on the patterns of genetic variation in barnacle populations. Our results are consistent with the hypotheses that the migration of loggerhead turtles helps maintain genetic homogeneity among western Atlantic *C. testudinaria* populations and is responsible for expansion of the range of *C. testudinaria* into the Mediterranean. In contrast, host migration appears to have less of an impact on the genetic structuring of *C. testudinaria* populations in the Pacific. The difference between ocean basins may stem from the fact that host-mediated dispersal in the Atlantic depends on advanced stage juveniles and adults while any host-mediated dispersal in the Pacific would have to involve early 'pelagic' stage juvenile loggerheads and highlights the need for additional studies on the epibiont community associated with migrating juvenile turtles.

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