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The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae)

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Abstract

The mode of development of marine invertebrates is thought to influence levels of population structure and the location of species range endpoints via differences in dispersal ability. To examine these effects, populations of three sympatric clades of sedentary, marine gastropods in the genus *Crepidula* were sampled along the Atlantic and Gulf coasts of North America. A haplotype tree was constructed for each clade based on 640 bp sequences of mitochondrial cytochrome oxidase *c* subunit I. Examination of the tree topology, and AMOVA analysis show that species with direct development (those hatching as benthic juveniles) have higher levels of population structure than do species with planktonic development. Both species in the direct-developing *C. convexa* clade have high levels of geographical differentiation, with most populations representing a discrete clade of haplotypes. The planktotrophic species *C. fornicata* contains two major haplotype clades, both of which include samples from throughout the Atlantic coast. In this species there is no geographical differentiation among haplotypes but AMOVA analysis detects a small but statistically significant level of geographical structure. The population structure within the *C. plana* species complex appears also to vary with mode of development: *C. atrasolea*, a direct-developing species, has higher levels of population structure than does *C. depressa*, a sympatric planktotrophic species. The coincident occurrence of range endpoints and genetic breaks along the east coast of Florida in both direct-developing species and species with planktonic development indicates that this biogeographic break is not due to development-specific mechanisms such as hydrographic effects on larval recruitment.

Keywords: *Crepidula convexa*, *Crepidula plana*, *Crepidula fornicata*, gastropod phylogeny, sibling species

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Introduction

The life cycles of benthic marine invertebrates generally include benthic direct development or planktonic (planktotrophic) larval development (see Strathmann 1985 for in-depth discussion of modes of development). Because planktonic larvae can remain suspended in the water column for weeks to months, where they may be at the mercy of ocean currents, it is commonly supposed that benthic species with planktonic larvae have higher levels of dispersal than do species with direct development (Crisp 1978; Hedgecock 1982). It follows that species with planktonic larvae are expected to have higher levels of gene flow and therefore lower levels of population structure than do species with direct development (Crisp 1978; Hedgecock 1982).

Of the numerous studies that have examined the population structure of marine invertebrates, only a handful have explicitly compared the population structure of species with differing modes of development (e.g. Berger 1973, 1977; Ament 1978; Ward 1990; McMillian *et al.* 1992; Duffy 1993; Hunt 1993; Hellberg 1996; Hoskin 1997; Arndt & Smith 1998; Ayre & Hughes 2000; Kyle & Boulding 2000) and only a few of these compare congeneric species from the same geographical range. The general result of these studies is that F_{ST} values are usually higher for species with direct development than for species with planktonic development. Among such published studies of gastropods, only

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those focusing on *Littorina* (e.g. Berger 1973; Janson 1987; Ward 1990; Kyle & Boulding 2000), and *Hydrobia* (Wilke & Davis 2000) have compared the population structure of closely related species over the same geographical range. The results of these studies are somewhat ambiguous. Allozyme work with *Littorina littorea*, *L. saxatilis* and *L. obtusata* have shown that the species with direct development have measurable population structure over a range of geographical distances, while those with planktonic development do not have well-defined population subdivisions (Berger 1973, 1977; Janson 1987). However, comparisons of two direct-developing species, *L. mariae* and *L obtusata*, show significant differences in population structure that are attributable to differences in generation time rather than mode of development (Rolán-Alvarez *et al.* 1995). Generation time and other life-history factors were not considered in the earlier studies of *Littorina* species. Recent analysis of mitochondrial cytochrome *b* sequence data of two planktotrophic and two direct developing species of *Littorina* from the North-eastern Pacific showed similar levels of significant population structure in one of the planktotrophic species (*L. plena*) and one of the direct-developing species (*L. subrotundata*) while the remaining two species (*L. sitkana* and *L. scutulata*) did not appear to have any significant levels of among-population genetic variance (Kyle & Boulding 2000). Finally, cytochrome oxidase I (COI) sequence data from two European species of *Hydrobia*, brackish-water gastropods, demonstrated that the species with a 1–3-day planktonic stage has significantly less population structure than does the direct-developing species (Wilke & Davis 2000). The authors, however, consider it unlikely that this effect is due to the difference in mode of development and attribute it instead to differences in adult habitat. Therefore, it is not clear that the paradigm drawn from the analysis of allozyme data, that differences in mode of development result in differences in population structure, will be consistently supported by DNA sequence data.

Differences in mode of development may also affect a species' geographical range (e.g. Gaylord & Gaines 2000). In marine systems, biogeographic boundaries often occur at boundaries between currents or other distinct hydrographic features (e.g. Briggs 1995; Gaylord & Gaines 2000). Previous studies have argued that the concordance of species range endpoints in numerous distantly related groups are the result of general processes (e.g. Fischer 1960; Avise 1992). The most common hypotheses explaining such biogeographic breaks in marine systems are that: (i) hydrodynamic factors limit recruitment or availability of planktonic larvae (e.g. Gaylord & Gaines 2000); and (ii) sharp gradients in other abiotic factors, such as temperature or salinity, limit ranges (e.g. Fischer 1960; Briggs 1995). Examination of clades of closely related species with differing modes of development across the same geographical range can be used to test between these alternative hypotheses. If limited larval recruitment results in coincident range endpoints, species with planktonic larvae will show concordant range endpoints or genetic breaks, but those species with direct development or extremely short-lived larvae will not. If sharp gradients in abiotic conditions cause biogeographic breaks then direct developers and planktotrophs should have similar absolute range boundaries.

One well-known biogeographic break occurs along the southeastern coast of Florida (Bert 1986; Avise 1992; Felder & Staton 1994; Schizas *et al.* 1999). Numerous marine invertebrates and fishes show either species-range boundaries or pronounced genetic breaks in this area. However, the distribution of closely related species that differ in mode of development across this region has not been examined.

In this study, mitochondrial DNA (mtDNA) sequence data are used to reconstruct haplotype phylogenies of three clades of *Crepidula* from the east coast of North America. Haplotype trees and analysis of molecular variance (AMOVA) are used to test the following hypotheses: (i) species with direct development exhibit more genetic population structure than do species with planktonic development; and (ii) genetic breaks are concordant across lineages and occur at the previously identified biogeographic break in southern Florida.

Materials and methods

Crepidula *biology and sampling design*

The three lineages of marine gastropods in the genus *Crepidula* that occur commonly along the east coast of North America are ideal animals with which to address these hypotheses. They exhibit the same generalized benthic filter-feeding lifestyle, inhabit similar geographical ranges, and are sedentary as adults. Most importantly, there are sympatric species with both planktotrophic and direct development. *Crepidula fornicata* (Linneaus 1758), a large (~2–4 cm) species with planktonic development, is reported from Prince Edward Island, Canada to the Bahamas (Hoagland 1977). *C. convexa* Say 1822, a small (< 2 cm), shallow-water species with direct development, ranges from Nova Scotia, Canada to Puerto Rico (Hoagland 1977). There is evidence that this range includes two cryptic species (Hoagland 1984, 1986) but they have not yet been formally described (Collin submitted for publication; here referred to as the northern and southern species). A third species, *C. plana* Say 1822, was believed to have an equally large range (Hoagland 1977) but recent developmental and molecular work has shown that along the east coast of the United States the *C. plana* species complex is composed of three distinct species (Hoagland 1984, 1986; Collin 2000): *C. plana*, *C. depressa* Say 1822, and *C. atrasolea* Collin 2000.

Live specimens of *Crepidula* were collected on both the east and west coasts of Florida, the Florida Keys and New Jersey and both live material from Panacea, Florida, and

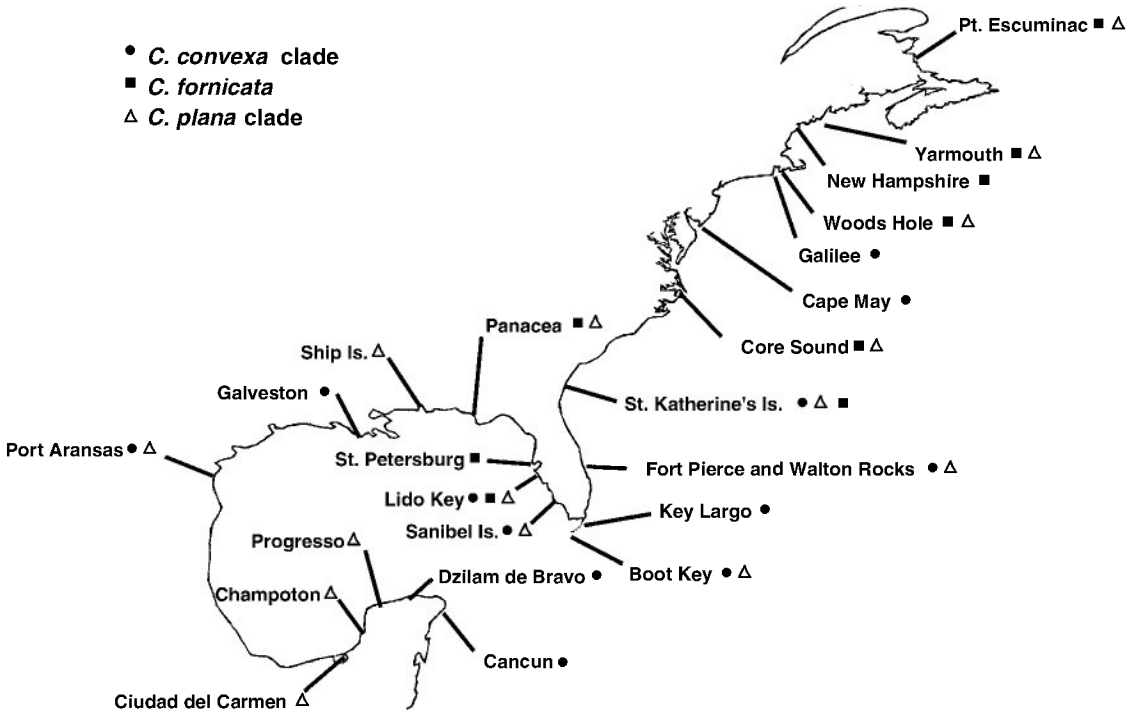

Fig. 1 Map of North America showing the collection localities for material used in this study.

preserved animals from a variety of other locations in North America were examined (Fig. 1; Appendix I). Species identification in the *C. plana* and *C. convexa* groups is treated in detail elsewhere (Collin 2000; Collin, submitted for publication) and *C. fornicata* was identified on the basis of Hoagland (1977). Vouchers are deposited at The Field Museum, Chicago, USA.

DNA sequencing and analysis

A 640-bp sequence of the mitochondrial COI was sequenced for multiple individuals from each locality. DNA was extracted from ethanol-preserved tissue with a Puregene® extraction kit (Gentra Systems), amplified using Ready-To-Go(tm) polymerase chain reaction (PCR) beads (Pharmacia Biotech), and the primers and PCR profile of Folmer *et al.* (1994). Both strands were cycle-sequenced using the amplification primers with dRhodamine, or Big Dyes cycle sequencing dye terminator kits (Perkin Elmer) on a ABI 377 automated sequencer. Sequences were aligned by eye using SEQUENCHER 3.0.

Outgroups were identified by examination of a preliminary phylogenetic analysis of 50 calyptraeid species. Neither *C. convexa* nor *C. fornicata* have closely related sister species amongst the species examined so far, but they appear to be closest to those outgroup species chosen here. The choice of outgroup does not affect the results of the analysis or the monophyly of each species complex.

Phylogenetic analyses were conducted using PAUP* version 4.0b2 (Swofford 1999). An equal-weighted parsimony analysis was performed using a heuristic search with tree bisection–reconnection (TBR) branch-swapping and 1000 random additions. Bootstrap support for each clade was assessed based on 100 bootstrap replicates with TBR branch-swapping and 10 random additions. The DNA sequences were translated to protein sequences with MACCLADE using the *Drosophila* mitochondrial genetic code, which has been shown to apply to other gastropod mitochondrial sequences (Yamazaki *et al.* 1997; Wilding *et al.* 1999).

The degree of population structure was evaluated using two methods. Samples from species with highly subdivided populations are likely to produce phylogenies where haplotypes from each locality form distinct clades, while samples from species with high gene flow are likely to produce phylogenies in which haplotypes from different sites are intermingled. Therefore tree topology, and branch lengths and bootstrap values of geographically distinct clades give a heuristic indication of population structure. Population structure was also assessed with analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) using ARLEQUIN version 2 (Schneider *et al.* 2000). Haplotypes were grouped into populations by geographical location and the genetic variance was partitioned into within- and among-population variance. The among-population component, the associated *P*-value of which is calculated with 10 000 replicate analyses of samples drawn randomly from the data, is

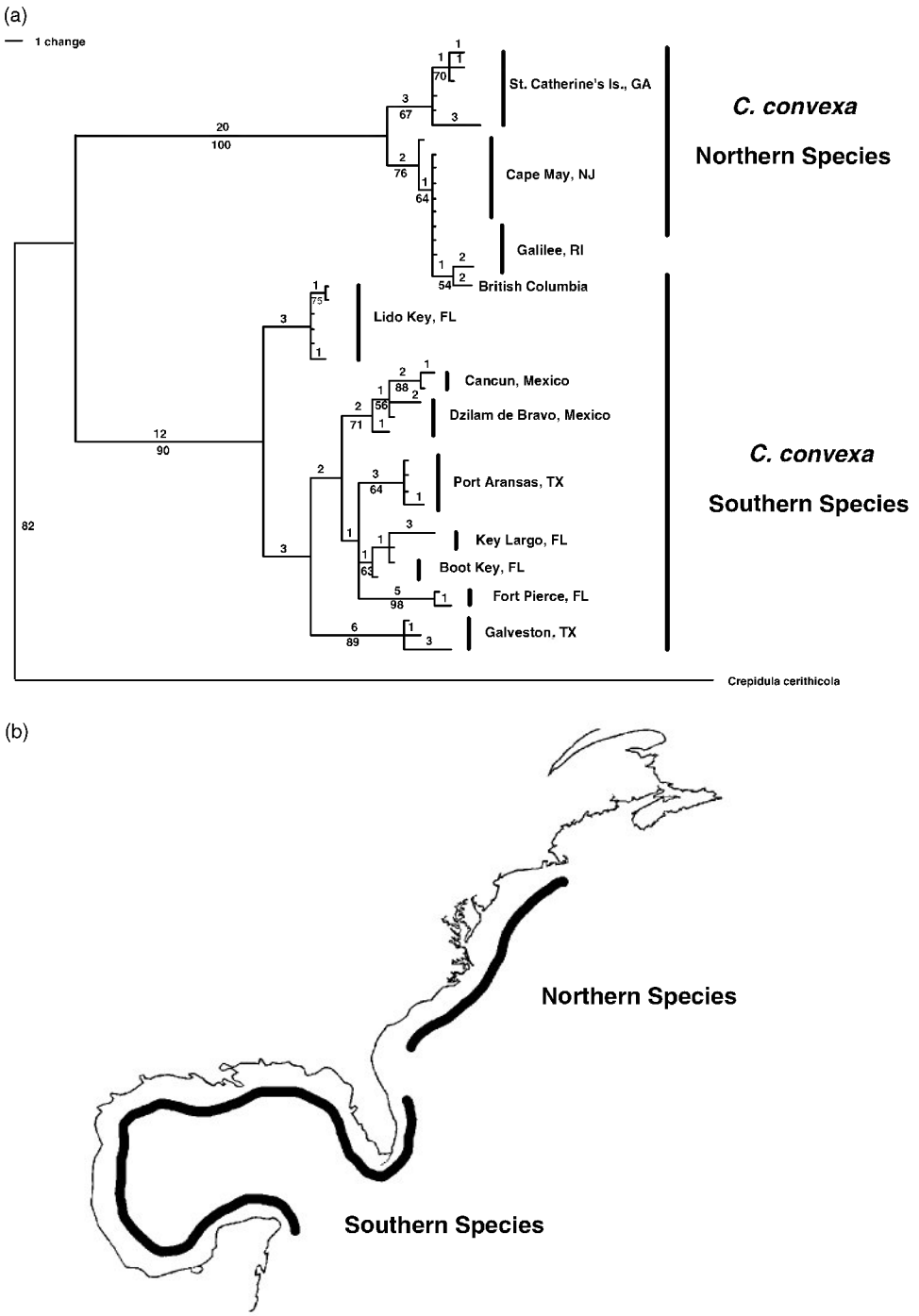

Fig. 2 (a) One of the 22 most parsimonious trees of *Crepidula convexa* species complex based on 640 bp of cytochrome oxidase I (Length = 175; CI = 0.82; RI = 0.95; 508 constant characters; 66 uninformative character; 66 informative characters). Numbers above the branches represent branch lengths and those below the branches represent values for branches with > 50% support from 100 bootstrap replicates. (b) The geographical ranges of the northern and southern clades of *C. convexa* as inferred from the molecular data from localities sampled in this study.

approximately equal to F_{ST} (Schneider *et al.* 2000). This analysis was performed separately for each species with samples from more than two populations, using only populations from which sequences from three or more individuals were available. Because genetic divergences are low, uncorrected genetic distances were used. Geographic distances were measured approximately along the coastline of a map.

Developmental stages for individuals from populations from Florida, Massachusetts and New Jersey were observed

and measured with a dissecting microscope. These data were supplemented with published data (Ament 1979; Hoagland 1984, 1986).

Results

Phylogenetic analyses

Parsimony analysis of 41 COI sequences from the *Crepidula convexa* clade produced a single island of 22 most parsimonious trees (Fig. 2; Length = 175; CI = 0.82; RI = 0.96; 508 constant characters; 66 uninformative characters; 66 informative characters). All informative substitutions but one (along the branch between the northern and southern clades) were synonymous. Two major clades which represent the two distinct species identified by Hoagland (1984) are clearly recovered. A northern clade includes haplotypes from Georgia to Rhode Island while a southern clade includes samples from the Gulf coast, the Atlantic coast of Florida and the Yucatan Peninsula (Fig. 2). Each clade shows considerable population structure, with mixing of haplotypes from different sites occurring only between the most closely spaced sampling sites. The relationship between geographical distances among the sites (Table 1) and genetic distance between individuals is shown in Fig. 3. Haplotypes from sites separated by less than 200 km in the southern species (and 400 km in the northern species) have not coalesced into separate monophyletic clades. Haplotypes from sites farther apart all form monophyletic clades with haplotypes from the same site. The biogeographic relationships among these clades is not clear and the pairwise genetic distance among individuals does not correlate with geographical distance between sites (Fig. 3). Some clades from the coast of Texas are more closely related to clades on the Atlantic coast of Florida than they are to other clades from the Gulf coast, and animals from the Florida Keys are genetically more similar to animals from east Texas and Yucatan than they are to animals from western Florida. The single individual collected from British Columbia groups closest to *C. convexa* from Rhode Island, which verifies the previously unpublished observations that *C. convexa* from New England has been introduced into the Vancouver area.

Parsimony analysis of 30 COI sequences from *C. fornicata* produced a single island of 2766 equally parsimonious trees (Fig. 4; length = 153; CI = 0.87; RI = 0.89; 517 constant characters; 94 uninformative characters; 29 informative characters). Two distinct clades were recovered with a mean divergence of about 2%. These clades showed little geographical variation, with northern (New England) and southern (Florida) individuals occurring in each clade. The large number of parsimonious trees is the result of different topological arrangements of nearly identical sequences.

Table 1 Geographic distances between *Crepidula convexa* sampling sites

Localities	Distance (km)*
C. convexa Northern species	
Rhode Island to New Jersey	400
New Jersey to Georgia	1100
C. convexa Southern species	
Harbor Branch to Key Largo, Florida	280
Key Largo to Boot Key, Florida	120
Key Largo to Lido Key, Florida	370
Lido Key, Florida to Galveston, Texas	1700
Galveston to Port Aransas, Texas	370
Port Aransas, Texas to Dzilam de Bravo, Mexico	2100
Dzilam de Bravo to Cancun Mexico	190

*Distances are treated as additive.

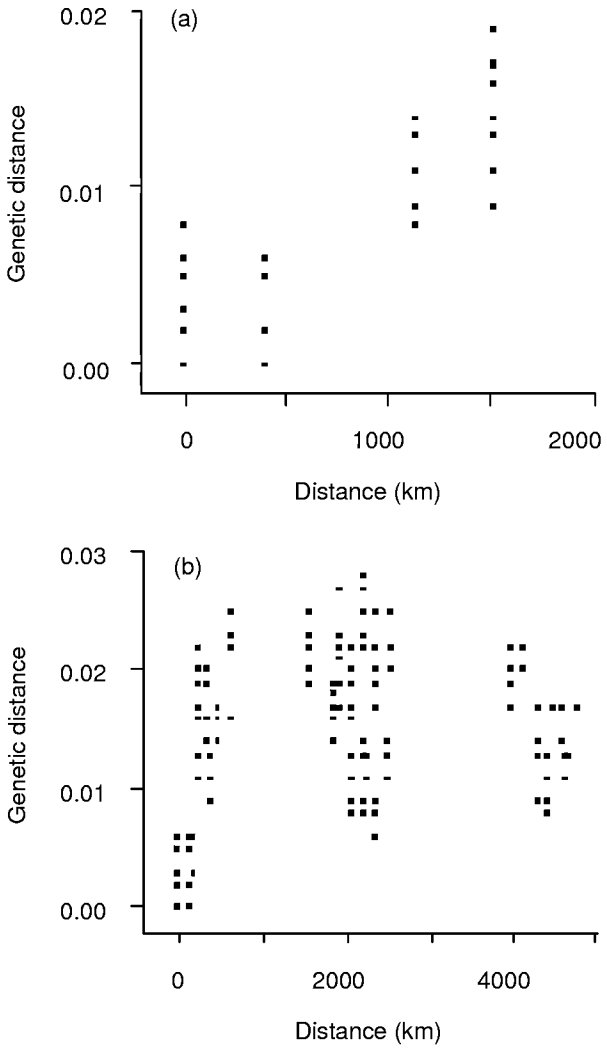

Fig. 3 Scatter plot of pairwise geographical distance between sampling sites and pairwise genetic distance for individuals of *Crepidula convexa* northern species (a) and southern species (b).

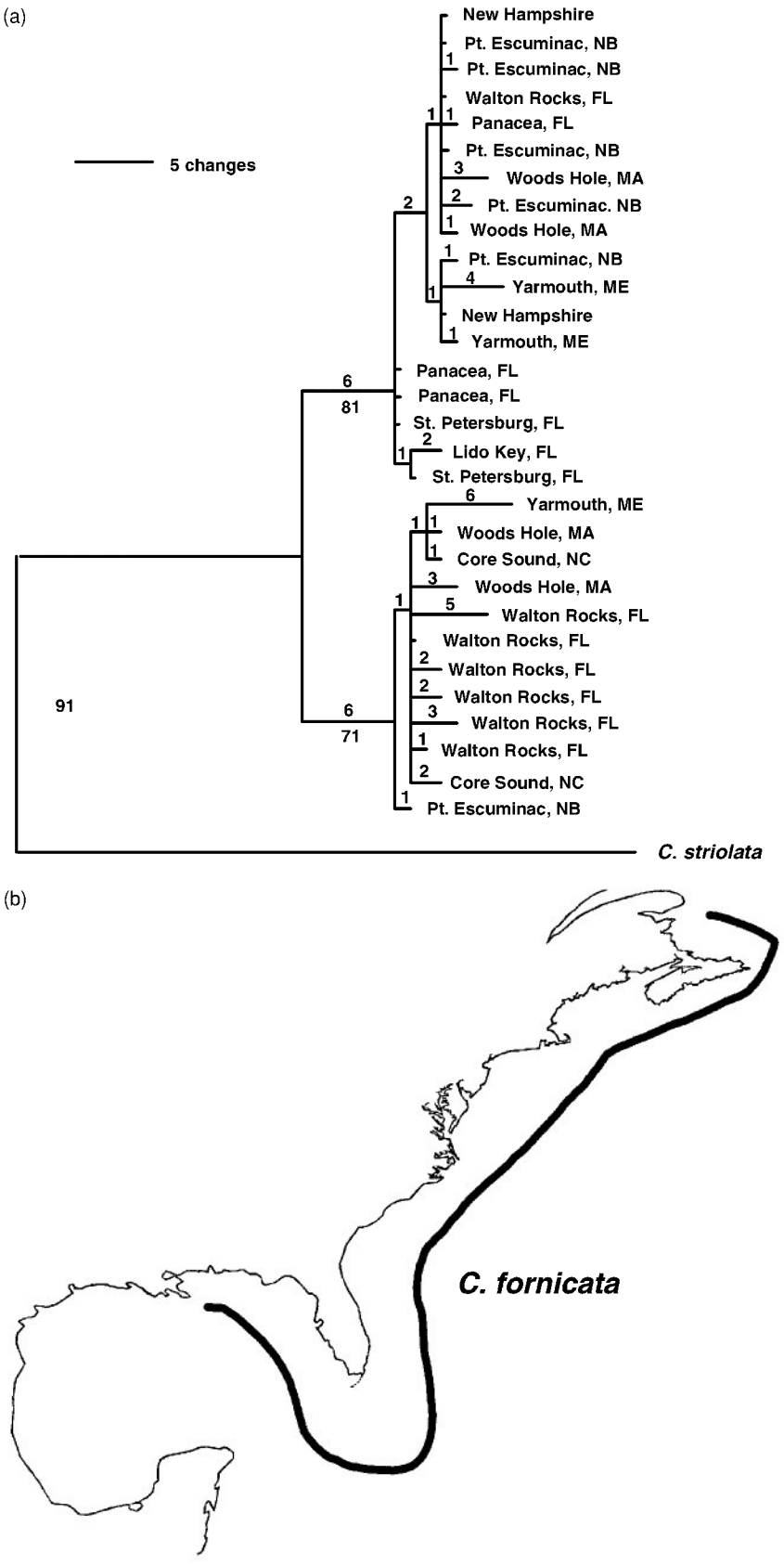

Fig. 4 (a) One of the many most parsimonious trees of *Crepidula fornicata* based on 640 bp of cytochrome oxidase I (length = 153; CI = 0.87; RI = 0.89; 517 constant characters; 94 uninformative characters; 29 informative characters). Numbers above the branches represent branch lengths and those below the branches represent values for branches with > 50% support from 100 bootstrap replicates. (b) Geographic ranges of the two haplotype clades of *C. fornicata* as inferred from the molecular data.

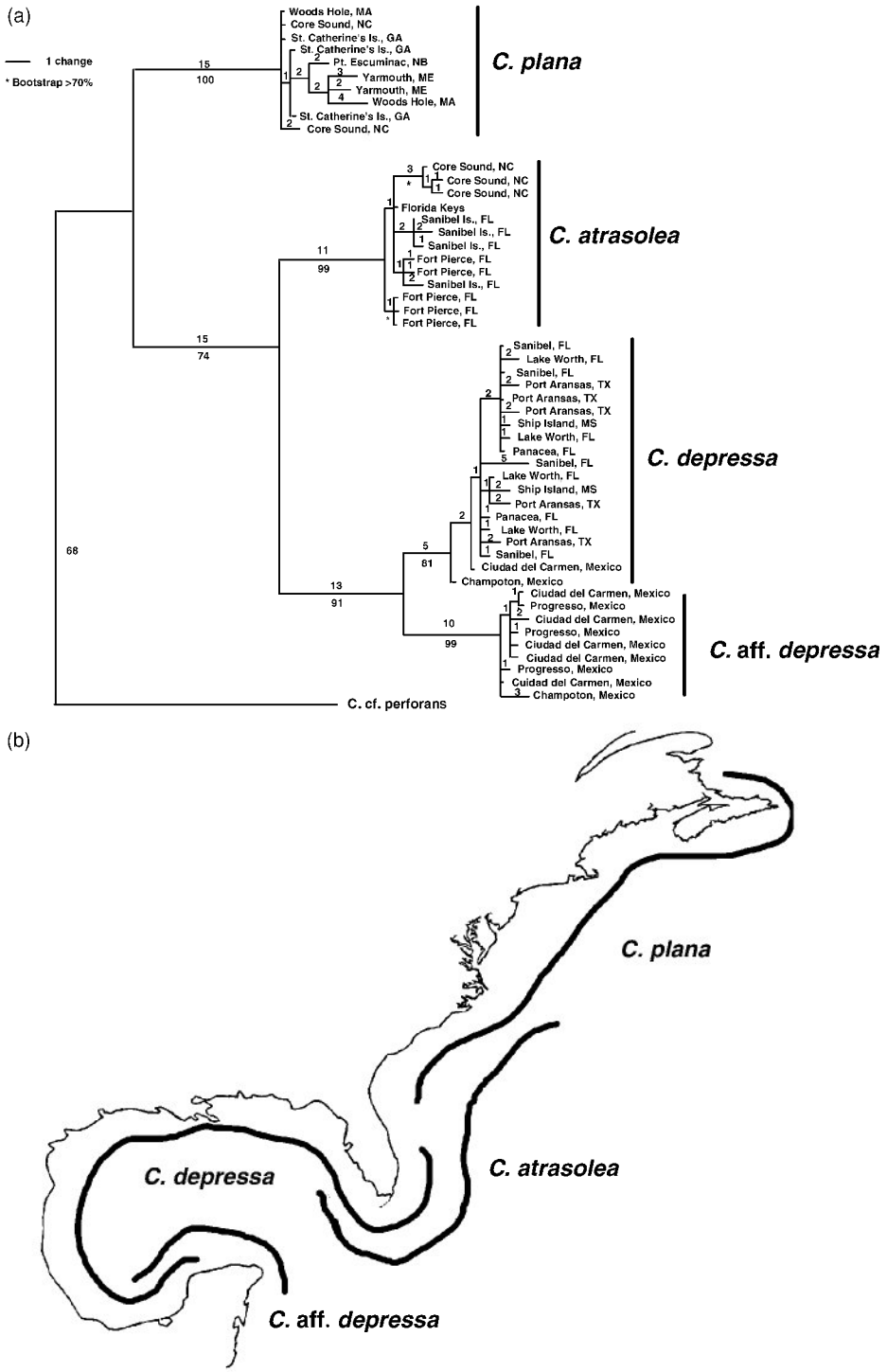

Fig. 5 (a) One of the many most parsimonious trees of *Crepidula plana* species complex based on 640 bp of cytochrome oxidase I (length = 209; CI = 0.76; RI = 0.95; 503 constant characters, 57 uninformative and 80 informative characters). Numbers above the branches represent branch lengths and those below the branches represent branches with > 50% support values from 100 bootstrap replicates. (b) Geographic ranges of *C. plana*, *C. depressa*, *C. atrasolea* and *C.* aff. *depressa* inferred from these molecular data.

No geographical groupings of haplotypes are well supported by bootstrapping, indicating little population structure. However one clade was composed mostly of northern individuals while the other clade was predominantly southern. All of the differences between the two haplotype clades are synonymous.

Analysis of 51 COI sequences from the *C. plana* species complex produced a single island of 19 439 equally

Table 2 Mode of Development in North Atlantic *Crepidula* Species

Locality	Mode of development	Reference
C. convexa Northern		
Woods Hole, MA	direct development	Hoagland (1986)
Long Island, NY	direct development	Hoagland (1986)
Cape May, NJ	direct development	Pers. Observation 1999
Delaware Bay, NJ	direct development	Ament (1978)
C. convexa Southern		
Lido, FL	lecithotrophic larvae and direct development	Pers. Observation 1997
Key Largo, FL	lecithotrophic larvae	Pers. Observation 1997
Gulf Breeze, FL	lecithotrophic larvae	Hoagland (1986)
Fort Pierce, FL	lecithotrophic larvae	Pers. Observation 1997 and Hoagland (1986)
C. fornicata		
Woods Hole, MA	planktotrophic larvae	Pers. Observation 1995 and Hoagland (1986)
Kettle Cove, ME	planktotrophic larvae	Hoagland (1986)
Delaware Bay, NJ	planktotrophic larvae	Ament (1978)
C. plana		
Woods Hole, MA	planktotrophic larvae	Pers. Observation 1995 and Hoagland (1986)
Long Island, NY	planktotrophic larvae	Hoagland (1986)
Delaware Bay, NJ	planktotrophic larvae	Ament (1978)
C. depressa		
Sanibel Is., FL	planktotrophic larvae	Collin (2000)
Lake Worth, FL	planktotrophic larvae	Collin (2000)
C. atrasolea		
Fort Pierce, FL	direct development	Collin (2000) and Hoagland (1986)
Sanibel Is., FL	direct development	Collin (2000)
Florida Keys, FL	direct development	Collin (2000)
C. aff. *depressa*		
Campeche, Yucatan	lecithotrophic larvae?	Field Observations: R. Collin and T. Griffin 2000
Ciudad del Carmen, Yucatan	lecithotrophic larvae?	Field Observations: R. Collin and T. Griffin 2000

parsimonious trees (Fig. 5; length = 213; CI = 0.74; RI = 0.94; 502 constant characters, 57 uninformative and, 81 informative characters). The large number of parsimonious trees is the result of different topological arrangement of nearly identical sequences which do not affect the well-supported aspects of the tree topology. This tree is similar to that produced by previous analysis of a smaller data set Collin (2000) with the addition of *C.* aff. *depressa* from the Yucatan Peninsula. The four clades have distinct geographical distributions (Fig. 4): *C. plana* ranges from New Brunswick to Georgia; *C. depressa* ranges around the Gulf coast and along the Atlantic coast of Florida; *C.* aff. *depressa* has been sampled only from Yucatan; and *C. atrasolea* ranges along the Atlantic coast from North Carolina, south to western Florida. None of these clades contain well-supported geographical subdivisions, but there is some haplotype sorting in the direct-developing *C. atrasolea*. All of the synapomorphic differences are synonymous.

Mode of development

Available information on mode of development for populations of these *Crepidula* species is presented in Table 2. *C. fornicata*, *C. depressa* and *C. plana* have planktotrophic development and larvae probably spend at least 2 weeks in the water column before settling. *C. atrasolea* and most populations of *C. convexa* have direct development. Three southern populations of *C. convexa* produce offspring that hatch as swimming pediveligers (lecithotrophic larvae) which settle and metamorphose less than 3 hr after hatching. This extremely short planktonic stage probably contributes little to dispersal in comparison to species that

Table 3 Results of the AMOVA analyses of each species group after excluding populations represented by fewer than three individuals

Species group	Clade	Level	d.f.	Sums of squares	Variance components*	% Variance*	*P*	Mode of development
C. convexa	Northern species	among populations	2	23.3	2.15	76.1	< 0.0005	direct
		within populations	13	8.7	0.65	23.9		
	Southern species	among populations	4	66.4	4.58	87.2	< 0.0005	lecithotrophic
		within populations	13	8.8	0.67	12.8		
C. fornicata	Entire clade	among populations	4	38.1	1.19	22.1	< 0.029	planktotrophic
		within populations	18	75.8	4.21	78.0		
C. depressa	Entire clade	among populations	2	3.0	0.15	−7.4	> 0.5	planktotrophic
		within populations	10	21.5	2.15	107.4		
C. atrasolea	Entire clade	among populations	2	14.6	1.63	54.3	< 0.0005	direct
		within populations	8	11.0	1.38	45.7		

*Among population variance is approximately equal to F_{ST} (Excoffier *et al.* 1992).

spend several weeks in the plankton. Mode of development has not been documented for *C.* aff. *depressa*. When egg capsules were collected in the field they contained large mid-stage embryos that are capable of swimming. However, laboratory observations are necessary to verify that the embryos do not form large planktotrophic veligers or alternately absorb the velum prior to hatching as juveniles.

Tests of population structure

AMOVA analysis shows that a higher percentage of genetic variance is explained by the among-population component in direct developing species than in species with planktotrophic development (Table 3). There is a significant among-population component of variance in the direct-developing *C. convexa* (76% northern species; 87% southern species) and *C. atrasolea* (54%) and in the planktotrophic *C. fornicata* (22%) (Table 3). There is no among-population contribution to variance in the planktotrophic *C. depressa* (Table 3). The negative value obtained for *C. depressa* reflects that this statistic is actually a covariance (Excoffier *et al.* 1992) and negative values can occur when the actual values are close to zero. *C. plana* and *C.* aff. *depressa* were not included in these analyses because too few populations of each had sequences from more than three individuals.

Discussion

Effects of development on population structure

Analysis of COI sequence data for *Crepidula* species along the east coast of North America shows that species with direct development have more population structure than do species with planktonic development. Not only is a greater percentage of genetic variance due to the among-population variance in direct developers, but the haplotypes form geographically distinct monophyletic clades. There is also a small but statistically significant among-population component of variance in *C. fornicata*, a planktotrophic species. The relatively greater number of sequences and larger geographical range of *C. fornicata* give this data set the power to detect the small but significant effect. Increased sample sizes for *C. depressa* and *C. plana* might also uncover a significant among-population component of variance. Similarly, the among-population component of variance in *C. atrasolea* might also increase with increased sampling.

The results presented in this study are similar to those reported from allozyme studies of the same species by Hoagland (1984) and Ament (1978). Based on one enzyme locus (PHI), Ament (1978) found high levels of population differentiation in *C. convexa* and *C. fornicata* along the east coast from Cape Cod to North Carolina while populations of *C. plana* were genetically homogeneous. Hoagland's (1984) analysis of 24 allozyme loci showed that samples of *C. convexa* and *C. plana* from Florida were genetically distinct from New England samples, with numerous fixed differences between the northern and southern *C. convexa* species. At a population level she found similar genetic distances among New England populations of *C. fornicata*, *C. plana* and *C. convexa*, and *C. convexa* from Florida. But the two geographically close populations of *C. atrasolea* (*C.* cf. *plana* of Hoagland 1984, 1986) from Florida are separated by almost three times the Roger's distance as populations of the other species. The limited geographical sampling (New England and Florida only) and unrecognized cryptic species (*C. depressa* and *C.* aff. *depressa* were not distinguished from *C. plana*) limit the conclusions that can be drawn from these results.

The differences in population structure detected in the present study may be due to differences in other aspects of the biology of these animals. For example Rolán-Alvarez *et al.* (1995) concluded that differences in levels of population structure between two direct-developing species of *Littorina* are due to differences in generation time. It is possible that the small *C. convexa* has a shorter generation time than do the larger species examined in this study. This could contribute to the greater population differentiation in *C. convexa*. Individuals living in the south may also have faster growth rates and shorter generation times than do individuals from colder northern waters. However, comparative data on generation times are not currently available for these species.

Planktonic larvae may not be the only cause of dispersal in benthic species. Small juveniles of many species may be carried around by currents (e.g. Martel & Chia 1991). *C. convexa* are often found living on blades of seagrass or on small gastropods living in seagrass beds. Rafting on seagrass has been shown to aid dispersal of other sedentary marine invertebrate species (Worcester 1994). However, the high levels of population structure in *C. convexa* suggests that rafting on seagrass could only be a significant cause of among-population gene flow over short distances. The larger species *C. fornicata*, *C. plana* and *C. depressa* are usually found attached to rocks or oyster shells, which do not facilitate adult dispersal. However, these species are also known to occur on the carapaces of horseshoe crabs (personal observation and Botton & Ropes 1988), which could result in occasional long distance dispersal. The animals from Ship Island, Mississippi and Champotón, Mexico were all collected from horseshoe crabs, but their genotypes are similar to other animals collected from nearby populations from less mobile substrates. Because all the species that occur on horseshoe crabs have planktonic development it will be difficult to distinguish between the effects of larval dispersal and dispersal via horseshoe crabs. These effects could be measured if genetic markers that can distinguish among source populations were available.

Coincidence of genetic discontinuity, range endpoints and biogeographic boundaries

The biogeographic patterns of marine organisms along the east coast of North America are complex. Two distinct biogeographic breaks have been well characterized by both species-range boundaries (Fischer 1960) and genetic structure within species (Reeb & Avise 1990; Avise 1992). At Cape Hatteras, there is a biogeographic break defined by the concordance of many species-range endpoints (Fischer 1960), which is usually explained by hydrographic patterns. The Gulf Stream veers east here and sea-surface temperatures can differ significantly to the north and south of Cape Hatteras. The second break occurs in the vicinity of eastern Florida and is the result of both species-range endpoints (Fischer 1960) and sharp genetic breaks in species with continuous distributions along the coast (Reeb & Avise 1990; Avise 1992; 1994). The reason for the rough concordance of biogeographic breaks in eastern Florida is not clear but it has been hypothesized to result from interactions of historical changes in sea level and in current patterns (Bert 1986; Reeb & Avise 1990; Avise 1992; Iturralde-Vinent & MacPhee 1999).

The three species groups of *Crepidula* examined in this study show genetic discontinuities or species-range endpoints at these previously defined biogeographic boundaries. Six genetic breaks or range endpoints occur within the sampled area. One (the northern limit of *C. atrasolea*) occurs near Cape Hatteras and four (the northern limits of *C. depressa* and the southern clade of *C. convexa* and the southern limit of *C. plana* and the northern clade of *C. convexa*) occur along the east coast of Florida. The final apparent range endpoint is that of *C. atrasolea* along the west coast of Florida. The coincidence of range boundaries for both direct-developing species and species with planktonic development suggests that the eastern Florida biogeographic break is not the result of hydrographic factors influencing larval recruitment. Avise (1992) reports that numerous other marine animals representing a variety of ecologies and life histories also show genetic breaks in this area. The *C. convexa* clade shows a 5% genetic divergence across the break while a range of 1–10% is reported for marine species (Avise 1992). The fact that three species range across the Cape Hatteras biogeographic break suggests that *Crepidula* species tolerate the geographically abrupt changes in water temperature. This is not surprising, since *Crepidula* are often abundant in habitats such as shallow bays and the intertidal regions, where they may be exposed to rapid fluctuations in temperature and salinity. Habitat choice may ameliorate the changes in temperature, since *C. fornicata* are common in the intertidal and shallow subtidal regions in New England and Canada, while they are exclusively subtidal in Florida.

Although analysis of mtDNA sequence data demonstrates that levels of population structure and population differentiation are related to mode of development in *Crepidula* from the east coast of North America, this pattern has still not been strongly demonstrated for many marine molluscs. This consensus view, reached from allozyme studies, has not been clearly supported by the few existing studies of DNA sequence data (e.g. Kyle & Boulding 2000; Wilke & Davis 2000). Further studies of closely related species with overlapping ranges are needed, not only to determine if mode of development commonly plays a strong role in defining the population structure of marine animals, but also to examine how it influences species-range endpoints at other well-known biogeographic boundaries.

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Rachel Collin is a graduate student working on the causes and consequences of mode of development in *Crepidula*. Her current work combines a phylogeny based on molecular and morphological data with observations of development to examine ecological and phylogenetic patterns in mode of development.

Appendix I Material sequenced for this study

Species	Locality	No. of individuals sequenced	Field museum Lot numbers for Vouchers	GenBank Numbers	Collector
C. convcxa North					
C. convexa	Bluff Hill Cove, Galilee, Rhode Island 41°23 N, 71°30 W	4	282300	AF388726–AF388729	R. Bullock
C. convexa	Wildwood Crest, Cape May, New Jersey 38°50 N, 74°59 W	6	282261, 282262, 282299	AF388720–AF388725	R. Collin and S. Padowitz
C. convexa	St. Catherine's Is., Georgia 31°60 N, 81°15 W	6	282259, 282260	AF388714–AF388719	J. Slapcinsky
C. convexa South					
C. convexa	Mustang Is., Port Aransas Bay, Texas 27°55 N, 97°08 W	4	282257, 282258	AF388730–AF388735	J. Wise and Houston Shell Club
C. convexa	Galveston, Texas 29°02 N, 94°53 W	3	282307	AF388736–AF388738	S. Harrison
C. convexa	Lido Key, Florida 27°20 N, 82°42 W	6	282190, 282191, 282253, 282254, 282268	AF388706–AF388711	R. Collin
C. convexa	Boot Key, Florida 24°41 N, 81°05 W	2	282255	AF388739, AF388740	R. Cipriani and R. Bieler
C. convexa	Fort Pierce, Florida 28°30 N, 81°20 W	2	282251, 282252	AF388704, AF388705	R. Collin
C. convexa	Key Largo, Florida 25°05 N, 80°25 W	2	282249, 282250	AF388712, AF388713	R. Collin and T. Rawlings
C. convexa	Dzilam de Bravo, Yucatan 21°20 N, 88°55 W	3	282316	AF388699–AF388701	R. Collin and T. Griffin
C. convexa	Cancun, Quintana Roo 21°10 N, 86°48 W	2	282315	AF388702, AF388703	R. Collin and T. Griffin
C. convexa	Blackie Spit, BC, Canada 49°04 N, 122°53 W	1	282247	AF388741	T. and R. Forsyth
C. fornicata					
C. fornicata	Woods Hole, MA 41°30 N, 70°40 W	4	282306	AF353126–AF353129	MBL Biological Supply
C. fornicata	St. Petersburg, Florida 27°30 N, 82°42 W	2	282286	AF353140–AF353141	T. Bert
C. fornicata	Lido Key, Florida 27°20 N, 82°42 W	1	282267	AF353148	D. Brumbaugh
C. fornicata	Point Escuminac, New Brunswick 47°06 N, 64°49 W	6	282288	AF353142–AF353147	D. Véliz
C. fornicata	Yarmouth, Maine 43°46 N, 70°09 W	3	282312	AF353149–AF353151	P. Willink
C. fornicata	New Hampshire 43°00 N, 70°50 W	2	282283	AF353124–AF353125	E. Lovely
C. fornicata	Core Sound, NC 35°33 N, 76°48 W	2	282263, 282264	AF353152–AF353154	E. Sotka
C. fornicata	Walton Rocks, Florida 27°00 N, 81°30 W	7	282314	AF353130–AF353136	T. Griffin
C. fornicata	Panacea, Florida 30°00 N, 84°30 W	3	282282	AF353137–AF353139	Gulf Specimen Co.
C. plana group*					
C. depressa	Ship Is., MS 30°15 N, 88°55 W	2	282285	AF387867, AF387868	R. Overstreet
C. depressa	Champotón, Campeche 19°23 N, 90°42 W	1	282318, 282319	AF387869	R. Collin and T. Griffin
C. depressa	Ciudad del Carmen, Campeche 18°40 N, 91°52 W	1		AF387870	R. Collin and T. Griffin

Appendix I *Continued*

Species	Locality	No. of individuals sequenced	Field museum Lot numbers for Vouchers	GenBank Numbers	Collector
C. aff. *depressa*	Champotón, Campeche 19°23 N, 90°42 W	1	282318, 282319	AF387871	R. Collin and T. Griffin
C. aff. *depressa*	Ciudad del Carmen, Campeche 18°40 N, 91°52 W	5		AF387875–AF387879	R. Collin and T. Griffin
C. aff. *depressa*	Progresso, Yucatan 21°14 N, 89°47 W	3	282317	AF387872–AF387874	R. Collin and T. Griffin
C. plana					
C. plana	Yarmouth, Maine 43°46 N, 70°09 W	2	282313	AF388696, AF388697	P. Willink
C. plana	Point Escurinac, New Brunswick 47°06 N, 64°49 W	1	282290	AF388695	D. Véliz
Outgroups					
C. cf. *perforans*	Naples Reef, Santa Barbara, CA 34°20 N, 120°01 W	1	282243	AF178155	S. Anderson and R. Collin
C. striolata	Rio Mar, Panama 08°18 N, 79°50 W	1	282331	AF353123	R. Collin
C. cerithicola	Punta Charmé, Panama 08°30 N, 79°40 W	1	282332	AF388698	R. Collin

*Material from Collin (2000) (GenBank accession numbers AF178119–AF178155) is not listed again here.