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Evolutionary origins of novel conchologic growth patterns in tropical American corbulid bivalves

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SUMMARY We conducted a combined sclerochronologic and phylogenetic analysis to document patterns and rates of shell accretion in several subclades of related corbulids, and to explore the evolutionary origins of novel conchologic developmental patterns. We found three disparate patterns of valve development in Neogene tropical American corbulid genera. These patterns include growth through primarily radial accretion along the sagittal plane, and two derivative patterns: one characterized by initial deposition of a thin shell followed by valve thickening with little increase in valve height, and another producing a well-defined nepioconch through a marked change in the primary growth direction. We conducted a species-level phylogenetic analysis of the taxa surveyed for growth patterns, focusing on the ([*Bothrocorbula*+*Hexacorbula*]+*Caryocorbula*) clade. The phylogenetic distribution of shell growth patterns suggests that this clade is characterized by derivative patterns of growth. Oxygen-isotope calibrated ontogenetic age estimates of species in the derived *Bothrocorbula* subclade further suggest that transitions from the ancestral radial (sagittal) growth pattern to a derived pattern of growth are a function of heterochrony (peramorphosis by acceleration). These findings are significant because they link previously observed patterns of morphological constraint with a specific evolutionary process, demonstrate how morphologic constraint and innovation can be interrelated, and serve as a model for understanding the evolution of morphologic diversity in the clade as a whole. Furthermore, this study highlights the utility of sclerochronologic records as an important component of evolutionary developmental research on organisms with accretionary skeletal growth.

INTRODUCTION

The diversity of evolutionary histories within the Bivalvia is evident in the clade's tremendously varied shell morphology, soft-anatomy, larval ecology, trophic strategies, and habitats. Developmental processes are fundamental to that variation. Nevertheless, whereas a great deal is known regarding the development of bivalve larvae and adult soft anatomy (e.g., Baker and Mann 1997; Le Pennec et al. 2003; Rupert et al. 2003), the phylogenetic framework of postlarval shell development remains poorly understood.

Various approaches have been used to understand the development and evolution of the bivalve shell. For example, mineralogic and conchologic characters have been used alone or in combination with other character sets to generate hypotheses of general bivalve evolution (e.g., Waller 1998; Carter et al. 2000; Giribet and Wheeler 2002), as well as phylogenetic hypotheses at lower taxonomic levels (e.g., Roopnarine 2001a; Anderson and Roopnarine 2003; Anderson et al. 2006; Graf and Cummings 2006; Mikkelsen et al. 2006). Morphometric techniques have been used to discriminate species on the basis of shell variation (Roopnarine 1995; Anderson 1996; Roopnarine and Vermeij 2000; Marko and Jackson 2001; Anderson and Roopnarine 2005; Guralnick 2005; Kosnik et al. 2006) and to examine patterns of shell development (Roopnarine 2001b; Anderson and Roopnarine 2005; Tang and Pantel 2005).

In addition to these traditional approaches, sclerochronologic investigations have been used effectively to determine patterns of shell growth, as well as other features of bivalve life-histories (Jones and Gould 1999; Goodwin et al. 2001; Buick and Ivany 2004; Schöne et al. 2006b). Sclerochronology, the mineralogic equivalent of dendrochronology, is the study of physical and chemical variations in the skeletons of organisms with accretionary growth (e.g., Wefer and Berger 1991). Traditionally, sclerochronologic investigations have focused on paleoenvironmental and paleoecologic questions (e.g., Jones and Allmon 1995; Goodwin et al.

2001) or to constrain the life-histories of commercially important species (Jones et al. 1983). The recent surge in sclerochronologic studies notwithstanding, few have addressed evolutionary developmental questions despite the obvious potential to do so (Jones and Gould 1999 is a notable exception). Here we combine sclerochronologic analysis with phylogenetic analysis, as well as previously determined patterns of morphometric variation and allometry, to describe the evolutionary origin of novel conchologic growth patterns in corbulid bivalves.

The Corbulidae comprises small to moderately sized bivalves, generally <3 cm in length. Corbulid shells range from subequal to greatly inequivalved (left and right valves can differ in size, shape and/or ornamentation) with the left valve fitting partially to completely into the right valve (e.g., Anderson and Roopnarine 2003). The nesting of Corbulidae within the larger clade of Heterodonta (Bernard 1895, 1897; Purchon 1990; Starobogatov 1992; Adamkewicz et al. 1997; Waller 1998; Campbell 2000; Steiner and Hammer 2000; Canapa et al. 2001; Giribet and Wheeler 2002) suggests that the inequivalve condition, although present in other bivalves (e.g., scallops, oysters, rudists), is a derived condition in Corbulidae. In addition, several authors have noted unusual patterns of valve accretion within this family (e.g., Wrigley 1946; Coan 2002). These patterns include an initial period of growth, primarily along the commissure, that produces a very thin shell, which subsequently thickens via accretion predominantly on valve inner surfaces. This transition is accompanied by a major change in growth direction in some species, with growth becoming highly oblique or perpendicular to the commissural plane (Wrigley 1946; Coan 2002).

In their study of the tropical American genera *Caryocorbula*, *Bothrocorbula*, and *Hexacorbula*, Anderson and Roopnarine (2005) speculated that these genera, and perhaps other corbulids, are subject to historical morphologic constraints due to their inequivalved condition and/or complex developmental histories. Specifically, adult valve shape may be constrained by a need to maintain sufficient internal space and functional articulation when valves differ in size and shape and undergo changes in shell-accretion direction as they grow. For Neogene *Caryocorbula*, these constraints are made evident by a strong interspecific pattern of allometry that persists for over 20 million years, through speciation and extinction events, and across environmental gradients. For *Bothrocorbula* and *Hexacorbula*, taxa endemic to the Neogene of tropical America, size range is truncated relative to that of *Caryocorbula* with neither of the former including small species (<8 mm) and with juveniles within species very rarely preserved. The lack of small species suggests that larger size is a critical morphologic feature of *Bothrocorbula* and *Hexacorbula*, whereas the rarity of small individuals indicates that juveniles have a very low preservation potential, perhaps due to a multiphase developmental pattern.

Valve asymmetry, together with apparent changes in the location of primary shell accretion during development, suggest a complicated pattern of mantle function in corbulids relative to other heterodonts. Further, constrained patterns of interspecific allometry indicate that variation in the relationship between valve size and valve shape also is constrained (Anderson and Roopnarine 2005), suggesting a complex developmental context for shell accretion. Identifying the specific process(es) responsible for these patterns requires a more intimate knowledge of the ontogeny of shell accretion than is available only from comparative phylogenetic and gross morphometric analyses. We therefore used sclerochronologic analysis to survey patterns of accretion in several clades of related corbulids, as well as to test directly for evolutionary developmental modifications within the monophyletic *Bothrocorbula*.

MATERIALS AND METHODS

Taxon selection

Our study focused on groups of taxa for which phylogenetic hypotheses exist. This essentially limits our study to tropical American Neogene Corbulidae genera (Anderson and Roopnarine 2003; Anderson et al. 2006). We surveyed developmental patterns of 22 species of Neogene corbulids and incorporated these species into a phylogenetic analysis (Table S1). Detailed sclerochronologic analyses were performed for 10 of these species, and sclerochronologic results were calibrated to ontogenetic ages using oxygen-isotope (δ^{18}O) profiles for seven of the species. Those seven species included exemplars of three developmental patterns present in the species examined as well as all *Bothrocorbula* species for which we had sufficient material to conduct destructive sampling (Table S2).

Sclerochronologic analysis

Shells were sectioned from the umbo to the commissure along the axis of maximum growth using a low-speed saw. Sectioned shells were glued to glass slides and trimmed to approximately 1 mm thickness. These "thick-sections" were polished using standard petrographic techniques (Goodwin et al. 2001; Schöne et al. 2006a). Growth patterns were determined by identifying periodic growth increments in thick-section using reflected light microscopy and digital photography. In addition, the cross-sections of some specimens were examined from fresh fractures along the axis of maximum growth.

For exemplars of growth patterns and for four *Bothrocorbula* species (Tables S1 and S2), we sampled selected thick-sections for isotopic analyses in order to determine ontogenetic age and timing of changes in shell accretion. Because oxygen isotopic variation in biogenic carbonates is a function of water temperature and isotopic composition, both of which show strong annual cyclicity in Neogene tropical American oceans (e.g., Jones and Allmon 1995; Bemis and Geary 1996; Teranes et al. 1996), patterns of within-shell δ^{18}O variation can be used to establish the ontogenetic age of individual specimens (Jones et al. 1983; Wefer and Berger 1991; Jones and Gould 1999; Goodwin et al. 2003).

Before isotopic sampling, fossil specimens were subjected to rigorous diagenetic screening to test for secondary mineralogic alteration, which could cause systematic changes in the primary isotopic composition of shell carbonate (See Supporting Information: Diagenetic Screening). Unaltered carbonate samples were subsequently collected from thick-sections using either point-sampling or micromilling techniques. Point-samples (~50–100 μg) were taken from the outermost shell layer using a 300-μm diameter drill bit (see Goodwin et al. 2001). Micromilled samples (~20–100 μg) were collected using a computer-controlled X–Y–Z motorized microdrill (Dettman and Lohmann 1995). All carbonate isotopic analyses were performed on a Finnigan MAT 252 mass spectrometer equipped with a Kiel III automated sampling device (Department of Geosciences, University of Arizona). Samples were reacted with 100% orthophosphoric acid at 70°C. Repeated measurement of standard carbonates resulted in standard deviations of 0.08‰. Results are presented in permil notation with respect to the VPDB carbonate standard.

Phylogenetic analysis

We conducted a species-level phylogenetic analysis, focusing on relationships within *Bothrocorbula+Hexacorbula* (see Anderson and Roopnarine 2003). Also included in the ingroup were five representatives of tropical American *Caryocorbula*; the western Pacific *Caryocorbula zelandica* (placed in *Anisocorbula* by a number of workers) and *Notocorbula vicaria*; the eastern Atlantic *Corbula sulcata* and *Bicorbula gallica*; the western Atlantic *Lenticorbula*? *idonea* (placed in *Bicorbula* by previous workers); and the tropical American *Corbula gatunensis* and *C. speciosa* (Table S1). In an analysis combining conchologic and anatomic characters, these genera form a subclade with *Bothrocorbula+Hexacorbula* within the Corbulidae (L. C. Anderson, unpublished data). In addition, a Miocene species from Venezuela (*Hexacorbula*? sp.), with morphologic similarities to both *Hexacorbula* and *Caryocorbula*, was included in the ingroup. The 20 ingroup species represent 13–19% of estimated total species diversity for the ingroup genera (Table S3), although most of this diversity (65–67%) is within *Caryocorbula*. In *Caryocorbula*, species are typically distinguished on the basis of subtle differences in valve shape and size, providing few discrete characters for coding, especially when counfounded by intraspecific and ontogenetic variation. Nevertheless, characters used in the phylogenetic analysis are conservative across individuals within species for the genera examined. Two corbulid species that fall outside the selected ingroup, *Juliacorbula scutata* and *Panamicorbula ventricosa* (Anderson and Roopnarine 2003; Anderson et al. 2006), were designated as outgroup taxa.

Sixty-nine multi-state conchologic characters (of which nine were autapomorphies), describing aspects of external ornament, valve shape, hinge, pallial line and sinus, and adductor muscle scars, were used in the analysis (Supplementay Material: Phylogenetic Analysis). Most characters used had discontinuous character states. For those describing the degree of expression of a trait, only characters with states that we could consistently distinguish because of morphologic gaps were retained. In addition, we coded the largest individuals available for species (assuming these correspond to adult ontogenetic stages) to minimize ontogenetic variability in character states. Phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2002). Characters were unordered and given equal weight, and polarized using the outgroup taxa. Analyses were conducted using branch and bound searches and maximum parsimony. Character state transformations were determined using both accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN). Both gave equivalent results for phylogenetic patterns of growth forms, which are summarized in the results. We calculated Bremer decay indices (Bremer 1988, 1994; Källersjö et al. 1992) and bootstrap values (1000 replicates) (Felsenstein 1988) with the branch and bound algorithm to characterize the robustness of cladogram nodes.

RESULTS

Patterns of corbulid growth

Three distinct patterns of shell development were observed, and the species *Lenticorbula*? *idonea*, *Caryocorbula amethystina*, and *Corbula speciosa* are used as exemplars in the following section.

Lenticorbula? *idonea* exemplifies a mode of development common in heterodont bivalves where valve growth is dominated by commissural accretion of new material in a primarily radial (sagittal) direction (herein referred to as Growth Form 1, [GF1]). The result is a valve that does not substantially alter its direction of growth, or overall shape, during ontogeny. For example, using growth lines as a guide (Fig. 1A), the growth history of a *L.*? *idonea* valve was arbitrarily divided into four intervals (Fig. 2A: T_{1-4}). Rotating and enlarging the cross-section at T_1 so that it can be compared with the shapes at T_{2-4} indicates that its cross-sectional shape did not change substantially as its size increased. This developmental pattern likely accommodates an increasing volume of internal soft-tissues. Isotopic data for the *L.*? *idonea* specimen (Fig. 1, A and B) indicate that the individual was at least 8 years old at its time of death: seven complete $\delta^{18}O$ cycles are present (Fig. 1C: positive peak to positive peak) as is the latter portion of a cycle nearest to the umbo (sample nos. 1 and 2) and the beginning of a cycle at the commissure (sample nos. 35–37).

The second exemplar, *Caryocorbula amethystina*, possesses one of two types of development apparently derived from GF1. Examination of growth lines (Fig. 3A) suggests that this species initially deposited a thin, high shell via radial accretion, followed by an interval of valve thickening. We designate this pattern as Growth Form 2 (GF2). Sampling only the outer shell layer of *C. amethystina* from umbo to commissure (as in *L.*? *idonea*) would likely fail to capture all annual increments, as well as the complete range of $\delta^{18}O$ variability, due to time-averaging in the later portions of shell growth where growth lines are very closely spaced (Goodwin et al.

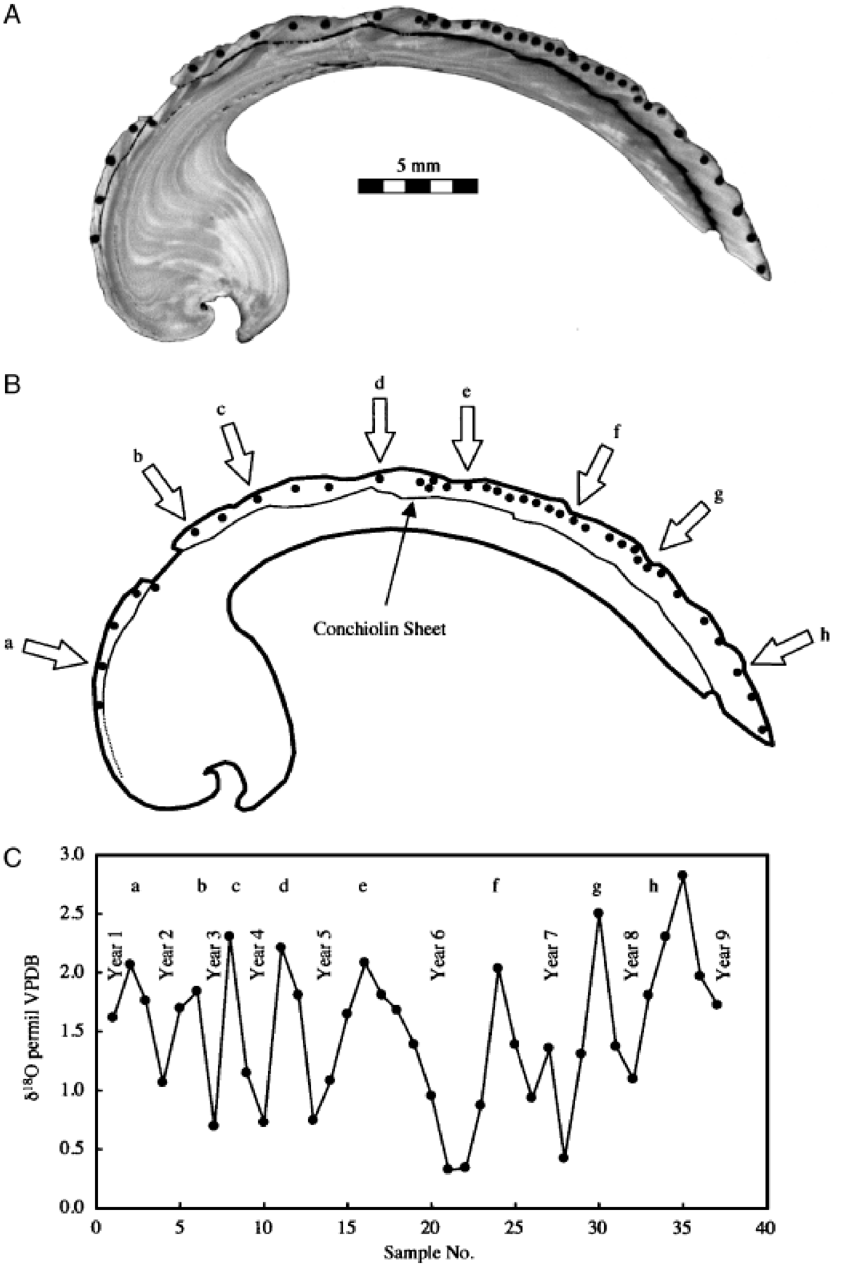

Fig. 1. Cross-sections and isotopic profile from *Lenticorbula*? *idonea*. (A) Photograph of a point-sampled shell. (B) Line drawing of 1A showing the position of 300-μm point-samples. The lettered arrows above the cross section correspond to the labeled peaks in the isotope profile. (C) $\delta^{18}O$ profile. This specimen was ~8 years old at the time of its death.

2003). Therefore, we collected eight point samples from the umbo to the point where shell accretion changes direction (Fig. 3B: filled circles), and micromilled 43 samples from shell material deposited during later ontogenetic stages (Fig. 3B: shaded area). Results from the two sampling techniques were then concatenated to construct a composite record of isotopic variability through ontogeny (Fig. 3C). The pattern of isotopic variation in the composite $\delta^{18}O$ profile suggests this two-phased sampling strategy is a valid approach. That is, the annual $\delta^{18}O$ amplitude attenuation observed in the isotope profile is similar to other modeled and observed profiles in which the complete ontogenetic series is obtained (see Goodwin et al. 2003 for a detailed discussion).

The developmental pattern of *C. amethystina* clearly deviates from GF1. The composite $\delta^{18}O$ profile indicates that this specimen was 3.5 years old at the time of its collection (Fig. 3C). The shell achieved ~80% of its ultimate height in the first year of the clam's life (Fig. 3C: samples 1–8). The subsequent 2.5 years of growth served to thicken the valve, via a ~ 90° shift in the direction of growth from parallel (T_1) to perpendicular to the commissural plane ($T_{2\text{–}4}$). As a result, valve thickness at T_4 is proportionally

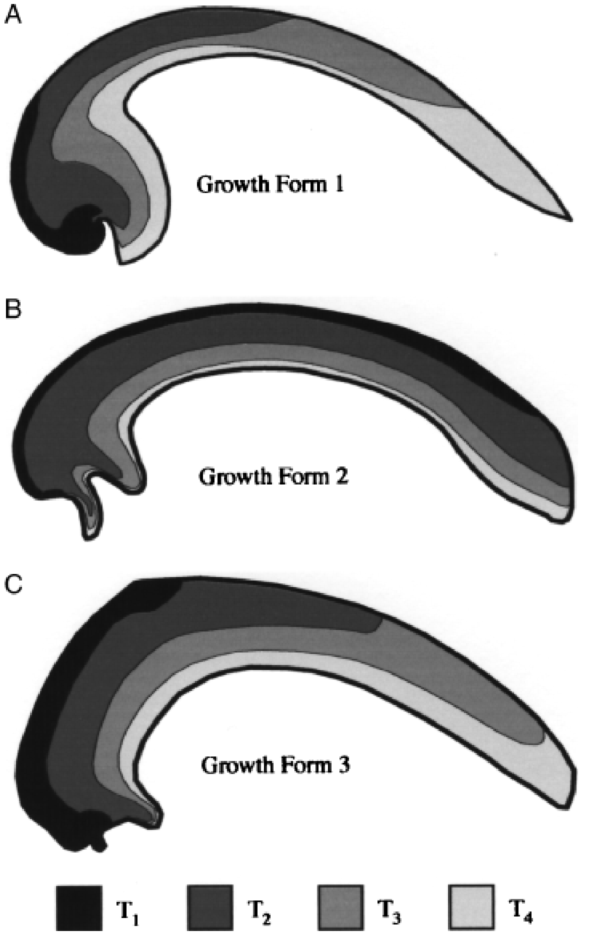

Fig. 2. Schematic illustrations of corbulid growth patterns. (A) Growth Form 1 (i.e., *Lenticorbula*? *idonea*). (B) Growth Form 2 (i.e., *Caryocorbula amethystina*). (C) Growth Form 3 (i.e., *Corbula speciosa*). In each case, development is divided into four stages. The ontogenetically youngest growth material (T_1) is shown in black. Successively older shell material (T_2-T_4) is shown in progressively lighter shades of grey.

greater than after its first year of growth (T_1) (Fig. 2B). In other words, if the size of the shell at T_1 was scaled so that it was the same thickness as the shell at T_4, the *height* of the resulting cross-section would be five times that of T_4. This shift in the direction of shell accretion differs from that commonly seen in other bivalves (e.g., venerids), where shell accretion at the commissure shifts increasingly to a direction perpendicular to the radial late in ontogeny (authors' personal observations), presumably when internal soft-tissue growth has ceased. Instead, thickening is not restricted to the commissural edge in *C. amethystina*, but occurs over the entire internal valve surface.

Valves of the third exemplar, *Corbula speciosa*, exhibit the second type of development derived from GF1 (Growth Form 3, [GF3]). The early portion of the postlarval shell is distinct from the remainder of the valve, thus forming a nepioconch (i.e., the earliest part of the dissoconch demarcated from the remainder by marked changes in growth direction, shape, sculpture, and/or color pattern) (Fig. 4, A and B). This is the only growth form which displays a distinct nepioconch. Isotope samples were micromilled from the trapezoidal shaded region on the cross section (Fig. 4B). Four complete cycles (samples 5–36) as well as two partial cycles (samples 1–5, 36–38) are present on the isotope profile (Fig. 4C), indicating a lifespan of $\sim$5 years. Because samples 1–10 were collected from the nepioconch (Fig. 4B), we can infer that it was accreted during the animal's first year (Fig. 4C). Subsequent growth served to thicken the shell. Unlike GF2, however, where thickening was relatively uniform across the inner valve surface, *C. speciosa* preferentially added material to the ventral margin of the valve. Deposition of this "ventral process" continued throughout the remainder of the animal's life, significantly increasing the inflation and height of the valve. That is, if the cross-section of the nepoiconch is enlarged to the height at T_4, its maximum thickness would be approximately one-half that of the valve at T_4 (Fig. 2C). Furthermore, the ratio of height to inflation is 2.6 for the nepioconch, but decreases to 2.1 at T_4.

Phylogenetic results

Two major clades are identified within the ingroup in our phylogenetic results: one includes *Caryocorbula* spp. (Clade A), the other assigns *Corbula* spp.+*Notocorbula vicaria* as sister to (*Bothrocorbula* spp.+*Hexacorbula*? sp.)+*Hexacorbula* spp. (Clade B) (Fig. 5). The node defining the ingroup is not robust, although it is supported by character states that include modifications to concentric ribs, the presence of a pallial sinus (also seen in *Bicorbula gallica*), adductor muscle scars that are oriented at a low oblique angle to the commissure and are more deeply embedded in the valve's inner surface, and derivations from GF1 (Tables S4 and S5). *Lenticorbula*? *idonia*, and *B. gallica*, which were identified as ingroup taxa in the analysis, form a subclade with one of the two outgroup taxa (*P. ventricosa*), although the topology of this subclade is not robust. The topology of Clade A also is not robust, and only one character state strongly supports (CI ≥ 0.5) the *Caryocorbula* spp. node: ornament on the posterior slope is an extension of the ribs, rather than striae, anterior to the keel.

Nodes within the *Corbula* spp.+*Notocorbula vicaria* subclade of Clade B are relatively well supported, particularly the basal node. A large number of character states define the subclade, including a distinct chondrophore and cardinal tooth/socket, and a strongly inequivalved shell (the latter also is present in *L.*? *idonea* and *B. gallica*). In the other subclade of Clade B, *Bothrocorbula* spp. form a derived clade relative to *Hexacorbula*? sp. and *Hexacorbula* spp. Recall that our phylogenetic analysis was performed specifically

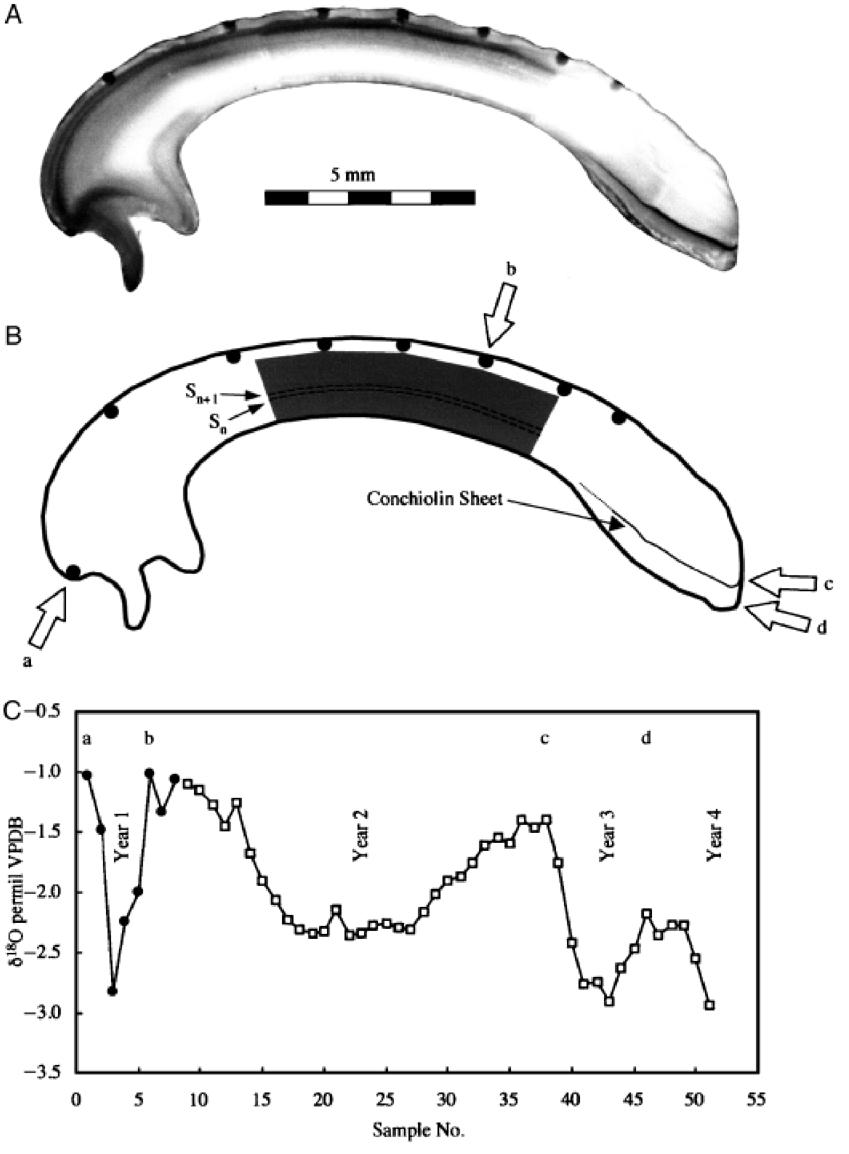

Fig. 3. Cross-sections and isotopic profile from *Caryocorbula amethystina*. (A) Photograph of a sampled shell. (B) Line drawing of 3A. Filled circles mark the position of 300-μm $\delta^{18}O$ samples. The shaded area indicates the micromilled region. S_n and S_{n+1} schematically show the relative position of two micromilled samples. Each micromilled sample was approximately 7 mm long, 20 μm wide, and 225 μm deep. Because micromilling began on the inner surface of the shell and progressed toward the outer surface, samples were collected in reverse ontogenetic order. The sequence of $\delta^{18}O$ values was, therefore, reversed to reconstruct the pattern of isotopic variability. The lettered arrows above the cross section correspond to the labeled peaks in the isotope profile. (C) Composite $\delta^{18}O$ profile. This specimen was ~3.5 years old at the time of its death.

to examine evolutionary relationships within *Bothrocorbula+Hexacorbula*. Our phylogenetic reconstruction indicates, however, that *Hexacorbula* is paraphyletic with respect to *Bothrocorbula+Hexacorbula*? sp. Character states that support the *Bothrocorbula* spp. subclade (CI ≥ 0.5) include modifications to valve shape; the presence of sharp, coarsely beaded radial striae on both valves; and a more robust cardinal tooth. The presence of a lunule, an inferred diagnostic trait of the genus, is instead an apomorphy for *Bothrocorbula* spp.+*Hexacorbula*? sp. Due to low support within basal nodes of this subclade, we chose to limit our coupled sclerochronologic–phylogenetic examination to the well supported *Bothrocorbula* derived subclade (see Evolutionary Mechanisms within Bothrocorbula).

The topology of *Bothrocorbula* agrees well with the stratigraphic occurrences of species within the genus (Fig. S1 and Table S2). The two stratigraphically highest taxa, Pliocene *B. viminea* and Plio-Pleistocene *B. wilcoxii*, form the most derived pair of sister taxa. This pair, in turn, forms a polytomy with the Mio-Pliocene *B.* sp. cf. *B. viminea* and middle Miocene *B. radiatula*. *Bothrocorbula synarmostes* is the most basal and oldest member of the clade (early Miocene). All nodes within this subclade are relatively robust (bootstrap ≥ 75 and/or Bremer ≥ 2) (Fig. 5), and topology relates to further modification of the hinge in response to increased development of the lunular pit, changes in valve shape, and a reduced number of radial striae toward the anterior in both valves.

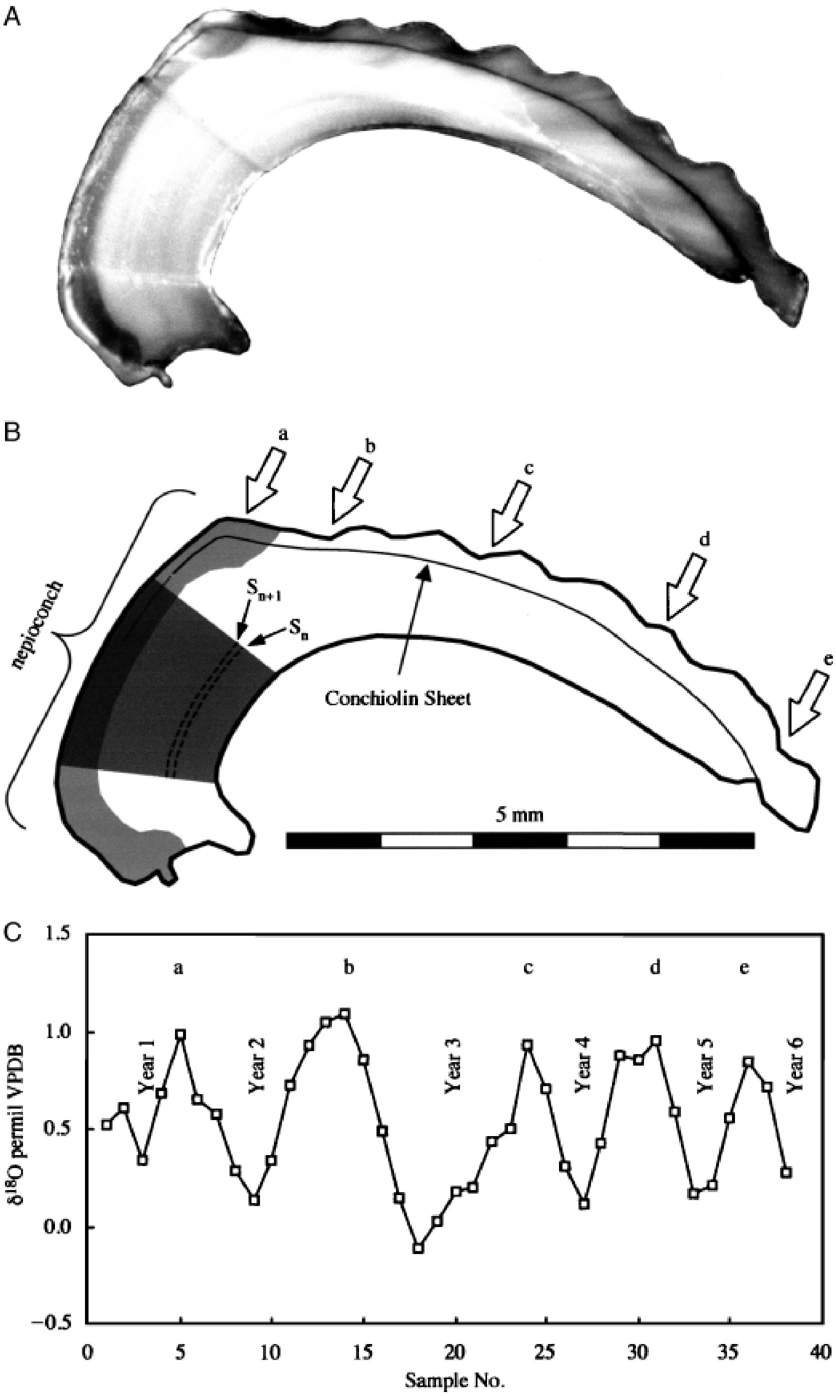

Fig. 4. Cross-sections and isotopic profile from *Corbula speciosa*. (A) Photograph of the micromilled shell. (B) Line drawing of 4A. Micromilled samples, each approximately 2 mm long, 40 μm wide, and 150 μm deep, were collected from the shaded trapezoidal region on the cross section. S_n and S_{n+1} schematically show the relative position of two samples. The lettered arrows above the cross-section mark the approximate position of the positive isotopic peaks observed in the $\delta^{18}O$ profile. (C) $\delta^{18}O$ profile. This specimen was ~5 years old at the time of its death.

DISCUSSION

Phylogenetic analysis of developmental patterns

The two outgroup taxa, *Juliacorbula scutata* and *Panamicorbula ventricosa*, possess GF1 (Fig. 5), with new shell material primarily added radially at the commissure. We infer, therefore, that GF1 is the ancestral character state of the Corbulidae, a reasonable assumption given the ubiquity of this type of accretionary growth in the Heterodonta (authors' personal observations). The ingroup clade is characterized by deviations from GF1 (Fig. 5). For Clade A, the transition is from GF1 to GF2. Two of the taxa in this clade, *Caryocorbula caribaea* and *Caryocorbula barrattiana*, were too small ($\ll$10 mm average shell length) to confidently determine their

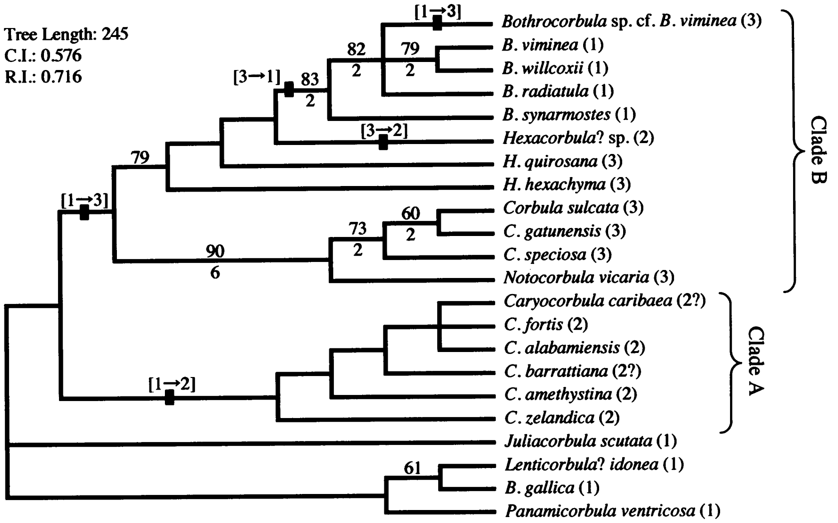

Fig. 5. Strict consensus of six most parsimonious trees, using *Juliacorbula scutata* and *Panamicorbula ventricosa* as outgroup taxa. Bootstrap values and Bremmer Decay Indices are shown above and below individual nodes, respectively. Growth form character state transitions are shown in brackets on nodes, and growth form for each species is indicated in parentheses after each taxon name. See text for discussion.

growth form. Their cross-sectional shape, however, is similar to other *Caryocorbula*, suggesting GF2 characterizes the entire subclade.

The distribution of growth forms in Clade B is more complex (Fig. 5). The basal node of the clade is characterized by a transition from GF1 to GF3. All the taxa in the *Corbula* spp.+*Notocorbula* subclade are characterized by GF3. The two basal-most taxa in the (*Bothrocorbula* spp.+*Hexacorbula*?+*Hexacorbula* spp.) subclade, *H. quirosana* and *H. hexachyma*, also are characterized by GF3. The next most-derived taxon, *Hexacorbula*? sp. is characterized by GF2. This character state transition is somewhat surprising given that it is the only instance where a transition between GF2 and GF3 occurs. Nevertheless, it may reflect an evolutionary propensity of this sublcade to modify patterns of growth. For example, the node at the base of the *Bothrocorbula* spp. clade reverses from GF3 back to the ancestral GF1. Four of the five species in this genus possess GF1. *B.* sp. cf. *B. viminea*, in contrast, possesses a well-defined nepioconch, and is best characterized by GF3. This character state transition therefore represents another reversal, from GF1 to GF3.

Evolutionary mechanisms within Bothrocorbula

In documenting the phylogenetic distribution of growth forms, we focused on *Bothrocorbula* for several reasons. First, the genus is monophyletic and all known *Bothrocorbula* species are included in our phylogenetic analysis (Fig. 5). Second, we have robust support for the phylogenetic topology of this subclade. These phylogenetic criteria are critical to identifying the process(es) responsible for observed evolutionary patterns (Fink 1982). Third, the character reversal at the base of the clade suggests that deviation from GF1 is a synapomorphic character state within the *Bothrocorbula*, whereas GF3 is an apomorphic character state in *B.* sp. cf. *B. viminea*. This pattern is similar to that observed in tropical American corbulids as a whole, where GF1 is symplesiomorphic and deviations are synapomorphic. Thus, evaluation of evolutionary processes in *Bothrocorbula* serves as a model system in which to investigate evolutionary processes that may be operating in the larger clade. Fourth, species of *Bothrocorbula* tend to be relatively large, allowing growth lines to be easily identified under low magnification, and micromilled at high resolution (Goodwin et al. 2003). Finally, geographic and stratigraphic distributions of *Bothrocorbula* span an interval of environmental change in the tropical western Atlantic associated with the emergence of the Isthmus of Panama and the closure of the Panama Seaway (Jackson et al. 1996; Coates et al. 2004). Thus, the potential exists to link evolutionary patterns observed in the fossil record with specific paleoenvironmental changes (e.g., Anderson 2001; O'Dea et al. 2007).

To better understand the evolution of shell development in *Bothrocorbula*, we examined the hypothesis that heterochrony was responsible for generating the distribution of growth forms within the genus. Heterochrony, changes in timing or rate of development through ontogeny from an ancestor to a descendant (de Beer 1958), seems a likely controlling mechanism because the initial phases of both derivative growth patterns resemble that of GF1 (i.e., primary radial accretion) (see Alberch et al. 1979). Failure to support the null hypothesis—i.e., heterochrony did not occur—permits us to suggest that heterochronic processes were responsible for morphologic evolution using the time, size, and shape framework developed by Alberch et al. (1979). Failure to reject the null hypothesis suggests that a mode of ontogenetic modification other than heterochrony (e.g., heterotopy) may have been responsible for the distribution of growth forms in this clade (Webster and Zelditch 2005).

Two criteria must be met to evaluate the role heterochrony plays in the morphologic evolution of a clade (i.e., heterochronic patterns of paedomorphosis vs. peramorphosis), whereas a third, if met, will identify the specific heterochronic *process* responsible for that pattern (see Roopnarine 2001b for discussion). The first criterion requires that a clear hypothesis of phylogenetic relationships within a clade be available so that putative ancestor and descendent taxa can be identified (Fink 1982). For *Bothrocorbula* we have a hypothesis of evolutionary relationship that is robust and with low stratigraphic debt (Fig. 5; Fig. S1). In this hypothesis, *B. synarmostes*, with GF1, is the basal-most and stratigraphically lowest taxon in *Bothrocorbula*, serving as a putative ancestor for other species in the genus.

The second criterion, that ancestors and descendants have coincident morphologic (shape) histories through their respective development (Roopnarine 2001b), also can be demonstrated for *Bothrocorbula* through three lines of evidence. First, conchologic features, with characters describing traits that range from valve ornament to dentition, strongly support the monophyly of *Bothrocorbula* (see also Supporting Information: *Bothrocorbula* species descriptions). Second, combined internal and external landmarks from geometric morphometric analysis indicate that *Bothrocorbula* is distinct morphologically in both shape and size from closely related taxa, including *Hexacorbula*, *Hexacorbula*?, and *Caryocorbula* (Anderson and Roopnarine 2005). Third, and most critical for our data on valve accretion patterns, the growth form present in *B.* sp. cf. *B. viminea* (GF3) recapitulates growth of the ancestral *B. synarmostes* (GF1), as illustrated in Fig. 6. Specifically, the cross-section of a *B.* sp. cf. *B. viminea* nepioconch (Fig. 6A: shaded area; 6B) is remarkably similar to the cross-section of a putative adult *B. synarmostes* (Fig. 6C), with valve heights, thicknesses and inflation virtually identical. Furthermore, both are characterized by strong commarginal ridges on the valve exterior and weak reflection of these ridges on the valve interior. This similarity suggests a peramorphic heterochronic pattern underlying the transition from GF1 to GF3 in *Bothrocorbula* (Alberch et al. 1979).

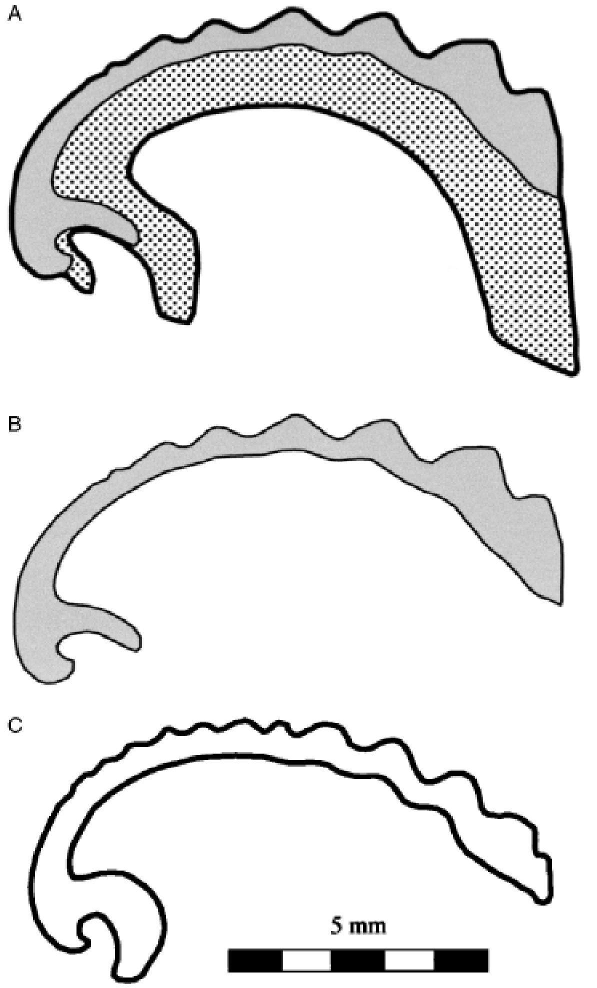

Fig. 6. Cross-sections of two species of *Bothrocorbula*. (A) *B.* sp. cf. *B. viminea*. The shaded area is the nepioconch and the stippled area marks shell material deposited after the nepioconch. (B) Cross-section of the nepioconch from *B.* sp. cf. *B. viminea*. (C) Cross-section of an adult *B. synarmostes*. Note the similar shape of the nepioconch of the *B.* sp. cf. *B. viminea* cross-section (nepoiconch) to that of the adult *B. synarmostes*.

The third criterion, which permits identification of heterochronic *process*, requires reliable estimates of ontogenetic age in order to identify changes in timing or rate of development between ancestor and descendent (Alberch et al. 1979; Jones and Gould 1999; Roopnarine 2001b). We established age estimates through sclerochronologic analysis. Representatives of each of four *Bothrocorbula* species examined were sampled to determine their ontogenetic age (Table S1). (For the fifth *Bothrocorbula* species, *B. viminea*, we determined its growth form but did not section valves due to a paucity of available material.) To establish maximum ontogenetic ages, specimens selected for isotopic analysis were drawn from the largest size class of each species. Micromilled profiles were chosen because they provide more complete records of $\delta^{18}O$ variability, and accordingly more accurate age estimates, than point-sampling approaches (Goodwin et al. 2003). Nevertheless, the point-sampled $\delta^{18}O$ profiles show similar patterns of variation (Fig. S2).

Isotopic variation in *B. synarmostes* indicates that the lifespan of this individual was approximately one year, likely representing shell deposition from late winter/early spring through late fall/early winter (Fig. 7A). The sinusoidal profile of *B. radiatula* $\delta^{18}O$ samples indicates that this individual also lived for approximately 1 year (Fig. 7B). Shell accretion probably began in the late summer and continued through the next year, with death occurring late in the second summer. Similarly, analysis of *B. wilcoxii* suggests these animals lived for approximately 1 year. Shell accretion likely began in the mid- to late summer, continued through the fall, winter and

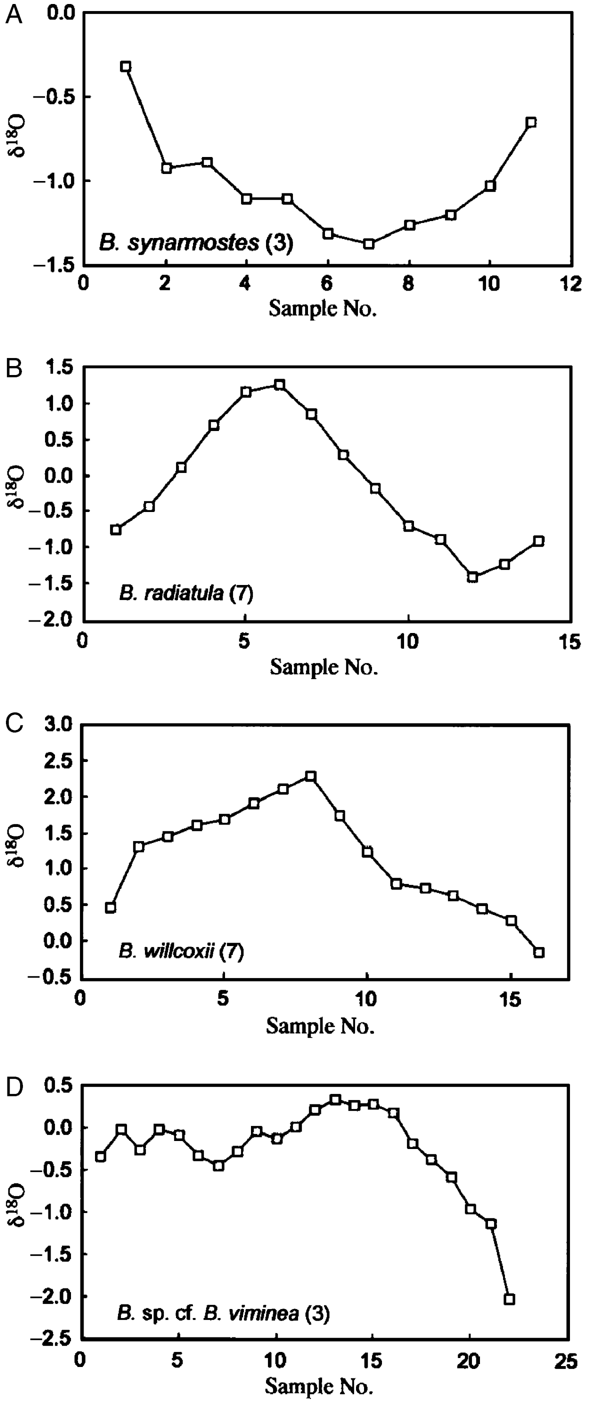

Fig. 7. Micromilled oxygen isotope profiles from specimens representing four species of *Bothrocorbula*. The parenthetic numbers on each graph refer to specimen number. See text for discussion.

spring, and ceased the following summer (Fig. 7C). Oxygen isotope analysis of *B.* sp cf. *B. viminea*, unlike those from the other *Bothrocorbula* species, does not show well-developed enriched peaks (Fig. 7D). Rather, the profile is characterized by relatively constant values at approximately 0‰ (samples 1–16), followed by a rapid decline to approximately – 2‰ (samples 17–22). This suggests rapid growth during the winter months, followed by slower growth in the spring and early summer. Accordingly, this individual lived for slightly less than one year. For this species, the first half of the $\delta^{18}O$ profile represents material sampled from the nepioconch, indicating that the nepioconch is deposited over several months (i.e., less than a year).

In summary, within the *Borthocorbula*, GF1 is a synapomorphic character state, while GF3 is an autapomorphic character state in *B.* sp. cf. *B. viminea* (Fig. 5). The nepioconch of *B.* sp. cf. *B. viminea* is the same size and shape as adult shells of other *Bothrocorbula* species (i.e., peramorphic) (Fig. 6). Finally, *B. synarmostes*, *B. radiatula*, and *B. wilcoxii* deposit their shell in approximately 1 year. In contrast, *B.* sp. cf. *B. viminea*, deposits its nepioconch in approximately half that time (Fig. 7; Fig. S2). These observations indicate that the peramorphic pattern observed is a function of increased growth rates in B. sp. cf. *B. viminea*. In other words, the morphological pattern is accomplished by acceleration (see Alberch et al. 1979).

The evolutionary origin of shell material deposited after the nepioconch in *B.* sp. cf. *B. viminea*, however, remains uncertain. If the GF3 developmental mode evolved independently within the *Bothrocorbula* clade, then this material (Fig. 6A: stippled area) represents a novel morphology. As such, this expansion into new morphospace would be best characterized as a type of heterotopy (see Roopnarine 2001b). If, on the other hand, GF3 is plesiomorphic within the greater corbulid clade, then its expression would be peramorphic by hypermorphosis (Alberch et al. 1979). The latter explanation may be more plausible because transitions between growth types are numerous within the clade, including an identical GF1 to GF3 transition at the node defining Clade B.

Evolutionary implications

Ontogenetic evolution within the Corbulidae is a nexus of contraints and innovations. Whereas a previous study (Anderson and Roopnarine 2005) documented constrained patterns of morphometric allometry throughout the genera *Caryocorbula*, *Bothrocorbula*, and *Hexacorbula*, this study suggests that modes of shell accretion are quite labile. These two phenomena, however, may be related. Specifically, the ubiquity of GF2 within *Caryocorbula* explains the constrained pattern of interspecific allometry present in Neogene Caribbean members of the genus. If valves grow quickly to near-maximum height before internal thickening, the shape of the valve outline parallel to the commissure will vary minimally with age and valve size, especially in fossil assemblages where thicker-shelled specimens could be preferentially preserved. The result is that both the adult valve outline and the positions of internal features are fixed early in postlarval ontogeny, and remain fixed even as the direction of valve accretion changes. This finding is particularly significant because it links patterns of morphological constraint with a specific evolutionary process (heterochrony), and serves as a model for understanding the evolution of morphologic diversity in the clade a whole. More fundamentally, it suggests that patterns of developmental and morphological constraint are not necessarily exclusive or independent of processes which permit or promote evolutionary change.

CONCLUSIONS

The combined sclerochronologic/phylogenetic approach used in this study reveals a wealth of developmental and evolutionary information retrievable from the bivalve shell. Corbulids demonstrate patterns of bivalve shell accretion that are more diverse than generally considered to occur. These patterns include development by primarily radial accretion along the sagittal plane (GF1), and two derivative patterns: one characterized by initial deposition of a thin shell followed by valve thickening with little increase in valve size (GF2), and the other producing a well-defined nepioconch through a marked change in the primary growth direction (GF3). Our phylogenetic results identified two major clades within the ingroup: one includes *Caryocorbula* spp., the other assigns *Corbula* spp.+*Notocorbula vicaria* as sister to (*Bothrocorbula* spp.+*Hexacorbula*? sp.)+*Hexacorbula* spp. The phylogenetic distribution of growth forms suggests that GF1 is the ancestral character state, while the two ingroup clades are characterized by deviations from the ancestral state. The results of our investigation of *Bothrocorbula* indicate that this diversity is, in part, produced by heterochronic modifications of development via peramorphosis. Specifically, the nepiochonch of *B.* sp. cf. *B. viminea* is a product of acceleration, and its post-nepionic development is a product of hypermorphosis. Documentation of various growth forms may also help explain previously recognized patterns of morphological constraint in tropical American corbulids. Finally, these growth forms apparently are widespread throughout the clade, and are present in both fossil (e.g., *Caestocorbula costata*; Wrigley 1946) and modern (e.g., *Solidicorbula*) taxa. Furthermore, multiphased patterns of shell accretion have also been observed in the Veneridae (e.g., *Callocardia nitidula*; Wrigley 1946). Together, these observations suggest that complex patterns of valve accretion are relatively common in heterodont bivalves. This study, therefore, serves as a model for understanding the evolution of disparity in this diverse and long-lived taxon.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Bothrocorbula *species descriptions*

All *Bothrocorbula* are roughly ovate with a pointed posterior and strong, rounded commarginal ribs. Species are primarily distinguishable based on size, expression of the lunular pit, subtle differences in valve shape, shape of the commissural margin, and distribution of radial ribs.

Bothrocorbula synarmostes (Supplemental Fig. S3) (Dall 1898) was collected from the Lower Miocene (Burdigalian) Chiploa Formation, the only known stratigraphic occurrence of this species. *Bothrocorbula synarmostes* valves have a diamond-shaped outline, with the deepest part of the ventral margin at the same point along the antero-posterior axis as the umbo. This species also possesses a distinct lunular area with only a slight depression opening toward the anterior; radial ribs that decrease in expression toward the anteroventral margin; a concave left-valve commissure; and an undulating right-valve commissure. Mature shells are relatively small (shell height approximately 10 mm).

Bothrocorbula radiatula (Supplemental Fig. S4) (Dall 1898) is know only from the Oak Grove Sand of the Alum Bluff Group, which is stratigraphically above the Chipola Formation. Randazzo and Jones (1997) date Oak Grove Sand deposition from the Burdigalian to Langhian, whereas Ward (1998) restricts it to the lower middle Miocene (Langhian). *Bothrocorbula radiatula* is the smallest of the *Bothrocorbula* species, typically with a maximum height <10 mm. Valves of *B. radiatula* are diamond-shaped; radial ribs are evenly spaced across valve surfaces; the lunular depression is an anteriorly open, moderately deep pit; the left-valve commissure is planar; and the right-valve commissure is undulating.

Bothrocorbula sp. cf. *B. viminea* (Supplemental Fig. S5) (Guppy, 1866) first appears in the Baitoa Formation of the Dominican Republic, which was dated as later early or early middle Miocene (Budigalian or Langhian) using molluscan assemblages (Saunders et al. 1986). This species ranges through the Miocene to the Lower Pliocene (Zanchlean) in the Dominican Republic, and the shells used in this study were collected from the Upper Miocene Cercado Formation (Saunders et al. 1986). As in *B. synarmostes* and *B. radiatula*, *B.* sp. cf. *B. viminea* has a diamond-shaped valve outline. *Bothrocorbula* sp. cf. *B. viminea* is characterized by a well developed nepioconch; a deep U-shaped lunular pit; strong, beaded radial ribs that are evenly spaced across the surface of both valves; a left-valve commissure that has a distinct stepped transition between a deeper posterior than anterior; and a planar right-valve commissure.

Bothrocorbula willcoxii (Supplemental Fig. S6) (Dall 1898), which first appeared in the in the middle Pliocene (Piacenzian) and ranges through the middle Pleistocene, is known from the Pinecrest sand (Tamiami Formation), the Caloosahatchee Formation, and the “Bermont Formation” of Florida. The material examined here was collected from the middle Pliocene Pinecrest sand. *Bothrocorbula wilcoxii* valves are ovate with a deep antero-ventral margin; and posses a deep U-shaped pit in the lunular area, radial ribs that decrease in number and expression toward the anterior of both valves, a concave left valve commissure, and a planar right-valve commissural margin. *Bothrocorbula viminea* (Supplemental Fig. S7) from the middle Pliocene (Piacenzian) Bowden Formation of Jamaica (Aubry 1993), is very similar morphologically to *B. wilcoxii*, differing in having a less produced anterior margin and a gently undulating right-valve commissure.

Diagenetic Screening

The presence/absence of diagenetic alteration was thoroughly tested using a combination of cathodoluminescence (CL) microscopy, x-ray diffraction (XRD) analysis, and scanning electron microscopy (SEM). These techniques are applied in concert because (1) CL identifies the presence of diagenetically introduced elements such as Mn^{2+}, Pb^{2+}, and Fe^{2+}, which cause luminescence (see Bathurst 1975; Flügel 2004; and references therein); (2) XRD analysis detects recrystallization of skeletal carbonate from aragonite to calcite; and (3) SEM makes evident any neomorphism of skeletal aragonite to sparry aragonite through distortion or destruction of original microstructures (Bathurst 1975).

For CL, we examined polished thick-sections using a Technosyn Cold Cathode Luminescence Model 8200 Mk II microscope (School of Earth Sciences, The Ohio State University). For XRD analyses, powdered samples were x-rayed using a Siemens (Bruker) D5000 theta/two theta diffractometer with solid state Si(Li) detector (Department of Geology and Geophysics, Louisiana State University). Sample spinning was used to perform the analyses. The Cu-target tube was operated at 40 KV and 30 ma. Samples were step-scanned with 0.02 degrees two theta increments and counting times of 5.0 seconds over the interval from 24–36 degrees two theta. For SEM, specimens were broken and fresh, fractured surfaces were examined using a FEI (formerly Philips) Quanta 200 Environmental SEM (Department of Geology & Environmental Science, University of Akron).

Diagenetic screening of fossil specimens confirmed a lack of secondary alteration and we can infer that all geochemical profiles represent original isotopic (environmental) variation. In all cases, the CL failed to produce luminescence, suggesting that diagenetically introduced activator elements are not present in our specimens. In addition, XRD analysis indicates that fossil shells are composed of $\sim 100\%$ aragonite. Finally, SEM images reveal exquisitely preserved skeletal microstructure (Supplemental Fig. S8). Supplemental Fig. S8A shows valve interior and fractured surfaces of the dorso-anterior quadrant of a specimen of *B. radiatula.* Two shell layers are separated by the trace of main conchiolin layer. Lewy and Samtleben (1979) note that decomposition of the main conchiolin layer causes the shell to weaken and separate along its trace. Supplemental Fig. S8B is a close up of the boxed region in Supplemental Fig. S8A. The surface marking the position of the main conchiolin layer is visible as a step perpendicular to the fractured surfaces. Complex(?) crossed-lamellar microstructure, characteristic of the Corbulidae (Taylor et al. 1973), is clearly visible on the fresh, fractured surfaces. Supplemental Fig. S8C shows the inner and outer shell layers of a fractured specimen of *B.* sp. cf. *B. viminea.* The inner shell layer (foreground) is separated from the outer shell layer by the trace of the main conchiolin sheet. The internal surface of the valve is also visible on inner shell layer. Supplemental Fig. S8D shows complex(?) crossed-lamellar microstructure preserved in the outer shell layer from the same specimen. Supplemental Figs S8E and S8F show complex(?) crossed-lamellar microstructure preserved in *B. synarmostes* and *B. wilcoxii*, respectively.

Phylogenetic Analysis

Character state descriptions and the character state matrix are provided in Supplementary Tables S4 and S5, respectively. Because corbulids are inequivalved, any trait that differed between valves in at least one taxon was coded as two characters, one for left and a second for right valves. If that trait was constant in one valve and varied in the other, only the character with variable states was included. When we used more than one character to describe a trait, we paid particular attention to coding taxa that lacked that trait in one or both valves. For example, a few taxa lack commarginal ribs on one or both valves (e.g. *Panamicorbula*). For these taxa we coded their character state as "ribs absent" for the left and/or right valve (Characters 2 and 3, which describe the shape and distribution of ribs on the left and right valves), respectively. For other characters related to commarginal ribs (Characters 1, 4, and 5), we coded the absence of ribs as missing (that is, as '?') to avoid building redundancy into the matrix through coding the same character multiple times (see also Mikkelsen 1998; Strong and Lipscomb 1999). Nonetheless, $<1\%$ of character states were coded as missing in the matrix (15 of 1518). Of these missing states, 8 were coded to counteract redundancy (all missing states in *Panamicorbula ventricosa* and *Bicorbula gallica*, and 2 or 3 states in *Caryocorbula amethystina*). The remaining seven characters were coded as missing because available material was either insufficient, too poorly preserved, or individuals were too small (i.e., it was not possible to observe growth lines in the two smallest *Caryocorbula* species) to allow character states to be coded with confidence.

Table S1. Species of Corbulidae examined for patterns of shell accretion (Growth Form), with the numbers of specimens that were examined externally (External), cut and thick sectioned (Sectioned), and/or sampled for isotopic analysis (Isotope) also indicated. For *Caryocorbula barrattiana* and *C. caribaea*, specimens were too small to identify growth form will full confidence.

Table S2. Species for which schlerochronologic data were calibrated to ontogenetic ages using δ^{18}O profiles. The first three taxa serve as exemplars of the growth forms observed in the Corbulidae surveyed. The remaining taxa are known species of *Bothrocorbula* for which we had sufficient material to sample destructively. Total age range for each taxon is provided (Age Range), as is locality (Locality) and stratigraphic unit (Unit; fossil material only) from which the specimens were collected.

Table S3. Estimated species diversity of ingroup taxa. Diversity estimates for genera based on taxonomic evaluations of validly described species. Estimated diversity minima represent counts of species confidently placed by the second author in each respective genus. Estimated diversity maxima also incorporate species that are placed in each genus, however the validity of these assignments is less certain because type material has not been examined by the second author.

Table S4. Character state descriptions for characters used in the phylogenetic analysis.

Table S5. Character matrix for phylogenetic analysis of *Bothrocorbula* clade.

Fig. S1. Phylogenetic relationships and stratigraphic ranges of *Bothrocorbula*. Note that the topology of the *Bothrocorbula* clade is identical to that on Fig. 5. An inferred range extension for *B. radiatula* is indicated in lighter grey with a "?".

Fig. S2. Point-sampled δ^{18}O profiles from specimens representing four species of *Bothrocorbula*. The parenthetic numbers on each graph refer to specimen number. Note that these patterns of isotopic variation are similar to the micromilled profiles. See text for further discussion.

Fig. S3. External and internal views of *Bothrocorbula synarmostes*. The left valve is shown on the left side of the figure, and the right valve is to the right. Scale bars in centimeters.

Fig. S4. External and internal views of *Bothrocorbula radiatula*. The left valve is shown on the left side of the figure, and the right valve is to the right. Scale bars in centimeters.

Fig. S5. External and internal views of *Bothrocorbula* sp. cf. *B. viminea*. The left valve is shown on the left side of the figure, and the right valve is to the right. Scale bars in centimeters.

Fig. S6. External and internal views of *Bothrocorbula wilcoxii*. The left valve is shown on the left side of the figure, and the right valve is to the right. Scale bars in centimeters.

Fig. S7. External and internal views of *Bothrocorbula viminea*. The left valve is shown on the left side of the figure, and the right valve is to the right. Scale bars in centimeters.

Fig. S8. Environmental scanning electron micrographs of fresh fractured, cross sectional surfaces from representatives of *Bothrocorbula*. A) Dorso-anterior quadrant of *Bothrocorbula radiatula*. B) Close up of highlighted region in A. Note the presence of well-preserved complex(?) crossed-lamellar microstructure characteristic of the Corbilidae. The trace of a conchiolin sheet (not preserved) is visible as a step (down to the left) perpendicular to the fractured surfaces. C) *Bothrocorbula* sp. cf. *B. viminea*. Inner (foreground) and outer (background) shell layers separated by the trace of a conchiolin sheet (not preserved). The interior surface of the valve is visible on the inner shell layer. D) Close up of the highlighted region in C. As in B, note the well-preserved complex(?) crossed-lamellar microstructure. E) and F) Complex(?) crossed-lamellar microstructure form *Bothrocorbula synarmostes* and *Bothrocorbula wilcoxii*, respectively.