# PATTERNS OF MORPHOLOGICAL DIVERSITY AMONG AND WITHIN ARCID BIVALVE SPECIES PAIRS SEPARATED BY THE ISTHMUS OF PANAMA

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ABSTRACT—Geminate species are morphologically similar sister-species found on either side of the Isthmus of Panama. The existence of all geminates in the tropical Eastern Pacific ocean and the Caribbean Sea is most often explained by vicariance: closure of the Central American Seaway 3.1 to 3.5 Ma simultaneously isolated populations of species with amphi-American distributions. In this paper, we test the potential of morphological measurements for discriminating between Recent geminate species pairs from three genera (*Arca, Arcopsis,* and *Burbatia*) in the bivalve family Arcidae and examine the prospects for distinguishing nominal species in the fossil record. Fourteen morphological variables were used to characterize shell shape and multivariate methods were used to discriminate between five Recent species pairs. Collection sites were also used as *a priori* groups for discrimination to describe patterns of intraspecific morphological variation and to evaluate differences among samples from different geographic regions.

On average, 84 percent of specimens within geminate pairs are classified correctly following five separate discriminant analyses with nominal species as the grouping variable. Although all but one arcid species pair are discriminated with high statistical significance, some collection sites within species are highly morphologically distinct. Overall, a large proportion of specimens from each collection locality (79 percent on average) can be classified correctly to site although no single site possessed a multivariate centroid that was significantly different from all other conspecific centroids. The distinctiveness of some collection sites, however, raises the possibility that some nominal species may harbor cryptic species, indicating the need for wider geographic surveys of both molecular and morphological variation within geminate species pairs.

The eigenvalue coefficients derived from the Recent samples of one geminate pair (*Arca mutabilis* and *A. imbricata*) were used to assess the potential for identifying arcid species in the fossil record. Discriminant analyses of fossil *Arca* indicate that the forms that characterize Recent *A. mutabilis* and *A. imbricata* are present in the fossil record as far back as the Late Early Miocene, in the Cantaure Formation of Venezuela. Because a deep water connection between the Eastern Pacific and Western Atlantic existed until the Middle Miocene, the morphological differences associated with Recent *A. mutabilis* and *A. imbricata* likely existed well before the rising Isthmus affected ocean circulation patterns in tropical America. Therefore, despite great overall morphological similarity, these putative geminate species likely have a time of divergence that is at least four times older than final seaway closure. The geographic distribution of fossils also suggests that morphological forms associated with each Recent species had amphi-American distributions both before and after isthmus formation but are now geographically restricted to either side of the isthmus in the Recent fauna.

## INTRODUCTION

UNDERSTANDING THE relative timing of environmental change and species diversification presents a challenge for developing hypotheses regarding the processes that generate biological diversity in the world's oceans. The rise of the Isthmus of Panama and the environmental changes associated with the closure of the Central American seaway provide a focal point for recent studies characterizing patterns of speciation, extinction, and morphological evolution in the context of tectonic, climatic, and oceanographic change over the last 20 Ma (e.g., Gunther, 1868; Rosenblatt, 1963; Vermeij, 1978; Stanley, 1986; Lessios, 1981, 1998; Cronin, 1985; Vermeij and Petuch, 1986; Duque-Caro, 1990; Lessios and Cunningham, 1990; Knowlton et al., 1993; Cronin and Dowsett, 1996; Budd et al., 1996; Jackson and Cheetham, 1999; Jackson et al., 1996, 1999; Roopnarine, 1996a; Knowlton and Weigt, 1998; Collins and Coates, 1999; Jackson and Johnson, 2000). "Geminate" species pairs (Jordan, 1908), in which one species inhabits the eastern Pacific (EP) and the other the Western Atlantic (WA), have served as pivotal examples of the role that geographic barriers and the disruption of gene flow play in the process of allopatric speciation (Mayr, 1954, 1963; Woodring, 1966; Olsson, 1972; Vermeij, 1978; Knowlton et al., 1993; Bermingham et al., 1997; Knowlton and Weigt, 1998; Lessios, 1998). Geminate pairs are hypothesized to have descended from single biological species that were broadly distributed throughout the tropical EP and WA prior to the closure of the Central American

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seaway (Mayr, 1954; Jones, 1972; Humphries and Parenti, 1986). Because the timing of final seaway closure is so well characterized in the geological record, geminate species provide both a useful and unusual paleobiological system for evolutionary study. Complete cessation of water exchange between the WA and EP occurred 3.5 to 3.1 Ma (Keigwin, 1982; Duque-Caro, 1990), setting a lower limit on the amount of time that geminate pairs have been physically isolated.

Although final seaway closure is well constrained in the geological record, the divergence times of living geminates are typically assumed to fall within the range of dates ascribed to final seaway closure (for a review see T. M. Collins, 1996). Recent molecular and paleontological data, however, strongly suggest that the isolation of the faunas of the EP and WA was a protracted rather than abrupt event (Coates et al., 1992; Jackson et al., 1993, 1996; Knowlton et al., 1993; Coates and Obando, 1996; L. S. Collins et al., 1996; T. M. Collins, 1996; Knowlton and Weigt, 1998). These data call into question the assumption that morphologically similar taxa found on either side of the isthmus were necessarily isolated at the time of final seaway closure. Most notably, varying amounts of DNA sequence and protein divergence among putative geminate pairs suggest that some pairs were formed before water flow between the WA and EP was disrupted by the rising Isthmus (for reviews see T. M. Collins, 1996; Lessios, 1998). Although molecular sequences demonstrate that calibration of the molecular clock with geminate species may result in overestimates of the rate of molecular evolution (e.g., Knowlton and Weigt, 1998) other hypotheses can potentially explain why molecular divergence varies among putative geminate pairs (T. M. Collins, 1996). Even though some of these hypotheses can

be addressed with neontological data (e.g., Bermingham and Lessios, 1993; Knowlton and Weigt, 1998), the most direct evidence available for determining the timing of divergences between EP and WA marine species resides in the fossil record.

Collection of paleontological data regarding the timing of morphological divergence of geminate species has lagged behind the molecular data. Surprisingly few geminate species have been studied from a morphological perspective, even in the Recent (but see Mayr et al., 1953; Rubinoff, 1963; Lessios, 1981; Weinberg and Starczak, 1989; Lessios and Weinberg, 1994; Schneider, 1995). The most difficult problem that must be solved by any paleontological study of speciation is the recognition of the morphological boundaries of genetically distinct and presumably reproductively isolated species. Cryptic species (see Knowlton, 1993), discrete polymorphisms (e.g., Palmer, 1985), phenotypic plasticity (e.g., Trussell and Smith, 2000), and geographic variation can all easily confound interpretations of morphological changes among stratigraphic samples (Smith, 1994; Jablonski, 2000). Fossilizable taxa with living representatives, however, hold great promise for paleontological studies of speciation, because of the potential to characterize patterns of intraspecific morphological variation in extant species prior to interpretation of morphological change in the fossil record (e.g., Stanley and Yang, 1987; Michaux, 1989; Jackson and Cheetham, 1994; Roopnarine, 1996b). Therefore, by identifying traits, in the context of intraspecific geographic variation, that morphologically discriminate extant species currently isolated by the isthmus, a morphological approach can be used to determine at what point both forms associated with Recent geminates first appear in the fossil record. Failure to identify both geminate species in the fossil record, however, does not necessarily allow rejection of the hypothesis of pre-isthmian speciation because morphological evolution may not necessarily be coupled with reproductive isolation. Nevertheless, identification of both species in pre-isthmian fossil deposits would provide evidence that geminates diverged before isolation of the EP and WA oceans.

A second problem for paleontological studies of speciation is that sister-taxa are typically morphologically similar and can be difficult to distinguish with morphological characters alone (e.g., Murphy, 1978; Mastro and Hedgecock, 1982; Palmer et al., 1990). Because a preliminary survey of Recent shell material indicated that no discrete morphological differences existed between any arcid geminate pairs, we opted to use a multivariate morphometric approach to describe arcid shell shape. Multivariate methods have proven indispensable in providing taxonomic resolution at the species level, particularly when coupled with some biological information about the specimens of interest (e.g., Stanley and Yang, 1987; Michaux, 1989; Budd et al., 1994; Jackson and Cheetham, 1994; Kowalewski et al., 1997).

In this paper, we use multivariate morphometrics to describe geographic patterns of shell shape variation in five geminate pairs in the bivalve family Arcidae (Figs. 1, 2). Seven geminate pairs of tropical American Arcidae have been recognized (by all authors below) although none have been subjected to morphometric, phylogenetic, or population genetic analyses (Olsson, 1961; Abbott, 1974; Keen, 1974; Vermeij, 1978). The majority (six) lives attached by a byssus to hard substrata, although all live in cryptic microhabitats (Olsson, 1961; Brusca, 1980). We limited our morphological survey to five geminate pairs for which we have collected 586 base pairs of the mitochondrial gene cytochrome c oxidase I from 26 Recent specimens (GenBank accession numbers: AF253475-94 and AF345641-47). Phylogenetic trees, rooted with either *Mytilus trossulus* or the infaunal arcid *Anadara grandis* and constructed with both parsimony and maximum likelihood

methods, indicate that all five of the nominal geminate pairs considered here are sister-taxa with respect to other living taxa sampled (Marko, personal commun.). The molecular phylogenetic analysis will be the subject of a forthcoming paper. Our primary goals are to characterize patterns of morphological variation within and between geminate arks and evaluate the utility of shell shape for distinguishing between Recent geminate species. After describing patterns of intra- and interspecific morphological variation in shell morphology, we then investigate the potential for discriminating geminate species in the fossil record for one geminate pair for which we have a small sample of fossils.

## MATERIAL

The 10 Recent species considered in this study (Figs. 1, 2) are all members of the bivalve family Arcidae (see Abbott, 1974; Keen, 1974). Collections of Recent specimens (Table 1) are a combination of personal collections made by PBM in Panama between September 1997 to March 1999, collections housed at the Naturhistorisches Museum of Basel (NMB), collections from the Natural History Museum of Los Angeles County (LACM), and the Smithsonian Institution (USNM). Recent NMB arcid collections focus on the eastern Caribbean, LACM material is from Mexico and the Galapagos, and PBM's collections focus on both coasts of Panama. Fossil *Arca* specimens were obtained from NMB/PPP collections (Table 2). An additional geminate pair (*A. zebra* and *A. pacifica*) not considered here which is likely the sister group to the *A. mutabilis*/*A. imbricata* pair, is easily distinguished by 1) the number of grooves on the cardinal surface of the shell (fewer than five grooves between the shell apex and the posterior end of the hinge plate in *A. imbricata* and *A. mutabilis*); and 2) the absence of cancellate sculpture (absent in *A. zebra* and *A. pacifica*) as well as several other morphological characters (see Olsson, 1961).

## METHODS

*Morphological variables measured.*—All shells were photographed using a Polaroid digital video camera attached to either a 55 mm macro lens or a dissecting microscope, depending on the size of the specimen. Images were saved to computer files and measured with ObjectImage (ver. 1.62) for Macintosh, a version of NIH Image modified for morphometrics (available from N. Vischer at http://simon.bio.uva.nl/object-image.html).

Fourteen morphometric variables were used to characterize shell shape (Fig. 3). Most variables consisted of linear measurements and angles, several derived from Stanley and Yang (1987). Other variables were chosen because they described unique aspects of arcid shell shape. Because one goal of this study was to compare patterns among geminate pairs, selection of morphological variables was limited to those that could be measured in all 10 species in the three genera studied. Consequently, we could not use other characters including ornamentation, rib dimensions, and characters of the shell margin that are unique to single genera and would probably increase our ability to discriminate species. The right valve was arbitrarily chosen for all measurements. We could not apply more powerful landmark-based methods (see Strauss and Bookstein, 1982; Bookstein, 1990, 1991; Budd et al., 1994) because we found that few landmarks can be reliably identified from specimen to specimen in arcids (i.e., unambiguously homologous sites).

*Morphometric analyses.*—The morphological variables measured on arcid shells are best analyzed using multivariate methods (see Reyment, 1990). Prior to analysis all data were log-transformed, and principle component analysis (PCA) was used first to explore Recent arcid shell morphospace. We next used discriminant analysis (DA) on Recent shell material. To remove size and
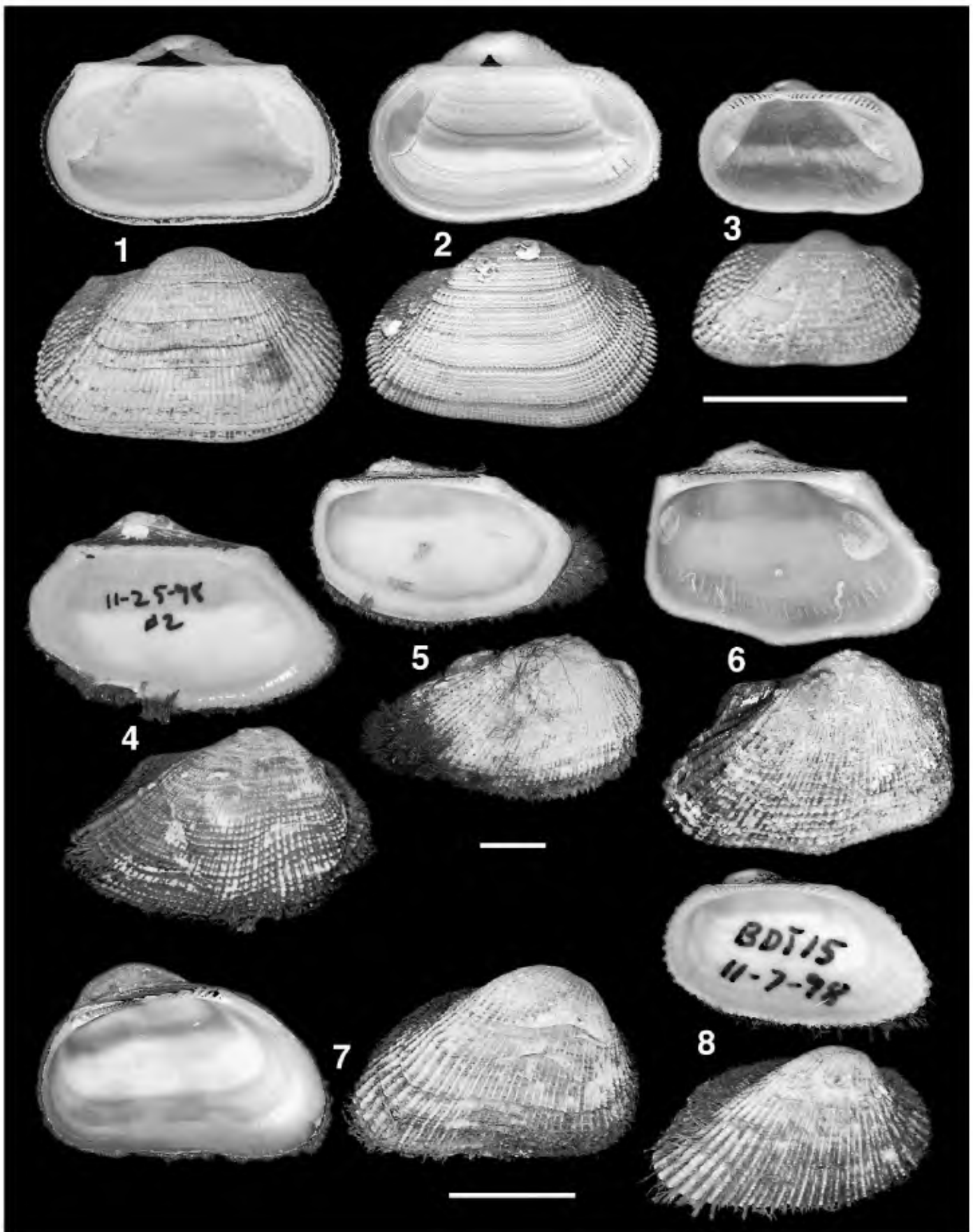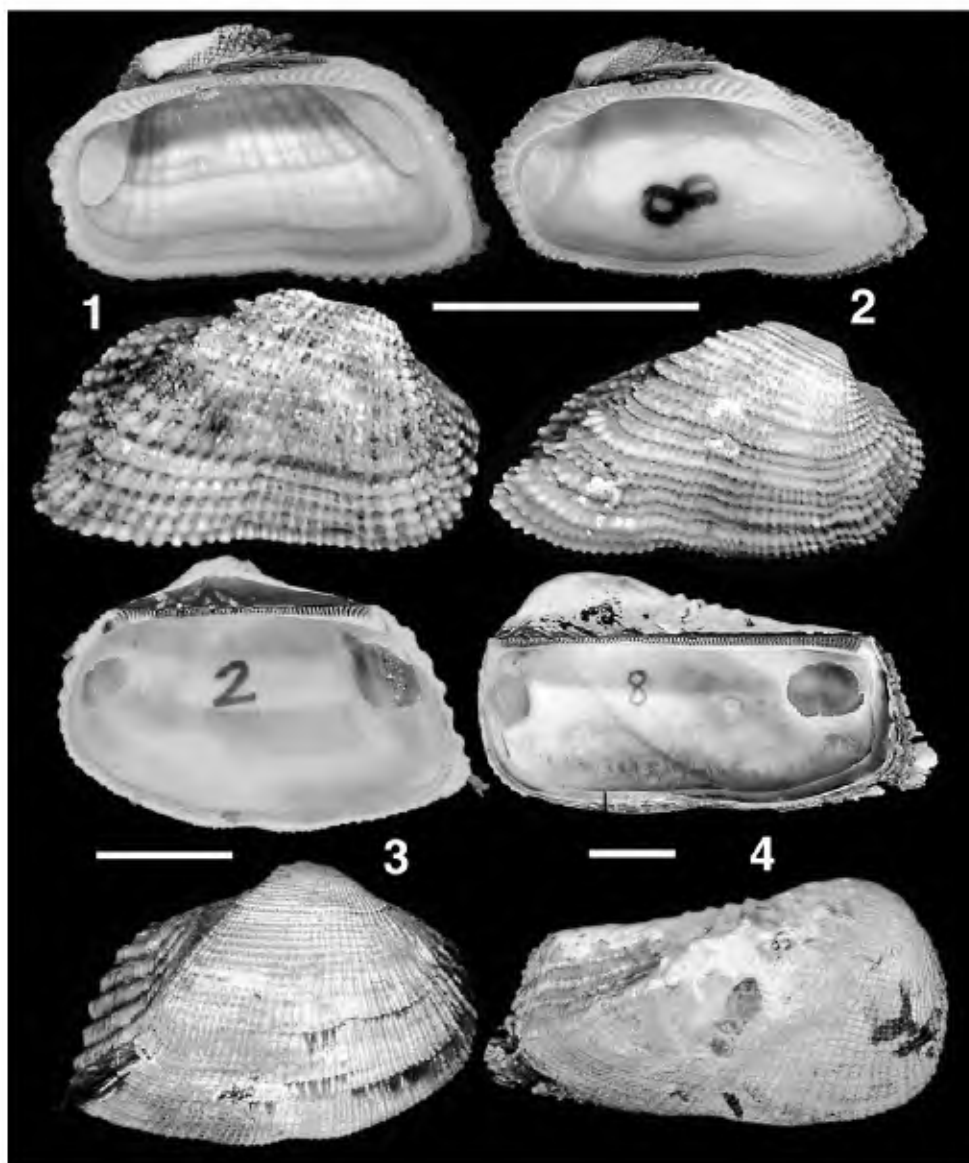
FIGURE 2—Shells of Recent geminate species in the bivalve family Arcidae. *1, Barbatia graduta,* Venado; *2, B. domingensis,* Bocas; *3, Arca mutabilis,* Rey; *4, A. imbricata,* Frio. Scale bars = 1 cm.

size related variation in shell shape, prior to each DA we zero-centered groups and regressed all observations on PC1, which described variation in shell size for all five geminate pairs (see Results). DAs were then conducted on the residuals obtained from each regression analysis (see Reis et al., 1990; Hutcheson et al., 1995; Kowalewski et al., 1997; also see Roopnarine, 1996b).

We first used the nominal species (found on either side of the isthmus) as the *a priori* groups in five separate two-group DAs. We then conducted a second set of DAs (with canonical analyses) in which collection sites were the *a priori* groups. Because each

species pair had a minimum of eight collection sites (which generates seven canonical variate axes in a DA) we present only exemplary plots of canonical variate (CV) axes scores for some geminate pairs. The use of collection site as a grouping variable allowed us to assess differences among different geographic regions and to compare the extent of geographic variation to transisthmian morphological differences. Significance of each discriminant function as whole was tested with F-tests for Wilks' λ. Squared Mahalanobis distances between group centroids were also calculated and F-tests (MANOVA) were used to determine

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FIGURE *1*—Shells of Recent geminate species in the bivalve family Arcidae used in this study. Both the interior and exterior view of the right valve of each specimen is photographed. Locality names are defined in Table 1. *1, Arcopsis solida,* Baja California-1; *2, A. udamsi,* Borburata; *3, A. adamsi,* Federal; *4, Barbatia reeveana,* Venado; *5, B. candida,* Bocas; *6, B. candida,* Federal; *7, B. illota,* Taboga; *8, B. tenera,* Bocas. Scale bars = 1 cm.

TABLE 1—Collection sites for Recent geminate arcid bivalves species. See Table 4 for sample sizes.

| | Site | Collection and lot number[1] | Name used in text/figures | Collector[2] |
|---|---|---|---|---|
| **I. Eastern Pacific** | | | | |
| *Arcopsis solida* | 1) Isla Venado, Panama City, Rep. of Panama | PBM | Venado | PBM |
| | 2) Bahia Santa Maria, Baja California, Mexico | LACM 67-68 | Baja-1 | JM |
| | 3) Isla Santa Margarita, Baja California, Mexico | LACM 66-8 | Baja-2 | JM |
| *Barbatia reeveana* | 1) Isla Pedro Gonzalez, Islas Perlas, Rep. of Panama | PBM | Gonzalez | PBM |
| | 2) Isla Venado, Panama City, Rep. of Panama | PBM | Venado | PBM |
| | 3) Isla Cedros, Baja California | LACM 71-151, 71-92 | Baja | JM |
| | 4) Isla Pinta, Galapagos Islands | LACM 84-41 | Galapagos | JM |
| *Barbatia illota* | 1) Isla Taboga, Panama City, Rep. of Panama | USNM 204107 | Taboga | Unknown |
| | 2) Isla Perico, Panama City, Rep. of Panama | PBM | Perico | PBM |
| | 3) Isla Venado, Panama City, Rep. of Panama | PBM | Venado | PBM |
| | 4) Bahia Cuastocomate, Jalisco, Mexico | LACM 68-41 | Jalisco | JM, PO |
| | 5) Puerto Penasco, Sonora, Mexico | LACM 63-56, 63-10 | Sonora | JM |
| *Arca mutabilis* | 1) Isla Del Rey, Islas Perlas, Rep. of Panama | PBM | Rey | PBM |
| | 2) Isla Perico, Panama City, Rep. of Panama | PBM | Perico | PBM |
| | 3) Isla Venado, Panama City, Rep. of Panama | PBM | Venado | PBM |
| | 4) Isla San Jose, Islas Perlas, Rep. of Panama | PBM | Jose | PBM |
| | 5) Isla Taboga, Panama City, Rep. of Panama | PBM | Taboga | PBM |
| | 6) Bahia Banderas, Jalisco, Mexico | LACM 68-18 | Jalisco | PO |
| | 7) Isla Isabel, Baja California | LACM 37-152 | Baja | Unknown |
| *Barbatia gradata* | 1) Isla Del Rey, Islas Perlas, Rep. of Panama | PBM | Rey | PBM |
| | 2) Isla San Jose, Islas Perlas, Rep. of Panama | PBM | Jose | PBM |
| | 3) Isla Taboga, Panama City, Rep. of Panama | PBM | Taboga | PBM |
| | 4) Bahia Cuastocomate, Jalisco, Mexico | LACM 68-41 | Jalisco | JM, PO |
| | 5) Punta Palmilla, Baja California | LACM 66-11 | Baja | JM, PO, LM |
| **II. Western Atlantic** | | | | |
| *Arcopsis adamsi* | 1) Isla La Orchilla, Venezuela | NMB 17701 | Orchilla | GS |
| | 2) Chichiriviche de la Costa, Distrio Federal, Venezuela | NMB 17678 | Federal | GS |
| | 3) Borburata, Estado Carabobo, Venezuela | NMB 17675 | Carabobo | GS |
| | 4) Isla la Tortuga, Venezuela | NMB 17702 | Tortuga | GS |
| | 5) Isla de Aves, Venezuela | NMB 17704 | Aves | GS |
| | 6) Bocas del Toro, Rep. of Panama | PBM | Bocas | PBM |
| *Barbatia candida* | 1) Bocas del Toro, Rep. of Panama | PBM | Bocas | PBM |
| | 2) Los Taques, Paraguana Peninsula, Venezuela | NMB 17662 | Paraguana | GS |
| | 3) Borburata, Estado Carabobo, Venezuela | NMB 17675 | Carabobo | GS |
| | 4) Playa Grande, Distrio Federal, Venezuela | NMB 17679 | Federal | GS |
| | 5) Isla la Tortuga, Venezuela | NMB 17702 | Tortuga | GS |
| | 6) Cabo Frio, Brazil | PBM | Brazil | NK |
| *Barbatia tenera* | 1) Bocas del Toro, Rep. of Panama | PBM | Bocas | PBM |
| | 2) Playa Grande, Distrio Federal, Venezuela | NMB 17679 | Federal | GS |
| | 3) Bahia Los Totumos, Estado Miranda, Venezuela | NMB 17686 | Miranda | GS |
| | 4) Mariguitar, Golfo de Cariaco, Venezuela | NMB 17690 | Cariaco | GS |
| *Arca imbricata* | 1) Bocas del Toro, Rep. of Panama | PBM | Bocas | PBM |
| | 2) Borburata, Estado Carabobo, Venezuela | NMB 17675 | Carabobo | GS |

TABLE *1*—Continued.

| | Site | Collection and lot number[1] | Name used in text/figures | Collector[2] |
|---|---|---|---|---|
| | 3) Playa Grande, Distrio Federal, Venezuela | NMB 17679 | Federal | GS |
| | 4) Buccaneer Bay, St. James Coast, Barbados | NMB 17724 | Barbados | GS |
| | 5) Laguna de Buche, Estado Miranda, Venezuela | NMB 17685 | Miranda | GS |
| | 6) Islas Chimanas, Estado Anzoategui, Venezuela | NMB 17692 | Anzoategui | GS |
| | 7) Isla Margarita, Juan Greigo, Venezuela | NMB 17696 | Margarita | GS |
| | 8) Isla la Tortuga, Venezuela | NMB 17702 | Tortuga | GS |
| | 9) Isla de Aves, Venezuela | NMB 17704 | Aves | GS |
| | 10) Key Biscaine, Florida, USA | NMB 17729 | Florida | GS |
| *Barbatia domingensis* | 1) Viente Frio, Colon, Rep. of Panama | PBM | Frio | PBM |
| | 2) Playa Grande, Distrio Federal, Venezuela | NMB 17679 | Federal | GS |
| | 3) Bocas del Toro, Rep. of Panama | PBM | Bocas | PBM |

[1] NMB = Naturhistorisches Museum, Basel; LACM = Natural History Museum of Los Angeles County; PBM = Primary author's collection; [2] PBM = P. B. Marko; JM = J. McLean; PO = P. Oringer; LM = L. Marincovich; GS = J. and W. Gibson-Smith; PJ = P. Jung; NK = N. Knowlton.

the significance of distances between group centroids. All multivariate analyses and tests were completed with Statistica ver. 5.1 (StatSoft, Inc.). Bonferroni corrections were applied when multiple statistical tests were conducted.

All DAs were followed by classification of observations and estimation of the *a posteriori* error rates (probability of misclassification). Because each discriminant function is optimized for the observations that are being classified, the proportion of misclassifed individuals will likely provide an underestimate of the actual error rate associated with a new sample. Therefore, to calculate a more accurate estimate of the classification error rates, we jacknifed across all observations in each DA (Hora and Wilcox, 1982; Kowalewski et al., 1997).

CV axis scores for fossil *A. mutabilis* and *A. imbricata* were generated using the eigenvalue coefficients derived from the Recent samples of the two species (see Michaux, 1989). Following the DA that included fossils, fossils were classified as one of the two Recent species. We then calculated the 95 percent confidence interval for the mean CV1 axis score for each group of fossils classified to either species within each sampling period.

RESULTS

*PCA on Recent specimens.*—The PCAs produced similar results for all five geminate species pairs (Table 3). When the data

were pooled across all localities within a nominal geminate pair, PC1 explained greater than 60 percent of the total variation. All variables exhibit a significant positive correlation with PC1 for all five geminate pairs (Table 3). Therefore, PC1 represents size variation. Either two or three additional PC axes explained nearly all the variation (91 percent on average). Additional PC axes describe shape variation because only some morphological variables load heavily and positively on additional PC axes (loadings not shown).

With the exception of the *Barbatia reeveana/B. candida* species pair (Fig. 5), PC ordinations provide evidence that individuals on either side of the isthmus are distinct rather than a single homogeneous groups of observations (Figs. 4–8). However, all geminate pairs exhibit some overlap in PC scores. The nominal species pairs that are separated along PC axes differ with respect to size and shape. For example, the *Arcopsis solida/A. adamsi* pair (Fig. 4) differs primarily along PC1, indicating that *A. solida* tends to be larger with respect to all 14 morphological variables (owing to larger PC1 scores for *A. solida* and a positive correlation between the measured variables and PC1). On average, specimens of *A. solida* are 58 percent larger in shell length than *A. adamsi,* the largest difference in shell length between any nominal geminate pair. In contrast, *B. illota* and *B. tenera* are separated primarily along PC2 (Fig. 6) and the *B. gradata/B. domingensis* pair

TABLE *2*—Locality information for fossil *Arca* material.

| | Locality (N)[1] | Formation | Collector[2] | Museum and lot number[3] | Approx. age |
|---|---|---|---|---|---|
| a) Eastern Pacific | 1) Playa Cocalito, Nicoya Peninsula, Costa Rica (3) | Montezuma | PPP | NMB 17471, PPP 825 | Plio-Pleistocene[4] |
| b) Western Atlantic | 1) Lake Enriquillo, Dominican Republic (10) | Enriquillo | PJ | NMB 16905 | Holocene[5] |
| | 2) Point-a-Pierre foreshore, Trinidad (2) | Point-a-Pierre | PJ | NMB 10187 | Holocene[5] |
| | 3) Matura Point, Manzanilla Coast, Trinidad (2) | Talparo | PJ | NMB 18572 | Pleistocene[6] |
| | 4) Casa Cantaure, Paraguana Peninsula, Venezuela (1) | Upper Cantaure | GS | NMB 17520 | Late Early Miocene[7] |
| | 5) Casa Cantaure, Paraguana Peninsula, Venezuela (17) | Lower Cantaure | GS | NMB 17516 | Late Early Miocene[7] |

[1] N = Sample size. [2] PPP = Panama Paleontology Project; PJ = P. Jung; GS = J. and W. Gibson-Smith. [3] NMB = Naturhistorisches Museum, Basel. [4] Coates et al (1992); [5] P. Jung, personal communication; [6] Jung (1989); [7] Díaz de Gamero (1974); Hunter and Bartok (1974); Gibson-Smith and Gibson-Smith (1979).
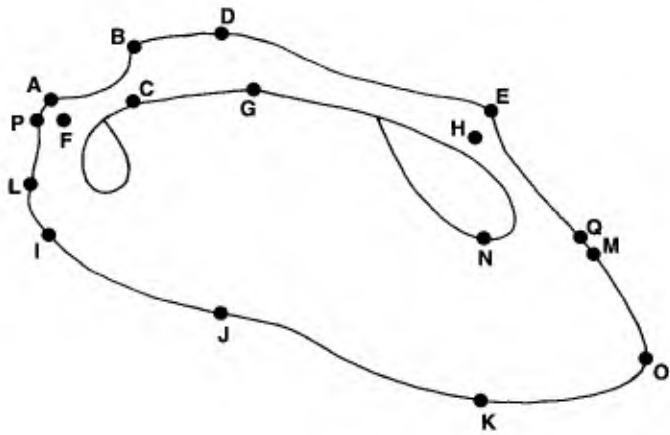
FIGURE 3—Morphological features used to characterize shell shape variation in arcid bivalves: A, anteriormost point of dorsal hinge margin; B, beak; C, intersection of inner shell margin of line perpendicular to A and B; D, dorsalmost point of umbo; E, posteriormost point of dorsal hinge margin; F, ventralmost position of anteriormost hinge tooth; G, dorsalmost point of ventral margin of hinge; H, ventralmost point of posteriormost hinge tooth; I, intersection of line with ventral shell margin that also passes through A and is perpendicular to AE; J, intersection of line with ventral shell margin that also passes through D and is perpendicular with AE; K, intersection of line with ventral shell margin that also passes through E and is perpendicular to AE; L, anteriormost point of shell margin; M, intersection of line that passes through L and intersects the ventralmost point of both adductor muscle scars; N, intersection point between the line LM and EK; O, posteriormost point of shell ventral to the hinge; P, tangent of a line on anterior shell margin that bisects A; Q, tangent of a line on posterior shell margin that bisects E. Morphological variables measured: hinge length (AE), shell length (LM), beak position (AC), beak height (CB), anterior shell height (AI), middle shell height (DJ), posterior shell height (EK), posterior shell length (NO), anterior slope (angle of EAP), posterior slope (angle of AEQ), umbonal angle (angle of ADE), ventral hinge angle (angle of FGH), byssal gap angle (angle of IJK), shell area (projected area of shell).

TABLE 3—Correlation of morphometric variables with principal component 1. All correlation coefficients are significant at $P < 0.01$. Variables are defined in Figure 1. Sample sizes in Table 4.

| Variable | A. solida/ A. adamsi | B. reeveana/ B. candida | B. illota/ B. tenera | A. mutabilis/ A. imbricata | B. gradata/ B. domingensis |
|---|---|---|---|---|---|
| | Correlation of variables with PC1 | | | | |
| Hinge Length | 0.965 | 0.989 | 0.966 | 0.959 | 0.963 |
| Shell Length | 0.992 | 0.992 | 0.990 | 0.984 | 0.989 |
| Beak Position | 0.944 | 0.967 | 0.893 | 0.892 | 0.892 |
| Beak Height | 0.828 | 0.836 | 0.716 | 0.892 | 0.753 |
| Anterior Height | 0.989 | 0.970 | 0.978 | 0.825 | 0.956 |
| Middle Height | 0.985 | 0.991 | 0.991 | 0.984 | 0.980 |
| Posterior Height | 0.988 | 0.979 | 0.987 | 0.972 | 0.981 |
| Posterior Length | 0.852 | 0.916 | 0.936 | 0.778 | 0.902 |
| Anterior Slope | 0.828 | 0.721 | 0.737 | 0.855 | 0.861 |
| Posterior Slope | 0.857 | 0.663 | 0.635 | 0.748 | 0.807 |
| Umbo Angle | 0.887 | 0.799 | 0.880 | 0.892 | 0.744 |
| Hinge Angle | 0.732 | 0.801 | 0.786 | 0.676 | 0.535 |
| Byssal Gap Angle | 0.867 | 0.858 | 0.880 | 0.884 | 0.772 |
| Shell Area | 0.992 | 0.970 | 0.994 | 0.992 | 0.995 |

differ largely with respect to PC2 and PC3 (Fig. 8), indicating that these species differ largely with respect to shell shape.

Some separation within species is apparent. For example, four individual *B. reeveana* exhibit smaller PC1 and PC2 scores than other conspecifics (Fig. 5). Although these individual are all from near Panama City (Isla Del Rey and Playa Venado; Table 1), they are not restricted to a single collection site. *A. adamsi* also exhibits some intraspecific discontinuities in PC scores (four points with low PC scores, Fig. 4), but in this case these individuals are restricted to a single collection site (Federal, Venezuela; Figs. 1–3). Inter-locality differences will be considered in more detail below.

*DA on Recent samples with species as groups.*—By regressing all observations on PC1, the correlation coefficients in Table 3 were reduced to values not significantly greater than zero ($P > 0.7$ for all cases). Therefore, the results from all DAs may be regarded as size-free.

For all five geminate pairs, values of Wilks' λ were significant at the 5 percent level (Table 4a) and most nominal species are separated along the first CV axis (Figs. 9–13). Discrimination of *B. reeveana/B. candida* (Table 4a) is not significant at a Table-wide Bonferroni corrected critical value of α = 0.01 (5 tests), in accordance with the broad overlap in observed PC scores (Fig. 5). Squared Mahalanobis distances between the centroids of each of five geminate pairs are all also significantly different at the 0.01 level for all five geminate pairs with the exception of *B. reeveana* and *B. candida* (Table 5).
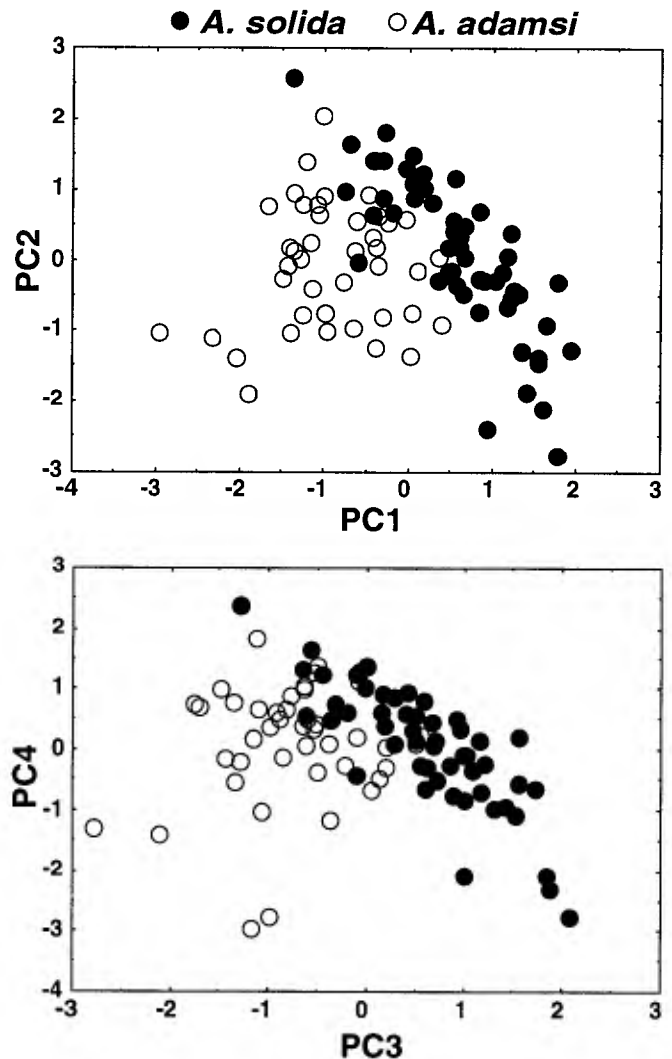


FIGURE 4—Principal component (PC) ordination of Recent shells of *Arcopsis solida* and *A. adamsi*.
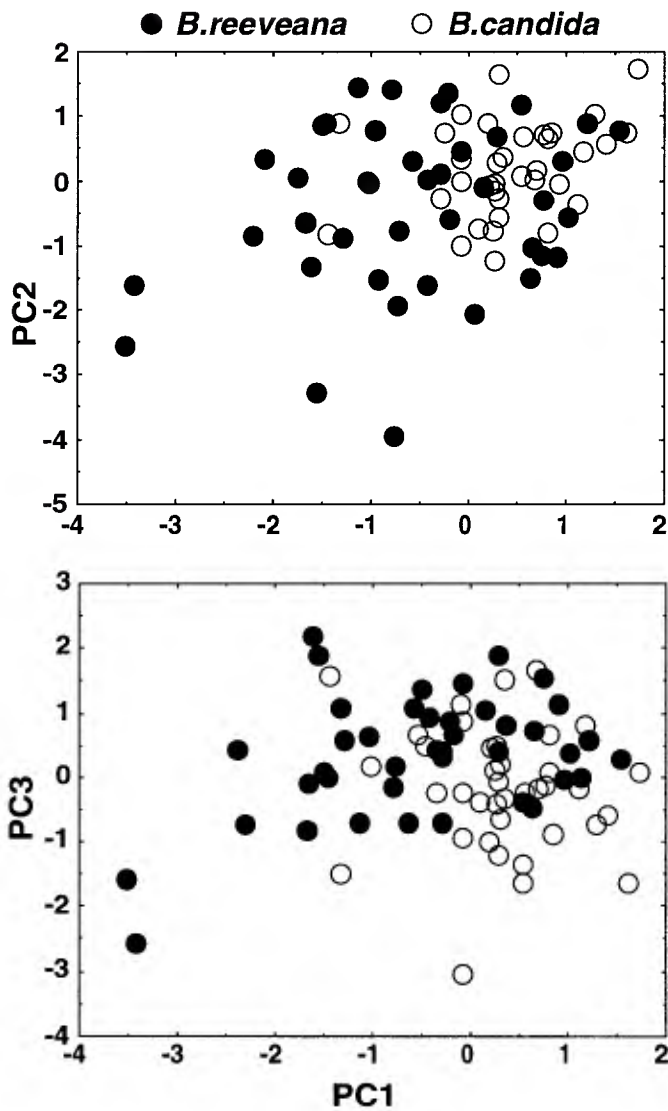
FIGURE 5—Principal component (PC) ordination of Recent shells of *Barbatia reeveana* and *B. candida*.
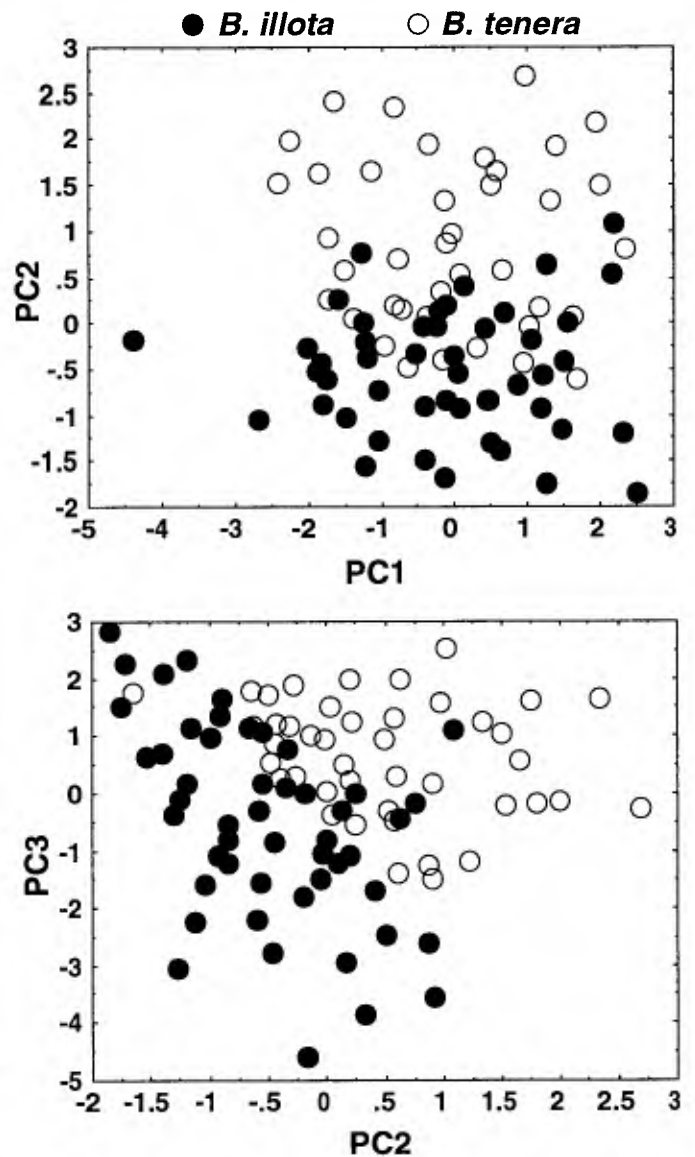


FIGURE 6—Principal component (PC) ordination of Recent shells of *Barbatia illota* and *B. tenera*.

Classification error rates (percentage of misclassified individuals, Table 4a) are variable, ranging from a low of seven percent (*B. gradata*) to a high of 24 percent (*A. adamsi*). On average across the five geminate pairs, 84 percent of the Recent specimens were classified correctly to species. Three species pairs that were distinct in the PCAs *B. gradata*/*B. domingensis*, *A. mutabilis*/*A. imbricata*, and *B. illota*/*B. tenera* (Figs. 6–8), all exhibited the greatest separation along CV1 (Figs. 11–13), the lowest classification error rates (Table 4a), and highly significant Mahalanobis distances (Table 5).

Size adjustment increased the classification error rate by 5 percent on average (Table 4a) although the impact of size correction varied substantially among species. Size-adjustment appears to reduce some spurious discriminations based on size-related differences across the isthmus: the DA for the species pair that appeared to differ most in shell size (*Arcopsis*; Fig. 4) is affected the most by the size-adjustment procedure (Table 4a, see values in parentheses).

*DA on Recent samples with collection sites as groups.*—Using collection site as the *a priori* grouping variable for Recent samples, classification success for Recent specimens allocated into collection sites (Table 4b) was variable among geminate pairs. For example, only slightly more than half of the specimens of *A. solida* could be correctly classified to site (Table 4b). In contrast, 94 percent of *B. illota,* and 90 percent of *B. gradata* individuals were correctly classified to site (Table 4b).

A wide range of patterns of differentiation exists among collection sites. Although the error rates for classifications to site were low for some species, no single site was significantly different from all other conspecific sites (based on Mahalanobis distances among sites, F-tests not shown). Some sites, however, are highly morphologically distinct. For example, even though *A. adamsi* exhibited the highest classification error rate (Table 4b), the Federal site in Venezuela (Fig. 14a, open squares; Figs. 1–3) is clearly separated from all other sites on both sides of the isthmus (this site is also distinct in the PCA for *A. adamsi*; Fig. 4); the Federal site of *B. candida* was also similarly distinct with respect to CV scores (Fig. 14b, Figs. 1–6). The species pair that
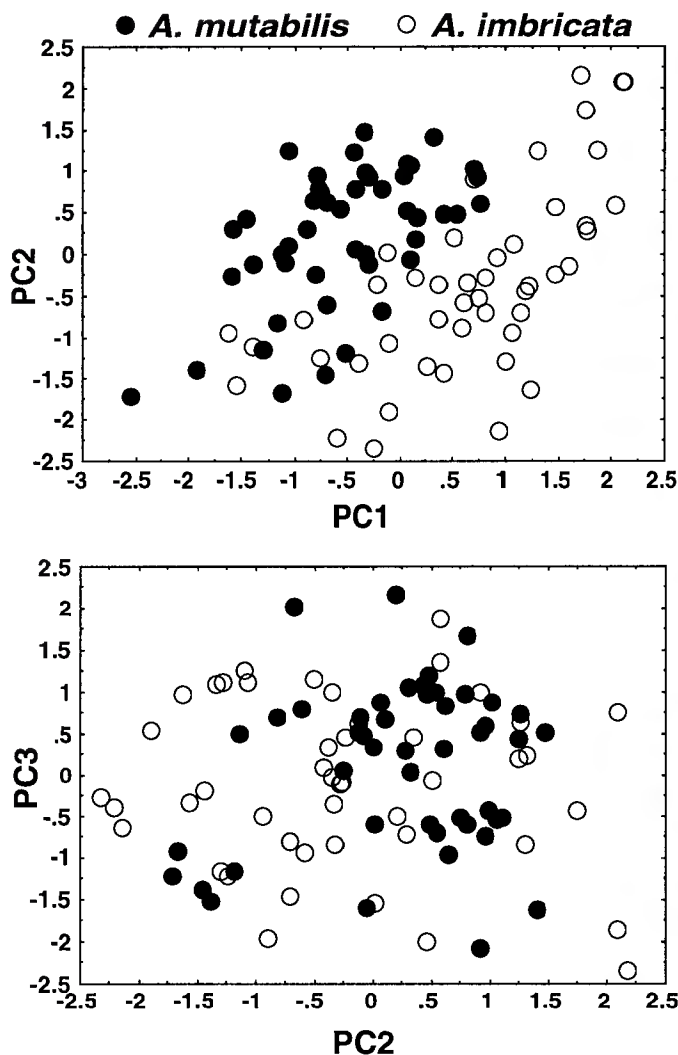
FIGURE 7—Principal component (PC) ordination of Recent shells of *Arca mutabilis* and *A. imbricata.*



FIGURE 8—Principal component (PC) ordination of Recent shells of *Barbatia gradata* and *B. domingensis.*

was most distinct in the DA in which species was the grouping variable, *B. gradata* and *B. domingensis,* also exhibited the most separation across all collection sites (Fig. 15a); collection sites wi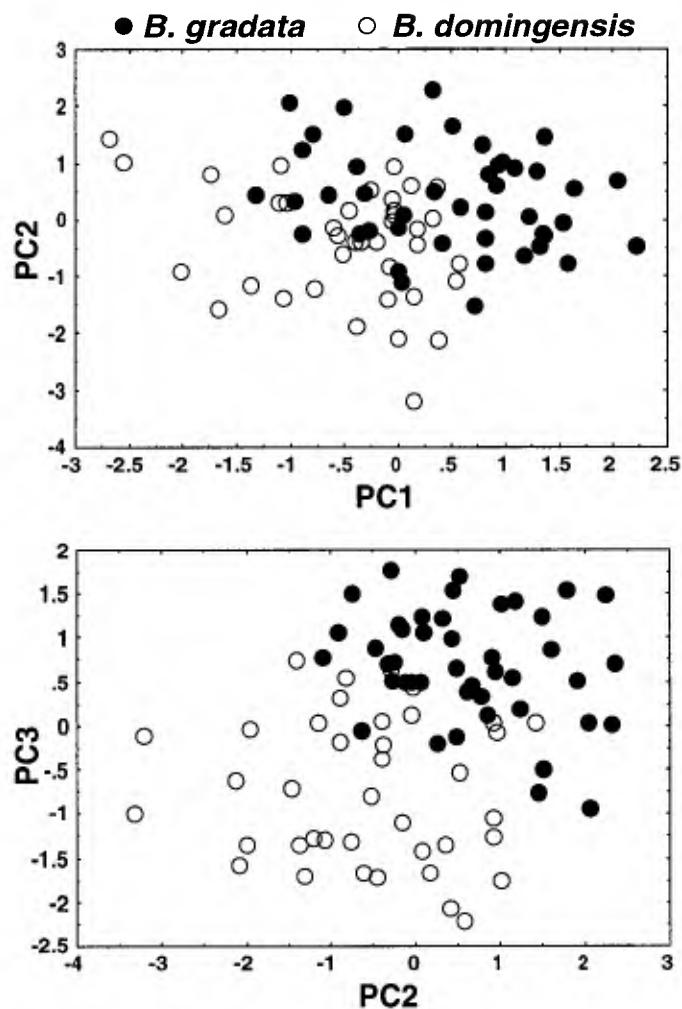thin each ocean also appear to be more similar to each other than sites on opposites sides of the isthmus for both *B. gradata* and *B. domingensis* (Fig. 15a). Collection sites of *A. mutabilis* and *A. imbricata* (Fig. 15b) also appear to separate with respect to ocean, but overall, individual collection sites are not as distinct as in *B. gradata* and *B. domingensis.*

For each species pair, intraspecific morphological distances

TABLE 4—Classification of specimens. Results are based on five discriminant analyses that either used species (a) or collection sites (b) as the *a priori* groupings. The percentage of correctly classified individuals was based on a jackknifing across specimens (see Methods). Values in parentheses are percentages from measurements that were not size-adjusted. F-tests are for values of Wilks' λ.

| | Sample size | a) Species as groups | | | b) Sites as groups | | |
|---|---|---|---|---|---|---|---|
| | | Percent correct to species | F-value | *P*-value | Percent correct to site | F-value | *P*-value |
| 1) *A. solida* | 52 | 83 (90) | | | 59 (64) | | |
| *A. adamsi* | 44 | 76 (89) | 2.40 | <0.01* | 83 (85) | 2.50 | <0.0001* |
| 2) *B. reeveana* | 40 | 83 (88) | | | 66 (66) | | |
| *B. candida* | 37 | 83 (83) | 2.39 | <0.05 | 81 (80) | 1.53 | <0.0001* |
| 3) *B. illota* | 52 | 82 (91) | | | 94 (97) | | |
| *B. tenera* | 35 | 87 (84) | 4.28 | <0.001* | 84 (89) | 3.00 | <0.0001* |
| 4) *A. mutabilis* | 47 | 89 (91) | | | 85 (89) | | |
| *A. imbricata* | 50 | 85 (86) | 6.08 | <0.0001* | 86 (90) | 2.17 | <0.0001* |
| 5) *B. gradata* | 45 | 93 (96) | | | 90 (90) | | |
| *B. domingensis* | 39 | 90 (95) | 7.35 | <0.0001* | 83 (83) | 2.27 | <0.0001* |

* Significant with a Bonferroni correction for five tests (α = 0.05/5).
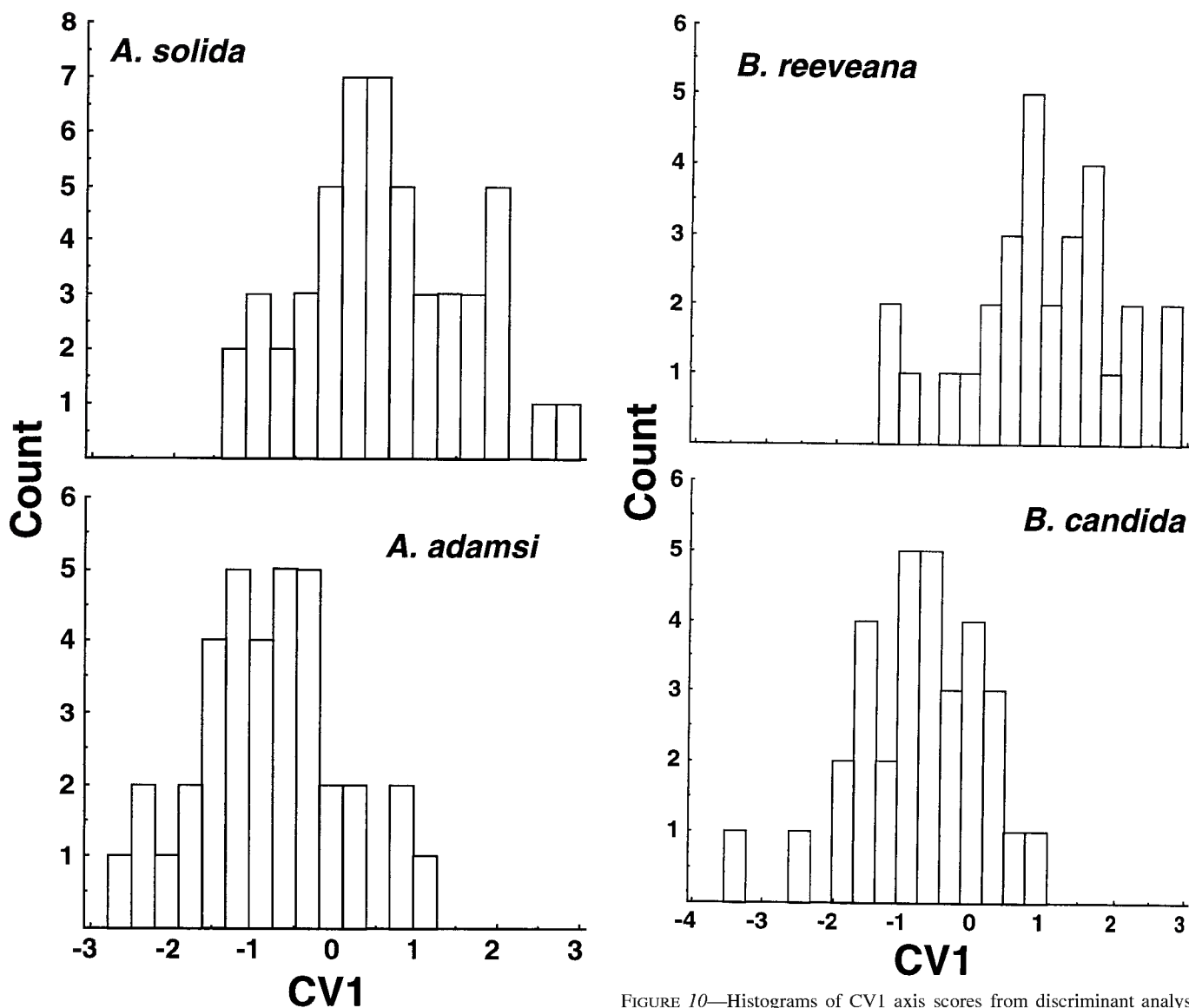
FIGURE 9—Histograms of CV1 axis scores from discriminant analysis for Recent *A. solida* (EP, closed symbols) and *A. adamsi* (WA, open symbols) using nominal species as the *a priori* groups.



FIGURE 10—Histograms of CV1 axis scores from discriminant analysis for Recent *B. reeveana* (EP, closed symbols) and *B. candida* (WA, open symbols) using nominal species as the *a priori* groups.

were as large as distances between collection sites on either side of the isthmus (Fig. 16), suggesting that morphological variability within oceans was generally as large as morphological variation across the isthmus. Among EP taxa, intra- and interspecific distances were significantly correlated (r = 0.916, $P$ < 0.05); no correlation existed for WA species (r = 0.245, $P$ > 0.5).

*Two-group DA including fossil Arca.*—Using the eigenvectors from the DA in which Recent nominal species separated by the isthmus were the *a priori* groups, morphological forms corresponding to both *A. mutabilis* and *A. imbricata* were identified in the fossil record (Fig. 17). In total, 12 fossils are classified as *A. mutabilis* and 23 are classified as *A. imbricata*. Except in the Plio-Pleistocene, at least one fossil was classified to each of *A. mutabilis* and *A. imbricata* from every sampling period with $P$ > 0.8 (Fig. 18). The maximum individual classification probability associated with a misclassified Recent specimen of either species (across all jackknife replicate data sets) was 0.779. Therefore, at least one fossil was classified to each species during each of the

Holocene, Pleistocene, and Early Miocene with larger classification probabilities than the largest probability associated with a misclassified Recent shell. The 95 percent confidence intervals for fossils classified as *A. mutabilis* and *A. imbricata* overlap in the Holocene, but do not overlap in either the Pleistocene or the Early Miocene (Fig. 18).

In the oldest sample of fossils (Cantaure Formation) six fossils were classified as *A. mutabilis* and 12 as *A. imbricata* (Fig. 18). Among these Early Miocene fossils at least one specimen was classified to both species with $P$ > 0.95 and at least three were classified to each with $P$ > 0.8 (Fig. 18).

### DISCUSSION

*Morphological variation and discrimination between Recent species.*—Discriminant analyses of Recent arcid shells indicate that shell shapes of geminate species are morphologically distinct and most specimens can be identified correctly with multivariate methods. Squared Mahalanobis distances between all but one geminate pair are highly significantly different and, on average,
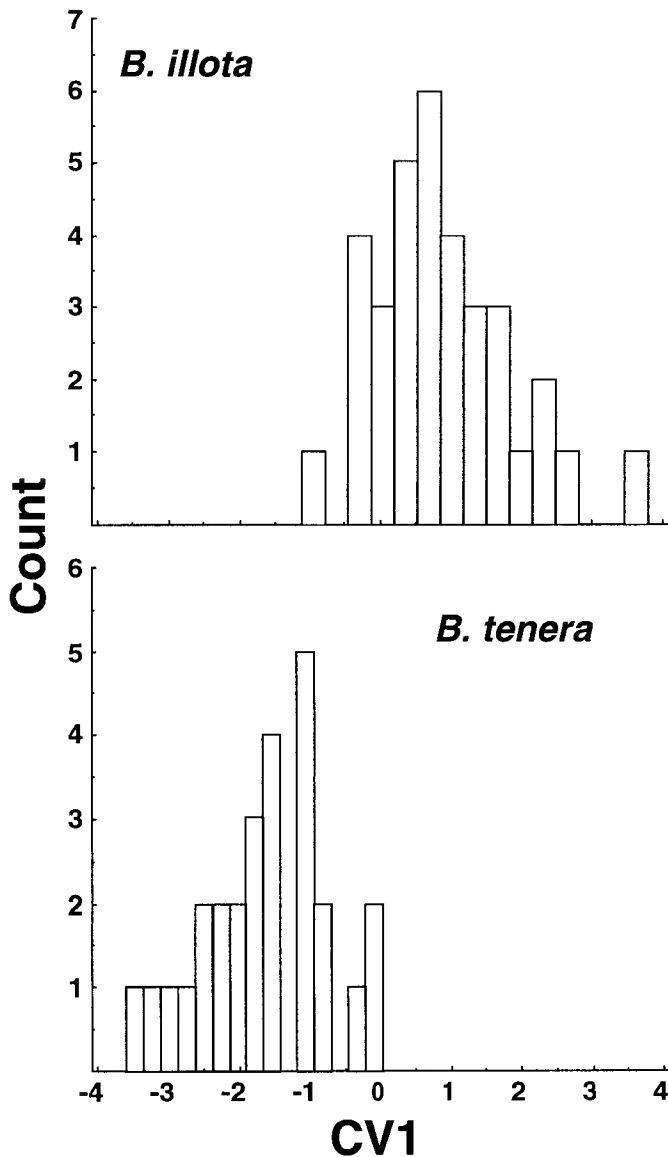
FIGURE *11*—Histograms of CV1 axis cores from discriminant analysis for Recent *B. illota* (EP, closed symbols) and *B. tenera* (WA, open symbols) using nominal species as the *a priori* groups.



FIGURE *12*—Histograms of CV1 axis scores from discriminant analysis for Recent *A. mutabilis* (EP, closed symbols) and *A. imbricata* (WA, open symbols) using nominal species as the *a priori* groups.

over 85 percent of Recent specimens are correctly classified to species when species are used as the *a priori* groups in discriminant analyses. Therefore, despite a high degree of morphological similarity between arcid sister-species on either side of the Isthmus of Panama, multivariate morphometrics appear to provide sufficient taxonomic resolution to apply discriminant analyses to fossil material to determine when and where most species occurred in the tropical Americas during the late Cenozoic.

Some geminates, however, are clearly more morphologically distinct than others are. For example, *Barbatia gradata* and *B. domingensis* are easily separated with respect to PC scores and a significant DA resulted in correct classification of greater than 90 percent of all Recent specimens. In addition to *B. gradata* and *B. domingensis,* relatively low classification error rates for the *Arca mutabilis/A. imbricata* and *B. illota/B. tenera* species pairs indicate that these three geminate pairs provide tractable systems for studies of speciation in the fossil record. In contrast, the PC scores of *B. reeveana* and *B. candida* overlap substantially, and between
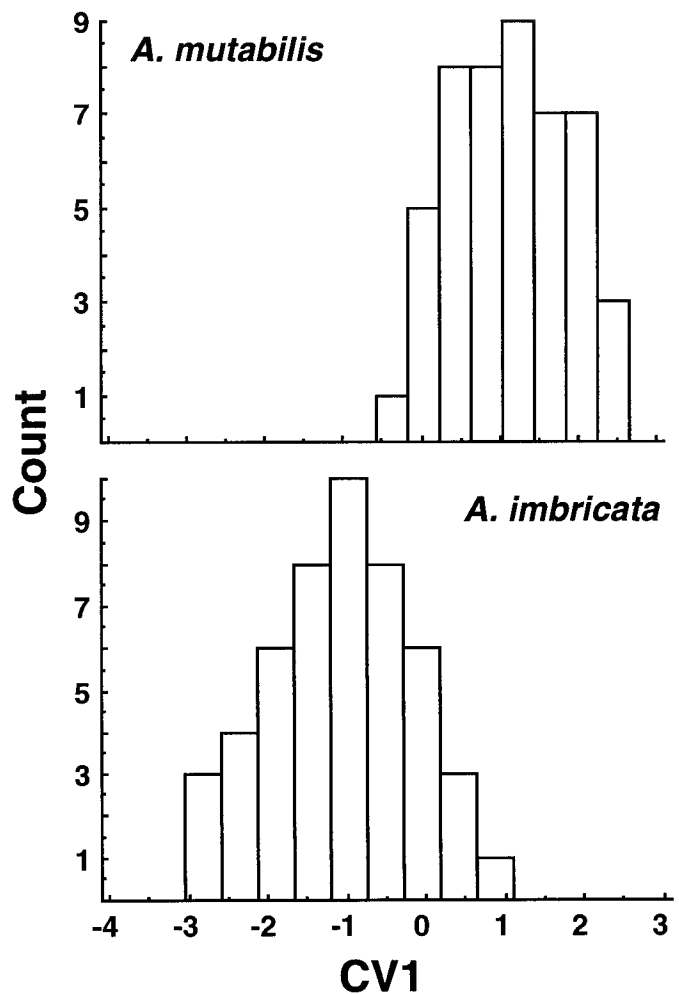
both *B. reeveana/B. candida* and the *Arcopsis solida/A. adamsi,* nearly 20 percent of Recent individuals misclassified to species in DAs. Because the classification error rates of the two most similar species pairs are relatively higher, reliable identification of these species in the fossil record will likely be more difficult. Moreover, because each species pair may be more morphologically similar in the past, we expect that actual classification error rates could be higher with fossil material.

We can also likely increase our capability to discriminate species in the future by adding additional characters. Because our primary objective was to comparatively analyze patterns of variation among five nominal species pairs, our characters were limited to measurements of the shell that could be made in all three genera. The exterior of arcid shells, however, contain an abundance of shell microstructural features that are unique to some subgeneric groups (e.g., Bartsch, 1931; Olsson, 1961). The distribution of radial cords on the exterior of the shell, the width of cords on different parts of the shell, the height of concentric lamina, and measurements of hinge teeth dimensions are all characters which exhibit considerable morphological variation within genera and subgenera. Increasing the number of morphological variables will likely improve morphological resolution between putative geminate species and may provide additional resolution
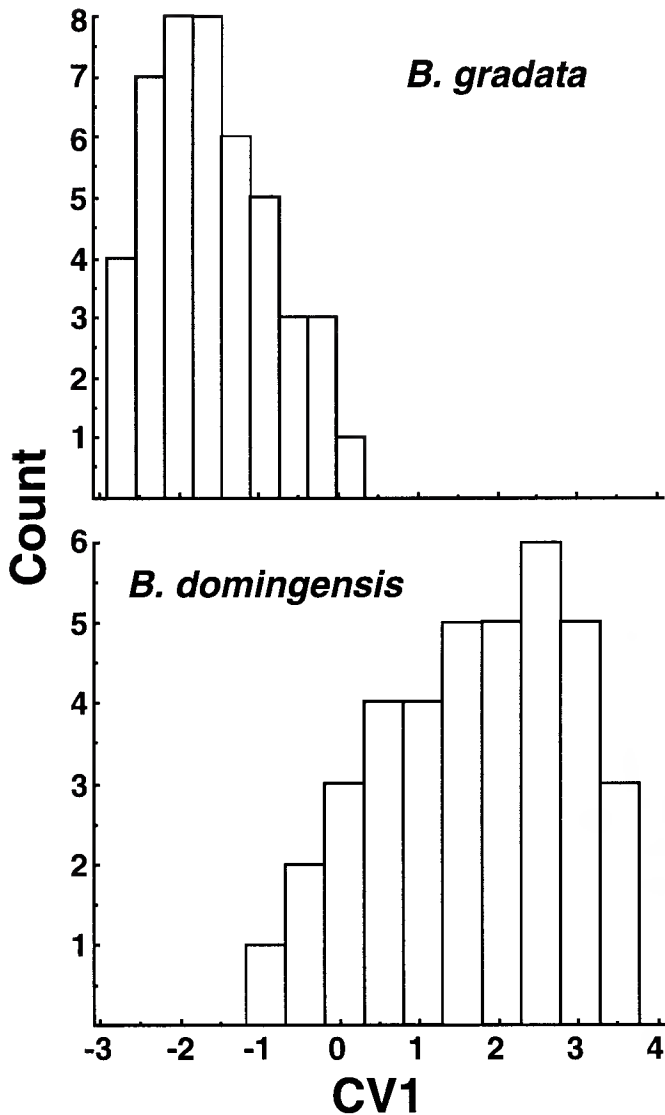
FIGURE 13—Histograms of CV1 axis scores from discriminant analysis for Recent *B. gradata* (EP, closed symbols) and *B. domingensis* (WA, open symbols) using nominal species as the *a priori* groups.

TABLE 5—Squared Mahalanobis distances between nominal arcid geminate species pairs.

| Species pair | N | Maha-lanobis D | F-value | P-value |
|---|---|---|---|---|
| A. *solida*/A. *adamsi* | 52/44 | 2.17 | 2.41 | <0.01* |
| B. *reeveana*/B. *candida* | 40/37 | 2.92 | 2.40 | <0.05 |
| B. *illota*/B. *tenera* | 52/35 | 6.75 | 4.25 | <0.001* |
| A. *mutabilis*/A. *imbricata* | 47/50 | 5.85 | 6.08 | <0.00001* |
| B. *gradata*/B. *domingensis* | 45/39 | 9.36 | 7.35 | <0.00001* |

* Significant with a Bonferroni correction for five tests ($\alpha$ = 0.05/5).

to two meters), attached to live corals on patch reefs in relatively calm water. Although these are only relatively coarse habitat descriptions, the degree to which arcid geminate pairs differ morphologically does not appear to be related in any obvious way to habitat similarities and differences on either side of the isthmus.

Significant differences in shell shape between *A. mutabilis* and *A. imbricata* allowed us to test the potential for morphological
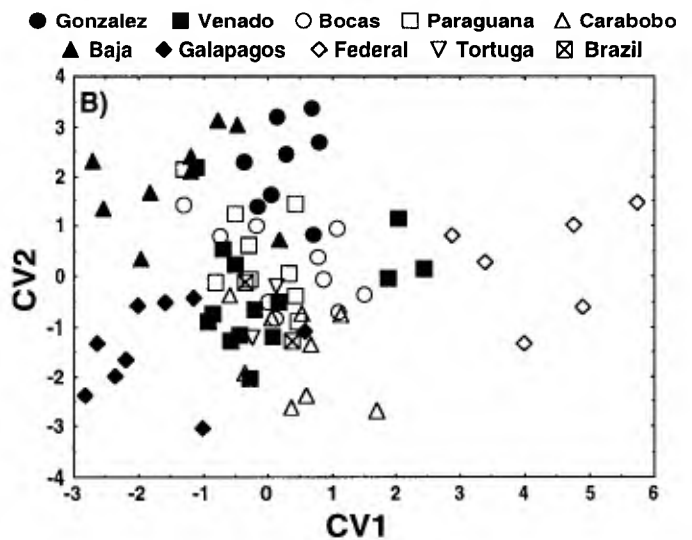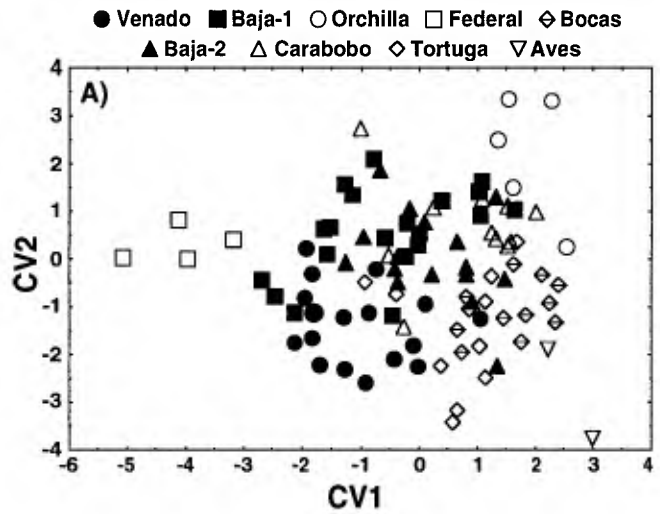




FIGURE 14—CV1 and CV2 scores for Recent a) *A. solida* and *A. adamsi* and b) *B. reeveana* and *B. candida.* Completely closed symbols = EP sites; open symbols = WA sites. Names of collection sites defined in Table 1.
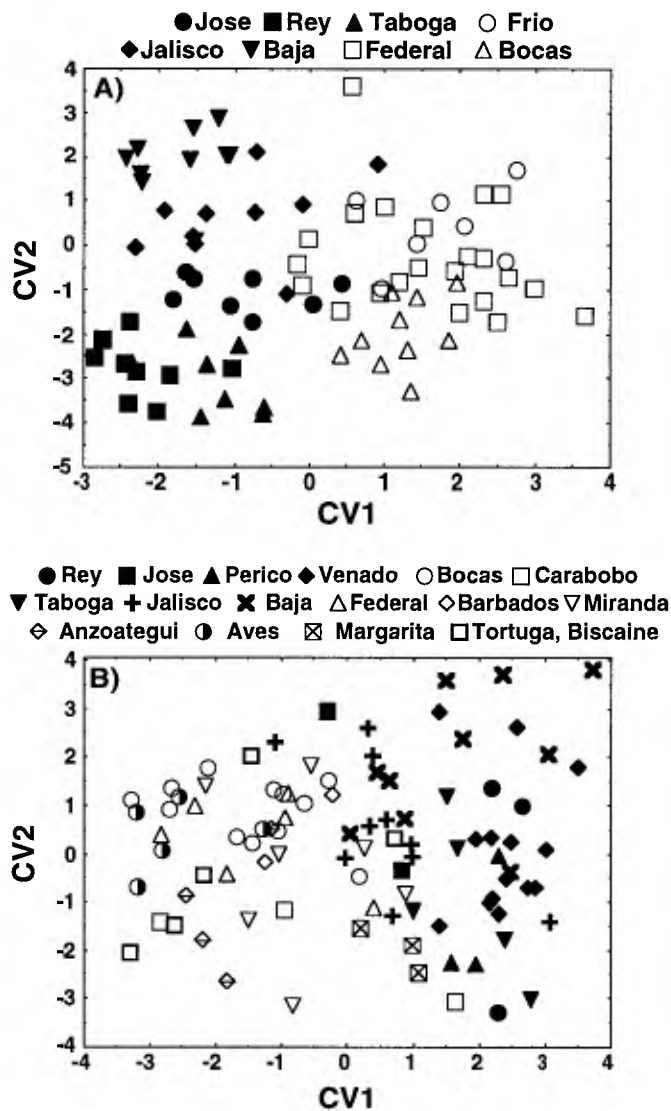
among morphological forms within species that we detected when collection site was the grouping variable in DAs.

Given that all of the species considered here have nestling lifestyles (Olsson, 1961; Abbott, 1974; Keen, 1974), morphological differences on either side of the isthmus may reflect habitat differences that have evolved since the lineage splitting event that produced each species pair. However, we routinely observed the second most morphologically similar species pair, *A. solida* and *A. adamsi,* and the most morphologically distinct pair, *B. gradata* and *B. domingensis,* in nearly identical microhabitats. Both pairs of species are found in the low intertidal of rocky shores, attached by their byssus to the undersides of large flat stones on both the EP and Caribbean coasts of Panama. In contrast, *B. reeveana* and *B. candida,* whose PC scores broadly overlap, are found in vastly different environments on either side of the isthmus. *B. reeveana* nestles in rock crevices in the mid-intertidal of the EP coast of Panama, exposed to relatively extreme conditions of wave action and desiccation for several hours daily. In the Caribbean, however, *B. candida* is typically found in the shallow subtidal (one

FIGURE 15—CV1 and CV2 scores for Recent a) *B. graduta* and *B. domingensis* and b) *A. mutabilis* and *A. imbricata*. Completely closed symbols = EP sites; open symbols = WA sites. Names of collection sites defined in Table 1.



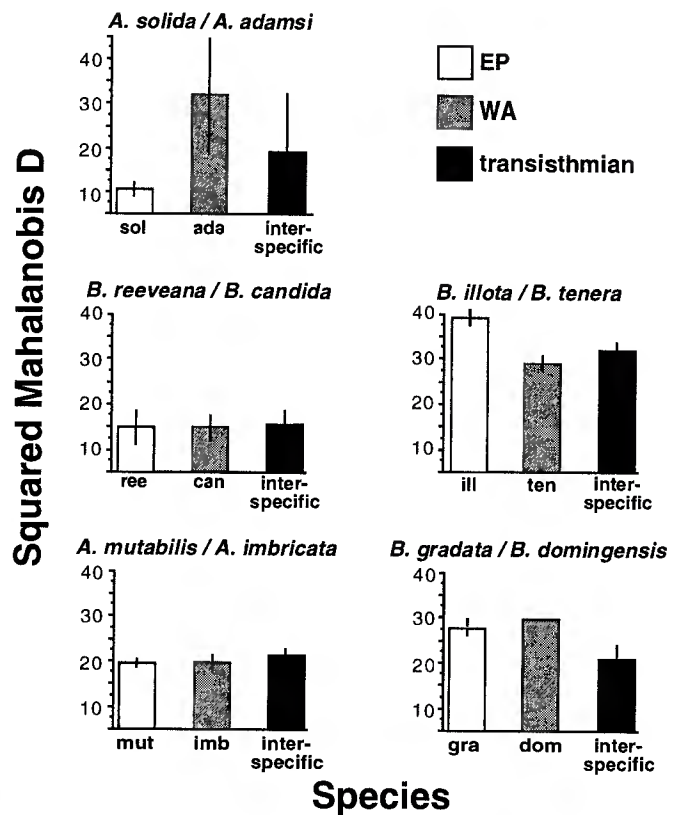FIGURE 16—Mean squared Mahalanobis distance (D) among collection sites. EP = Eastern Pacific; WA = Western Atlantic. Error bars are standard errors.

discrimination of geminate species in the fossil record. In the DA that included fossil material, fossils from nearly all of the sampling periods were classified to both species. Most significantly, the presence of forms associated with both Recent species are present in the Late Early Miocene of Venezuela. This sample of fossils from the Cantaure Formation is of Burdigalian age (Locality GS1PGNA of Gibson-Smith and Gibson-Smith, 1979), which is no younger than 16.3 Ma (Harland et al., 1990). Formation of the isthmus likely had biogeographic effects earlier than 3.1 to 3.5 Ma: some snapping shrimp, gastropods, and foraminiferans associated with deeper water were affected as early as 7 to 9 Ma (Cunningham and Collins, 1994; L. S. Collins et al., 1996; Knowlton and Weigt, 1998). However, given the presence of an abyssal depth seaway between the EP and WA until the beginning of the Middle Miocene (Coates and Obando, 1996), an Early Miocene divergence attributable to isthmus formation is unlikely. Given that both *A. mutabilis* and *A. imbricata* are shallow water nearshore species, divergences greater than 16.3 Ma most likely represent pre-isthmian events.

Both the Pleistocene (Talparo, Pt.-à-Pierre) and Holocene (Enriquillo) samples from the WA contain at least one individual classified as the EP species *A. mutabilis* with $P > 0.8$. Because these samples are all post-isthmian, these fossils (in addition to the Venezuelan fossils from the Early Miocene) indicate that the morphological form associated with the Recent *A. mutabilis* existed in both ocean basins before and after isthmus formation. Similarly, classification of fossils from the Montezuma Formation (Nicoya Peninsula, Costa Rica) suggests that the geographic range of the morphological form associated with *A. imbricata* extended into the EP during the Plio-Pleistocene. If ocean conditions in the Caribbean and the EP were more similar during the Miocene and Pliocene than today (Vermeij, 1978; Petuch, 1981; Vermeij and Petuch, 1986; Allmon, 1992; Teranes et al., 1996), the presence of both species in both oceans prior to closure of the Central American seaway is not a surprise considering that both appear to be distinct species in Early Miocene times. Many taxa that had amphi-American distributions during the Miocene are now restricted to either the EP or WA, including several arcid subgenera (see Woodring, 1966; Vermeij, 1978; Jung, 1989; Jackson et al., 1996). *A. mutabilis* and *A. imbricata* most likely represent species that are geographically restricted to one side of the isthmus rather than the descendants of an ancestral species whose geographic range was disrupted by the formation of the isthmus; differential extinction on each side of the isthmus, rather than speciation, best explains their modern distributions.

Because 11 and 15 percent of the Recent shells of *A. mutabilis* and *A. imbricata*, respectively, were misclassified we expect at least the same proportion of fossils to be classified to the wrong species. Because the maximum classification probability associated with any misclassified Recent shell was 0.779, the possibility
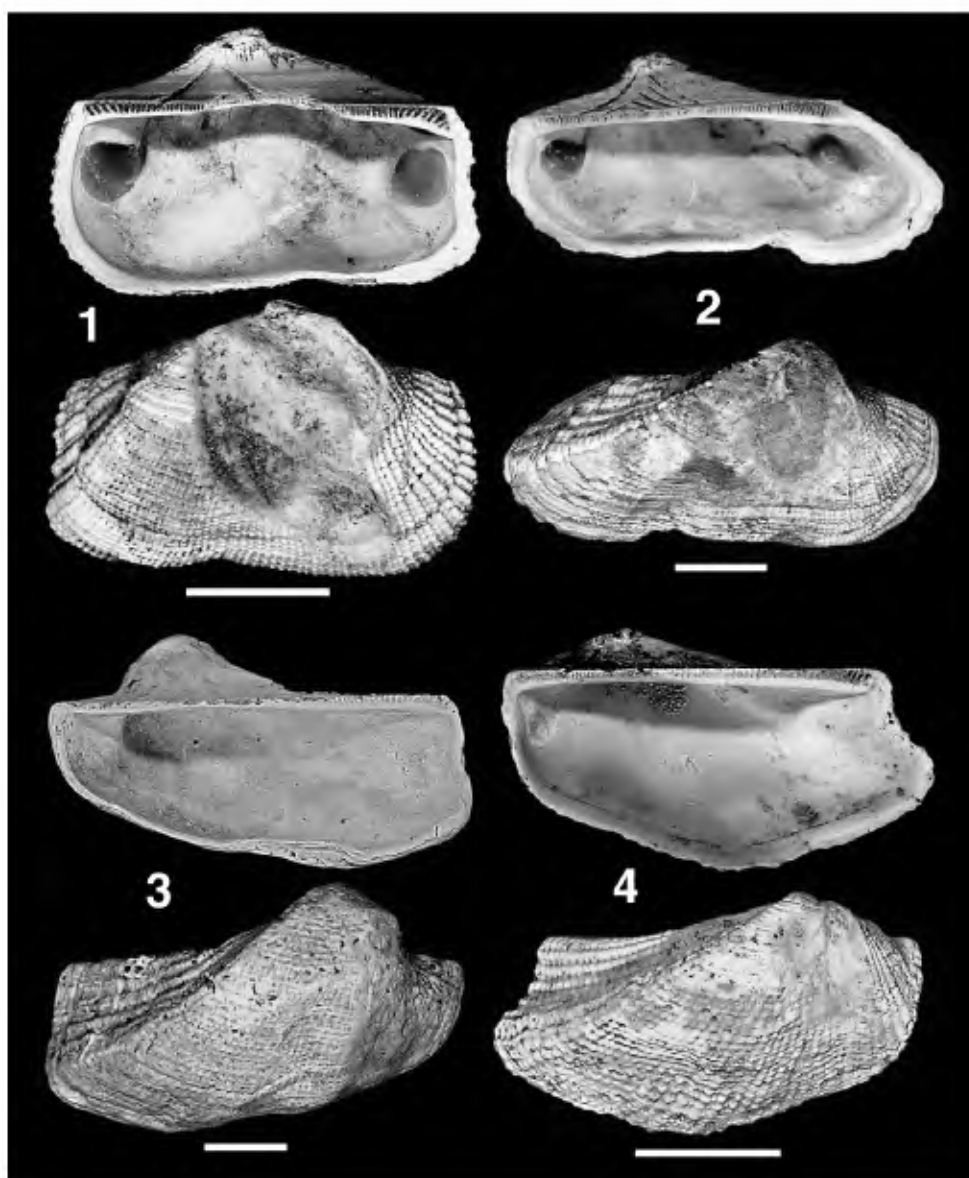
FIGURE 17—Representative fossils from the Cantaure Formation (late early Miocene) identified following discriminant analysis. 1–2, specimens classified as *A. mutabilis*; 3–4, specimens classified as *A. imbricata*. Scale bars = 1 cm.

that fossils classified with $P > 0.95$ are statistical artifacts of misclassification is doubtful; outliers are presumably rare in small samples of individuals. Fossils were also classified with classification probabilities that are comparable to those of the Recent shells. The average classification probability for fossil *A. mutabilis* ($0.75 \pm 0.03$) is nearly the same as the average classification probability for Recent *A. mutabilis* ($0.73 \pm 0.04$). Similarly, fossil *A. imbricata* were classified with approximately the same probability ($0.72 \pm 0.04$) as Recent specimens of *A. imbricata* ($0.71 \pm 0.04$). The disparity of forms found in single fossil samples (i.e., fossils classified to each species with $P > 0.95$ in the same sample and non-overlapping 95 percent confidence intervals) is also probably best explained by the presence of more than one distinct species. Among the Recent shells, no single locality of either *A. mutabilis* and *A. imbricata* (Fig. 15b) exhibits the range of morphological variation exhibited by either species, and does not begin to approach the range of variation between both species.

Our conclusions regarding the origins of *A. mutabilis* and *A.*

*imbricata*, however, rest on several important assumptions. First, our methods assume that the Recent survey of geographic variation adequately describes the morphospace occupied by each Recent species. For example, if increased sampling of each Recent species increases the amount of overlap in morphological form between nominal species, our ability to discriminate between species may be reduced, thereby reducing our ability to identify fossils (although increasing our character database for each putative geminate pair may compensate for this, see above). Second, although we can assign fossils to each Recent nominal species, the reproductive compatibility of fossils can never be known with certainty.

Lastly, and probably most importantly, we have yet to rule out the possibility of cryptic species, either in the Recent or in the fossil record. The ubiquitous pattern of extensive intraspecific morphological variation among collection sites (which rivals that observed across the isthmus in some cases) may reflect the presence of cryptic sibling species. Surveys of the tropical American
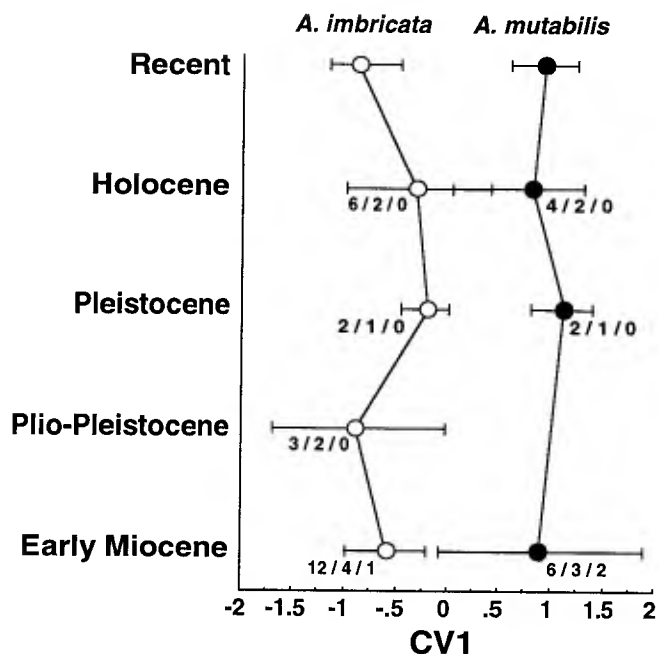
A. imbricata    A. mutabilis



FIGURE 18—CVI means for Recent and fossil A. mutabilis and A. imbricata. Horizontal bars are upper and lower 95 percent confidence limits for the mean CV1 score. The Pt.-à-Pierre and Talparo samples from Trinidad (Pleistocene) and the Upper and Lower Cantaure samples from Venezuela (late early Miocene) were each combined. Integers beside symbols indicate the total number of individuals classified, the number classified with $P > 0.8$, and the number classified with $P > 0.95$, respectively. The vertical axis does not reflect the relative ages of the samples.

molluscan fauna indicate that both Recent and fossil subgeneric diversity has been substantially underestimated (Jung, 1989; Jackson et al., 1993; Allmon et al., 1993) and because speciation in marine environments clearly does not require strong permanent geological barriers to dispersal (e.g., Kwast et al., 1990; Knowlton, 1993; Kessing, 1991; Duffy, 1996; Marko, 1998; Hellberg, 1998), speciation events in the tropical American fauna both before and after final seaway closure are possible. Changing the taxonomic framework within which we identified fossils, by recognizing additional living and/or extinct species within the nominal taxa, could potentially change our results regarding the timing of divergence of transisthmian taxa in *Arca*. Characterization of the genetic structure of Recent species (e.g., Michaux, 1989) plus integration of exploratory morphological analyses that do not rely on the Recent taxonomy (e.g., Budd et al., 1994; Jackson and Cheetham, 1990) will likely be necessary to consider this hypothesis more explicitly in the future.

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