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ONTOGENETIC CHANGES IN THE CARAPACE OF TYRRHENOCYTHERE AMNICOLA (SARS) A HEMICYTHERID OSTRACODE[1]

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Abstract.—Detailed description and illustration of the carapace of *Tyrrhenocythere amnicola* (Sars, 1888) at eight different growth stages show morphological changes during ontogeny and lay groundwork for a better understanding of its relationships to similar ostracodes. Regression of log size on developmental stage indicates that growth rate is constant; the area enclosed by the outline of the carapace increases by a factor of 1.74 from one instar to the next. Biorthogonal analysis graphically shows the amount and direction of change of shape in homologous regions of the carapace between successive instars. Eigenshape analysis and qualitative evaluation indicate that only adults, not juveniles, are sexually dimorphic in shape. Both eigenshape and biorthogonal analyses indicate that change of shape during growth is primarily monotonic and is concentrated posteriorly. More change occurs early than late, and the greatest change occurs between instars A-6 and A-5.

Most recent ostracode species have nine growth stages or instars if one includes the initial larval stage and the adult. Unfortunately, juvenile instars are often poorly preserved, and most taxonomic descriptions cover only adults. Study of juvenile instars has too often been limited simply to recognizing them as immature so as not to mistake them for new species. Knowledge of ontogeny is important in understanding evolutionary processes and phylogenetic relationships, however, especially those that are indicated by heterochrony of the carapace (Gould, 1977).

Ostracodes are ideal candidates for study of

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heterochrony. Such studies require determination of the developmental stage of an organism, which is possible with ostracodes because they molt a fixed number of times in life. A prerequisite to the search for heterochrony among ostracodes is detailed descriptions of the ontogenies of individual species, including quantification of changes in size and shape.

We have studied ontogenetic changes and morphological variability in the carapace of *Tyrrhenocythere amnicola* (Sars, 1888) (Maness and Kaesler, 1985), a brackish-water species of the Mediterranean area that is used in biostratigraphy and paleoenvironmental analysis. *T. amnicola* is a member of the Hemicytheridae, an important Cenozoic, predominantly marine family. The ontogenies of hemicytherid species are poorly known, and the ontogeny of *T. amnicola* may be representative of hemicytherid ontogeny in general. While the immediate goal was a better understanding of the ontogeny of *T. amnicola,* this study also lays groundwork for a better understanding of this species's phylogenetic relationship to similar ostracodes. Moreover, it demonstrates some of the methods that are useful in studying ontogeny of ostracodes.

This study focused on taxonomically useful features of the carapace: muscle-scar pattern, shape, hinge, surface ornamentation, marginal area, and normal-pore canals. Hazel (1967) used muscle scars and shape to delineate subfamilies of Hemicytheridae and primarily shape, muscle scars, hinge, and surface ornamentation to delineate genera. *Tyrrhenocythere* is distinguished primarily by bundled radial-pore canals on its marginal area (Ruggieri, 1955). Krstič (1977) recognized three groups of *Tyrrhenocythere* species based on shape and surface ornamentation.

Note that shape, particularly shape of the outline, is used at several taxonomic levels in the identification of *T. amnicola,* as it is in the identification of ostracodes in general. Qualitative descriptions, however, are inadequate for rigorous comparisons of shape, because although the human eye and brain discriminate shapes remarkably well, description fails when differences are subtle, "sample sizes large, variation multidimensional, and groups ill defined" (Scott, 1980:758). To avoid these problems in the present study of ontogenetic differences in shape, we used quantitative methods to describe changes of shape.

Shape analysis has recently stirred interest of scientists in diverse fields, and many techniques have been developed. Researchers must decide which technique to use, depending on their objectives and the questions to be answered (Kaesler and Foster, in press). Our objectives in this study of the shape of *T. amnicola* were (1) to show graphically how shape changes during ontogeny and (2) to archive a representative shape for each instar in such a way that shape and shape change of this species are available for comparison with those of other ostracodes. In addition, we wanted to know (3) whether change of shape is unidirectional, (4) at what stage or stages shape changes the most, and (5) if sexual dimorphism is apparent in early instars.

Material

More than 2,000 specimens of *T. amnicola* were picked from sediment samples of a piston core taken by Lamont-Doherty Geological Observatory's *Vema* (Cruise 10, 1956). The core site is in the Gulf of Corinth, Greece, lat 38°21′40″ N., long 22°25′35″ E., in 73.2 m of water. The 8.95-m-long core was sampled at 50-cm intervals except for the top half meter, which was sampled at 10-cm intervals. Clay, fine to medium sand, and shell debris comprised the sediment; and *T. amnicola* was abundant throughout the core. The entire cored interval is assumed to be recent in age, although precise stratigraphic control is lacking. No morphological differences of *T. amnicola* from top-to-bottom of core were observed.

Specimens were sorted into instars by size and morphology. Seven instars and adults were present. Most species of podocopid ostracodes have eight preadult instars, but some have fewer. If *T. amnicola* is typical, only the earliest instar was absent. A histogram of number of specimens per instar (Fig. 1) shows a pattern typical of ostracode assemblages unsorted by current action (Whatley, 1983): late instars (A-1 and A-2) dominate the assemblage; adults are common; and early instars are rare.

Methods

Three kinds of ontogenetic change in the carapace of *T. amnicola* were examined: change

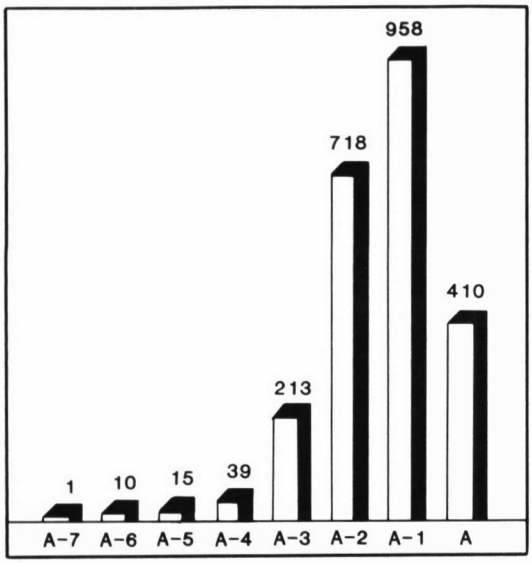

Fig. 1. Frequency histogram of number of specimens occurring in each instar. Adult is labelled A; first instar from adult is A-1; etc. Pattern is typical of ostracode assemblages unsorted by current action.

of such morphological features as pore canals and hinge, change of size, and change of shape. The methodology for analyzing the first two of these kinds of change is straightforward. To determine at what stage features of the carapace develop and to describe their changes in ontogeny, each instar was studied and illustrated using both light and scanning electron microscopy. To determine whether size increases at a constant rate during ontogeny and, if so, at what rate, log size was regressed on instar number. Size was measured by digitizing the outline of a camera-lucida image of each specimen and computing the area enclosed by the outline. A logarithmic transformation of size prior to statistical analysis is common practice in biological measurements because of allometric growth (see, e.g., Gould, 1966) and was also necessary here because a nonlinear measure of size was used.

The methods used to analyze change of shape and variability of shape are less straightforward. Two fundamental problems complicate comparison of shapes: choosing appropriate variables for the measurement of shape and accounting for differences in size. In traditional multivariate morphometrics, a number of point-to-point distances are measured. After these measures of distance are subjected to various techniques to reduce the dimensionality of multivariate space, contrasts in shape may become apparent in bivariate plots. Alternatively, distance measurements are converted to ratios; for example, length-height ratios of ostracodes are often calculated. The problem with using distance measurements is that their selection is *ad hoc,* and valuable information on shape may be lost (Scott, 1980; Bookstein, 1982). The distances selected do not necessarily correspond with the directions of greatest differences of shape. An oval and an oblong carapace may have identical length-height ratios, which are totally inadequate as descriptors of these shapes. Similar problems are inherent in many multivariate data sets.

Differences in size complicate comparisons of shape. Two groups of organisms that differ in shape also typically differ in size, and traditional multivariate morphometric techniques typically confound shape and size. Although various schemes to factor out size have been proposed (see discussion by Humphries and others, 1981), there is no consensus among morphometricians on how best to deal with size when shape is of primary interest.

To study change of shape in ontogeny, we needed a method that is capable of comparing shape independently of size and detecting subtle differences in shape. Furthermore, because shape of the outline itself is a critical feature in ostracode taxonomy, we wanted a method that deals directly with the outline rather than a combination of distance measures that describe the outline only imperfectly. Eigenshape analysis was used because it has decided advantages over such other techniques as Fourier analysis (Lohmann, 1983). Our primary concern was with the outline itself, but as Bookstein (1978:64) pointed out, "The boundary represents the shape, but it is not the boundary which grows." To show better how shape changes from one instar to the next, we have also used biorthogonal analysis (Bookstein, 1978, 1980, 1982). This technique, which is also independent of size, operates on homologous landmarks, such as occur over much of the ostracode's carapace rather than on the outline.

Eigenshape analysis.—We followed the method of Schweitzer and others (1986) to examine quantitatively the change of outline with growth

using eigenshape analysis. For analysis, approximately 30 well-preserved specimens were selected from each instar except the earliest, of which fewer specimens were available. The first step in the analysis was to digitize points along the outline of each specimen to record their Cartesian coordinates. Although eigenshape analysis is not based on homologous landmarks, it presupposes that one is comparing broadly homologous regions of the outline. To insure this geometrical homology from form to form, we used the same starting point on each specimen, which was the point where selvage and outline become coincident posteriorly (Fig. 2). Location of this point was estimated in the earliest instars. Each specimen was mounted in water, and its image was projected with transmitted light onto a digitizing pad. We used a hand-held digitizer, which has the advantage over a microprocessor-controlled system that irregularities due to dirt on the edge of the carapace are avoidable. Of course, a hand-controlled system is slower, and a shaky hand can introduce irregularities into the outline. To eliminate irregularities, we smoothed each outline using parametric cubic splines (Evans and others, 1985). Each outline was interpolated into 128 segments of equal length, a convenient number that is sometimes used in Fourier analysis of outlines. Outlines were then plotted and inspected.

The next step was to represent each outline using the phi-star function, a normalized version of Zahn and Roskies's (1972) phi function, which measures net angular change in direction at every point along an outline. The phi function itself is obtained by taking tangents to the 128 points spaced equally around the curve of the outline and calculating angles between the tangents and the tangent at an arbitrary starting point. To normalize the phi function of an outline, the phi function of a circle of equal area is subtracted. The resulting phi-star function is an exact description of how the outline differs from a circle, a description that is independent of size. Advantages of using phi-star functions as descriptors of shape include the fact that all information necessary for precise reconstruction of an outline is retained, reentrants along an outline do not affect the function, and computing the center of the shape is not necessary (Lohmann, 1983).

At this step in the analysis each specimen

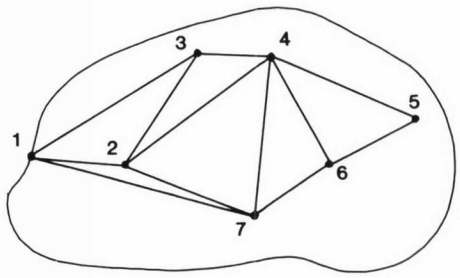

Fig. 2. Homologous landmarks used in quantitative analysis of shape. Point 1 is where selvage and outline become coincident posteriorly; points 2 to 7 are normal-pore canals. Point 1 was the starting point for digitizing outlines for eigenshape analysis, and points 1 to 7 define the six homologous triangles used in biorthogonal analysis.

was represented by 128 variables, each measuring the value of the phi-star function at a specific point on the outline. We emphasize that these points are not necessarily biologically homologous. Within each instar these variables were highly correlated with each other because each specimen has nearly the same shape. Specimens were grouped by instar, and a separate eigenshape analysis (Lohmann, 1983) was performed on each group to extract the basic shape of the instar and to examine variability within the instar. Eigenshape analysis is simply principal component analysis on a correlation matrix of shape functions. It reduces the number of variables necessary to account for the variation in shape of a group of specimens by constructing eigenshapes (principal components), which are new, independent variables that are linear combinations of the original correlated variables. An eigenshape is in the form of a phi-star function and, like the phi-star function of an original form, can be reconstructed into a physical shape. In any principal component analysis, the first few components typically account for most of the original variation, and later components are difficult or impossible to interpret (Morrison, 1976). Here the first three eigenshapes from each analysis were interpreted.

The first eigenshape summarizes the general or shared shape of an instar, and all specimens were positively correlated with it. It can be thought of as the average shape of an instar, and when reconstructed, it looks like the

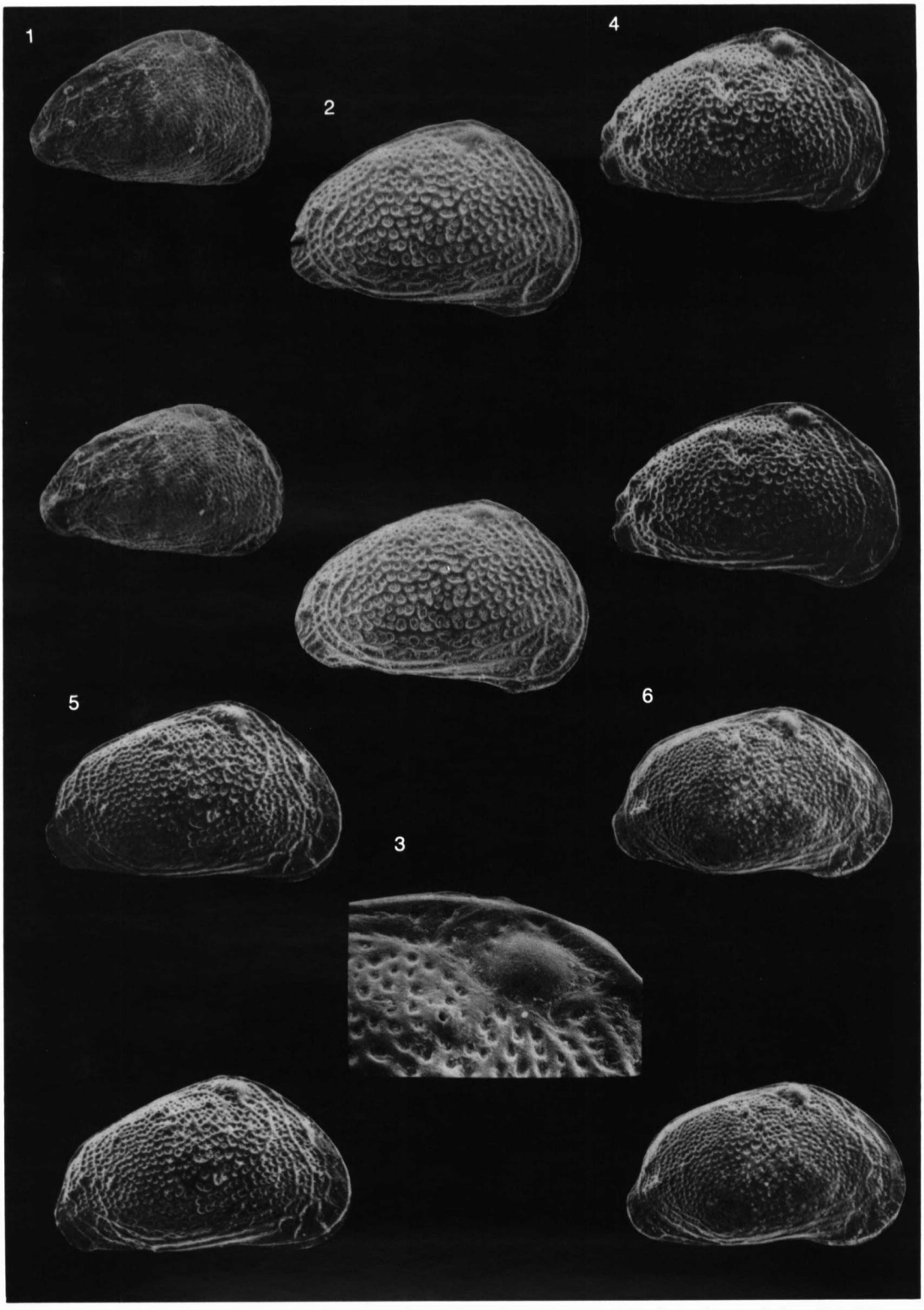

Fig. 3. SEM's, mostly stereoscopic, of right valves of A-7 through A-3 instars of *Tyrrhenocythere amnicola*.—*1*. A-7 instar, KUMIP 1101285, ×217.—*2*. A-6 instar, KUMIP 1101273, ×196.—*3*. Eye tubercle of A-6 instar, ×547.—*4*. A-5 instar, KUMIP 1101273, ×156.—*5*. A-4 instar, KUMIP 1101281, ×108.—*6*. A-3 instar, KUMIP 1101282, ×86.

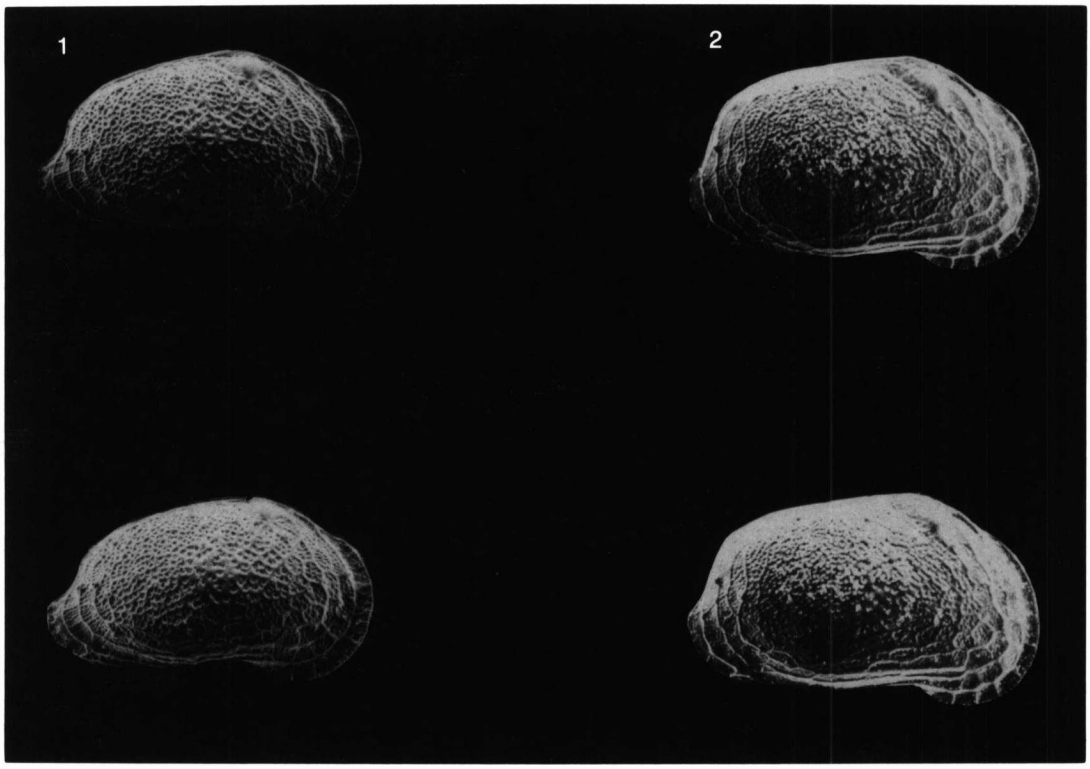

Fig. 4. Stereoscopic SEM's of right valves of A-2 and A-1 instars of *Tyrrhenocythere amnicola*.—*1*. A-2 instar, KUMIP 1101283, ×58.—*2*. A-1 instar, KUMIP 1101284, ×50.

outline of the instar. Subsequent eigenshapes represent contrasts in shape because, while some specimens are positively correlated with a given eigenshape, others are negatively correlated with it. Bivariate plots of each specimen's correlation with the second and third eigenshapes show the major differences in shape within an instar. These plots were used to look for dimorphism, shown by clumping, and aberrant specimens, which plot as outliers.

The first eigenshape from each of the previous analyses was taken as the best representation of each instar's outline. A second eigenshape analysis was performed on these representative shapes for a quantitative comparison of the shape of each instar to the others that would demonstrate a developmental pathway for the species.

Biorthogonal analysis.—D'Arcy Thompson (1961) used transformation grids to show graphically the change from one form to another. In using his method, one superimposes a Cartesian coordinate grid on one form, and points on this grid are matched with homologous points on the other form, a process that deforms the grid and produces a map of the deformation of one form onto another. Thompson's method, however, was never formulated mathematically and has been little used because of the imprecision in constructing the grids.

Bookstein's (1978, 1980, 1982) biorthogonal analysis, a modification of Thompson's method, is mathematically precise. Thompson's method started with a square grid over the original form that was subsequently transformed into a curvilinear grid on the new form. At specific, homologous points on the forms, Bookstein's method aligns a grid with the directions of greatest and least change of shape. These directions are perpendicular to each other, producing a grid that is rectilinear on both forms.

To construct the grid, triplets of homologous landmarks are connected to make triangles. Each triangle on one form is then deformed onto its homolog on the other form.

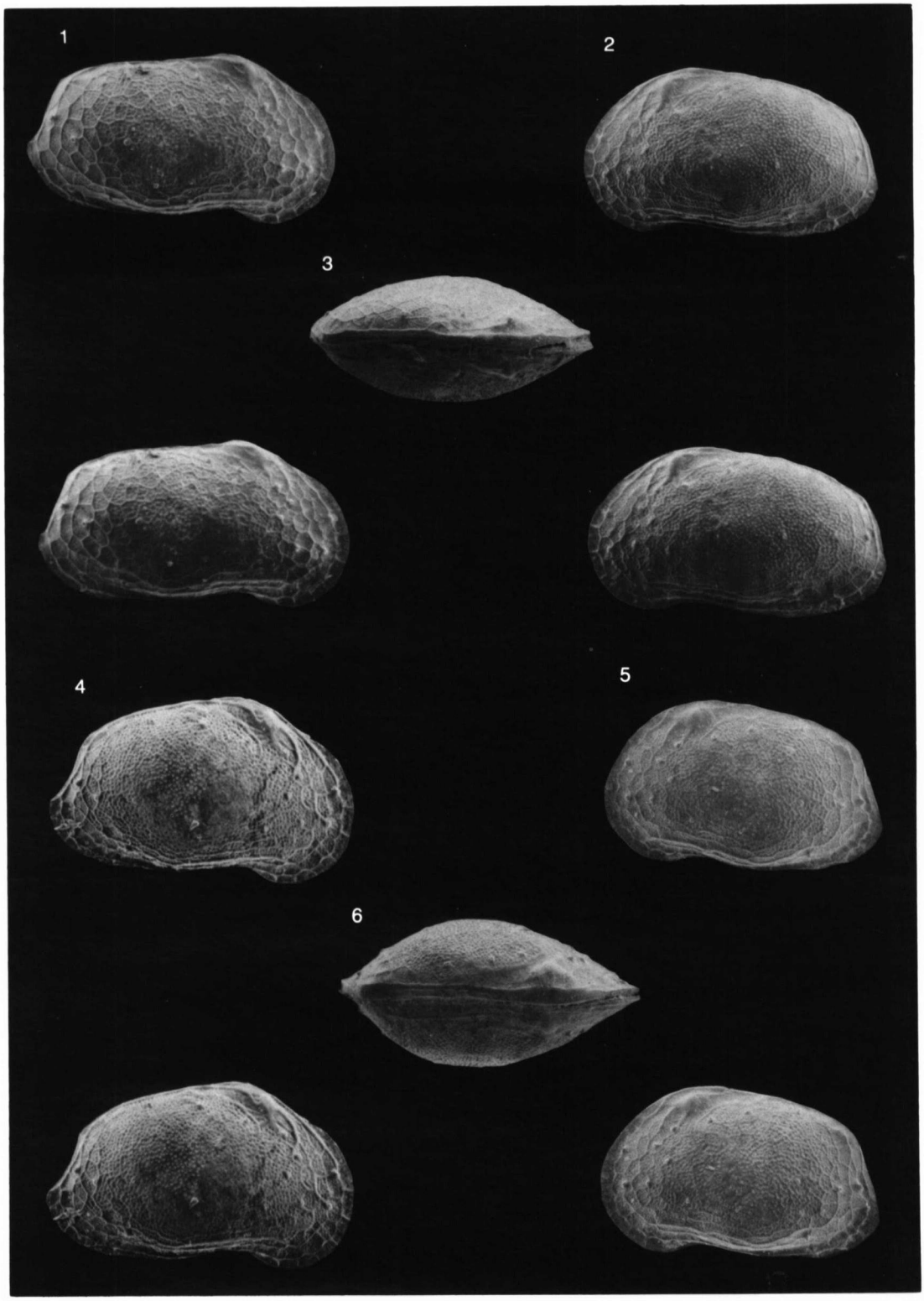

Fig. 5. SEM's, mostly stereoscopic, of adult *Tyrrhenocythere amnicola*.—*1*. Male right valve, KUMIP 110251, ×38.—*2*. Male left valve, KUMIP 1101250, ×37.—*3*. Dorsal view of male, KUMIP 1101293, ×37.—*4*. Female right valve, KUMIP 1101253, ×41.—*5*. Female left valve, KUMIP 1101269, ×39.—*6*. Dorsal view of female, KUMIP 1101292, ×35.

Circles within triangles of the original form are deformed to ellipses. Each ellipse has a long axis and a perpendicular short axis, the directions of greatest and least dilation respectively (specific rate of change of length). Dilations are plotted as crosses centered within the triangles. For each triangle, they show directions of greatest and least change of shape, and their lengths show relative amount of stretch or shrink in those directions. What results is a graphic representation of the stretch and shrink necessary to deform one form onto another, independent of differences in size.

The first step in applying Bookstein's method to shape change in *T. amnicola* was selecting homologous morphological features to use as vertices of triangles. Ideally, one would like complete coverage of a form, with many points on the outline itself. Establishing homology of points from instar to instar proved difficult, however, and we selected seven points (Fig. 2) that were connected to form six homologous triangles using the method of Kaesler and Foster (in press). The seven points on each specimen were digitized, and lengths of all sides of triangles were computed. The average configuration of each instar was computed and used as a representative form for biorthogonal analysis. A separate biorthogonal analysis was then performed on each pair of successive instars.

ONTOGENETIC CHANGES IN THE CARAPACE

Correct identification of the earliest known instar (A-7) of *Tyrrhenocythere amnicola* by comparison only with an adult specimen would be difficult. Its identity, however, is apparent when the ontogenetic sequence of instars, A-7 through adult, is studied (Figs. 3, 4, and 5). The appearance of *T. amnicola* does not change dramatically from one instar to the next; most apparent are changes in shape and surface ornamentation and the addition of pore canals with growth.

Shape.—Adults are sexually dimorphic; males are generally larger, more elongate, and more laterally compressed than females (Fig. 5). Sexual dimorphism, however, is subtle; and separating the sexes is sometimes quite difficult. We observed no differences in ornamentation of the carapace between the sexes; dimorphism is apparently limited to shape of the carapace. In addition to sexual dimorphism, the posterior region of the right valve has a more strongly developed caudal process than the left valve (Fig. 5), and the right valve is slightly smaller.

The primary change of shape in ontogeny is a decrease in slope of the dorsal margin from A-7 through adult. The most dramatic change occurs from A-6 to A-5; note that the carapace becomes more elongate and that positions of eye and dorsal normal-pore canals, although homologous from instar to instar, appear to shift anteriorly relative to the rest of the carapace due to expansion of the posterior part of the carapace (Fig. 3,*2,4*). Shape is discussed quantitatively in a later section.

Surface ornamentation.—All instars are covered densely with fine pits. A faint reticulum near the free margin is weakly developed in early instars, becoming stronger later. Well-preserved specimens are translucent, but an

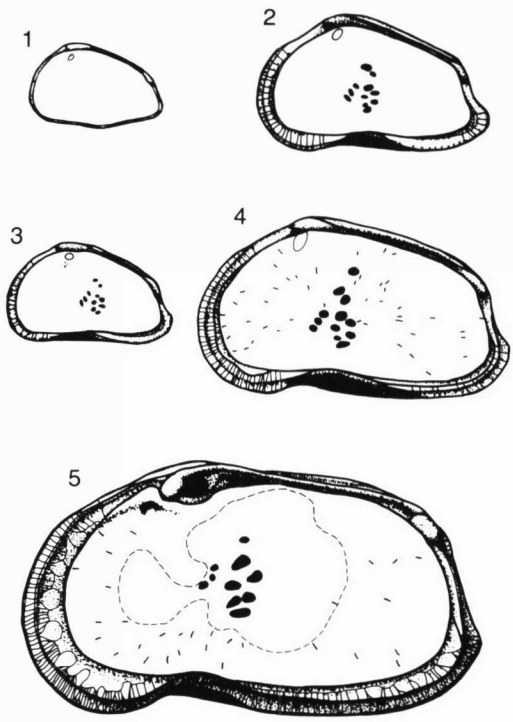

Fig. 6. Camera-lucida drawings of interiors of right valves of *Tyrrhenocythere amnicola*, A-4 through adult. All approximately ×100.—*1*. A-4 instar.—*2*. A-2 instar.—*3*. A-3 instar.—*4*. A-1 instar.—*5*. Adult male.

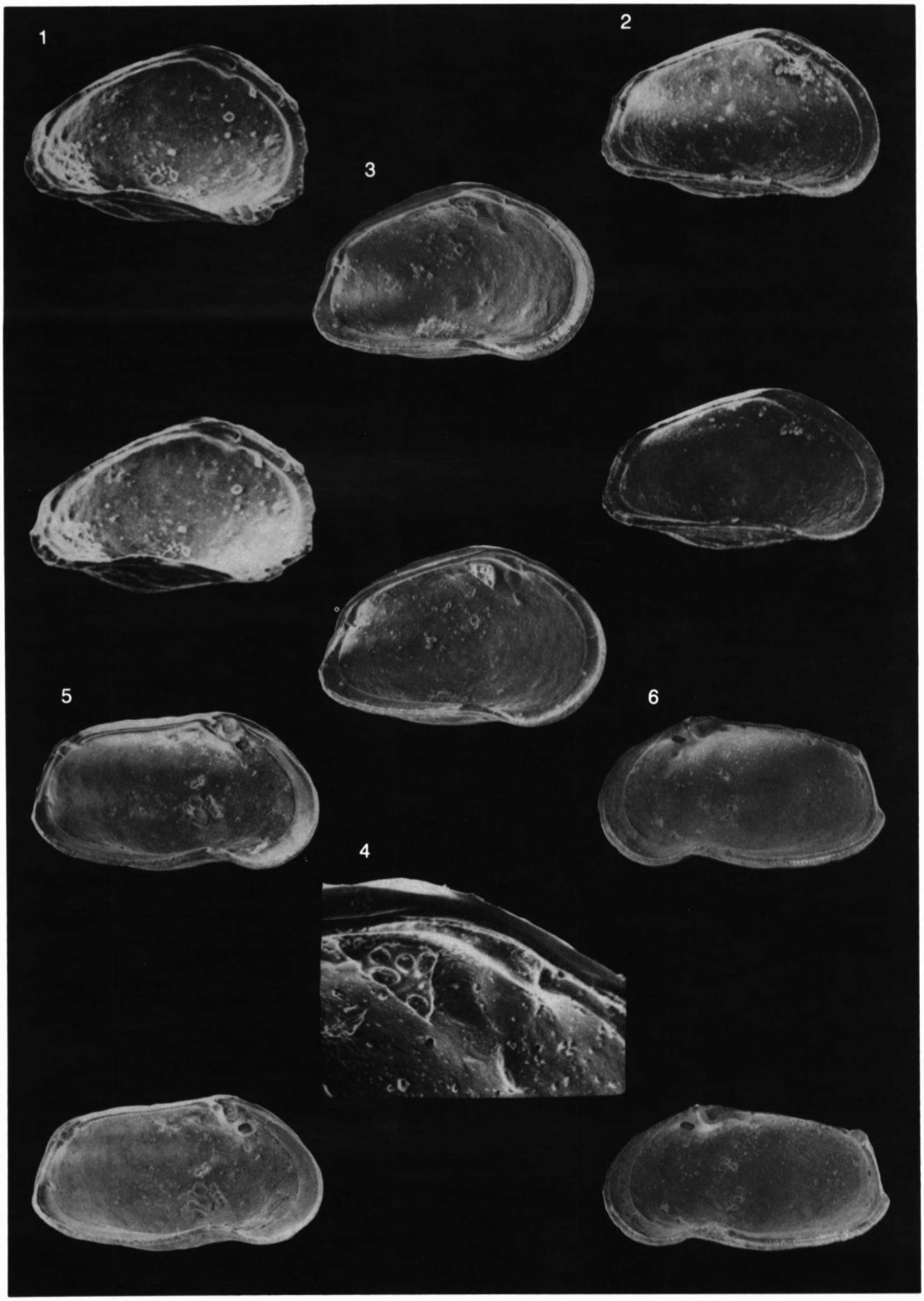

Fig. 7. SEM's, mostly stereoscopic, of interiors of *Tyrrhenocythere amnicola*.—*1.* Left valve of A-6 instar, KUMIP 1101286, ×169.—*2.* Left valve of A-4 instar, KUMIP 1101290, ×110.—*3.* Left valve of A-2 instar, KUMIP 1101289, ×58.—*4.* Dorsal muscle scars, near eye pit, A-2 instar, ×200.—*5.* Adult male left valve, KUMIP 1101256, ×31.—*6.* Adult male right valve, KUMIP 1101257, ×31.

opaque area is usually present in the central region of each valve. A distinct eye tubercle is present at least from A-6 to the adult. Adults have 15 raised, simple normal-pore canals, some of which are present in early instars. Many less-prominent, simple normal-pore canals are scattered over the carapace. Number of normal-pore canals increases in ontogeny from approximately 10 at A-7 to between 40 and 50 at adult.

Muscle scars.—The central muscle-scar pattern in A-3 through adult is typically hemicytherid: a vertical row of four adductor scars, with dorsomedian scar divided and ventromedian scar usually divided, and an oblique row of three frontal scars anterior to the adductors (Fig. 6). No muscle scars were observed in earlier instars. A lone scar, sometimes divided, is located dorsomedially. Dorsal muscle scars were observed in one specimen (Fig. 7,*4*) but are generally not preserved.

Marginal features.—A vestibule is lacking until the A-3 instar; it becomes wider in subsequent instars and is partitioned in the adult (Fig. 6). Each pocket is the base of a fanlike bundle of radial-pore canals. This unusual vestibule is the primary distinguishing feature of the genus.

A selvage is also lacking until the A-3 instar, where it is weakly developed. A much more prominent selvage is present from A-2 through adult (Fig. 7).

Radial-pore canals first appear in the A-4 instar and increase from about five to more than a hundred in adults. The adult hinge is holamphidont and that of juveniles is merodont or lophodont in A-1 and lophodont from at least as early as A-6.

Change of Size in Ontogeny

We regressed size on instar number to determine the rate of growth of *T. amnicola* using log of area (as projected onto a plane surface) enclosed by outline as a measure of the dependent variable size. Choice of a scale to express instar number is arbitrary; for convenience, adult specimens were coded as zero, A-1 as negative one, A-2 as negative 2, etc. A plot of log area against growth stage yields a straight line (Fig. 8), and regression statistics indicate that the model fits the data well (Maness, 1986). Area of the carapace increases by a constant factor of 1.7 from one instar to the next. Disregarding allometric growth, this means that linear dimensions of the carapace increase by a factor of 1.3 and volume by a factor of 2.3, slightly greater than the doubling of size often predicted for crustacean growth.

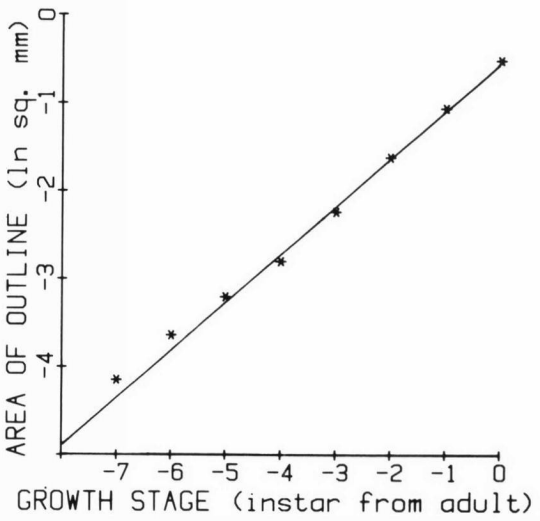

Fig. 8. Plot of size against growth stage. X-axis is instar number, where adult is 0, first instar from adult is −1, etc. Y-axis is log of area enclosed by outline in mm². Points plotted correspond to mean area of outline of each instar, and line is best-fit linear regression. Plot and regression statistics indicate that size increases by a constant factor from one instar to the next.

QUANTITATIVE ANALYSIS OF SHAPE

Results of eigenshape analysis.—In eigenshape analysis, the first eigenshape or principal component represents the general or shared shape of the specimens. All specimens are positively correlated with it, while subsequent eigenshapes represent contrasts in shape. The purpose of grouping specimens belonging to the same instar and performing a separate eigenshape analysis on each of these groups was twofold: (1) to obtain a representative outline of each instar (the first eigenshape) and (2) to examine variation within an instar using bivariate plots of subsequent eigenshapes. If in fact nonrandom variation occurs within an instar, then the first eigenshape from the analysis is not as good a representation of the instar as it might be because it is a composite of two or more

groups with different shapes.

Results of eigenshape analyses of each instar, which are summarized in bivariate plots of each specimen's correlation with the second and third eigenshapes, indicate that specimens belonging to the same instar are roughly the same shape; nonrandom variation is lacking (Maness, 1986). The adult is an exception, however, as a strong systematic variation in shape among specimens is indicated (Fig. 9). Males and females plot separately because of sexual dimorphism.

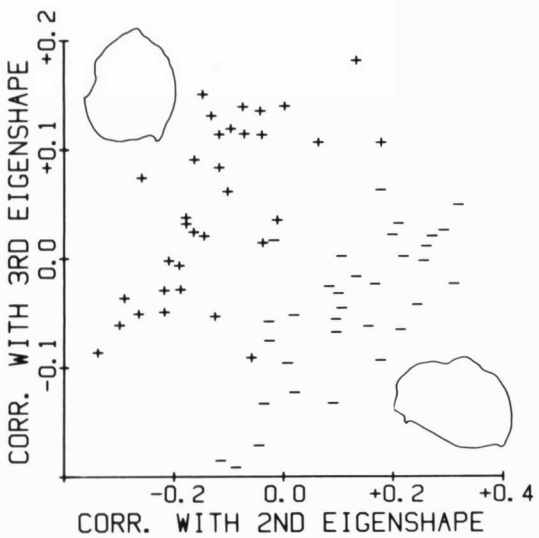

Fig. 9. Variation in shape of outline of adult *Tyrrhenocythere amnicola*. Each specimen's correlation with the second and third eigenshapes is plotted; these eigenshapes summarize most of the contrast in shape of adults. Eigenshapes are reconstructed at the ends of axes. Specimens were sorted by sex prior to eigenshape analysis (males are −, n = 33; females are +, n = 33). Note clear separation of the sexes.

Only two specimens, one male and one female, plot in the wrong groups. Note that both of these specimens plot at the edge of their respective groups because they are not strongly dimorphic. We originally had trouble grouping precisely such specimens as these according to sex and needed to study them in dorsal view, note relative sizes, and make side-by-side comparisons to identify them. Using quantitative descriptions of outlines alone, we undoubtedly would have misidentified more specimens. With eigenshape analysis, however, and using only information from the outline that is independent of size, the sexes are clearly separated. This indicates that the technique is capable of distinguishing subtle differences in shape. The lack of nonrandom variation in shape within juvenile instars indicates that sexual dimorphism is not manifest until the adult stage.

We can see how males and females differ by looking at the reconstructed second and third eigenshapes (Fig. 9). Males are generally positively correlated with the second eigenshape and negatively with the third; females are generally negatively correlated with the second eigenshape and positively with the third. These correlations result from the fact that males are relatively drawn out posterodorsally, whereas females are more rounded posteroventrally.

How does eigenshape analysis compare with more traditional methods of showing dimorphism? A length-height plot shows that although males and females generally plot separately, the separation is not great. Separating the groups without a priori knowledge of their sex is impossible (Fig. 10). Eigenshape analysis does a much better job of detecting dimorphism.

First eigenshapes from analyses of each instar of *T. amnicola* (Maness, 1986), including

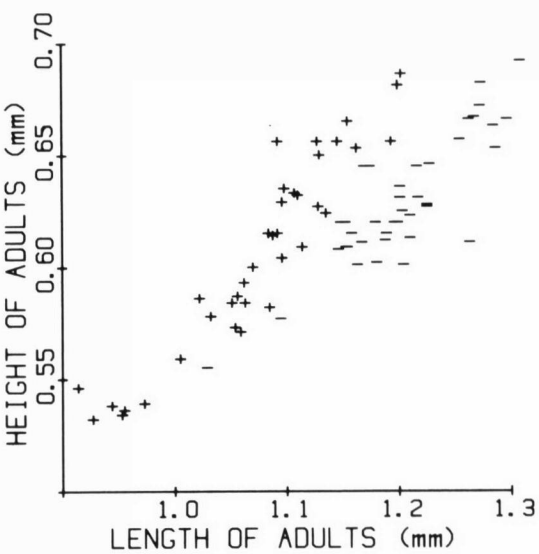

Fig. 10. Plot of length vs. height of adults (males are −, n = 33; females are +, n = 33). Note that although males and females plot separately, the separation is not great.

males and females analyzed separately, were taken as the best representation of each instar's outline. The use of first eigenshapes, which account for 92 percent of the total variation, as representative outlines allowed for comparison of the instars without considering any within-instar variation.

To facilitate comparisons among instars, another eigenshape analysis (Maness, 1986) was performed on these first eigenshapes, a total of nine shapes (7 juvenile instars and both male and female adults). The first eigenshape again represents general or shared shape and is of no interest here. The second eigenshape represents the major contrast in shape of the instars and accounts for 5 percent of the total variation. The third eigenshape, which accounts for 1 percent of the total variation, is not interpreted because its associated eigenvalue does not differ significantly from that of the fourth eigenshape. Principal components associated with eigenvalues that do not differ significantly from each other are meaningless because such eigenvalues indicate a region of spherical variation in the data, and an infinite number of orthogonal axes is possible in spherical space (Neff and Marcus, 1980).

The second eigenshape is considered the only one of biological significance, although the third eigenshape helped to discriminate between sexes (Fig. 9). It is plotted against growth stage to show the developmental pathway of *T. amnicola* (Fig. 11). Earliest instars show a positive correlation with this shape, which has high height relative to length and a subtriangular posterior portion. Latest instars show a negative correlation with this shape because they are more elongate and have more rounded posterior ends. The plot shows that change of shape is monotonic because all instars are in proper ontogenetic sequence with the exception of the adult male, which plots between the A-2 and A-1 instars. It also shows the relative amount of change between growth stages. Between A-7 and A-6 there is little change in shape, while relatively large changes occur from A-6 to A-3. Instars A-3 and A-2 are similar in shape, but a fair amount of change of shape occurs between A-2 and A-1. A-1 is intermediate in shape between males and females.

Biorthogonal analysis of instars.—A separate biorthogonal analysis was performed on each pair of successive instars using an average form computed for each instar (Maness, 1986). Poor preservation of the A-7 specimen precluded its use. Deformation grids (Fig. 12) show the stress field necessary to transform each instar to its next growth stage. Each grid is plotted within the earlier of the two instars being compared. Relative lengths of each arm in a cross indicate amounts of change of shape within each of the homologous triangles (Fig. 12,*1*). The long arm of a cross is in the direction of greatest change of shape and the short arm is in the direction of least change. If arms are of nearly equal length, that triangle has changed little in shape during the transformation from one instar to the next. If one arm is much longer than the other, the triangle has significantly changed its shape.

A major change of shape occurs between A-6 and A-5 (Fig. 12,*2*). Axes of maximum strain are unaligned, indicating that deformation occurs in many directions. During growth, points 3, 4, and 7 shift anteriorly relative to the others. The change from A-5 to A-4 (Fig. 12,*3*) shows all axes aligned in a direction that indicates posterodorsal-anteroventral stretching.

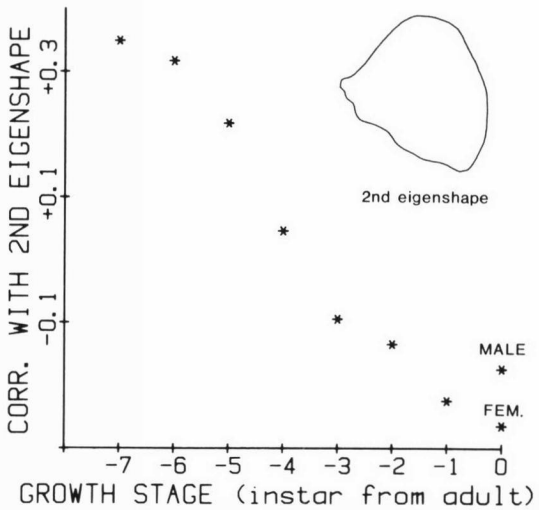

Fig. 11. Variation in shape among instars. Each instar's correlation with the second eigenshape is plotted; this eigenshape summarizes the major contrast in shape of outline of all instars. Plot shows that change of shape in ontogeny is monotonic. It also shows the relative amount of change of shape between successive instars.

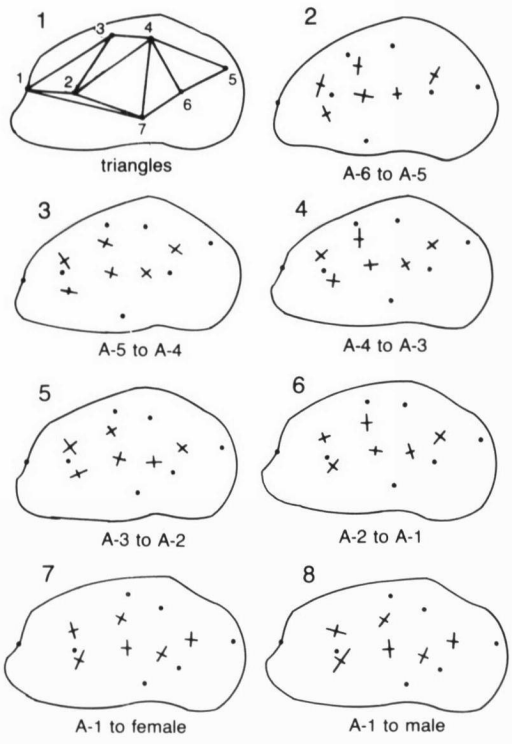

Fig. 12. Graphic representation of biorthogonal analyses of successive instars.—*1*. Homologous triangles used in analysis.—*2–8*. Strain field necessary to deform an instar to its next stage.

This stretching causes points 3 and 4 to shift posteriorly and point 7 to move anteriorly. Changes from A-4 to adult are less pronounced. The anterior region is nearly stable in shape from A-4 to the adult except that distance between points 5 and 6 widens relative to that between points 6 and 7. Little change takes place between instars A-4 and A-3 (Fig. 12,*4*). From A-3 to A-2, however, the triangle defined by points 1, 2, and 7 becomes more elongate. Growth from A-2 to A-1 causes point 2 to shift anteriorly (Fig. 12,*6*). Note the difference in change of shape from A-1 to adult female and A-1 to adult male (Figs. 12,*7,8*). Anterior regions are virtually identical, but the transition from A-1 to the male results in a greater and more posteriorly localized change than the transformation to the female. Apparently sexual dimorphism is manifest only in the posterior region of the carapace.

Discussion.—Both eigenshape and biorthogonal analyses show that the posterior region changes most in ontogeny, presumably because ostracodes add appendages and reproductive organs posteriorly as they grow. Both methods show that shape changes most in early instars, although there are some differences in results from the two methods. Eigenshape analysis showed a major change between A-4 and A-3 (Fig. 11), whereas biorthogonal analysis showed little change (Fig. 12,*4*). Eigenshape analysis showed little change between A-3 and A-2 (Fig. 11), whereas biorthogonal analysis showed a fair amount of change in the posterior region (Fig. 12,*5*).

Such differences should be expected because the analyses operated on different kinds of data. Eigenshape analysis used segments along the outline; biorthogonal analysis used segments between homologous points away from the outline. If homologous points along the outline could have been identified and used in biorthogonal analysis, there might have been closer agreement between the methods. Even so, differences are to be expected, as the methodologies and underlying philosophies of these techniques differ.

As is generally true, a trade-off is made in choosing one method over the other. With eigenshape analysis, we deal with the outline itself in its entirety, and the outline is important in distinguishing shapes and identifying ostracodes. We lose strict homology, however, because there is no guarantee that a given segment on one form is homologous with the same segment on another. The homology used in eigenshape analysis is geometrical rather than biological. With biorthogonal analysis, we have strict biological homology but lack complete coverage of the outline. The shapes we compare are not outlines—only imperfect representations of them. We think that geometrical homology generally corresponds closely to biological homology in closely related organisms and that eigenshape analysis is the best way to compare ostracode outlines. The problem with biorthogonal analysis of ostracode outlines is that outlines generally lack easily recognized homologous points. This method, however, is excellent for comparing configurations of such interior points as normal-pore canals. Both methods are applicable to studies of heterochrony in ostracodes.

The representative shapes of *T. amnicola*

archived here could be compared with those of similar ostracodes. In particular, we would like to see a comparison of ontogenies of species of *Tyrrhenocythere* and *Aurila*. Krstič (1977) noted that shapes of carapace and types of surface ornamentation in *Tyrrhenocythere* are almost identical to those in its close relative *Aurila*. Indeed, some species of the two genera can be distingushed only on the basis of size and the vestibule of adults, which is unpartitioned in *Aurila*. This similarity indicates a possible heterochronic relationship between the genera. *Tyrrhenocythere* may show recapitulation with respect to *Aurila* both because its morphology is similar to that of *Aurila* until the adult stage and because it is larger.

CONCLUSIONS

1. Size increases at a constant rate in the ontogeny of *T. amnicola*; area enclosed by outline of the carapace increases by a factor of 1.7 from one instar to the next.

2. Change of shape is for the most part monotonic during growth and is concentrated in the posterior region of the carapace. More change occurs early in ontogeny than late, and the biggest change occurs between the A-6 and A-5 instars.

3. Sexual dimorphism in the carapace of adults is limited to shape and does not affect other morphological features. The primary differences in shape between the sexes are in the posterior region of the carapace. Juvenile instars show no evidence of sexual dimorphism.

4. Eigenshape analysis is a powerful technique for extracting the basic shape of a group of biological outlines and comparing two or more groups of outlines.

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Repository.—Specimens have been reposited with The University of Kansas Museum of Invertebrate Paleontology and given numbers KUMIP 1,101,248 to KUMIP 1,103,837.

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