ORDINATION IN THE STUDY OF MORPHOLOGY, EVOLUTION AND SYSTEMATICS OF INSECTS:

APPLICATIONS AND QUANTITATIVE GENETIC RATIONALS

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ELSEVIER

Amsterdam – London – New York – Tokyo 1992

PRONOTAL SHAPE: A SOURCE OF CONFUSION OR PANACEA IN SYSTEMATIC STUDIES OF TREEHOPPERS (HOMOPTERA: MEMBRACIDAE)?

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Abstract

Treehoppers are easily distinguished at the family level by their swollen pronotum, but their identification and taxonomic separation below the tribal level is often difficult because frequently pronotal shape is the only attribute that characterizes their genera. Within the tribe Smiliini, we explore the potential use of pronotal shape, in profile, to distinguish genera and deduce a phylogenetic network. We digitized the pronotal outline of members of the tribe, using 20 spaced nodal points along each of four pronotal contours. Cubic splines were used to calculate distances between the analogous nodal points among the contours, and the angles of the resulting segments were determined. The data were size scaled and subjected to discriminant function analysis, after which the centroid values for the taxa were networked using a Euclidean distance phylogenetic networking algorithm. The resulting networks, as phylogenetic hypotheses, produce credible evidence for the evolution of host plant utilization by the treehoppers, and for their historical biogeography.

1 Introduction

Shape of an individual or a characteristic structure is often one the most diagnostic features that confers membership in a taxon, but it is also the most elusive feature to measure. The elegant shape transformations by D'Arcy Thompson 1961) of fish, or Raup (1962, 1966) for snail shells, provides

convincing evidence not only of the importance of shape analysis in evolutionary biology (Futuyma, 1986) but also its potential in systematic studies. Bookstein et al. (1985) have formalized this approach to shape by using trusses that can be transformed through a biorthogonal grid system. At present, the analysis can be used to transform pairs of taxa, but it has not been developed to the point where it can be applied to a large array of taxa.

Systematists, in general, do not deal with shape directly in this manner, but rather look for suites of homologous, discrete multistate characters that can be polarized or characterize variations in shape as a multistate condition (Wiley, 1981); such discrete state characters may then be used in a cladistic analysis. After cladistic analysis, the result can be a cladogram in which the array of taxa visually resemble a shape transformation series. Such results are not surprising, because morphological, multistate characters in monophyletic lineages, in theory, are an external measure of shared and derived developmental processes that are controlled by genetic loci. How these characters are spatially arranged is dictated by intraspecific allometric relations, although interspecific differences may reflect shared or derived patterns of allometry (Futuyma, 1986).

One of the confusing aspects of allometric patterns, and that of size, is that although there is a strong genetic component, the phenotype potentially can be influenced by environmental factors (Atchley and Rutledge, 1980). Without techniques or direct experimental evidence that permit the elimination of environmental influences on size measures, they can provide misleading results in systematic studies (Humphries et al. 1981). Here we consider how to deal with groups of organisms that can be distinguished by the overall shape and size of a particular structure, but which, nevertheless, appear to have a dearth of characters that can be categorized into discrete, multistate conditions.

2 Pronotum

The Membracidae are easily distinguished from other families of auchenorrhynchus Homoptera by the dorsal expansion of their pronotum. The variety and complexity of pronotal shapes and associated color patterns are as visually striking as the spectacular color patterns of some butterflies and birds. To naturalists, such as Poulton (1903), pronotal shapes and color patterns suggested crypsis, mimicry and aposematic functions. At present, there is no experimental evidence to support either cryptic or mimic functions in any treehopper species.

Experimental evidence for aposematic coloration exists for adults of several species (Wood, 1975a, 1977). These species change color as they mature and lose their defensive chemical qualities, but in others the shape and hardness of the pronotum provide a physical defense (Wood, 1975a). Perforations in the pronotum of one species provides an easily detached segment that could provide the basis for an escape from some types of predators (Mann, 1912; Strumpel, 1983).

Early workers (Funkhouser, 1951) thought the pronotum had no physiological function and was simply a hollow dorsal extension of the prothorax. At least in one species, the presence of trachea, nerves and neurosecretory tissue suggests that it is far from physiologically inert (Wood

1975b). The surface architecture of many species is covered with articulating setae which protrude over deep conical pits that penetrate to the inner wall of the pronotum (Wood and Morris, 1974). Although direct evidence is lacking, the dorsal pronotal expansions may increase the surface area for the reception of sensory information (Wood and Morris, 1974), or act as a dispersal site for pheromones. Sexual dimorphism is common in the Membracidae, raising the possibility that pronotal shape and color may be involved in the location and recognition of mates. Males, after locating a female, spend a considerable amount of time on the side of the female pronotum during precopulatory pairing (Wood, 1974, 1976; Wood et al. ,1984; Wood and Dowell, 1985).

Although the exact functions of the pronotum are at present conjecture, it is clear that it is structurally complex. As might be expected, the pronotum has played a central role in the taxonomy and classification of the group. Early workers (Funkhouser, 1951; Goding, 1926, 1928a, 1928b, 1929; Van Duzee, 1908) used the shape of the pronotum, in addition to other characters, to define subfamilies and tribes. They clearly saw shape transformations but had no way to measure or compare shape, except through written descriptions or line drawings. The end result was confusion at all levels of classification. With the advent of Hennig (1966), modern workers have tended to avoid pronotal shape in an attempt to find more definitive characters that would permit a cladistic analysis.

The only pronotal trait used in higher classification in the Membracoidea, is whether the scutellum is covered. Strumpel (1972) used this character to divide the Membracidae into two subfamilies, the Centrotinae and the Membracinae. Although not defined in a quantitative sense, Strumpel (1972) recognized that within each subfamily there appeared to be a transformation series of pronotal shapes. In contrast, Deitz (1975) used the same pronotal trait as one of a suite of characters to divide the New World Membracoidea into seven subfamilies and 27 tribes. Although he recognizes that pronotal shape may be useful at some levels of classification (L. Deitz, personal communication), he defines subfamilies and tribes without the vagueness associated with written descriptions of shape.

Even though subfamilies and tribes can be defined on the basis of other characters, most generic and specific definitions depend a great deal on pronotal shape. Early monographs relied heavily on elaborate written descriptions and line drawings to describe shape (e.g., Ball, 1932). Caldwell (1949) was the first to attempt a classification of the Ceresini using male genitalic characters. Kopp and Yonke (1979), in the most recent revision of this group, relied heavily on male genitalia, but also used a number of pronotal characters. Although genitalia are potentially very useful characters, they are only helpful in identifying one sex, or in distinguishing complexes of species.

In spite of recent work, diagnostic features for species are often body measurements, such as length, or a series of outline drawings of the pronotum. Regional faunal monographs that provide keys which attempt to minimize the use of pronotal shape (Kopp and Yonke, 1973a, 1973b, 1973c, 1974; Dennis, 1952, 1964, 1965), ultimately have to depend on line drawings, size and color patterns for accurate identification. In general, this works rather well for a given region because of the limited array of species present. However, this approach has limited value in generic revisions and phylogenetic investigations. The quest to find a suite of discrete characters that define genera and species is laudable, but may be unattainable because divergence at

these levels may be the result of selection on aspects of pronotal shape that are difficult to define. We argue here that to ignore pronotal shape is to ignore a major and important aspect of the genome that has undergone transformation in the same way as a suite of discrete characters. The major difficulty with shape is that it is difficult to measure and translate into a set of discrete, multistate conditions that are operational for traditional cladistic analyses.

Because many species descriptions are based on pronotal shape and size, we have been examining techniques to measure these variables. The overall objective of this work is to develop a technique that: (1) provides rapid, accurate, defendable identifications and classifications; (2) can be used to examine intraspecific morphological variation that is related to host plants and geography; and (3) can provide a basis to infer phylogenetic relationships. In this chapter, we report a technique that we have developed to meet these objectives, and we explore its use in defining groups of species in the tribe Smiliini.

3 Tribe Smiliini

Approximately 46% of North American treehoppers are in the Smiliini, and a majority of the genera are restricted to oaks (*Quercus*). Historically, this tribe, as delineated by Deitz (1975), was divided into two tribes, the Telamonini and Smiliini. This early division was based upon size, the shape of the pronotum, and the presence or absence of a cross vein on the hind wing. Unfortunately, the cross vein character is unreliable because Ball (1932), Deitz (1975) and TKW (unpublished data) have found bilaterally differing specimens with the vein present on one wing but not the other. At the generic and specific levels, Ball (1932), Woodruff (1919, 1920, 1924) Dennis (1952, 1964, 1965), and Kopp and Yonke (1973a, 1973b, 1973c, 1974), all failed to find consistent, discrete characters, and without exception, all relied on the shape of the pronotum, color pattern and size measurements for diagnostic features.

At present, genera are characterized by several attributes of the pronotum and size: the presence or absence of a dorsal crest, the origin of the dorsal crest in relation to the humeral hump, the location of the crest's highest point relative to the humeral hump, the presence or absence and location of various surface depressions; descriptive features of the posterior process; and length of the pronotum or body (Fig. 1.). With practice, good illustrations and available types, species identifications are possible, but not particularly reliable among the 22 genera and 200+ species in this tribe of membracids. Both identifications and rigorous defense of apparent pronotal shape transformations between and within genera would be considerably easier and more dependable if sufficient tools were developed to that reflect the ability of the specialist's "gestalt."

4 Generic Assignments

We selected what appeared to be a typical female specimen from each of 168 described and undescribed species within the Smiliini. Each specimen was assigned initially to one of 22 recognized genera (Metcalf and Wade, 1965; Deitz, 1975). Because the generic limits within the tribe are rather vague, we

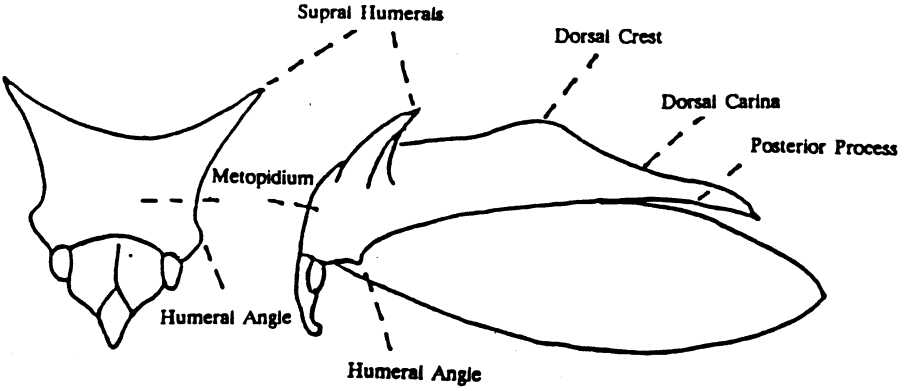

Figure 1. Topographical features of the pronotum that have been historically used to define genera and species of membracids.

combined some genera and reassigned some species accordingly. These reassignments were made on the basis of TKW's interpretation of present generic limits. After reassignments were made, this reduced the number of genera or groups to 16 (Appendix 1).

5 General Morphometric Considerations

With the advent of digitizing equipment, a number of workers with other taxa have experimented with complex equations to define shape. Fourier series have been used to describe the shape of snails (Ferson et al., 1985), bryozoans (Anstey and Delmet, 1973), and diatoms (Younker and Ehrlich, 1977; Stoermer and Ladewski, 1982; Ehrlich et al., 1983). This approach has been criticized (Bookstein et al., 1982, 1985) on the grounds that the coefficients used to characterize shape have no homology and are of little value in understanding morphological transformations in systematic studies. These views are not shared by Ehrlich et al. (1983). Our initial approach was to use coefficients from Legendre polynomials to describe the contours of the pronotum. These coefficients provided very good data reconstruction, but failed dismally to reveal distinguishable groups, in either principal components analysis or discriminant analysis, simply because the coefficients, as Bookstein et al. (1985) pointed out, are neither analogous or homologous among species.

An approach that Bookstein et al. (1985) advocate is the use of homologous landmarks, construction of truss networks and biorthogonal grids for the analysis of morphological transformations. This approach seems to work rather well with organisms whose contours or outlines are marked with homologous landmarks, such as fins. The problem with the membracid pronotum is that the only landmarks are its beginning and end, with no

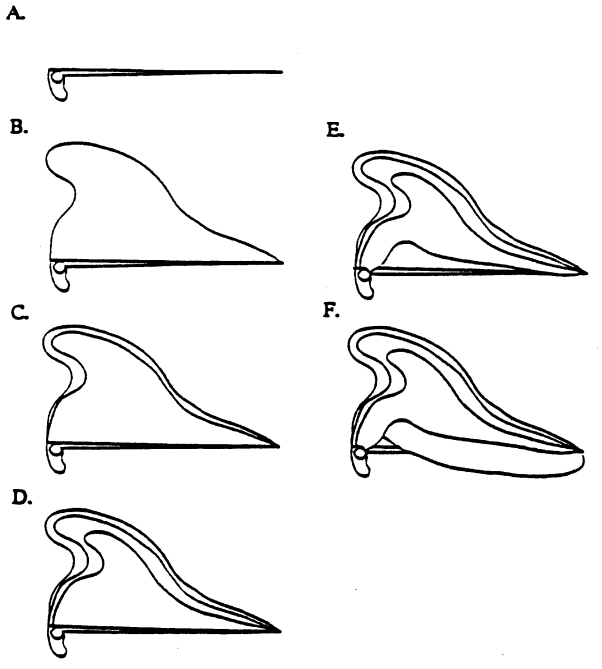

Figure 2. Diagrammatic representation of the pronotum and the contours traced. (A) The dorsal expansion of the pronotum removed, leaving the mid-line and lateral edge. (B) The dorsal expansion along the mid-line (contour 1). (C) The second contour begins lateral to the mid-line, following changes in the surface topography to the tip of the posterior process. (D) The third contour begins above the eye, following the surface topography to the tip of the posterior process. (E) The fourth contour begins at the outside of the eye, continuing along the lateral edge of the pronotum to the tip of the posterior process. (F) Representation of all contours relative to the attachment of the wing.

identifiable, homologous landmarks between them.

Others, such as Lohmann (1983) and Ray (in press) working with leaf shape, identify contours that extend between landmarks. Ray divides these "homologous contours" into line segments of equal length and uses the angles between segments in principal components analysis. Although, initially, we were unaware of Lohmann's or Ray's work, the technique we developed is similar to their analyses of outlines, but also incorporates the spirit of Bookstein's truss analysis.

Our procedure can be envisioned as follows. If the dorsal portion of the pronotum is excised and viewed laterally, it resembles a triangle (Fig. 2A). From this triangular base, a series of lateral contours rise dorsally; these contours extend from above the head and run ultimately to the posterior, terminating at the same point at the tip of the posterior process. Four points above the head were selected (Figs. 2B-2E) at progressively greater lateral distances from the mid-line: (1) the point at the mid-line (Fig. 2B) where the dorsal carinae rises from the head [contour 1]; (2) the point where the dorsal

carina is separated from the humeral hump (Fig. 2C) [contour 2]; (3) the point above the inner-most corner of the eye (Fig. 2D) [contour 3]; and (4) the point defined by the outer-most edge of the eye and the pronotum (Fig. 2E) [contour 4].

6 Digitizing

Specimens were aligned under a Wilde 420 macroskop, with the longitudinal profile of the pronotum in focus along the mid-lateral line. This could be clearly defined as the point where the dorsal carinae rises from above the head and passes along the dorsal surface of the pronotum to the tip of the posterior process. Specimens were then tilted to bring the lateral humeral angle into focus. When this image was transmitted, via a television camera, to the computer screen, it was possible to discern not only silhouette outlines, but also surface topography consisting of bulges and depressions that extended laterally from the mid-line of the specimen.

With the use of a digitizing pad (connected to a Zeiss Videoplan II) set to record x- and y-coordinates every 0.1 mm and a mouse, traces of the contours were made from each of the four landmarks above the head to the tip of the posterior process (Figs. 2B-2E). The Zeiss Videoplan II was directly wired to an IBM PC computer, so that when a given trace was completed, the x- and y-coordinates were captured in a form that could be transferred directly to a IBM (3090 300E) mainframe computer for analysis. Before a trace was made, the following data were entered into the file for each specimen: contour number, sex, species code, individual code, and generic code. These data were carried along with all subsequent data manipulations and statistical analyses. Because the treehoppers in this tribe vary greatly in length and height of the pronotum, magnifications on the macroskop were changed so that the image filled the screen on the Zeiss Videoplan II; when magnifications were changed, the digitizing pad was rescaled. This assured that for each contour trace there would be at least 85 x- and y-coordinates.

7 Measurement of Shape and Size Components

To analyze size and shape successfully, a Fortran program (Appendix 2) was written for the IBM mainframe which, in sequence:

(A) Established a hypothetical reference line between the first point in contour 1 and the tip of the posterior process to provide a common perspective. The original, digitized x- and y-coordinates were translated, so that the first point on contour 1, which began above the head at the mid-line, was considered to be the origin, with $x,y = 0,0$.

(B) Rotated the new coordinates, so that reference line became the horizontal axis (the tip of the posterior process then had a y-coordinate of 0).

(C) Scaled all coordinates, so that all pronota, regardless of species, had reference line lengths of 1; thus, the new coordinates became for the first point on contour 1 ($x,y = 0,0$) and the last ($x,y = 1,0$). The remaining three contours were also similarly scaled.

(D) Translated contours 2 through 4, so that their coordinates for the tip of the posterior process coincided with that for contour 1.

(E) Calculated the arclength from the first point of each contour to successive, adjusted x- and y-coordinates along that same contour. After this was done, the arclength for each contour was normalized (the arclength between points was divided by total arclength) so that the distance from the first point to the last was 1.

(F) Calculated separately, for the x and y-coordinates of each of the four contours, a cubic spline that was relative to the normalized arclength. Using these splines, the x and y-coordinates of points at 0, 5, 10, 15, 20, 25, 30 . . . 100% intervals along each contour (as measured by normalized arclength) were calculated. The resulting 21 nodes divided each contour into 20 line segments, with the terminal node at the tip of the posterior process common among all four contours.

(G) Calculated the (two-dimensional) distance between each of 20 nodes (excluding the terminal node) in contour 1 and analogous nodes in contour 2. Similar, analogous, internodal distances were calculated between contours 2 and 3, and 3 and 4.

(H) Calculated, for each contour, the angle between a reference line and each generated internodal segments. These angles were output to a SAS program, which used them to determine the angles between adjacent internodal segments along each contour.

(I) Calculated, using the unscaled, digitized coordinates, the actual arclength (in mm) of each of the four contours, plus the length (in mm) from the first point of contour 1 to its last point, at the tip of the posterior process.

(J) Calculated, using the unscaled, digitized coordinates, pronotal area (in mm^2) between contours 1 and 4 by using Green's theorem and the trapezoidal rule.

8 Analysis of Essential Shape and Size Components

Strumpel (1972) was the first to attempt an analysis of the relationship between pronotal area and body length. He examined seven species in the genus *Membracis* and found that they formed an allometric series. Because the Smiliini (current limits) was previously divided on the basis of size and pronotal shape by early workers into the Telamonini and Smiliini, we evaluated whether pronotal area (square root), length and the perimeter could be used to partition the tribe. Although there is a tendency for the Telamonini to be larger in all three of these measurements (Fig. 3), there is considerable overlap with the Smiliini and no clear separation between these taxa using these variables. Thus, raw size (length) and general shape measurements, such as pronotal area and perimeter, are insufficient to discriminate among subtribes.

We used canonical discriminant analysis (SAS Institute Inc., 1985) to determine which shape and size measurements, or combinations thereof, if any, could be used to discriminate groups of species. The analysis reduces the number of variables that summarize between-class (group) variation by deriving linear combinations of variables that have the highest multiple correlation with groups. Canonical correlations are normalized so that within-group variance of the canonical variable is one (unity). The second correlation is obtained by finding linear combinations that are uncorrelated with the first canonical variable. Subsequent variables are extracted until the

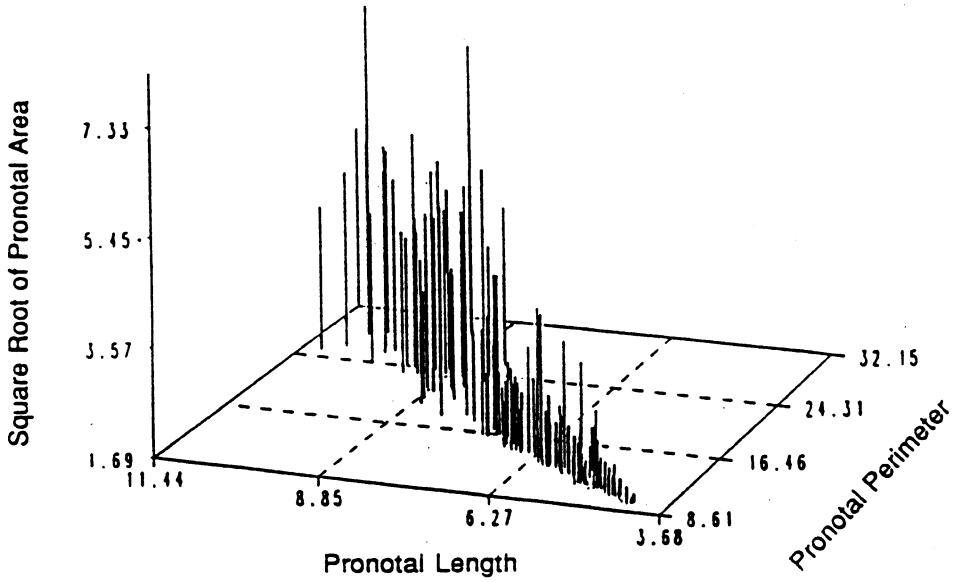

Figure 3. Three dimensional plot of the pronotal area (square root), length and perimeters to determine if the Smiliini can be divided into the Telamonini and Smiliini.

number of new variables equals the number of original variables or one less than the number of groups [n - 1] (SAS Institute, 1985).

We approached the analysis in two ways, using separate analyses on two parallel sets of variables, and sequentially increasing the number of variables within each series as it was analyzed. For the first set of variables, we used measurements that included components of both shape and size (e.g, area [= square root], contour length[s], pronotal length), plus variables that were size-independent (e.g., the angles and distances between spline nodes among contours from the normalized and scaled pronota). The measurements of variables with shape and size variance were transformed using logarithms (base 10), as suggested by Bookstein et al. (1985) to make them comparable to the dimensionless measurements of the scaled, normalized pronota. In the second set of variables, we attempted to directly measure shape, independent of size, by using only the angles and distances between spline nodes among the contours on normalized, scaled pronota.

Our first analysis was conducted using 23 variables; these were: area, pronotal length, length of contour 1, and the 20 angles from contour 1 (Fig. 4A). The results showed that discrimination was rather poor whether size measurements were included (Fig. 4A') or not (Fig. 4A"). When the angles from, and length of, contour 4 were added (Fig. 4B), the results showed a tendency to partition the data plots into two major groups, whether size measurements were included (Fig. 4B') or not (Fig. 4B"). In both the latter cases, the three-dimensional plots of various combinations of canonical variates seemed to separate some genera, and two major, but rather diffuse,

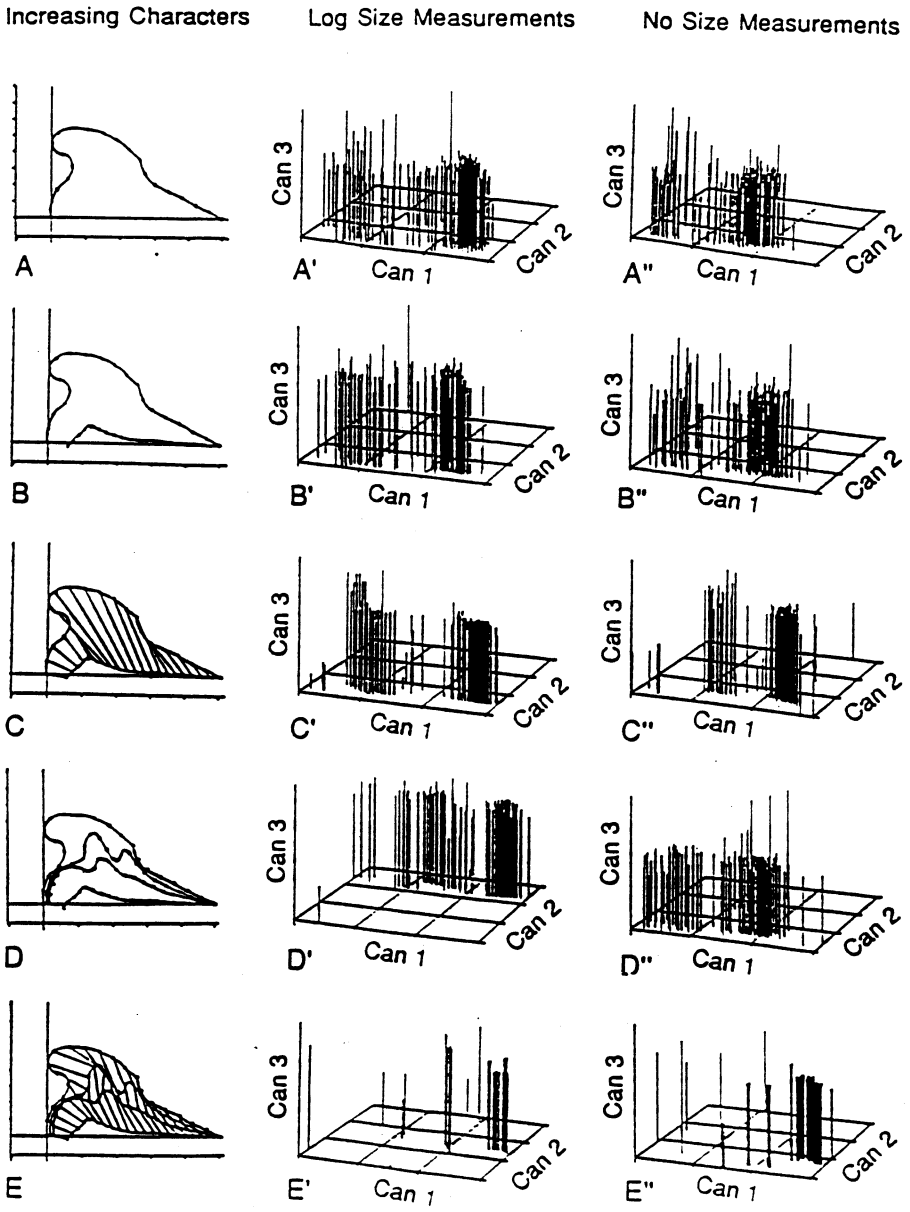

Figure 4. The effect of progressively using more variables on the discrimination among the sixteen groups in this study. The left column (A-E) diagrammatically represents an increasing number of angles and distances between analogous pronotal spline nodes. The middle column (A'-E') represents the degree of group discrimination when log square root area and the appropriate contour lengths are included with those represented in the left column. The right column (A"-E") represents discrimination when size measurements are excluded.

"clouds" of taxa within the tribe. Thus, simple measures of the pronotal outline at the mid-line or the outer boundary, combined with size measurements, were insufficient to describe and define shape differences.

We then modified our program to calculate the distance from the spline nodes on contour 1 to analogous nodes on contour 4; this increased the maximum number of measurements (variables) analyzed to 64 when size variables were included (Fig. 4C). With the inclusion of size variables (Fig. 4C'), relatively tight clouds resulted which represented the Telamonini and Smiliini; within the Telamonini cloud, even a few smaller groups appear as relatively tight clouds. When the size measurements were excluded (Fig. 4C"), there was slightly better discrimination. The distance measures between analogous spline nodes represented the degree of expansion and contraction between the two contours that were not reflected by any of the other measurements.

We expanded the number of measurements to 86, which included area, pronotal length, the lengths of contours 1 through 4, and all the angles between the line segments along each of four contours (Fig. 4D). When size measurements (Fig. 4D') where included in these analyses, two major clouds, and some relatively tight groupings of species that represent genera, were evident. This pattern was also evident when size measurements were excluded (Fig. 4D").

As a last step, we calculated the distances between the analogous spline nodes on contour pairs 1 and 2, 2 and 3, and 3 and 4 (Fig. 4E). These measurements were added to those described above to make a total of 146 variables for each individual, when size was included. With size variables included (Fig. 4E'), all 16 species groups form tight clouds when the first three canonical variables are plotted against each other. The analysis reduces the 146 variables, so that 92% of the between-group variation occurs in the first six canonical variables. When the six size measurements are excluded (Fig. 4E") only 13 of the 16 groups can be delineated on the same plot by the first three canonical variables. However, when the scale is expanded to delete outlying groups, 15 of the 16 groups were separated.

These analyses indicate that within the Smiliini, as currently defined, there appear to be three major subdivisions. When we plot only the Telamonini (Fig. 5A) (but adding *Smilia*), there appear to be two divisions. One group (*Hemicardiacus*, *Archasia*, *Smilia*, *Antianthe*, and *Telamonanthe*) have high, rounded, foliaceous pronota (Fig. 6: lineage group 2), while the other group (*Telamona*, *Heliria*, *Glossonotus*, *Thelia*) have elevated pronota that are more triangular (Fig. 6: lineage groups 1 and 3). The third division (Fig. 5C) reflects, in part, the older concept of the Smiliini (which originally included *Smilia* but not *Carynota*), although here it also includes: *Atymna*, *Grandolobus*, *Godingia*, *Cyrtolobus*, *Xantholobus*, *Ophiderma*, and *Carynota*. As a major grouping, these genera resemble each other in shape and size (Fig. 6: lineage groups 4 and 5).

The inclusion of size measurements in the analyses had relatively little effect in discriminating the groups in what are defined here as the Telamonini (Figs. 5A, 5B). The genera of that division are morphologically distinct and both analyses reflect differences in shape. The necessity of including size measurements is clearly evident in the old Smiliini (Figs. 5C, 5D). The exclusion of size produces discernable, but relatively diffuse, clusters of species in contrast to those produced when size is included. Therefore, when overall

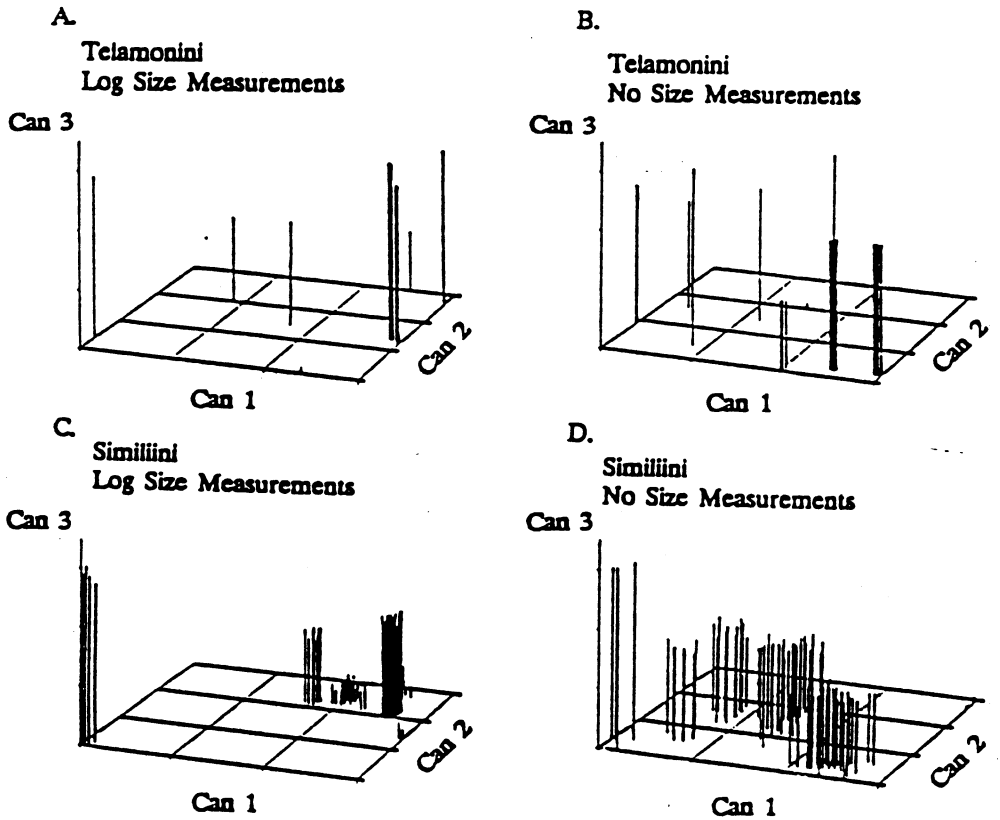

Figure 5. Expanded three dimensional plots of Fig. 4 E'-E" to show two major divisions of the Smiliini. (A) and (B) Groups that have been historically assigned to the Telamonini (but here includes *Smilia*). (C) and (D) Groups that have been historically assigned to the Smiliini (but here includes *Carynota* and *Tropidarnis*).

shape is similar, size variables become essential for group discrimination.

9 Phylogenetic Inferences

The canonical discriminant analysis generated a square Mahalanobis' distance matrix that is free of correlations among quantitative measurements (Felsenstein, 1988). Several Mahalanobis' distance matrices that resulted from our canonical analyses of the 16 groups were down-loaded to an IBM AT personal computer, and served as input data for the Fitch distance matrix program (options: P = 2, branch lengths unconstrained) in PHYLIP (version 2.7) (Felsenstein, 1984).

Our first phylogenetic analysis (sensu Sorensen, 1987, this volume; Sorensen and Foottit, this volume) was conducted to determine if the shape

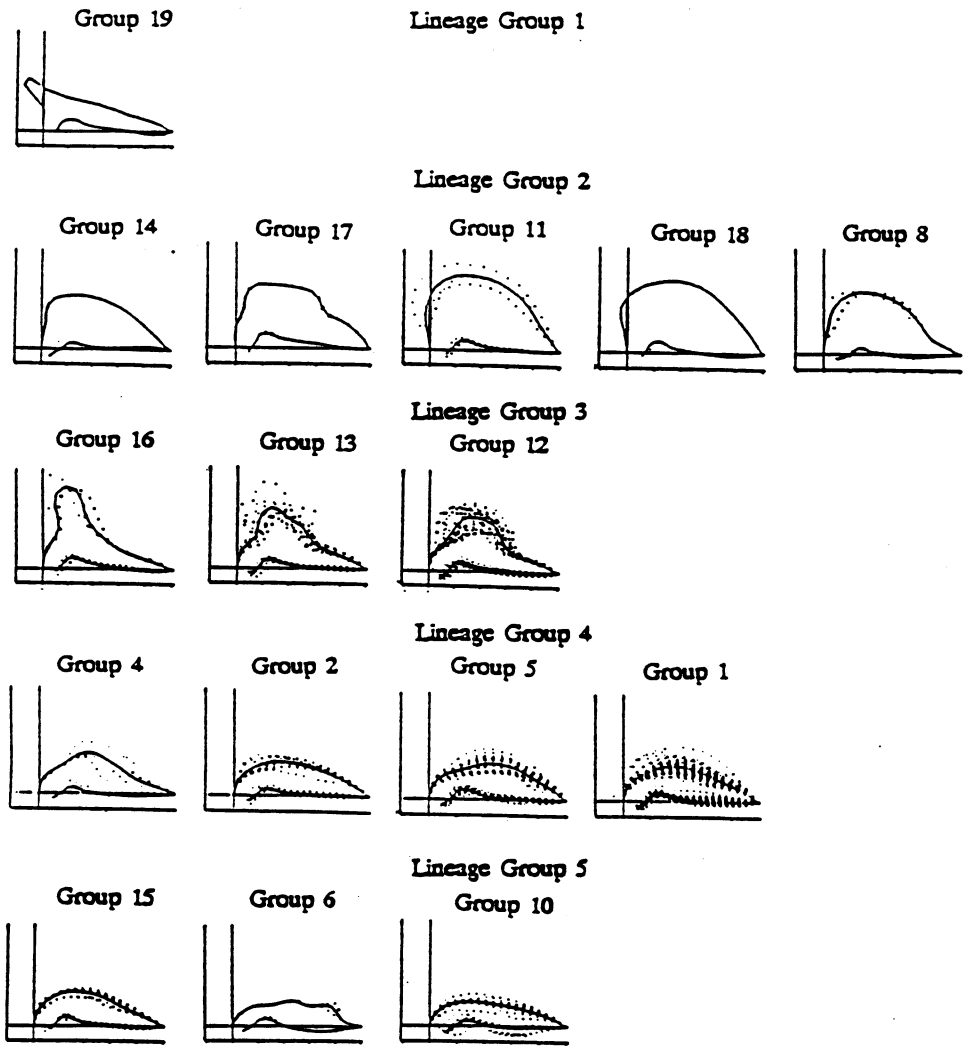

Figure 6. The average shape of the array of species in each group. The shape is designated by solid lines along contours 1 and 4. Dots represent the scatter at analogous spline nodes of species within each group. The five lineage groups were defined from dendrograms in Figs. 7 and 8, and are used to examine allometric relationships in Fig. 9.

variables of angles and distances between spline nodes (140 measurements) on scaled, normalized individuals would provide a possible transformation array for our 16 groups. The program produced and examined 414 trees; these had a sum of squares of 1.12 and an average standard deviation of 6.88%. After examining the lengths between nodes, we rooted the best tree as shown in Fig.

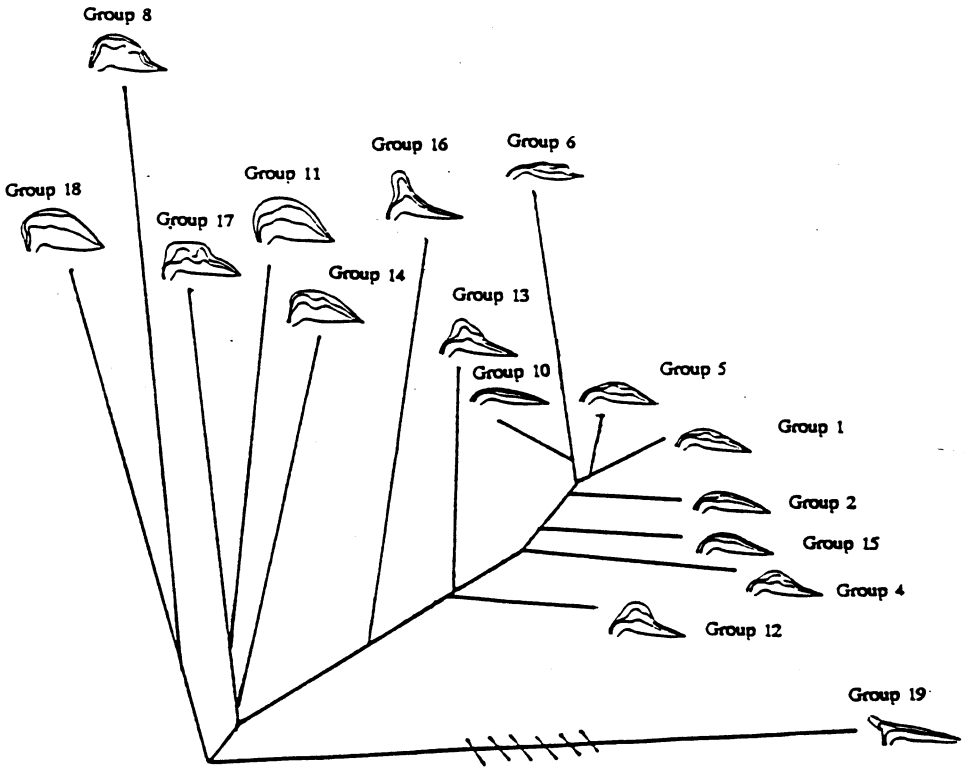

Figure 7. A Fitch-Margoliash tree generated from Mahalanobis' distances resulting from canonical discriminate analysis of 140 pronotal angles and distances, but *excluding* size variables.

7. In general, this network provided a credible transformation array for shapes, wherein the most distinctive pronotal shapes are located at the base of the tree. This general transformation array shows an initial divergence of forms with a frontal horn, from those with dorsally elevated pronota. The apex of the tree (Fig. 7) also shows that the transformation for the remainder of the groups is from high, foliaceous pronota to low, conical pronota.

A subsequent phylogenetic analysis included the six size measurements; PHYLIP produced 382 trees with a sum of squares of 1.21 and an average standard deviation of 7.13%. We rooted the best tree (Fig. 8) after examining the lengths between nodes. This size-included tree also provided, at least visually, a credible transformation series of pronotal shapes very similar to that on the size-excluded tree (Fig. 7). This tree separates groups with high foliaceous rounded pronota as an independent lineage. The remaining groups show a progressive transformation where the elevation of the pronotum is shifted posteriorly, from anteriodorsad of the head to behind it; this occurs as the elevation is decreased until the pronotum becomes conical (Fig. 8).

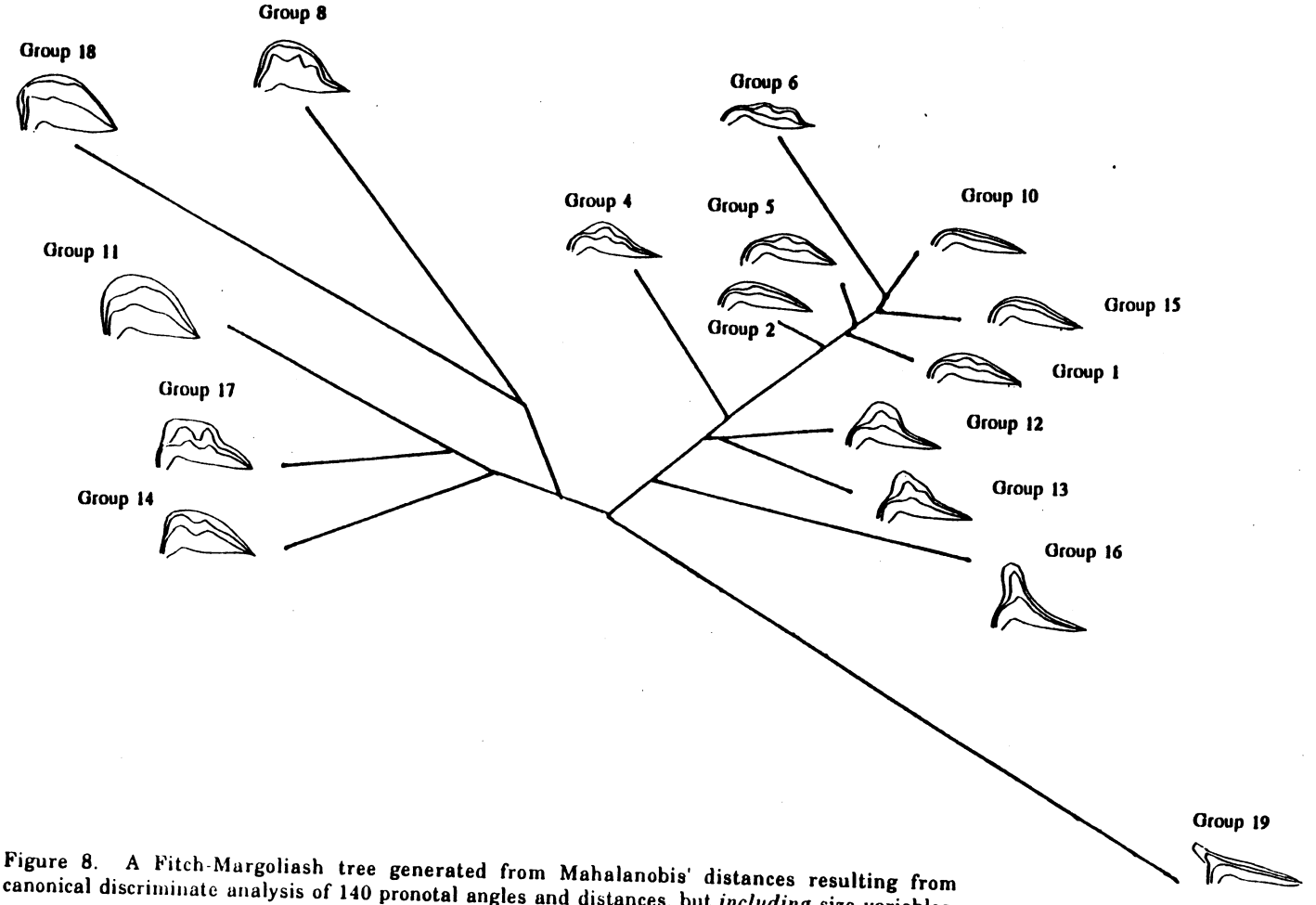

Figure 8. A Fitch-Margoliash tree generated from Mahalanobis' distances resulting from canonical discriminate analysis of 140 pronotal angles and distances, but *including* size variables.

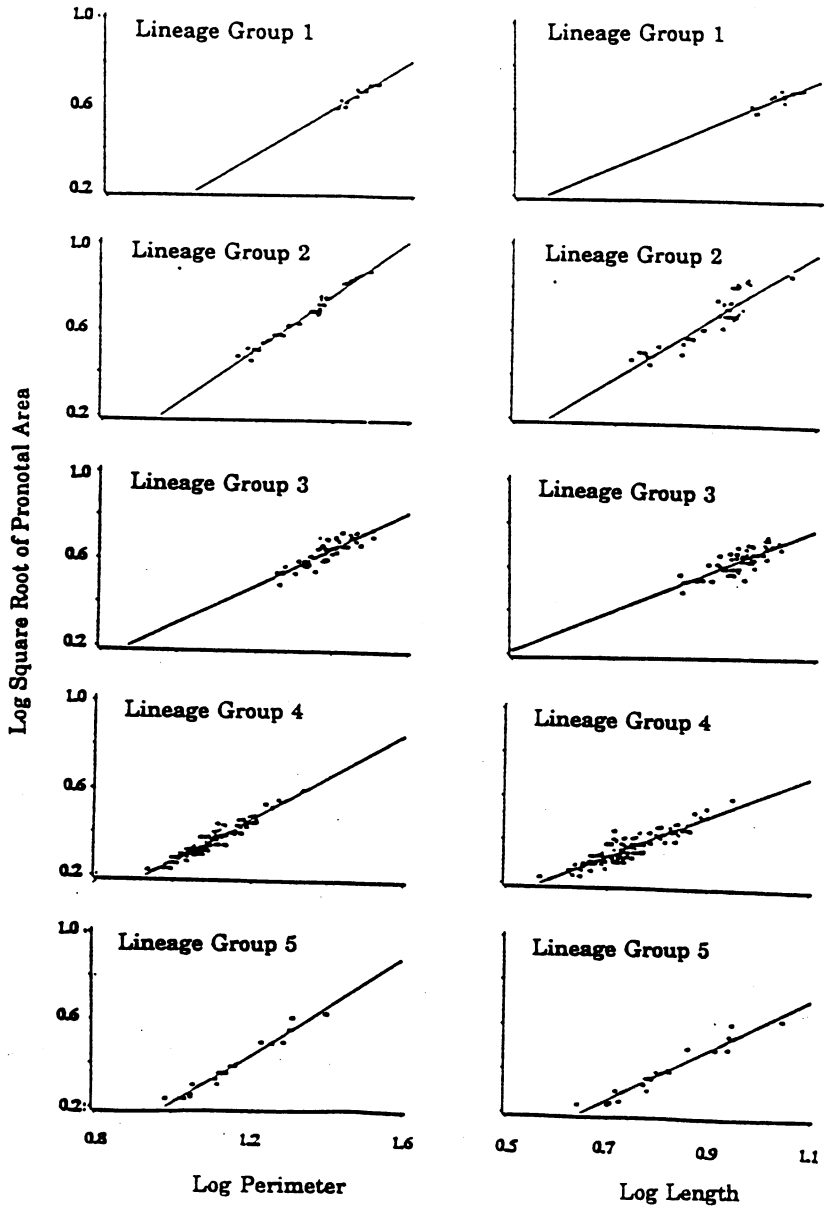

Figure 9. Regression of the log square root of pronotal area against the log perimeter (left column) and the log length (right column) for each of five lineage groups defined in Fig. 6. Vertical axes in all figures = Log Square Root of Pronotal Area; horizontal axes of left column figures = Log Perimeter; horizontal axes of right column figures = Log Length. Comparison of slopes and intercepts are in Tables 1 and 2.

10 Associated Correlates with a Size Related Phylogeny

Some authors have made a considerable effort to develop techniques that remove general-size from morphometric analyses to examine shape variation (Humphries et al., 1981). They imply that general-size is a confounding variable for systematic studies because of potential environmental effects. However, in many groups of organisms, major shifts in body size are associated with evolutionary divergence leading to radiation; therefore, size should not be ignored in phylogenetic studies.

Treehopper pronota show two components of size, vertical and horizontal. To demonstrate the within-group variation in the vertical axis, we calculated the average for contours 1 and 4 for each group (using scaled, normalized pronota) and then plotted the range of variation in that group (Fig. 6) along the mean contour. Although there is variation around the two contours within the groups, there is a consistent pattern between groups.

To determine the importance of allometric relations in defining groups, we took the original 16 groups and divided them into five lineage groups, based on their position on the tree in Fig. 8; these are defined in Fig. 6. Because lineage groups 1 and 2 contained few individuals, additional specimens were measured. The relationship between the square root of area and the perimeter incorporated not only a measure of size but also shape. We transformed (log base 10) each of these variables and performed a regression analysis for each of the five groups. Although there is some overlap among groups along the regression lines, which are presented in Fig. 9, three of the groups are characterized by high area/perimeter relationships, and two by low area/perimeter relationships; the regression values for the lines in Fig. 9 are given in Table 1. When the slopes of the lines were compared (Table 2), lineage group 1, although morphologically the most distinct, did not differ from any of the lineage groups. In contrast, the remaining lineage groups all differed from each other, indicating differences in allometry. On the tree that included size, this group diverged at the base as a separate lineage. The remainder of the groups on that tree have similar, but different, slopes; this suggests that they share common developmental pathways. An almost identical pattern emerged for pronotal area and length (Fig. 9) The only difference is that the slope for lineage group 3 did not differ from groups 4 and 5 (Table 3). This suggests that major changes in pronotal length are associated with gradual, but substantial, changes in shape.

There are two alternative interpretations of these allometric relationships. Strumpel (1972) interpreted similar regressions as an evolutionary tendency to increase the area of the pronotum in *Membracis*. There are, however, several arguments against this interpretation for the Smiliini. First, Fig. 7, which is based on scaled, normalized pronota and, therefore, contains no actual size related measurements, represents a progression from larger to smaller values for pronotal areas and lengths. Thus, the analysis objectively polarized these shapes toward decreasing pronotal area and length. Second, the number of points on the respective regression lines (Fig. 9) is a relative measure of extant species (with the exception of lineage groups 1 and 2); it seems clear that with a major reduction in pronotal area, there is a dramatic increase in the number of species. This is consistent with the concept that major reductions in body size may have permitted radiation within the tribe. Third, a reduction in pronotal area should be a developmentally and energetically more realistic

Table 1.
Values for regressions of lineage groups shown in Fig. 9.

Regression Values for Log $\sqrt{\text{Pronotal Area}}$ to *Log Perimeter* (*Left column*, Fig. 9)

Lineage Group	log $\sqrt{\text{area}}$	F	R^2	n
1	-0.89 + 1.05 log (perimeter)	89.40	0.88	13
2	-0.98 + 1.24 log (perimeter)	1377.06	0.98	41
3	-0.53 + 0.84 log (perimeter)	188.98	0.77	57
4	-0.70 + 0.97 log (perimeter)	1069.47	0.92	100
5	-0.87 + 1.09 log (perimeter)	443.64	0.96	19

Regression Values for Log $\sqrt{\text{Pronotal Area}}$ to *Log Length* (*Right column*, Fig. 9)

Lineage Group	log $\sqrt{\text{area}}$	F	R^2	n
1	-0.36 + 1.00 log (length)	42.34	0.78	13
2	-0.61 + 1.44 log (length)	160.37	0.80	41
3	-0.25 + 0.92 log (length)	87.38	0.61	57
4	-0.33 + 0.94 log (length)	463.00	0.82	100
5	-0.54 + 1.15 log (length)	263.12	0.94	19

evolutionary response for groups that feed upon nutritionally difficult host plants, such as *Quercus*. Smaller body sizes could facilitate the shorten developmental periods that are characteristic of these oak-inhabiting groups, by allocating energy to earlier reproduction.

The life history of oak-inhabiting groups is, in general, confined to the early spring flush of foliation; the majority of adults in such groups appear to die by midsummer, when oak leaves are fully tanned (T.K. Wood, personal observation) and become nutritionally poor (Feeny, 1970). With the exception of one species, all of the groups at the apex of the generated tree are restricted to *Quercus*. The overall selection pressure that is imposed by host plants appears to be for rapid adult maturation, and would favor reductions in body size. Thus, our interpretation of the polarity of the regressions is that evolution occurs with a decrease in pronotal area, so that the most derived species within each group are those with low area/perimeter relationships.

11 Discussion

The sensitivity of pronotal shape to environmental effects is perhaps the most important potential factor limiting its use to infer phylogenies. Although

Table 2.
Comparison of intercepts and slopes from regression of pronotal log square root area against log length (Fig. 9) for each of the five lineage groups defined in Fig. 6. Results of the analysis of covariance (F = 1342.9, P = 0.0001, R^2 = 0.98, n = 234), test for heterogeneity of intercepts (F = 8.7, P = 0.0001) and slope (F = 11.7, P = 0.0001) suggested differences among the five groups. Paired comparisons below suggest a pattern of allometry that is associated with the dendrogram in Figs. 7 and 8.

NS = not significant; * = $P < 0.05$; ** = $P < 0.009$; *** = $P < 0.0009$.

		Comparison to Lineage Group				
Lineage Group	Comparison for	1	2	3	4	5
1	Intercept	--	NS	NS	NS	NS
	Slope	--	NS	NS	NS	NS
2	Intercept		--	***	***	NS
	Slope		--	***	***	**
3	Intercept			--	*	***
	Slope			--	*	*
4	Intercept				--	**
	Slope				--	*
5	Intercept					--
	Slope					--

few people would suggest that phenotypic shape does not have a genetic component, many would argue that it could be influenced by nutritional differences associated with different host plant species, or by other environmental variables, and that it should be, therefore, of dubious value in systematics. Wood and Datz (unpublished data) have completed a series of experiments designed to determine the sensitivity of pronotal shape to environmental influences. When photoperiod and temperature were held constant but the species of host plant was varied, six species of the *Enchenopa binotata* complex retained their characteristic pronotal shape, although inappropriate host species in some cases had an effect on body size. Thus, pronotal shape, as measured by the techniques presented here, appears to be phenotypically stable, and should serve as a indicator of the genotype.

We believe the phylogenetic tree that incorporates both shape and size, provides the best tentative transformation series for pronotal shape within the tribe. This tree reflects at least two evolutionary patterns. In the first, a shift occurs from highly elevated pronota at the basal portion of the tree, toward lower, rounded, conical pronota at the tree's terminal branches. In the second, pronotal area and length decrease with increased evolutionary distance from the tree's basal node. These general patterns of morphological evolution are reflected within each divergent group, suggesting that once the groups separated, selection favored similar trends within each lineage. The tree is consistent with the notion that the most derived groups are morphologically the most similar, suggesting convergence and less time for differentiation. These derived groups are extremely rich in species compared

Table 3.
Comparison of intercepts and slopes from regression of pronotal log square root area against log length (Fig. 9) for each of the five lineage groups defined in Fig. 6. Results of the analysis of covariance ($F = 498.82$, $P = 0.0001$, $R^2 = 0.95$, $n = 234$), test for heterogeneity of intercepts ($F = 4.97$, $P = 0.0007$) and slope ($F = 7.98$, $P = 0.0001$) suggested differences among the five groups. Paired comparisons below suggest a pattern of allometry that is associated with the dendrogram in Fig's. 7 and 8.

NS = not significant; * = $P < 0.05$; ** = $P < 0.009$; *** = $P < 0.0009$.

		Comparison to Lineage Group				
Lineage Group	Comparison for	1	2	3	4	5
1	Intercept	--	NS	NS	NS	NS
	Slope	--	NS	NS	NS	NS
2	Intercept		--	***	***	NS
	Slope		--	***	***	**
3	Intercept			--	NS	*
	Slope			--	NS	NS
4	Intercept				--	**
	Slope				--	*
5	Intercept					--
	Slope					--

to the more primitive (basal) groups which are either monotypic, or contain less than six species (Table 4). This suggests a recent radiation among derived, morphologically similar groups, and either extinction or developmental constraints to divergence among plesiomorphic (basal) groups that are more morphologically distinct.

Congruent with the above trends are patterns of host plant utilization (Table 4). Although it is impossible to identify the ancestral host plant for this tribe, the inferred phylogeny suggests a credible candidate. *Thelia*, which is the first group to diverge at the base of the tree, is unique because of the presence of a frontal horn and because one species (*Thelia bimaculata*) is the only member of the tribe that is restricted to a leguminous host plant (*Robinia pseudoacacia*) (Funkhouser, 1915). A major portion of the African (Capener, 1962), Indian (Ananthasubramanian and Ananthakrishnan, 1975), Central American (Wood, 1984) and South American (Richter, 1942) treehopper faunas are either restricted to, or utilize, host plants in the Fabaceae.

Old World membracids are restricted to the Centrotinae and related subfamilies, which are considered to be the most plesiomorphic of the Membracidae (Strumpel, 1972; Deitz, 1975). The majority of the African fauna appears to be restricted to legumes (Capener, 1962). With only a few minor exceptions, in one subfamily, there is no overlap between the Old and New World treehopper faunas (Strumpel, 1972; Deitz, 1975). This suggests that association with leguminous host species may have preceded the separation of the continents.

Table 4.
Lineage group composition: host plants and distributions. Grp = assigned lineage group, # Sp = number of described species in genus, Q = *Quercus* as host plant, O = other non-*Quercus* host plant, U = unknown host plant, NW = Pacific Northwest, CA = California, RM = Rockie Mountains, SW = Arizona and New Mexico, MX = Mexico, CSA = Central and South America, E = eastern North America.

			Host Plants			Geographic Region						
Grp	Genus	# Sp	Q	O	U	NW	CA	RM	SW	MX	CSA	E
1	*Thelia*	2	0	2	0	0	0	0	0	0	0	2
2	*Hemicardiacus*	1	1	0	0	0	0	0	0	1	0	0
2	*Archasia*	3	3	0	0	0	0	1	1	0	0	3
2	*Telamonanthe*	2	2	0	0	0	1	2	2	2	0	0
2	*Antianthe*	6	0	2	4	0	1	0	1	4	6	0
2	*Smilia*	3	2	0	1	0	0	0	0	1	0	2
3	*Telonaca*	2	2	0	0	0	1	0	0	0	0	1
3	*Glossonotus*	5	4	1	0	1	0	1	0	0	0	5
3	*Helonica*	1	1	0	0	0	0	0	0	1	0	1
3	*Heliria*	12	5	5	2	1	0	6	2	3	0	10
3	*Palonica*	6	2	3	1	0	0	3	1	1	1	3
3	*Telmona*	29	15	6	8	2	3	6	2	3	4	22
4	*Grandolobus*	5	5	0	0	0	0	0	1	3	1	0
4	*Atymna*	12	9	1	2	0	0	1	2	2	2	6
4	*Ashmeadea*	1	1	0	0	0	0	0	1	1	0	0
4	*Xantholobus*	12	12	0	0	0	0	3	6	0	0	6
5	*Godingia*	1	1	0	0	0	0	0	0	1	0	0
5	*Tropidarnis*	1	1	0	0	0	0	0	1	1	0	0
5	*Carynota*	4	1	3	0	0	0	0	0	0	0	4
5	*Ophiderma*	16	16	0	0	0	0	1	4	0	2	9

If we assume that a legume was the ancestral host of the Smiliini, the tree suggests that the colonization of *Quercus* led to extensive radiation. If the basal node (Fig. 8) represents the colonization of *Quercus*, lineage group 2, with high foliaceous pronota, diverged and one member (*Antianthe*) moved to *Solanum* (Wood, 1984). *Antianthe* is unique in the tribe not only in the host plants that it uses but also in its presocial, multivoltine life history.

The progression in the main lineage is toward restriction to *Quercus*. The first groups to diverge are primarily on *Quercus*, but a number of species are restricted to other deciduous host trees in several different families (Table 4). Because taxonomic revisions have not been done for these groups, we cannot determine whether the utilization of oak is a derived condition for them. Many of the species, which occur on other genera of hosts, in these groups tend to have lower, rounded pronota which, according to our tree, is a derived condition. If the species using hosts other than oaks are derived, it suggests that the primitive positions of these groups reflects a sufficient time interval to permit the colonization of new host plant genera.

Of the remaining seven terminal groups, six are restricted to *Quercus* (one *Atymna* species is found on *Castanae dentata* [Fagaceae]). The single exceptional group (14) has a species on *Quercus* and the remaining three are on other deciduous trees. Its position on the tree suggests that it is the oldest of the three terminal groups, which is consistent with longer time intervals for the colonization of new host genera.

The last point in support of the hypothesized phylogeny relates to the broad geographic distributions of members of this tribe and of *Quercus*. *Quercus* has a worldwide distribution, but with the exception of several records in Pakistan (I. Ahmad, personal communication), it has not apparently accumulated the extensive treehopper fauna that is characteristic of North America. During the Tertiary, the North American *Quercus* flora became regionally differentiated and isolated from that of Asia (Axelrod, 1983). Major centers of oak diversity were established in the central highlands of Mexico, California, Arizona and the eastern United States. The greatest diversity of species is in central Mexico, followed sequentially by the eastern U.S., California, and Arizona (Trelease, 1924).

Treehoppers in the Smiliini are also regionally differentiated (Table 4): of the 164 described species (Metcalf and Wade, 1965), 104 are found in the eastern U.S., 32 are in Mexico, 31 are in Arizona, 27 are in the Rocky Mountains, 16 are in Central and South America, and 7 are in California. At first glance, this suggests that many regions may be under sampled. This does not appear to be the case, however, because TKW has collected extensively in each region during the last five years and examined many insect collections, finding little evidence to indicate a large number of species remain undiscovered in any of these regions.

The similarities between the distributions of the treehoppers and *Quercus* suggest that both have been biogeographically affected by the same geological events. Outlined below is our speculation of a possible historical reconstruction for the Smiliini.

(1) Because no other members of the subfamily Smiliinae occur in the Old World (Asia or Africa), differentiation of the tribe had to have been within the last 65 million years, after the separation of South America.

(2) Possible relict species in some genera in South America (Table 4) support this time of divergence, and suggest that the tribe moved into North America within the last five million years, when these areas in the Americas became connected.

(3) The pervasive use of leguminose host plants by primitive subfamilies in all major zoogeographic regions suggests that members of this plant family served as the ancestral hosts in North America.

(4) Treehoppers, such as *Thelia bimaculata* (restricted to the eastern U.S., leguminose hosts and the most morphologically differentiated), appear to be relicts of the early treehopper fauna.

(5) The lack of an extensive treehopper fauna on *Quercus*, an extremely old genus (Axelrod, 1983), in any other region of the world suggests that historically, it is a recently colonized host.

(6) The pan-United States distribution of a few species in primitive (basal) groups of the tribe (those with representatives in California and the Pacific Northwest) suggests their broad distribution prior to the last glaciation.

(7) The colonization of *Quercus* may have occurred prior to the last glaciation, because all of the California Smiliini, except *Antianthe* (which may

have been introduced, [R.J. Gill, personal communication]), occur on this host genus.

(8) Because *Cyrtolobus* has a single representative in California, all genera showing divergence nodes on the phylogeny below *Cyrtolobus* must have also diverged by the last glaciation.

(9) The effect of the last glaciation was to restrict the eastern deciduous forest (Webb, 1981; Davis, 1983), and the treehoppers associated with these hosts, to the southern portions of the U.S.

(10) These glaciers appear to have created disjunctions in treehopper and *Quercus* distributions across the U.S.

(11) Such geographic isolation during glaciation may have promoted much of the species richness found in the more derived (terminal) taxa in this tribe.

(12) With the retreat of the ice shield, beginning 18,000 years ago, oaks dispersed throughout the Eastern U.S. in a roughly northeast direction (Davis, 1983; Webb, 1981).

(13) The treehoppers followed the northeasterly dispersal of the oaks, which reached their northern limit within the last 6,000 years.

(14) With the gradual aridification of mid-Texas and northern Mexico during the last 5,000 years, complete regional separation between these faunas was achieved.

12 Future Considerations

The technique, as presented here, is being used to understand geographic variation, sexual dimorphism, and the underlying genetics of pronotal shape, not only in the Smiliini but in other groups. When inventories of tropical forests are made, collecting and maintenance of specimens will be critical limitations, but without faster techniques for the identification and analysis of data, it will be several generations before we know what has been found and what it means. The technique presented here has promise, at least for treehoppers, for coping with the logistical problems that will face systematists.

We are beginning to explore the following possible avenue for handling specimens from massive faunal surveys. First, establishing calibration data sets for each level of classification within the family. Second, as described in this chapter, digitizing specimens and associating that data with relevant collection information. Third, the input of this digitized data directly into a series of programs that allow identification within seconds. Fourth, to set the probabilities of a correct match (identification) to a member taxon of the training data set high enough, so that potentially undescribed species can be immediately recognized as such and brought to a specialist's attention. Once new species are identified, their associated digitized information can be quickly added to the calibration data to reduce the interval between the recognition of a new taxa (description, publication) and its use in further identifications. This approach will provide an almost instant access to large data sets, not only for systematists, but also ecologists and biogeographers. Only through an approach to systematics that is similar to this procedure will it be possible to know what is there (or was), but more importantly to understand the relationships between taxa, trophic levels and geographic regions.

13 Acknowledgements

We thank K. Kruluts and M. Hardin for their help in the early portions of this work, G. Pratt for commenting on various drafts of the manuscript, and T. Green for general assistance in the preparation of figures. This work was supported by National Science Foundation Grant BSR-8400245. It is published as a miscellaneous paper of the Delaware Agricultural Experiment Station; contribution number 604 of the Department of Entomology and Applied Ecology, University of Delaware, Newark; and contribution number 128 of the Ecology Program, School of Life and Health Sciences, University of Delaware, Newark.

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15 Appendices

Appendix 1.
List of 168 species in the tribe Smiliini (as defined by Deitz, 1975) and their assignment to the 16 groups referred to in this paper. Generic and specific names are those used in the Metcalf and Wade catalogue (1965). Specific names are tentative, because synonymies have not been worked out. Unlisted species names are specimens collected by TKW that cannot, with some degree of assurance, be matched with an existing species description or are known to be new species. Code numbers are those that we are presently using to designate morphospecies, while one of us (TKW) revises the genera. Area = the square root of pronotal area (mm), length = the distance at the mid line of the pronotum between the first point above the head to the tip of the posterior process (mm), contour 1 = is the length of the mid line outline (mm).

Group	Code	Genus	Species	Area	Length	Contour 1
1	1	*Cyrtolobus*	*vau*	2.28	5.33	6.33
1	2	*Cyrtolobus*	*puritanus*	1.94	4.66	5.48
1	4	*Cyrtolobus*	*parvulus*	2.03	4.91	5.77
1	5	*Cyrtolobus*	*maculifrontis*	2.10	5.12	6.01
1	6	*Cyrtolobus*	*gratiosus*	2.01	4.89	5.80
1	8	*Cyrtolobus*	*pictus*	2.04	5.13	5.82
1	10	*Cyrtolobus*		2.33	5.55	6.70
1	11	*Cyrtolobus*	*frigidus*	1.79	4.22	5.16
1	12	*Cyrtolobus*	*togatus*	1.82	4.44	5.27
1	14	*Aeshmeadea*	*carinata*	2.70	6.51	7.67
1	15	*Cyrtolobus*	*vanduzeei*	2.60	5.71	7.06
1	16	*Cyrtolobus*	*woodruffi*	2.56	5.40	7.18
1	17	*Cyrtolobus*		2.06	5.28	6.08
1	18	*Cyrtolobus*		2.97	7.23	8.46
1	20	*Cyrtolobus*		2.22	5.78	6.70
1	21	*Cyrtolobus*		1.97	5.25	5.88
1	27	*Cyrtolobus*		2.39	5.37	6.38
1	28	*Cyrtolobus*		2.20	5.65	6.48
1	29	*Cyrtolobus*		2.02	4.60	5.54
1	32	*Cyrtolobus*	*limus*	2.19	4.86	5.88
1	34	*Cyrtolobus*		2.06	5.12	6.03
1	45	*Cyrtolobus*	*inermis*	2.16	5.22	6.19
1	46	*Cyrtolobus*	*pallidifrontis*	2.08	5.40	6.30
1	47	*Cyrtolobus*	*fuliginosus*	2.72	6.08	7.90
1	48	*Cyrtolobus*	*fuscipennis*	2.21	5.40	6.39
1	49	*Cyrtolobus*	*griseus*	2.40	5.68	6.69
1	50	*Cyrtolobus*	*gramatanus*	2.05	4.92	5.77
1	52	*Cyrtolobus*	*rufulus*	2.29	5.69	6.52
1	53	*Cyrtolobus*		2.00	4.68	5.61
1	56	*Cyrtolobus*	*fenestratus*	2.52	6.07	7.57
1	57	*Cyrtolobus*	*clarus*	2.65	6.25	8.26
1	58	*Cyrtolobus*	*discoidalis*	2.40	5.80	7.07
1	64	*Cyrtolobus*		2.41	5.33	6.64
1	70	*Cyrtolobus*		1.93	4.53	5.37
1	72	*Cyrtolobus*		2.67	6.92	8.08
1	73	*Cyrtolobus*		2.33	5.59	6.99
1	74	*Cyrtolobus*		2.33	5.44	6.64
1	76	*Cyrtolobus*		2.11	5.49	6.44
1	79	*Cyrtolobus*		1.86	4.94	5.63

1	81	*Cyrtolobus*	*funkhouseri*	2.43	5.59	7.07
1	82	*Cyrtolobus*	*flavolatus*	2.12	5.31	6.30
1	83	*Cyrtolobus*	*ovatus*	2.76	5.16	7.36
1	86	*Cyrtolobus*	*arcuatus*	2.92	6.39	8.28
1	87	*Cyrtolobus*	*accuminatus*	2.81	7.08	8.74
1	88	*Cyrtolobus*	*auroreus*	2.45	5.81	7.07
1	90	*Cyrtolobus*		2.39	5.91	7.11
1	92	*Cyrtolobus*		2.78	6.78	8.28
1	93	*Cyrtolobus*		2.56	5.64	6.97
1	94	*Cyrtolobus*		2.80	7.24	8.39
1	96	*Cyrtolobus*		3.27	7.68	9.81
1	97	*Cyrtolobus*		3.02	6.91	8.65
1	98	*Cyrtolobus*		1.73	4.28	4.90
1	99	*Cyrtolobus*		1.81	4.63	5.37
1	100	*Cyrtolobus*		2.70	5.78	7.37
1	129	*Cyrtolobus*		1.92	4.32	5.32
1	130	*Cyrtolobus*		2.22	5.89	6.76
1	132	*Cyrtolobus*	*celsus*	2.39	4.99	6.38
1	134	*Cyrtolobus*		2.48	5.20	7.16
1	135	*Cyrtolobus*		2.10	4.69	5.79
1	139	*Cyrtolobus*		1.99	5.39	6.27
1	140	*Cyrtolobus*		2.19	5.50	6.58
2	9	*Atymna*		2.79	7.32	8.50
2	42	*Atymna*	*helena*	2.45	6.10	7.12
2	55	*Atymna*	*querci*	1.96	4.73	5.46
2	60	*Atymna*	*simplex*	2.87	7.13	8.79
2	65	*Atyminia*	*elongata*	2.50	6.84	7.78
2	67	*Atymna*		2.11	4.88	5.98
2	80	*Atymna*	*castanae*	2.87	6.28	7.94
2	89	*Cyrtolobus*	*dixianus*	2.67	6.42	7.83
2	156	*Cyrtolobus*	*maxeini*	2.39	5.71	6.81
2	166	*Atymna*		2.26	5.15	6.63
4	13	*Grandolobus*	*grandis*	2.99	6.81	8.25
4	22	*Grandolobus*		2.75	6.74	8.16
4	23	*Grandolobus*		2.21	5.86	7.34
4	59	*Cyrtolobus*	*tuberosus*	3.39	7.47	9.42
4	71	*Cyrtolobus*	*cristiferus*	3.96	8.89	12.16
5	41	*Xantholobus*	*nigrocinctus*	2.48	6.50	7.48
5	44	*Xantholobus*	*muticus*	2.80	6.65	8.11
5	54	*Xantholobus*		3.55	7.89	10.24
5	68	*Xantholobus*	*lateralis*	2.57	6.39	7.69
5	102	*Xantholobus*		2.12	4.64	5.95
5	103	*Xantholobus*		1.98	5.14	5.90
5	104	*Xantholobus*		2.75	6.84	8.06
5	105	*Xantholobus*		2.79	6.86	8.14
5	106	*Xantholobus*		1.96	4.48	5.64
5	107	*Xantholobus*		2.14	4.80	5.99
5	108	*Xantholobus*		1.83	4.20	5.25
5	110	*Xantholobus*		1.89	4.49	5.51
5	111	*Xantholobus*		2.00	4.95	5.68
5	114	*Xantholobus*		1.69	3.68	4.62
5	124	*Xantholobus*		2.18	5.34	6.46
5	125	*Xantholobus*		2.89	6.69	8.11
5	152	*Xantholobus*		1.70	4.43	5.13

5	153	*Xantholobus*		2.08	5.60	6.33
5	154	*Xantholobus*		1.97	4.49	5.64
5	155	*Xantholobus*		1.93	4.89	5.70
5	161	*Xantholobus*		2.40	5.10	6.53
5	162	*Xantholobus*		2.41	4.99	6.39
5	163	*Xantholobus*		2.53	5.14	6.99
5	165	*Xantholobus*		2.08	5.27	6.08
6	91	*Godingia*		2.29	6.04	7.23
6	128	*Godingia*	*guerreroensis*	3.14	8.22	9.35
8	84	*Smilia*	*camelus*	4.79	8.39	13.27
8	85	*Smilia*	*fasciata*	4.13	7.51	11.21
10	159	*Ophiderma*	*flavicephala*	2.28	5.95	6.81
10	167	*Ophiderma*	*pallida*	2.00	5.20	5.76
10	168	*Ophiderma*	*definita*	1.73	5.01	5.51
10	169	*Ophiderma*	*evelyna*	2.27	6.12	6.97
10	170	*Ophiderma*	*pubescens*	2.28	6.12	6.88
10	173	*Ophiderma*		2.02	6.00	6.65
10	175	*Ophiderma*	*flava*	2.47	6.67	7.50
10	177	*Ophiderma*	*salamandra*	2.52	6.60	7.54
10	178	*Ophiderma*	*grisea*	2.14	5.92	6.59
10	179	*Ophiderma*		1.76	5.07	5.78
10	180	*Ophiderma*		1.80	5.28	5.59
10	181	*Ophiderma*		1.73	4.37	5.04
10	182	*Ophiderma*		2.45	6.25	7.43
11	212	*Archasia*	*belfragei*	5.15	8.46	13.97
11	213	*Archasia*	*auriculata*	7.00	9.19	19.20
11	214	*Archasia*	*pallida*	5.43	8.22	14.30
12	158	*Telamona*	*monticola*	4.44	9.25	14.19
12	183	*Palonica*	*pyramidata*	4.39	9.12	14.54
12	184	*Palonica*	*portola*	4.48	9.13	15.32
12	189	*Telamona*	*maculata*	5.03	9.35	15.32
12	190	*Telamona*	*compacta*	3.44	7.35	10.08
12	191	*Telamona*	*dubium*	3.68	8.75	11.78
12	192	*Telamona*	*collina*	4.33	9.48	14.20
12	193	*Telamona*	*tristis*	3.66	6.80	11.51
12	194	*Telamona*	*concava*	4.62	8.61	14.19
12	196	*Telamona*	*reclivata*	3.88	8.32	11.58
12	197	*Telamona*	*salvini*	3.48	7.55	10.31
12	198	*Telamona*	*spreta*	5.15	10.71	16.49
12	199	*Telamona*	*ampelopsis*	4.77	10.01	15.42
12	200	*Telamona*	*extrema*	4.89	8.93	14.56
12	201	*Telamona*	*gibbera*	3.74	8.56	12.60
12	202	*Telamona*	*barbara*	4.23	9.09	14.18
12	203	*Telamona*	*vestita*	4.42	8.88	13.24
12	204	*Telamona*	*decorada*	3.89	8.46	12.32
12	205	*Telamona*	*unicolor*	5.27	10.24	15.62
12	206	*Telamona*	*lugubrus*	3.80	8.93	12.12
12	207	*Telamona*		4.80	10.81	14.52
12	218	*Telamona*		3.43	7.90	10.06
12	230	*Telamona*		4.36	10.13	13.33
12	239	*Telamona*		3.04	6.87	10.73
13	185	*Heliria*		4.19	9.30	13.59
13	209	*Telonaca*	*ramona*	4.59	8.90	14.11
13	215	*Helonaca*	*excelsa*	5.27	10.10	18.21

13	224	*Heliria*	*scalaris*	4.49	8.10	16.86
13	225	*Heliria*	*cristata*	4.98	10.46	17.25
13	226	*Heliria*	*praelata*	3.96	9.59	14.02
13	227	*Heliria*	*gemma*	4.93	10.14	15.06
13	228	*Heliria*	*sinuata*	4.46	9.29	13.13
13	229	*Heliria*	*strombergi*	4.18	9.51	12.94
14	211	*Antianthe*	*expansa*	4.10	7.48	11.18
15	186	*Tropidarnis*		4.25	11.10	13.39
15	187			4.08	8.84	11.14
15	195	*Telamona*	*wescotti*	3.15	8.70	10.09
15	216	*Carynota*	*mera*	3.61	8.75	10.84
15	217	*Carynota*	*marmorata*	3.16	7.25	8.99
16	210	*Telonaca*	*alta*	4.95	9.54	17.62
16	219	*Glossonotus*	*crategei*	3.41	6.89	12.51
16	220	*Glossonotus*	*univittatus*	4.01	8.21	15.08
16	221	*Glossonotus*	*accuminatus*	4.62	9.28	19.87
17	222	*Telamonanthe*	*pulchella*	3.43	6.07	9.72
17	223	*Telamonanthe*	*reilyi*	3.20	5.71	9.39
18	188	*Hemicardiacus*		7.33	11.18	19.43
19	208	*Thelia*	*bimaculata*	4.92	11.44	19.83

Appendix 2.
Fortran program for calculating pronotal shape of treehoppers.

```
C
C     From digitized data this program computes a grid of
C     analogous points using spline interpolation. Using
C     this grid, the program computes distances, angles,
C     lengths and area for use in canonical discriminant
C     analysis and discriminant function analysis.
C
C     The program conforms to the full ANSI 1977 Fortran
C     standard. However, the subroutine DCSINT which does
C     the cubic spline interpolation is in the IBM
C     Engineering and Scientific Library.
C
C     1. Declarations.
C
      INTEGER NMAX, NVALS, MAXC, NVM1
      DOUBLE PRECISION ONE, ZERO
      PARAMETER (NMAX=400, NVALS=21, MAXC=4*NMAX, NVM1=NVALS-1,
     *     ONE=1.0D0, ZERO=0.0D0)
      CHARACTER CODE*15, CONTUR*1 REST*29, REST2*29
      INTEGER I, J, INIT, MCOUNT
      DOUBLE PRECISION PI
      DOUBLE PRECISION DNVALS
      DOUBLE PRECISION XSMC, YSMC
      DOUBLE PRECISION XB, YB, SLOPE, SLOPE2, COSTH, SINTH, DMC
      DOUBLE PRECISION DIMAG, DSQRT, DATAN, DABS
      DOUBLE PRECISION CRENDX, CRENDY
      DOUBLE PRECISION X(NMAX), Y(NMAX), XS(NMAX), YS(NMAX)
```

```
      DOUBLE PRECISION T(NVALS), C(MAXC), S(NMAX)
      DOUBLE PRECISION XH(NVALS), YH(NVALS)
      DOUBLE PRECISION XC1(NVM1), XC2(NVM1), XC3(NVM1)
      DOUBLE PRECISION YC1(NVM1), YC2(NVM1), YC3(NVM1)
      DOUBLE PRECISION D12(NVM1), D23(NVM1), D34(NVM1)
      DOUBLE PRECISION XMIN, XMAX, XDIF, SCALE, WIDTH
      DOUBLE PRECISION ANGLE(NVALS,4)
      DOUBLE PRECISION LC(4), TLC
      DOUBLE PRECISION AREA, AREA1, AREA2
C
C     First executable statement
C
C     Compute pi for later use
C
      PI=4.0D0*DATAN(1.0D0)
C
C     Compute values at which spline interpolation is done.
C
      DNVALS=NVALS
      DO 700 I=1,NVALS
      T(I)=(DFLOAT(I)-ONE)/(DNVALS-ONE)
700   CONTINUE
C
C     2. Read data.
C
C     (The input starts with an identification line. The first
C     column indicates the  contour after which is information
C     identifying the specimen. After that the digitized
C     coordinates follow one point to a line. Coordinates
C     greater than or equal to 99 signal the end of the
C     contour. Contours 2, 3, and 4 are recorded the same
C     way except that there is no information after column
C     1 in the identification line.)
C
900   READ (30,1,END=9999) CONTUR, REST
1     FORMAT(A1,A29)
C
      IF(CONTUR.EQ.'1')REST2=REST
      I=1
151   READ (30,*) X(I), Y(I)
C
C     Check for end of contour
C
      IF (X(I) .GE. 99.0)  GO TO  150
C
C     Check data for duplicate points. (Duplicates are ignored).
C
      IF ( I .GT. 1 ) THEN
            IF  ( DABS(X(I)-X(I-1) ).LT. 1.0D-03.AND.
     *            DABS(Y(I)-Y(I-1)).LT. 1.0D-03 ) GO TO 151
      END IF
      MCOUNT=I
       I=I+1
      GO TO 151
```

```
150   CONTINUE
C
C     3. Translate, rotate and scale coordinates
C
      IF (CONTUR .EQ. 111) THEN
            XDIF=DSQRT( (X(MCOUNT)-X(1) )**2 + (Y(MCOUNT)-Y(1))**2 )
            XB=X(1)
            YB=Y(1)
            SLOPE=(Y(MCOUNT)-Y(1))/(X(MCOUNT)-X(1) )
            SLOPE2=SLOPE*SLOPE
            COSTH=ONE/DSQRT(ONE+SLOPE2)
            SINTH=SLOPE*COSTH
      ENDIF
      DO 701 I=1, MCOUNT
            XS(I) = SCALE*(COSTH*(X(I)-XB)+SINTH*(Y(I)-YB))
            YS(I) = SCALE*(-SINTH*(X(I)-XB)+COSTH*(Y(I)-YB))
701   CONTINUE
C
C     4. Translate contours 2, 3 & 4 so tip of posterior process
C     coincides with that of contour 1.
C
            IF (CONTUR .EQ. '1') THEN
                  CRENDX=XS(MCOUNT)
                  CRENDY=YS(MCOUNT)
            ELSE
                  XSMC=XS(MCOUNT)
                  YSMC=YS(MCOUNT)
                  DO 702 I=1, MCOUNT
                        XS(I)=XS(I)+ CRENDX-XSMC
                        YS(I)=YS(I)+ CRENDY-YSMC
702               CONTINUE
            END IF
C
C     5. A. Compute contribution of line integral for area from
C     contours 1 and 4. (By Green's theorem the area
C     computation is reduced to numerical integration of
C     a line integral).
C
      IF(CONTUR.EQ.'1')THEN
            AREA1=ZERO
            DO 703 I=2, MCOUNT
                  AREA1=AREA1-.5D0*(XS(I)+XS(I-1))*(YS(I)-YS(I-1))
703         CONTINUE
      END IF
      IF(CONTUR.EQ.'4')THEN
            AREA2=ZERO
            DO 704 I=2, MCOUNT
                  AREA2=AREA2+.5D0*(XS(I)+XS(I-1))*(YS(I)-YS(I-1))
704         CONTINUE
      END IF
C
C     6. Compute arclength and normalize.
C
      S(1)=ZERO
```

```
      DO 705 I=2, MCOUNT
            S(I)=S(I-1)
     *      +DSQRT( ( XS(I) - XS(I-1))**2 +  (YS(I)-YS(I-1))**2 )
705   CONTINUE
      TLC=XDIF*S(MCOUNT)
      DO 706  I=1, MCOUNT
            S(I)=S(I)/S(MCOUNT)
706   CONTINUE
C
C     7. Spline Interpolation.
C
      INIT=0
      CALL DCSINT(S, XS, C, MCOUNT, INIT, T, XH, NVALS)
      INIT=0
      CALL DCSINT(S, YS, C, MCOUNT, INIT, T, YH, NVALS)
C
C     8. Compute distances.
C
      IF(CONTUR.EQ.'1' ) THEN
            DO 707 I=1, NVM1
                  XC1(I)=XH(I)
                  YC1(I)=YH(I)
707         CONTINUE
      ELSE IF(CONTUR.EQ.'2' ) THEN
            DO 708 I=1, NVM1
                  XC2(I)=XH(I)
                  YC2(I)=YH(I)
708         CONTINUE
      ELSE IF(CONTUR.EQ.'3' ) THEN
            DO 709 I=1, NVM1
                  XC3(I)=XH(I)
                  YC3(I)=YH(I)
709         CONTINUE
      ELSE  IF(CONTUR.EQ.'4')THEN
            WIDTH=XDIF*DSQRT((XC1(1)-XH(1))**2+(YC1(1)-YH(1))**2)
            DO 710  I=1, NVM1
                  D12(I)=DSQRT((XC1(I)-XC2(I))**2+(YC1(I)-YC2(I))**2)
                  D23(I)=DSQRT((XC2(I)-XC3(I))**2+(YC2(I)-YC3(I))**2)
                  D34(I)=DSQRT((XC3(I)-XH(I))**2+(YC3(I)-YH(I))**2)
710         CONTINUE
      ENDIF
C
C     9. Compute angles of segments with the reference segment.
C
C     (Note: We chose to compute the angle by forming a complex
C     number whose real and imaginary parts are the differences
C     of the X-coordinates and of the Y-coordinates of the
C     endpoints respectively of the segment. The angle (in
C     radians) is then the imaginary part of the logarithm
C     of this number.)
C
      IF(CONTUR.EQ.'1' ) THEN
            DO 711 I=1, NVM1
            LC(1)=TLC
```

```
                ANGLE(I,1)=180.0D0/PI*
     *                DIMAG(CDLOG(DCMPLX(XH(I+1)-XH(I),YH(I+1)-YH(I))))
 711            CONTINUE
               ELSE IF(CONTUR.EQ.'2' ) THEN
                LC(2)=TLC
                DO 712 I=1, NVM1
                ANGLE(I,2)=180.0D0/PI*
     *                DIMAG(CDLOG(DCMPLX(XH(I+1)-XH(I),YH(I+1)-YH(I))))
 712            CONTINUE
               ELSE IF(CONTUR.EQ.'3' ) THEN
                DO 713 I=1, NVM1
                LC(3)=TLC
                ANGLE(I,3)=180.0D0/PI*
     *                DIMAG(CDLOG(DCMPLX(XH(I+1)-XH(I),YH(I+1)-YH(I))))
 713            CONTINUE
               ELSE  IF(CONTUR.EQ.'4')THEN
                DO 714 I=1, NVM1
                LC(4)=TLC
                ANGLE(I,4)=180.0D0/PI*
     *                DIMAG(CDLOG(DCMPLX(XH(I+1)-XH(I),YH(I+1)-YH(I))))
 714            CONTINUE
C
C       5. B. Complete computation of area by adding contribution
C       of gap between starts of contours 1 and 4. Then
C       multiply by the length of the reference segment
C       squared to get square millimeters.
C
                AREA=AREA1+AREA2-.5DO*(XC1(1)+XH(1))*(YC1(1)-YH(1))
                AREA=XDIF*XDIF*AREA
C
C       10. Write results to a file.
C
C       Identification.
C
                WRITE (31,2) REST2
 2              FORMAT(A29)
C
C       Length, distance from beginning of contour 1 to that of
C       contour 4 and area.
C
                WRITE (31,*) XDIF, WIDTH, AREA
                DO 715 I=1, NVM1
C
C       Distances.
C
                WRITE (31,*) D12(I), D23(I), D34(I)
 715            CONTINUE
C
C       Lengths of contours.
C
                WRITE (31,*) (LC(J),J=1,4)
C
C       Angles.
C
```

```
            DO 716 I=1, NVM1
            WRITE (31,*) (ANGLE(I,J),J=1,4)
716         CONTINUE
        END IF
        GO TO 900
C
C       11. End of computation.
C
9999    CONTINUE
        STOP
        END
```