Evolutionary Relationships among the Salt Marsh *Aedes* (Diptera: Culicidae)[1]

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ABSTRACT. This study delineated the phylogeny of common salt marsh *Aedes* mosquitoes through phenetic and phylogenetic analyses of electrophoretic data. The results were subsequently used to determine if existing classifications based on morphology were consistent with the derived phylogeny. Electromorphs were coded by allele frequency and quantified by one Manhattan and six Euclidean distance measures. Phenetic assessment was accomplished by UPGMA cluster analysis. Phylogenetic treatments included the distance Wagner procedure in producing both midpoint and out-group rooted trees, and by construction of the most parsimonious cladogram.

INTRODUCTION

Salt marsh *Aedes* mosquitoes comprise an ecological assemblage of approximately a dozen species in the world. Collectively, these species exhibit variable taxonomic affinities, but all appear to have descended from ancestors which have left more terrestrial counterparts. A few have successfully invaded inland where their unique adaptations generally restrict them to saline sites.

Most of the salt marsh species are clearly distinguishable morphometrically. They are thus amenable to systematic analysis in

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comparing their taxonomic affinities based on both morphometric and biochemical data sets. For this study, six salt marsh and ten out-group species were examined (Table 1). Out-groups included taxa with a broad range of presumed evolutionary relationships to the salt marsh species and with specific systematic questions regarding their own phylogeny.

Objectives. - This project was designed to discover whether or not existing classifications of the salt marsh *Aedes* reflected their evolutionary history. Since these classifications have been based on phenetic analysis of morphology and the phylogeny of the *Aedes* was unexplored, the primary objective of this study subsequently became threefold: (1) to examine an alternative data set through multiple analytical techniques, (2) to reconstruct a phylogenetic history based on molecular data, and (3) to evaluate existing classifications for congruency with a reconstructed phylogeny.

Current Biosystematics. - The classification scheme of Edwards (1932) as described by Steward (1968) is illustrated in Figure 1 and was used as an initial reference point for this study. Alternative classifications developed by Rohlf (1963a, 1963b) and Steward (1968) are depicted as generalized phenograms in Figures 2 and 3 respectively for subsequent comparisons and discussion. With few exceptions the salt marsh *Aedes* occur north of the equator and they have historically been placed among the *communis*, *dorsalis*, and *stimulans* groups, and in the subgenus *Culicelsa*.

The *Culicelsa* is a well-defined assemblage believed to include *Aedes sollicitans* (WALKER) and *Aedes taeniorhynchus* (WIEDEMANN), which are salt marsh species, and two freshwater species, *Aedes nigromaculis* (LUDLOW) and *Aedes mitchellae* (DYAR). All are Nearctic species believed by Ross (1964) to have originated from a common ancestor within the United States. Although the taxonomy of each species has been very stable, classification of the group has ranged from consideration as remnants of a primitive and distinct subgenus (Edwards, 1932) to unique but more recently derived species within the subgenus *Ochlerotatus* (e.g., Rohlf, 1963b).

The taxonomy of the mosquitoes collectively belonging to the *dorsalis* group has been widely interpreted (Gutsevich et al., 1974; Richards, 1956; Rohlf, 1963b; etc.). The ancestor of this group is thought to have arisen in central Asia and later have extended its range into western Europe and North America with evolutionary descendants either restricted to the New or Old World, or ubiquitously Holarctic (Ross, 1964). The group is generally believed to consist of eight species (Gutsevich et al., 1974; Knight and Stone, 1977; etc.), four of which are subjects of this study: *Aedes campestris* DYAR & KNAB, *Aedes caspius* (PALLAS), *Aedes dorsalis* (MEIGEN), and *Aedes melanimon* DYAR. All except *campestris* and *melanimon* are typically salt marsh forms.

Controversy has especially persisted regarding the taxonomic rank of *dorsalis*. Holarctic in distribution, this organism has been reclassified nine times since it was first described in 1830 (Knight and Stone, 1977; Knight, 1978). Opinion has recently been sharply divided as to whether *dorsalis* is a distinct species, or a subspecies of *caspius*. Until 1978,

Knight and Stone listed *dorsalis* as a full species based on the works of Richards (1956) and others who believed that American *dorsalis* and Eurasian *caspius* possessed characters sufficiently different to warrant consideration as separate species. Although these two taxa were also known to be morphologically distinct and sympatric in western Europe, Knight (1978) reclassified *dorsalis* as a subspecies of *caspius* following the discovery of intermediate forms in eastern Europe and Asia (Gutsevich et al., 1974). More recently, Rioux et al. (unpublished) could not find molecular evidence of introgression between these two species from sympatric populations sampled in eastern France. Their conclusion of full-specific status for both was supported by the production of sterile hybrids through forced copulation experiments. In subsequent geographic works Darsie and Ward (1981) and Wood et al. (1979) continued to hold the opinion that differential characteristics were sufficient to retain *dorsalis* as a full species.

Other than the possible inclusion of *dorsalis*, a total of thirteen Palearctic subspecies have been described for *caspius* (Knight, 1978). The taxonomy of *campestris* has been very stable but this species was believed to be strictly Nearctic until Danilov (1980) reported the discovery of populations in Russia. *Ae. melanimon* thus remains the sole member of the group limited in distribution to the Western Hemisphere, but it has also experienced systematic uncertainties. Shortly after being described in 1924, this organism was considered as either a synonym or subspecies of *dorsalis* (Knight and Stone, 1977). *Ae. melanimon* was later resurrected to a full species based on differences in the structure of the male terminalia (Barr, 1955), and additional morphological differences in larvae and adult females are currently recognized.

A Palearctic species, *Aedes detritus* (HALIDAY) seems to be the only member of the *communis* group adapted to salt marshes and appears to comprise a species complex (Pasteur et al., 1977). The remaining salt marsh species included in this study is the Nearctic *Aedes cantator* (COQUILLETT) which, although clearly described on a species level, has not been hierarchically ranked with ease. This species has been closely allied with both the *communis* (Rohlf, 1963b) and *stimulans* (Steward, 1968) groups, but in this study will initially be treated as a member of the latter group as originally suggested by Edwards (1932).

Most of the out-groups selected for study have a confusing taxonomic history above the species level. One of two Nearctic subspecies, *Aedes c. canadensis* (THEOBALD) has alternatively been placed in the *dorsalis* (Edwards, 1932) and *communis* (Rohlf, 1963b) groups, but Ross (1964) and Steward (1968) were of the opinion that *canadensis* was the sole member of a unique group. Holarctic in its range, *Aedes cataphylla* DYAR has consistently been placed in the *communis* group and has an unremarkable taxonomic history. A Nearctic species which may exist in the Old World (Danilov, 1981), *Aedes niphadopsis* DYAR & KNAB was originally believed to also be a member of the *communis* group. However, *niphadopsis* was considered by Rohlf (1963b) to be a member of an expanded *dorsalis* group which additionally included *Aedes flavescens* (MUELLER). The latter species has long been regarded as a close relative of *Aedes excrucians* (WALKER) (e.g., Edwards, 1932; Wood et al., 1979) with which it has been placed in

the *stimulans* group. The uncertain taxonomic position of *flavescens* is further marked by Steward's (1968) conclusion that this species is the sole representative of a primitive lineage.

Aedes vexans (MEIGEN) is Holarctic in distribution, but is the only member of over a hundred species in the subgenus *Aedimorphus* known to occur in the Western Hemisphere. This species has been known under a dozen synonyms since it was first described and is believed to have a subspecies in western Asia (Knight and Stone, 1977). Both *Culex tarsalis* COQUILLETT and *Anopheles freeborni* AITKEN are limited in range to southwestern Canada, the United States, and Mexico, and their taxonomy has been very stable.

METHODS AND MATERIALS

Experimental Design. - The general utility of electrophoresis to assess genetic variability is well-known and has frequently been used to resolve species problems within the Culicidae. Since the development and improvement of phylogenetic methods, however, a great deal of debate has occurred regarding the relative merits, applications, and limitations of these methodologies. Controversies have especially continued in discussions of taxonomic congruence (Mickevich, 1978, 1980; Mickevich and Farris, 1981; Rohlf et al., 1983; etc.) and in estimations of phylogenetic trees using genetic distance data (e.g., Farris, 1981; Fitch and Margoliash, 1967; Prager and Wilson, 1976, 1978). Although no single congruence measure has received general acceptance (Berlocher and Bush, 1982), high levels of consistency between different data sets have been reported when analyzed using phylogenetic techniques (Bullini and Sbordoni, 1980; Ferguson, 1980; Mickevich and Johnson, 1976; etc.).

Contrary results from the joint application of controversial theories can be minimized when methods are selected which theoretically accomplish specific objectives (Simon et al., 1982). This guidance was generally followed throughout this project. However, when the completion of objectives for this study necessitated the combined use of methods which generated conflicting results, resolution was obtained through selection of the most statistically consistent procedures. In summary, this project was designed on the following theoretical basis: (1) molecular techniques will effectively and consistently differentiate species of mosquitoes; (2) phenetic and phylogenetic methods are equally applicable to both molecular and morphometric data; (3) results obtained from the use of phenetic and phylogenetic methods are comparable when their theoretical constraints are not exceeded; (4) for any group of organisms there is only one true phylogeny which can best be estimated from the combined use of analytical techniques; (5) dendrograms which are least homoplastic, best conform to available goodness-of-fit criteria, exhibit the shortest tree length, and are most consistent with one another are preferred for phylogenetic reconstruction; and (6) classifications which reflect evolutionary history are preferred for their heuristic value.

Sampling Procedure. - Thirty-two populations of mosquitoes were sampled in 1983 for this study. Collection sites, sampling dates, and population codes for subsequent reference are listed in Table 2. Based on the works of previous investigators (Gorman and Renzi, 1976; Nei, 1978; Nei and Roychoudhury, 1974; Sarich, 1977), a minimum of five individuals from each population were considered sufficient for systematic study. Adult female mosquitoes over forty-eight hours in age (i.e., host seeking) were selected for analysis since they (1) do not contain pupal enzymes which may obscure accurate genetic distance determination, (2) are genetically more precisely defined than immatures of a given species, and (3) are easier to locate and/or identify than larvae, pupae, and adult males (Pasteur, personal communication).

Specimens were captured through the use of an aspirator as they sought to bite, in CDC miniature light traps baited with dry ice, or reared from larvae. Fully active mosquitoes were subsequently rendered immobile by exposure to either dry ice or liquid nitrogen, quickly identified to species, and then frozen and transported in a portable liquid nitrogen container. Specimens were later stored in an ultra low freezer at the University of New Mexico until they underwent electrophoresis in the autumn following collection. All electrophoresis was completed within six weeks in laboratory facilities of Dr. Terry Yates. Frozen voucher specimens were retained in the same facility.

Electrophoresis. - In preliminary studies seventeen polymorphic loci (i.e., isozymes) from eleven enzyme systems were found to stain for diagnostic allozymes in all species examined, and are listed in Table 3. The electrophoretic equipment, materials, and procedures employed are summarized in Schultz (1984). Prior to electrophoresis each mosquito was homogenized in an equivalent volume of ultra distilled water which provided sufficient sample material for two gels. As noted in Table 3, protein stains fell into one of two buffer systems. Since a gel was comprised of a single buffer and a maximum of six slices could be obtained from each gel, it was possible to assay each individual mosquito for all loci without the need for (and complication of) pooling specimens.

A homozygous strain of *taeniorhynchus* maintained in laboratories of the Gulf Coast Mosquito Research Center (Lake Charles, Louisiana) was used as a control for all gels. Two control specimens were run with every population sample of five individuals. Numerical designation of alleles basically followed the format used by Sites et al. (1981). Allozymes at each locus of a *taeniorhynchus* control were measured in millimeters from the point of origin and designated as 100% mobility. Allozymes from population samples were then measured and designated by per cent migration relative to the 100 allele of controls. When more than one locus appeared at a protein stain, loci were successively designated by a subscript as fast (f), medium (m), or slow (s) as appropriate.

Data Analysis. - Analysis of electrophoretic data was facilitated through the use of BIOSYS-1 (Swofford and Selander, 1981), a FORTRAN IV program available at the University of New Mexico. The program was run to categorically integrate determination of genetic variability, genetic distance, and dendrogram typology.

Genetic variability measures evaluated the strength of associations among taxa and were thus of systematic interest. For this project variability measures were limited to the *Aedes* (i.e., no generic out-groups) and included calculations of allele frequencies, alleles per locus, percentage of loci polymorphic at 0.95 and 0.99 criteria, and unbiased, normal, and Hardy-Weinberg expectations of mean heterozygosity. Normal and unbiased estimations (Nei, 1972) were calculated in order to assess differences between these two measures based on sample size. Hardy-Weinberg expectations of mean heterozygosity indicate possible influences of selection, genetic drift, and other factors which may cause populations to deviate from equilibrium.

Seven indices were utilized to measure genetic distance. Nei's original (1972) and unbiased minimum (1978) distances were used as a direct means of comparing genetic distance based on sample size. The latter measure is especially useful in determining if the use of small samples is justified. The remaining distance measures applied were those of Cavalli-Sforza and Edwards (arc and chord)(1967), Prevosti (Wright), Rogers (1972), and the modified Roger (i.e., with reduced weighting of loci exhibiting slight differences in allele frequency)(Wright, 1978). All but the two measures of Nei were used to construct both phenograms and phylogenetic trees. A summary of the applications of distance measures in this study is presented in Table 4.

Multiple genetic distance measures were employed since they exhibit various theoretical constraints when used in phylogenetic analysis. The indices of Nei (1972, 1978) incorporate an extended Pythagorean theorem, but are not metrics. The arc, chord, Prevosti, Rogers, and modified Rogers are metrics and are thus amenable to tree construction. All but the Prevosti are Euclidean. The latter has been applied as a Manhattan metric (e.g., Berlocher and Bush, 1982).

The distance Wagner procedure was selected for this study due to its established utility as a numerical phylogenetic technique (Swofford, 1981; Wiley, 1981; etc.). The "F" value of Prager and Wilson (1976) was chosen for selection of partial networks. "Criterion III" (Farris, 1972) was selected as the primary additive criterion for choosing between operational taxonomic units (OTU's) during tree construction.

Each of the five (metric) distance measures selected for phylogenetic analysis were used to construct dendrograms through the distance Wagner procedure by midpoint and out-group rooting. The arc, Prevosti, and Rogers measures were additionally used to produce directed trees through hierarchical out-group rooting. This was primarily done to assess the effects of utilizing generic (*tarsalis*) and subfamilial (*freeborni*) out-groups, but also served to compare results based on either Euclidean or Manhattan metrics. Rooted trees produced by both midpoint and out-group methods underwent branch-length optimization designed to minimize Prager and Wilson's "F" values (i.e., producing a more parsimonious tree through adjustment of hypothetical ancestoral-character states)(Swofford, 1981).

The conventional unweighted pair-group method of cluster analysis (UPGMA)(Sneath and Sokal, 1973) was applied to six distance measures (see Table 4) in order to provide strictly phenetic comparisons. Both clustered phenograms and distance Wagner dendrograms were evaluated by Farris (1972) "F," Fitch and Margoliash (1967) per cent standard deviation, and cophenetic correlation (Swofford, 1981) goodness-of-fit statistics.

Although distance measures provided full use of allele frequency information, discrete character analysis was additionally included in this study to achieve greater confidence in an estimated phylogeny. Allozymes coded by their relative mobilities were subjected to classical Hennigian cladistics using methods described by Wiley (1981). Allozymes from each out-group taxon were thus examined for synapomorphic, autapomorphic, and symplesiomorphic character states on a per locus basis, and these electromorphs were subsequently used to generate the most parsimonious cladogram.

RESULTS

A total of seventeen loci from thirty-two populations (sixteen species) was assayed in this project. All 134 allozymes recorded migrated anodally. Electrophoretic mobilities were rounded to the nearest five per cent for clarity and were actually adjusted by less than plus or minus two per cent. A detailed account of genetic and biochemical variablity, genetic distances, phenograms, and phylogenetic trees resulting from this study is presented in Schultz (1984). Allele designations and frequencies for all species examined are summarized in Table 5.

Genetic Variability. - Allele frequency, polymorphism, and heterozygosity values were very uniform throughout the range of taxa examined, but were conservative estimates relative to the results from previous genetic studies (see Bullini and Coluzzi, 1978; Ferguson, 1980). The mean number of alleles per locus calculated (1.1) is one-half to one-third that previously reported for mosquitoes (Bullini and Coluzzi, 1978), reflecting the low number of populations rather than individuals sampled for this study. Unlike the mean number of alleles, low heterozygosity estimates (per locus) are due to the limitations inherent in sampling five individuals per population.

Observed and expected heterozygosities were very similar, indicating that populations were in Hard-Weinberg equilibrium. G-tests for each of nine species with multiple populations demonstrated that there were no significant differences between observed and either standard or unbiased Hardy-Weinberg expectations at high alpha levels (e.g., 0.5). An additional G-test at alpha equal to 0.4 resulted in complete congruence between observed and expected heterozygosities at all thirty-two populations sampled. As anticipated, heterozygosity values obtained through Nei's (1978) unbiased estimate for correction of small sample size were always higher than results obtained from the usual method due to the larger squared deviations derived by the former.

Genetic Distance. - Over 5,000 distance coefficients computed by seven distance measures are listed in Schultz (1984). These results indicated that the use of the Prevosti and either arc or chord measures minimized data redundancy and maximized statistical "fit" while providing a basis of comparison in evaluating Manhattan and Euclidean metrics respectively. The coefficients derived from the Prevosti and arc measures are presented in Table 6. Justification for the use of small samples was supported by Nei's (1978) minimum distance of 0.729 ± 0.268 averaged for all population and 0.175 as the lowest value calculated between any two species (*caspius* and *campestris*).

Phenetic Analysis. - All phenograms were in agreement on the relative magnitude of associations among individual populations. In no case did the clustering level of any intra-specific association exceed that between species (Schultz, 1984). Phenograms using the arc and Prevosti measures are illustrated in Figures 4 and 5 respectively. Goodness-of-fit statistics based on all distance measures used in phenetic analysis are summarized in Table 7.

Phylogenetic Analysis. - Dendrograms constructed by the distance Wagner procedure varied by the choice of out-group and method (e.g., midpoint) selected. Midpoint and out-group rooted dendrograms using the arc and Prevosti measures are illustrated in Figures 6 - 13. Summary goodness-of-fit statistics are presented in Table 8. Figure 14 and Table 9 depict the most parsimonious cladogram and synapomorphic character states used in construction respectively. Branch-length optimization is ommitted from subsequent discussions since use of this procedure frequently distorted the shape of trees by allowing path-length (i.e., patristic) distances to be less than observed (i.e., phenetic) distances between taxa.

DISCUSSION

Phenograms basically fell into one of two patterns differing qualitatively only in phenetic relationships within the *dorsalis* group. The arc, chord, and modified Rogers measures placed *caspius* phenetically closest to *campestris*, while the remaining measures indicated that *caspius* was most similar to *dorsalis*. The "best fit" phenograms were those employing arc and chord distances, lending support to Wright's (1978) opinion that the measures of Cavalli-Sforza and Edwards (1967) may be the most preferred for electrophoretic data sets. The modified version of Rogers distance closely follows the previous two statistically and suggests that the basic typology described by these three measures is most phenetically representative. In this case one may assume that *caspius* is genetically most similar to *campestris*, since this is the only point of departure among the various distance measures.

Dendrograms produced by the midpoint rooting method, especially those based on arc and chord distances, superficially resembled biochemically derived phenograms. As with phenograms, midpoint-rooted trees essentially

divided taxa into one of two general groups. One group typically included the *Culicelsa* as the most derived taxa, while the *dorsalis* group was the most consistently derived assemblage in the other. Taxa within the *Culicelsa* displayed branching patterns equivalent to those of phenograms but differed in delineation of *dorsalis* members. While retaining the close phenetic relationship between *caspius* and *campestris*, midpoint rooting using the Prevosti and Rogers measures placed *dorsalis* rather than *melanimon* ancestoral to the group.

The midpoint rooting method always located *cataphylla*, *flavescens*, and *vexans* closest to the *Culicelsa*, although the relative position of each varied. Dendrograms based on the arc, chord, Prevosti, and Rogers placed *vexans* most near the *Culicelsa*, while the modified Rogers measure considered *vexans* to be within a subgroup which included *cataphylla* and *flavescens*. The salt marsh species *cantator* and *detritus* were consistently placed adjacent to each other in all phenograms and dendrograms. In midpoint-rooted trees, however, the position of both was closer to the *Culicelsa* than to the *dorsalis* group according to all measures accept the arc and chord. The latter two measures also differed from the others in situating *canadensis* closer than *niphadopsis* to the *dorsalis* group.

The basic patterns of wide diversification between the *Culicelsa* and *dorsalis* group is repeated when dendrograms are rooted to *vexans*. However, these out-group rooted trees differed in general placement of *detritus* and *cantator*, the relative positions of *canadensis* and *niphadopsis* to the *dorsalis* group, and depiction of the most ancestoral member of the latter group. As with midpoint rooting, these dendrograms placed *melanimon* antecedent to *dorsalis* using the Prevosti and Rogers measures, while those constructed by the arc, chord, and modified Rogers distances suggested that *dorsalis* speciated first.

Except for the dendrogram based on the modified Rogers distance, *cantator* and *detritus* are consistently placed between *canadensis* and a lineage which included *cataphylla* and *flavescens*. When rooted to *vexans*, the modified Rogers distance measure additionally suggested that the ancestor of *cataphylla* gave rise to a subsequent lineage which included the *Culicelsa*, but which was earlier a member of a lineage including *flavescens* as a descendant species. The arc and chord measures also indicated that *cataphylla* descended from the lineage of *flavescens*, but differ from the modified Rogers version in placing these two latter taxa closer to the *dorsalis* group than to the *Culicelsa*.

All other *vexans*-rooted dendrograms indicated that *cataphylla* and *flavescens* are sister taxa which underwent divergence before the *Culicelsa* speciated. Midpoint rooting based on arc and chord distances alternatively suggested that *cataphylla* is actually ancestoral to *flavescens*. As with midpoint rooting, the arc and chord measures reverse the positions of *canadensis* and *niphadopsis* when dendrograms are rooted to *vexans* by placing the former species closest to the *dorsalis* group.

Generic out-group rooting to *tarsalis* using the arc, chord, Prevosti, and Rogers measures produced similar results to their subgeneric

counterparts. The dendrogram constructed by Prevosti distances considered *vexans* to be the most primitive member of a lineage which subsequently diverged into two sister groups, one including the *Culicelsa* and the other all remaining taxa examined. The arc, chord, and Rogers metrics indicated that *vexans* was a more derived species which, although ancestoral to the *dorsalis* group and most other species, diverged after a lineage developed which led to the *Culicelsa*. The dendrogram based on the arc measure differed from all other tree typologies in placing *canadensis* rather than *niphadopsis* closest to the *dorsalis* group, and located *melanimon* ancestoral to the remaining members of the latter assemblage.

Out-group rooting to *freeborni* resulted in a very different set of dendrograms from those based on the other two out-groups, but presented trees which only differed from each other in the continual reversal of *niphadopsis* with *canadensis* and *dorsalis* with *melanimon* (based on arc distances). The arc, Prevosti, and Rogers measures each displayed a tree typology in which the presumed sister taxa of *cantator* and *detritus* were the first to diverge due to the relatively high number of electromorphs shared between these two species and *freeborni*. In this case *vexans* was depicted as a far more derived species than previously indicated and as an intermediate ancestor to the *Culicelsa*.

The statistics listed in Tables 7 and 8 are within the range reported from previous phylogenetic studies (e.g., Case, 1978; Prager and Wilson, 1978). Overall, trees which were constructed through either midpoint or subgeneric rooting generated the "best fit" statistics. However, these results must be viewed with a certain degree of caution.

As noted by Farris (1981), values of each statistic cannot be directly compared with one another since they each involve different scales. Consequently, in evaluation of a single tree a statistic is not preferred over one derived through a different procedure because its result is "smaller." Instead, a number of trees are assessed for "best fit" using a single statistical procedure for comparison. For this study each of the goodness-of-fit statistics was alternatively evaluated since no single statistical procedure has received general acceptance.

Midpoint rooting assumes equal patristic distance between two taxa and an ancestoral form based on equivalent rates of evolution. This assumption was not validated but this rooting method is known to be applicable in elucidating relationships among poorly understood groups. Out-group rooting is more advantageous in the delineation of phylogenies, but subgeneric rooting to *vexans* was justified only if this species is monophyletic relative to the other species examined. Excluding dendrograms in which *vexans* was chosen as an out-group, no phenogram or phylogenetic dendrogram except a single tree rooted to *tarsalis* using Prevosti distances clearly indicated that *vexans* was not potentially paraphyletic.

Although dendrograms rooted to *freeborni* appear to confirm consistently demonstrated patterns within certain groups as the *Culicelsa* and *dorsalis*, these trees should not be used as a sole basis from which to definitively place highly variable taxa. Convergence of character states greatly

increase above the generic level (Ferguson, 1980). Higher category relationships indicated by branching patterns of trees rooted to *Anopheles* species are difficult to defend due to the primitive relationship (i.e., at least subfamilial) of this genus to both the *Culex* and *Aedes*.

With these comments in mind, dendrograms rooted to *tarsalis* would seem to provide the most accurate phylogenetic portrayal even though they generated the poorest statistics. The single "best fit" dendrogram was that using the modified Roger distances rooted to *vexans*. However, this tree suggested a branching scheme at higher levels inconsistent with any other derived in this study. There are thus two most probable patterns typlified by the dendrograms which incorporated the arc and Prevosti measures. These two trees essentially differed in the most ancestoral species within the *dorsalis* group, the relationship between *cataphylla* and *flavescens*, and the justification for using *vexans* as a monophyletic out-group.

Using the phylogenetic method originally described by Hennig (Wiley, 1981), electromorphs were viewed as either apomorphic or plesiomorphic character states, and numerous cladograms were constructed to test hypotheses of presumed synapomorphies. Of the 134 electromorphs identified in this project, twenty-four were unequivocally synapomorphic in the most parsimonious cladogram developed. Overall, the cladogram illustrated in Figure 14 lends additional evidence to support associations between taxa.

The placement of *niphadopsis* nearest to the *dorsalis* group appeared most representative, especially since less parsimonious cladograms invariably situated *canadensis* even farther from the *dorsalis*. The population of *caspius* from Egypt was selectively factored from the remaining populations of that species as it was in all phenograms and phylogenetic trees. The Cairo population is most likely indicative of an intermediate form linking *caspius* and *campestris*.

The strong associations between *cataphylla* and *flavescens*, and between *cantator* and *detritus*, seem to be confirmed, as are those among the *Culicelsa*. The illustrated cladogram places *vexans*, *flavescens*, and *cataphylla* closest to the *Culicelsa* as do most midpoint-rooted dendrograms and all phenograms. This differs from most out-group rooted dendrograms which located these former three species nearest to the *dorsalis* group. According to the cladogram, the lineage terminating in the *dorsalis* group arose earlier than a lineage including *vexans* and the *Culicelsa*.

The most parsimonious cladogram appeared to delineate probable lines of descent, but underestimated the magnitude of associations between taxa. This deficiency is evident by the two unresolved trichotomies shown in Figure 14. Within the *Culicelsa* two different sets of potential synapomorphies alternatively linked *nigromaculis* with either *sollicitans* or *taeniorhynchus* (see Table 5). Although there is no doubt that these species are closely related based on three uniquely shared electromorphs, additional allozymes are necessary to parsimoniously differentiate members of this group. Similarly, *cantator* and *detritus* shared five potential synapomorphies, but did not share unique alleles with either of the other

two lineages. However, these two species are probably closely related to the *dorsalis* group, some members with which they shared twice as many alleles than with the lineage which included *vexans*, *cataphylla*, *flavescens*, and the *Culicelsa*.

CONCLUSIONS

Phylogenetic Reconstruction. - A synthesis of goodness-of-fit tests, phenetic similarities, dendrogram typologies, and synapomorphic associations permit reconstruction of the most probable phylogeny depicted in Figure 15. Rationale for this representation was based on the observations that: (1) the Manhattan distance measure did not generate a branching pattern distinct from trees based on Euclidean metrics; (2) in conjunction with theoretical limitations inherent to the use of Manhattan distances in analysis of gene frequency data, all dendrograms were evaluated as metrically equivalent; (3) the use of distance data to construct phylogenetic trees could not be defended with greater confidence than phenetic treatments of electrophoretic data in this study; (4) assumptions of constant or equivalent evolutionary rates inherent to the midpoint rooting method are limiting, but can be viewed as supportive to a single phylogenetic representation when congruent with out-group rooted dendrograms and the most parsimonious cladogram; (5) although the most parsimonious cladogram posed unresolved trichotomies, the occurrence of multiple synapomorphies at branch points lent considerable weight to the strength of specific associations, and (6) a pattern of evolutionary history consistently depicted by the most parsimonious cladogram and "best fit" phenograms and phylogenetic trees was most defensible.

The cladogram, phylogenetic dendrograms, and most phenograms indicated that the pattern illustrated in the reconstructed phylogeny between *caspius* and *campestris* is probably an accurate representation. Based on genetic distance coefficients alone, *caspius* from Egypt is only slightly more similar to other populations from France than to *campestris*. As noted in Schultz (1984), Nei (1972) distance results indicated at least subspecific status for the Cairo population. However, all rooted dendrograms and the most parsimonious cladogram suggested that the latter population was more closely related to *campestris* than to the other *caspius* populations. *Ae. caspius* thus appears to comprise a complex, at least one population of which (i.e., Cairo) is transitional between this species and *campestris*. The results of this project weigh in favor of considering the Cairo population as a distinct species.

Figure 15 depicts *dorsalis* closer than *melanimon* to *caspius* and *campestris* on the basis of congruency among all phenograms, parsimony, and the two "best fit" dendrograms. All methods except dendrograms constructed from either arc or chord distance coefficients using the distance Wagner procedure placed *niphadopsis* closer than *canadensis* to the *dorsalis* group. A close relationship between *detritus* and *cantator* was clearly established, and these two species have been located closest to *canadensis* since all methods indicated this position except three midpoint-rooted dendrograms and an unresolved cladogram.

With the exceptions of the cladogram and phylogenetic trees based on the modified Rogers measure, *nigromaculis* was situated closest to *sollicitans*. However, precise delineation of the relationships among the species comprising the *Culicelsa*, *vexans*, *cataphylla*, and *flavescens* was difficult. All methods except those using arc, chord, and modified Rogers measures indicated that *flavescens* and *cataphylla* were sufficiently close to be considered sister taxa. However, the patterns illustrated by the measures based on arc and chord coefficients rooted to either *vexans* or *tarsalis* were selected as most representative since their "best fit" dendrograms seemed to differentiate these two taxa in greater detail. *Ae. cataphylla* was additionally believed to be more closely related than *flavescens* to a genealogy terminating in the *dorsalis* group due to a number of alleles shared with members along this lineage.

The descendant rather than ancestoral position of *vexans* relative to the *Culicelsa* seemed justified for several reasons. The results from cluster analysis, parsimony, and simple inspection of distance coefficients and electromorphs indicated that *vexans* was not distinctly monophyletic. Consequently, greater emphasis was placed on branching patterns which precluded its function as an out-group. The typology shown in Figure 15 was consistently evident, although dendrograms rooted to *tarsalis* were not the "best fit." The great genetic distance of the *Culicelsa* from all other taxa and relatively close associations between *vexans*, *cataphylla*, and *flavescens* strongly suggested that the *Culicelsa* comprise the most primitive *Aedes* species examined in this study and is ancestoral to a lineage which subsequently diverged into what are currently regarded as the *Aedimorphus* and *Ochlerotatus* subgenera.

Comparative Morphology. - The basic purpose of this project was to determine if existing (morphometric) classifications represent the evolutionary history of the salt marsh *Aedes*. A comparison of the reconstructed phylogeny illustrated in Figure 15 with examples of existing classifications noted in Figures 1 - 3 and Table 10 indicates that major incongruencies exist:

1. The species *canadensis*, *cantator*, *cataphylla*, *flavescens*, and *niphadopsis* have not been consistently categorized by phenetic analysis of morphometric data alone. Phylogenetic techniques and phenetic treatment of molecular data demonstrated that these taxa exhibit consistent associations at lower levels but tended to increase in variablity hierarchically. The confused morphological groupings of these and other taxa at lower levels seem to most probably be due to failure to distinguish homoplasous and/or plesiomorphic characters from those uniquely derived, not weighing evolutionary important characters as differentially significant, or both. Inconsistencies in groups joined by molecular data at higher categories are most likely due to a failure to recognize convergence.

2. The *Culicelsa* warrant consideration as not only a distinct subgenus but as a category ancestoral to possibly several currently accepted subgenera. If the popular categorizations

(e.g., Knight and Stone, 1977) of subgenera are maintained, then the *Culicelsa* need to be elevated to a higher rank. The species within this assemblage may represent a lineage of the most primitive *Aedes* to leave evolutionary descendants.

3. *Ae. vexans* and perhaps its entire subgenus (*Aedimorphus*) is biochemically more derived than the *Culicelsa* and possibly other currently accepted members of the *Ochlerotatus*.

4. Intra-group relationships between the salt marsh *Aedes* and their kin are highly correlated both biochemically and morphologically, but members of some groups (e.g., the *dorsalis*) which are similar phenotypically are more easily and consistently distinguished biochemically than by their morphology.

5. There is no molecular evidence to support the belief that *dorsalis* is a subspecies of *caspius*. *Ae. caspius* does appear to comprise a species complex, however, one population of which (i.e., Cairo, Egypt) is transitional between *campestris* and other populations of *caspius* based on molecular criteria.

6. No single morphometric classification of the salt marsh *Aedes*, their kin, and selected out-groups reflected the evolutionary pathways concluded by molecular data analysis. However, the results of specific phenetic treatments of molecular data closely parallel the same treatment of morphometric data for lower taxonomic categories.

Classifications. - Assignment of appropriate taxonomic levels for mosquitoes through phylogenetic classification is difficult for two principal reasons. First, the entire family Culicidae is in need of hierarchical revision. Inter-category differentiation and intensive speciation by many forms are sufficient to elevate the rank of most mosquito taxa. Second, too few taxa and characters were examined in this study to generate an unambiguous classification at higher categories. However, the results of this study lend support to certain presumed associations and seriously question others.

The reconstructed phylogeny depicted in Figure 15 and previously constructed phenograms from other studies are indicative of the necessity to elevate ranking within the Culicidae. Phylogenetically, each branch point represents a speciation event. Since all supraspecific taxa originate as species, each terminal lineage must represent either ancestoral species which survived a speciation event or the descendant species of higher taxa. At each branch point subsequent lineages are sister groups which should be ranked at equivalent taxonomic levels. Future phylogenetic analyses will undoubtedly continue to demonstrate insufficient categories by present convention to explain a plethora of branch points between taxa.

The reconstructed phylogeny represents an estimate of natural associations and indicates that a classification can be constructed which depicts evolutionary history. Elevated ranking is primarily a problem at the lowest hierarchical level (i.e., genus). Generic and familial levels must be

distinguished from those of other taxa by a significant gap. Although the illustrated phylogeny does not present serious problems in elevating taxa to new familial levels, assignment of some taxa to generic levels from their current "group" status would be difficult.

Dispersal Routes. - The reconstructed phylogeny additionally presents a model to describe dispersal routes. The belief by Ross (1964) that the ancestor of current representatives of the *dorsalis* group migrated to North America via the Bering Strait from Asia is not supported by the results of this project. Rather, a theory is proposed in which a single Holarctic *dorsalis* ancestor initially existed. The first lineage to diverge from this form could have been that of the Nearctic speciation by *melanimon* to freshwater habitats. However, it is also possible that *melanimon* represents a stem species from which a salt tolerant form proliferated into Holarctic salt water habitats while *melanimon*, in far greater competition with other freshwater species, remained restricted in range. In either event, two Holarctic forms seemed to later develope, one including a descendant *dorsalis* and the other a *caspius* and *campestris* ancestoral form. Incomplete reproductive isolation could account for areas, as in eastern Europe, where introgression may currently occur between *caspius* and *dorsalis*, a phenomenon which could suggest a primary location for speciation of these two forms. Further selective pressures resulting in habitat partitioning or some other manner of either sympatric or allopatric speciation may have led to the divergence of *campestris* and *caspius*. A vicariant event could easily explain the present distributions of these latter two species by which *caspius* is relegated to Palearctic zones while *campestris* is limited to relic populations in both Asia and North America.

The four species *cantator*, *detritus*, *canadensis*, and *niphadopsis* seem to form a loose assemblage which may comprise the framework for the *communis* group. The salt marsh species *cantator* and *detritus* appear so closely related and ecologically similar that they most likely shared a Holarctic ancestor which adapted to temperate saline habitats and subsequently speciated into Nearctic and Palearctic forms respectively. These two species may be typical members of the *communis*, but the only ones to successfully invade salt marshes. *Ae. canadensis* and *niphadopsis*, especially the latter species, are possibly transitional forms linking the *communis* and *dorsalis* groups. The relatively isolated (Great Basin) distribution and restricted habitats of *niphadopsis* suggest that, although this species is more closely related to the *dorsalis* group than the other species examined, it probably represents a unique lineage terminating in a highly derived taxa. However, it may represent relic populations of a primitive Holarctic form if, as with *campestris*, its occurrence in Eurasia was to be confirmed.

Although sharing few morphological and ecological similarities with each other, *flavescens* and *cataphylla* appear to be the most primitive of the bona fide members of the *Ochlerotatus* members examined. The fact that they are relatively closely associated biochemically should not obscure the possibility that their mutual affinity is strictly relative to the taxa included in this study. There is little doubt that species as these

two with divergent hierarchical branch points represent distinct assemblages which may include complexes as the *excrucians* and *stimulans* groups.

The remaining assemblage which included salt marsh species, the *Culicelsa*, very likely evolved in North America as theorized by Ross (1964). *Ae. sollicitans* occasionally utilizes inland saline sites for breeding, but it and *taeniorhynchus* typically inhabit coastal salt marshes and are easily distinguished morphologically. *Ae. sollicitans* and *nigromaculis* are very similar phenotypically, however, and are largely allopatric in distribution (usually inhabiting eastern and southern coastal salt marshes and western inland freshwater sites respectively). Through various isolating mechanisms, selective differentiation would be competitively favored within a sympatric speciation model (e.g., a localized deme of the ancestor of *sollicitans* originating within or peripheral to the range of *taeniorhynchus*). This theory leads to the conclusion that the ancestor of *nigromaculis* invaded inland freshwater sites from a common line of descent with *sollicitans* after the latter species differentiated from *taeniorhynchus*. An allopatric model, on the other hand, could propose that the ancestor of *nigromaculis* and *sollicitans* speciated in geographic isolation from *taeniorhynchus*, and that the latter two species subsequently developed a secondary zone of contact. The reconstructed phylogeny presented in this study supports either possibility, but it seems fairly certain that the ancestor of the *Culicelsa* was a salt marsh form.

In summary, three distinct lineages appear to have contributed to the current salt marsh *Aedes* fauna of the world. The *Culicelsa* were the most primitive *Aedes* examined, and seem to be typically subtropical and temperate species with evolutionary descendants confined to the New World. A second group consisted of salt tolerant members of the *communis* group which diverged into temperate salt marshes of eastern North America and Eurasia, and are represented by *cantator* and *detritus*. The third and most diverse group is the *dorsalis* which apparently left a freshwater representative (*melanimon*) before undergoing a high level of speciation into salt marsh habitats.

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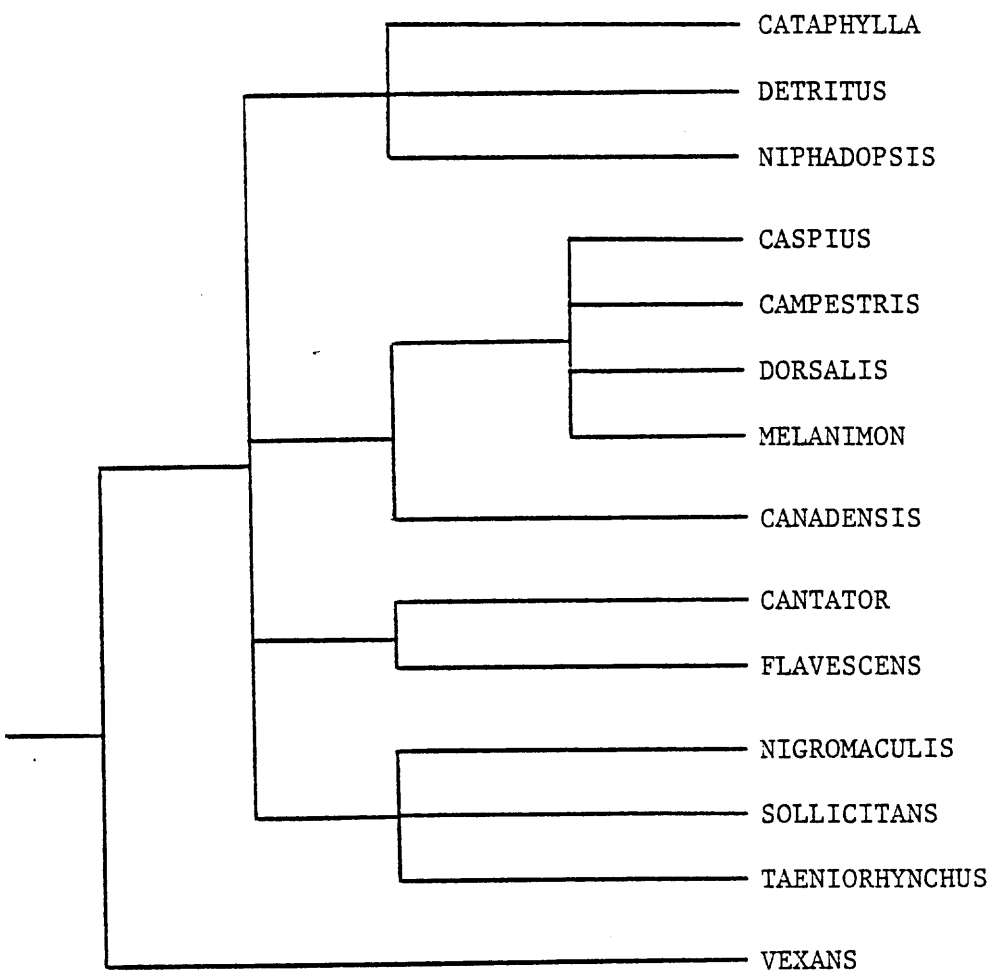

FIGURE 1. - Natural classification (*sensu* Edwards, 1932) of the *Aedes* examined in this study. Representation modified from dendrogram of Steward (1968) to include additional taxa.

FIGURES 2-3. Numerical Classification of the Salt Marsh *Aedes*, their Kin and Selected Out-groups Based on External Morphology

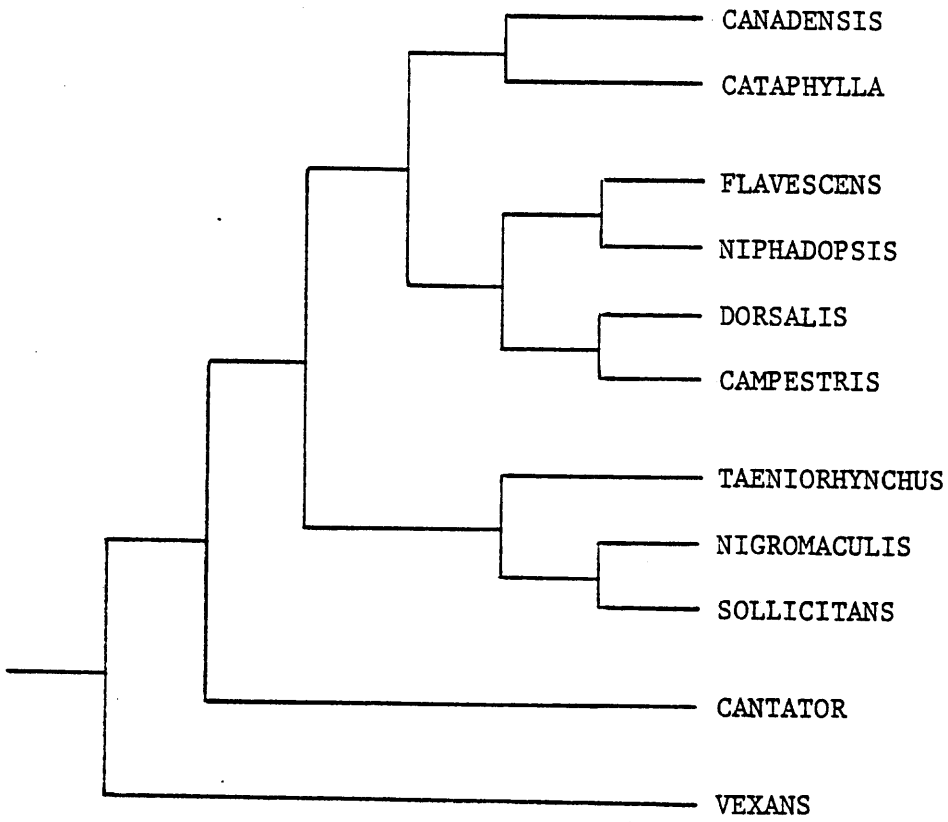

FIGURE 2a. - UPGMA classification of selected *Aedes* by Rohlf (1963b) based on adult and larval morphology. *Ae. caspius*, *Ae. melanimon* and *Ae. detritus* not included in original study. Branch lengths are not to scale.

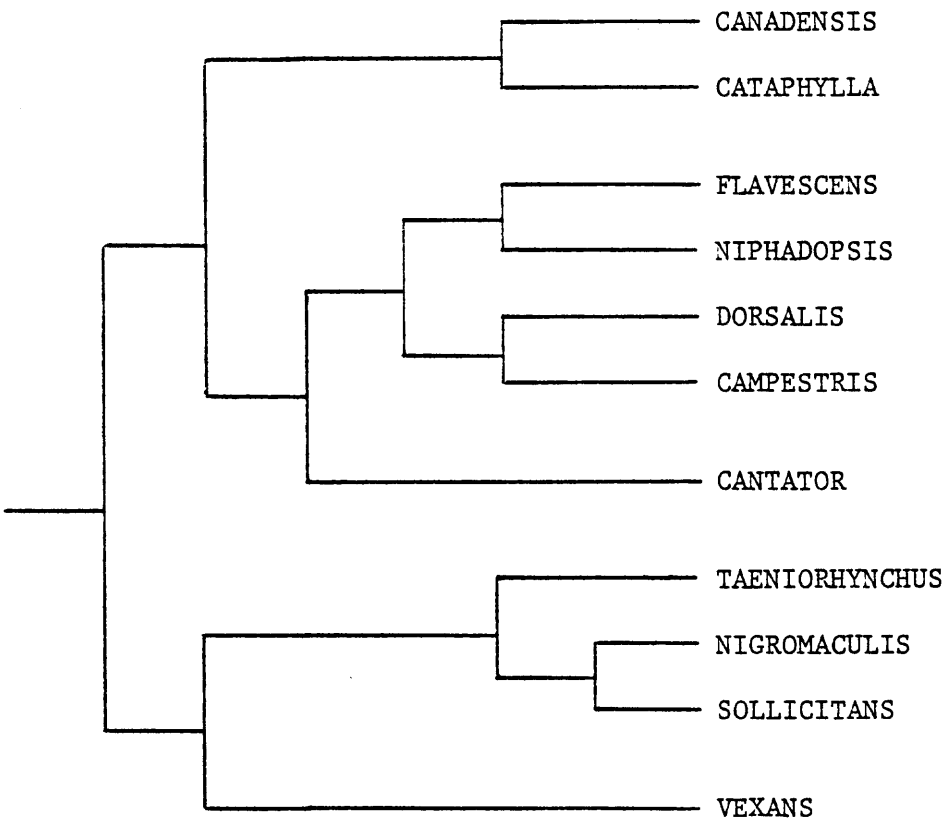

FIGURE 2b. - WPGMA classification of selected *Aedes* by Rohlf (1963a) using adult characters. *Ae. caspius*, *Ae. melanimon* and *Ae. detritus* not included in original study. Branch lengths are not to scale.

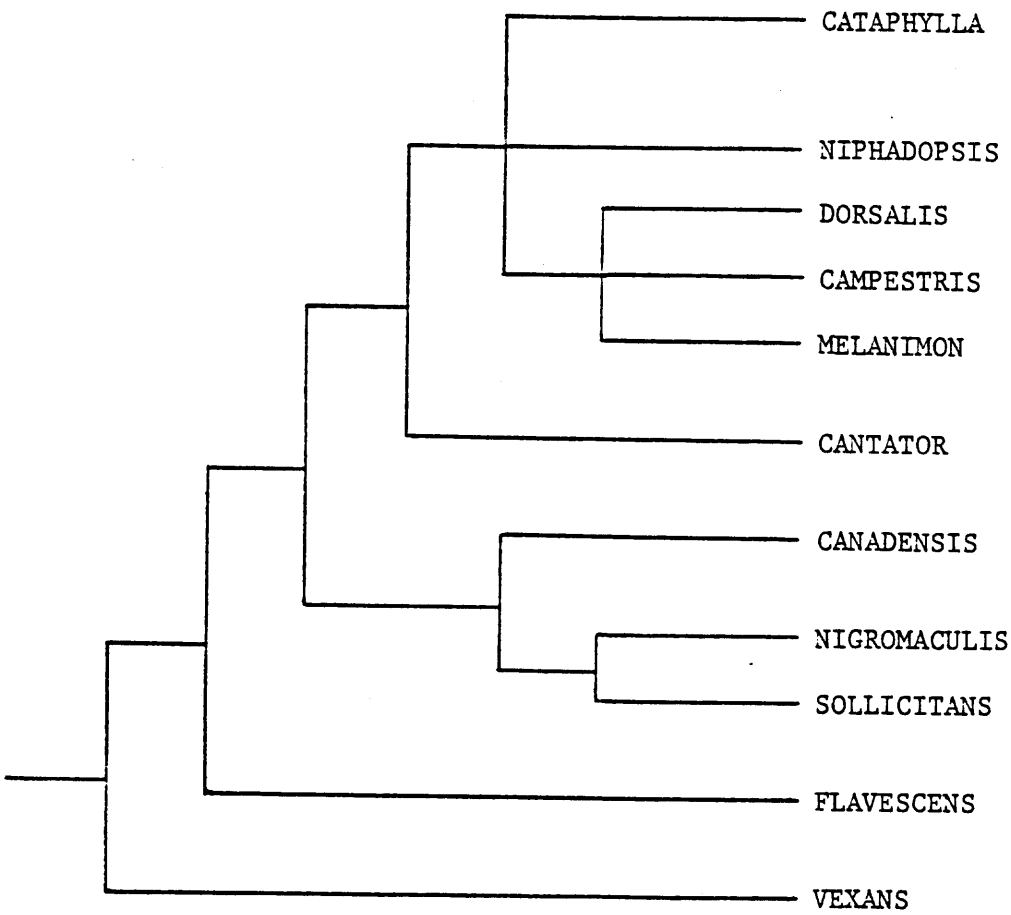

FIGURE 3. - Classification of selected *Aedes* by Steward (1968) using matching coefficients of adult characters. *Ae. caspius*, *Ae. detritus* and *Ae. taeniorhynchus* not included in original study. Branch lengths are not to scale.

FIGURES 4-5. - UPGMA Cluster Analysis of Distance Measures

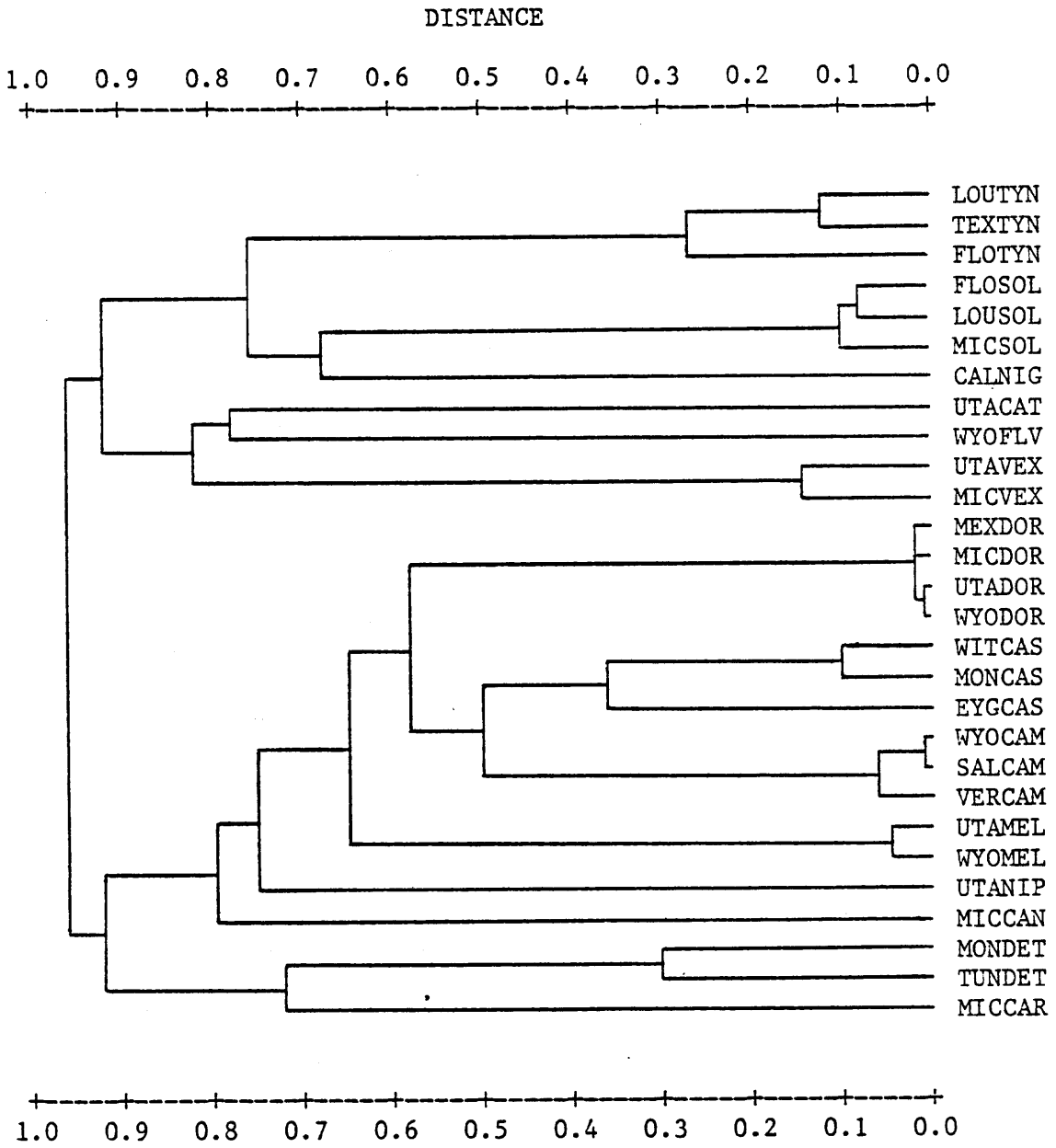

FIGURE 4. - Phenogram generated using Cavalli-Sforza and Edwards (1967) arc distance coefficients.

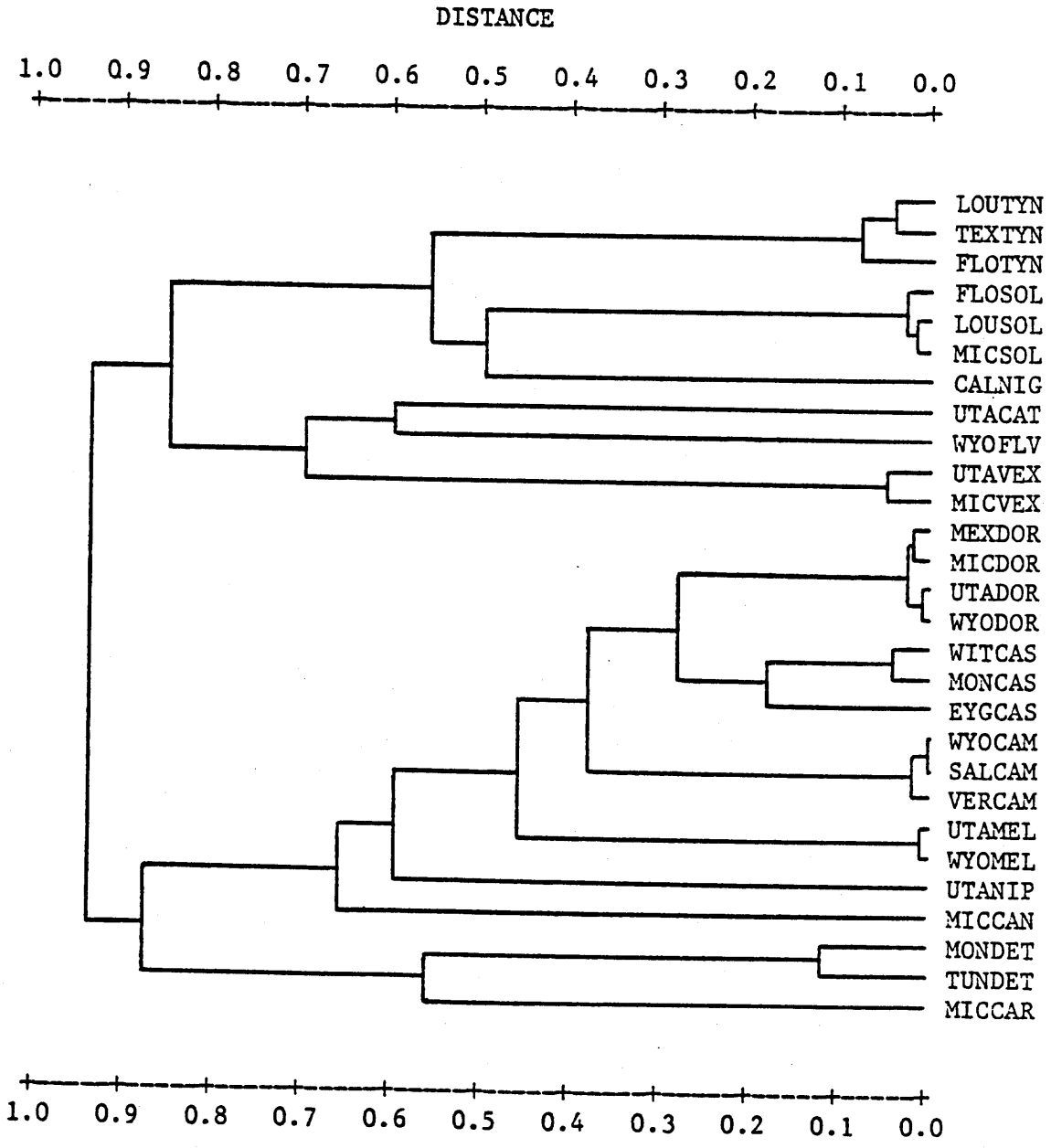

FIGURE 5. - Phenogram generated using Prevosti (Wright, 1978) distance coefficients.

FIGURES 6-7. - Dendrograms Rooted by Midpoint Method

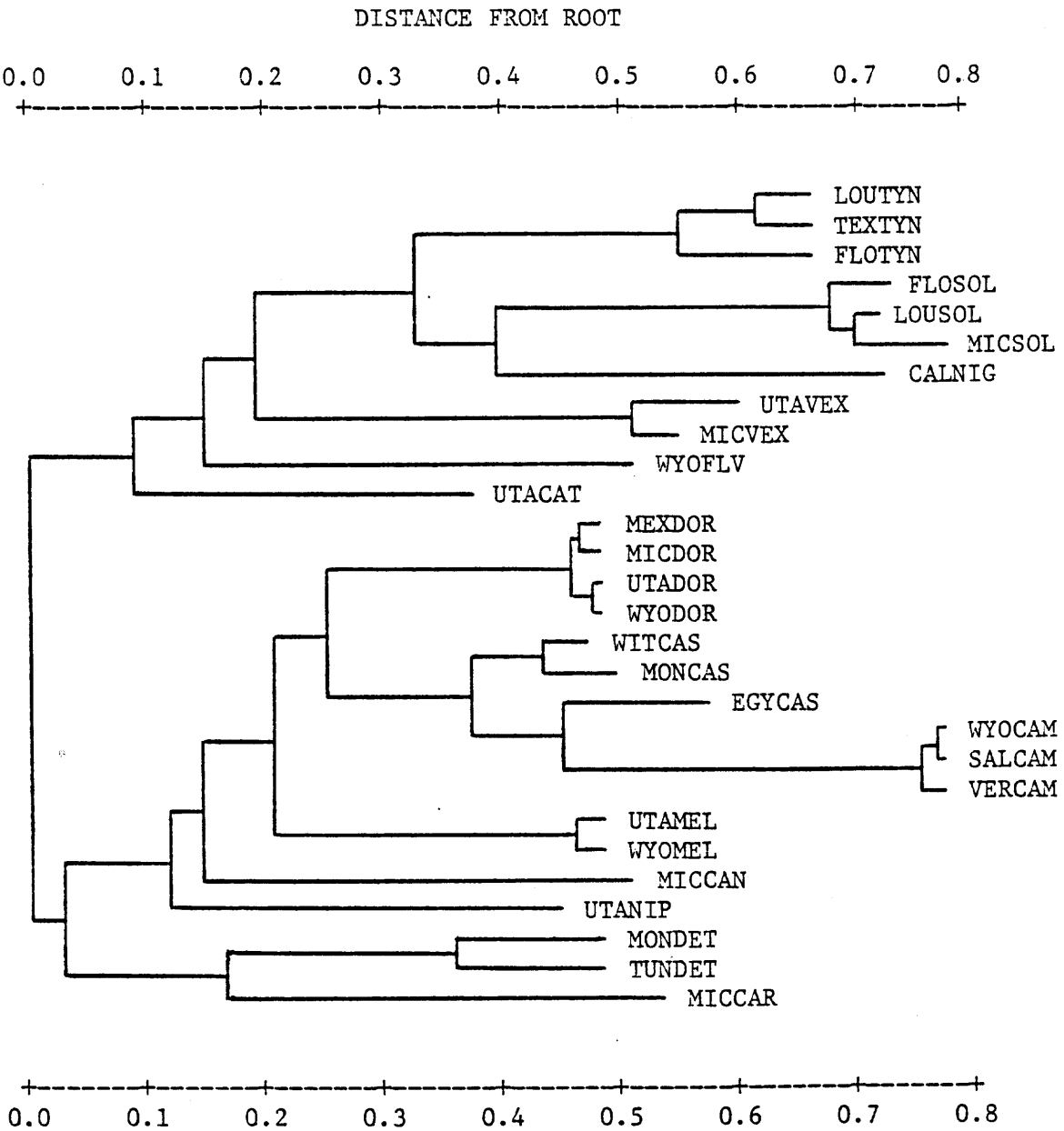

FIGURE 6. - Dendrogram rooted by midpoint method using Cavalli-Sforza and Edwards (1967) arc distance coefficients.

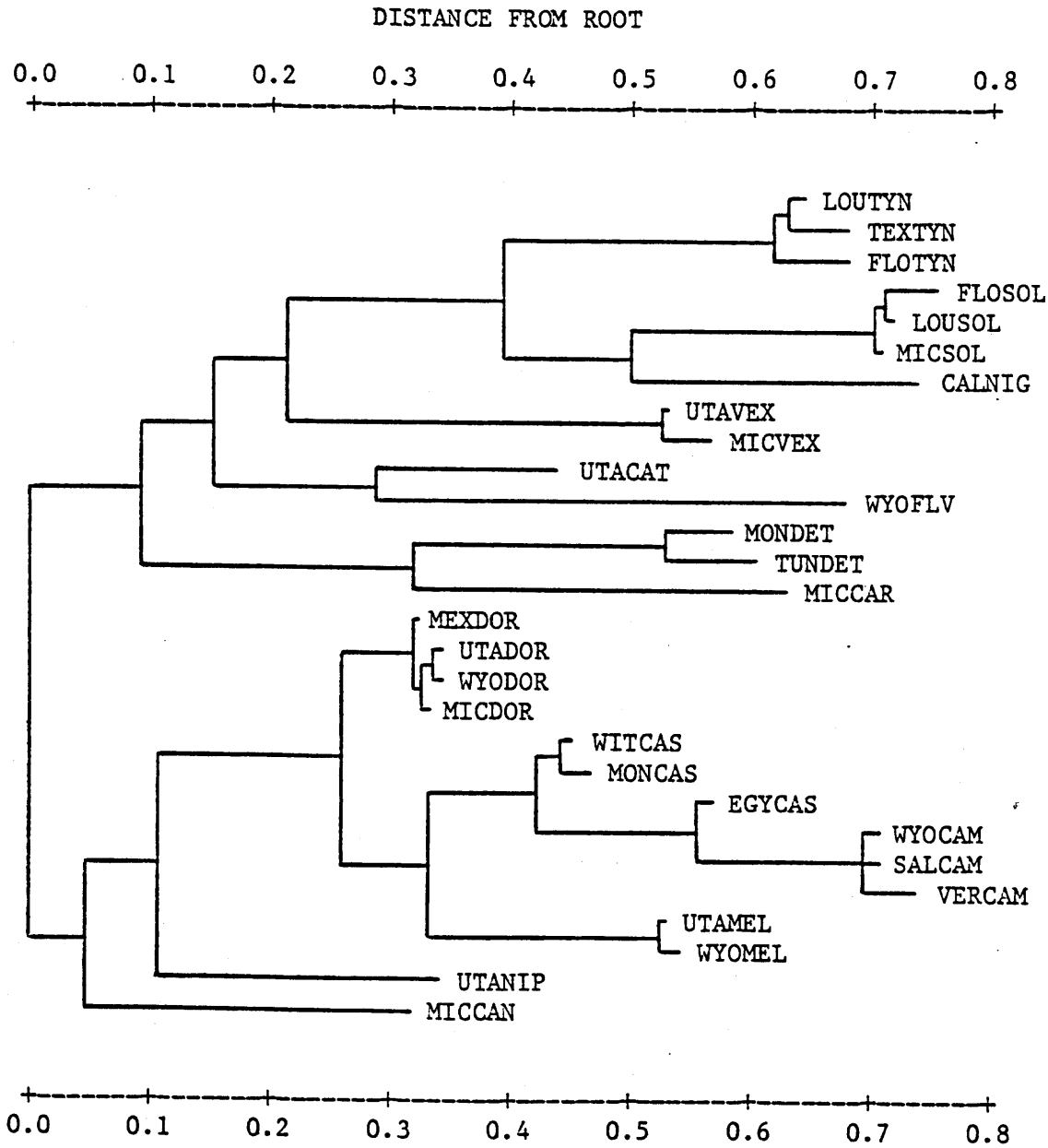

FIGURE 7. - Dendrogram rooted by midpoint method using Prevosti (Wright, 1978) distance coefficients.

FIGURES 8-13. - Dendrograms Rooted by Out-group Method

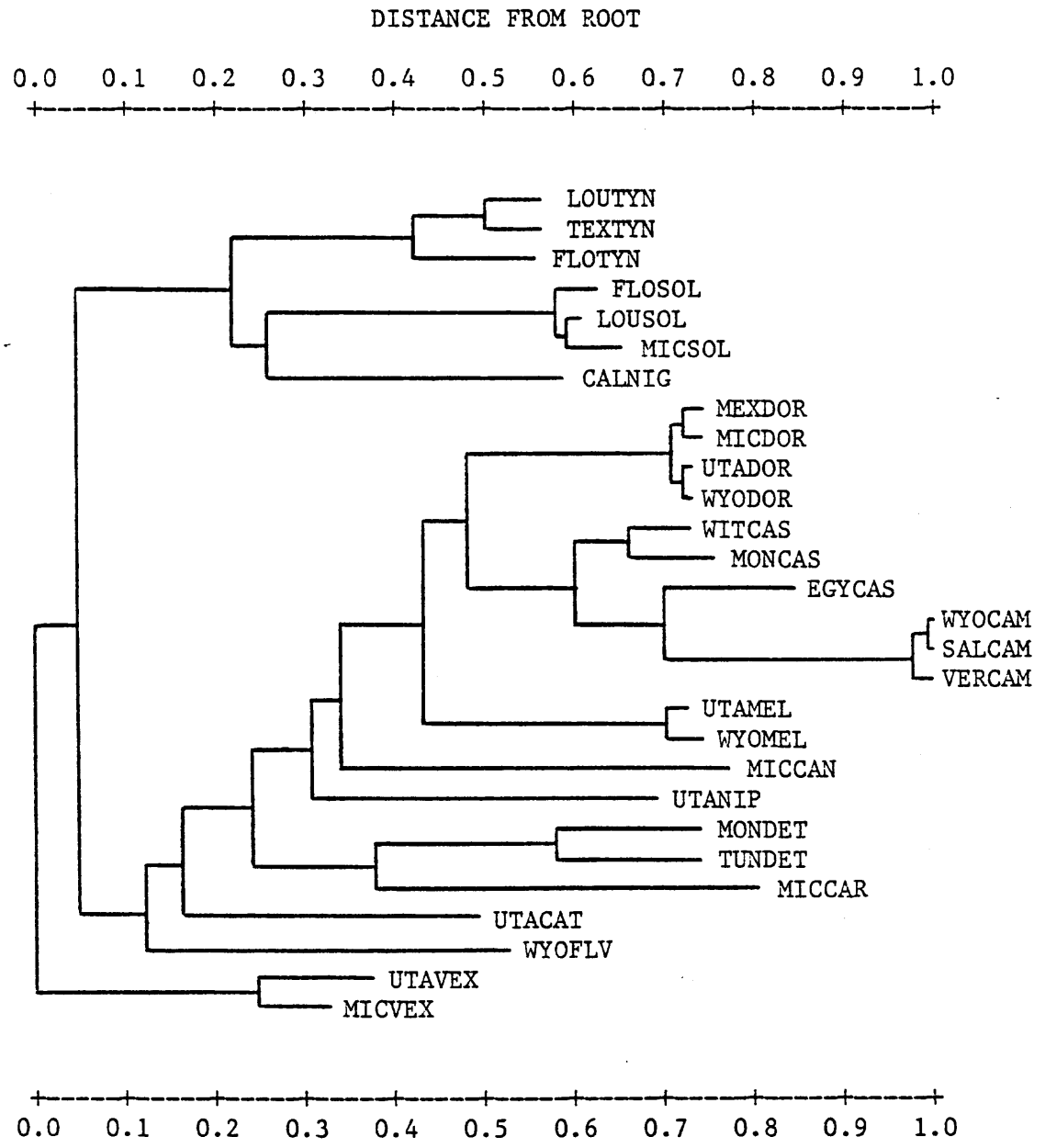

FIGURE 8. - Dendrogram rooted by out-group method to *Ae. vexans* using Cavalli-Sforza and Edwards (1967) arc distance coefficients.

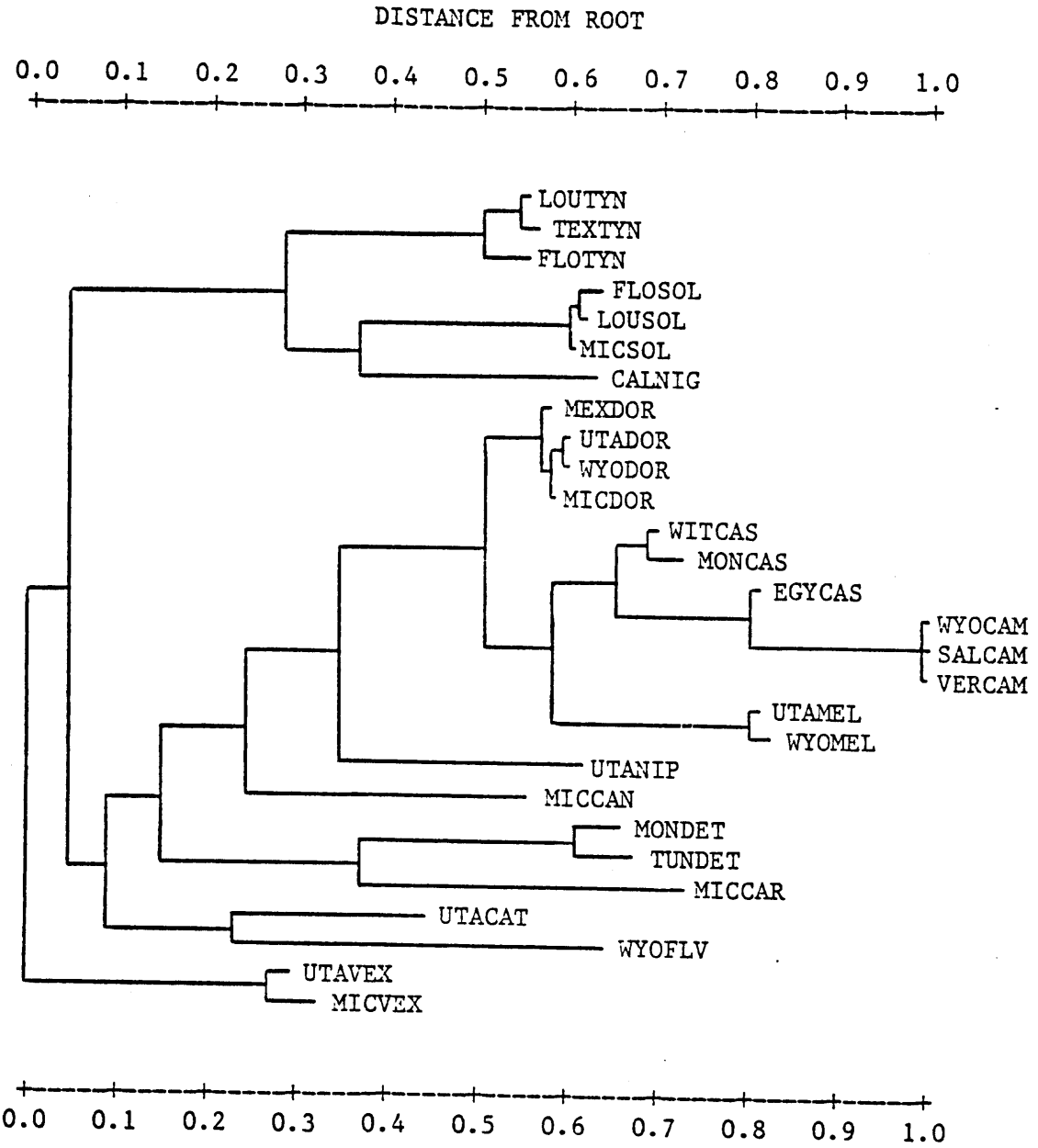

FIGURE 9. - Dendrogram rooted by out-group method to *Ae. vexans* using Prevosti (Wright, 1978) distance coefficients.

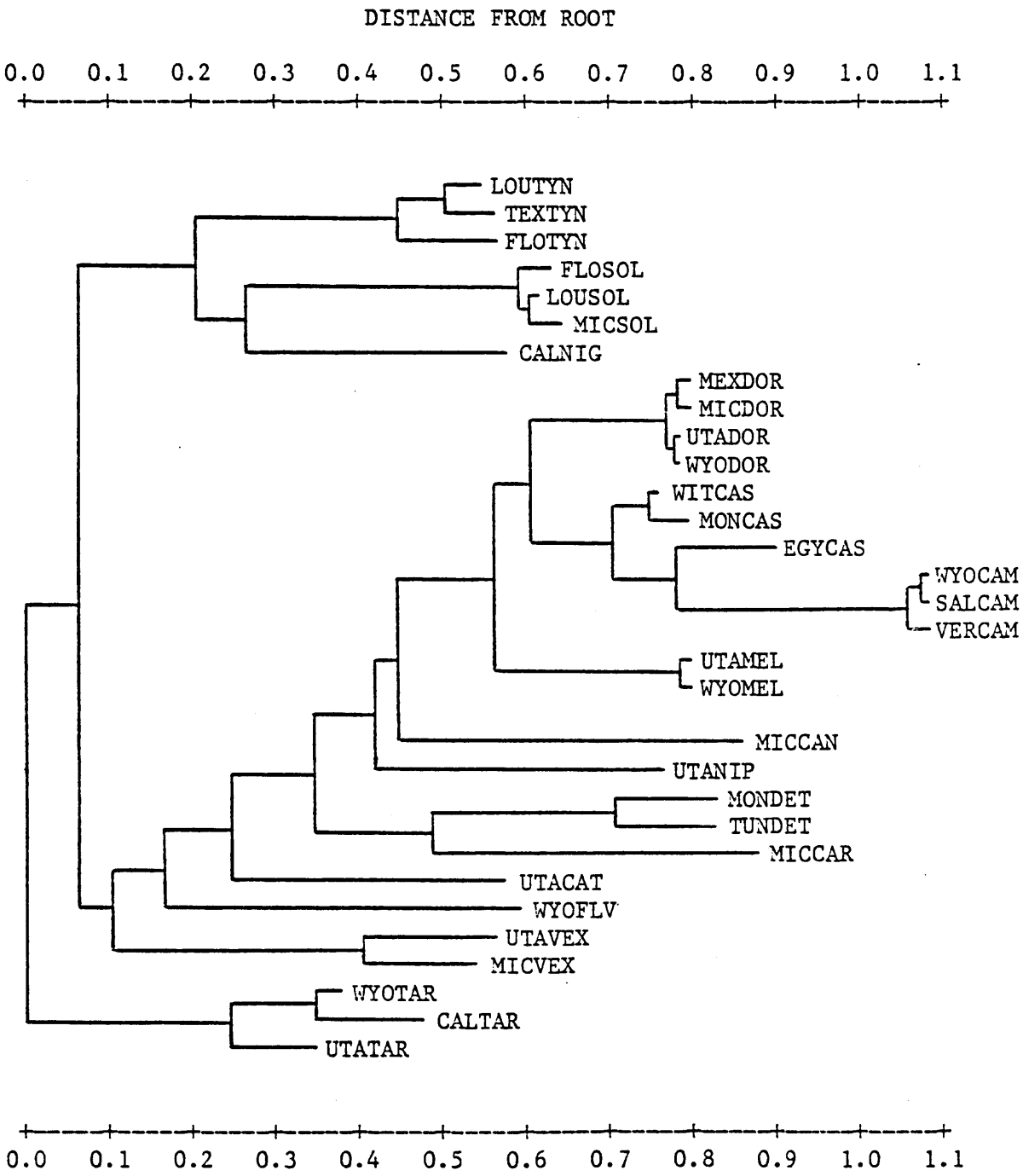

FIGURE 10. - Dendrogram rooted by out-group method to *Cx. tarsalis* using Cavalli-Sforza and Edwards (1967) arc distance coefficients.

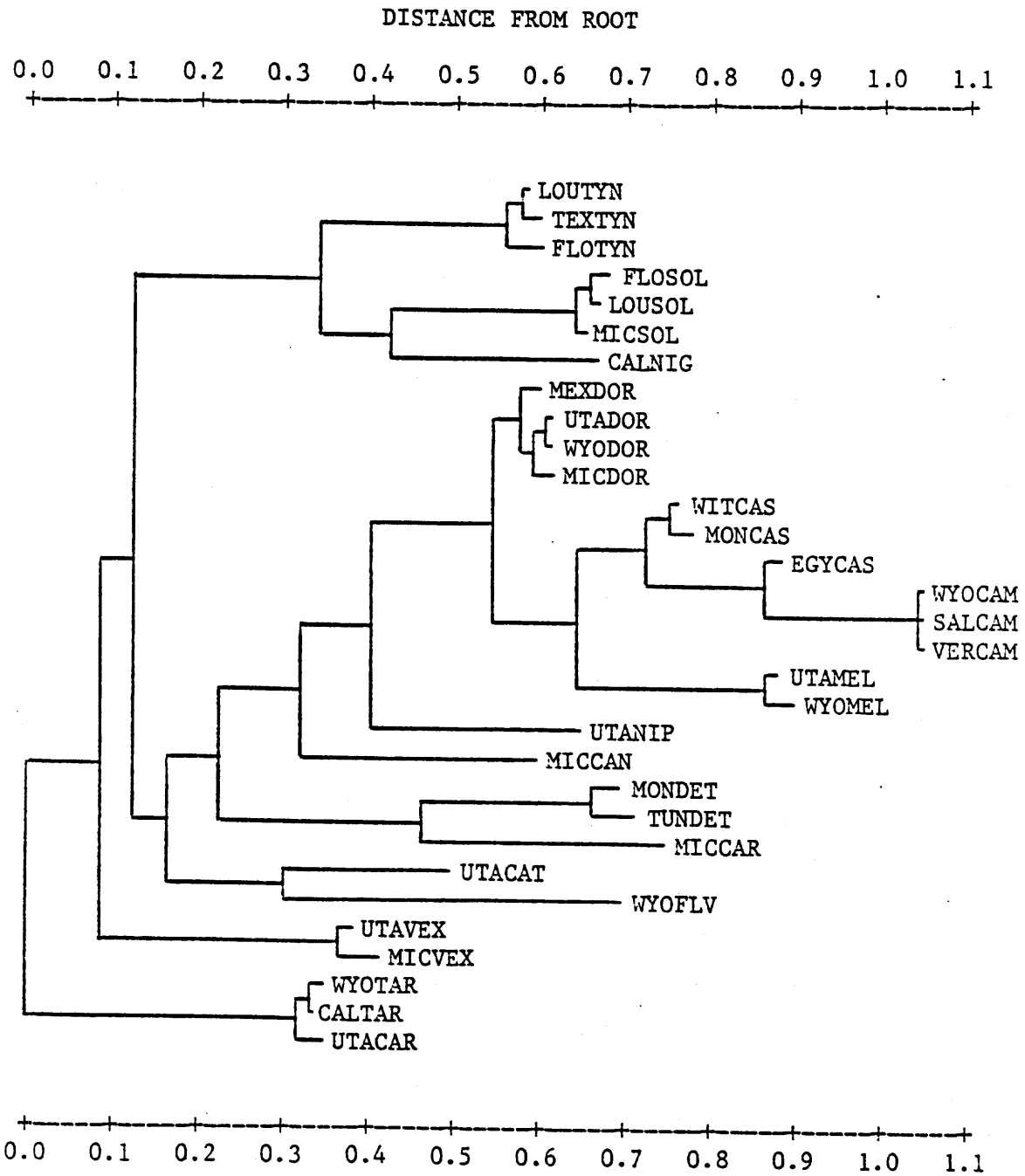

FIGURE 11. - Dendrogram rooted by out-group method to *Cx. tarsalis* using Prevosti (Wright, 1978) distance coefficients.

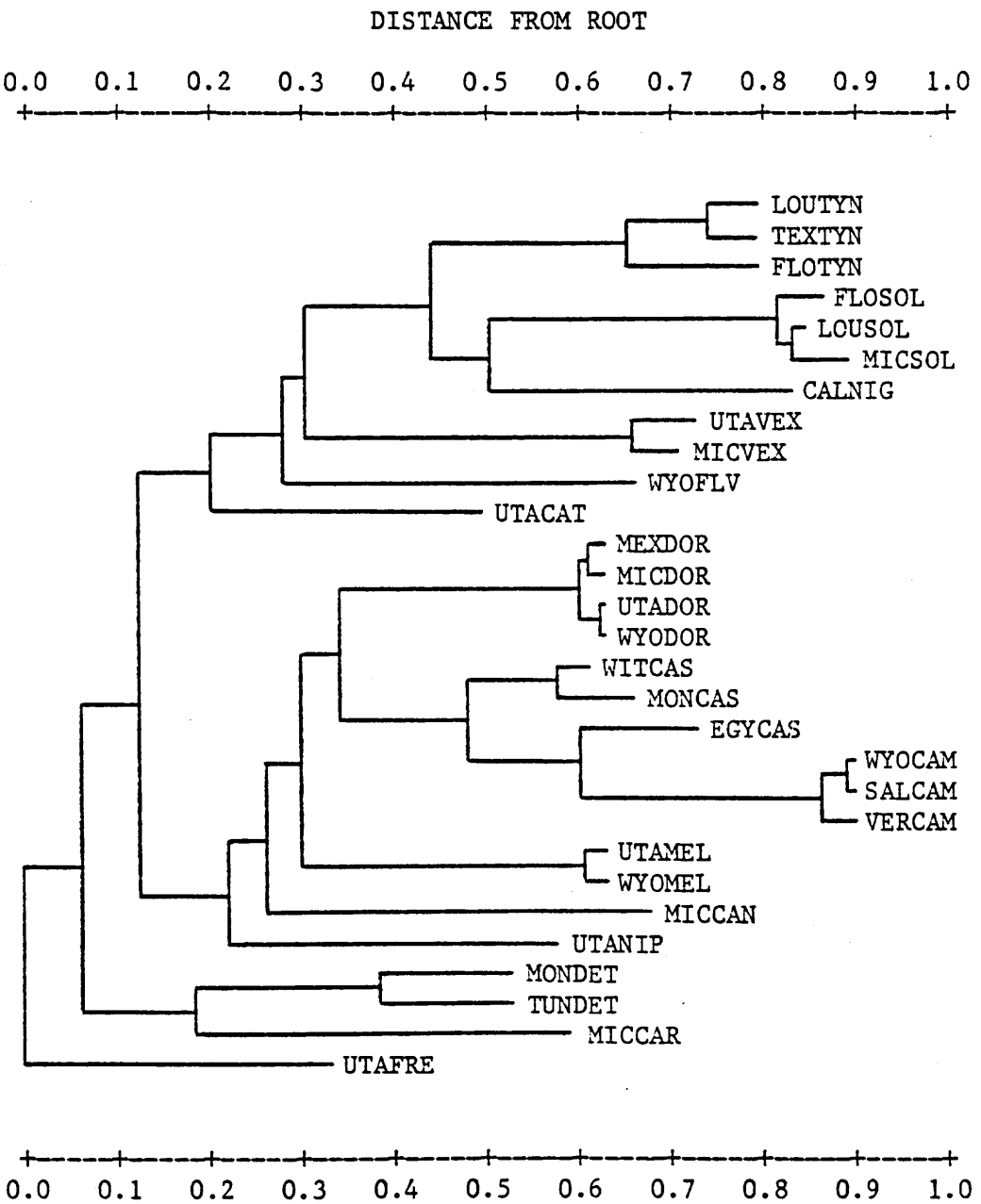

FIGURE 12. - Dendrogram rooted by out-group method to *An. freeborni* using Cavalli-Sforza and Edwards (1967) arc distance coefficients.

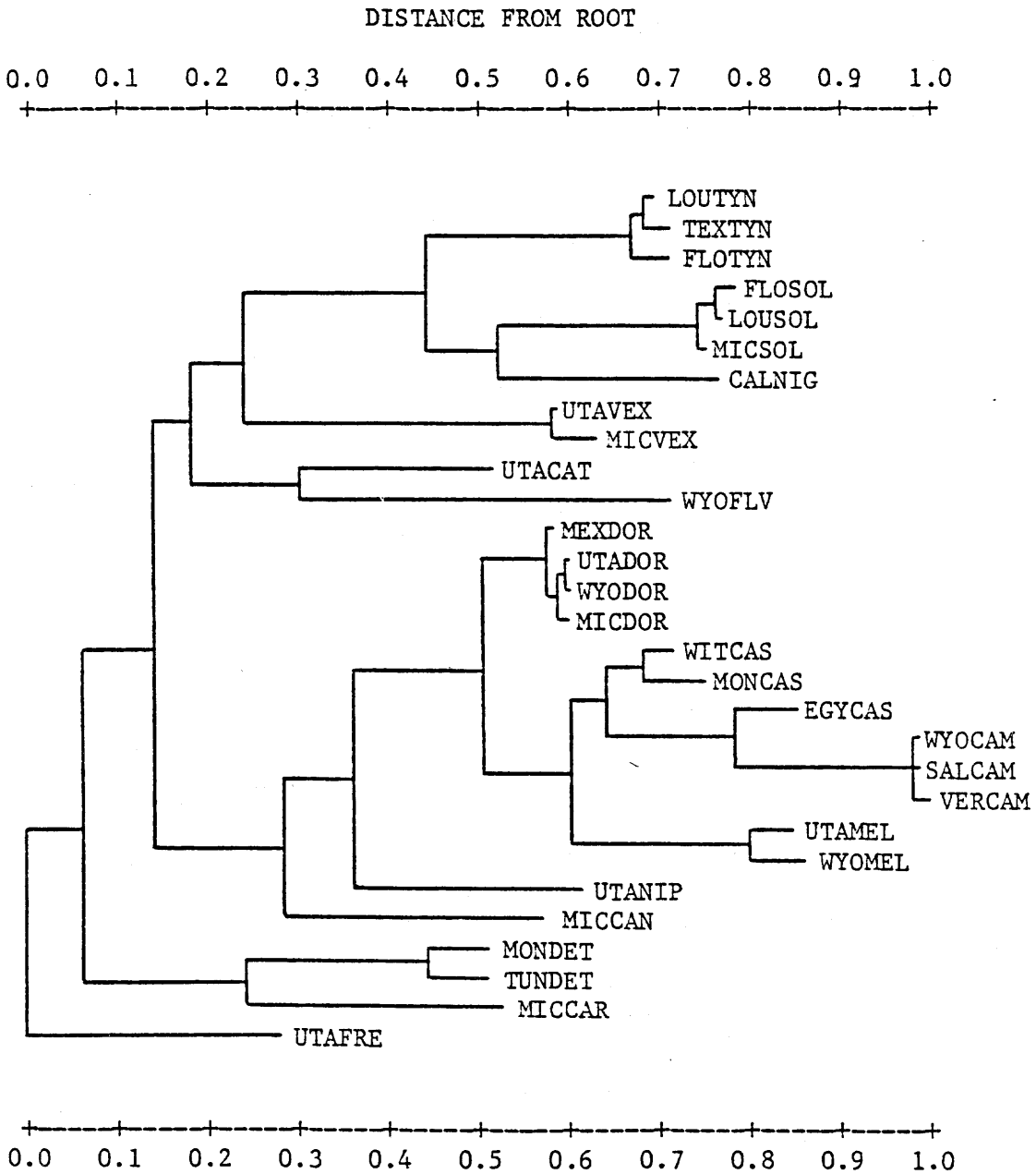

FIGURE 13. - Dendrogram rooted by out-group method to *An. freeborni* using Prevosti (Wright, 1978) distance coefficients.

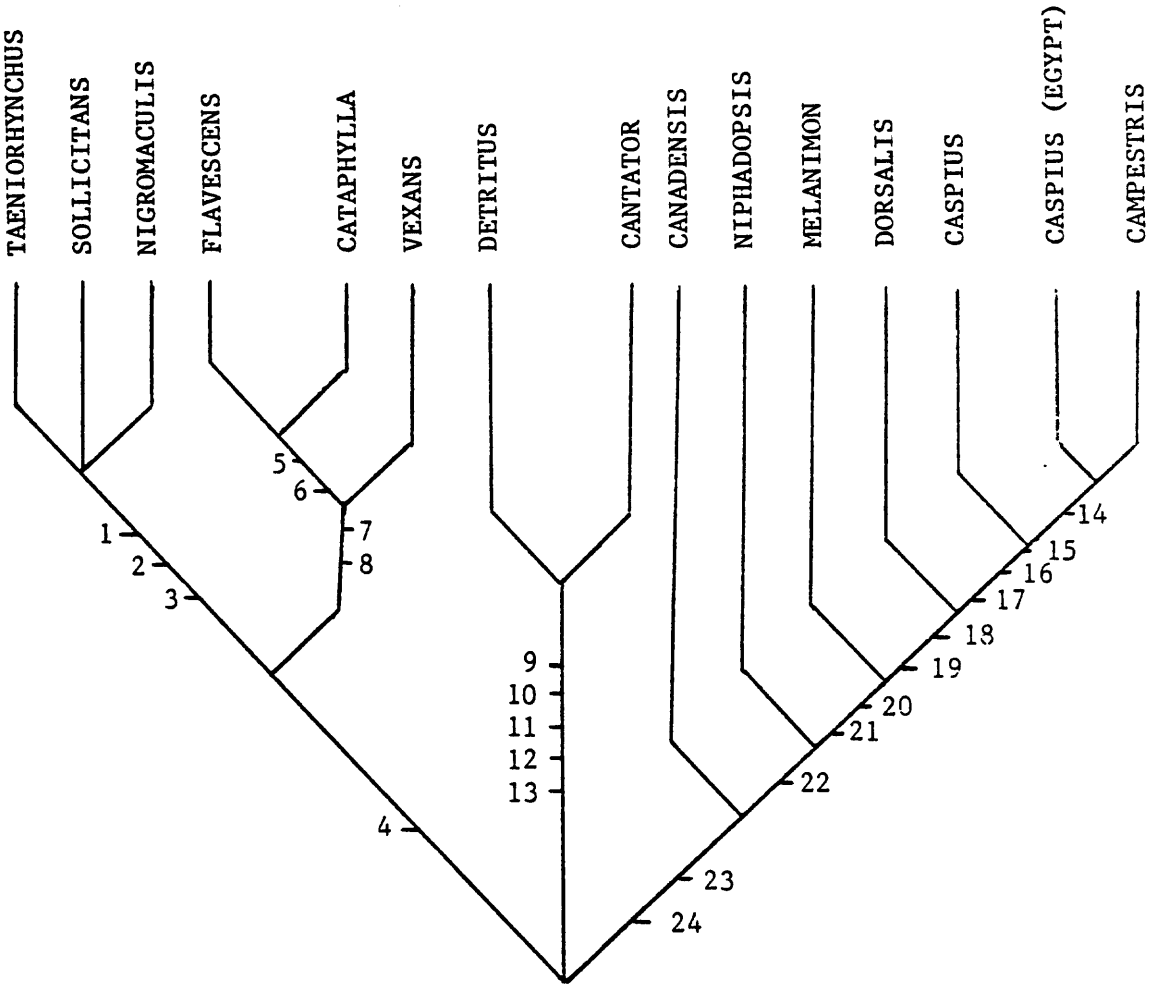

FIGURE 14.- Most parsimonious cladogram of the *Aedes* examined in this study. Only hypothesized synapomorphies are noted. (Character states described in Table 9).

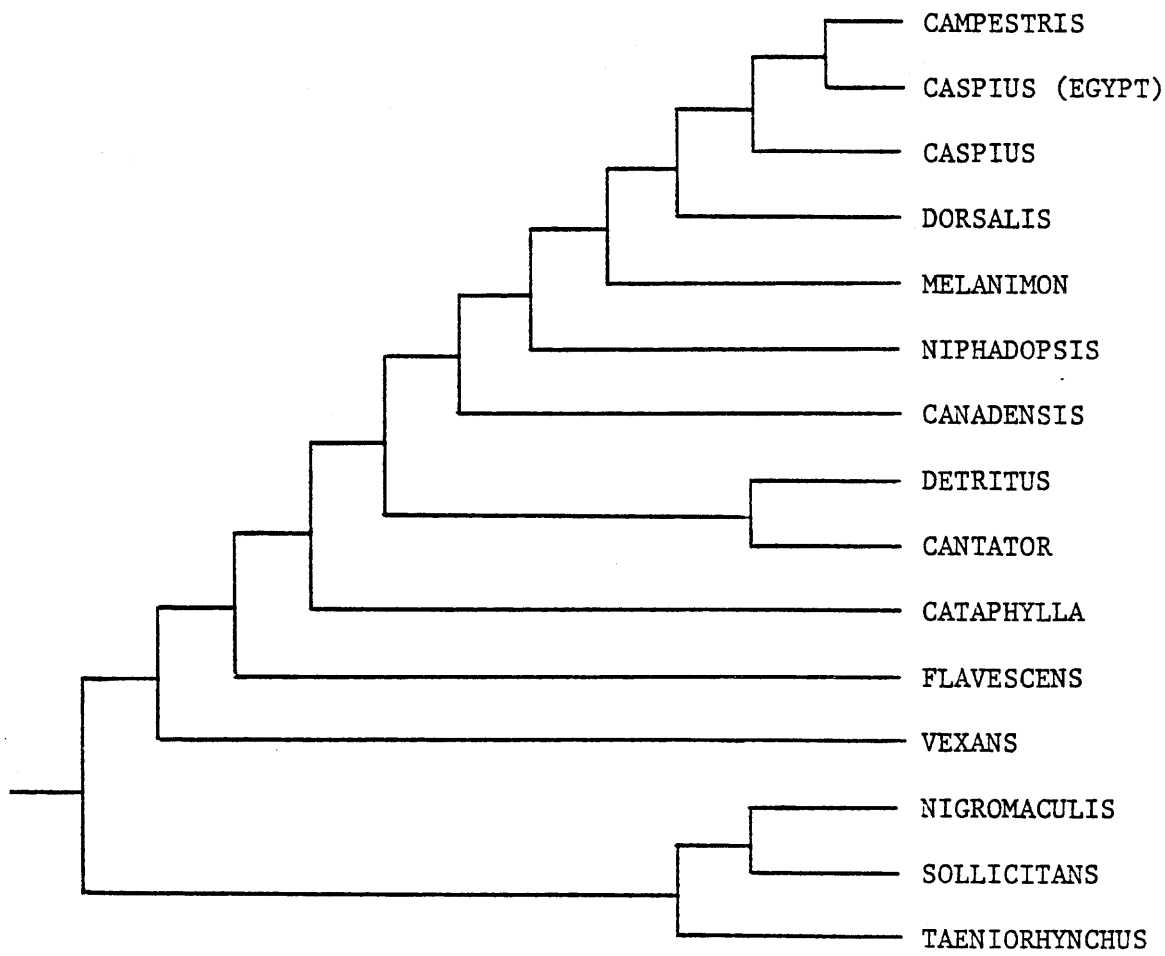

FIGURE 15.- Reconstructed phylogeny of the salt marsh *Aedes*, their kin, and selected out-groups.

TABLE 1. - Salt Marsh Species and Out-groups Selected for Study

SPECIES	CATEGORICAL ASSIGNMENT*
Aedes campestris DYAR & KNAB	salt marsh sister group
Aedes c. canadensis (THEOBALD)	supraspecific out-group
Aedes cantator (COQUILLETT)	salt marsh
Aedes caspius (PALLAS)	salt marsh
Aedes cataphylla DYAR	supraspecific out-group
Aedes detritus (HALIDAY)	salt marsh
Aedes dorsalis (MEIGEN)	salt marsh
Aedes flavescens (MUELLER)	supraspecific out-group
Aedes melanimon DYAR	salt marsh sister group
Aedes nigromaculis (LUDLOW)	salt marsh sister group
Aedes niphadopsis DYAR & KNAB	supraspecific out-group
Aedes sollicitans (WALKER)	salt marsh
Aedes taeniorhynchus (WIEDEMANN)	salt marsh
Aedes vexans (MEIGEN)	subgeneric out-group
Culex tarsalis COQUILLETT	generic out-group
Anopheles freeborni AITKEN	subfamial out-group

* hierarchical rank based on current convention (see text)

TABLE 2. - Collection Sites, Sampling Dates, and Population Codes of Salt Marsh Species and Selected Out-groups

SPECIES	LOCALITY[1]	CODE[2]	COLLECTION DATE (1983)
campestris	Vernal, Utah	VERCAM	02 May
	Salt Lake City, Utah	SALCAM	06 May
	Laramie, Wyoming	WYOCAM	15 July
canadensis	Franklin, Michigan	MICCAN	05 July
cantator	St. Clair, Michigan[3]	MICCAR	06 July
caspius	Wittlesheim, France[4]	WITCAS	11 June
	Cairo, Egypt	EGYCAS	20 June
	Montpellier, France	MONCAS	21 June
cataphylla	Park City, Utah	UTACAT	21 May
detritus	Tunis, Tunisia[5]	TUNDET	20 June
	Montpellier, France	MONDET	22 June
dorsalis	Albuquerque, New Mexico	MEXDOR	20 April
	Vernal, Utah	UTADOR	02 May
	St. Clair, Michigan	MICDOR	02 June
	Laramie, Wyoming	WYODOR	14 July
flavescens	Laramie, Wyoming	WYOFLV	14 July
melanimon	Vernal, Utah	UTAMEL	23 May
	Laramie, Wyoming	WYOMEL	14 July
nigromaculis	Fresno, California	CALNIG	11 May
niphadopsis	Grantsville, Utah	UTANIP	20 May
sollicitans	St. Clair, Michigan	MICSOL	06 July
	Lake Charles, Louisiana	LOUSOL	02 August
	Jacksonville, Florida	FLOSOL	05 August
taeniorhynchus	Houston, Texas	TEXTYN	31 July
	Lake Charles, Louisiana[6]	LOUTYN	02 August
	Jacksonville, Florida	FLOTYN	05 August

TABLE 2 (continued)

SPECIES	LOCALITY[1]	CODE[2]	COLLECTION DATE (1983)
vexans	Vernal, Utah	UTAVEX	23 May
	St. Clair, Michigan	MICVEX	02 June
tarsalis	San Diego, California	CALTAR	05 May
	Vernal, Utah	UTATAR	23 May
	Laramie, Wyoming	WYOTAR	14 July
freeborni	Vernal, Utah	UTAFRE	23 May

1. field collections listed by nearest incorporated city unless noted otherwise
2. population codes used for reference in this study
3. state record
4. laboratory colony maintained for seven years (E.I.D., Montpellier, France)
5. laboratory colony maintained for over five years (E.I.D., Montpellier, France)
6. laboratory colony maintained for ten years (Gulf Coast Mosquito Research Center, Lake Charles, Louisiana)

TABLE 3. - Buffer Systems and Protein Stains Examined by Electrophoresis

BUFFER SYSTEM	ENZYME NUMBER	PROTEIN STAIN	LOCI ENCODED
DH	1.1.1.8	∝-Glycerophosphate dehydrogenase (∝-GPD)	1
	1.2.1.37	Xanthine dehydrogenase (XDH)	1
	1.15.1.1	Superoxidase dismutase (SOD)	2
	2.7.3.2	Creatine kinase (CK)	2
	2.7.4.3	Adenylate kinase (AK)	2
CA-7	1.1.1.37	Malate dehydrogenase (MDH)	1
	1.1.1.40	Malic enzyme (ME)	1
	1.1.1.42	Isocitrate dehydrogenase (ICD)	2
	1.1.1.44	6-Phosphogluconate dehydrogenase (6-PGD)	1
	2.7.1.1	Hexokinase (HK)	3
	2.7.5.1	Phosphoglucomutase (PGM)	1

TABLE 4. - Applications of Genetic Distance Measures

DISTANCE MEASURE	GENETIC DISTANCE DETERMINATION	UPGMA CLUSTER ANALYSIS	MIDPOINT & SUBGENERIC OUT-GROUP ROOTING *	GENERIC OUT-GROUP ROOTING *
Cavalli-Sforza and Edwards (arc)(1967)	+	+	+	+
Cavalli-Sforza and Edwards (chord)(1967)	+	+	+	
Nei (1972)	+			
Nei (1978) unbiased minimum	+	+		
Prevosti (Wright, 1978)	+	+	+	+
Rogers (1972)	+	+	+	+
Modified Rogers (Wright, 1978)	+	+	+	

* distance Wagner procedure

TABLE 5a. - Allele Designations and Frequencies in the Subgenus *Culicelsa*

LOCUS	FLOTYN	LOUTYN*	TEXTYN	FLOSOL	LOUSOL	MICSOL	CALNIG
AK_f	100	100	100	100	100	100	100
AK_s	100	100	100	100	100	100	100
CK_f	100	100	100	100	100	100	100
CK_s	100	100	100	120	120	120	120
∝-GPD	100	100	100	100	100	100	100
HK_f	100	100	100	85	85	85	105
HK_m	100	100	100	85	85	85	105
HK_s	100	100	100	85	85	85	105
ICD_f	100	100	100	80	80 (.9) 105 (.1)	80	105
ICD_s	100	100	45 (.4) 100 (.6)	115	115	115	45
MDH	100	100	100	95	95	95	110
ME	100	100	100	70	70	70	80
6-PGD	100	100	100	115	115	115	115
PGM	75 (.1) 100 (.6) 125 (.3)	100	100 (.8) 125 (.2)	75 (.1) 100 (.5) 125 (.4)	100 (.8) 125 (.2)	100	75 (.3) 100 (.3) 125 (.4)
SOD_f	100	100	100	100	100	100	100
SOD_s	100	100	100	100	100	100	100
XDH	100	100	100	90	90	90	100

* control group

TABLE 5b. - Allele Designations and Frequencies in the *Dorsalis* Group

LOCUS	MEXDOR	MICDOR	UTADOR	WYODOR	EGYCAS	MONCAS	WITCAS
AK_f	95	95	95	95	95	95	95
AK_s	70	70	70	70	70	70	70
CK_f	90	90	90	90	90	90	90
CK_s	60	60	60	60	60	60	60
∝-GPD	90	90	90	90	90	90	90
HK_f	115	115	115	115	95	95 (.1) 115 (.9)	95 (.2) 115 (.8)
HK_m	120	120	120	120	95	95 (.1) 115 (.9)	95 (.2) 115 (.8)
HK_s	110	110	110	110	80	110	110
ICD_f	120	120	120	120	90	90	90
ICD_s	130	130	130	130	130	130	130
MDH	85	85	85	85	85	85	85
ME	90	90	90	90	90	90	90
6-PGD	65	65	65	65	75	75	75
PGM	100 (.5) 125 (.5)	100 (.6) 125 (.4)	100 (.7) 125 (.3)	100 (.7) 125 (.3)	100 (.8) 125 (.2)	75 (.1) 100 (.9)	75 (.2) 100 (.4) 125 (.4)
SOD_f	90	90	90	90	65	65	65
SOD_s	170	170	170	170	170	170	170
XDH	120	120	120	120	120	120	120

TABLE 5b (continued)

LOCUS	SALCAM	VERCAM	WYOCAM	UTAMEL	WYOMEL	MICCAN
AK_f	95	95	95	95	95	95
AK_s	70	70	70	50	50	80
CK_f	90	90	90	90	90	100
CK_s	60	60	60	60	60	60
α-GPD	90	90	90	90	90	90
HK_f	95	95	95	115	115	115
HK_m	95	95	95	115	115	120
HK_s	80	80	80	110	110	115
ICD_f	90	90	90	95	95	90 (.5) 130 (.5)
ICD_s	130	130	130	130	130	145
MDH	130	130	130	85	85	120
ME	90	90	90	95	95	80
6-PGD	65	65	65	50	50	40
PGM	75 (.1) 100 (.7) 125 (.2)	100 (.5) 125 (.5)	75 (.1) 100 (.7) 125 (.2)	100 (.8) 125 (.2)	75 (.1) 100 (.7) 125 (.2)	75 (.2) 100 (.8)
SOD_f	65	65	65	105	105	105
SOD_s	170	170	170	170	170	115
XDH	130	130	130	120	120	120

TABLE 5c. - Allele Designations and Frequencies in the *Communis* and *Stimulans* Groups*

LOCUS	MONDET	TUNDET	UTANIP	UTACAT	MICCAR	WYOFLV
AK_f	85	85	95	100	95	100
AK_s	60	20 (.8) 60 (.2)	60	80	60	80
CK_f	115	115	95	90	110	85
CK_s	35	35	60	60	35	90
∝-GPD	90	90	90	90	110	95
HK_f	90 (.1)	110	115	120	110	125
HK_m	110	110	110	125	110	125
HK_s	115	115	110	120	115	120
ICD_f	90	90	120	110	95	70
ICD_s	115	115	130	100	145	115
MDH	90	90	120	70	80	125
ME	75	75	75	75	80	85
6-PGD	35 (.7) 65 (.3)	150	65	80	35	80
PGM	125	125	75 (.9) 100 (.1)	100 (.8) 125 (.1) 145 (.1)	75 (.1) 100 (.4) 125 (.5)	85
SOD_f	85	85	70	70	120	70
SOD_s	65	65	65	65	65	100
XDH	140	140	120	130	140	130

* *communis*: MONDET, TUNDET, UTANIP, UTACAT; *stimulans*: MICCAR, WYOFLV

TABLE 5d. - Allele Designations and Frequencies in Subgeneric and Generic Out-groups

LOCUS	MICVEX	UTAVEX	CALTAR	UTATAR	WYOTAR	UTAFRE
AK_f	100	100	110	110	110	105
AK_s	80	80	40	40	40	100
CK_f	105	105	85	85	85	110
CK_s	60	60	60	60	60	60
α-GPD	80	80	70	70	70	90
HK_f	75	75	90	90	90	80
HK_m	80	80	90	90	90	75
HK_s	75	75	90	90	90	70
ICD_f	110 (.5) 120 (.5)	120	85	70	70 (.3) 85 (.7)	75
ICD_s	145	145	100	100	100	15
MDH	90	90	75	75	75	105
ME	85	85	85	85	85	65
6-PGD	80	80	30	30	30	80
PGM	100 (.7) 125 (.3)	85 (.1) 100 (.9)	100 (.3) 125 (.5) 145 (.2)	100 (.1) 125 (.3) 145 (.6)	100 (.1) 125 (.5) 145 (.4)	100 (.3) 125 (.7)
SOD_f	115	115	50	50	50	75
SOD_s	100	100	100	100	100	65
XDH	110	110	110	110	110	140

TABLE 6. - Arc (Cavalli-Sforza and Edwards, 1967) and Prevosti (Wright, 1978) Genetic Distance Coefficients (*Aedes*). Example only - see Schultz (1984) for comprehensive list.

POPULATION	MEASURE	LOUTYN	FLOTYN	TEXTYN	FLOSOL
LOUTYN	ARC	+			
	PREVOSTI	+			
FLOTYN	ARC	0.265	+		
	PREVOSTI	0.082	+		
TEXTYN	ARC	0.128	0.270	+	
	PREVOSTI	0.035	0.094	+	
FLOSOL	ARC	0.776	0.728	0.770	+
	PREVOSTI	0.618	0.535	0.606	+
LOUSOL	ARC	0.770	0.730	0.767	0.081
	PREVOSTI	0.600	0.541	0.588	0.024
MICSOL	ARC	0.767	0.735	0.770	0.121
	PREVOSTI	0.588	0.553	0.600	0.029
CALNIG	ARC	0.744	0.688	0.708	0.687
	PREVOSTI	0.571	0.488	0.535	0.482
MEXDOR	ARC	0.978	0.972	0.971	0.971
	PREVOSTI	0.971	0.953	0.959	0.947
UTADOR	ARC	0.974	0.971	0.970	0.972
	PREVOSTI	0.959	0.947	0.947	0.953
WYODOR	ARC	0.974	0.971	0.970	0.972
	PREVOSTI	0.959	0.947	0.947	0.953
MICDOR	ARC	0.976	0.971	0.971	0.971
	PREVOSTI	0.965	0.947	0.953	0.947
WITCAS	ARC	0.980	0.971	0.974	0.970
	PREVOSTI	0.976	0.953	0.965	0.947
MONCAS	ARC	0.971	0.974	0.974	0.976
	PREVOSTI	0.947	0.959	0.953	0.965
EGYCAS	ARC	0.973	0.972	0.970	0.972
	PREVOSTI	0.953	0.953	0.941	0.959

TABLE 7. - Goodness-of-fit Statistics for UPGMA Cluster Analysis*

MEASURE	FARRIS "F"	P-W "F"	F-M % S.D.	COPH. CORR.
ARC	11.071	3.529	7.170	0.983
CHORD	9.916	3.505	7.136	0.983
MINIMUM	18.152	6.599	22.925	0.971
PREVOSTI	17.052	6.021	13.744	0.973
ROGERS	17.338	6.162	13.824	0.972
MODIFIED	11.579	3.708	7.682	0.982

* statistics are the Farris (1972) "F," Fitch and Margoliash (1967) per cent standard deviation, Prager and Wilson (1976) "F," and cophenetic correlation coefficient (Swofford, 1981)

TABLE 8. – Goodness-of-fit Statistics for Distance Wagner Procedure[1,2]

MEASURE	OUT-GROUP	FARRIS "F"	P-W "F"	F-M % S.D.	COPH. CORR.	LENGTH OF TREE
ARC	*VEXANS*	57.594	18.356	23.581	0.913	6.454
	TARSALIS	86.683	22.054	27.476	0.890	7.099
	FREEBORNI	63.559	18.726	23.669	0.912	6.898
CHORD	*VEXANS*	51.492	18.199	22.418	0.915	5.858
PREVOSTI	*VEXANS*	54.251	19.157	23.871	0.930	5.015
	TARSALIS	69.521	19.371	24.334	0.923	5.524
	FREEBORNI	59.716	19.441	24.346	0.924	5.394
ROGERS	*VEXANS*	63.886	22.703	27.052	0.927	4.963
	TARSALIS	93.602	26.266	31.214	0.907	5.446
	FREEBORNI	69.163	22.656	27.249	0.921	5.338
MODIFIED	*VEXANS*	42.669	13.663	17.744	0.926	6.381

1. midpoint and out-group statistics equivalent

2. statistics are the Farris (1972) "F," Fitch and Margoliash (1967) per cent standard deviation, Prager and Wilson (1976) "F," and cophenetic correlation coefficient (Swofford, 1981)

TABLE 9. - Synapomorphic Character States Noted in the Most Parsimonious Cladogram Illustrated in Figure 14

CHARACTER STATES	
1. α-GPD^{100}	13. XDH^{140}
2. 6-PGD^{115}	14. HK_s^{80}
3. SOD_f^{100}	15. HK_f^{95}
4. AK_f^{100}	16. HK_m^{95}
5. HK_m^{125}	17. SOD_f^{65}
6. HK_s^{120}	18. ME^{90}
7. 6-PGD^{80}	19. AK_s^{70}
8. AK_s^{80}	20. SOD_f^{170}
9. 6-PGD^{35}	21. CK_f^{90}
10. HK_f^{110}	22. ICD_s^{130}
11. HK_m^{115}	23 HK_f^{115}
12. CK_s^{35}	24. XDH^{120}

TABLE 10. - Existing Classifications of the *Aedes* Examined in this Study, Based on Morphology

EDWARDS (1932)[1]	ROHLF (1963B)[1,2]
GENUS *AEDES*	GENUS *AEDES*
SUBGENUS *OCHLEROTATUS*	SUBGENUS *OCHLEROTATUS*
(*COMMUNIS* GROUP)	(*COMMUNIS* GROUP)
CATAPHYLLA	*CATAPHYLLA*
(*DORSALIS* GROUP)	*CANADENSIS*
CANADENSIS	(*DORSALIS* GROUP)
CAMPESTRIS	*CAMPESTRIS*
DORSALIS	*DORSALIS*
MELANIMON	*FLAVESCENS*
(*STIMULANS* GROUP)	*NIPHADOPSIS*
CANTATOR	(*CANTATOR* GROUP)
FLAVESCENS	*CANTATOR*
SUBGENUS *CULICELSA*	(*TAENIORHYNCHUS* GROUP)
NIGROMACULIS	*NIGROMACULIS*
SOLLICITANS	*SOLLICITANS*
TAENIORHYNCHUS	*TAENIORHYNCHUS*
SUBGENUS *AEDIMORPHUS*	SUBGENUS *AEDIMORPHUS*
VEXANS	*VEXANS*

1. *caspius* and *detritus* not included in original work
2. *melanimon* not included in original work