The Genus *Toxorhynchites* (Diptera: Culicidae); Numerical Phylogenetic Analysis of *Tx. splendens* and Allies with Phenetic Comparisons[1]

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This paper describes a numerical phylogenetic study of 32 species within the mosquito genus *Toxorhynchites* Theobald. These selected species occur in the Oriental and Australasian Regions. Moss et al. (1979) explored the phenetic similarity among 25 of these species but did not construct a phylogenetic tree. Prior to 1979, phylogenetic relationships within the genus *Toxorhynchites* were largely unexplored. Accordingly, Moss et al. sampled widely for possible characters and used these to establish phenetic groupings. The phylogenetic results reported here are based on a data set slightly modified from theirs. In both studies adult characters predominated and all data were taken from exemplars (single male and female specimens).

This paper is intended as an instructional model for the use and comparison of numerical techniques for data examination and phylogeny construction. We, therefore, include a detailed discussion of the historical and theoretical development of numerical phylogenetic techniques, a step-by-step explanation of relevant numerical procedures, and mention of phenetic techniques useful for data examination (Appendix 1).

This study provides an example of the application of numerical techniques and also makes some generalizations about the phylogenetic relationships of *Tx. splendens* and allies. In addition, given the preliminary assessment of overall similarity presented by Moss et al. (1979), repeated here for a modified data set, we ask whether the groups found using overall (phenetic) similarity are the same as groups found using a numerical phylogenetic procedure. Congruence of phylogenies for adults and immatures is also explored.

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MATERIALS AND METHODS

The present study uses the Wagner algorithm (WAGNER-78, J. S. Farris, S.U.N.Y. Stony Brook) to calculate phylogenetic trees. Readers not familiar with this procedure and its rationale should refer to Appendix 1.

The 25-species taxonomic study (Moss et al. 1979) uses 77 female (51 adult, 26 pupal), and 79 male (53 adult, 26 pupal) morphometric characters. The present study includes seven new species (Appendix 2) and uses a modified character set (Appendix 3). The data were modified as follows: 1) characters with low loadings on the first three principal component axes (see Moss et al. 1979) were discarded because they contained less information useful in separating the groups; 2) new characters were added that were useful in separating groups (i.e., such characters were helpful in identifying groups as opposed to suggesting phylogenetic groupings of taxa); 3) additional pupal characters were added; and 4) larval characters were added. The resulting 133-character set comprised 94 adult characters (48 female, 46 male), 30 pupal characters (12 female, 17 male), and 9 larval characters (4 female, 5 male). Characters were standardized to zero mean and unit variance for all analyses in order to eliminate weighting due to absolute size of a given character. A reduction of this data set was necessary since the Wagner program available to us could handle only 100 characters. Only the number of adult characters was reduced because there were many fewer immature characters. In order to reduce the adult data set and still retain, as nearly as possible, the same information on evolutionary relationships contained in the original data, a Wagner tree was constructed from the original 94 adult characters and the 33 characters which least agreed with the tree were removed. Agreement of the characters to the tree was judged using the consistency index of Kluge and Farris (1969; discussed in Appendix 1). The remaining 61 adult characters were added to the 39 immature characters to constitute the 100-character data set used in this study.

To convince ourselves that the 61 adult characters contained approximately the same phylogenetic information as the original 94 adult characters, we compared phylogenetic trees constructed from each data set. The two data sets produced essentially the same tree topology (shape) or branching order (Figs. 1, 2). We quantified the agreement between the two adult-character tree shapes by comparing the lengths of the two trees when each was fitted to the other's character set; that is, the character states of the HTU's and the sum of the branch lengths were calculated for each of the different character sets constrained to a given tree shape. The Wagner program calculates lengths by summing the number of character state changes necessary to evolve from the character states of the ancestral node (chosen arbitrarily) to the character states possessed by each terminal taxon, following the branching paths described by the tree. The length of a particular character on a tree is determined by the tree shape.

When the 94 adult characters were fitted to the 61-character tree shape and the total length calculated, the sum of the branch lengths was only 0.7% longer than that of the 94-character tree shape. Similarly, when the 61 adult characters were fitted to the 94-character tree shape and the total length of the tree

calculated for that character set, the sum of the branch lengths was only 3.6% longer (Table 2). The lack of identity between the two shape/length comparisons was due to the generally low consistency of most characters in the data set (discussed below).

Once the 100-character data set was established, comparisons were made of Wagner trees for the following character sets: the 39 immature characters; the 61 adult characters; the the 100-character combined data set. In addition, Wagner trees were compared to phenetic clusterings of the 100-character data set (UPGMA on distance, DIST, and correlation, CORRL, matrices; NT-SYS, F. J. Rohlf, J. Kishpaugh, and D. Kirk, S.U.N.Y. Stony Brook) to an intuitive classification prepared by W.A.S. and N.L.E. in the manner described by Moss et al. (1979).

RESULTS AND DISCUSSION

Adult, larval, and combined phylogenies are shown in Figures 2, 3, and 4. The results of phenetic clustering procedures are shown in Figures 5 and 6. A dendrogram produced by an "intuitive" grouping of taxa is shown in Figure 7. This dendrogram is not identical to the intuitive grouping of Moss et al. (1979); seven new taxa have been added and several taxa have been repositioned as the result of new taxonomic findings (Evenhuis and Steffan, in prep.).

For each of the seven tree shapes (Figs. 1-7), comparisons were made using the three character sets. Trees were compared, as discussed above, by fitting the three character sets to the seven tree shapes and calculating their respective lengths (Tables 1-3). Tables 1-3 are arranged such that tree shapes are column headings and the character sets for which each of the shapes was fitted are row labels. Only columns within rows can be compared in Table 1 because the tree lengths are dependent on the number of characters used to calculate those lengths. Tables 2 and 3 are percentages of total length and can be compared across rows. Trees were also compared by computing consensus measures (Table 4). Many such measures have been proposed (reviewed by Rohlf 1982). We used Rohlf's first consensus index, CI(r1), calculated from a strict consensus tree. A strict consensus tree is a dendrogram that consists of those subsets of objects that are held in common by a pair of trees. The RI consensus index is calculated by weighting counts based on subset size and by adjusting the measure to reflect the number of multifurcations contained in the consensus tree (Rohlf 1982).

It is not surprising that the 61 adult character tree shape is most similar to the 100-character tree shape because the 61 characters are a subset of the 100 (Tables 2, 4). As mentioned above, the 61 character tree shape is also quite similar to the 94 character tree shape (Tables 2, 4). The 61 characters were purposefully chosen to most closely duplicate the information contained in the 94 character data set. These two trees are not perfectly congruent because, as shown in Table 3, the data contain many inconsistencies and do not fit any tree shape very well. The tree shape produced from the 39 immature characters is less congruent with the 100-character tree shape than are the adult comparisons (Tables 2, 4). The 39 characters comprise a smaller subset of the data and alone suggest one of the most divergent patterns (Tables 2, 4).

The intuitive tree shape shows the least congruence when compared to the Wagner trees. It agrees more closely with the adult character tree shapes than with the immature. The originators of the intuitive tree (W.A.S. and N.L.E.) agree that they were largely influenced by adult characteristics in constructing the tree.

One of the phenetic procedures (CORRL-UPGMA) produced a branching form which was very similar to the 100-character Wagner tree (Tables 2, 4). The other phenetic procedure (DIST UPGMA) was less congruent by both consensus (Table 4) and length/shape (Table 2) comparison methods.

The fact that the 100-character analysis based on overall similarity (CORRL-UPGMA) produced results similar to the 100-character analysis based on derived similarity (WAGNER) might be interpreted as an indication of low homoplasy (convergences, parallelisms, and reversals) in the data. Table 3 demonstrates that the opposite is, in fact, the case. All trees and data sets show very low consistency values (20.8-32.2%). The data are so full of conflicting evidence that tree shapes produced by WAGNER and CORRL-UPGMA might be said to be equally poor summaries of the information contained in the original data. Rolph and Sokal (1981) predict this result: "Somewhat counterintuitively, one would expect data sets with much homoplasy to yield phenetic classifications that approximate those based on estimated cladograms reasonably well. This is so because the resultant noise in the data would affect both approaches more or less equally." This point is discussed in more detail by Simon (1983).

These low consistencies do not mean, however, that our data are of no value. On the contrary, in all analyses and in the intuitive classification, the same species-groups are more or less repeatedly recognized. The intuitive dendrogram recognizes four clear-cut monophyletic species-groups (*Tx. leicesteri* group, *Tx. splendens* group, *Tx. acaudatus* group, and *Tx. christophi* group). The one group which is recognized in all the analyses is the *Tx. christophi* group (not including *Tx. quasiferox*). The *Tx. leicesteri* group is recognized in all classifications except that of the immature's. The *Tx. acaudatus* group is closely allied to the *Tx. leicesteri* group in all classifications but is never separated as an autonomous group; in fact, it is sometimes interspersed with the *Tx. leicesteri* group (as in the Wagner tree for the immatures). *Tx. minimus*, *Tx. gigantulus*, *Tx. magnificus*, *Tx. funestus*, and *Tx. nepenthis* are most closely associated with the *Tx. leicesteri* group. The *Tx. splendens* group is the least well defined. It is separated as an autonomous group only in the CORRL-UPGMA analysis which is not a phylogenetic procedure. It is questionable whether the *Tx. splendens* group, as defined here, should be considered a taxonomic unit.

Toxorhynchites quasiferox is a problem species; it is placed in the *Tx. christophi* group in the intuitive analysis but, according to all other analyses, does not belong there. It is placed near *Tx. splendens* in the CORRL-UPGMA phenogram and the 61- and 100-character Wagner trees, and it is placed in the *Tx. acaudatus* group in the 39-character analysis.

Tx. nepenthicola occurs either within the *Tx. acaudatus* group (Figs. 1, 5), within the *Tx. splendens* group (Figs. 7, 3), or between the two (Figs. 2, 4, 6). Its taxonomic position is unclear.

In addition to the consistently recognized species-groups, certain pairs of species are always joined: *Tx. manicatus* and *Tx. yaeyamae; Tx. acaudatus* and *Tx.* sp. I; *Tx. ater* and *Tx. nigripes; Tx. magnificus* and *Tx. funestus;* and *Tx. minimus* and *Tx. gigantulus. Toxorhynchites leicesteri* and *Tx. gravelyi* are commonly paired. One relationship not recognized in the intuitive classifications, present in the other four analyses, is the species pair sp. W and sp. X. Intuitively, sp. D. was paired with sp. X.

The *Tx. acaudatus* Group

The *Tx. acaudatus* group, revised by Evenhuis and Steffan (in prep.), occurs solely in pitchers of *Nepenthes* throughout Malaysia, Sumatra, Borneo, and the Philippines. These species are characterized by the relatively bare and ovate to subquadrate pupal paddles and the usual presence of brown scales on Mks. of the adults. There are two subgroups within the *Tx. acaudatus* group. These are distinguished, in adults, by the presence (*Tx. ater* subgroup) or absence (*Tx. acaudatus* subgroup) or caudal tufts on the abdominal terga VI-VIII. The *Tx. ater* subgroup contains *Tx. ater, Tx.* sp. LL, *Tx. nigripes* and one new species. The *Tx. acaudatus* subgroup contains *Tx. acaudatus, Tx.* sp. I, *Tx. coeruleus, Tx. nepenthis,* and three new species. Not all of the above species were available for this investigation.

According to the intuitive analysis and to the numerical analyses, *Tx. nepenthis* is more closely allied to *Tx. minimus* and *Tx. gigantulus* than it is to any species in *Tx. acaudatus* group (sensu Evenhuis and Steffan, in prep.). Evenhuis and Steffan (in prep.) place *Tx. minimus* and *Tx. gigantulus* in their own group and *Tx. nepenthis* in the *Tx. acaudatus* group because of the pitcher dwelling habit of the larvae of *Tx. nepenthis (Tx. minimus* and *Tx. gigantulus* larvae are found in bamboo). *Tx. magnificus* is placed in a group with *Tx. funestus.* The intuitive tree (Fig. 7) reflects the above classification.

Most of the analyses presented here either do not recognize a monophyletic *Tx. acaudatus* group (Figs. 1, 2, 4) or if they recognize *Tx. acaudatus* as monophyletic, they do not indicate a close affinity between it and *Tx. minimus, Tx. gigantulus, Tx. nepenthis, Tx. magnificus,* and/or *Tx. funestus* (Figs. 3, 5, 6).

In all analyses, *Tx. nepenthis, Tx. minimus, Tx. gigantulus, Tx. magnificus,* and *Tx. funestus,* are closely allied to the *Tx. leicesteri* group. Some analyses place them within the *Tx. leicesteri* group proper (Figs. 2, 3, 6). Some analyses indicate that the *Tx. leicesteri* group plus *Tx. minimus, Tx. gigantulus, Tx. nepenthis, Tx. magnificus,* and *Tx. funestus,* belong to the larger monophyletic group which includes the *Tx. acaudatus* group (Figs. 1, 2, 4). The results of the two phenetic analyses are quite different from the Wagner analyses and the intuitive analysis. They indicate a close relationship between the *Tx. acaudatus* group and the *Tx. splendens* group. In other words, the *Tx. acaudatus* group is evolutionarily most similar to the *Tx. leicesteri* group but must contain homoplasious character states or retain certain ancestral character states which link it phenetically with the *Tx. splendens* group.

CONCLUDING REMARKS

The present study suggests that when analyzing large numbers of taxa, it may be difficult to construct classifications highly consistent with the data. Characters which are highly consistent within one monophyletic group of a tree may be inconsistent within others. Even characters which are consistent within many or all subgroups of a tree may be inconsistent across subgroups. Possibly, when inter- and intraspecific variation are studied in more detail, modification of the data set will improve the consistency of the classification. Hopefully, additional characters will be found which exhibit high consistency relative to the entire tree. Analyses of character variation within species is an important aspect of choosing additional characters for phylogenetic analysis. Ideal characters should be relatively constant within species and different among species. Character variation could not be evaluated in the present study because data were taken from single male and female specimens.

Despite the low consistencies observed in this study, certain subgroups appeared to be natural taxa as they were grouped together by all the taxonomic procedures tried. These natural groups were the *Tx. leicesteri* group, and the *Tx. christophi* group. The *Tx. leicesteri* group may contain more species than were initially recognized. Neither the *Tx. acaudatus* group nor the *Tx. splendens* group could be identified as separate monophyletic entities in these analyses. The classification and phylogeny of these two groups requires additional character analysis and more samples of each of the included species.

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APPENDIX 1: A PRIMER OF PHYLOGENETIC ANALYSIS

The preceding paper used techniques associated with two schools of systematic thought: the numerical phenetic school and the numerical phylogenetic (or cladistic) school. Numerical phylogenetics was emphasized because our goal was to construct an evolutionary tree. This primer is intended to serve as an historical summary and simplified guide to the theory and methodology of numerical phylogenetic tree construction, specificatlly, the Wagner method (Farris 1970). We discuss phenetic methodology only insofar as it relates to homoplasy analysis and multivariate techniques for viewing data.

Definition of Terms

Phylogenetic procedures group taxa based on the possession of shared derived character states. *Phenetic* procedures group taxa based on overall similarity: the sum of shared derived, shared ancestral and homoplasious similarities.

The term *homoplasy* is useful in that it is a summary term meaning any or all of the following: parallelism, convergence, or reversal. In short, homoplasy is any *repetitive* evolutionary change which can lead to an over estimation of the similarity of two taxa. Phenetic methods are different from phylogenetic methods in that they do not distinguish between similarity due to common ancestry and similarity due to homoplasy. The adjective *phenetic* has, unfortunately, been confused with the adjective *phenotypic* which is used to differentiate visible features of an organism from the genetically coded (genotypic) expression of those same features. Whether characters are coded based on their genotypic or phenotypic expression has no bearing on choice of phenetic versus phylogenetic analyses.

The results of a phylogenetic study are usually represented as a phylogenetic *tree*. The term tree, by convention in numerical phylogenetics, is used only when referring to a rooted phylogeny. The term *phenogram* is used to describe the branching diagram produced by phenetic analysis (always rooted). The term *dendrogram* can refer to any kind of rooted branching diagram.

A Wagner tree without a root is called a *Wagner network*. (These are not the strict mathematical definitions of tree and network but rather common phylogenetic-systematic usages.) Phylogenetic networks and trees are assumed to have no anastomosing branches (i.e., no closed loops). Thus, taxonomic assemblages suspected of having hybrid taxa should not be analysed using this standard phylogenetic methodology.

The term *monophyletic* (sensu Hennig 1965; 1966) implies the inclusion of *all* taxa descended from a common ancestor (referring only to those taxa under consideration and not to any as yet undiscovered or extinct taxa). Figure A-1 demonstrates the distinction. The definition of Hennig (1966), although not universally accepted (Ashlock 1972), is widely used because of its heuristic value. The terms *paraphyletic* and *polyphyletic* will not be discussed here but the concepts (reviewed by Platnick 1977) are critical to the construction of classifications from phylogenetic trees.

Development of Phylogenetic Methods

The paucity of the fossil record, especially for insects, requires that inferences of evolutionary history be based on studies of recent taxa. Evolution is a historical process involving successive changes in character states. "As a result, it is not the extent of resemblance or difference between various organisms that is of significance in constructing phylogenies but rather the connection of agreeing or divergent characters with earlier conditions" (Hennig 1965). Characters which remain unchanged from their ancestral state contain no information about genealogical relationships. Characters which have changed (i.e., possess derived states) record evolutionary events. For example, let Figure A-2 represent the known phylogenetic history of taxa A, B, C, D, E, and F. Three characters are represented on the tree. All three characters have state 0 in the ancestor. The character states of each of the three characters for each terminal taxon are as indicated in the figure. All characters are perfectly consistent with the tree (i.e., no character state reversals, convergences, or parallelisms are required to explain the distribution of states observed in the terminal taxa), yet some characters contain more information than others. For character 1, the derived state 2 defines the group AB. The derived state-1 defines the group DE. Assuming that the character states represent a sequentially ordered transition series as follows ($-1 \rightarrow 0 \rightarrow 1 \rightarrow 2$), the evolution of character 1 can be explained most simply as a three-step process in which a $0 \rightarrow 1$ transition occurs at a point "a," a $1 \rightarrow 2$ transition occurs at point "b," and a $0 \rightarrow -1$ transition occurs at point "c." Taxon F possesses the ancestral state for character 1.

Character 2 contains less information than 1, since it tells us only that taxa A, B, and C are a group (defined by state 1). We know nothing of the relationship between D, E, and F; the ancestral states possessed by these three taxa reveal no record of evolutionary change.

Character 3 contains the least information of the three characters, indicating only that taxa D and E share the derived state 1. The 0 (ancestral) state, in general, tells us nothing of relationships but here can be used to demonstrate the danger of using ancestral states to define groups. Taxa A, B, C, and F all share ancestral state 0. This shared state does not define the group ABCF but rather represents a lack of change; no evolution has taken place. Monophyletic groups can only be recognized with respect to shared derived characters.

The more derived characters shared between taxa, "the better founded is the assumption that these species form a monophyletic group" (Hennig 1966:121). Convergences and parallelisms can result in apparent sharing of character states which do not reflect a common evolutionary pathway but are rather the result of adaptation to common environmental circumstances. It is unlikely that a large number of characters will show the same exact pattern of convergence unless they are functionally related (Lundberg 1972). Thus, a presumed monophyletic group which is defined by many shared derived characters is more likely to be monophyletic than a group defined by fewer shared states.

The Camin/Sokal Method

Camin and Sokal (1965) outline a procedure for systematically and objectively quantifying the number of evolutionary steps (transitions from one character state to another) present on a phylogenetic tree. Their method is conceptually identical to the cladistic approach of Hennig (1966). Its advantage is that it facilitates computerization and can therefore deal efficiently with large numbers of characters and taxa.

The Camin/Sokal method is based on the criterion of evolutionary *parsimony*. The most parsimonious phylogenetic tree is the one of shortest length, i.e., the tree containing the fewest postulated evolutionary steps necessary to produce the character state distributions observed in the terminal taxa. Camin investigated a variety of techniques for the construction of phylogenies. Each technique was used to construct a genealogy of a hypothetical group of organisms (Caminalcules) whose "true evolutionary history" was known only by their creator, Joseph Camin. Comparison of these genealogies with the "truth" led to the observation that "those trees which most closely resemble the true cladistics invariably required for their construction the least number of postulated evolutionary steps for the characters studies" (Camin and Solak 1965:311; Sokal and Rohlf 1980).

Camin and Sokal admit, but do not explicitly describe the close relationship between their cladistic numerical procedure and that of classical phylogenetics. Farris, Kluge, and Eckhardt (1970) subsequently demonstrated

that the criterion of parsimony used by Camin and Sokal (1965), as well as their models for character state changes, conformed closely to classical Hennigian phylogenetic principles summarized in the following axioms:

I. A character is a collection of mutually exclusive states with a fixed order of evolution such that a) each state is derived directly from just one other state, and b) a unique state exists from which every other state is eventually derived. In other words, there are no anastomosing branches on the tree.

II. A monophyletic group, G, is distinguished by the joint possession by all its taxa of the derived state(s) of at least one character, for which no species outside the monophyletic group possess a state derivable from any state present in G.

III. In the absence of evidence to the contrary, any state corresponding to a step shared by a group, G, of taxa is taken to have arisen just once in G.

IV. The larger the number of derived character states possessed in common by a group of species, the better founded is the assumption that this group is monophyletic.

These axioms are consistent with the mathematics of evolutionary tree construction (Ferris et al. 1970).

The most important link between Hennigian cladistics and numerical phylogenetics is that the shortest length evolutionary tree agrees with axiom IV. On a shortest length (most parsimonious) tree, taxa which share many steps are generally placed together such that all monophyletic groups contained on a tree will, on average, share a maximum number of evolutionary steps and therefore a maximum number of derived character states. The number of steps shared by two species can be computed from a measure of overall similarity as follows:

$$s(A,B) = 1/2\ [p(A,Q) + p(B,Q) - p(A,B)]$$

where A and B are two terminal taxa, Q is a taxon with all ancestral character states, and p is the phenetic distance between species (Manhattan distance, discussed below) (Farris et al. 1970).

Critics of the parsimony criterion (e.g., Rogers et al. 1967) claim that its use in tree construction implies a belief that evolution itself is parsimonious. Kluge and Farris (1969) point out that trees constructed using the parsimony criterion often show large numbers of convergent and parallel changes, as well as reversals. Such non-parsimonious character state changes are minimized but expected. In fact, these homoplasious changes can *only* be detected once an evolutionary pattern most consistent with available data is established. Therefore, the use of the parsimony criterion, they reason, does not require a belief that all evolution is parsimonious. Critics still contend that if the true evolutionary tree could be known, it would not necessarily

be one in which homoplasious events were absolutely minimized. This argument has little heuristic value. The reason for choosing parsimony as a criterion is that true evolutionary histories cannot be known with certainty and, therefore, the most reasonable assumption to work from is that evolution of character states is not unnecessarily complicated. This methodology does not prevent a taxonomist from choosing a phylogeny which is only a few percent longer than the shortest possible tree. There are no statistical tests which can prove that a tree of, say, 45 units is significantly different, statistically, from one of, say, 48 units. That is, no one has yet determined the best objective method for choosing between trees of similar lengths. Often such trees differ by only minor rearrangements of the tips. Character choice, character coding, and interpretation of final results remain a taxonomic "art"; an art with methods and principles, not an art based on intuition. (We do not mean to rule out the possibility that some scientists are more perceptive than others.)

The Wagner Method

The Wagner method (Farris 1970) is another numerical cladistic tree-building procedure based on the criterion of parsimony. It has advantages over the Camin/Sokal procedure in that it incorporates the realistic assumption that character state reversals are permitted. In order to incorporate reversibility, axioms I-IV must be modified such that it can no longer be guaranteed "that a particular state is everywhere derived, but only that a state is locally derived in some part of the tree" (Farris et al. 1970). Otherwise all four axioms hold.

The Wagner method circumvents the problem of *a priori* identification of ancestral states for each character. Instead, an unrooted shortest-length network is created, using all available characters. The position of the root is determined by including a more distantly related group (out-group) which will, by definition, branch from the tree at a point closest to the ancestor. Inherent in this method is the assumption that the group chosen is actually an out group and not a rapidly evolved derivative of the monophyletic group under investigation.

The Wagner method proceeds as follows: STEP 1. For a set of taxa, the phenetic distance between all pairs of taxa is calculated. This phenetic distance is a measure of overall similarity based on all characters. The distance metric recommended by Farris (1970) for use in phylogeny construction is the Manhattan (= city block or lattice) metric. The Manhattan distance between species A and B, d(A,B), is equal to the sum over all characters of the absolute value of the state possessed by taxon A minus the state possessed by taxon B:

$$d(A,B) = \sum \left| X(A,i) - X(B,i) \right|$$

where X(A,i) = the character state value of the ith character in the Ath species and n is the total number of characters. The Manhattan distance is chosen instead of the typical Euclidean (straight line) distance for the following reason. In Figure A-3, the dashed line "c" represents Euclidean distance,

which is equal to the square root of (a + b) (or the square root of the difference between the state "3" in character 1 minus the state "1" in character 1, squared, plus the state "3" in character 2 minus the state "2" in character 2, squared. The Euclidean distance between taxa A and B is a function of the sum of the distances based on characters 1 through n. The Manhattan distance is merely the sum of the difference between the states of each character, 1 through n, for each taxon (Fig. A-3: d(A,B) = a + b + ... + n). Characters can be more easily envisioned as evolving independently using this metric and individual evolution steps can be counted by the Wagner algorithm. However, the choice of a distance measure will have much less effect on the branching form of a dendrogram than the choice of the dendrogram building algorithm.

STEP 2. The pair of taxa A,B with the smallest distance between them is joined to form an interval AB [INT(A,B)]. In case of ties, select one pair arbitrarily and proceed; when the analysis is complete, return to the step in which there was a tie and try the alternative pathway. Ideally, one would follow this procedure for every instance of a tie at any step of the procedure.

STEP 3. The distance from each remaining taxon (1) to the interval A,B is computed as follows:

$$d[I, INT(A,B)] = 1/2\ [d(I,A) + d(I,B) - d(A,B)]$$

STEP 4. The unplaced taxon closest to the interval A,B is connected to it via a hypothetical ancestor (HTU).

STEP 5. The character states for the HTU are then calculated as the median values of the states of the three taxa connected to the HTU. The median value is used because it best approximates a minimum length network (cf. Farris 1970: 86, for proof).

STEP 6. Add the character states of the HTU calculated in STEP 5 to the data table and then calculate the distance from each remaining taxon to the newly established network (i.e., to each branch of the network) using the equation from STEP 3.

STEP 7. Add the taxon which is closest to the network by joining it to the nearest branch via an HTU whose states are the median values of the three taxa connected to the HTU.

STEP 8. If any taxa remain, return to STEP 6 and proceed from there until all taxa have been placed on the tree.

STEP 9. Optimize the tree (see below).

The end result is a tree which approximates a tree of shortest length. This tree was constructed using phenetic distances, but the process of constructing a tree of shortest length is not a phenetic one in the sense that the distance between taxa as measured along tree branches (the patristic distance)

will be greater than the distance between taxa calculated by a phenetic distance metric (such as Manhattan distance) by an amount equal to the sum of all homoplasious evolution (discussed later).

The Wagner procedure becomes more complicated when more than one of the taxa to be added is/are equidistant from the existing network, or one species to be added is equidistant from two branches. In such cases, ties are resolved by constructing alternative networks, optimizing them as discussed below and choosing the shortest length solution. Rather than investigating all possible ties, a computer program may simply rearrange the branches of the first-obtained network hoping to find an even shorter solution.

There may, of course, be more than one shortest length solution, necessitating an educated judgment on the part of the taxonomist and perhaps analysis of more characters. Since there are no significance tests for phylogenetic trees, there is no way of knowing whether a tree which is a few steps longer is actually significantly longer than the tree to which it's being compared. Therefore, a taxonomist is justified in choosing a tree which is not the absolute shortest if there is sufficient reason. In the absence of any compelling data, the best choice is the shortest length solution.

In addition to the difficulty of investigating ties, another problem must be overcome in order to find a shortest length solution. Farris (1970) notes that, "the HTU's produced during the stepwise addition of [taxa] to the tree may not be the optimal ones for the complete tree." A simple "optimization procedure" adjusts the character states of the hypothetical ancestors to produce a more parsimonious tree or network.

Optimization

Optimization proceeds as follows:

1. An HTU is chosen arbitrarily as the root of the tree.

2. An HTU is assigned a character state or set of character states as follows:

   A) Usually beginning at the end farthest from the "ancestor," a terminal cluster consisting of two of the original taxa and their immediate common ancestor (an HTU) is chosen arbitrarily.

   B) For each character in turn, the HTU is assigned the character state value(s) of the two terminal taxa (its descendants). If the two character state values are different, the HTU is assigned the range of values bounded by the two states and this range becomes known as its *state set*. For example, if one descendant possesses state 1 for character 1, and the other descendant possesses state 0 for character 1, the HTU would be assigned the state set (0,1).

3. The two original terminal taxa are dropped from consideration temporarily, and another cluster of two taxa (one of which could be an HTU on the network)

connected by an immediate common ancestor is chosen. The HTU is assigned character states for each character in turn as follows (until no HTU's are left):

A) If both of the descendant taxa possess but a single state, return to step 2B, and continue from there.

B) If one of the descendant taxa possesses a single state and the other possesses a state set, the HTU is assigned the value of the intersection of the two sets. For example, if one descendant possesses state 2 and the other possesses state set (1,2), the HTU would be assigned state 2. If the intersection is empty, the HTU is assigned the set equal to the "smallest closed interval" connecting the states of the two descendants; that is, the range of values defined by the single state of the one character and the most numerically similar state of the state set. For example, if one of the descendants possesses state 0, and the other possesses state set (1,2), the HTU is assigned state set (0,1). Return to 2B and continue from there.

C) If both descendants possess state sets, the HTU is again assigned the set equal to the intersection of the two sets. If no intersection exists, the HTU is assigned the value of the smallest closed interval connecting the states of the two descendants. For example, if the descendants possess state sets (0,4) and (2,7), the HTU would be assigned state set (2,4). Return to 2B and continue from there.

4. Once all HTU's have been assigned states or state sets, a second pass of the tree is made, beginning at the end nearest to the ancestor. The purpose of this pass is to reduce all state sets (which represent possible alternative character states) to a single state. An HTU is assigned a state equal to the intersection of its state set with its immediate ancestor's state set. Once this is completed, each HTU should possess only one state for each character, and the number of evolutionary steps represented on the network should be approximately minimized. There may be other equally parsimonious optimizations of the data.

The length of a tree reflects only the data used to construct the tree. That is, the relative lengths of the branches represent the average evolution that has taken place for those characters studied. It is unlikely that the small character sets typically used by taxonomists (usually less than 200 characters) represent a random sample of the genome of the taxa under investigation. Therefore, generalizations about overall rates of evolution for a particular taxon are likely to be invalid.

Rooting

The Wagner network must be rooted to produce a tree. The simplest way to root a network is to choose an out-group, i.e., a taxon not belonging to the monophyletic group under consideration but which is closely enough related that homologous characters can be recognized. This taxon will by definition join the network at the most ancestral point.

Farris (1970) suggests two additional methods for rooting Wagner networks: 1) Place the root halfway between the two most distnat taxa (*patristic or phyletic distance* measured as distance along the tree branches); and 2) select a root so as to minimize either the range or the variance of the patristic distance of each terminal taxon from the ancestor. Implicit in the first technique is the assumption of homogeneity of evolutionary rates. It is presumed that the two most divergent taxa have had the same average rate of evolution. As a result, the patristic distance from either of them to the ancestor would be equal; the root is halfway between. The assumption of homogeneity of evolutionary rates used here is not strict. It requires only that, in cases with large amounts of divergence, the length of time since divergence be well correlated with the amount of divergence. "A Wagner tree whose root has been estimated using this method remains independent of the assumption that amounts of divergence are correlated with times of divergence for very similar and moderately similar pairs of [taxa]" (Farris 1970).

Tree Evaluation and Comparison

To judge how well data fit a tree, a measure of consistency was defined (Kluge and Farris 1969):

$$C = R/L$$

where R = the sum of the ranges of each character and L = the length of the tree (the sum of the branches). The range, r, of each character is defined as "the difference between the numerically largest and numerically smallest states of the character .... The value of C lies between 0 and 1. It is 1 if there is no convergence on the tree, and tends to 0 as the amount of convergence on the tree increases." This definition, of course, assumes that one has a reasonably good estimate of the true tree. If all of the data were to reflect one extremely convergent pattern, they could fit the tree perfectly (Consistency = 1) and one would still not have the true tree. Such a situation is unlikely.

Consistency is the ratio of the phenetic distance among taxa to their patristic distance, where

PATRISTIC DISTANCE = PHENETIC DISTANCE + HOMOPLASIOUS SIMILARITY

A tree with high consistency might be said to be "better" than a tree with lower consistency because it is based on many agreeing characters; there are fewer contradictions in the data.

A poor consistency value could be an indication of either a poor data set (many homoplasious character states) or a poor tree building procedure (i.e., an algorithm which creates more homoplasious situations than necessary for a particular data set).

Other measures for comparing trees have been suggested. The most commonly used methods, called consensus analyses, examine opposing trees for correspondence by counting common subsets of taxa defined by a single stem. Several slightly different consensus indices have been suggested (Adams 1972; Mickevich 1978; Nelson 1979; Rohlf 1982; Colless 1980; Schuh and Farris 1981). There is no general agreement on which index has the most desirable properties but some (Mickevich 1978) have been shown to have undesirable properties such as the favoring of asymmetrical trees (Rohlf 1982).

In addition to judging the agreement between two trees, measures have been proposed to judge the "goodness" of a particular tree-building algorithm. These include stability to the addition of new characters and taxa, high predictive value, high information content, naturalness, high cophenetic matrix correlation with original data, etc. These optimality criteria have been reviewed and discussed in detail (Archie 1980; and Archie in prep.).

Numerical Phenetic Analyses

Overall (phenetic) similarities among taxa are most often expressed visually in the form of a phenogram, ordination diagram, or minimum spanning network (Sokal 1974; Moss 1979). These illustration techniques can summarize, in a controlled manner, a large amount of multivariate information about similarities among taxa. A graphical illustration of this kind is more easily assimilated than a table of numerical data. Each phenetic technique can offer different insights and each can suffer from inaccuracies or distortions arising from its method of construction.

Any technique designed to reduce the dimensionality of data (i.e., to create a few composite variables which summarize the distinguishing information contained in a large number of variables) is called an *ordination technique*. Characters are grouped into summary variables on the basis of their correlations with each other. The most commonly used ordination techniques are: principal components analysis, factor analysis, multidimensional scaling, and discriminant analysis. Groups of taxa can be graphed in two or three dimensions based on their scores for each summary statistic.

Phenograms are dendrograms resulting from cluster analyses. *Cluster analyses* join taxa and groups of taxa hierarchically based on the overall similarity among them. Various measures of similarity are used (association coefficients, distance coefficients, correlation coefficients, etc.) and these, in combination with the diverse methods for joining groups together based on these similarities (unweighted pair-group method, weighted pair group method, single linkage method, complete linkage method, and many more), are the reason that no one unique phenetic dendrogram is ever expected.

Phenograms accurately depict the phenetic similarity among closely related taxa but tend to distort higher level connections. Ordination diagrams, especially principal components diagrams, depict differences among distantly related groups more accurately than they depict distances among closely related

taxa (Rohlf 1972). Because of these recognized weaknesses, phenetic classifications are recommended to be composites of several of these analytical techniques (Colless 1980).

Phenetic methodologies are not based on phylogenetic principles and are not intended to be interpreted phylogenetically (Sneath and Sokal 1973). Nevertheless, because of the branching shape of phenograms many biologists have mistaken them for phylogenies. With this problem in mind, Colless (1970) has attempted to define conditions under which a phenogram is a reasonable representation of a phylogeny. This should occur when levels of homoplasy are low and rates of evolution are more or less constant for all branches of the evolutionary tree. The problem of discovering whether evolutionary rates are constant and exactly how much homoplasious evolution has occurred is difficult and requires the comparison of a phenogram to a phylogenetic tree.

The best advice to follow when making a classification is to choose a method which theoretically accomplishes your stated purpose. If you desire a phylogenetic solution, use phylogenetic methodologies. If you have no desire to incorporate phylogenetic information in your classification use phenetic techniques. Not all techniques advocated by numerical pheneticists (historically called 'numerical taxonomists' as opposed to numerical systematists in general) are dendrogram forming techniques. Other techniques include multivariate statistical analyses used for viewing in a simplified manner, or for studying character variation within and among taxa. The use of these latter techniques may be helpful in character choice, the most important phase of phylogenetic analysis.

Table 1. Lengths of trees for a specified tree shape and character set using the Wagner optimization procedure (see Appendix 1). Method= the particular classification used to produce the tree shape or branching pattern. No. Chars.(columns)= the number of characters used to produce a given tree shape; Fitted Chars.(rows)= the number of characters fitted to a given tree shape. Life stages for the various character sets are as follows: 61-adult; 94-adult; 39-immature; 100-both adult and immature characters. C-UPGMA= Correlation UPGMA. D-UPGMA= Distance UPGMA.

	Tree Shapes						
Method:	WAGNER	WAGNER	WAGNER	WAGNER	INTUITIVE	C-UPGMA	D-UPGMA
No. Chars.:	100	94	61	39	100	100	100
Fitted Chars.							
100	1171.39	1229.51	1177.86	1284.38	1286.65	1200.26	1256.77
94	1157.86	1133.24	1141.69	1361.00	1246.37	x	x
61	646.05	632.71	610.96	788.85	701.76	x	x
39	525.34	596.80	566.91	495.53	649.35	x	x

Table 2. The percent of length by which the tree specified is longer than the most parsimonious tree for a given data set. The most parsimonious tree is indicated by an *. Abbreviations are the same as in Table 1.

	Tree Shapes						
Method:	WAGNER	WAGNER	WAGNER	WAGNER	INTUITIVE	C-UPGMA	D-UPGMA
No. Chars.:	100	94	61	39	100	100	100
Fitted Chars.							
100	*	5.0%	0.6%	9.6%	9.8%	2.5%	7.3%
94	2.2%	*	0.7%	20.1%	10.0%	×	×
61	5.7%	3.6%	*	29.1%	14.9%	×	×
39	6.0%	20.4%	14.4%	*	18.0%	×	×

Table 3. Consistency for a given tree shape and a given data set. Abbreviations are as in Table 1.

	Tree Shapes						
Method:	WAGNER	WAGNER	WAGNER	WAGNER	INTUITIVE	C-UPGMA	D-UPGMA
No. Chars.:	100	94	61	39	100	100	100
Fitted Chars.							
100	29.7	28.3	29.6	27.1	27.0	29.0	27.7
94	24.4	25.0	24.8	20.8	22.7	x	x
61	30.4	31.0	32.2	24.9	28.0	x	x
39	28.8	25.4	26.7	30.6	23.3	x	x

Table 4. Rohlf's First Consensus Index , CI(R1), for tree topology comparisons of interest (see text).

		Tree Shapes						
	Method:	WAGNER	WAGNER	WAGNER	WAGNER	INTUITIVE	C-UPGMA	D-UPGMA
	No. Chars.:	100	94	61	39	100	100	100
Tree Shapes								
WAGNER	100	*	.242	.350	.060	.026	.178	.059
WAGNER	94	*	*	.280	.035	.028	x	x
WAGNER	61	*	*	*	.035	.011	x	x
WAGNER	39	*	*	*	*	.006	x	x

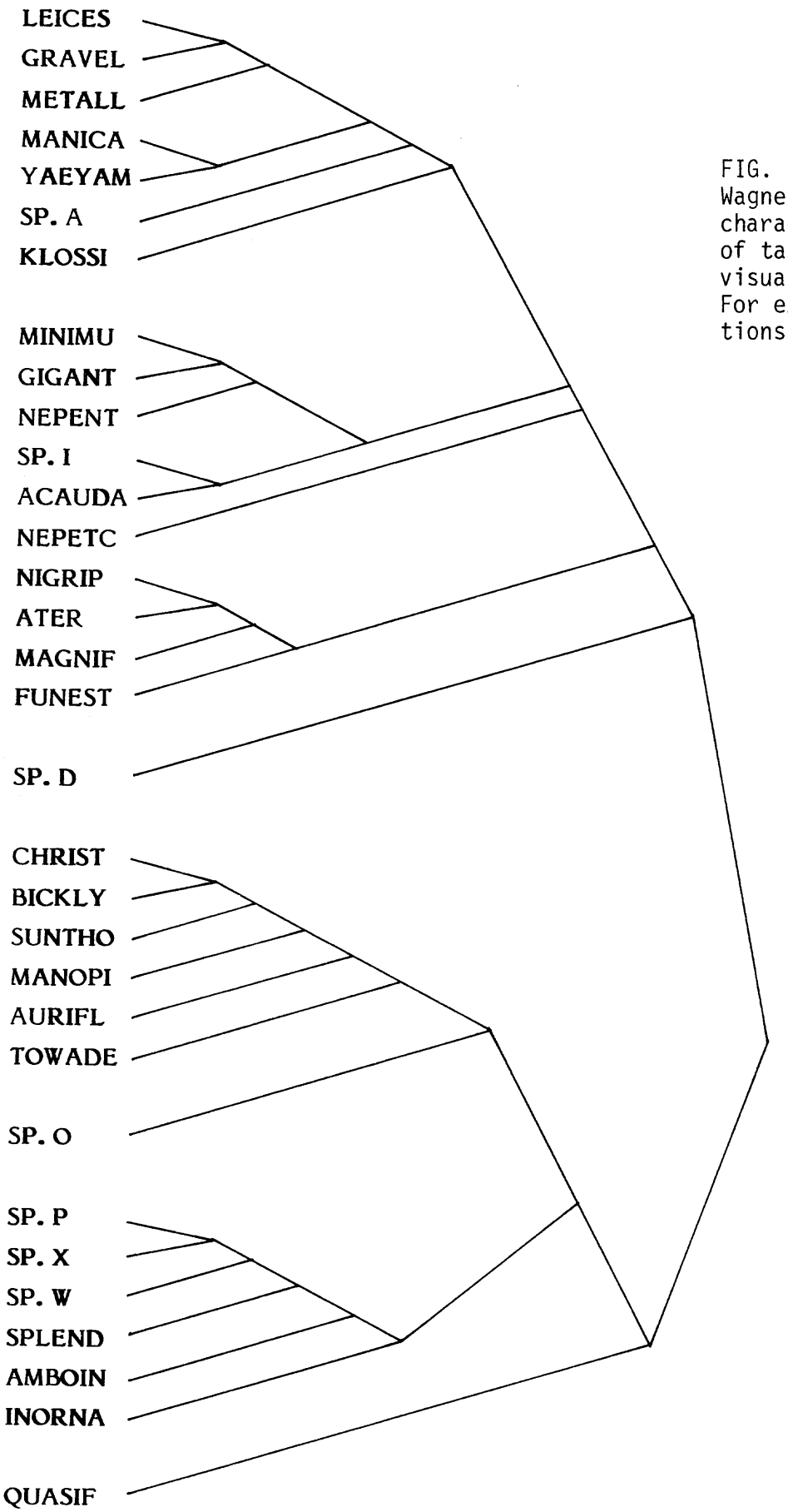

FIG. 1. Tree topology based on Wagner analysis of 94 adult characters. Gaps between groups of taxa are inserted to facilitate visual comparisons of tree shapes. For explanations of taxon abbreviations see Appendix 2.

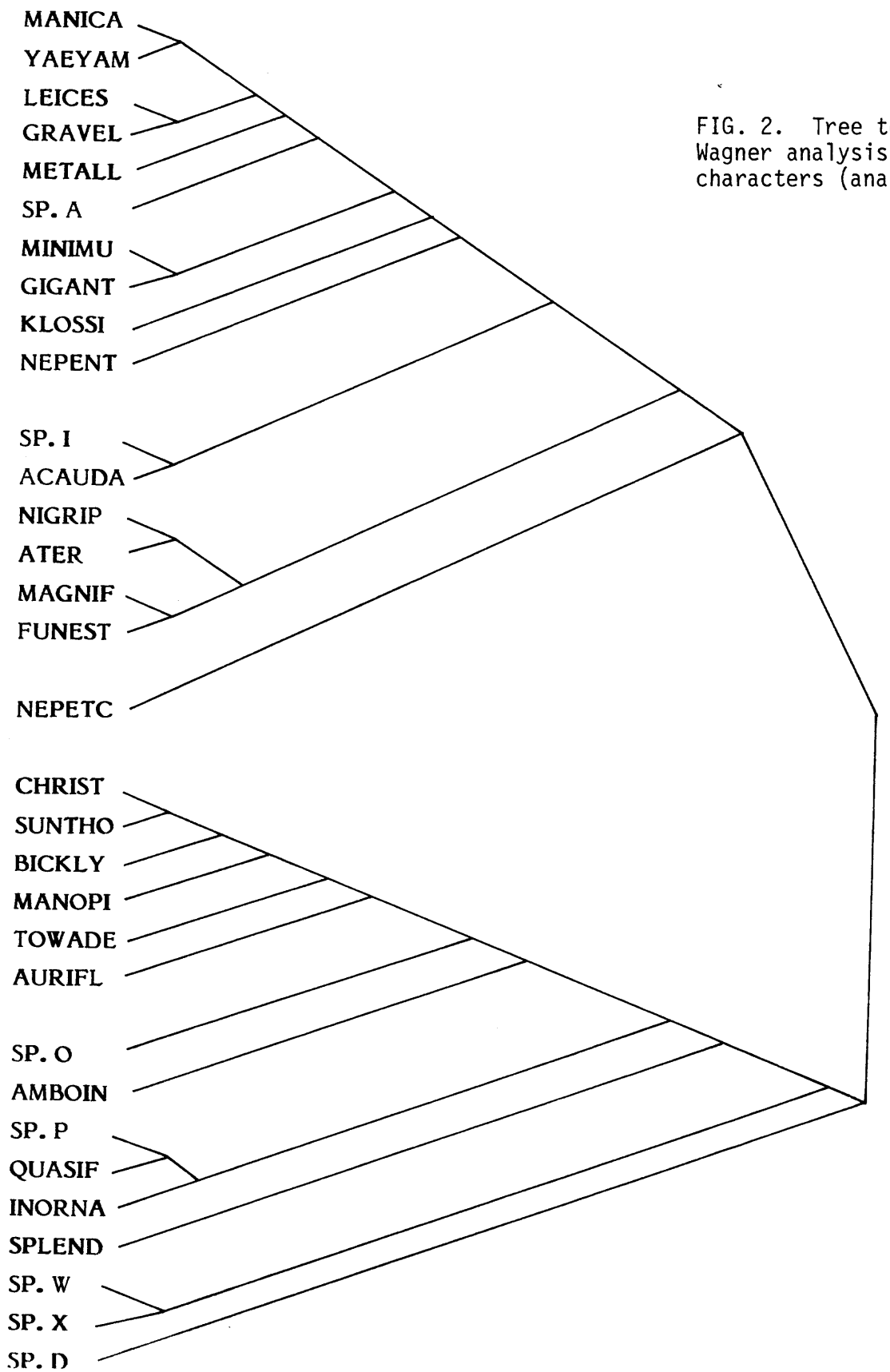

FIG. 2. Tree topology based on Wagner analysis of 61 adult characters (analogous to Fig. 1).

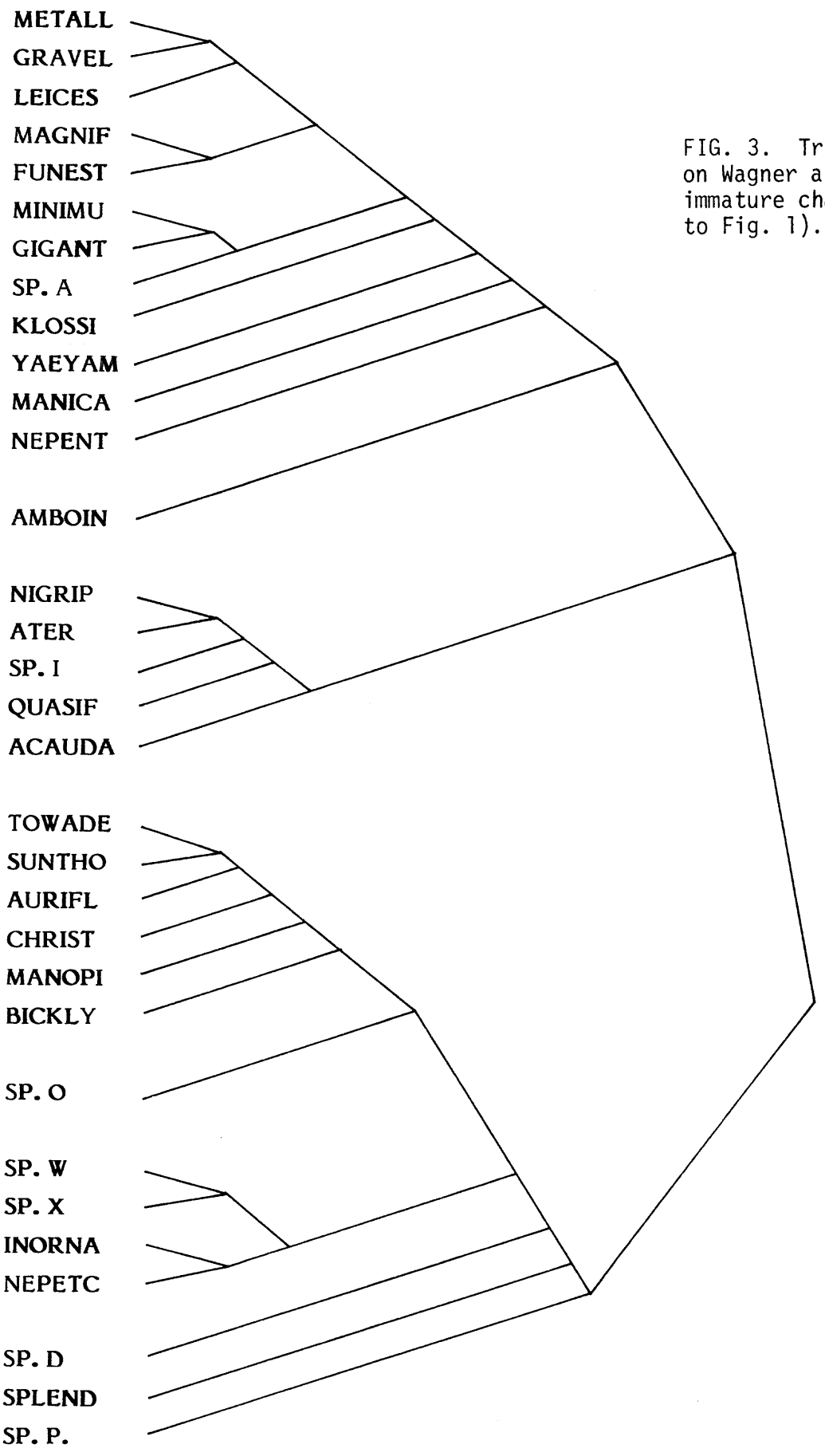

FIG. 3. Tree topology based on Wagner analysis of 39 immature characters (analogous to Fig. 1).

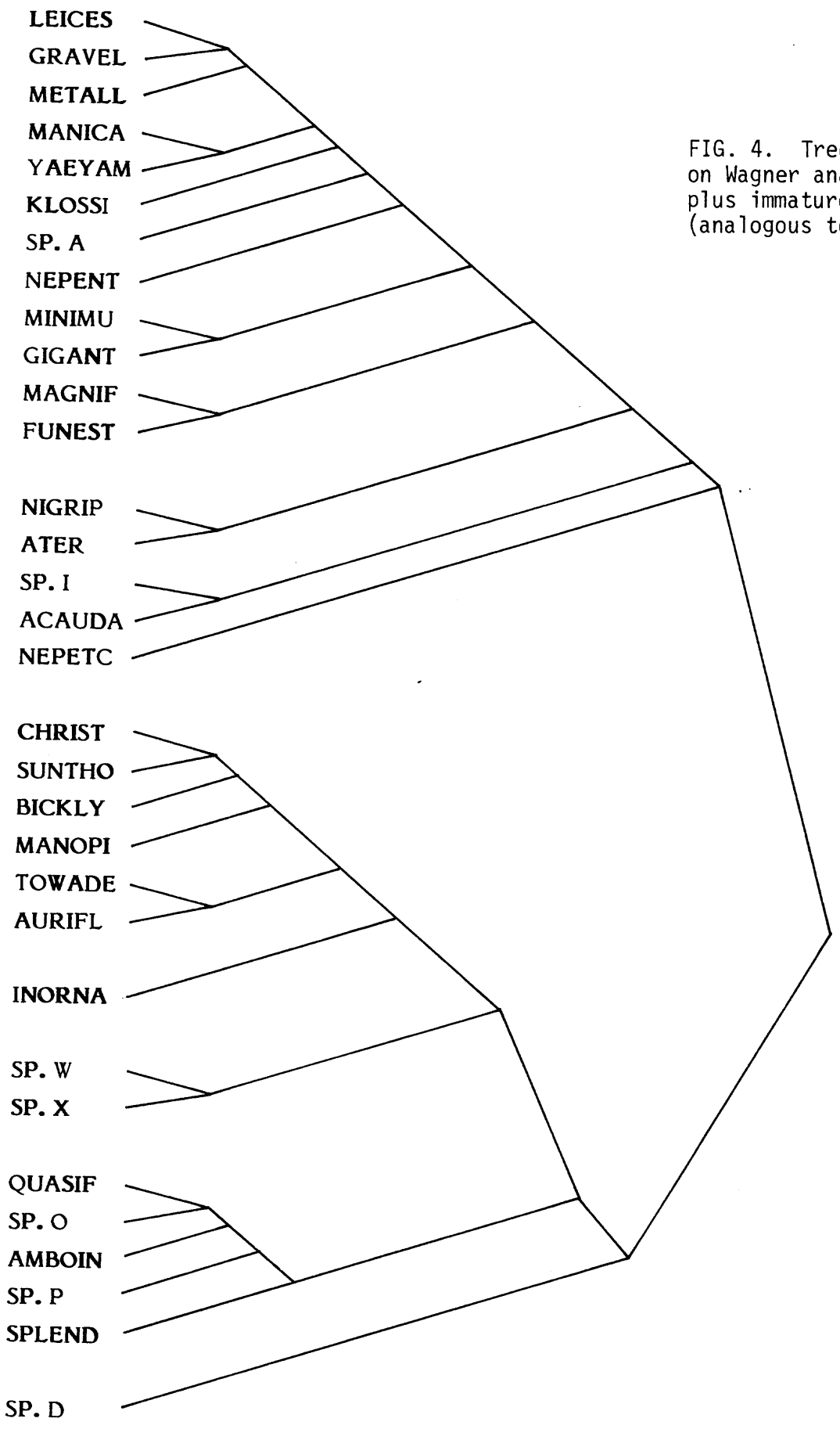

FIG. 4. Tree topology based on Wagner analysis of 100 adult plus immature characters (analogous to Fig. 1).

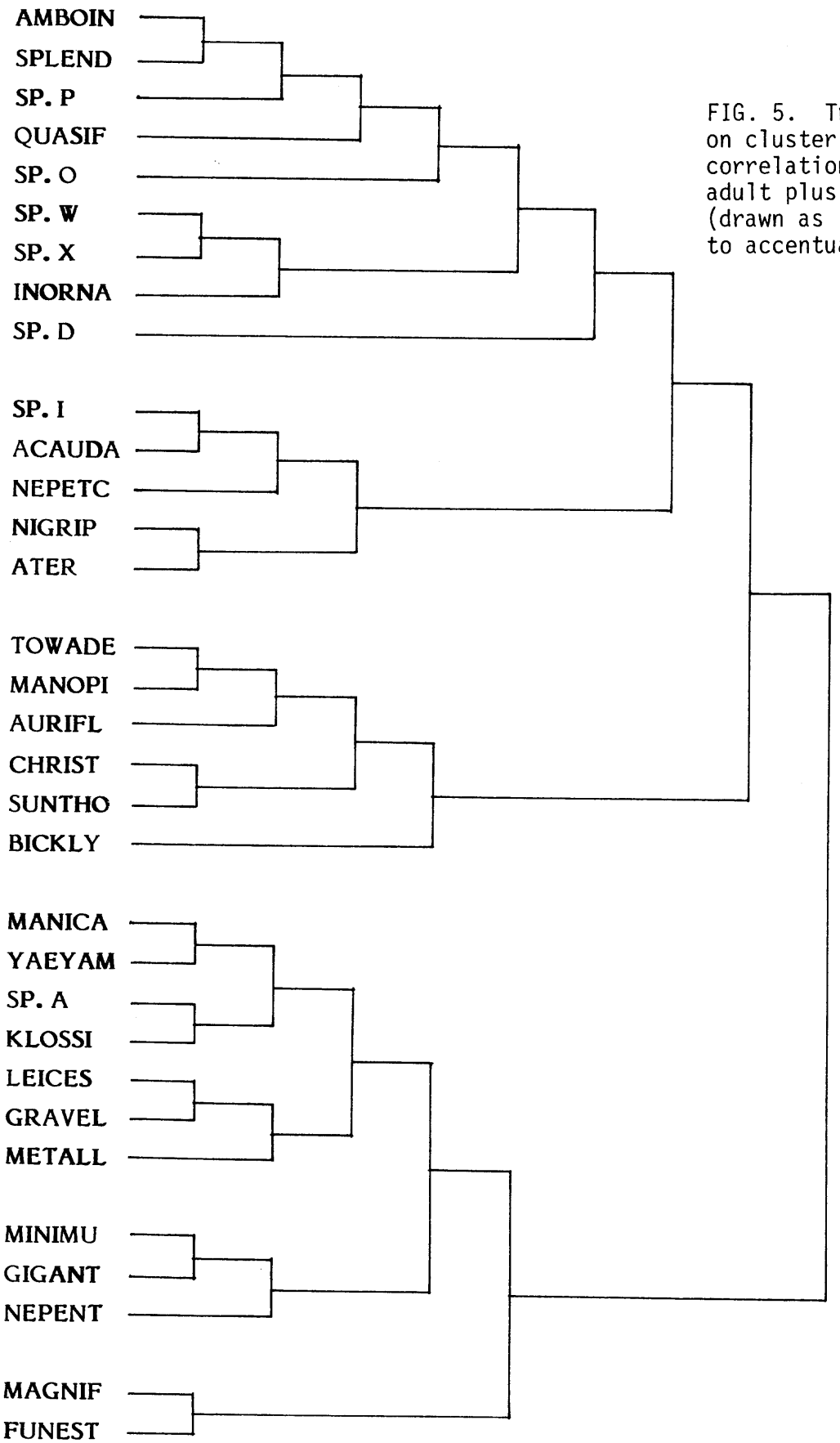

FIG. 5. Tree topology based on cluster analysis (UPGMA, correlation matrix) of 100 adult plus immature characters (drawn as in Fig. 1 with gaps to accentuate groups of taxa).

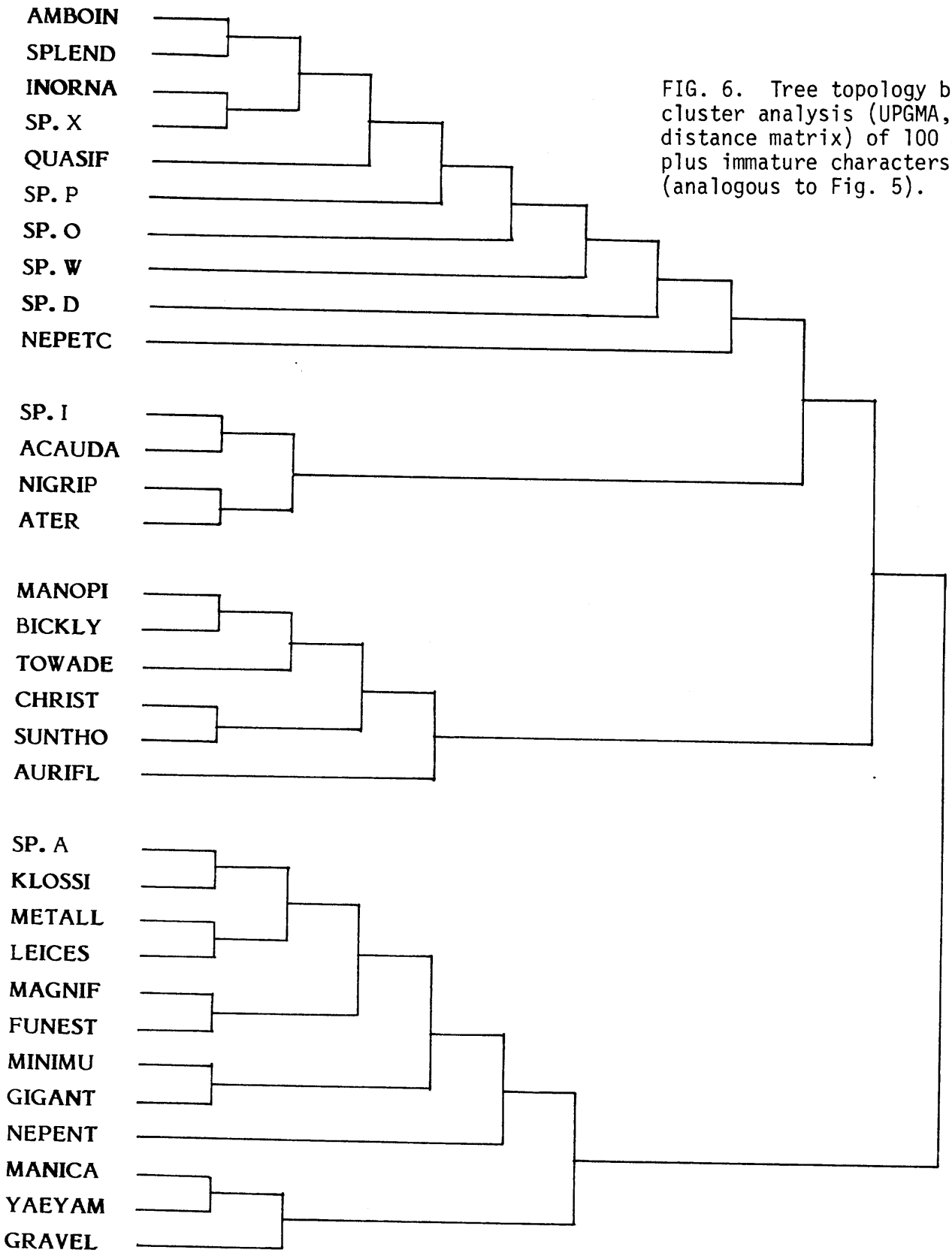

FIG. 6. Tree topology based on cluster analysis (UPGMA, distance matrix) of 100 adult plus immature characters (analogous to Fig. 5).

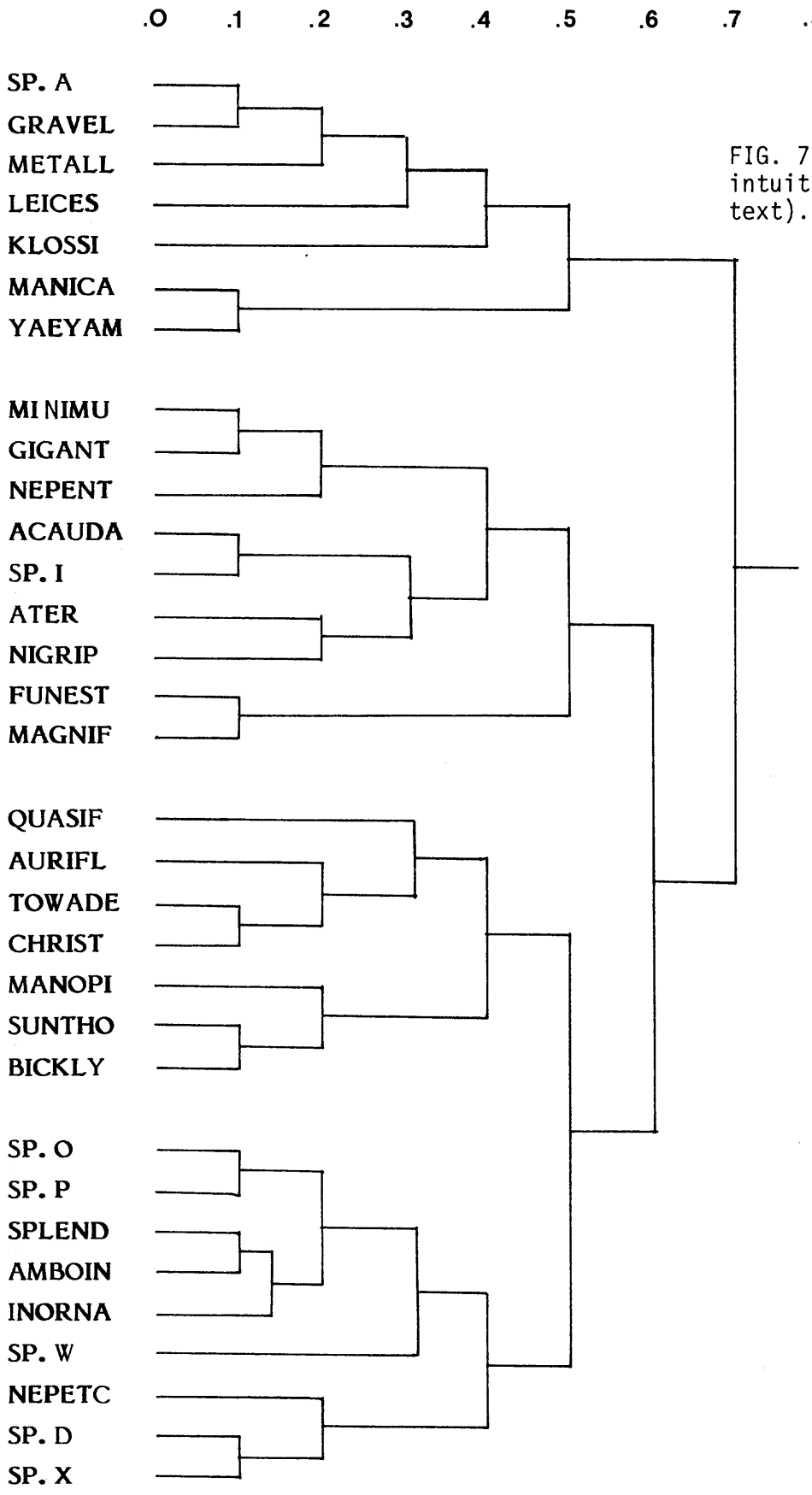

FIG. 7. Tree topology based on intuitive classification (see text).

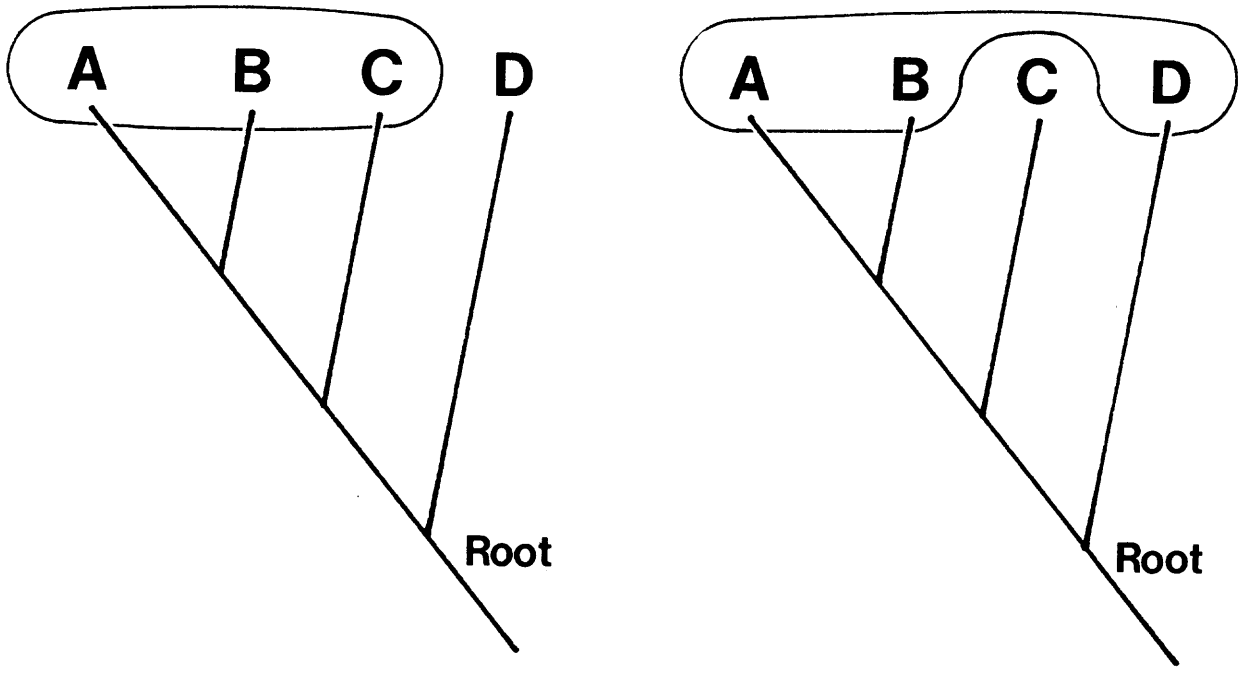

FIG. A-1. Definition of monophyly sensu Hennig (1966). The encircled group on the left is monophyletic. The encircled group on the right is not.

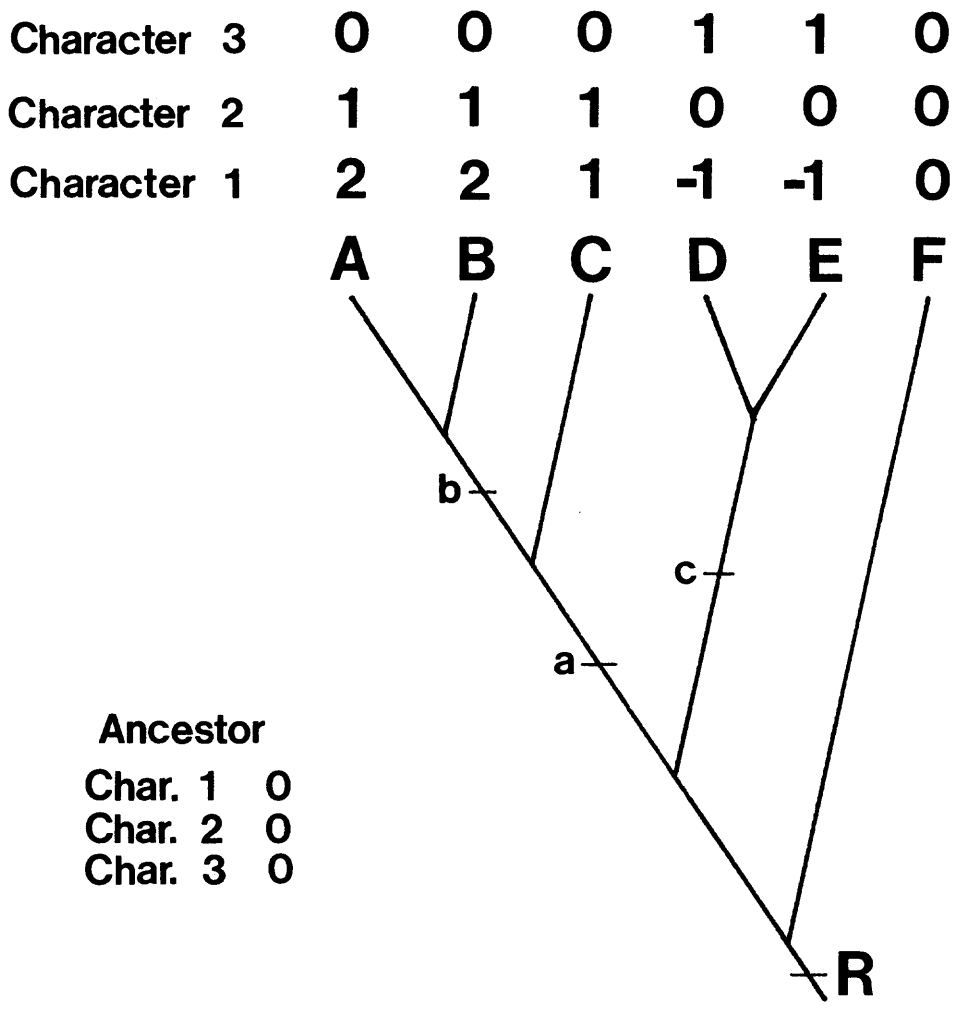

FIG. A-2. Hypothetical phylogenetic tree (see text for explanation).

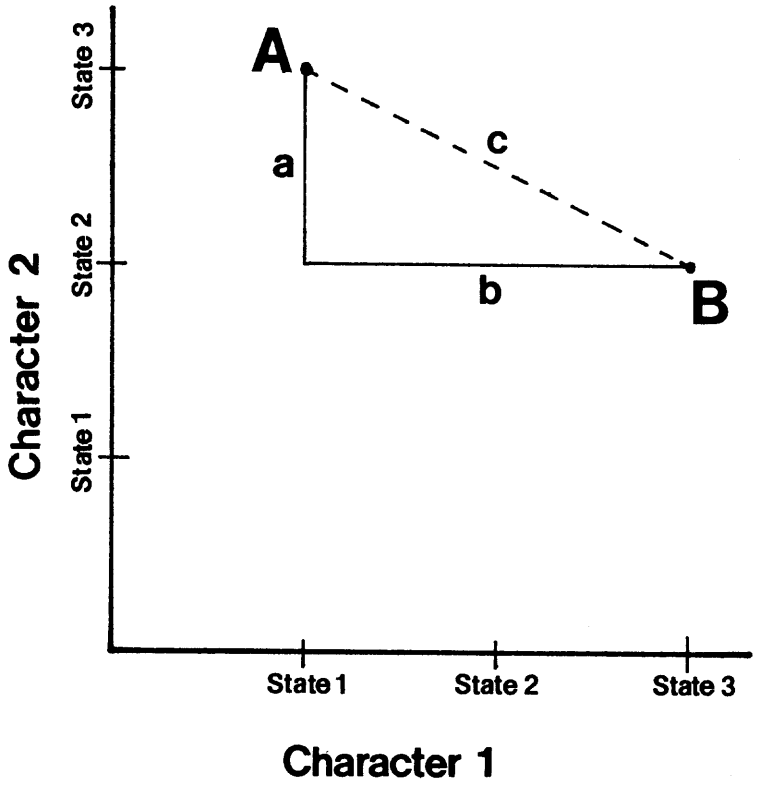

FIG. A-3. Euclidean and Manhattan distances (see text for explanation).

Appendix 2. List of species used in this analysis. (The last seven species were not included in Moss et al. 1979.)

	Taxa	Abbreviation	Source
1.	*Tx. (Tox.)* sp. A.	SP. A	Malaysia
2.	*Tx. (Tox.) acaudatus*	ACAUDA	Singapore
3.	*Tx. (Tox.) amboinensis*	AMBOIN	Singapore
4.	*Tx. (Tox.) ater*	ATER	Malaysia
5.	*Tx. (Tox.) aurifluus*	AURIFL	Taiwan
6.	*Tx. (Tox.) bickleyi*	BICKLY	Thailand
7.	*Tx. (Tox.)* sp. D	SP. D	Malaysia
8.	*Tx. (Tox.) funestus*	FUNEST	Malaysia
9.	*Tx. (Tox.) gigantulus*	GIGANT	Philippines
10.	*Tx. (Tox.) gravelyi*	GRAVEL	Thailand
11.	*Tx. (Tox.) klossi*	KLOSSI	Malaysia
12.	*Tx. (Tox.) leicesteri*	LEICES	Thailand
13.	*Tx. (Tox.) magnificus*	MAGNIF	Malaysia
14.	*Tx. (Tox.) manicatus*	MANICA	Taiwan
15.	*Tx. (Tox.) manopi*	MANOPI	Thailand
16.	*Tx. (Tox.) metallicus*	METALL	Malaysia
17.	*Tx. (Tox.) minimus*	MINIMU	Malaysia
18.	*Tx. (Tox.) nepenthis*	NEPENT	Philippines
19.	*Tx. (Tox.) nigripes*	NIGRIP	Borneo (Kalimantan)
20.	*Tx. (Tox.) quasiferox*	QUASIF	Malaysia
21.	*Tx. (Tox.) splendens*	SPLEND	Philippines

22.	*Tx. (Tox.) sunthorni*	SUNTHO	Thailand
23.	*Tx. (Tox.) towadensis*	TOWADE	Japan
24.	*Tx. (Tox.) yaeyamae*	YAEYAM	Ryukyus
25.	*Tx. (Tox.)* sp. X	SP. X	Thailand
26.	*Tx. (Tox.) christophi*	CHRIST	Korea
27.	*Tx. (Tox.)* sp. I	SP. I	Malaysia
28.	*Tx. (Tox.) inornatus*	INORNA	New Britan
29.	*Tx. (Tox.) nepenthicola*	NEPETC	Papua New Guinea
30.	*Tx. (Tox.)* sp. O	SP. O	Thailand
31.	*Tx. (Tox.)* sp. P	SP. P	India
32.	*Tx. (Tox.)* sp. W	SP. W	Vietnam

Appendix 3. List of characters used in the present study and in the previous (Moss et al., 1979) study. Characters include measurements, counts, and qualitative ordered states. The measurements and the estimates of percent light scaling were made using an ocular micrometer. Numbered characters were used in the present study. Unnumbered characters were used by Moss et al. (1979) but eliminated in the process of reducing the data set from 133 to 100 characters (see text). * denotes characters added since 1979. Characters used by Moss et al. (1979) which were not considered here due to low principal component scores (see text) are listed at the end of this appendix section.

Character Number	Abbreviation	Sex	Description of character
			ADULT CHARACTERS
	FLAG1SC	♂	color of scales on first flagomere of antennae
1	OCSETCOL*	♂	color of occipital setae
2	OCSETCOF	♀	color of occipital setae
	PROMSCAL*	♂	color of promontory scales
	PROMSCAF*	♀	color of promontory scales
	UPANSCA	♂	color of upper anterior pronotum scales
	UPANSCF	♀	color of upper anterior pronotum scales
3	WINGL	♂	wing length
4	WINGLF	♀	wing length
5	WINGW*	♂	wing width
6	WINGWF*	♀	wing width
7	RMRATIO	♂	ratio of longitudinal vs. transverse portion of R-M wing vein
8	RMRATIOF	♀	ratio of longitudinal vs. transverse portion of R-M wing vein

9	FORET1	♂	foretarsus segment 1	presence (=1) or absence (=0) of light scaling
10	FORET2	♂	foretarsus segment 2	
11	FORET3	♂	foretarsus segment 3	
12	FORET4	♂	foretarsus segment 4	
13	MIDT1	♂	mid tarsus segment 1	
	MIDT2	♂	mid tarsus segment 2	
14	MIDT3	♂	mid tarsus segment 3	
15	MIDT4	♂	mid tarsus segment 4	
16	MIDT5	♂	mid tarsus segment 5	
17	HINDT1	♂	hind tarsus segment 1	
18	HINDT2	♂	hind tarsus segment 2	
19	HINDT3	♂	hind tarsus segment 3	
20	HINDT4	♂	hind tarsus segment 4	
	FORET1F	♀	foretarsus segment 1	
	FORET2F	♀	foretarsus segment 2	
21	FORET3F	♀	foretarsus segment 3	
22	FORET4F	♀	foretarsus segment 4	
23	FORET5F	♀	foretarsus segment 5	
24	MIDT1F	♀	mid tarsus segment 1	
	MIDT2F	♀	midtarsus segment 2	
25	MIDT3F	♀	mid tarsus segment 3	
	MIDT4F	♀	midtarsus segment 4	
26	MIDT5F	♀	mid tarsus segment 5	
	HINDT2F	♀	hind tarsus segment 1	
27	HINDT2F	♀	hind tarsus segment 2	
	HINDT3F*	♀	hind tarsus segment 3	
28	HINDT4F	♀	hind tarsus segment 4	
29	HINDT5F	♀	hind tarsus segment 5	

30	HINDTFR*	♂	presence or absence of hind tarsal fringe (only in male)	
31	MESSC*	♂	presence or absence of brown scales on mesokatepineuron	
	TERGCOL1*	♂	color of tergal segment 1	
	TERGCOL2*	♂	color of tergal segment 2	
	TERGCOL3*	♂	color of tergal segment 3	
	TERGCOL4*	♂	color of tergal segment 4	
	TERGCOL5*	♂	color of tergal segment 5	
	TERGCOL6*	♂	color of tergal segment 6	
32	TERGCOL7*	♂	color of tergal segment 1	
	TERGCOL8*	♂	color of tergal segment 8	
33	TERGCL1F*	♀	color of tergal segment 1	
34	TERGCL2F*	♀	color of tergal segment 2	
35	TERGCL3F*	♀	color of tergal segment 3	
36	TERGCL4F*	♀	color of tergal segment 4	
37	TERGCL5F*	♀	color of tergal segment 5	
	TERGCL6F*	♀	color of tergal segment 6	
	TERGCL7F*	♀	color of tergal segment 7	
38	TERGCL8F*	♀	color of tergal segment 8	
	TLSCACOL*	♂	tergolateral scale color	
39	SLSCACOL*	♂	sternolateral scale color	
	TLSCCOLF*	♀	tergolateral scale color	
40	SLSCCOLF*	♀	sternolateral scale color	
	TLATPAT2	♂	tergolateral patch 2	percent of light scaling
	TLATPAT3	♂	tergolateral patch 3	

	TLATPAT4	♂	tergolateral patch 4	
	TLATPAT5	♂	tergolateral patch 5	
	TLATPAT6	♂	tergolateral patch 6	
41	TLATPAT7	♂	tergolateral patch 7	
42	SLATPAT 2	♂	sternolateral patch 2	
43	SLATPAT3	♂	sternolateral patch 3	
	SLATPAT4	♂	sternolateral patch 4	
44	SLATPAT5	♂	sternolateral patch 5	
45	SLATPAT6	♂	sternolateral patch 6	
	SLATPAT7	♂	sternolateral patch 7	
46	TLAPAT2F	♀	tergolateral patch 2	percent of light scaling
47	TLAPAT3F	♀	tergolateral patch 3	
48	TLAPAT4F	♀	tergolateral patch 4	
49	TLAPAT5F	♀	tergolateral patch 5	
50	TLAPAT6F	♀	tergolateral patch 6	
51	TLAPAT7F	♀	tergolateral patch 7	
52	SLAPAT2F	♀	sternolateral patch 2	
53	SLAPAT3F	♀	sternolateral patch 3	
	SLAPAT4F	♀	sternolateral patch 4	
54	SLAPAT5F	♀	sternolateral patch 5	
55	SLAPAT6F	♀	sternolateral patch 6	
	SLAPAT7F	♀	sternolateral patch 7	
	SLAPAT8F	♀	sternolateral patch 8	
56	CAUD6	♀	color of caudal scale tuft 6	
57	CAUD7	♂	color of caudal scale tuft 7	
58	CAUD8	♂	color of caudal scale tuft 8	
59	CAUD6F	♀	color of caudal scale tuft 6	

60	CAUD7F	♀	color of caudal scale tuft 7
61	CAUD8F	♀	color of caudal scale tuft 8

PUPAL CHARACTERS

62	GONCLAW	♂	gonostylar claw
63	PADL	♂	length of pupal paddle
64	PADW	♂	width of pupal paddle
65	PADLF	♀	length of pupal paddle
66	PADWF	♀	width of pupal paddle
67	PADSHAPE	♂	pupal paddle shape
68	PADAPSET	♂	presence or absence of apical setae on paddle
69	TRUMPL	♂	trumpet length
70	TRUMPLF	♀	trumpet length
71	TRUMSHAP	♂	trumpet shape
72	6II	♂	abdominal segment II
73	6III	♂	abdominal segment III
74	6IV	♂	abdominal segment IV
75	6V	♂	abdominal segment V
76	6VI	♂	abdominal segment VI
77	6VII	♂	abdominal segment VII
78	5VII	♂	abdominal segment VII, length of seta 5
79	6VIIBR*	♂	abdominal segment VII, #branches, seta 6
80	5VIIBR*	♂	abdominal segment VII, #branches, seta 5
81	6IIF	♀	abdominal segment II
82	6IIIF	♀	abdominal segment III
83	6IVF	♀	abdominal segment IV
84	6VF	♀	abdominal segment V
85	6VIF	♀	abdominal segment VI
86	6VIIF	♀	abdominal segment VII

87	5VIIF	♀	abdominal segment V, length of seta 5
88	6VIIBRF*	♀	abdominal segment VII, # branches, seta 6
89	5VIIBRF*	♀	abdominal segment VII, # branches, seta 5
90	PADPIGM	♂	paddle pigmentation, 0 = light, 1 = medium, 2 = dark
91	1IX*	♂	presence or absence of seta #1, of abdominal segment IX

LARVAL CHARACTERS

92	ANTL*	♂	antenna length
93	ANTW*	♂	antenna width
94	ANTLF*	♀	antenna length
95	ANTWF*	♀	antenna width
96	SIPHL*	♂	siphon length
97	SIPHW*	♂	siphon width
98	SIPHLF*	♀	siphon length
99	SIPHWF*	♀	siphon length
100	STIRSHAP*	♂	stirrup shape

CAUDAL TUFT COLOR CODE:

0 = absent

1.2 = yellow

1.3 = orange

1.7 = black

2.1 = white and yellow

2.2 = white and orange

2.4 = white and black

2.6 = yellow and black

2.7 = orange and black

3.1 = yellow, white and black

COLOR CODES FOR REMAINING COLOR CHARACTERS:

1 = white silver

2 = yellow

3 = golden

4 = green

5 = blue-green

6 = blue

7 = brassy

8 = brown

9 = magenta

10 = purple

Characters used by Moss et al. (1979) which were not used here (their numbering scheme): 1, 3-6, 22-27, 28-41, 45, 47, 52, 55-63, 71-79; 1-6, 25-43, 47, 49-51, 53-55, 57, 58-61, 71-77 (see text for explanation).