The Genus *Toxorhynchites* (Diptera: Culicidae); Analysis of *T. splendens* and Allies Using Techniques of Numerical Taxonomy[1]

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INTRODUCTION

This paper describes phenetic similarities within a group of Oriental species of the mosquito genus *Toxorhynchites* Theobald. Our analysis represents one facet of a broad biosystematic study of *Toxorhynchites* that will lead eventually to a monographic treatment on a worldwide basis. We intend to reach this goal by combining conventional taxonomy, numerical phenetics and cladistics. The analysis in this paper is based on a morphometric character set, but we will eventually include data from morphology, geography, ecology, cytogenetics, and biochemistry.

Toxorhynchites has never been revised; only Theobald (1901) and Edwards (1932) included this genus (as *Megarhinus*) in their treatises of the mosquitoes of the world. Regional studies since then have contributed substantially to our knowledge of local faunas (reviewed by Steffan 1975), but species of *Toxorhynchites* are strikingly similar throughout the world and difficult to identify, even with characters used successfully for other groups of mosquitoes. Currently there are 70 recognized species assigned to three subgenera (Knight & Stone 1977, Knight, 1978; Belkin, 1977) and 102 available names (Steffan 1977). Many synonyms are suspect because detailed comparisons with types were not made and the extent of intraspecific variation is poorly understood, even for widespread species such as *Tx. splendens* (Wiedemann) and *Tx. amboinensis* (Doleschall).

The relative homogeneity of this group of mosquitoes and the numerous characters available for taxonomic study led us to the techniques of numerical taxonomy as a viable approach for assessing interspecific relationships. Our collaboration involved the efforts of a numerical taxonomist (WWM) to supplement the more conventional approach initially undertaken by WAS. All of us participated to some degree in the recognition and descriptions of characters, with data recording and processing carried out primarily by NLE and DLM. Species were identified by WAS and NLE.

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We chose the predominantly Oriental *Tx. splendens* and 24 related species as a preliminary test case (Appendix Table I). This analysis allowed us to familiarize ourselves with trends of morphometric variation, to gain experience in dealing with large numbers of characters, to test numerical methods, and to compare numerical and conventional classifications of the same taxa.

We carried out two studies involving different numbers of characters. Species similarities obtained in the first numerical study were generally reasonable but puzzling in some cases when compared with intuitive estimates of similarity. Questionable placements in our intuitive and numerical analysis were clarified in the second numerical study by redefinition of characters and by the inclusion of additional data. Further modifications of the data will be made as we define additional characters and analyze them. Interaction between conventional and numerical approaches has proved to be a key element in our collaborative approach.

Other applications of numerical taxonomic procedures to culicids include analyses of *Aedes* by Rohlf (1977) and earlier papers listed by Sneath and Sokal (1973).

MATERIALS AND METHODS

The Operational Taxonomic Units (OTUs) in this study were males and females of 25 species of Oriental *Toxorhynchites* (Appendix Table I). Individuals deficient in one or more characters were supplemented by observations from additional specimens. Data were gathered from male and female adults and pupae; the sexes were analyzed separately because of pronounced dimorphism. Larvae were not available in sufficient numbers for this study, but will be examined later. Our intuitive impressions of similarity were based on all available stages.

Initially, 56 females and 54 male morphometric characters were chosen. This number was increased to 77 female (51 adult, 26 pupal) and 79 male (53 adult, 26 pupal) characters in the second study (Appendix Tables II and III). Characters were defined as features that differed in their expression from one species to another and which could not be subdivided logically (Sneath & Sokal 1973); they included measurements, counts, color of scales, percentage of pale scaling, presence or absence of certain morphological features, and ordered multi-state characters from all regions of the adult and pupal body. We avoided characters that would require missing values because the principal component program required a complete data table. Data were punched on cards; listings or card copies are available upon request.

Data were standardized and analyzed using procedures of Q- and R-mode analysis described by Sneath & Sokal (1973) and followed by Moss, Peterson, and Atyeo (1977). Techniques included character standarization by expressing each state as a deviation from the mean in standard deviation units, computation of character correlations and taxonomic distances between species, principal component analysis, and nonmetric multidimensional scaling. The results were combined in a reasonably distortion-free ordination diagram produced by multidimensional scaling of standardized Q-mode taxonomic distances, using the projections of the species onto the first three principal components as an initial configuration. A minimum spanning network and subsets were superimposed to show closest relatives and most coherent groups (Rohlf et al. 1974). The findings described below are based primarily on these ordination diagrams.

The data from both studies were processed by NT-SYS (Rohlf et al. 1974) and an IBM 370/168 VS2 at the Uni-Coll Corporation, accessed via a remote job entry station at the Academy of Natural Sciences of Philadelphia. The second study was repeated and its results confirmed using the same program on the University of Hawaii IBM 370-158 VS2.

Comparison of the intuitive and numerical classifications was considered essential both as a check of the numerical results and as an important interactive phase of the project. The 25 species were classified intuitively by expressing our impressions of similarity in the form of a dendrogram or phenogram (Fig. 4). The application of an arbitrarily-coded, equal-interval dissimilarity scale to this phenogram enabled us to generate a table of phenetic distances comparable to those obtained through the numerical analysis. This enabled us to compare both approaches in several interesting combinations (cf. Moss and Wojcik 1978), and to clarify the characters used to classify intuitively.

RESULTS

Male Adult and Pupal Characters

A principal component analysis of the 79-character correlations yielded 24 components to account for all the variation in the study; of these, 12 had eigenvalues greater than 1.0. The first 2 components accounted for 45% of the variation (Table 1). The relatively large number of components indicated a pattern of richly divergent variation, but a 2-dimensional multidimensional scaling ordination diagram was adequate to display patterns of relationships satisfactorily as judged by the high matric correlation (0.950) and low stress (0.242); Rohlf (1972) discusses different ordination techniques and their relative effectiveness.

Two groups were evident in Fig.1: a *Tx. sunthorni-Tx. minimus* cluster (group II) running from upper left to lower right and a *Tx. manicatus-Tx. leicesteri* cluster (group II) in the upper right corner. Group I was difficult to characterize because it overlapped in both directions along axes I and II. A definite size trend was evident in group I from *Tx. sunthorni* (the largest) to *Tx. minimus* (the smallest) and in group II from *Tx. manicatus* to *Tx. leicesteri*. These trends ran along axis I, but diagonally as the diagram was viewed from above.

The ordination diagram was related to the original characters by correlating the coordinates of each species along the nonmetric multidimensional scaling axis with the original data matrix. Adult characters with the highest loading on the first component (axis I, Fig. 1) were length of wing and of gonostylus, percentage of pale scales on sterna II-IV, VI, banding on tarsomere 2 of proleg, midleg and hindleg, and caudal tufts on abdominal segments VI and VIII. Pupal characters with the highest loading were: length of setae 6-1-IV, VI, setae 1-III-V, and width of the paddle. The species at the left end of group I (*Tx. bickleyi-Tx. manopi*) were large and had tarsomere 2 of the proleg, midleg and hindleg all pale or banded, whereas species at the right end of group I were small and most had dark tarsomere 2 of the proleg, midleg, and hindleg. The *Tx. splendens* subgroup (*Tx. splendens, Tx. amboinensis, Tx. quasiferox, Tx.* sp. D, and *Tx.* sp. X) were intermediate in size, had a dark tarsomere 2 on the proleg and usually a partially

banded tarsomere 2 on the hindleg. Species of group II (*Tx. manicatus, Tx. yaeyamae, Tx. metallicus, Tx. klossi, Tx. gravelyi, Tx. leicesteri*, and *Tx.* sp. A) tended to have almost entirely pale sterna II-IV, VI, tarsomere 2 of the proleg and hindleg usually dark and caudal tufts on abdominal segments VI-VIII absent. There were exceptions to these trends as some species showed a mosaic of character state expression.

Adult characters with highest loadings on the second component (axis II, Fig. 1) were: length of the r-m crossvein, banding on tarsomere 1 and 3 of the midleg, and color of the scales of the upper postpronotum. Pupal characters with the highest loading were: length of the midrib of the paddle, and length of the pupal abdomen. Species near the bottom of axis II (*Tx. nigripes, Tx. ater*, etc.) tended to have a short pupal paddle midrib, long r-m and short pupal abdomen. Species at the top of axis II (group II) tended to have a long pupal paddle midrib, and all had a short r-m and long pupal abdomen.

Female Adult and Pupal Characters

As in the male study, a principal component analysis of the female 77-character correlations showed that 24 components accounted for all the variation in the study; 14 of these components had eigenvalues greater than 1.0. The relative amounts of trace explained by the various components were similar to the pattern shown in Table 1. The first 3 components accounted for 52.14% of the variation.

Three groups were evident in Fig. 2: a compact *Tx. towadensis-Tx. sunthorni* cluster (group I) in the upper left corner, a less distinct *Tx. nigripes-Tx. funestus* cluster (gr.II) restricted primarily to the lower left corner, and a diffuse *Tx. leicesteri-Tx. gigantulus* cluster (group III) running from upper right to lower right of the ordination diagram. There were obvious similarities between species placements in the two ordination diagrams. Taxonomic distances based on male and female characters were correlated at $r = 0.704$. Male numerical and intuitive estimates were correlated at $r = 0.608$, female numerical and intuitive estimates at $r = 0.531$.

Adult characters with the highest loadings on the first component (axis I, Fig. 2) were: length of the antenna and r-m crossvein, banding on tarsomere 2 of the midleg and hindleg, percentage of pale scales on sternum VI, and caudal tufts on abdominal segments VI and VIII. Pupal characters with the highest loadings were: length of setae 6-I-VI, setae I-III-V, VII, and width of paddle. Species to the right of axis I (group III) lacked banding on tarsomere 2 of the hindleg (except *Tx. minimus* and *Tx. gigantulus*), had a short r-m crossvein, lacked caudal tufts (except *Tx. gigantulus*), and were smaller. The species in groups I and II were more difficult to characterize and seemed to differ primarily in the larger size of the species in group I.

Adult characters with the highest loadings on the second component (axis II, Fig. 2) were: length of wing, banding of tarsomere 2 and 4 of the foreleg, tarsomeres 3-5 of the midleg and tarsomere 4 of the hindleg and color of the pile of the forecoxa. Pupal characters with the highest loadings were: length of the paddle midrib, abdomen, and trumpet. Species below axis I tended to be smaller and to have unbanded tarsomeres 3 and 4 of the midleg (except *Tx. yaeyamae*) and unbanded tarsomere 5 of the midleg. Species above axis I tended to be larger and have completely pale or banded tarsomeres 2-5 of the midleg.

The females showed a greater mosaic of character states in both components than did the males.

DISCUSSION

No clearly-delimited groups were observed in the ordination diagrams of the first numerical study, reflecting in part the mosaic of variation shown by the species and the inadequacies of the preliminary data tables. Sections of the ordination diagrams of both the males and females did show similar species placed together; this was obvious when models of the ordination diagrams (components I and II) were made by placing pinned specimens in their respective locations on the diagrams. The visible size trend was somewhat surprising considering the small number of measurement characters.

The placement of each species pair was examined for correspondence with our intuitive impressions of relationships and the characters recorded in our data tables. Some differences between the numerical results and our intuitive classification (Fig. 4) were due to differences in handling the pronounced sexual dimorphism in this taxon. The intuitive study was based on an analysis of both sexes and all stages, whereas the numerical study treated the males and females separately. Our impressions of intuitive similarity in some cases proved to be based on characters not included in the data table. For example, grouping of *Tx. splendens, Tx. amboinensis, Tx. quasiferox, Tx.* sp. D and *Tx.* sp. X was based primarily on similar characteristics in the male and pupa, and geographical distribution. The crucial evidence for the grouping of *Tx. nigripes, Tx. ater* and *Tx. nepenthis* was the pitcher plant habitat occupied by these species, plus the sharing of common states for morphological characters such as humeral stripe, banding of tarsomeres, lateral pale patches on the abdominal terga and pupal paddle hairs.

Toxorhynchites splendens-Tx. amboinensis and *Tx.* sp. D-*Tx.* sp. X were pulled out as separate subsets in the male ordination diagram (Fig. 3), with *Tx.* sp. D-*Tx.* sp. X appearing to be more similar to the *Tx. acaudatus-Tx. funestus* subset. *Tx. nepenthis* was well separated from the *Tx. ater-Tx. nigripes* subsets.

The second numerical study included some additional adult characteristics and a larger number of pupal characters. The resulting ordinations (Figs. 1 & 2) were closer to our intuitive impressions. The cluster of species (group I) in the upper right quadrant of Fig. 1 coincides with our intuitive group of the species with the short r-m crossvein. Relationships within this group need to be clarified, especially the species from *Tx. metallicus* to *Tx. leicesteri*. Two species, *Tx. manicatus* and *Tx. yaeyamae*, form a distinct subset, which agrees with our intuitive impressions and those of Dr. Tanaka (personal communication) who will elaborate on this relationship in his publication on the mosquitoes of Japan and Korea.

The numerical analysis of the females of group I showed a much more diffuse pattern of relationships (Fig. 2) with no clearly delimited groups. In fact, none of the subsets coincided with those in the male, e.g., even *Tx. manicatus* and *Tx. yaeyamae* were widely segregated. This may be due to sexual dimorphism or to misidentification of one of the sexes. The specimens available to us and identified by other workers as *Tx. manicatus* are

different from the type of *Tx. manicatus*. The unique holotype female also differs from the descriptions published by Lien (1965) in his revision of the Taiwan *Toxorhynchites*. As is the case with most *Toxorhynchites*, a detailed analysis needs to be done of intraspecific variation and the degree of sexual dimorphism. Progeny rearings are particularly needed.

The *Tx. splendens* species group formed a clearly delimited subset in the numerical analyses of the male data, generally agreeing with our overall intuitive impressions. However, in the female analysis (Fig. 2) *Tx. amboinensis* was pulled out of the group and placed closer to *Tx. sunthorni*. This does not agree at all with our intuitive analysis which includes a fairly large sample size, detailed geographical data, and progeny rearings of *Tx. splendens* and *Tx. amboinensis*. We feel that the relationships of these two species need further clarification. Progeny rearings from colonies in Bangkok indicate that some key characters used to separate *Tx. splendens* and *Tx. amboinensis* are variable. A more detailed analysis of this group will include genetic and biochemical studies.

The second numerical study again separated *Tx. nepenthis* from the *Tx. ater-Tx. nigripes* subset in both the male and female analysis. Subsequent examination of the types of these species and more detailed examinations of other specimens available to us revealed additional characters that separated *Tx. nepenthis* from the other two species breeding in pitcher plants of the genus *Nepenthes*. Individuals of *Tx. nepenthis* were found in the Philippines while those of *Tx. ater* were recorded from Peninsular Malaysia and *Tx. nigripes* from Borneo (Malaysian and Indonesian). In this case, the numerical study revealed the interspecific relationships of this group before the group was discovered by the intuitive method. This is another group that we shall examine in greater depth when we include all species found in *Nepenthes*.

The absence of a linkage between *Tx. manopi* and the *Tx. bickleyi-Tx. sunthorni* pair in the upper left quadrant of the ordination diagram (Fig. 1) does not coincide with our intuitive impressions; *Tx. bickleyi*, *Tx. sunthorni* and *Tx. manopi* (all from Thailand) are probably close relatives. In this case, the numerical analysis of the female data set (Fig. 2) agreed more with our intuitive interpretations than did the numerical analysis of the male data set. *Tx. aurifluus* (from Taiwan) and *Tx. towadensis* from Japan also appear to be more closely related than indicated in either of the ordination diagrams.

GENERAL COMMENTS

Interest in collaborative and multidisciplinary approaches was expressed at the Washington symposium on mosquito systematics. This interest is encouraging and tends to corroborate views expressed earlier, following the study of other taxa (Moss & Hendrickson 1973, Moss, Peterson & Atyeo 1977). Surely it is preferable to cast the net widely for data. Developments in various disciplines, e.g., genetics, biochemistry, electronic data processing (both hardware and software), and electron microscopy have accelerated to the point where most systematists have neither the time, equipment nor expertise to utilize efficiently all the sources of data potentially available to them. A classification based on an analysis of the broadest possible information base should be more stable; thus, monographic studies of complex taxa should

incorporate information from a wide range of disciplines. The methods of numerical taxonomy incorporated into our biosystematics study at an early stage should assist in the development of a sound data base for other methods of taxonomic analysis.

Some benefits of using numerical taxonomy in conjunction with an ongoing intuitive study were discussed by Crovello (1969). These include first, the development of a complete data table. All characters must be examined and recorded for every taxon. This avoids the intuitive tendency to skip from one prominent character to another, producing a convenient but incomplete mosaic of character values across the set of OTUs. To state that "Species B is similar to Species A except..." is not very informative unless the reader knows exactly which characters the author has examined. We found it helpful in this study to define our characters in depth; redefinition proved necessary at times to clarify some relationships. It might be possible eventually to develop a baseline set of characters that would be usable for all mosquitoes, supplemented in special cases with additional characters needed to distinguish species within a particular genus. This baseline data set would permit a very large overall classification (Rohlf, pers. comm.).

Second, numerical techniques allow the efficient summarization of phenetic similarities among species. It is difficult for the human mind to manipulate efficiently a large volume of multivariate data for any sizable taxonomic group. Relationships within closely related taxa tend to be stressed more than the equally important relationships between distantly related groups, and the mind can exaggerate the close or distant nature of such relationships (Moss 1970, Moss & Hansell MS). In the present study we found instances in which our earlier, intuitive impressions of similarity were modified following numerical classification; the reverse also held true in certain cases where characters had been poorly defined.

Third, a numerical analysis provides the opportunity to reexamine existing or proposed classifications using different methodologies. Systematists can become polarized with respect to a particular method of classification, but in fact a variety of approaches and possible classifications exist. Surely the taxon is more important than the taxonomic method; eclecticism is a virtue in classification. A monographic study of any complex taxon will benefit from a combination of methodologies, especially if collaborators participate in the early stages of the study.

The present analysis is a starting point, one of several approaches that will be used for a monographic treatment of *Toxorhynchites*. Our preliminary work has shown interesting agreements and discordances in conventional and numerical results. These have in turn stimulated a closer look at the species, which has clarified relationships in most cases. Character sets will be modified considerably as the study progresses, both in the selection of characters and in the determination of character states; we are now looking critically at color characters and their states for the degree of pale scaling on the abdomen and tarsomeres because of dissatisfaction with our initial selection of character states in these areas. Additional OTUs will also be added as we expand our study of the genus.

We encourage and welcome comments and suggestions regarding the various aspects of this study undertaken to date and planned for the future.

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Table 1. Principal component analysis of 79-character study, male adult and pupal characters

Component*	Accumulated % of Trace
1	29.38
2	45.49
3	54.88
4	61.57
5	66.51
6	71.26
7	74.94
8	78.15
9	81.10
10	83.82
11	86.16
12	88.36

*only components with eigenvalues > 1.0 are listed.

Appendix I. List of species (OTU's) used in this analysis and source of specimens.

1.	*Toxorhynchites (Toxorhynchites) acaudatus*	Singapore
2.	*Tx. (Tox.) ater*	Malaysia
3.	*Tx. (Tox.) aurifluus*	Taiwan
4.	*Tx. (Tox.) bickleyi*	Thailand
5.	*Tx. (Tox.) funestus*	Malaysia
6.	*Tx. (Tox.) gigantulus*	Philippines
7.	*Tx. (Tox.) gravelyi*	Thailand
8.	*Tx. (Tox.) klossi*	Malaysia
9.	*Tx. (Tox.) leicesteri*	Thailand
10.	*Tx. (Tox.) magnificus*	Malaysia
11.	*Tx. (Tox.) manopi*	Thailand
12.	*Tx. (Tox.) metallicus*	Malaysia
13.	*Tx. (Tox.) minimus*	Malaysia
14.	*Tx. (Tox.) nepenthis*	Philippines
15.	*Tx. (Tox.) nigripes*	Borneo (Kalimantan)
16.	*Tx. (Tox.) quasiferox*	Malaysia
17.	*Tx. (Tox.) sunthorni*	Thailand
18.	*Tx. (Tox.) towadensis*	Japan
19.	*Tx. (Tox.) yaeyamae*	Ryukyu
20.	*Tx. (Tox.) manicatus*	Taiwan
21.	*Tx. (Tox.) splendens*	Philippines
22.	*Tx. (Tox.) amboinensis*	Philippines
23.	*Tx. (Tox.)* sp. D	Malaysia
24.	*Tx. (Tox.)* sp. X	Thailand
25.	*Tx. (Tox.)* sp. A	Malaysia

Appendix II. List of characters, including measurements, counts and qualitative ordered states. Males. Measurements and degree of light scaling determined by counts of ocular micrometer units.

1. Antennal length (ANTLENGT): measured from base of flagellomere I to tip of flagellomere XIII.
2. Flagellomere I. mesal scaling (FLAGSCAL): bare (0), light (1), dark (2).
3. Proboscis length (PROBLENG): measured along the entire length from clypeus to tip of labellum.
4. Maxillary palpus, ventral scale color (PLPVENCL): yellow (2), blue (6), brown (8), magenta (9), purple (10).
5. Humeral stripe scale color (HUMSTRIP): white/silver (1), green (4), blue-green (5), brown (8).
6. Mesonotal scale color (MESSCOL): golden (3), green (4), brassy (7), brown (8), magenta (9).
7. Antepronotum, scale color (APNSCACL): white/silver (1), yellow (2), green (4), blue-green (5), blue (6), brown (8), magenta (9), purple (10).
8. Wing length (WINGLENG): measured at longest point from base of wing to tip.
9. Wing vein length (RMLENGTH): length $\geq$ 2 X width (1), length < 2 X width (2).

10. Tarsomere 1 of proleg, banding (PTAR1): % light scaling.
11. Tarsomere 2 of proleg; banding (PTAR2): % light scaling.
12. Tarsomere 3 of proleg, banding (PTAR3): % light scaling.
13. Tarsomere 4 of proleg, banding (PTAR4): % light scaling.
14. Tarsomere 1 of midleg, banding MTAR1): % light scaling.
15. Tarsomere 2 of midleg, banding MTAR2): % light scaling.
16. Tarsomere 3 of midleg, banding (MTAR3): % light scaling.
17. Tarsomere 4 of midleg, banding (MTAR4): % light scaling.
18. Tarsomere 5 of midleg, banding (MTAR5): % light scaling.
19. Tarsomere 1 of hindleg, banding (HTAR1): % light scaling.
20. Tarsomere 2 of hindleg, banding (HTAR2): % light scaling.
21. Tarsomere 4 of hindleg, banding (HTAR4): % light scaling.
22. Abdominal transverse crossbanding on tergum II (TRBD2): absent (0), white/silver (1), yellow (2).
23. Abdominal transverse crossbanding on tergum III (TRBD3): absent (0), white/silver (1), yellow (2), green (4).
24. Abdominal transverse crossbanding on tergum IV (TRBD4); absent (0), white/silver (1), yellow (2).
25. Abdominal transverse crossbanding on tergum V (TRBD5): absent (0), white/silver (1), yellow (2), green (5).
26. Abdominal transverse crossbanding on tergum VI (TRBD6): absent (0), white/silver (1), yellow (2).
27. Abdominal transverse crossbanding on tergum VII (TRBD7): absent (0), white/silver (1), yellow (2).
28. Abdominal lateral scaling on tergum II (TRLP2): % light scaling.
29. Abdominal lateral scaling on tergum III (TRLP3): % light scaling.
30. Abdominal lateral scaling on tergum IV (TRLP4): % light scaling.
31. Abdominal lateral scaling on tergum V (TRLP5): % light scaling.
32. Abdominal lateral scaling on tergum VI (TRLP6): % light scaling.
33. Abdominal lateral scaling on tergum VII (TRLP7): % light scaling.
34. Abdominal lateral scaling on tergum VIII (TRLP8): % light scaling.
35. Abdominal lateral scaling on sternum II (STLP2): % light scaling.
36. Abdominal lateral scaling on sternum III (STLP3): % light scaling.
37. Abdominal lateral scaling on sternum IV (STLP4): % light scaling.
38. Abdominal lateral scaling on sternum V (STLP5): % light scaling.
39. Abdominal lateral scaling on sternum VI (STLP6): % light scaling.
40. Abdominal lateral scaling on sternum VII (STLP7): % light scaling.
41. Abdominal lateral scaling on sternum VIII (STLP8): % light scaling.
42. Tergum VI, caudal tuft color (CAUDTUF6): absent (0), white (1,10), orange (1,30), black (1.40), black and white (2.10), black and yellow (2.20).
43. Tergum VII, caudal tuft color (CAUDTUF7): absent (0), orange (1.30), black (1.40), black and orange (2.30).
44. Tergum VIII, caudal tuft color (CAUDTUF8): absent (0), yellow (1.20), orange (1.30), black (1.40).
45. Gonostylus length (GONSTYLN): measured from base to apex along a straight line.
46. Gonostylus claw length (GONCLAWL): measured from socket to apex.
47. Gonostylar lobe setae (SETGONLB): number of strong setae at apex of gonostylar lobe.
48. Pupal paddle shape (PADDSHAP): quadrate (1), round, no lobe (2), ovate, no lobe (3), ovate, small lobe (4), large, round, lobed (5), ovate, lobed (6), ovate, long lobe (7).

49. Pupal paddle apical fringe hairs (PADDHAIR): absent (0), present (1).
50. Pupal paddle length (MIDRIBLN): measured along midrib.
51. Pupal paddle pigmentation (PADDPIGM): light (1), medium (2), dark (2).
52. Pupal abdominal length (ABDLENGT): measured from anterior middorsal edge of segment I to posterior middorsal edge of segment VIII.
53. Trumpet shape (TRUMSHAP): meatus extremely short, cylindrical, pinna almost perpendicular to meatus (1), meatus long, thin, cylindrical, pinna slightly angled (2), meatus slightly flared, pinna slightly angled (3), meatus slightly flared, pinna at 45° angle (4), meatus curved, flared, pinna angled near 45° (5), meatus curved, flared, anterior surface grooved, pinna strongly angled (6), meatus large, flared, pinna at 45° angle (7), meatus long, thin, flared, pinna greater than 45° angle (8).
54. Trumpet length (TRUMPLEN): measured from base of meatus to apex of pinna.
55. Coxa of proleg, seta color (COXIPILE): white/silver (1), yellow (2), brown (8).
56. Decumbent-head scale color (OCCSCCOL): golden (3), green (4), blue-green (5), blue (6), brassy (7), brown (8), magenta (9), purple (10).
57. Postalar callus scale color (POSTALSC): white/silver (1), green (4), blue-green (5), blue (6), brassy (7), brown (8), magenta (9), purple (10).
58. Postpronotum, dorsal scale color (DORPPNSC): white/silver (1), green (4), blue-green (5), blue (6), brassy (7), brown (8), magenta (9), purple (10).
59. Postpronotum, ventral scale color (VENPPNSC): white/silver (1), yellow (2).
60. Proboscis, light scale banding (PROBBAND): absent (0), present (1).
61. Pupal seta 2-CT (2CBRANCH): number of branches.
62. Pupal seta 3-CT (3CBRANCH): number of branches.
63. Pupal seta 6-I length (6-ILN): measured from socket to tip.
64. Pupal seta 6-II length (6-IILN): measured from socket to tip.
65. Pupal seta 6-III length (6-IIILN): measured from socket to tip.
66. Pupal seta 6-IV length (6-IVLN): measured from socket to tip.
67. Pupal seta 6-V length (6-VLN): measured from socket to tip.
68. Pupal seta 6-VI length (6-VILN): measured from socket to tip.
69. Pupal seta 6-VII length (6-VIILN): measured from socket to tip.
70. Pupal seta 5-VII length (5-VIILN): measured from socket to tip.
71. Pupal body pigmentation (BODYPIGM): light (1), medium (2), dark (3).
72. Pupal paddle width (PADDWIDT): measured at widest point.
73. Pupal seta 8-CT length (8-CTLN): measured from socket to tip.
74. Pupal seta 1-II length (1-IILN): measured from socket to tip.
75. Pupal seta 1-III length (1-IIILN): measured from socket to tip.
76. Pupal seta 1-IV length (1-IVLN): measured from socket to tip.
77. Pupal seta 1-V length (1-VLN): measured from socket to tip.
78. Pupal seta 1-VI length (1-VILN): measured from socket to tip.
79. Pupal seta 1-VII length (1-VIILN): measured from socket to tip.

Appendix III. List of characters, including measurements, counts and qualitative ordered states. Females. Measurements and degree of light scaling determined by counts of ocular micrometer units.

1. Antennal length (ANTLENGT): measured from base of flagellomere I to tip of flagellomere XIII.
2. Maxillary palpus length (PALPLENG): measured from base to tip.
3.-13. Same as male characters 3-13.
14. Tarsomere 5 of proleg, banding (PTAR5): % light scaling.
15. - 23. Same as male characters 14.-21.
24. Tarsomere 5 of hindleg, banding (HTAR5): % light scaling.
25. Abdominal transverse crossbanding on tergum II (TRBD2): absent (0), white/silver (1), yellow (2), green (4), blue-green (5).
26. Abdominal transverse crossbanding on tergum III (TRBD3): absent (0), white/silver (1), yellow (2), green (4), blue-green (5).
27. Abdominal transverse crossbanding on tergum IV (TRBD4): absent (0), white/silver (1), yellow (2).
28. Abdominal transverse crossbanding on tergum V (TRBD5): absent (0), white/silver (1), yellow (2), golden (3).
29. Abdominal transverse crossbanding on tergum VI (TRBD6): absent (0), white/silver (1), yellow (2), golden (3).
30. Abdominal transverse crossbanding on tergum VII (TRBD7): absent (0), white/silver (1), yellow (2), golden (3).
31.-36. Same as male characters 28.-33.
37.-43. Same as male characters 35.-41.
44. Tergum VI, caudal tuft color (CAUDTUF6): absent (0), white (1.10), Yellow (1.20), black and white (2.10), black and yellow (2.20), black and orange (2.30).
45. Tergum VII, caudal tuft color (CAUDTUF7): absent (0), orange (1.30), black (1.40), black and orange (2.30).
46. Tergum VIII, caudal tuft color (CAUDTUF8): absent (0), yellow (1.20), orange (1.30), black (1.40).
47. Postalar callus scale color (POSTALSC): yellow (2), green (4), blue-green (5), blue (6), brassy (7), purple (10).
48. Decumbent head scale color (OCCSCCOL): yellow (2), golden (3), green (4), blue-green (5), blue (6), brassy (7), magenta (9), purple (10).
49. Coxa of proleg, seta color (COXLPILE): white/silver (1), yellow (2), golden (3), brown (8), black (11).
50.-56. Same as male characters 48.-54.
57. Postpronotum, dorsal scale color (DORPPNSC): white/silver (1), green (4), blue-green (5), blue (6), brassy (7), brown (8), magenta (9), purple (10).
58. Postpronotum, ventral scale color VENPPNSC): white/silver (1), yellow (2), green (4).
59.-77. Same as male characters 61.-79.

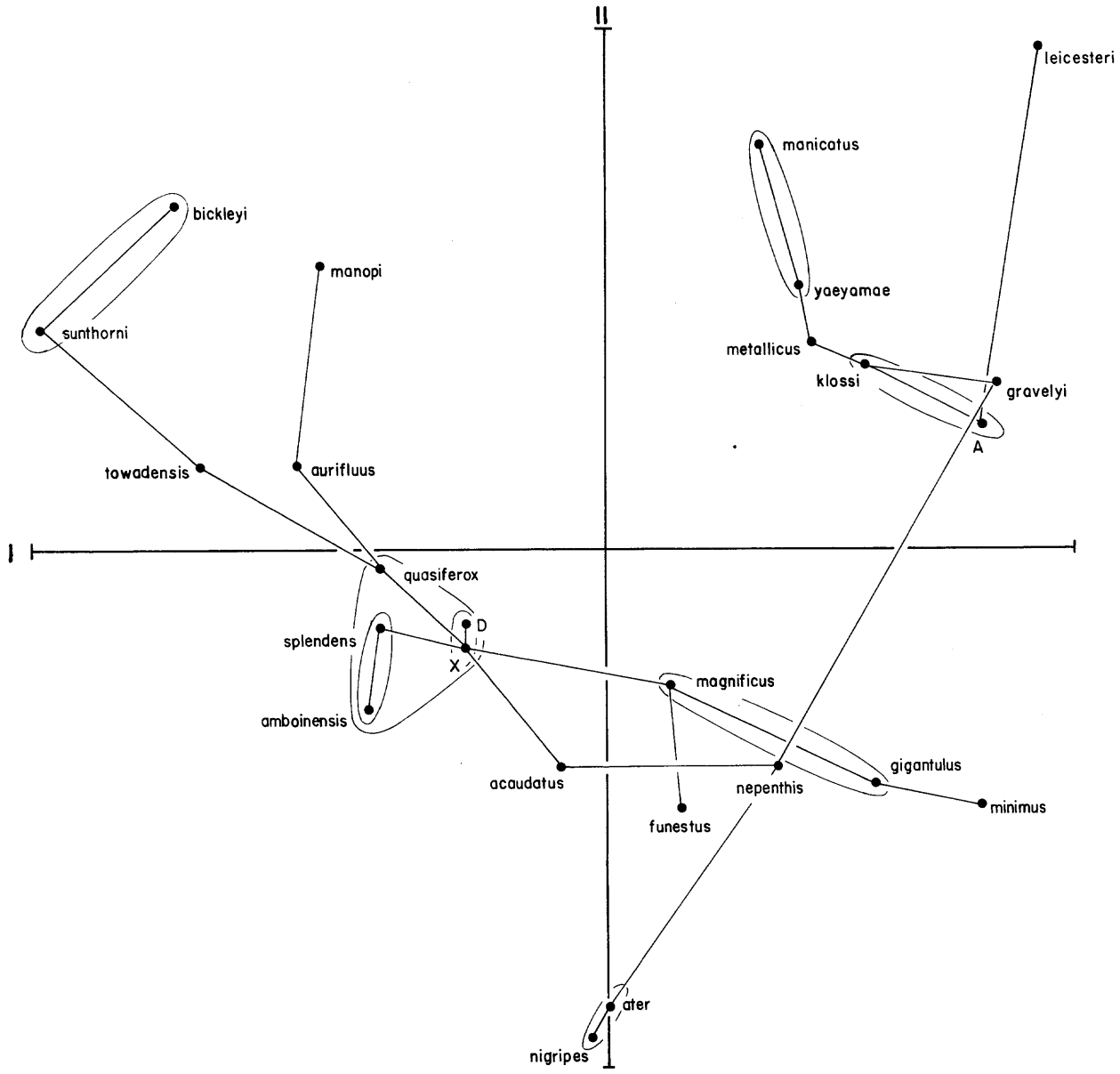

Fig. 1. Ordination diagram of taxonomic distances between males of 25 species of *Toxorhynchites*, based on 79 characters from adults and pupae. Taxonomic distances, minimum spanning network and closest relatives (subsets) are shown (Rohlf et al. 1974). Placement of species is by nonmetric multidimensional scaling using an initial configuration derived from principal component analysis of character correlations. Matrix r = 0.950, stress = 0.242.

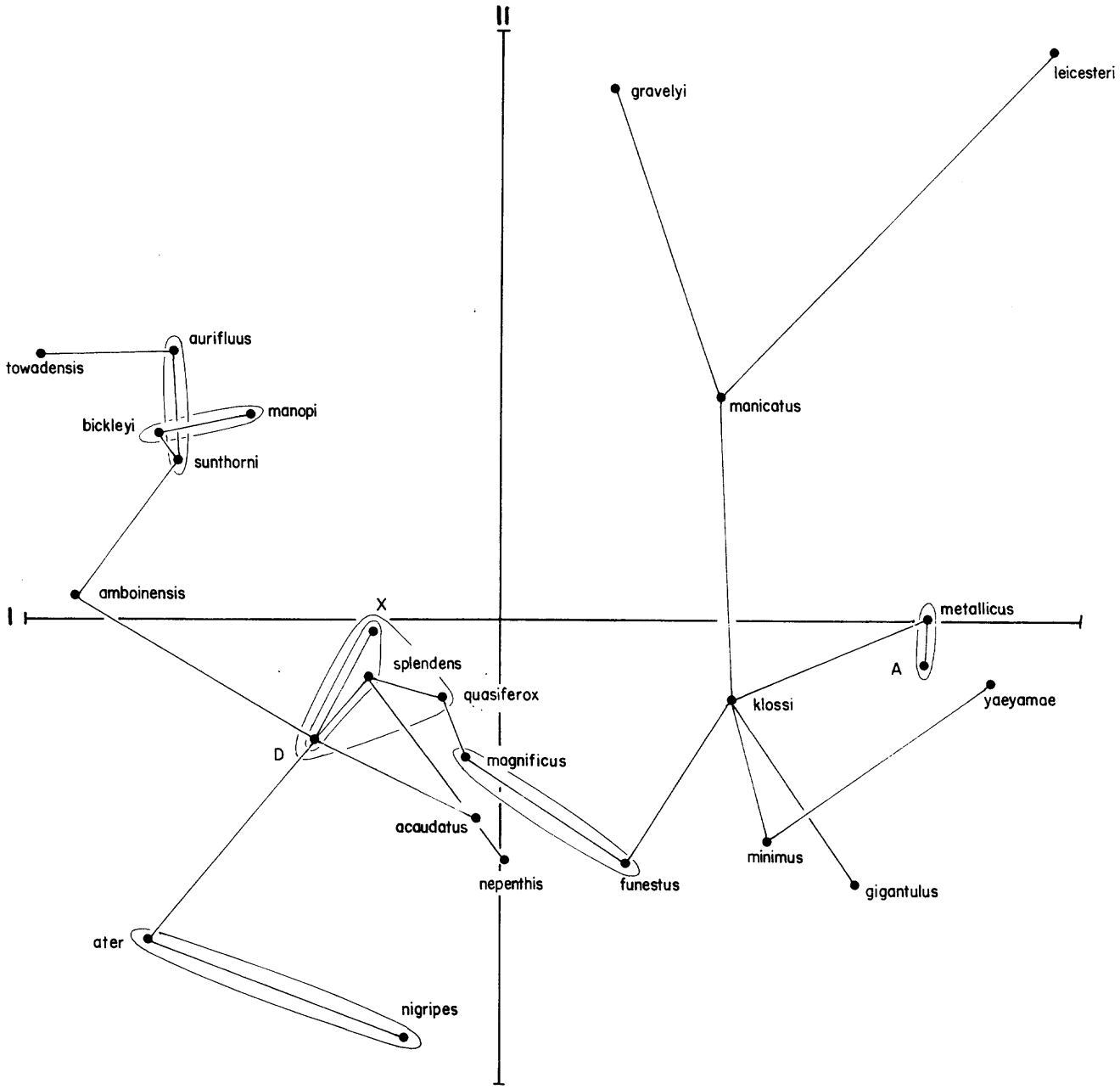

Fig. 2. Ordination diagram of taxonomic distances between females of 25 species of *Toxorhynchites*, based on 77 characters from adults and pupae. Representation as in Fig. 1. Matrix r = 0.930, stress = 0.283.

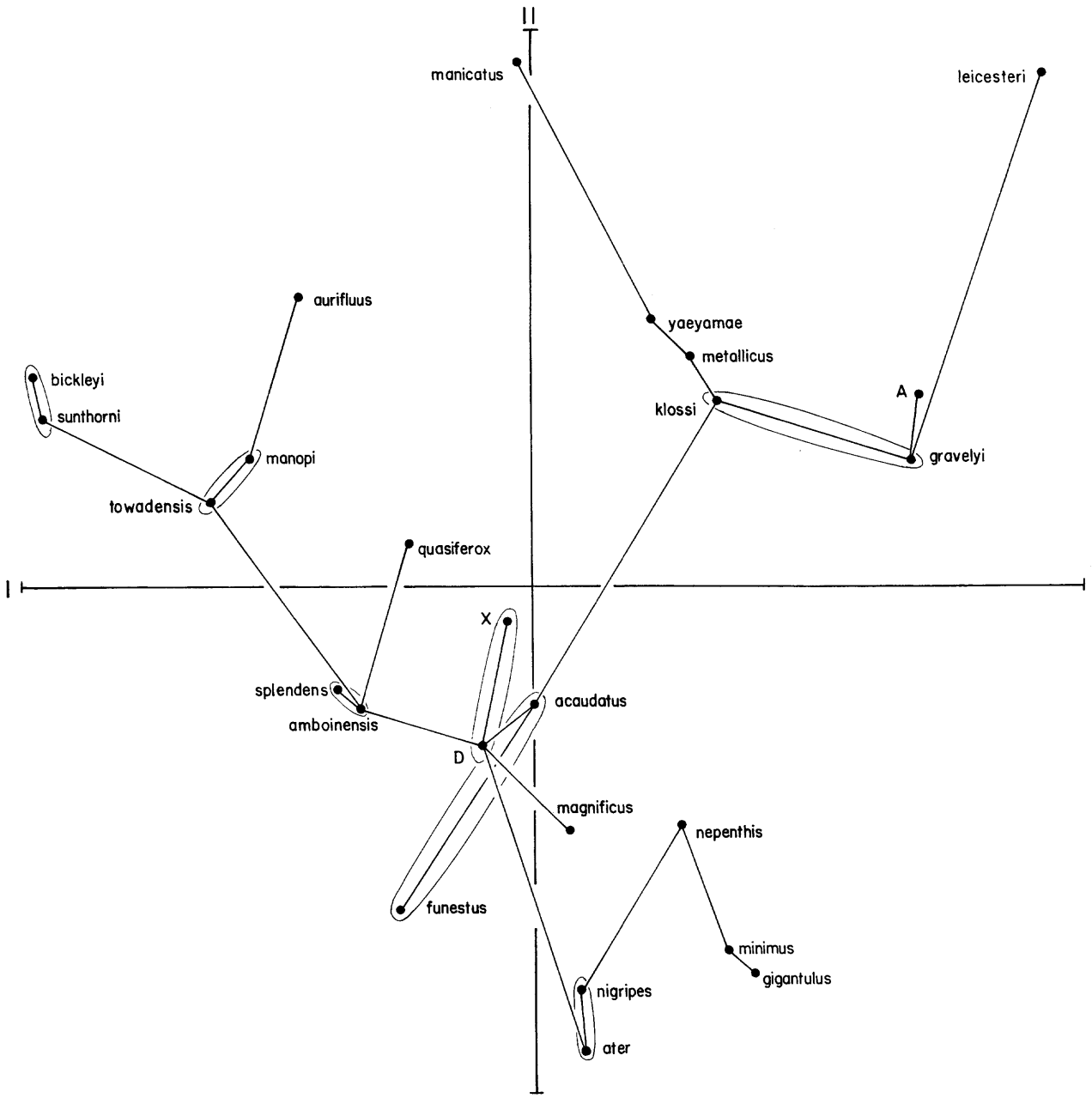

Fig. 3. Ordination diagram of taxonomic distances between males of 25 species of *Toxorhynchites* based on 54 characters from adults and pupae. Representation as in Fig. 1. Matrix r = 0.943, stress = 0.249.

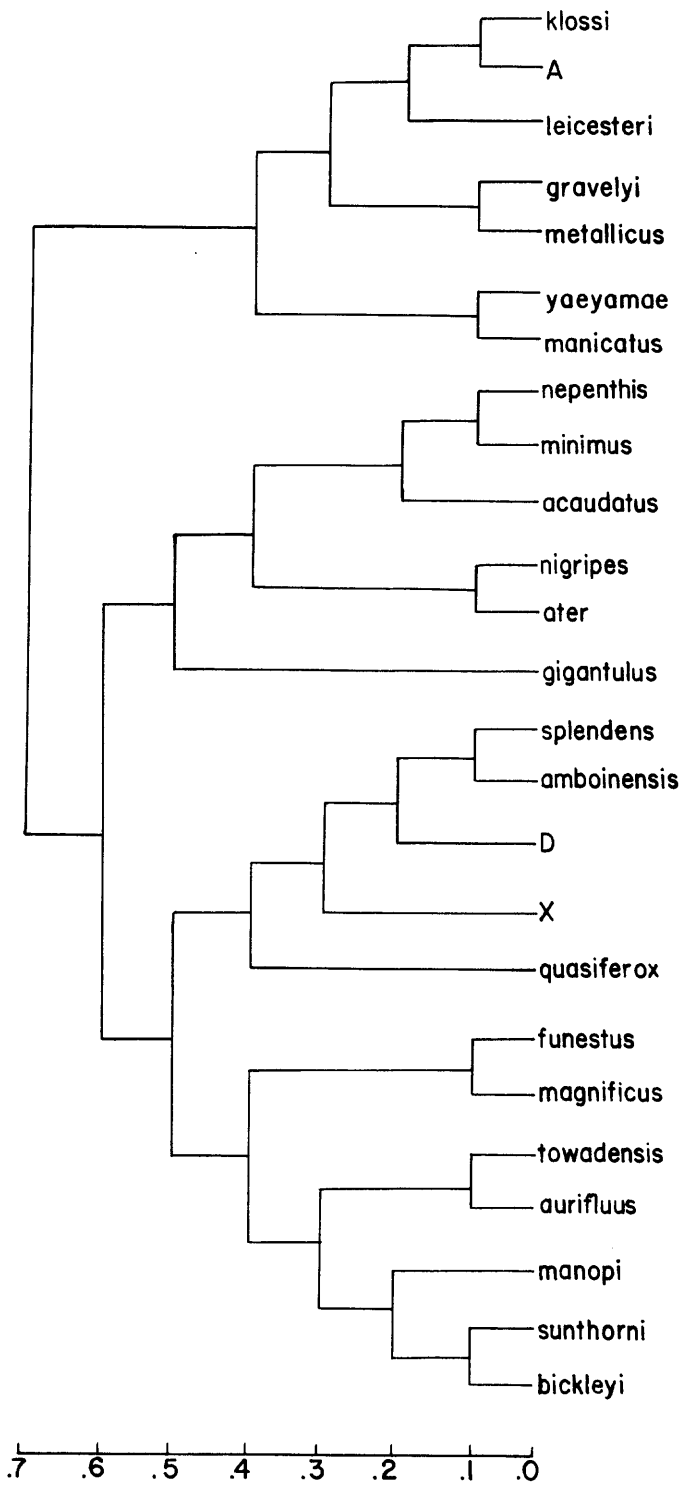

Fig. 4. Phenogram based on a matrix of intuitively-assigned dissimilarities between species of *Toxorhynchites*.