

COMMENTS ON THE SYSTEMATIC STATUS OF VAMPIRE BATS (FAMILY DESMODONTIDAE)

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Abstract

Immunologic analyses of serum proteins, studies of karyotypes, and morphology of spermatozoa reveal that vampire bats (family Desmodontidae) are more closely related to members of the family Phyllostomatidae than is suggested by conventional morphological characters. Immunologic tests show *Desmodus* to be related to the Phyllostomatidae through the subfamilies Phyllostomatinae and Glossophaginae. When fundamental and diploid numbers of chromosomes are plotted, two monotypic desmodontid genera (*Desmodus* and *Diaemus*) have karyotypic values that fall in the area of highest concentration of phyllostomatids. Spermatozoa of *Desmodus* and the third monotypic desmodontid genus, *Diphylla*, are indistinguishable in general morphology from those of representatives of five subfamilies of phyllostomatids. It is suggested that the vampires may represent only a subfamily of the Phyllostomatidae.

Adaptations of bats to specialized feeding habits are reflected by changes in dental, cranial, and other gross morphological features. In some cases, the resultant modifications have been extensive, making it necessary to employ other than conventional morphological criteria to determine phylogenetic relationships. The three monotypic genera (*Desmodus*, *Diaemus*, and *Diphylla*) of vampire bats comprising the New World family Desmodontidae have undergone extreme modifications associated with their sanguineous food habits, and these structural adaptations have been the basis for assignment of vampire bats to a distinct family. However, recently acquired evidence suggests that vampires are much more closely related to members of the New World family Phyllostomatidae than implied by the current classification of bats.

Machado-Allison (1967) noted host-ectoparasite relationships that closely allied *Desmodus rotundus* with the Phyllostomatidae and suggested that a re-evaluation of the status of desmodontids might be in order. Immunologic and electrophoretic analysis, karyotype studies, and comparison of sperm morphology also indicate that desmodontids are closely allied with the Phyllostomatidae. The findings recorded below further suggest that a reappraisal of the familial status of Desmodontidae is neces-

sary. Of the studies here reported, Gerber is responsible for the serological work, Baker for the karyotypic analysis, and Forman for the comments on sperm morphology. Specimens mentioned by Gerber and Forman are deposited in the Museum of Natural History at The University of Kansas; those recorded by Baker are housed in the collections at Texas Technological College or the University of Arizona.

IMMUNOLOGIC AND ELECTROPHORETIC COMPARISONS

Serum samples from 25 species of New World bats, representing six families, were compared by immunoelectrophoresis and two-dimensional, micro-Ouchterlony immunodiffusion tests. The 25 species were studied using antiserum prepared against the sera of 7 species representing 4 families. In a second series of tests, sera from 16 species, including 15 of Phyllostomatidae and 1 of Desmodontidae, were studied using antisera prepared against the sera of 10 species of Phyllostomatidae and antiserum against the serum of *Desmodus rotundus*.

Bats were bled by cardiac puncture, the sera separated by centrifugation, and preserved by freezing to -15°C or by adding "Merthiolate" to inhibit bacterial growth. Antisera against whole bat sera were prepared in rabbits.

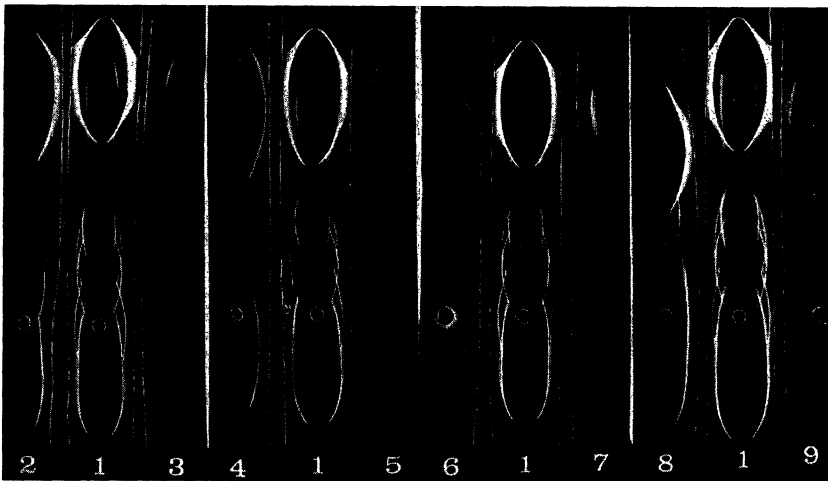


FIG. 1.—Immunoelectropherograms of reactions of anti-*Desmodus rotundus* antiserum with sera of five families of bats: phyllostomatid sera—*Sturnia lilium* (no. 2), *Glossophaga soricina* (no. 4), *Phyllostomus discolor* (no. 8); emballonurid serum—*Saccopteryx bilineata* (no. 3); vespertilionid sera—*Plecotus townsendii* (no. 5), *Myotis velifer* (no. 6); noctilionid serum—*Noctilio leporinus* (no. 7); molossid serum—*Tadarida brasiliensis* (no. 9). The reference antigen, *Desmodus* serum (no. 1), is in the center of each slide, and the anode is toward the top. Note that all families, except the Phyllostomatidae, give a weak cross-reaction with anti-*Desmodus rotundus* antiserum.

Protein-nitrogen concentrations for immunoelectrophoresis tests were determined by using the Aloe-Hitachi hand protein-refractometer. Protein-nitrogen determinations for immunodiffusion tests, where concentration is critical, were made using the Dittebrandt modification of the Biuret method and the Beckman 151 Spectro-Colorimeter, which is specially designed for micro-samples (0.1 ml).

Micro-immunoelectrophoresis was carried out in Michalis buffer, pH 8.7, for 50 minutes at 40 volts (Fig. 1). Equal amounts of standardized serum (1% protein per ml) were added to each well. For micro-Ouchterlony immunodiffusion tests, all serum samples were standardized to 400 μ g protein-nitrogen/ml. To the antigen well, 8 lambda of serum were added; to the antiserum well, 16 lambda of antiserum were added in two equal portions. Diffusion of the reactants in both the immunoelectrophoresis and immunodiffusion tests was allowed to proceed for 20 to 24 hours after which the slides were washed in three changes of borate-buffered saline for three

days, rinsed in distilled water for one day, and allowed to dry. Precipitin arcs were stained with Amido-Schwartz dye. The unbound stain was removed from agar on the slides by two washes in acid-alcohol (70% ethanol, 1% acetic acid) and one wash in distilled water. To prevent cracking and peeling of the agar, slides were soaked 30 minutes in a 2% glycerol solution.

Immunoelectrophoretic relationships were demonstrated in two ways: (1) by comparing the number of arcs in the cross-reaction with those in the reference reaction, and (2) by considering the differences in density of the arcs, assigning a value of five if the arcs in the reference and cross-reaction were the same, and a value of four if the corresponding cross-reacting arc was less dense than the reference arc. Summated values for the cross-reactions were divided by the summated values for the reference-reactions and multiplied by 100 to give a per cent immunological correspondence.

The zones of precipitate of immunodiffusion tests were scanned by using transmitted light in a Joyce-Loebl densitometer

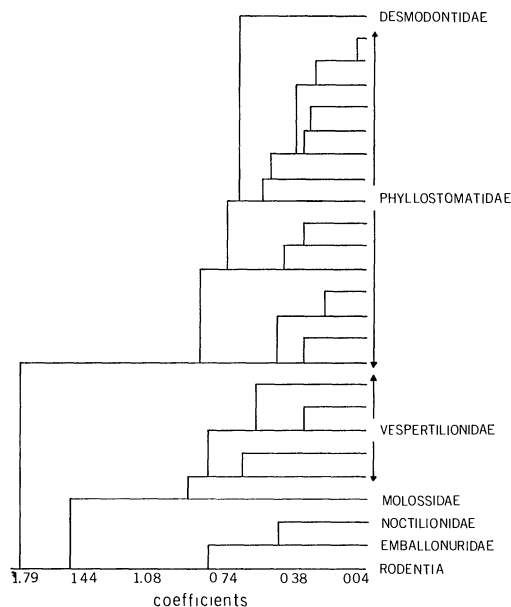


FIG. 2.—Phenogram representing distance coefficients of similarity of sera from six families of Chiroptera and a rodent, *Neotoma floridana*. The lowest distance coefficient represents the closest relationship. Note that *Desmodus* has a high affinity for species of Phyllostomatidae.

(40° cam, sensitivity 2.5 to 3.0) to obtain a quantitative evaluation of the relative similarities of the cross-reacting sera to the reference serum.

Both immunoelectrophoretic and immunodiffusion data were analyzed on a GE 625 computer using programs for multivariate analysis devised by Rohlf, Kishpaugh, and Bartcher at The University of Kansas. The results of the “classical numerical taxonomy” program were expressed as distance coefficients, and a cluster analysis was performed. Phenograms were constructed from the distance coefficients, illustrating immunological relationships among bats (Figs. 2 and 3). A factor analysis was performed on the data, and a three-dimensional configuration of relationships was constructed (Fig. 4).

Fig. 1 shows immunoelectrophoresis tests illustrative of the 175 analyzed. The reference reaction is *Desmodus rotundus* and the cross-reacting sera represent five addi-

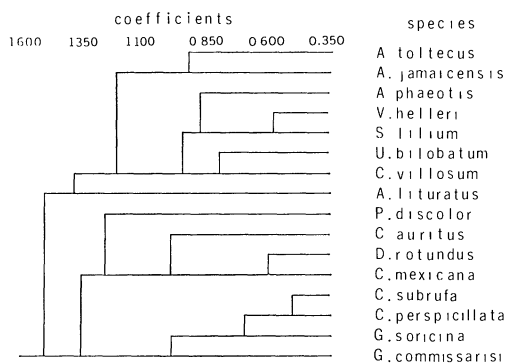


FIG. 3.—Phenogram representing distance coefficients of immunological affinities of sera from 15 phyllostomatids and *Desmodus rotundus*. The lowest distance coefficient represents the closest relationship.

tional families of Chiroptera. The three phyllostomatids had a high degree of cross-reactivity with *Desmodus rotundus*, whereas the sera from species of Noctilionidae, Molossidae, Emballonuridae, and Vespertilionidae had little cross-reactivity. Distance coefficients, computed using all of the immunoelectrophoresis data suggest to us that *Desmodus rotundus* is more closely related to some members of the Phyllostomatidae than are certain phyllostomatids to each other.

Interfamilial affinities based on immunodiffusion tests can be seen in Figs. 2 and 4. Except for *Desmodus rotundus*, all the families studied showed a marked immunologic separation from the phyllostomatids. To analyze more precisely the affinities of *Desmodus* and the phyllostomatids, sera from 15 species of Phyllostomatidae and *Desmodus* were compared using antisera prepared against the sera of 10 species representing five subfamilies of Phyllostomatidae and *Desmodus*. Again *Desmodus rotundus* showed a high immunological affinity for the phyllostomatids, being more similar to some species of phyllostomatids than other phyllostomatids are to each other (Fig. 3). Distance coefficients within the Phyllostomatidae ranged from 0.500 to 1.520. *Desmodus* showed a distance coefficient of 0.600 to *Choeronycteris mexicana*,

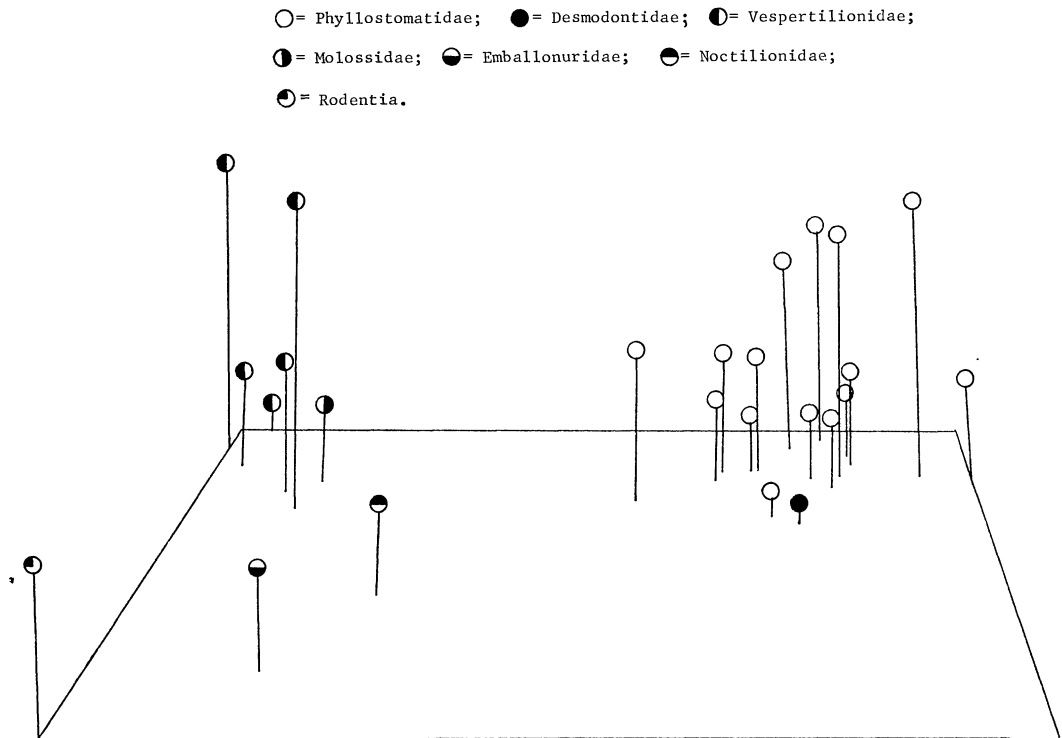


FIG. 4.—Three-dimensional plot illustrating immunological affinities of 25 species of six families of bats studied.

1.00 to *Chrotopterus auritus*, and 1.275 to *Phyllostomus discolor*. Distance coefficients for the immunoelectrophoresis data ranged from 0.475 to 1.575. *Desmodus* was definitely within this range having a distance coefficient of 0.825.

ANALYSIS OF KARYOTYPES

The rate of chromosomal change in bats, based on the amount of variation found within genera and between closely related genera, seems to be low (Baker, 1967; Baker and Patton, 1967; Hsu, Baker, and Utakoji, 1968). For this reason, similarities in chromosome morphology deserve serious consideration as possibly being the result of close phylogenetic relationships. The chromosomes of specimens of *Desmodus rotundus* ($2N = 28$) from Veracruz, Mexico, were illustrated by Hsu and Benirschke (1967). In this study, additional specimens

of *Desmodus* have been examined from Oaxtepec, Morelos (two males, two females), Ojo de Agua del Rio Atayac, Veracruz (two females), 42 km west of Cintalapa, Chiapas (three males), and Guayaguayare, Trinidad (one male, three females). The chromosomes of these individuals were indistinguishable from those illustrated by Hsu and Benirschke. The chromosomes of a female from Guayaguayare, Trinidad (Fig. 5) show only one autosome has an obvious nucleolus organizer (see arrow), the same thing being found in some specimens from all localities studied.

The fundamental number (FN) of *Desmodus* is 52 (see Baker and Patton, 1967). All autosomes are banded and, except for one medium-sized pair of submetacentrics, are metacentrics or submetacentrics. The X chromosome is the largest submetacentric.

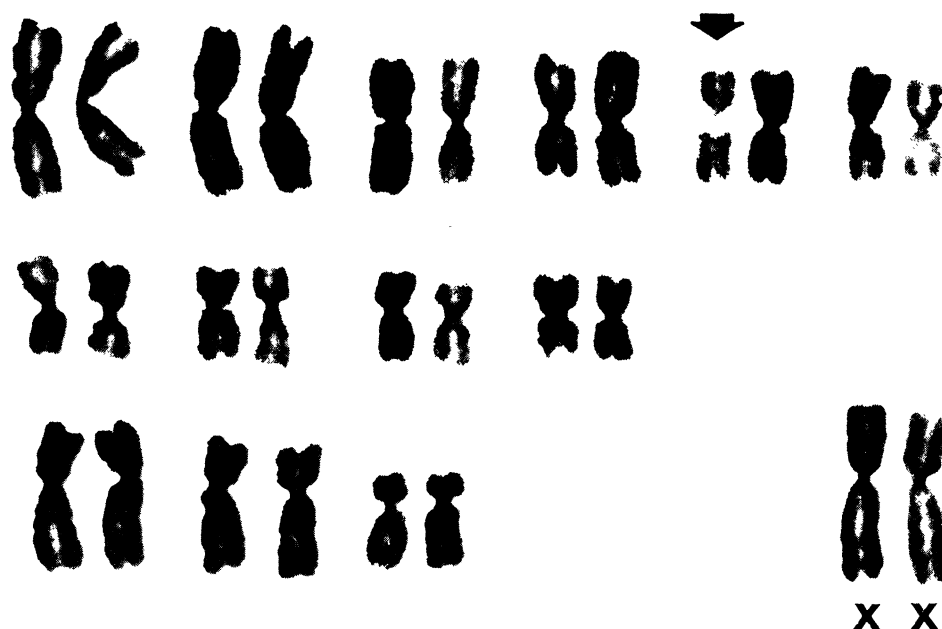


FIG. 5.—Representative karyotype of a female *Desmodus rotundus* from Guayaguayare, Trinidad (TT 6502).

The Y chromosome usually appears as a minute acrocentric; however, in one Trinidadian specimen it had a biarmed appearance.

Four specimens of *Diaemus youngi* ($2N = 32$, $FN = 60$, Fig. 6) were examined. Chromosomes were biarmed and, except for one pair of medium-sized subtelocentrics, all were metacentric or submetacentric in nature. The X chromosome was a large submetacentric and the Y chromosome a minute acrocentric. The smallest pair of autosomes had a nucleolus organizer on the longest arm near the centromere.

Karyotypic data are available for two of the three known species of desmodontids. The diploid number varies between the two species by four, and the fundamental number varies by eight. An analysis of karyotypic variation at various taxonomic levels has been made for two families of bats, Phyllostomatidae (Baker, 1967) and Vespertilionidae (Baker and Patton, 1967). In both these families karyotypic variation between closely related genera was found

to be both greater and less than that illustrated by the two desmodontids, although the degree of variation in fundamental number usually was less.

In the family Vespertilionidae (33 species examined) the diploid number varies from 20 to 50, and the fundamental number varies from 28 to 56 (Baker and Patton, 1967; Capanna and Civitelli, 1964). In the family Phyllostomatidae (22 species examined) the diploid number varies from 16 to 46 and the fundamental number from 26 to 68 (Baker, 1967). Although there is broad overlap between the two families in both diploid number and fundamental number, when the two are plotted against each other little overlap occurs (see Fig. 7). Because diploid number limits the fundamental numbers possible, the degree of separation of the plotted values for the two families is significant.

The values of the phyllostomatid *Uroderma bilobatum* ($2N = 44$, $FN = 50$) fall in the area occupied by most members of the Vespertilionidae; the values of the

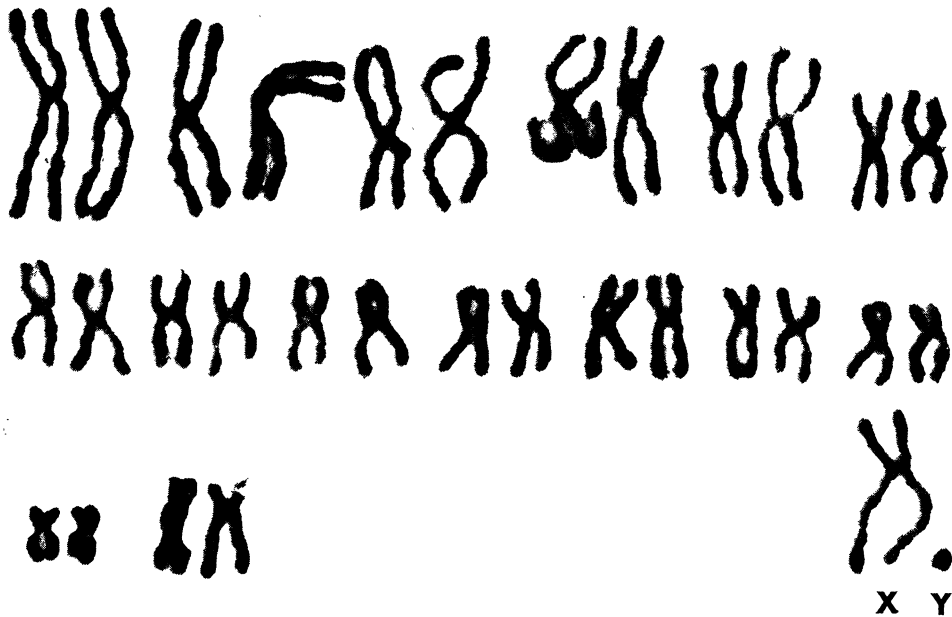


FIG. 6.—Representative karyotype of a male *Diaemus youngi* from Las Cueveus, Trinidad (TT 5411).

vespertilionid *Pipistrellus subflavus* ($2N = 30$, $FN = 56$) fall in the area of most members of Phyllostomatidae. Vespertilionidae is characterized by relatively lower fundamental numbers and higher diploid numbers than Phyllostomatidae. Values plotted for the few members of Rhinolophidae that have been studied (Dulic, 1967; Capanna and Civitelli, 1964) do not fall in the area of either family because of their high fundamental and diploid numbers.

Desmodus and especially *Diaemus* have karyotypic values that fall in the area of the highest phyllostomatid concentration. These data imply that the desmodontids are more closely related to the Phyllostomatidae than to the other two families for which information is available. In fact, the values for *Desmodus* and *Diaemus* are well within the variation already known for the Phyllostomatidae, and vampires share with species of the latter group the chromosomal characteristics of relatively high fundamental number and lower diploid number.

MORPHOLOGY OF SPERMATOZOA

Morphology of spermatozoa has proved useful in systematic studies of the Chiroptera (Forman, 1968). The following account compares gross morphology of spermatozoa of *Desmodus rotundus* and *Diphylla ecaudata* to that of selected representatives of three other families of North American bats.

Testes were taken from freshly killed males and preserved in a propio-alcohol fixative. Smears were prepared by smashing a short section of tubule on a slide and staining the sperm with a lacto-phenol cotton blue stain. Measurements (in microns) were taken from photographs on which 1.082 mm equaled 1 micron. Two specimens of *Desmodus rotundus murinus* and two specimens of *Diphylla ecaudata*, all from Nicaragua, were examined and are described below.

Desmodus rotundus.—**Head** (measurements based on 20 spermatozoa) with apex narrowly rounded but blunt, generally egg-shaped or ovate; tapering abruptly in lateral

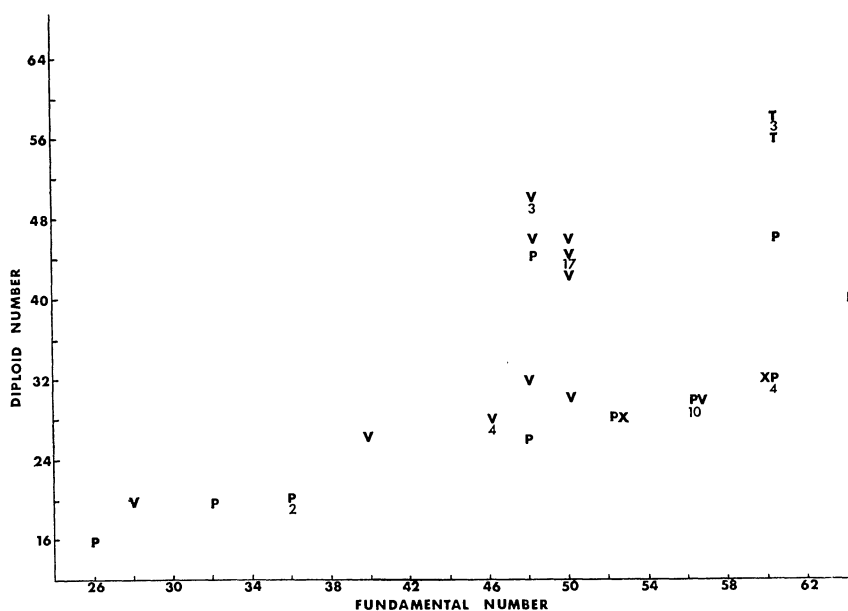


FIG. 7.—Chromosomal values (diploid number plotted against fundamental number) for species of four families of bats: Vespertilionidae (V), Phyllostomatidae (P), Desmodontidae (X), and Rhinolophidae (T). Numbers below symbols indicate number of species at that coordinate.

view to a fine point at apex; base symmetrical and concave; length 4.66 (range 4.44–4.81), width 3.27 (3.19–3.38), depth 0.97 (0.92–1.02). **Neck** region not observed, indistinct or absent. **Midpiece** (measurements based on 15 spermatozoa) tapering posteriorly; junction with head indistinct but recognizable neck region probably absent; demarcation with tail distinct in dorsal and ventral view; length 11.71 (11.23–12.29), width 0.84 (0.83–0.88). **Tail** tapers gradually to narrow filament; length about 78 in four measurements.

Diphylla ecaudata.—**Head** (measurements based on 20 spermatozoa) rounded with blunt but rounded apex, generally more circular than *Desmodus rotundus*; broad in basal half; tapering abruptly to fine point at apex in lateral view, sometimes with slight curvature; base concave, appearing symmetrical; length 5.10 (4.90–5.27), width 4.06 (3.88–4.15), depth 0.53 (0.51–0.54). **Neck** region not observed; midpiece appears continuous with base of head. **Midpiece** (measurements based on

10 spermatozoa) tapering posteriorly although sides nearly parallel in anterior two-thirds; not centrally attached to head; helical configuration observed throughout length; length 9.77 (9.18–10.64), width 0.79 (0.75–0.83). **Tail** not observed as complete.

The sperms of *Desmodus rotundus* (Fig. 8A) and *Diphylla ecaudata* (Fig. 8B) are similar in general morphology and dimensions of head and midpiece to spermatozoa of representative species of five subfamilies of the Phyllostomatidae previously examined (Forman, 1968). The head of *Desmodus rotundus* appears slightly more compressed laterally, and the midpiece somewhat longer than in sperms of *Phyllostomus*, *Glossophaga*, *Anoura*, *Carollia*, *Sturnira*, and *Artibeus* (Fig. 8C–G), but is otherwise indistinguishable from them in gross structure.

The sperm of *Diphylla ecaudata* is similar to phyllostomatids previously examined, differing only in point of attachment of the midpiece to the head. In *Diphylla*, the midpiece is not centrally attached. Point

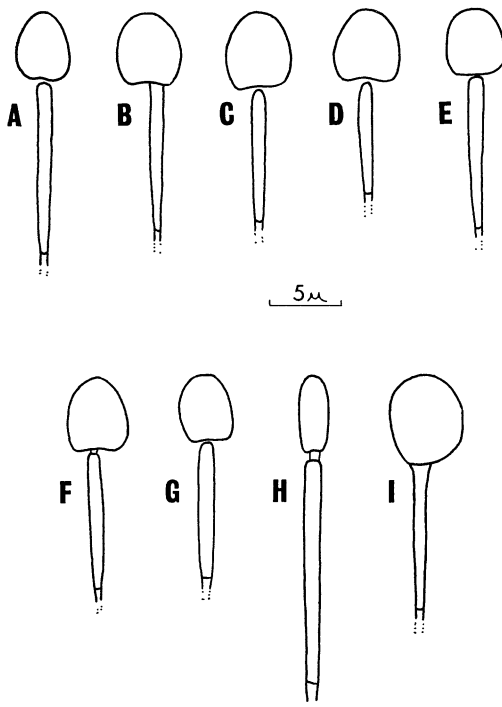


FIG. 8.—Spermatozoa of selected New World bats. **A**, *Desmodus rotundus* (KU 106263); **B**, *Diphylla ecaudata* (KU 115129); (**C-G**, Phyllostomatidae) **C**, *Phyllostomus discolor*; **D**, *Anoura cultrata*; **E**, *Sturnira ludovici*; **F**, *Carollia castanea*; **G**, *Artibeus jamaicensis*; **H**, *Myotis volans* (Vespertilionidae); **I**, *Molossus molossus* (Molossidae). Note that the midpieces of some spermatozoa are shown without connection to the heads. Although the anterior limits of the midpieces were demonstrable, the exact nature of the midpiece-head connection could not be resolved in these species.

of attachment of head to midpiece of spermatozoa is variable within families of mammals as illustrated by Friend (1936) and Hughes (1965), although this condition has not previously been demonstrated in Chiroptera.

Examination of sperms of other families of North American bats (e.g., Vespertilionidae and Molossidae) clearly reveals familial distinctness in general morphology (Fig. 8H–I) far exceeding the differences that separate *Desmodus rotundus* and *Diphylla ecaudata* from species of the Phyllostomatidae.

SUMMARY

Immunologic and electrophoretic tests show *Desmodus* to be related to the Phyllostomatidae through the subfamilies Phyllostomatinae and Glossophaginae. The glossophagine serum for which *Desmodus* serum shows the highest affinity is that of *Choeronycteris mexicana*. The phyllostomatine serum for which *Desmodus* serum shows the highest affinity is that of *Chrotopterus auritus*. The remaining four families studied show a distinct immunological separation from the Phyllostomatidae.

Karyotypic data are yet so meager for Chiroptera that much additional work needs to be done before relationships can be properly analyzed. Nonetheless, it can be stated that the available karyotypic data generally support, and in no way refute, the assignment of the vampire bats as a subfamily of the Phyllostomatidae. Three subfamilies of phyllostomatids (Phyllostomatinae, Glossophaginae, Stenoderminae) have members with karyotypic values similar to those of *Desmodus* and *Diaemus*. From chromosome studies, a relationship of vampires to one of these three subfamilies appears likely.

In addition, spermatozoan morphology shows *Desmodus rotundus* and *Diphylla ecaudata* to be notably similar in general structure to the phyllostomatids examined, when compared to the morphological distinctness of spermatozoa of North American representatives of two other families of bats. The sperms of *Desmodus* and *Diphylla* resemble those of *Artibeus*, *Phyllostomus*, *Anoura* and other genera representing five subfamilies of the Phyllostomatidae to such a degree that the familial distinctness of the Desmodontidae appears questionable. Evidence from immunologic and karyotypic comparisons and studies of morphology of spermatozoa suggests that vampire bats should be classified as a subfamily within the Phyllostomatidae.

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