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Geographic variation in the frog genus *Vanzolinius* (Anura: Leptodactylidae)

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Abstract.—*Vanzolinius discodactylus,* a forest-dwelling frog species of western Amazonia in South America, varies in characters of color pattern, morphology, and advertisement call. Analysis of this variation indicates that very local (site) differentiation results in mosaic patterns of differentiation, largely obfuscating larger geographic patterns. Comparison of available genetic estimates of differentiation for *V. discodactylus* are consistent with the morphologically and advertisement call-based conclusions. A previously studied forest-dwelling lizard and another forest-dwelling frog also demonstrate local differentiation patterns suggesting that the variation in *V. discodactylus* may represent a general pattern for forest-dwelling amphibians and reptiles in Amazonia.

During examination of specimens for a study of *Leptodactylus* species (Heyer 1994), several *Vanzolinius* specimens were encountered. Dr. Claude Gascon found *Vanzolinius* to be relatively common along the Rio Juruá in Brazil and used the species to test the riverine barrier hypothesis (Gascon et al. 1996). A cursory examination of these additional materials suggested that there was considerable variation, which might profitably be studied. The purpose of this paper is to analyze geographic variation in *Vanzolinius.*

Materials and Methods

As many adults, larvae, and recordings of advertisement calls as possible were assembled from major museum collections (Appendix 1).

The sex of individuals was determined either by examination of vocal slits, or dissection to examine gonads. The following categories are used: adult male—vocal slits present; juvenile male—testes present, but vocal slits not broken through; adult female—oviduct folded at least in part; juvenile female—ovaries present, but oviduct straight; juvenile—condition of gonads indeterminate (in some cases, gonads had been removed by previous workers).

Analyses differ depending on the type of data gathered for the characters examined. The following descriptions of characters are arranged by analytical groups.

Color patterns and external morphological features of adult form individuals.—These qualitative traits are categories recorded as either binary or multistate characters. For the latter, states were added to the series as they were encountered during the data-taking phase. The states within each series have no intended or implied relationships or transformation series. Intermediate conditions between states were recorded with the first letter of the state that most nearly approached the condition observed in the specimen examined.

Dorsal snout pattern: Three basic states were encountered: a relatively uniform dark pattern (Fig. 1A); a variegated pattern (Fig. 1B); and a uniform light pattern (Fig. 1C).

Light postorbital eye stripe: A series of symbols define the distinctiveness of the postorbital eye stripes: − [absent]; (+) [indistinct]; + [distinct]; +! [sharply defined].

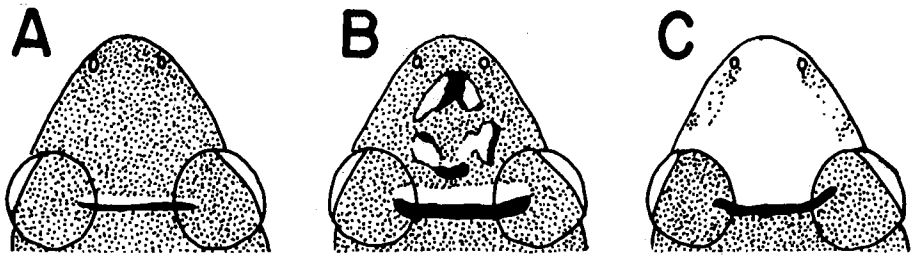

Fig. 1. Dorsal snout pattern standards.

In cases where the two sides of the head differed, both conditions were recorded.

Light subocular bar: The distinctiveness of the bar was noted by the same symbols as for the previous character, except the +! category was not encountered.

Dorsal pattern: Dorsal pattern variation forms a continuum among the more distinctive states recorded. The states recognized are: State A—either an uniform dorsum (brown or tan) or indeterminately blotched (Fig. 2A); State B—the dorsum with very distinct dark markings in the interorbital and interscapular areas (Fig. 2B); State C—distinct interorbital blotch, well defined chevron markings anteriorly and blotches posteriorly on the body (Fig. 2C); State C-1—as previous state except chevrons continuous; State D—a distinct darker straight edged band extending from behind eyes on full extent of dorsum (Fig. 2D); State D-1—as previous state, except sides irregular.

Dark mid-dorsal pin stripe: An interrupted dark mid-dorsal pin stripe was recorded as either present or absent.

Throat and chest pattern.—Variation in this character is continuous among the states encountered: State A—variegated pattern (Fig. 3A); State A-1—as previous state, but light; State B—uniform light pattern (Fig. 3B); State B-1—as previous state, but lateral portions darker; State C—dark speckled pattern (Fig. 3C); State C-1—as previous state, but dark spotting more extensive; State D—dark pattern (Fig. 3D); State E—dark pattern with light spots (Fig. 3E).

Belly pattern: Variation in this character is continuous among the states encountered:

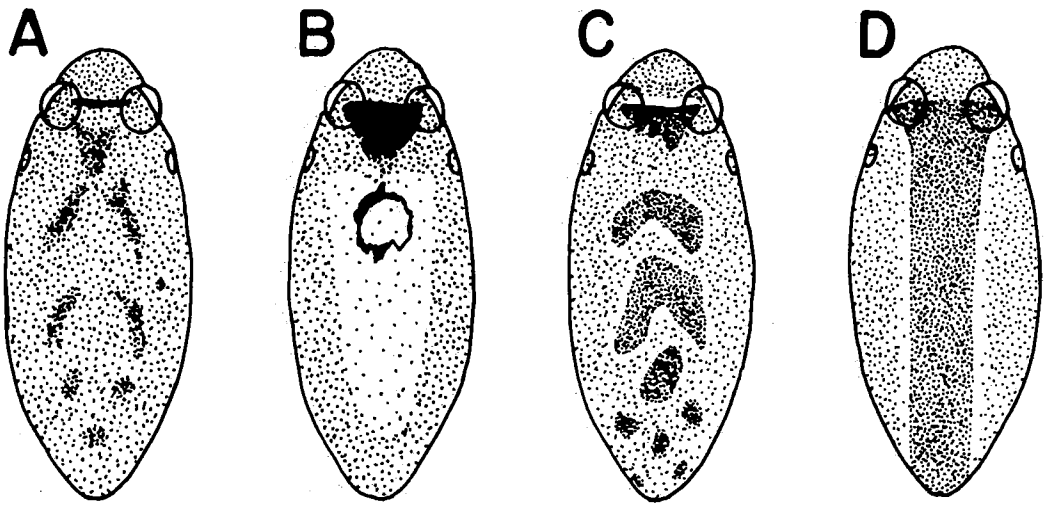

Fig. 2. Dorsal pattern standards.

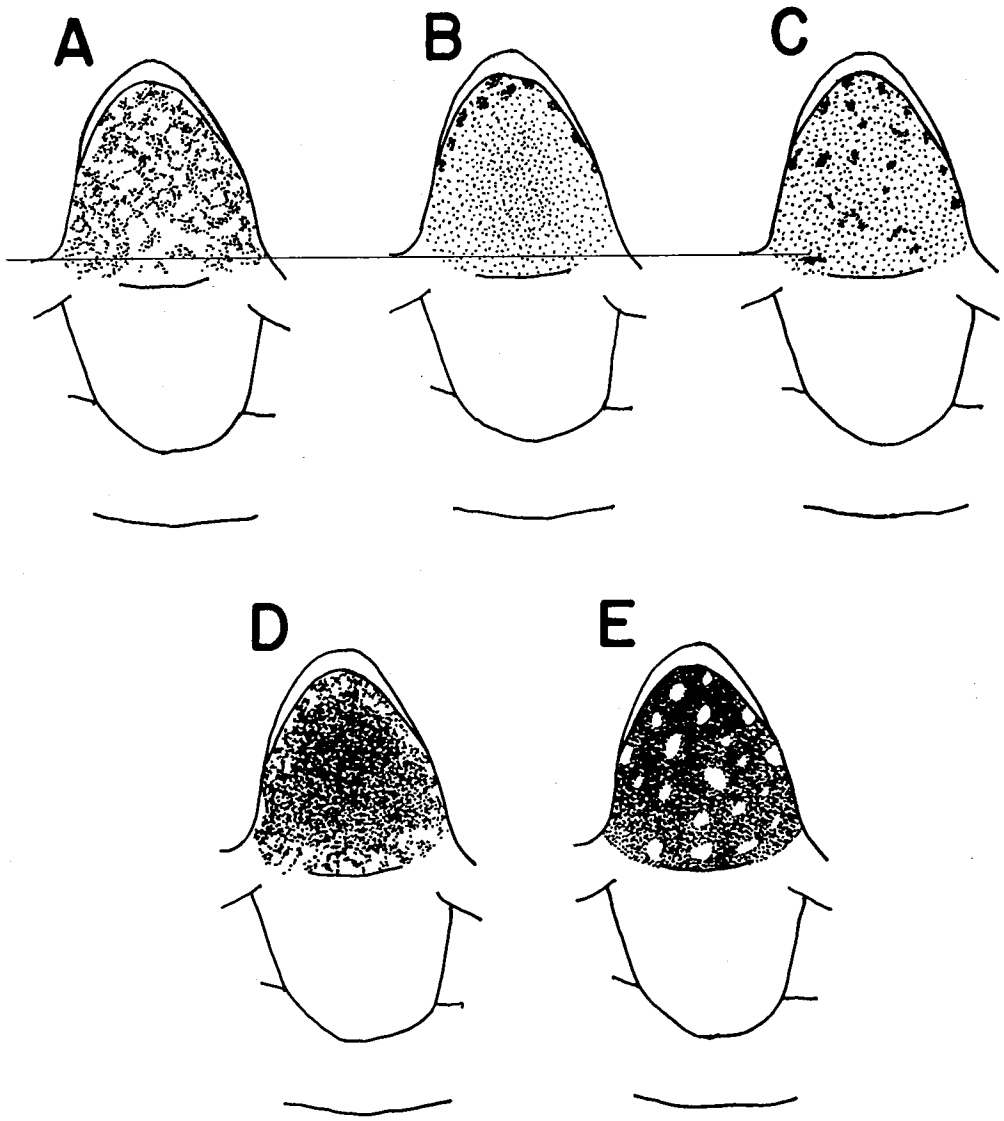

Fig. 3. Throat and chest pattern standards.

State A—speckled pattern (Fig. 4A); State A-1—almost uniform cream pattern; State B—indistinctly mottled, more intense anteriorly (Fig. 4B); State B-1, as previous state, but lighter; State C—distinctly mottled, rather uniform over belly (Fig. 4C); State C-1—as previous state, but lighter; State C-2—as State C but dark anteriorly and no melanophores posteriorly; State D—distinctly variegated dark and light pattern (Fig. 4D).

Posterior thigh pattern: Variation in this character is continuous among the states encountered: State A—indistinctly mottled (Fig. 5A); State B—indistinctly mottled with indistinct dark longitudinal band (Fig. 5B); State C—distinctly mottled (Fig. 5C); State D—speckled with indistinct dark longitudinal band (Fig. 5D); State E—speckled with distinct dark longitudinal band (Fig. 5E); State F—speckled with dark longitudinal band bordered above by light longi-

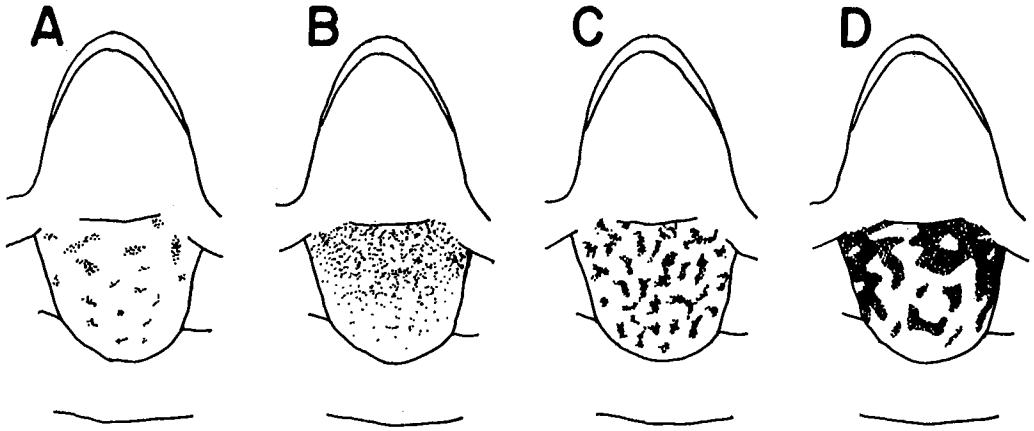

Fig. 4. Belly pattern standards.

tudinal stripe (Fig. 5F); State F!—as for State F except light stripe very distinct.

Outer tarsal pattern: Data were taken on the distinctiveness of the outer tarsal pattern relative to the dorsal tarsal pattern. However, variation turned out to be minimal and scoring could not be done consistently. These data are not analyzed further.

Dorsolateral fold condition: There is relatively little variation in this character and the variation that exists is difficult to evaluate in terms of the impact preservation has on recognition of fold condition. Most individuals have no dorsolateral folds. In a few individuals, a short ridge or elongated warts lie in the dorsolateral fold region posterior to the eye. The variation in this character is not analyzed further.

Male secondary sexual characters: All males lack secondary sexual characters of thumb or chest pads or spines or male arm hypertrophy as found in *Leptodactylus*.

Male vocal sac: Variation in this character is minimal and difficult to evaluate in terms of preservation artifact. In most males, the vocal sac is single and internal; in a few males, the single vocal sac has external indications of weak lateral folds. Variation of this character is not analyzed further.

Textures: Data were taken on textures of the dorsum, the upper shank, the outer tarsus, and sole of foot. In all cases the degree of development of shagreen and tubercles was difficult to categorize consistently and differentiate from preservation artifact. The

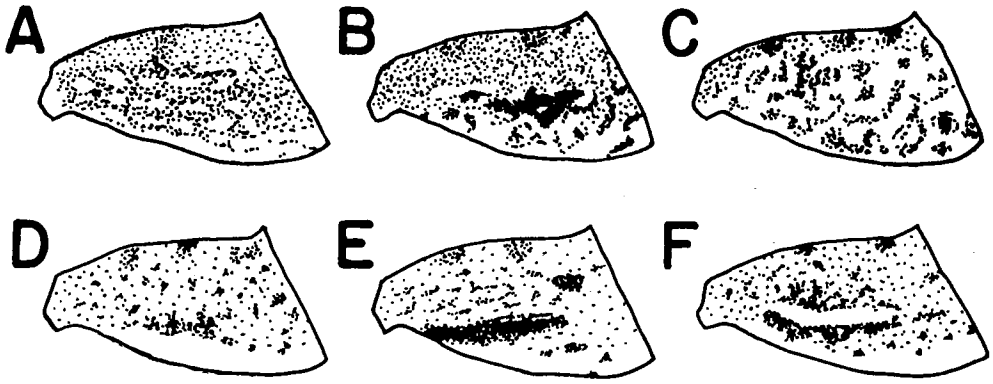

Fig. 5. Posterior thigh pattern standards.

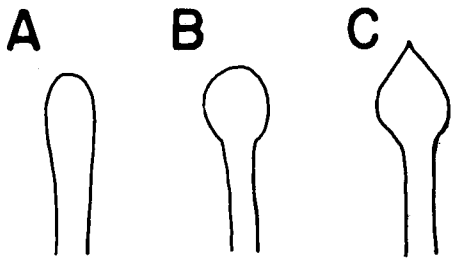

Fig. 6. Digit tip dorsal outline standards.

dorsum is tuberculate, with either small black-tipped or white-tipped tubercles, usually more densely packed posteriorly. The dorsum may also be somewhat granular, have a shagreen, or be smooth. The upper shank and outer tarsus consistently have tubercles, black and/or white tipped, and the surfaces may also be shagreened. The foot is either smooth or the outer margin has a few black/white tipped tubercles and/or a shagreen. Variation for these characters is not analyzed further.

Finger tip dorsal outline: For both finger and toe tip shapes, the outline shapes standardized by Savage (1987) for *Eleutherodactylus* were used. For both finger and toe tip dorsal outlines, only three of Savage's (1987) shapes were encountered corresponding to his unexpanded even (Fig. 6A), expanded even (Fig. 6B), and expanded pointed or lanceolate (Fig. 6C) states. The third finger tip is the most expanded and is the digit from which data were taken. Very little variation was encountered, suggesting that observer variation in interpreting shape was probably as large as actual variation. The conditions recorded ranged from not expanded, just A, A, A-B, or A-C. Variation is not analyzed further for this character.

Toe tip dorsal outline: The same standards were used for toe tips as for finger tips (Fig. 6). Data were recorded for both the third and fourth toe tips. The conditions for the third and fourth toe dorsal outlines are very similar. For 165 individuals, the conditions are identical, for 9 individuals the fourth toe dorsal outline is perceptibly more expanded than for the third, and for 75 individuals, the third toe dorsal outline is perceptibly more expanded than the fourth. As the third toe tip is a bit more expanded in the total sample, data are analyzed only for the third toe tip.

Dorsal toe grooves: The dorsal surfaces of the toes typically have from 2–5 grooves involving the epidermis and dermis. The grooves are almost parallel to each other along the long axis of the toe, but radiate slightly outward from the proximal toe tip to the distal tip. Usually the grooves extend almost the entire length of the expanded portion of the toe tip, but the grooves do not reach the tip of the toe. Counting the exact number of grooves is not always precise as the grooves are sometimes incomplete and preservation artifact can obscure the definition of the grooves. Data were taken for both the third and fourth toes. As for the dorsal outline, the conditions within individuals are similar, but typically the third toe has one more groove than the fourth. For 96 individuals, the third and fourth toes have the same number of grooves, for 18 individuals, the fourth toe has more grooves than the third, and for 132 individuals the third toe has more grooves than the fourth. Because the raw data indicate that the variation in the fourth toe mirrors that seen in the third, only the data for the third toe are analyzed further.

Analysis of preceding characters: The preceding characters are recorded as discrete entities even though variation is mainly continuous. Because the data are discrete, chi-square analyses are used to determine whether occurrence frequencies of states differ significantly. The 0.05 convention is used to determine significance. Data were adjusted, when necessary, to reach a minimum cell size of an average expected frequency of 5 (Hayek 1994:239). Data were first examined to determine whether states for adult males and females differed significantly. If they did not, then data recorded for juveniles were added to both the male and female data to provide more robust data sets for statistical analysis among geograph-

ic regions (see definitions of regions below).

Measurement data and analyses.—Measurements were taken on the following variables, as defined by Gascon et al. (1996): snout-vent length (SVL), nostril separation, eye width anterior, eye width posterior, head width, head length, eye to nostril distance, thigh length (=femur length of Gascon et al. 1996), shank length (=tibia length of Gascon et al. 1996), foot length, tympanum diameter (=tympanum height of Gascon et al. 1996), eye length, maximum width of third finger, and maximum width of fourth toe. Measurements were taken with a Helios dial calipers and recorded to the nearest 0.1 mm.

Only adult specimens are used for the measurement data analyses. As males and females are sexually dimorphic in size, they are analyzed separately (L. C. Hayek, C. Gascon, and W. R. Heyer (unpublished data) discuss multivariate analyses on morphometric data on *Vanzolinius*.) The data are analyzed using the software program SYSTAT 5 (Wilkinson et al. 1992).

Larval data.—To my knowledge, the only larvae available are those reported on by Duellman (1978) from a single locality in Ecuador (Mera, Pastaza). Specimens KU 121362–121363 are larvae ranging from Gosner stages 30–38. Specimens KU 121360–121361 are just metamorphosed individuals. There is no internal evidence from study of these specimens to either establish that they are *Vanzolinius* or they are not. Dr. John Lynch collected the specimens and informs me (pers. comm.) after he consulted his field notes, " . . . it appears that I guessed on the identification . . . on the basis of habitat selection. Hence, don't trust the identification." As nothing can be determined about geographic variation based on these specimens (even assuming they are *Vanzolinius discodactylus*), larvae are not treated further in this paper.

Advertisement call data and analyses.—Recordings of single individuals from five localities are available for analysis.

Brazil: Acre; Nova Vida, Rio Juruá, USNM Tape 256 Cut 12. Recorded at 1900 h on 17 March 1992 by Claude Gascon at a temperature of 25°C, no voucher specimen.

Brazil: Amazonas; Altamira, Rio Juruá, USNM Tape 255 Cut 2. Recorded at 1915 h on 17 November 1991 by Claude Gascon at a temperature of 25°C, voucher INPA 5021.

Brazil: Amazonas; Barro Vermelho, Rio Juruá, USNM Tape 254 Cut 5. Recorded at 1900 h on 27 October 1991 by Claude Gascon at a temperature of 24.4°C, voucher INPA 3352.

Brazil: Amazonas; Jainú, Rio Juruá, USNM Tape 254 Cut 13. Recorded at 1740 h on 2 November 1991 by Claude Gascon at a temperature of 26.1°C, no voucher specimen.

Ecuador: Napo; Limoncocha, USNM Tape 18 Cut 1. Recorded at 2000–2034 h on 9 July 1971 by Ronald Heyer at an air temperature of 23.4°C, water temperature 23.6°C, voucher LACM 92001.

Calls were analyzed using Canary 1.2 software (Charif et al. 1995) on a Power Macintosh 8500 computer. Calls were digitized for analysis at a sample rate of 22050 Hz and a sample size of 16 bits. Call rate was determined directly from recordings for periods ranging from 45 to 180 s per recording. Other call parameters were taken from a combination of waveform, audiospectrogram (=spectrogram as used in Canary manual), and spectrum analyses based on ten calls for each individual. Most of the recordings had considerable noise. Many parameters were taken from filtered calls. The filter around option was used for determining some parameters for USNM Tape 256 Cut 12 (filtered around 520–5000 Hz), USNM Tape 255 Cut 2 (filtered around 500–5000 Hz), USNM Tape 254 Cut 5 (filtered around 330–4000 Hz), and USNM Tape 18 Cut 1 (filtered around 500–4500 Hz).

Definition of geographic areas for analysis.—The primary purpose of this study is

to determine the nature of geographic variation found within *Vanzolinius discodactylus*. Sample sizes are insufficient to analyze the data from each locality independently. Localities were plotted on a map and localities were grouped on the basis of geographic proximity (Fig. 7). The rationale used for grouping localities involved trying to maximize three criteria simultaneously: to have as many groups as possible in order to characterize geographic variation; to have as many individuals as possible in each group to permit robust statistical analyses; and to maintain geographic integrity. With respect to geographic integrity, a rule of thumb of keeping localities within the same major river drainage basins was generally applied (Areas A, B, C, D, F, G, H), but not exclusively so (Area E). Initially 10 geographic area groupings were made. When the data were examined for these groupings to see if they were sufficient, three of the groups lacked sufficient data for analysis. Two Colombian localities were sufficiently isolated from each other as well as other samples to be placed in their own groups; unfortunately, the specimens from both localities are faded such that data are incomplete for them. Thus, data for the Colombian localities of Caldas; Villa María and Caquetá; Florencia, are not included in the geographic analyses (Fig. 7, upper two squares). A single Peruvian locality (Ucayali, Yarinacocha) also formed a distinct geographic group by itself and contains one faded specimen, unsuitable for further geographic analysis (Fig. 7, lower square). Eight geographic groupings remain and are identified by letter in further discussion: (A) northern Amazonian Ecuador; (B) southern Amazonian Ecuador; (C) Amazonian Peru; (D) the Brazil-Colombia border region; (E) easternmost known localities for *Vanzolinius* in Amazonian Brazil; (F) the mid-region of the Rio Juruá of Brazil; (G) the upper region of the Rio Juruá in the Brazilian State of Amazonas; and (H) the upper region of the Rio Juruá in the Brazilian State of Acre.

As there are but five individuals available from Region D, (1 female, 1 male, 3 juveniles), Region D data are omitted from the analyses by areas, unless otherwise noted.

Results

Dorsal snout pattern.—This was the only character for which some individuals clearly demonstrated two states (this exception involved only conditions B and C both occurring in the same individual), suggesting partial independent genetic control of this character. The states (Appendix 2) were collapsed for analysis to three: (1) pure A, (2) any B, and (3) any C. Because several individuals had both states (2) and (3), the total number of state conditions analyzed exceeds the number of individuals examined for this character only.

The chi-square analysis by sex was not significant ($\chi^2 = 1.35$; $df = 2$; $P = 0.50 > 0.30$). Thus, male, female, and juvenile data were combined to analyze by geographic area. The chi-square analysis by geographic area is significant ($\chi^2 = 69.92$; $df = 12$; $P < 0.001$). In partitioning the results, regions A+B are distinct from regions E+F+G+H. Region C is not distinct from either of the other two area groupings.

Light postorbital eye stripe.—For individuals having different states on either side of the head, the more distinctive state was scored for statistical analysis (e.g., an individual recorded as having state (+) on one side of the head and + on the other was treated as having the + state for statistical analysis). The chi-square analysis by sex was significant ($\chi^2 = 6.43$; $df = 2$; $P = 0.05 > 0.02$); therefore the variation among geographic areas has to be analyzed separately by sex. For females, the chi-square analysis by geographic area is significant ($\chi^2 = 42.43$; $df = 12$; $P < 0.001$). In partitioning the significance, regions B+E are distinct from regions A+C+F+G+H. In order to meet the minimum expected cell size criterion for statistical robustness for males, the data had to be collapsed by rec-

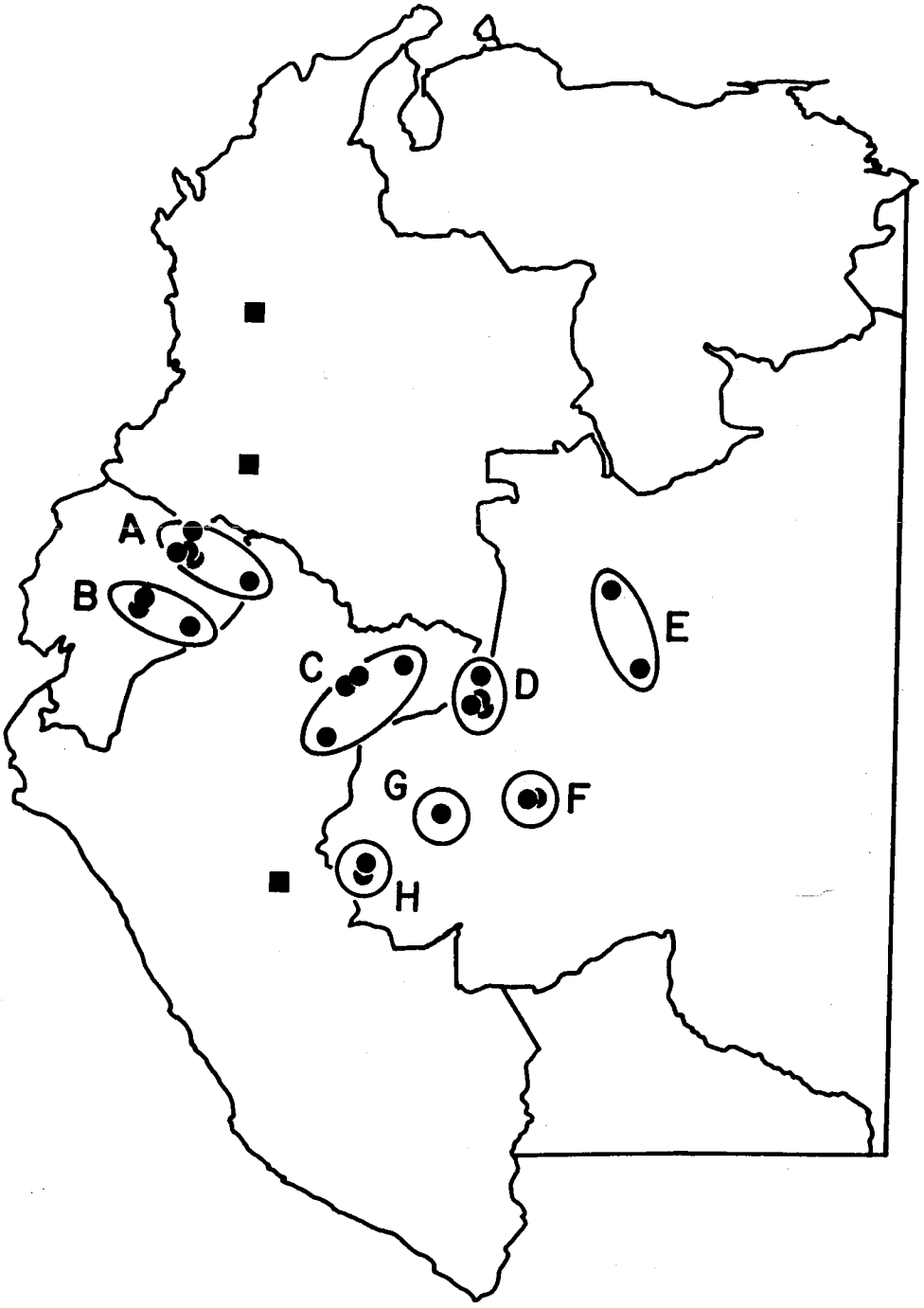

Fig. 7. Map of northwestern South America showing known distribution of *Vanzolinius discodactylus*. Guyana and Brazil are truncated by the 60°W meridian; Bolivia and Brazil by the 15°S parallel. Squares are single localities excluded from analysis of geographic variation. Circles and ellipses labelled A–H indicate groupings of localities (dots) used for analysis of geographic variation. A dot may represent more than one locality.