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Taxonomy and Ecology of *Dendrobates bombetes*, a New Andean Poison Frog With New Skin Toxins

CHARLES W. MYERS¹ AND JOHN W. DALY²

ABSTRACT

Dendrobates bombetes, new species, is a small, red-striped frog inhabiting the western Andes near Cali, Colombia. Nearest relatives are the Ecuadoran *D. abditus* and the northern Colombian *D. opisthomelas*; these three Andean species are considered a monophyletic unit of the "minutus group" because of a larval synapomorphy. The name *D. reticulatus* is resurrected from the synonymy of *D. quinquevittatus* for a small frog occurring sympatrically with *quinquevittatus* in Amazonian Peru.

Dendrobates bombetes was found in two forest types at localities separated by 30 km. distance and 800 m. elevation. Differences in population structure suggest the possibility that either reproductive success or juvenile survivorship may be inversely density dependent. Cool montane forest islands supported dense, presumably stable populations having few juveniles and a high proportion of large (old?) adults. Marginal habitat in relatively xeric gallery forest supported a small population having significantly more juveniles and smaller (younger?) adults, suggesting rapid turnover in a precarious habitat. One or two tadpoles

were carried by male nurse frogs, but free-living larvae were not found.

The call is a short, surprisingly loud and far-carrying, insect-like buzz influenced by ambient temperature. Rising temperature causes pulse rate to increase and call length to decrease; the second effect probably reinforces the first, since there seems to be an independent tendency for short calls to be pulsed faster than long ones. The call of a related species, *Dendrobates opisthomelas*, differs even at the same temperature in duration, pulse rate, and dominant frequency.

Defensive skin secretions of *Dendrobates bombetes* contained 22 piperidine alkaloids in the two sampled populations, with 15 or 17 compounds each. Interpopulational variation is partly due to minor differences in degree of saturation of some compounds, and the gas chromatographic profiles are therefore much alike even though the shared-alkaloid value is low (67%). Three new alkaloids form at least a natural subgroup in the pumiliotoxin-A class, to which they are tentatively assigned in spite of anomalous mass spectra; a fourth new alkaloid is placed in the pumiliotoxin-C class.

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INTRODUCTION

Some time ago we became aware of the existence of a small, undescribed poison frog that inhabits part of the Andes near Cali, Colombia. The first specimens had been taken in 1971 by Stephen R. Edwards and Jorge E. Ramos at Quebrada de la Chapa, a small tributary of the Río Dagua on the road to Buenaventura. When visiting the locality in 1974, we were astonished to see that it lies in one of the curious dry areas that punctuate the rain-drenched western slopes of the Cordillera Occidental; there was only a scrubby gallery forest, conspicuously fringed with columnar cacti. To find *Dendrobates* living in such circumstances on the Pacific side of Colombia was unexpected, even though we had collected a few other species in drier situations in southern Ecuador and along the Venezuelan Orinoco. Still, most species of this group are inhabitants of wet forest and so we gazed past the head of Quebrada de la Chapa at distant wooded uplands (fig. 6), and wondered whether we were sampling an outlying frog population that might have originated from those higher and wetter slopes. We never made that climb, but it became unnecessary when a few years later our colleague Eva Bartels de Bernal discovered the frogs on a mountain above Lago de Calima, a man-made lake some little distance past the high slopes that first roused our curiosity. Dr. Bartels guided us to the new locality, where the little frogs were abundant and



FIG. 1. In natural size, a "large" adult specimen of *Dendrobates bombetes*, new species, from the type locality.

where we were able to observe aspects of their behavior and to obtain additional samples of their defensive skin secretions, which contain several novel alkaloids.

In naming the species, we have chosen to call attention to the relatively loud, insect-like buzzes emitted by calling males. The specific epithet, a noun in apposition, is from the Greek *βομβητης* and means a "buzzer." The Latinized word should be stressed on its second syllable, whose vowel, being derived from the Greek eta, is long (pronounced bom *bā'* tes):

***Dendrobates bombetes*, new species**

Figures 1–5, 9A, 10A, 10B, 11

Dendrobates sp., Daly et al., 1978, p. 183 (skin toxins from Quebrada de la Chapa population).

HOLOTYPE: AMNH 102601 (field no. CWM 13774), an adult female obtained by Eva Bartels de Bernal, John W. Daly, and Charles W. Myers on November 21, 1976, in mountains above south side of Lago de Calima, 1580–1600 meters elevation, about 2 km., airline southwest of Puente Tierra (village), Department of Valle del Cauca, Colombia. The locality is roughly 50 km. north of Cali, on the mountain above kilometer post 23 on the present Loboguerrero-Buga road (about 3° 52' N, 76° 25' W).

PARATYPES: A total of 107 specimens, all from Department of Valle del Cauca, Colombia, as follows: AMNH 102602–102651, 102652–102656 (male nurse frogs with one or two tadpoles each), 102657–102670 (skinned carcasses), 102671–102676 (cleared and stained), collected at the type locality by Myers, Daly, and Bartels de Bernal on November 21, 1976, and by Myers and Daly on December 4, 1976. AMNH 89384–89391, 102677–102683 (skinned carcasses), KU 139644–139660, from Quebrada de la Chapa, 670–780 meters, 1 km. W Loboguerrero, collected by S. R. Edwards and J. E. Ramos (KU specimens) on April 21, 1971 and by Myers and Daly on February 21, 1974.

DEFINITION AND DIAGNOSIS: A small

dendrobatid having an adult snout to vent length of about 16–20 mm. Body black or brown, anteriorly with nonmetallic red or orange dorsolateral stripes that are posteriorly somewhat convergent and often ending by midbody; ventral surfaces mottled black on pale green, blue-green, or yellow; lacking concealed flash marks. Teeth absent; appressed first finger shorter ($\sim\frac{3}{4}$) than second; third finger disc of adults about 1.4–2.2 times wider than finger, largest in males. Piperidine skin alkaloids present, including pumiliotoxin-A and -C classes, but no histronicotoxins.

Dendrobates bombetes is readily distinguished from all other dendrobatids by its distinctive color pattern in combination with small size and first finger shorter than second. The smaller *D. minutus* (<16 mm. SVL) has dorsolateral stripes and a somewhat similarly patterned venter, but its stripes are yellowish or orangish gold, non-convergent, and extend the length of the body. *Dendrobates bombetes* is thought to be most closely related to *D. abditus* and *D. opisthomelas*, but the color patterns are dissimilar (see Systematics section).

MEASUREMENTS (IN MM.) OF HOLOTYPE:

The undissected holotype (fig. 2, top) is an adult female, as judged by its size and lack of vocal slits. Length from snout to vent 19.1; tibia length from heel to fold of skin on knee 7.6; greatest width of body 7.7; greatest head width (between angles of jaws) 6.1; head width between edges upper eyelids 5.5; approximate width of interorbital area 2.8; head length from tip of snout to angle of jaws 5.2; tip of snout to center of naris 0.6; center of naris to anterior corner of eye 1.6; distance between centers of nares 2.5; eye length from anterior to posterior corner 2.4; horizontal diameter of tympanum (posteriorly indistinct) about 1.4; corner of mouth to lower edge tympanic ring 0.5; length from proximal edge of large medial palmar tubercle to tip of longest (3rd) finger 4.6; width of disc of third finger 0.9; width of third finger (penultimate phalanx) below disc 0.5; width of discs of third and fourth toes both 0.7; width of third and fourth toes below discs both 0.5.



FIG. 2. *Dendrobates bombetes*, new species, showing minor variation in color pattern at type locality. From top to bottom, AMNH 102601 (holotype), 102602, 102603. Approximately $\times 3$.

TABLE 1
Size and Proportions of Adult^a *Dendrobates bombetes*, New Species, from Type Locality

| Character | N | Mean \pm 1 S.E. | S.D. | C.V.(%) | Range |
|---|-----|-------------------|-------|---------|-----------|
| Snout-vent length (SVL) in mm. ^a | 28♂ | 17.76 \pm 0.10 | 0.55 | 3.08 | 16.7-18.5 |
| | 19♀ | 18.63 \pm 0.14 | 0.59 | 3.18 | 17.2-19.8 |
| Tibia length/SVL | 28♂ | 0.424 \pm 0.003 | 0.014 | 3.29 | 0.39-0.45 |
| | 19♀ | 0.403 \pm 0.002 | 0.011 | 2.73 | 0.38-0.42 |
| Head width/SVL | 28♂ | 0.332 \pm 0.002 | 0.012 | 3.67 | 0.31-0.36 |
| | 19♀ | 0.325 \pm 0.002 | 0.008 | 2.38 | 0.31-0.34 |
| Center naris to edge eye/eye length | 28♂ | 0.724 \pm 0.007 | 0.037 | 5.17 | 0.65-0.77 |
| | 19♀ | 0.734 \pm 0.009 | 0.038 | 5.16 | 0.67-0.81 |
| Width 3rd-finger disc/ finger width below disc | 28♂ | 1.849 \pm 0.030 | 0.158 | 8.55 | 1.60-2.20 |
| | 19♀ | 1.650 \pm 0.035 | 0.154 | 9.31 | 1.40-2.00 |

^aParatopotypic juveniles excluded from table include six males (13.0-15.0 mm. SVL) that lack vocal slits, and three subadult females (16.5-16.7 mm. SVL) that have small albeit enlarging ova and oviducts.

DESCRIPTION

Size small, with maximum snout-vent length (SVL) of 19.8 mm.; females about 1 mm. longer than males in mean and maximum SVL (table 1). Widest part of head between jaw articulations, but distance between outer edges of upper eyelids nearly as great (to within 0.1 mm.) in some individuals. Head usually narrower than body, especially in gravid females; greatest head width averaging 93 percent of greatest body width in 24 adult males (range 81-109%), and 85 percent in 13 adult females (76-94%). Greatest head width averaging about 32-33 percent of SVL in adults (tables 1-2), and somewhat greater in juveniles (\bar{X} = 35.7%, range 35-37% in six paratopotypic juvenile males; \bar{X} = 34.7%, range 34-35% in three paratopotypic subadult females). Adult males with well-developed vocal slits (except that either side may fail to open in some individuals) but little or no external indication of the shallow subgular vocal sac; some females with dark creases on floor of mouth in same position as male vocal slits.

In life, top of body and limbs black or dark brown, with pair of bright red or reddish orange (rarely light orange) dorsolateral stripes arising from the similarly colored snout and extending posteriad over eyes and usually ending about midbody. Stripes less common-

ly extending back only to level of arm insertions or, contrarily, occasionally extending the full length of body; stripes not remaining parallel behind head, but becoming somewhat convergent on body. Rear of head often with a reddish blotch of same hue as dorso-lateral stripes, fused or not with the stripes; middorsum frequently with a slight suffusion of same bright color or, rarely, with faint indication of a thin, broken vertebral line. Upper lip and upper arm reddish like the body stripes, or lip pale green with some suffusion of red; small reddish or pale green spots sometimes present on anterodorsal side of thigh. Tops of hands and feet usually having a small blotch of pale green, bluish green, or pale yellow; digits light brown or grayish brown; palms and soles blackish gray. Sides of body black (even when dorsum and limbs are brown), with a few spots or bars of light yellow or greenish yellow. Ventral surfaces of head, body, and hind limbs boldly mottled or marbled black on pale green or very pale yellow or bluish green (these hues so pale as to seem white in poor light); undersides of arms mainly black with inconspicuous small spots of the pale color. Iris very dark brown in life, almost no contrast between it and pupil. Tongue and mouth lining blackish gray; flesh of freshly skinned specimens deep black. Small juveniles 12-13 mm. SVL col-

TABLE 2
Size and Proportions of Adult^a *Dendrobates bombetes*, New Species, from Quebrada de la Chapa

| Character | N | Mean \pm 1 S.E. | S.D. | C.V.(%) | Range |
|---|-----|-------------------|-------|---------|-----------|
| Snout-vent length (SVL) in mm. ^a | 4♂ | 17.00 | — | — | 16.6–17.8 |
| | 10♀ | 17.52 \pm 0.22 | 0.68 | 3.90 | 16.4–18.4 |
| Tibia length/SVL | 4♂ | 0.420 | — | — | 0.41–0.43 |
| | 10♀ | 0.416 \pm 0.004 | 0.012 | 2.82 | 0.40–0.44 |
| Head width/SVL | 3♂ | 0.320 | — | — | 0.32–0.32 |
| | 10♀ | 0.317 \pm 0.004 | 0.012 | 3.66 | 0.30–0.34 |
| Center naris to edge eye/eye length | 3♂ | 0.787 | — | — | 0.75–0.85 |
| | 10♀ | 0.745 \pm 0.013 | 0.042 | 5.63 | 0.68–0.81 |
| Width 3rd-finger disc/ finger width below disc | 4♂ | 1.775 | — | — | 1.50–2.00 |
| | 10♀ | 1.520 \pm 0.041 | 0.130 | 8.52 | 1.40–1.75 |

^aParatypic juveniles excluded from this table include one subadult male (15.6 mm. SVL) lacking vocal slits, one subadult female (also 15.6 mm. SVL) having small ova and oviducts, and nine unsexed froglets (10.5–13.7 mm. SVL).

ored like adults, but smallest two specimens (KU 139654, 139655) of about 10.5–10.7 mm. SVL appear to have had dorsa extensively suffused with bright pigmentation. In preservative, the bright body markings and pale ventral color fade to bluish gray.

Skin granular dorsally and ventrally, with granulation being particularly coarse and strong on lower back and hind limbs (fig. 2); granulation less pronounced or even lost in preservative. Snout sloping, rounded or obtuse in lateral profile, bluntly rounded to truncate in dorsal or ventral aspect. Naris situated near tip of snout and directed posterolaterally; both nares visible from front and from below but not from above. Canthus rostralis rounded; loreal region vertical and virtually flat. Interorbital area wider than upper eyelid. Length of snout nearly equal to eye length; distance from naris to eye distinctly shorter (by 19–35%) than eye length. Tympanum concealed posterodorsally, where it subcutaneously dips under anterior edge of m. depressor mandibulae; tympanum seen by dissection to be either circular or slightly vertically elliptical, with an area greater than 50 percent of eye.

Relative length of *appressed* fingers 3 > 4 \geq 2 > 1; appressed first finger three-fourths or more the length of second finger (longer than second if measured from base), reach-

ing in some specimens to base of disc of second finger. Discs expanded on all but first finger. In adults, disc of third finger 1.40–2.20 times wider than distal end of adjacent phalanx, averaging wider in males than in females (tables 1–2); discs relatively less expanded in juveniles, the third finger disc being only 1.25–1.67 times wider than finger in specimens <14 mm. SVL. A large circular to elliptical outer metacarpal tubercle on median base of palm, a smaller inner metacarpal tubercle on base of first finger, and one or two usually prominent subarticular tubercles on fingers (one each on fingers 1, 2, one or two on finger 4, two on finger 3); all tubercles low, with rounded surfaces.

Hind limbs of moderate length, with heel of appressed limb reaching tympanum or eye; tibia 38–44 percent of snout-vent length in adults, averaging longer in males than females (tables 1–2); tibia relatively longer in juveniles than in adults (41–45% of SVL, \bar{X} = 43.8% in six juvenile males; 44–46%, \bar{X} = 44.6% in three subadult females). Relative lengths of appressed toes 4 > 3 > 5 > 2 > 1; first toe very short, sometimes failing to reach the subarticular tubercle situated on base of second toe, and with unexpanded disc; other toes with distinct, slightly expanded discs (tending to be smaller than those on fingers). Small inner and outer

metatarsal tubercles, the outer one smaller but usually more prominent because of tendency to be slightly protuberant. One to three nonprotuberant subarticular tubercles (one each on toes 1 and 2, two each on 3 and 5, three on 4), one or more being indistinct on some specimens. A small, obliquely aligned tubercle present on inner side of tarsus, but often weak; a weak tarsal ridge present or absent, when present extending from tarsal tubercle toward inner metatarsal tubercle. Hands and feet lacking webbing, supernumerary tubercles, or lateral fringe.

MYOLOGY AND OSTEOLOGY

MYOLOGY: Some muscles were examined on AMNH 102671–102676, before these specimens were cleared and stained. *Dendrobates bombetes* has typical dendrobatid jaw and thigh musculature insofar as has been described (references in Myers and Daly, 1979, p. 8). The only minor difference from published descriptions is that, in *D. bombetes*, the distal tendon of the deep m. semitendinosus seems to pierce a few posterior muscle fibers of the mm. gracilis complex, passing very near to but not, as usually reported, actually passing through the common gracilis tendon. The flesh of *D. bombetes* is heavily pigmented, appearing deep black in freshly skinned specimens.

OSTEOLOGY: Six cleared and stained paratopotypic specimens were examined: AMNH 102671–102673 (adult males 16.8, 17.2, and 17.8 mm. SVL), AMNH 102674–102676 (adult females 18.2, 18.6, and 18.9 mm. SVL).

Skull slightly longer than wide. Frontoparietals long, fused along their entire length, except two smallest males having a distinct common frontoparietal suture. Sphenethmoid large, closely encircling anterior end of frontoparietal pair, and with a ventrolateral process extending toward but well separated from preorbital process of maxillary. Nasal bones widely separated. In smaller males (16.8–17.2 mm. SVL), the nasals, sphenethmoid, and anterior ends of frontoparietals are slightly separated or distinctly sutured, but in largest male (17.8 mm.) and females

(>18 mm.), these bones are partly or completely fused to form a solid unit. Palatine bones absent. Vestigial prevomers very small, widely separated, vertically aligned bones lying beneath nasals. Alary processes of premaxillae tipped slightly forward. Maxilla posteriorly shallow, anteriorly with a high, often poorly ossified facial lobe bearing a small ill-defined preorbital process. Eight presacral vertebrae, none fused above or below except in largest male (AMNH 102673), in which vertebrae 1 and 2 are fused dorsally and ventrally, and vertebrae 3 and 4 are fused ventrally. Neural spines of vertebrae not expanded into small dorsal shields. Omosternum an elongate, anteriorly tapering style, well defined but not ossified.

TADPOLES

The following description is based on seven larvae from the backs of male nurse frogs. The larvae are all in stage 25 by the Gosner system (external gills absorbed, no indication of hind limb buds).

HABITUS, PROPORTIONS, AND MEASUREMENTS: The tadpole has a somewhat globular shape when viewed from above (fig. 11), with body width averaging 79.4 percent (74–92%) of head-body length. The head and body are depressed (width much greater than depth), slightly convex above and flattened below. Eyes and nostrils are directed dorsolaterally from a dorsal position. The spiracle is sinistral and low and the anus dextral. The low-finned tail averages 64.3 percent (61–67%) of total length, and its greatest depth is only 13.8 percent (12–16%) of total length. The dorsal fin stops short of reaching the body; the end of the tail is rounded.

The measurements following are in millimeters; $N = 7$ for each. Head-body length 4.0–4.6 ($\bar{X} = 4.33$); greatest body width 3.1–3.7 ($\bar{X} = 3.44$); total length 11.1–13.7 ($\bar{X} = 12.23$); greatest tail depth from upper edge dorsal fin to lower edge ventral fin 1.6–1.8 ($\bar{X} = 1.71$).

PIGMENTATION: In life, the long-tailed larvae were grayish brown, turning clear on the end of the tail. In preservative, the body

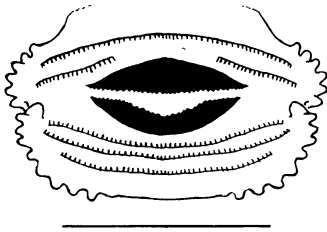


FIG. 3. Mouth of *Dendrobates bombetes* tadpole, stage 25 (semidiagrammatic, based on AMNH 102655). Line equals 1 mm.

and tail, including fins, are seen under magnification to be rather uniformly pigmented, with the tail being somewhat transparent towards the end. The venter is translucent.

MOUTHPARTS: The mouth is directed anteroventrally. The tooth-row formula is 2/3, with the second upper row being broadly broken above the beak (fig. 3); the other rows are essentially complete, although a short median gap may occur in either the first or third lower rows. The beak is massive, with serrate cutting edges; the lower beak is indented as seen in frontal view and broadly V-shaped in ventral aspect. The tooth-row denticles and, to a lesser extent, the beak, are variably keratinized in this sample. Most of the larvae have all the denticles keratinized but, of the two smallest (11.1, 11.2 mm. in total length), one has only the first upper row keratinized and the other lacks keratinization in the third lower row.

The oral disc is laterally indented. The posterior edge of the oral disc has a broad median gap in a single row of sparse papillae (fig. 3). Anteriorly, the oral disc lacks a definite edge, being smoothly confluent with the tip of the snout.

SKIN ALKALOIDS AND TOXICITY

We have previously listed, under the name "*Dendrobates* sp.," the alkaloids detected in an extract of seven skins from paratypic specimens (AMNH 102677–102683) of *D. bombetes* collected in February 1974, at Quebrada de la Chapa (Daly et al., 1978, p. 183). Analysis has now been made of two additional samples of 10 skins each from par-

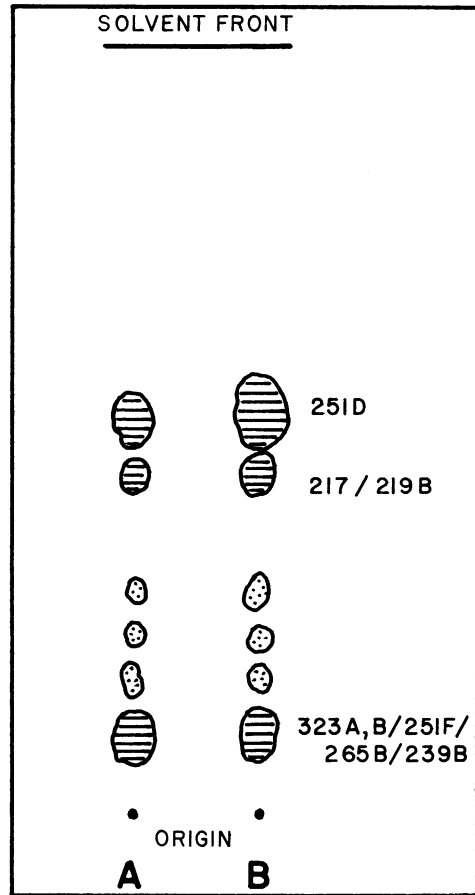


FIG. 4. Representation of a thin-layer chromatoplate of alkaloids from two population samples of *Dendrobates bombetes*. A. Quebrada de la Chapa (seven paratypes, February 1974). B. Lago de Calima (10 paratotypes, November 1976). A sample of 10 μ l of methanolic alkaloids equivalent to amount in 10 mg. wet skin was applied at each origin, and the silica gel GF plate (Analtech Co.) developed with 9:1 chloroform-methanol. Visualization, after chromatography and drying, by exposure to iodine vapor.

Designations are based on R_f values of some of the compounds identified in the unresolved alkaloid fraction by combined gas chromatography-mass spectrometry (fig. 5). Spot intensities depicted as follows: Horizontal pattern = moderate amounts; dots = small amounts (reference standards are figs. 1–2 in Myers and Daly, 1976a).

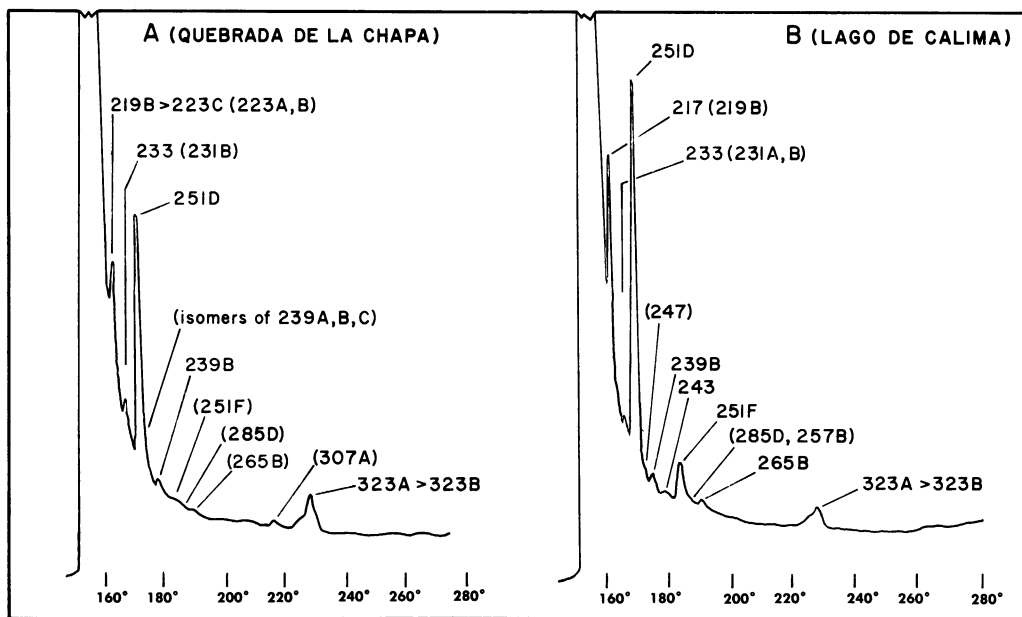


FIG. 5. Gas chromatograms of alkaloids from two population samples of *Dendrobates bombetes*. A. Quebrada de la Chapa (seven paratypes, February 1974). B. Lago de Calima (10 paratypes, November 1976). Chromatography was run with 2 μ l of methanolic extract containing concentrated alkaloids equivalent to amount in 2 mg. of wet skin (methodology same as in Myers and Daly, 1976a, Appendix 2).

Alkaloids are designated by their molecular weights; parentheses indicate trace compounds. The corresponding protonated molecular ions of these alkaloids were identified in separate computer-assisted analysis by combined gas chromatography-mass spectrometry.

atopotypic specimens (AMNH 102657–102676) collected in November 1976, above the south side of Lago de Calima. All specimens were skinned on the date of capture and the skins placed in 100 percent methanol for transport at ambient temperature and eventual storage at -5° C. The blotted wet-skin weights of the three samples were 0.4 g. ($N = 7$), 0.6 g. ($N = 10$), and 0.6 g. ($N = 10$), for a calculated weight of 60 mg. of skin per frog. Alkaloid fractions were prepared from the methanol extracts and analyzed by thin-layer chromatography (fig. 4) and by combined gas chromatography-mass spectrometry (fig. 5).

ALKALOIDS: The skin secretions of *Dendrobates bombetes* are rich in piperidine alkaloids, with 22 kinds having been detected in the samples studied (table 3). Inasmuch as 15–17 different alkaloids may be present in

samples of 7–10 skins (table 4), it seems likely to us that at least a dozen compounds, if not more, are being secreted by individual frogs.

Four of the 22 compounds have not been previously recognized and are not listed in our recent catalogue of dendrobatid piperidine alkaloids (Daly et al., 1978). Their empirical formulae are shown in table 3 and additional data are given in an appendix. Three of the new alkaloids (247, 251F, and 265B) have been detected only in *Dendrobates bombetes*, but a fourth (217) is now known to occur in several other species of *Dendrobates* (see Appendix).

With the exception of two unclassified compounds, all the alkaloids of *Dendrobates bombetes* are assigned to the several classes of pumiliotoxins. However, the new alkaloids 247, 251F, and 265B are particularly

TABLE 3

Tentative Classification of Skin Alkaloids of *Dendrobates bombetes*, New Species, Following System of Daly et al. (1978, table 1)^a

| Pumiliotoxin-C Class | Hydroxypumiliotoxin-C Class | Pumiliotoxin-A Class | Unclassified |
|--|------------------------------------|--|--------------------------|
| 217* C ₁₅ H ₂₃ N (152, H ₆) | Isomer of 239A ^b | 247 "C ₁₆ H ₂₅ NO" (109, H ₄) | 257B ^d |
| 219B | 239B | 251D* | 285D ^d |
| 223A | Isomer of 239B ^b | 251F* C ₁₆ H ₂₉ NO (111, H ₀) | |
| 223B | Isomer of 239C ^b | 265B ^c C ₁₇ H ₃₁ NO (125, H ₀) | |
| 223C | | 307A | |
| 231A | | 323A | |
| 231B | | 323B | |
| 233 | | | |
| 243 | | | |

^a Alkaloids are designated by molecular weight in boldface type, followed in some cases by a code letter to distinguish from other compounds of identical weight. Alkaloids occurring as major compounds in one or more populations of *D. bombetes* (see table 4) are designated by an asterisk (*). New compounds are followed by an empirical formula (quotation marks indicate a formula not confirmed by high resolution spectrometry) and, within parentheses, by a major electron-impact mass spectral fragment (the base peak) and the number of additional hydrogens in the apparent perhydro-derivative.

^b If these apparent isomers prove to be new compounds, they will be given the designations **239H**, **239I**, and **239J**, but **239A–239G** are a complex series of trace alkaloids that have also been confused with alkaloids "239AB" and "239CD" of the gephyrotoxin class (see Daly et al., 1978, pp. 170–171).

^c Alkaloid **265** in Daly et al. (1978) now becomes **265A**.

^d Has a very atypical mass spectrum for a dendrobatid alkaloid and might actually represent a degradation artefact (*vide* Daly et al., 1978).

worthy of further investigation because their mass spectra appear atypical for the pumiliotoxin-A class, to which they are tentatively assigned. If not actually members of a new class of dendrobatid alkaloids, these three compounds at least form a distinctive sub-unit of the pumiliotoxin-A class.

INTERPOPULATIONAL VARIATION: Geographic variation in skin toxins is common among species of *Dendrobates* (Daly and Myers, 1967;³ Myers and Daly, 1976a; Daly et al., 1978). *Dendrobates bombetes* is no exception, showing both quantitative and qualitative differences in skin alkaloids of two populations separated by 800 m. of elevation and some 30 km. of airline distance.

³Our initial study, on *Dendrobates pumilio*, demonstrated mainly interpopulational quantitative differences in skin toxins, as shown by thin-layer chromatography and bioassay. But follow-up studies with improved technology have revealed striking qualitative differences also, and we are subjecting *D. pumilio* to a new and more extensive analysis.

It should be noted that there were no discernible differences between the alkaloid profiles of two samples of 10 skins each from a single locality (Lago de Calima), and that intrasample variation is therefore unlikely to be pertinent to the following discussion.

Quantitative differences between the two population samples of *Dendrobates bombetes*, although not directly measured, seem obvious from inspection of figures 4 (cf., spot size) and 5 (cf., peak height). Frogs from the type locality seem to have a greater quantity of alkaloids than those from Quebrada de la Chapa.⁴ The latter, however, have a few

⁴Frogs from the type locality are also somewhat larger (cf., tables 1, 2), which probably accounts for part, if not all, of the quantitative differences in skin toxins. The calculated skin weight was, as already stated, the same in each sample (60 mg./frog), but weights of the pooled samples were rounded to the nearest 0.1 g. and cannot reflect such slight differences in body size, mainly because of the impossibility of obtaining "exact" weights of blotted wet skin.

TABLE 4
Alkaloid Profiles in Two Populations of
Dendrobates bombetes, New Species
(+++ = Present as Major Compound; ++ = Minor Compound; + = Trace; - = Not Detected)

| Alkaloid ^a | Que. de la Chapa | Lago de Calima |
|---------------------------------|------------------------|----------------------|
| PUMILIOTOXIN-A CLASS | | |
| 1) 247 | - | + |
| 2) 251D | +++ | +++ |
| 3) 251F | + | +++ |
| 4) 265B | + | ++ |
| 5) 307A (Pumiliotoxin A) | + | - |
| 6) 323A (Pumiliotoxin B) | ++ | ++ |
| 7) 323B (Allopumiliotoxin B) | ++ | ++ |
| PUMILIOTOXIN-C CLASS | | |
| 8) 217 | - | +++ |
| 9) 219B | ++ | + |
| 10) 223A | + | - |
| 11) 223B | + | - |
| 12) 223C | ++ | - |
| 13) 231A | - | + |
| 14) 231B | + | + |
| 15) 233 | ++ | ++ |
| 16) 243 | - | ++ |
| HYDROXYPUMILIOTOXIN-C CLASS | | |
| 17) 239B | ++ | ++ |
| 18) Isomer of 239A ^b | + | - |
| 19) Isomer of 239B ^b | + | - |
| 20) Isomer of 239C ^b | + | - |
| UNCLASSIFIED | | |
| 21) 257B ^c | - | + |
| 22) 285D ^c | + | + |
| TOTAL PER POPULATION | 17 | 15 |

^a Relatively few of the dendrobatid alkaloids have been formally named, but all can be designated by molecular weight plus an added code letter (if needed) to distinguish from other compounds of identical weight.

^b See table 3, note *b*.

^c See table 3, note *d*.

more kinds of alkaloids (17 vs. 15, table 4). The two population samples share 10 of a possible 15 alkaloids, for an alkaloid-similarity value of 67, as determined by the formula $100 C/N_1$ (fig. 13). Such similarity coefficients ranged from 38 to 100 among nine populations of the better studied *D. histrionicus*,

in which the highest values (≥ 75) were generally found among "nearest neighbors" (Myers and Daly, 1976a, p. 217). Two *histrionicus* populations having the same similarity value (67) as *bombetes* are separated by an airline distance of over 200 km.—compared with a distance of only some 30 km. between the two known populations of *bombetes*. Nonetheless, the skin alkaloids of the two *bombetes* populations are more similar than would appear to be indicated by their shared-alkaloid value. Whereas the gas chromatographic traces of the aforesaid pair of *histrionicus* populations (fig. 15E and 15G in Myers and Daly, 1976a, p. 221) look completely different, the chromatographic traces of the *bombetes* samples are very similar (fig. 5). The reason appears to be that some of the *unshared* alkaloids of *bombetes* have virtually the same carbon rings and ring substituents as certain other alkaloids present in one or both samples. Such alkaloids differ only in degree of saturation, usually in a ring substituent (unsaturated compounds have fewer hydrogen atoms owing to the presence of double and/or triple bonds and will yield the same hydrogenated derivative). For example, 217 is an unsaturated analog of 219B and of 223C. Similarly, 231A appears to be an unsaturated analog of 233, and 247 an unsaturated analog of 251F. Such analogs are likely to emerge in the same chromatographic peaks, thus contributing to (or at least not detracting from) the similarity of the chromatograms in figure 5. The Lago de Calima population of *Dendrobates bombetes* tends to have alkaloids with a greater degree of unsaturation than does the lower-elevation Quebrada de la Chapa populations, but we have no idea why this might be.

TOXICITY: Toxicity of the Lago de Calima frogs was tested by subcutaneously injecting, into a 20 gram white mouse, a concentrated methanolic extract equivalent to the amount of alkaloids in 100 mg. of wet skin (1 skin \sim 60 mg.). Both samples of 10 skins from this locality were so tested, with the following results: After displaying initial irritation at the injection site, the mice had severe locomotor difficulties, with wobbling

gaits, muscle spasms, and labored breathing. After 20 minutes, the mice moved with great difficulty, and there were occasional occurrences of gagging and minor convulsions. But recovery was nearly complete after 3 to 4 hours.

Therefore, *Dendrobates bombetes* is not particularly toxic in spite of the abundance of alkaloids in its skin toxins. In contrast, an equivalent-sized injection from the related *D. opisthomelas* caused loss of locomotor ability within 1 minute and death of the mouse at 8 minutes.

DISTRIBUTION, GEOGRAPHIC VARIATION, AND NATURAL HISTORY

DISTRIBUTION AND VARIATION: As presently known, *Dendrobates bombetes* occupies a small section of the Cordillera Occidental, at latitude 3° 47'–52' N, longitude 76° 25'–41' W. This is on the Pacific versant at 670–1600 m. above sea level. Inasmuch as the species seems to tolerate rather diverse habitats, its geographic range along the western cordillera of the northern Andes may prove to be much larger than now known. Nonetheless, its current distribution seems to be spotty even in the small area where known, and its range has almost certainly been thinned, if not actually trimmed, by extensive destruction of Andean forest (see Habitats and Population Structure).

The two known populations are separated by an airline distance of only about 30 km., which, in many species of *Dendrobates*, would be more than sufficient to lead to (or at least maintain) striking differences between populations (e.g., Daly and Myers, 1967; Myers and Daly, 1976a). However, interpopulational variation in *Dendrobates bombetes* is not conspicuous because populational differences in color pattern are not obvious in our samples. Intrapopulational variation in color pattern is relatively slight (e.g., fig. 2) and appears to be of roughly the same extent in each population. Field notes by Myers (AMNH) and Edwards (KU) suggest that there might be a slight difference in

the hue of the pale areas of the venter: It was described only as pale bluish green or blue-green for the Quebrada de la Chapa population (AMNH and KU paratypes), and, more variably, as "in most pale green, or, in some, very pale yellow, or, in a few, pale bluish green" in a sample from above Lago de Calima (AMNH 102601–102638). At the latter locality, the various hues were all so pale as to seem whitish in dim light of the forest, and it is difficult to imagine a significant function for such subtle differences.

Interpopulational variation in skin toxins is, as shown on previous pages, particularly evident when shared alkaloids are tabulated. The Lago de Calima population sample has fewer kinds of toxins, of which only 67 percent are shared with the Quebrada de la Chapa sample. But, as already discussed, the difference becomes less impressive when gas chromatographic traces are compared and when it is realized that some unshared alkaloids differ only in their relative degrees of saturation.

Comparison of tables 1 and 2 will reveal a few seemingly minor differences in body proportions, which will not be further discussed because the small Quebrada de la Chapa sample is comprised of two smaller subsamples having different preservational histories. However, the differences in snout-vent length clearly indicate that the Quebrada de la Chapa population is comprised of smaller frogs, as is evident from inspection of both means and ranges (comparing means of adult females, $t = 4.581$, $P < 0.001$). We think that the body-size differences between the samples do reflect some reality of nature, but we doubt that they reflect genetic differences. A hypothesis accounting for size differences will be presented in the following section on Population Structure.

HABITATS: The two known localities for the species differ considerably in vegetation and climate. At Quebrada de la Chapa, the adjacent grassy slopes have been cleared of original vegetation—probably a scrubby deciduous forest, since the area receives relatively little rain—and there is only a straggle of cactus-fringed gallery forest along the stream itself (fig. 6). Specimens of *Dendro-*



FIG. 6. Quebrada de la Chapa, looking upstream (northward) from about 610 m. elev. on Cali-Buenaventura Road, 1 km. W Loboguerrero (November 21, 1976). Specimens of *Dendrobates bombetes* were collected a short distance upstream at 670–780 m. elev. Locality is in rain-shadow section of upper Río Dagua Valley; forested uplands in distance receive more moisture (but the abrupt forest edge is artificial).

bates bombetes were found on the ground amongst dry reeds and in seepage areas, in shaded places near the clear-water rocky stream; they were not heard calling during our visit here.

The other locality is situated above the southern side of Lago de Calima in a cooler, rainier climate, about 30 km. northeast and 800 m. higher than the Quebrada de la Chapa location. Lago de Calima is in a region of humid montane forest that has been extensively cleared for pasture (fig. 7). Remnants of this forest are left on the lower slopes as isolated "islands," and the type locality of *Dendrobates bombetes* is two neighboring forest islands lying at about 1600 meters elevation, or about 200 m. higher than the surface of the lake.

These forest islands (fig. 8) are comprised

of tall, straight-boled trees; there is brushy undergrowth with many treelets and/or saplings and a few tree ferns, but no palms. Scattered large trees have buttressed roots; spiny trunks were not seen. The moss layer on trunks is scant, but broadleaf epiphytes (Araceae) are abundant; there are bromeliads growing high in the trees, but only a few are to be found as low as head height and none on the ground (save for an occasional fallen one). The ground layer is comprised of low herbaceous plants and ferns. There is an abundant leaf litter, which accumulates in brush at the bases of some trees to depths of 0.3–0.6 m. We did not estimate the sizes of the two islands, but they were "small" in the sense that one could stroll through the forest from end to end in perhaps 15 minutes or less.

One of the forest islands (fig. 8, top) straddles a ridge, lacks surface water, and is relatively drier than the other one. The latter island (fig. 8, bottom) has a small, mud-banked stream channel, with a partial rock bottom, that must hold water for at least part of the year (but not during our visit in November). This moister island differed also in having small groves of giant bamboo, a generally denser understory, and a better developed ground stratum of herbs and ferns. *Dendrobates bombetes* was abundant in both of these rather diverse islands, although other dendrobatids (*Colostethus* spp.) occurred only in the moister of the two. However, we did not find *D. bombetes* in a seemingly suitable forest island only about 5 km. to the east and 50 m. lower in elevation, nor have we encountered or heard it during very brief forays into forest on the northern side of the lake and below the dam at the western end. Our work in this region can hardly be called extensive, but it does seem that *D. bombetes* occurs spottily. It is not a difficult frog to find, especially when it is calling (although even biologists are initially likely to mistake the calls for those of insects).

POPULATION STRUCTURE: There are 108 frogs in the type samples, 32 from Quebrada de la Chapa and 76 from the two forest islands at Lago de Calima, including juveniles and specimens skinned for toxin analysis. There is no convincing evidence of unbalanced sex ratios. For Lago de Calima, where we might have been biased toward collecting calling males (but also often finding presumed females close by), the ratio of adult males to adult females is a close 36:31. For Quebrada de la Chapa, the ratio of adult males to females is 7:13, which, at this sample size, is not statistically significant ($\chi^2 = 1.800$, $P > 0.1$), although we certainly would like to see a larger sample from this locality for reasons that will become evident below.

As briefly discussed under Distribution and Variation, the Quebrada de la Chapa sample is comprised of frogs having a smaller average adult size than those at Lago de Calima; there also is a correlated difference in ranges of variation, with the smallest sexually mature adults being found in the small-

er Quebrada de la Chapa sample (tables 1, 2). It can be doubted that this reflects a genetically fixed size difference because the Quebrada de la Chapa sample also has a significantly larger number of sexually immature specimens. In this sample, 12 (37.5%) of 32 specimens are juveniles versus only 9 (11.8%) of 76 specimens from Lago de Calima. This difference is highly significant when chi-square is determined from a 2×2 table and Yates's corrected formula ($\chi^2 = 7.897$, $P = 0.005$).

What is to explain the smaller-sized adults and large proportion of juveniles in the Quebrada de la Chapa sample? Seasonality of reproduction is one possibility, since there was obvious breeding at one locality (Lago de Calima) in November and early December, and since males were not heard calling during our visit to Quebrada de la Chapa in February (the photograph in fig. 6 was taken in November, but no other field activities were conducted then). This hypothesis could be easily checked by a resident biologist, but we suspect that the explanation is not so simple and venture to predict that, at least in the evergreen forest at Lago de Calima (and probably elsewhere), *Dendrobates bombetes* will be found to breed yearlong.

Dendrobates bombetes corresponds to what seems to us to be a general pattern (with some exceptions) among *Dendrobates*, especially among the relatively apomorphic species of northwestern South America and lower Central America. When a species is very abundant at a locality, then juveniles tend to be scarce or rare even though there may be ample evidence of breeding activity in the population. Conversely, when a species is relatively uncommon, juveniles will tend to make up a larger proportion of one's sample. These and other considerations have recently led to a suggestion that, in *Phyllobates terribilis*, either reproductive success or juvenile survivorship may be inversely density dependent and somehow suppressed in large, stable populations (Myers, Daly, and Malkin, 1978, pp. 327-328). When the hypothesis is applied to *Dendrobates bombetes*, the populational differences may be explained as follows:



FIG. 7. Lago de Calima, looking northwest from 1490 m. elev., below forest islands shown in figure 8 (November 21, 1976). Extensive clearing around this man-made lake has left small islands of forest.

(1) Optimal habitat is found in the cool, montane forest islands above Lago de Calima, where *Dendrobates bombetes* maintains a dense, stable population. Individuals are conspicuous in both color and behavior and are richly endowed with skin alkaloids. From these facts and the marked abundance of the frogs, it may be supposed that predation is not a particular source of mortality for adults, allowing for relatively long lives and attainment of large average size, with occasional individuals being able to attain the maximum observed sizes of the species (table 1). Population turnover is slow.

(2) Only marginal habitat is provided by the lower-elevation Quebrada de la Chapa, which lies in a rain shadow. The sparse canopy and ground cover of the narrow gallery forest makes desiccation a real threat to this diurnal forest frog, which might also encounter predators to which it is not well adapted. Males, which frequently call from exposed low perches, might be more vulnerable than

females to such threats (note sex ratios above). Whatever the sources of mortality, adults have shorter lives and rarely, if ever, attain maximum size. The population cannot attain stability because of rapid turnover, and populational constraints on reproduction are not operable. It is a population which maintains a precarious occupancy.

These perceptions are an accidental product of a field program that was designed for other purposes, and are presented as encouragement for someone to investigate mechanisms of population control in dendrobatid frogs.

ACTIVITY: *Dendrobates bombetes* is a diurnal frog that appears to be strictly terrestrial based on our limited observations.⁵

⁵But, without having searched high-growing bromeliads for frogs and tadpoles, we could easily be drawing the wrong conclusion. For example, most available specimens of the small *D. fulguritus* from northwestern Colombia and central Panama have been found on the



FIG. 8. Type locality of *Dendrobates bombetes*: Neighboring forest islands at 1580–1600 m. elev., on mountain above south side of Lago de Calima (November 21, 1976). *Top*: Ridgetop island at 1600 m. *Bottom*: Moister island at about 1580 m., over the ridge and at a distance of about 100 m. downhill from above.

ground, but we also have obtained specimens from under moss on the limbs of a *madroño* tree (*Reedia choensis*) at a height of about 7.5 m. above ground. Elsewhere, we have noted interpopulational and even interdeme variation in extent of arborealism within a single species of *Dendrobates* (Daly and Myers, 1967), and we are in the process of describing a Panamanian species that seems almost completely arboreal. Other examples could be easily cited, as there are strong arboreal tendencies within the genus *Dendrobates*, a fact that is not generally appreciated even by field biologists.

One individual was on a log 0.5 m. above ground, but all others were on the ground or on leaf litter or other ground debris. In the forest islands above Lago de Calima, where all the following notes were made, the frogs would attempt to escape capture by going into the abundant leaf litter, but they were rather slow about it and were easily caught; they appeared docile and unexcited when handled. In late morning on November 21,

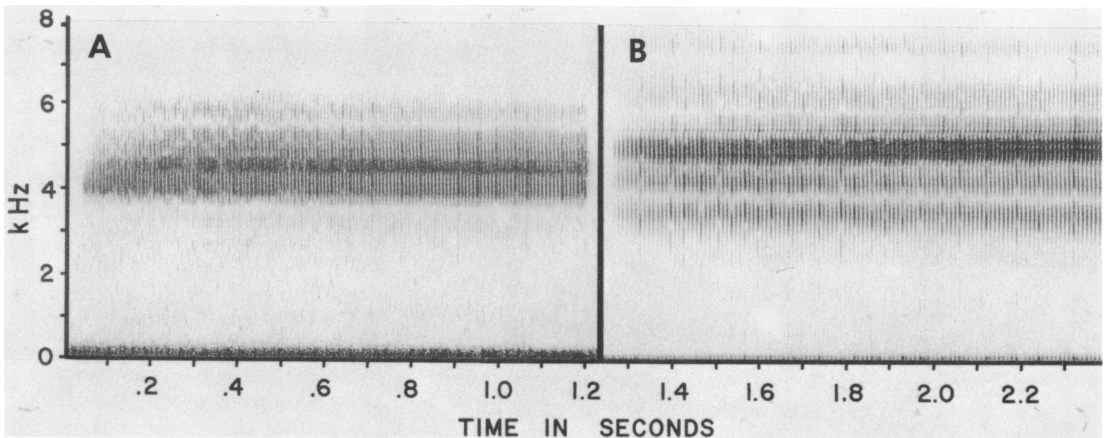


FIG. 9. Comparison of buzz calls of two species of Andean *Dendrobates* recorded at same temperature (ground level at calling site). Call on left has faster pulse rate (117/sec. vs. 99/sec.) and lower dominant frequency (4500 Hz vs. 5000 Hz), as well as being shorter (only part of right-side call is shown).

A. *Dendrobates bombetes*, new species. Complete 1.2-second-long buzz of AMNH 102639 from type locality at 1600 m. in Cordillera Occidental; recorded at 17.2° C. on December 4, 1976. B. *Dendrobates opisthomelas*. Beginning portion of 2.3-second-long buzz of specimen (seen but not caught) at Represa de Santa Rita, 1930 m. in Cordillera Central; recorded at 17.2° C. on November 30, 1976. (Both wide-band sound spectrograms, 300-Hz filter, made from field recordings on AMNH reel 209.)

there was frequent calling and individuals were moving about in the open, even though the top surface of the leaf litter was crispy dry. But after a brisk, ground-wetting rain, the frogs came out of the leaf litter in larger numbers and calling became more intense.

Males were calling from the forest floor or, more often, from atop or within leaf litter that had piled up in brush and around the bases of trees. Although many were calling from beneath leaves or within the loosely structured piles of litter, many others were in plain sight and we did not have the impression that they deliberately concealed themselves (compare remarks on *D. opisthomelas* under Systematics). They seemed to maintain small calling areas, although sometimes moving several centimeters between calls. Several were seen to snap at small insects or other food items between calls. Many males seemed to call frequently and then to quit for long periods before resuming; two seemingly undisturbed individuals had not resumed calling by the end of half-hour observation periods.

VOCALIZATION: As indicated by its name, *Dendrobates bombetes* is a "buzzer." Considering the small size of this species (fig. 1), males emit surprisingly loud sounds that are easily heard from 10 m. away and which, in relatively open forest, are audible even at 20 m. The call (fig. 9A) is heard as a short, loud and high-pitched insect-like buzz of about 0.9–1.7 seconds duration; the call is uniformly pulsed throughout its length, at a rate of 113–134 pulses per second and at a dominant frequency of about 4000–4800 Hz. These variables have smaller ranges when considering consecutive buzzes of individual frogs calling at one temperature (table 5), but the total range of variation doubtless could be extended by study of recordings taken of additional frogs and at different temperatures. Pulse rate is correlated positively with temperature: Whether mean pulse rates or either minimal or maximal rates are contrasted (table 5), a three degree increase in temperature resulted in an increase of 15–16 pulses per second, a significant positive correlation as shown by regression analysis of 43 calls re-

TABLE 5
Analysis of 43 Buzz Calls from Five Paratopotypic *Dendrobates bombetes*, New Species^a

| AMNH No. | Temperature ^a | N | Call Length (sec.) ^b Mean and Range | Pulses Per Second ^c Mean and Range | Dominant Frequency (Hz) ^d Mode and Range |
|---------------------|--------------------------|----|---|--|--|
| 102639 | 17.2° C. | 11 | 1.20 (1.1–1.3) | 115.8 (113–118) | 4500 (4300–4800) |
| 102640 ^e | 17.8° C. | 14 | 1.46 (1.4–1.7) | 116.1 (114–118) | 4200 (4000–4300) |
| 102641 | 18.0° C. | 4 | 1.45 (1.4–1.5) | 119.5 (114–125) | 4700 (4700–4700) |
| 102642 ^e | 19.4° C. | 11 | 1.31 (1.2–1.4) | 127.1 (123–130) | 4800 (4700–4800) |
| 102643 | 20.2° C. | 3 | 0.97 (0.9–1.0) | 131.0 (129–134) | 4500 (4500–4600) |

^a Recorded during rising temperature on morning of December 4, 1976. Air temperature measured at level of calling site on ground or leaf litter immediately after recording and capturing specimen.

^b Measured to nearest 0.1 second from sound spectrograms.

^c Pulse rate = number of pulses counted in 1.0-second-interval on sound spectrograms (wide-band, 300-Hz filter), except where necessarily extrapolated from shorter call segments (in three of four broken calls of AMNH 102641 and in one short call of AMNH 102643).

^d Rounded to nearest 100 Hz, from one or two "sections" of each spectrogram.

^e Specimens having only one vocal slit open (left in 102640, right in 102642).

corded at five temperatures ($r = 0.92$, $P < 0.001$). Call length, on the other hand, probably is correlated with temperature in a negative manner, although individual variability seems to complicate the pattern: Regression of call length on temperature produces a low and statistically not significant correlation coefficient when all 43 calls in table 5 are utilized ($r = -0.28$, $P > 0.05$), but, removing the one nonconforming individual (first specimen in table) produces a strong negative correlation ($r = -0.82$, $P < 0.001$). As a further complication, the pulse rate is perhaps negatively correlated with call length (i.e., longer calls tending to be pulsed more slowly than shorter calls), as has been shown for the buzz calls of *D. minutus* ($r = -0.67$, $P < 0.01$) at constant temperature (Myers and Daly, 1976a, p. 228): But in the present case, the correlation is weak albeit significant ($r = -0.38$, $P = 0.01$), being masked by overriding temperature effects and not evident from inspection of data from single frogs. To summarize the interactions as perceived above, rising temperature causes pulse rate to increase and causes call length to decrease, and, since shorter calls may tend to be pulsed faster, the decreasing call length probably reinforces the strong temperature influence on pulse rate. These correlations seem to be general tendencies

whose results, at least in small samples, are somewhat blurred by individual variability.

Further variability is introduced by differences in pitch, as reflected by the dominant frequency (table 5), and, rarely, by altering the call through omission of small groups of pulses. Occasional spectrograms of normal calls of *Dendrobates bombetes* have one to several single-pulse gaps that seem to have no audible effect on the call. However, one calling male (AMNH 102641) consistently had a variably wavering or stuttering aspect to each of its calls that was unmatched in any of the many other callers heard,⁶ and so it was purposely stalked and four of its calls recorded. The spectrograms (fig. 10A, 10B) show calls that begin normally but which acquire 7–15 gaps, each gap representing a time-space for 2–4 pulses. Similarly broken calls have been mentioned for *Dendrobates minutus* by Myers and Daly (1976a, p. 228). *Dendrobates opisthomelas* may also drop pulses, either throughout the call (fig. 10C) or toward the end (fig. 10D). Such variations seem anomalous, but, if there is a genetic basis, they might sometimes provide a basis for the evolution of new calls.

⁶This specimen is normal in having both of its vocal slits open. Individuals having but one slit (notes e, table 5) produced normal-sounding calls.

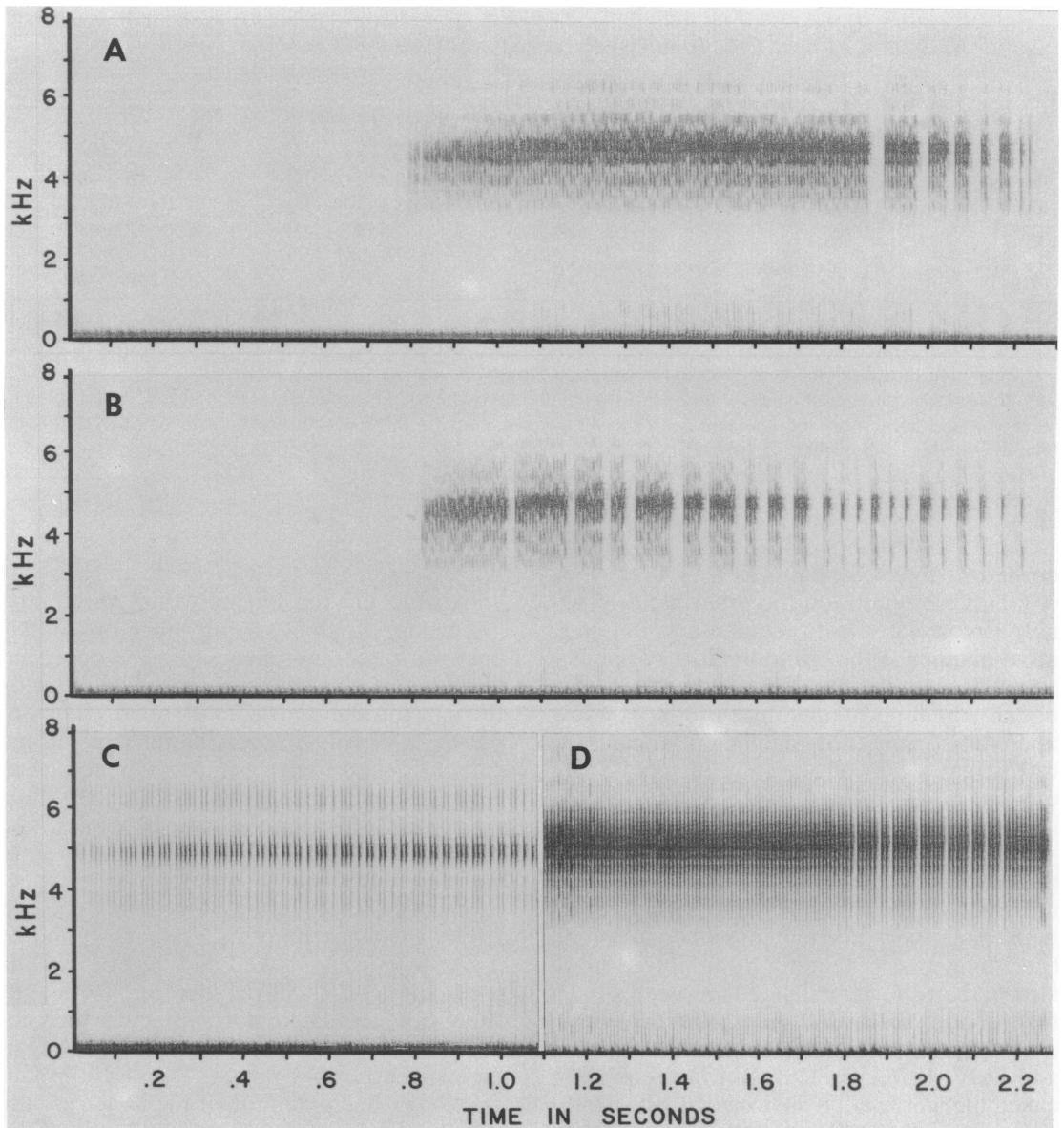


FIG. 10. Atypical buzz calls of two species of Andean *Dendrobates*, showing variability in omission of pulses. Compare with normal calls in figure 9. A, B. *Dendrobates bombetes*. Two complete calls from AMNH 102641 (18.0° C.). C, D. *Dendrobates opisthomelas*. Portions of calls from two frogs, as follows: C. Beginning of 2.1-second-long call of unpreserved specimen (at 17.4° C.). D. End of 1.9-second-long call of AMNH 102586 (19.0° C.). Locality and other data same as in figure 9.

The call of the closely related *Dendrobates opisthomelas* is a similarly loud buzz that differs from that of *D. bombetes* (cf., fig. 9A, 9B) in being longer (1.6–2.3 sec.) and in hav-

ing a slower pulse rate (~100/sec. at 17–19° C.) and a higher dominant frequency (~5000–5500 Hz).

TADPOLE CARRYING: Five nurse frogs,

all males, were found carrying stage-25 tadpoles in the Lago de Calima forest islands on November 21. Three of the frogs each carried a single larva, and two others each carried two larvae. The larvae were carried low on the nurse frogs' backs, with their heads positioned forward (fig. 11). This is the normal carrying position in the "minutus group" of *Dendrobates* (see also fig. 12B), whose tadpoles are relatively massive compared with the small body sizes of the adult frogs. The tadpoles are attached, not by their mouths, but by the probably universal dendrobatid method of sticking to a small patch of mucus on the nurse frog's back. In some dendrobatids, this attachment is accomplished solely by mere surface adhesion between the mucus and the tadpoles' flattened or slightly concave bellies, and the larvae are easily moved about and dislodged; but even so, a certain amount of soaking in water is required for the bond to loosen and for the tadpoles to be released normally. In other dendrobatids, including *Dendrobates bombetes*, the mucus attachment seems almost glue-like and the tadpoles are very resistant to being dislodged (even when the frog is roughly handled or dropped into formalin), and presumably the nurse frog must immerse itself for a longer period before the larvae swim free. When dorsal larvae of *D. bombetes* are gently pried from the live frog, the sticky mucus pulls out into definite strands, and a mucous patch (white in preservative) remains on the adult's back, as described by Stebbins and Hendrickson (1959, p. 509) for *Colostethus subpunctatus*. The degree of firmness of the attachment may be correlated with the length of time that the tadpoles are carried about by the nurse frog, which appears to us to be a variable trait among species of dendrobatids.

We did not find free-living tadpoles although a search was made of the few low-growing bromeliads that were present, and we examined water-containing axillae of arcaeous plants, looked in small pools caught in pockets of large dead leaves on the forest floor, and dredged a tiny net through a small tree hole. We would anticipate that the larvae are deposited in live, water-holding

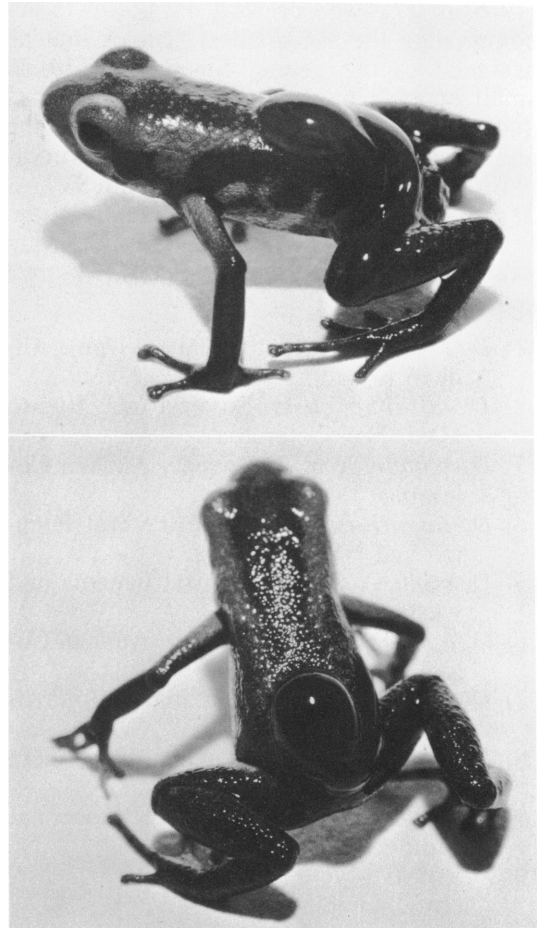


FIG. 11. Male nurse frogs of *Dendrobates bombetes*, new species, approximately $\times 3$. Top: AMNH 102653, with two tadpoles. Bottom: AMNH 102652, with one tadpole.

plants, and we would not omit high-growing bromeliads from consideration (see p. 14n), although our search near ground level was cursory. Larvae of the related *D. abditus* and *D. opisthomelas* have been found in ground bromeliads, which were absent or very rare at the *D. bombetes* localities.

SYSTEMATICS

Dendrobates bombetes belongs to the "minutus group" as defined by Silverstone

(1975, p. 27), who erected the group to accommodate the six smallest species that he assigned to the genus. Several additional small species have since been discovered and others are likely to follow. Based on the apomorphic (but not necessarily synapomorphic) features of small size (maximum SVL ~ 22 mm.), enlarged digital discs, and relatively shortened first finger, the *minutus* group would now consist of the following 11 known species:

- 1) *D. abditus* Myers and Daly (1976b), Andean Ecuador
- 2) *D. altobueyensis* Silverstone, Baudó Mts., NW Colombia
- 3) *D. bombetes* of this paper, Andean Colombia
- 4) *D. fulguritus* Silverstone, central Panama and NW Colombia
- 5) *D. minutus* Shreve, central Panama and western Colombia
- 6) *D. opisthomelas* Boulenger, Andean Colombia
- 7) *D. quinquevittatus* Fitzinger, Amazonian South America
- 8) *D. reticulatus*⁷ Boulenger, Amazonian Peru
- 9) *D. steyermarki* Rivero, Cerro Yapacana, southern Venezuela
- 10) *D. viridis* Myers and Daly (1976a), western Colombia
- 11) *D. species* (name in manuscript), western Panama

We have collected all 11 species and have obtained tadpoles of all save *D. fulguritus* and *D. viridis*. Based on a larval synapomorphy, we suggest that at least *D. abditus*, *D. bombetes*, and *D. opisthomelas* form a monophyletic subgroup. The character state is the median gap that interrupts the papillate fringe on the posterior (lower) edge of the

oral disc (fig. 3). Silverstone (1975, p. 32) first called attention to this trait in *D. opisthomelas*, and we later noted its presence in a tadpole assigned to *D. abditus* (Myers and Daly, 1976b, p. 11). Such a median gap is not unknown among anuran larvae, but it is rare in the Dendrobatidae. Therefore, it seems most probable that the median gap is apomorphic in the family and synapomorphic among the three small Andean *Dendrobates* known to possess it. *Dendrobates bombetes* has a broader median gap and larger and fewer labial papillae than either *abditus* or *opisthomelas*. All three species lack a distinct edge to the anterior part of the oral disc, which is broadly and smoothly confluent with the tip of the snout. Silverstone's (*loc. cit.*) illustration of the mouth of a stage-29 *opisthomelas* larva shows a pronounced posteriad curvature to the upper beak that is lacking in stage-25 *bombetes* (fig. 3); this seems to be an ontogenetic rather than a species difference, since young *opisthomelas* larvae resemble *bombetes* in this respect (older *bombetes* larvae are not available for comparison).

Dendrobates abditus (fig. 12A) is highly derived in being unicolor dark bronzy brown above and below, with vivid golden orange flash marks proximally on the arms and legs adjacent to axilla and groin. It is somewhat smaller than *D. bombetes*, attaining maximum known sizes of 17.3 (♂) and 17.7 (♀) mm. SVL (18.5 and 19.8 mm. in *bombetes*). Its call is unknown; considering the buzz calls of *bombetes* and *opisthomelas*, we likely were mistaken in tentatively associating a "tink-tink-etc." series to *abditus* (Myers and Daly, 1976b, p. 5).⁸ *Dendrobates abditus* is known only from its type locality at 1700 meters elevation on the Amazonian flank of Volcán Reventador, Napo Province, Ecuador.

⁷*Dendrobates quinquevittatus* (*sensu* Silverstone, 1975, p. 33) is a composite, and we are resurrecting one available name—*D. reticulatus*—for use above and in the Appendix. It is a morphologically and behaviorally distinct species that we found living sympatrically with *D. quinquevittatus* near the Río Nanay in Amazonian (Loreto) Peru. Documentation, based on AMNH specimens, will be provided elsewhere.

⁸Nonetheless, buzz calls do not characterize all members of the *minutus* group. *Dendrobates steyermarki*, a montane isolate in southern Venezuela, emits a soft "wheep." Calls are unknown for several species (*abditus*, *altobueyensis*, *fulguritus*, and *viridis*, although the last is suspected of having a buzz call *fide* Myers and Daly, 1976a, p. 252).

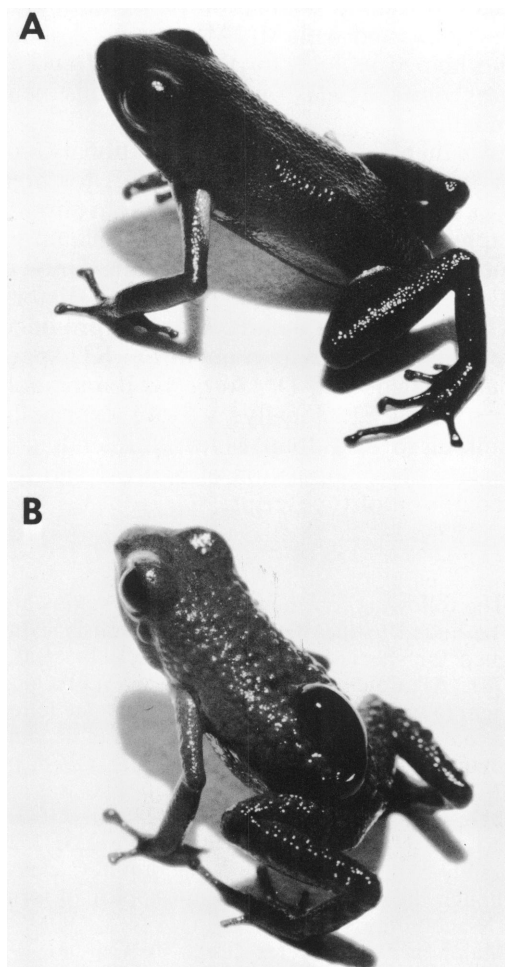


FIG. 12. Andean relatives of *Dendrobates bombetes*, approximately $\times 3$. A. *Dendrobates abditus*, adult male from Ecuador (AMNH 89603, holotype). B. *Dendrobates opisthomelas*, male nurse frog (AMNH 102589) with tadpole, from Represa de Santa Rita, Antioquia, Colombia.

Dendrobates opisthomelas (fig. 12B) is a small red frog, turning gray distally on limbs and mainly black on the venter (or chocolate brown *vide* Silverstone, 1975, p. 32); its skin is more granular than in *abditus* and *bombetes* (cf., figs. 2 and 12). It attains nearly as large a size as *bombetes* but seems to average smaller. The call of *opisthomelas* is a

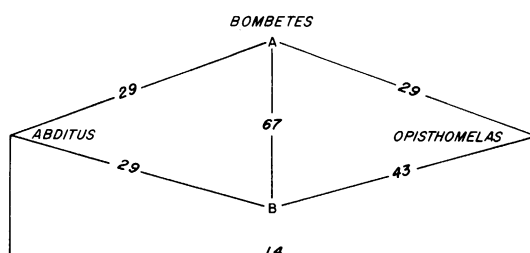


FIG. 13. Alkaloid-similarity values for paired samples in the *abditus-bombetes-opisthomelas* series of *Dendrobates*, as calculated by $100 C/N_1$ (where C = number of shared alkaloids, and N_1 = number of alkaloids in the population sample containing the smaller number). Sample sizes: *D. abditus* and *D. opisthomelas* each have 7 alkaloids and always represent N_1 ; *D. bombetes* populations A (Quebrada de la Chapa) and B (Lago de Calima) have, respectively, 17 and 15 compounds.

similarly loud buzz but it differs in some important respects from that of *bombetes* (see text with fig. 9). In our experience, *opisthomelas* is also a much more secretive frog than *bombetes* and usually calls from concealed places in leaf litter, or at bases of terrestrial bromeliads or under overhanging roots etc. It occurs in montane forest in the northern parts of the Cordillera Occidental and Cordillera Central of Colombia (see Silverstone, 1975, for known localities and map).

A total of 30 alkaloids has been found in the skin secretions of *Dendrobates abditus*, *D. bombetes*, and *D. opisthomelas*. Except for three unclassified compounds occurring only in trace amounts, all are tentatively assignable to the pumiliotoxin-A and (hydroxy)pumiliotoxin-C classes—a restriction in basic ring structures shared by most other *minutus*-group species (unpubl. data) and therefore not particularly informative for the present subgroup. Curiously, *D. bombetes* contains over twice the number of alkaloids (15 or 17 per population) found in samples of *abditus* and *opisthomelas* (seven alkaloids each), although, as stated earlier, *opisthomelas* is much more toxic than *bombetes* (toxicity of *abditus* not tested). Alkaloid 323B (allopumiliotoxin B) is the only compound detected in all three species. Consid-

eration of shared alkaloids gives results consistent with the "nearest neighbor" effect previously analyzed for populations of *D. histrionicus* and sibling species (Myers and Daly, 1976a). The geographically distant *abditus* and *opisthomelas* share only one alkaloid, compared with the two or three alkaloids that each shares with the intermediate *bombetes* (fig. 13). In this case, however, such results might be influenced by the large pool of alkaloids in *bombetes*, which intuitively might seem to make it more likely that the terminal species would share more compounds with *bombetes* than with each other.

The centrally situated *Dendrobates bombetes* probably has a more primitive color pattern (dorsally striped and ventrally pale with dark reticulum) than the southern *abditus* or northern *opisthomelas*. The relationships of the three species among themselves are not clear but are likely to become better understood with the discovery of additional species in poorly collected intervening areas. The discovery of *bombetes*, and collection and examination of most *minutus*-group tadpoles, now allows a reasonable choice between previously posed hypotheses concerning separate lowland versus highland origins of the widely separated *D. abditus* and *D. opisthomelas* (Myers and Daly, 1976b, pp. 11–12). We now propose the *abditus-bombetes-opisthomelas* series as probable remnants of a formerly widely dispersed (or dispersing) montane stock, the ultimate origin of which has yet to be sought.

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Lago de Calima population of *Dendrobates bombetes*, and with Mr. Michael G. A. Hill, who helped us look for the related *Dendrobates opisthomelas* in the mountains near Medellín.

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APPENDIX

The following four new dendrobatid toxins are to be added to the 90 alkaloids previously catalogued for species of *Dendrobates* (Daly et al., 1978). Alkaloids are designated by molecular weight in boldface type, with an added code letter where needed. Method of presentation of accompanying data is explained in Daly et al. (1978, p. 167).

217. C₁₅H₂₃N, 0.40, 166°, m/e 217(2), 216(3), 152(C₁₀H₁₈N, 100). H₆-derivative, m/e 223, 152. Possibly a member of the pumiliotoxin-C class, although the compound does not appear to form an N-acetyl derivative.

Major in *D. bombetes* (Lago de Calima), *D. opisthomelas* (Colombia, Represa de Santa Rita), and *D. tricolor* (Ecuador, 16 km. W Santa Isabel); trace in *D. reticulatus* (Peru, Mishana on Río Nanay). [Abbreviated localities are sufficient for identifying voucher specimens in American Museum amphibian collection.]

247. "C₁₆H₂₅NO," —, 175°, m/e 247(5), 246(3), 230(5), 170(25), 166(25), 150(40), 109(100), 70(50). H₄-derivative, m/e 251, 232, 111, 70. Probably an unsaturated analog of **251F**. Known only as trace compound in *D. bombetes* (Lago de Calima).

251F. C₁₆H₂₉NO, 0.25, 184°, m/e 251(20), 250(23), 236(10), 234(3), 222(C₁₄H₂₄NO, 13), 220(C₁₅H₂₆N, 25), 194(C₁₂H₂₀NO, 30), 166(10), 164(C₁₁H₁₈N, 14), 152(C₁₀H₁₈N, 32), 150(C₁₀H₁₆N, 16), 112(40), 111(C₇H₁₃N, 100), 98(C₆H₁₂N, 52), 70(15). H₀-derivative. O-acetyl derivative. Possibly in the pumiliotoxin-A class, although the major fragments at 111 and 98 and the lack of a *major*

fragment at 70 are not typical of this class. Known only in *D. bombetes*, as both a major (Lago de Calima) and trace (Quebrada de la Chapa) compound.

"265" (in Daly et al., 1978, p. 172) becomes **265A** because of following addition:

265B. $C_{17}H_{31}NO$, 0.28, 193°, m/e 265(18), 264(22), 250(12), 236(17), 234(20), 194(16), 166(25), 152(10), 126(30), 125(100), 112(28), 70(20). H_0 -derivative. Possibly of the pumiliotoxin-A class, but it has an anomalous mass spectrum and appears to be a higher homolog of **251F** (*vide supra*). Known only in *D. bombetes*, as a minor (Lago de Calima) or trace (Quebrada de la Chapa) compound.

The following entry replaces "*Dendrobates* sp: An undescribed species" in the section *Sources and Occurrence of Alkaloids* in Daly et al. (1978, top of p. 183):

Dendrobates bombetes

(A) Quebrada de la Chapa, Valle, Colombia, Feb. 1974, 7 skins, 0.4 g. Major alkaloid: **251D**. Minor alkaloids: **219B**, **223C**, **233**, **239B**, pumiliotoxin B's (**323A**, **B**). Trace alkaloids: **223A**, **B**, **231B**, **251F**, **265B**, **285D**, **307A**, and isomers of **239A**, **B**, **C**. [This is the same sample as reported in Daly et al. (1978, p. 379) with one correction and two additions: Alkaloid "379" is to be deleted, as it has been recently found to be a laboratory-conversion product of pumiliotoxin B (**323A**). Alkaloids **251F** and **265B**, which occur only in trace amounts in this population, were not previously recognized as new compounds.]

(B) Mountains above south side Lago de Calima, Valle, Colombia (type locality), Nov. 1976, 10 skins, 0.6 g. Major alkaloids: **251D**, **251F**, **217**. Minor alkaloids: **233**, **239B**, **243**, **265B**, pumiliotoxin B's (**323A**, **B**). Trace alkaloids: **219B**, **231A**, **B**, **247**, **257B**, **285D**. [A second sample, also 10 skins of 0.6 g., yielded the same results.]

It also is appropriate to list the following recently described species (Myers and Daly, 1979), which contained no new alkaloids:

Dendrobates silverstonei

Cordillera Azul, Huánuco, Peru (type locality), Nov. 1974, 4 skins, 2.0 g. Major alkaloids: Allo-

pumiliotoxin B (**323B**), **251D**, **205**. Minor alkaloids: **265**, **307C**.

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