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A NEW SPECIES OF POISON FROG (*DENDROBATES*) FROM ANDEAN ECUADOR, INCLUDING AN ANALYSIS OF ITS SKIN TOXINS

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In 1973, William E. Duellman called our attention to four specimens of a small poison frog that had been collected in Ecuador, by a University of Kansas field party working under his supervision. These specimens represented a previously unknown species, and we therefore visited the locality ourselves to obtain samples of skin toxins for an ongoing biochemical survey of dendrobatid frogs. The new species is described here on the basis of specimens preserved in The American Museum of Natural History (AMNH) and The University of Kansas Museum of Natural History (KU).

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Dendrobates abditus, new species

Figures 1-3

Holotype.—AMNH 89603 (field no. CWM 12356), an adult male obtained by John W. Daly and Charles W. Myers on February 16, 1974, in lower montane rain forest at 1700 meters elevation, southwest of the Río Azuela bridge on the Quito-Lago Agrío road,

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eastern base of Volcán Reventador, Napo Province, Ecuador (latitude 0° 05' S, longitude 77° 37' W).

Paratypes.—Eleven specimens, all from the type locality, as follows: AMNH 89604-89610 [89608-09 cleared and stained; 89610 skinned carcass], same data as holotype. KU 143090-143093, collected October 21-24, 1971, by Joseph T. Collins and William E. Duellman.

Etymology.—The specific epithet is the perfect participle of the Latin *abdo* and means something that is removed or secret, in allusion to the apparent isolation of this highland species from all other *Dendrobates*.

Definition and Diagnosis.—A small *Dendrobates* attaining a snout-vent length of about 18 mm. Body and limbs overall blackish or dark bronzy brown except for bright orange spots in region of axilla and groin. Teeth absent; omosternum present; first finger shorter than second; tarsal tubercle absent or weak. Skin alkaloids belonging to the pumiliotoxin-A class. *Dendrobates abditus* is readily distinguished from all other dendrobatids in having the body and limbs uniformly dark above and below, with orange axillary and inguinal flash marks being the *only* markings (Fig. 1).

Description (12 specimens).—Size small, snout-vent length (SVL) to 17.7 mm, with females being larger on the average than males: three adult males 16.3-17.3 mm SVL (\bar{x} = 16.73); four adult females 17.3-17.7 mm SVL (\bar{x} = 17.60); five other paratypes are unsexed juveniles 10.4-11.9 mm SVL (\bar{x} = 11.34). Well-developed vocal slits and shallow subgular vocal sac present in adult males. Enlarged ova present in the smallest and largest adult females.

In life, both juveniles and adults with vivid flash marks of metallic, golden orange in region of axilla and groin: "axillary" mark situated on posterior side of proximal one-half to two-thirds of upper arm; "inguinal" mark situated anteroventrally on proximal part of thigh; neither mark extends onto body. No other markings with all dorsal and ventral surfaces, including hands and feet, being otherwise uniformly dark bronzy brown (the slight metallic cast being produced by innumerable tiny specks of bronze, but in poor light specimens appeared black or dark brown). Iris very dark brown, little contrast between it and pupil. Color in preservative black, with white flash marks. [Description of live frogs based on Duellman's notes and color transparencies of KU specimens, and Myers' notes and transparencies of AMNH specimens.]

Skin nearly smooth on some individuals (*e.g.*, AMNH 89604), on others weakly or moderately granular on dorsum of body and venter (*e.g.*, holotype); top of head and/or snout finely pitted in a few individuals including holotype. Head about as wide as body, but body distinctly wider in one rotund female (AMNH 89604: head 5.7 mm, body 8.5 mm); widest part of head between outer

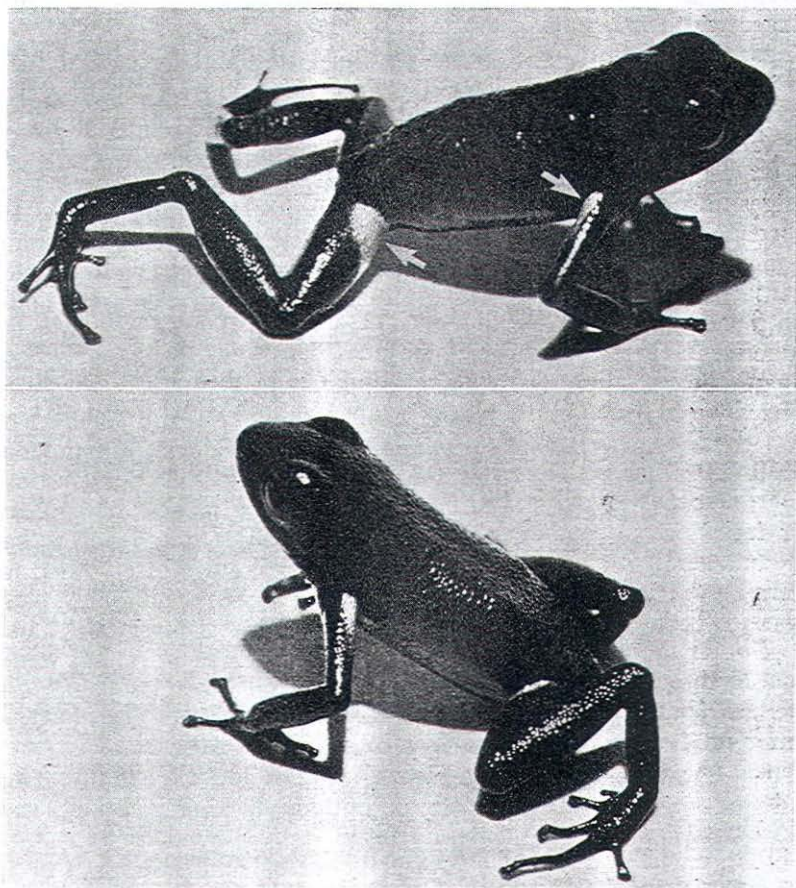


FIG. 1.—*Dendrobates abditus*, new species. Two views of the holotype in life, $\times 3.4$. Arrows denote the golden orange flash marks, which are the only markings on a frog that is otherwise unicolored dark bronzy brown above and below.

edges upper eyelids. Eyes prominent, nearly equal to length of snout. Snout short, sloping, truncate in dorsal or ventral aspect, and rounded in lateral profile. Naris directed laterally; both nares visible from front, barely visible from below, but not visible from above; nares much closer to tip of snout than to eye. Canthus rostralis rounded; loreal region flat or very slightly concave and nearly vertical. Tympanum a vertical oval, rather indistinct and no greater than one-half area of eye; not visible posterodorsally (where tympanic ring is subcutaneously overlain by anterior edge of *m. depressor mandibulae*).

Relative lengths of appressed fingers $3 > 4 \cong 2 > 1$, all except first with distinct discs expanded about one and one-half to twice

finger width in adults. Disc of first finger small, little wider than finger; disc of third finger 1.33-2.00 times wider than distal end of adjacent phalanx—smallest in juveniles, largest in adult males (disc width/finger width in five juveniles 1.33-1.67, mean 1.53; four females 1.60-1.75, mean 1.68; three males 1.80-2.00, mean 1.93). First finger more or less three-fourths length of appressed second finger; disc of first finger nearly reaching disc of second in a few specimens. A large medial (outer metacarpal) tubercle at base of palm, a smaller and less prominent (often indistinct) inner metacarpal tubercle, and one or two prominent subarticular tubercles on fingers (one each on fingers 1, 2, and 4, two on finger 3); all tubercles low, with rounded surfaces. Hind limbs of moderate length, heel of appressed limb reaching eye in all but one specimen (reaching between shoulder and tympanum in AMNH 89604, which has the lowest of following tibial ratios); tibia/snout-vent length = 0.41-0.45. Relative lengths of appressed toes $4 > 3 > 5 > 2 > 1$; first toe very short, only reaching base of second, with distinct or indistinct unexpanded disc; other toes with distinct, slightly expanded discs. A weak, obliquely elongated keel-like ridge discernible on inner side of most tarsi; tarsal tubercle (proximal end tarsal ridge) usually absent, or but weakly developed; small inner and outer metatarsal tubercles (outer one often more prominent); one to three often indistinct (especially on toe 4) subarticular tubercles (one each on toes 1 and 2, two each on 3 and 5, two or three on 4). Palms and soles fleshy. Digits of hands and feet flattened on bottom; lacking webbing, supernumerary tubercles, or lateral fringe.

Dorsal bones of skull not fused or sculptured; palatine bones absent; omosternum present; eight presacral vertebrae, the dorsum of each flattened and laterally expanded to form a small shield; no fusion of vertebrae or presacral shields (osteological description based on AMNH 89608 and 89609, cleared and stained females). Teeth absent. Muscle tissue of skinned specimens blackish, especially before preservation.

Measurements of Holotype (in mm).—The undissected holotype is the largest male in the type series and is considered to be sexually mature because of its size and well-developed vocal slits. Length from snout to vent 17.3; tibia length from heel to fold of skin on knee 7.3; greatest width of body 5.3; greatest head width (between outer edges upper eyelids) 5.5; head length from tip of snout to angle of jaws 5.2; tip of snout to center of naris 0.9; center of naris to edge of eye 1.5; diameter of orbit 2.1; horizontal diameter of tympanum (posteriorly indistinct) > 1.0 ; distance between centers of nares 2.4; length from proximal edge of large palmar tubercle to tip of longest (3rd) finger 4.5; width of disc of third finger 0.8; width of finger (penultimate phalanx) below disc 0.4.

Tadpole.—AMNH 89602 (one), referred to this species (see

Natural History), is a large free-living larva 7.5 mm head-body length, 20 mm total length, seemingly past stage 25 (because of size, but limb buds not detectable on damaged body [died before preservation]). Eyes and nostrils dorsolateral; spiracle sinistral and low; tail fins (damaged) narrow. Mouth (badly damaged) directed anteroventrally; beak massive, with serrated cutting edges, and median indentation on lower edge; tooth rows 2/3, about equal in length, with second upper row widely broken medially and first lower row narrowly broken medially; oral disc indented laterally (?); lower edge of oral disc with broad median gap in a single row of small papillae (upper edge not papillate).

Natural History.—The type locality is a forested ridge at 1700 meters elevation,³ a short climb southwest from the Río Azuela bridge (1650 m) on the gravel road from Quito to Lago Agrio. The ridge forest has a relatively low canopy (perhaps 15-20 m) and is exceedingly dense in most places, with close-spaced trees, treelets and saplings, and abundant low herbaceous plants and ferns. Many of the trees are buttressed; palms are present but not abundant. This forest is evidently evergreen and is cool and very damp, with a conspicuous moss layer and many epiphytes on the tree trunks. Ground bromeliads are present although not abundant; the brown soil bears a thin litter layer. We experienced a hard, drenching rain during the afternoon of February 16, 1974 (our only day at the site), and Duellman's party also experienced hard rains in October, 1971. The forest, which is being cleared in the vicinity of the road, is physiognomically classifiable as Lower Montane Rain Forest. Grubb *et al.* (1963) described and illustrated similar forest at an equivalent elevation (1710 m) in this zone, at a site about 40 km to the south-southwest.

All specimens of *Dendrobates abditus* were caught by day, on or close to the ground, in the forest and in adjacent new clearings. Two specimens were under a piece of tin, others were active amongst ground litter of leaves and sticks, and one specimen was coming out of a terrestrial bromeliad that contained a large tadpole tentatively assigned to this species (see below). The frogs attempted to escape capture by hopping into holes in bases of trees or by going into the ground litter. None was observed to call, but a short series of soft, closely spaced "tink-tink-tink-tink" notes was thought possibly to belong to this species. This call was given infrequently and had little carrying power; it was heard coming from beneath mats of litter and rootlets and once seemed to be emitted from a collecting bag.

³ Duellman obtained a reading of 1740 m at a camp nearly 50 m lower than this ridge. The difference (about 90 m) between our readings is insignificant, inasmuch as changes in atmospheric pressure frequently cause uncorrected altimeters to fluctuate ± 100 m at this altitude.

The only other dendrobatids that have been found at this locality are two forest-dwelling (*i.e.*, non-streamside) species of *Colostethus*: One species, *C. taeniatus*, is common; the other, a larger, less common frog, represents a new species currently being described by Stephen R. Edwards. A single dendrobatid tadpole found in a ground bromeliad (also containing an adult *Dendrobates*) must be assigned to *Dendrobates abditus* rather than to *Colostethus*, the larvae of which are usually deposited in small streams rather than standing water. The tadpole differs notably from descriptions of known larvae of *Colostethus*, including the two species occurring at this locality (Edwards, 1974), in having a broad median gap in the papillate fringe on the lower edge of the oral disc.

SKIN TOXINS

The unique skin alkaloids of dendrobatid frogs have been recently reviewed and grouped into three classes of compounds, with a number of individual alkaloids being left unclassified owing to a lack of data (Myers and Daly, 1976). Two of the three classes, histrionicotoxins and pumiliotoxins, were recognized as occurring within the genus *Dendrobates*.⁴ Now, after the accumulation of new data, the pumiliotoxins can be further subdivided, and some 80 *Dendrobates* alkaloids can be tentatively distributed among four classes of structurally related compounds. Most or all of the detectable alkaloids of *Dendrobates abditus* belong to the pumiliotoxin-A class, which is to be defined elsewhere (Daly *et al.*, MS).

Three paratypic specimens of *Dendrobates abditus* (AMNH 89608-89610) were skinned on the date of capture for subsequent toxin analysis. The three complete skins, which had a combined wet weight of 180 mg, were extracted in methanol. The extracts were fractionated and the alkaloid fraction analyzed by thin-layer chromatography, electron impact and chemical ionization mass spectrometry, gas chromatography, and combined mass spectrometry-gas chromatography. These procedures were explained in detail in Myers and Daly (1976). Mass spectral analyses of the major alkaloids, after separation by thin-layer chromatography (Fig. 2), provided independent confirmation of the results of combined gas chromatographic-mass spectral analysis of a sample of the unresolved alkaloid fraction (Fig. 3). Seven alkaloids were identified from the skin extracts of *D. abditus* and are listed below in order of their quantitative importance.

⁴ The third class, batrachotoxins, is known only from the true poison-dart frogs, genus *Phyllobates* (*sensu stricto*), which we are presently investigating. Batrachotoxins seem biosynthetically unrelated to other dendrobatid toxins, and are characterized by a steroidal ring structure, great toxicity, and different pharmacological properties.

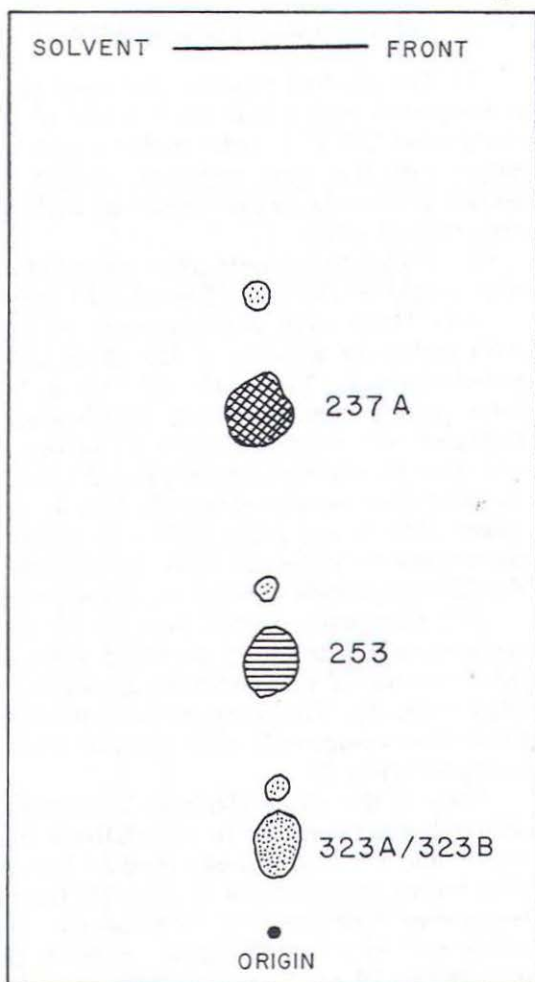


FIG. 2.—Representation of a thin-layer chromatoplate of alkaloids from a population sample of *Dendrobates abditus*, new species (three paratopotypes, February, 1974). A sample of 10 μ l of methanolic alkaloids equivalent to the amount in 10 mg wet skin was applied at the origin. For detailed methodology, see Appendix 1 in Myers and Daly (1976).

Numbers are molecular weights of the principal alkaloids, as determined by chemical ionization mass spectral analysis of material extracted from the corresponding spots; the trace alkaloids (Fig. 3) were inadequately represented for localization by this technique. Code letters differentiate alkaloids from other known dendrobatid toxins of the same molecular weight (Daly *et al.*, MS), and numbers separated by solidus indicate presence of two alkaloids in the same spot. Spot intensities are depicted as follows: Cross-hatched pattern = large amounts of alkaloids; horizontal pattern = moderate amounts; dots = small amounts (reference standards are Figs. 1 and 2 in Myers and Daly, 1976).

1) The alkaloid fraction contained relatively large amounts of a compound with a molecular weight of 237 (Figs. 2 and 3); it is designated "237A" in order to distinguish it from other dendrobatid toxins with the same molecular weight (Daly *et al.*, MS). The empirical formula, as determined by high resolution mass spectrometry, is $C_{15}H_{27}NO$.

2) Moderate amounts were present of an alkaloid with a molecular weight of 253, and a formula of $C_{15}H_{27}NO_2$ (Figs. 2 and 3).

3-4) There were small amounts of a mixture of two alkaloids with molecular weights of 323 (Figs. 2 and 3). One of these is pumiliotoxin B (323A); the other is an isomer (323B) that separates poorly from coexisting pumiliotoxin B. These compounds migrated only a short distance on the one thin-layer chromatoplate run from *D. abditus* extract (Fig. 2), and therefore gave a lower R_f value than usually obtained. But, as we have emphasized elsewhere (Myers and Daly, 1976), R_f values do vary and are not in themselves an adequate basis for alkaloid identification. Both of the 323 compounds have an empirical formula of $C_{19}H_{33}NO_3$.

5-7) Computer-assisted analysis by combined gas chromatography-mass spectrometry provided evidence for the presence of trace amounts of three additional alkaloids, namely 237B, 265, and 281A (Fig. 3). The amounts present were insufficient to identify these trace compounds with spots or areas on the thin-layer chromatoplate (Fig. 2).

None of the above alkaloids is unique to *Dendrobates abditus*, although most seem to be of relatively limited occurrence in the genus, and no other species studied has an identical spectrum of skin toxins. Pumiliotoxin B (323A), however, is an exception in being very widespread in *Dendrobates*. Since its initial discovery (Daly and Myers, 1967) in *D. pumilio*, this compound has been detected in 12 of 18 species studied to date; it often occurs as a major compound. With the possible exception of the trace compound 265, all the alkaloids of *D. abditus* belong to the pumiliotoxin-A class. The unclassified 265 substance shows a very atypical mass spectrum for a dendrobatid toxin and might prove to be a degradation artifact of some other alkaloid (Daly *et al.*, MS).

For a species that elaborates primarily pumiliotoxin-A class alkaloids, *Dendrobates abditus* produces skin toxins in relatively "large" amounts (as measured in micrograms per 100 mg of skin)—at least in the one population sampled by us. The areas represented by the various peaks in the gas chromatogram (Fig. 3) are roughly proportional to the total amount of each of the corresponding alkaloids. The mean weight of three *abditus* skins was 60 mg. However, dendrobatids vary greatly in size; therefore, it is convenient to extrapolate comparisons to a standard of 100 mg of skin. A rough estimate of the amounts of alkaloids contained in an extract equivalent to 100 mg of *abditus* skin was obtained by comparison

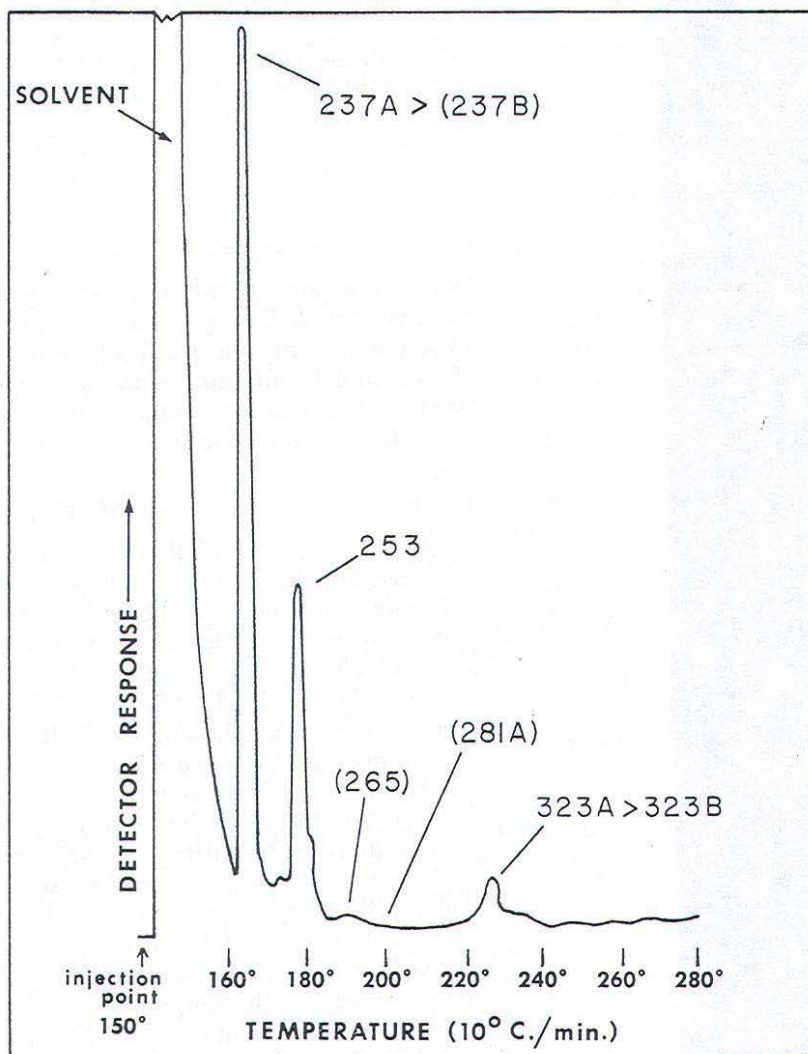


FIG. 3.—Gas chromatogram of alkaloids from a population sample of *Dendrobates abditus*, new species (three paratopotypes, February, 1974). Chromatography was run with 2 μ l of methanolic extract containing concentrated alkaloids equivalent to amount in 2 mg of wet skin.

The alkaloids are designated by their molecular weights; trace compounds are included within parentheses. The corresponding protonated molecular ions of these alkaloids were identified in separate computer-assisted analysis by combined gas chromatography-mass spectrometry. For details of instrumentation, see Appendix 2 in Myers and Daly (1976).

of the gas chromatographic peaks with the area of a peak of a known amount of histrionicotoxin. This estimate provides the following quantitative breakdown of the principal skin toxins of *Dendrobates abditus*:

Alkaloid (Molecular Weight)	Estimated Amount (μg) per 100 mg Skin	Percent of Total
237A	115	71
253	34	22
323A	8	5
323B	3	2
	Total 160 μg	100%

For comparison: *Dendrobates histrionicus* (Guayacana, Colombia) contains about 230 μg of alkaloids per 100 mg of skin; *D. pumilio* (Bastimentos, Panamá) 200 μg ; *D. auratus* (Isla Taboga, Panamá) 150 μg ; and *D. minutus* (Cerro Campana, Panamá) only 40 μg . Of these, *Dendrobates minutus* is the only species that is related to *D. abditus* at the species-group level (see below).

DISCUSSION

Although intrafamilial relationships and generic partitioning of dendrobatid frogs are in a confused state, the new species is clearly a *Dendrobates*, both in the restricted sense of Silverstone (1975) and in our own broader view (Myers and Daly, 1971, 1976). *Dendrobates abditus* is most readily assignable to the *minutus* species group as defined by Silverstone (1975:27). We would also add the recently described *Dendrobates viridis* Myers and Daly (1976) to the *minutus* group; it is a small, uniformly green species from western Colombia. Thus far we have obtained toxin samples of only four of the eight species now included in the *minutus* group—namely *abditus*, *fulguritus*, *minutus*, and *viridis*. Consequently, it is premature to speculate on the usefulness of biochemical data in defining the *minutus* group or in clarifying relationships among the contained species. It is worth noting, however, that skin extracts of the four species studied contained alkaloids only of the pumilio-toxin classes,⁵ with compounds of the pumiliotoxin-A class being qualitatively and quantitatively the most important. The less toxic histrionicotoxins have not been detected in the *minutus* group to date, although both classes of alkaloids occur within some natural assemblages of *Dendrobates* (e.g., the *histrionicus* group).

Among species of *Dendrobates* that produce primarily pumiliotoxin-A class alkaloids, *Dendrobates abditus* differs from most of the

⁵ Excluding a presently unclassified alkaloid from *Dendrobates abditus* (compound 265) and another from *D. minutus* (compound 267B, Daly *et al.*, MS).

others in that it contains relatively large amounts of skin toxins, and is therefore relatively more poisonous than most other species of similar size. However, we have shown elsewhere (Daly and Myers, 1967) that degree of toxicity may be geographically variable within a species; that this phenomenon occurs in the *minutus* group is evidenced by one Colombian sample of *D. minutus* that contained fairly large amounts of an alkaloid (alldihydropumiliotoxin B) of the pumiliotoxin-A class. Relative "toxicity" (as determined by bioassay) presumably is related in some way to relative differences in distastefulness (or other buccal effects) to predators and may be under selective control, but this has not been demonstrated and the significance of intraspecific variation in skin toxins is uncertain.

Dendrobates abditus is known only from its type locality on the eastern base of Volcán Reventador, a still-active Quaternary volcano on the Amazonian flank of the Andes (erupted as recently as 1960 *vide* Grubb *et al.*, 1963). The elevation (1700 m) of the type locality indicates that *abditus* is a strictly montane species; some primarily lowland species of *Dendrobates* do have elevational ranges that, in places, exceed 1000 meters, but none of these is known above 1300 meters (*e.g.*, see Silverstone, 1975:12). *Dendrobates abditus* therefore is probably isolated from various congeners that occur in the adjacent Amazonian lowlands, including *D. parvulus*, *D. pictus*, *D. trivittatus*, and *D. quinquevittatus* (only the last is considered a *Dendrobates* by Silverstone, 1975). Of these, *Dendrobates quinquevittatus* was tentatively placed in the *minutus* group by Silverstone (1975:34) and appears to be the geographically closest species-group relative of *D. abditus*. It differs most notably from *abditus* in having a boldly patterned dorsum and venter, in lacking axillary and inguinal flash marks, and in having noticeably larger finger discs. Because highland isolates may be derived from lowland ancestors, these two species will merit closer comparison, especially when the skin toxins and tadpole of *quinquevittatus* become known. At this time, however, we would only call attention to a singular correspondence between *D. abditus* and *D. opisthomelas*, a geographically remote member of the *minutus* group from the Andes of northwestern Colombia: The damaged mouthparts of an *abditus* tadpole, when carefully repositioned with fine teasing needles, could be seen to match almost exactly Silverstone's (1975:32) illustration for *opisthomelas* (compare our description with his figure 20). The points of resemblance include a broad, median gap in the papillate fringe on the lower edge of the oral disc; this break in the papillate fringe was, according to Silverstone (*loc. cit.*), heretofore "unique [to *opisthomelas*] among known tadpoles of *Dendrobates*."

Present evidence seems insufficient to allow for a reasonable choice between hypotheses of lowland versus highland ancestry of

Dendrobates abditus. In the former case, *abditus* presumably would represent a highland invasion separate from that of the geographically remote *opisthomelas*. In the latter case, these two species might be terminal relicts of an ancestor whose range was fragmented by climatic-vegetational changes that occurred in the Andes during Quaternary time. In either case, it seems likely that additional kinds of small *Dendrobates* remain to be found, inasmuch as five of eight species now comprising the *minutus* group were discovered within the last decade. Each new species, and additional data on those already known, can be expected to contribute toward a solution of the biogeographic puzzle.

RESUMEN

Se describe *Dendrobates abditus*, una nueva especie de ranas venenosas obtenida en la base del Volcán Reventador, Provincia del Napo, Ecuador, a una elevación de 1700 metros. El habitat es el bosque lluvioso montañoso bajo, del lado amazónico de los Andes orientales. Es una rana diminuta (16-18 mm) que se puede distinguir de otros dendrobátidos por medio de su coloración. La especie se caracteriza por una coloración dorsal y ventral uniformemente negruzca o pardo oscura, con manchas de color anaranjado brillante en la axila y en la ingle. Se asigna esta ranita al grupo *minutus* (*sensu* Silverstone, 1975) del género *Dendrobates*. El renacuajo de *D. abditus* tiene piezas bucales notablemente similares a las de *D. opisthomelas*, una especie del mismo grupo restringida a los Andes de Colombia septentrional. *Dendrobates abditus* produce en su secreción cutánea solamente alcaloides de la clase pumiliotoxina-A.

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