

Observations on the reproduction and ecology of the tropical montane toad, *Bufo holdridgei* Taylor in Costa Rica

by

Richard M. Novak* and Douglas C. Robinson**

(Received for publication June 30, 1975)

ABSTRACT: Reproductive behavior of the mute, tympanumless montane Costa Rican toad, *Bufo holdridgei* is described and discussed. The species has one of the smallest egg complements and one of the largest egg sizes reported for the genus. Eggs and larvae are described, and limited ecological observations reported. Size, pigmentation and number of eggs suggest less relationship with the monotypic genus *Crepidophryne* than has been postulated by some authors.

Bufo holdridgei was described from a single specimen, "...collected at an elevation of approximately 7500 ft on Volcán Barba¹, western slope,..." in Costa Rica by TAYLOR (26). Figures were included, and later (27) a photograph of the same specimen but no additional information. SAVAGE (20) described the type locality as being, "...near FINCA GEORGINA, western slope Volcán Barba (Pacific drainage) leading to the Desengaño pass above Los Cartagos. Lower Montane Pluvial bioclimate." The only other reported locality for the species is, "S slope Volcán Barba, Alto del Roble, 0.4 km N Cerro Chompipe, 2020-2040 m." (18). This is also stated to be in Lower Montane Pluvial bioclimate (20). The present investigation was conducted at the latter locality.

Bufo nids thus far known from the Costa Rican and Panamanian montane regions which share with *B. holdridgei* varying degrees of reduction or loss of auditory and/or vocalization structures are *Bufo fastidiosus*, *Bufo periglenes*, *Bufo peripatetes*, the monotypic *Crepidophryne epioticus*, several species

* Grinnell College, Grinnell, Iowa 50112, U.S.A.

** Escuela de Biología, Universidad de Costa Rica, Ciudad Universitaria, Costa Rica.

¹The names of both the town and volcano were designated as Barva by official decree on October 4, 1974.

of *Atelopus*, and *Rhombophryne acrolopha* (17, 18, 21, 19, 29). Savage concluded that *B. holdridgei*, *B. peripatetes* and *B. fastidiosus* are closely allied, in part on the basis of the lack of external and middle ear structures and on the presence of hands and feet forming fleshy pads. He mapped the localities for these and other species (18).

All additional references and a color photograph of *B. holdridgei* may be found in the compendium volume "Evolution in the Genus *Bufo*" (2). R. MARTIN (13) describes some osteological characteristics and concludes that the species cannot be conveniently placed in any grouping of toads on osteological evidence, accordingly assigning it to its own group. He notes, "There is general ecological and osteological similarity [of *B. periglenes*] with *B. holdridgei* but little additional information of real systematic value". He further states, "Morphological characters of *Crepidius* [= *Crepidophryne*] argue strongly for its retention in a separate taxon, but speculation may be entertained about the derivation of this genus from *B. holdridgei* or and ancestor." (sic). The skull of *B. holdridgei* is illustrated.

BOGART (6) examined the karyotype and states, "No hybrid evidence is available for this species. *Bufo holdridgei* could be derived from a number of different lines."

BLAIR (3, 5) notes the hypertrophied testes which he believes to contain some compound, as yet unidentified, that prevents fertilization or normal development of the eggs in attempted crosses with other species of *Bufo*. The testes of *B. periglenes* are not hypertrophied and do not inhibit fertilization. BLAIR (4) suggests that *B. holdridgei* possibly belongs to the *guttatus* group, presumably on the basis of the fertilization-inhibiting substance of the testes.

W. MARTIN (14) reports atrophy of the sound receiver apparatus and the laryngeal vocal structures in *B. holdridgei* and *Bufo rosei*. Both species retain the arytenoid valve and opercular muscle and cartilage, but all other structures are absent (*B. holdridgei*) or reduced (*B. rosei*).

In this paper, we offer data and observations on the reproduction and ecology of *B. holdridgei* which we attempt to relate to its morphological peculiarities, distribution and relationships.

MATERIAL AND METHODS

In the laboratory, amplexus and ovulation were induced in *B. holdridgei* using small doses of *Bufo marinus* or *B. holdridgei* pituitary. Success, obtained within 24 hours, cannot be directly attributed to the treatment since the toads were in an incipient breeding state. Egg complements were obtained from paired toads maintained in plastic bags at the breeding site and in jars at the study locality. Ovarian eggs were counted in preserved, ripe females in order to confirm the validity of the counts obtained from pairs. Eggs and tadpoles were reared through metamorphosis under various conditions of temperature in both the field and laboratory. These were preserved in 5% formalin. Eggs

were measured with an ocular micrometer to an accuracy of 0.01 mm. Larvae and adults were measured with vernier calipers to 0.1 mm accuracy. Eggs and larvae were staged according to the system of LIMBAUGH and VOLPE (12).

At two breeding puddles toads were counted and sexed twice daily, and their toes clipped to allow subsequent recognition. Berleze funnel samples were obtained from juvenile toad habitat ground litter, and stomach contents of toads compared with these samples. Vegetation samples were identified to characterize the habitat. Air and water temperatures were taken at intervals, and a rain gauge was installed at the study locality during the period of intensive observations and read twice daily, in the early morning and late afternoon.

A total of 21 trips were made to the study site at Alto del Roble between March 22 and June 29, 1975. Observations were made on 23 days. Novak spent seven uninterrupted days at the locality from April 19 through April 25, during the height of breeding activity at the study puddles.

HABITAT

Montane rain forest caps Volcán Barva's 2906 m summit, surrounded by a lower belt, the lower montane rain forest. To the north and east, this is bordered by premontane rain forest and to the south and west, by lower montane wet forest (28). *Bufo holdridgei* has only been recorded from lower montane rain forest. At the study locality, they were observed between 1920 and 2040 m elevation (Fig. 1).

Characteristic plants of the area include oaks, *Quercus* sp., tree ferns of the genus *Alsophila*, and the bamboo *Chusquea Lechmanii*. The cleared areas are overgrown with the rush *Juncus effusus* (Juncaceae), and various grasses. Plants found around the breeding sites which are indicators of the habitat include *Disterigma Humboldtii* (Ericaceae), *Alchemilla pectinata* (Rosaceae), *Monnina* sp. (Polygonaceae), *Eupatorium anisochromum* (Compositae), *Didymopanax Pittieri* (Araliaceae), *Gunnera insignis* (Haloragaceae), *Prunella vulgaris* (Labiatae), *Rhynchospora vulcani* (Cyperaceae), *Lycopodium* sp. (Lycopodiaceae) and the two abundant ferns, *Alsophila quadripinnata* and *Blechnum sessilifolium*, which overhang many of the breeding puddles.

There are several precipitous streams, along which natural vegetation still stands, but most of the area represents secondary growth or has recently been recut. Many drainage ditches along the roadside, paths, and through fields serve as reproductive sites for *B. holdridgei*.

The general weather syndrome can be described as very humid, with frequent strong winds bringing moist air masses during the nights from the Atlantic slopes. The locality is on the Atlantic versant and the local streams form part of the headwaters of the Río Chirripó. The Finca Georgina locality pertains to the Río Grande de Tárcoles drainage, on the Pacific watershed.

Rainfall recorded from 1:15 PM on April 14 through 8:20 AM, April 28, averaged 8.7 mm per day and totaled 111.3 mm. Average night and day

rainfall over the intensive study period was 7.7 mm and 4.3 mm, respectively. Air temperatures recorded twice daily when toads were censused averaged 15.2 C with extremes of 8 C and 20 C. Average water temperature was identical during this period with extremes of 13 C and 19 C, being generally higher than ambient air temperatures in the morning, and lower in the afternoon.

REPRODUCTION

ONSET AND DURATION: Males and egg-laden females were excavated from mossy banks near suitable but dry breeding sites on March 22. On March 31, adult toads of both sexes were seen in the wet ditches but no amplexus observed. Freshly laid eggs were found on April 4, in standing water in a ditch, representing perhaps three or four complements. No adults were seen. By April 8, a large batch of eggs representing an estimated 30 complements was found in a single puddle, again with no adults present. On April 14, however, no new eggs nor adults were seen in the ditches. Many eggs, but no adults, were found in a different ditch on April 17, and on April 19, massive reproductive activity had commenced at many sites. At this time, two puddles were selected for intensive study, where it was possible to follow the initial, peak and diminishing reproductive activity described below. By April 26, activity had ceased at the study puddles, but continued unabated at other sites. The same conditions obtained on April 28 and May 5, but on May 11, only seven toads were observed near the breeding puddles which were filled with eggs and larvae. On May 18, most eggs had hatched and only one female was observed on the road. Nonetheless, on May 25, considerable reproductive activity and many fresh eggs were found, mostly at the western edge of the study zone. Only one puddle near the intensive study area contained a few toads. On June 1, three males were found in a puddle, and on June 11, and subsequently, no adults were found, nor had tadpoles metamorphosed. Tadpoles metamorphosed on and about June 29 in the study puddles.

We subjectively place the height of the 1975 mating period on April 20 or 21. In 1968, similar intensity was observed on April 21, and again, in 1969, on April 20, when 813 toads were counted in a puddle measuring approximately 6 m² (Site no. 3 in Fig. 1). Typical puddles are shown in Figs. 2 and 3.

CHRONOLOGY OF REPRODUCTIVE ACTIVITY IN TWO SELECTED PUDDLES: Activity had commenced in Puddle 1 on April 19, and it began in Puddle 2 on April 20. These are indicated in Fig. 1 and were separated by a distance of about 376 m. Puddle 1 was a concavity, roughly tear-shaped with steeply sloped edges, overhung by ferns and other vegetation. It was located near the path through an overgrown field and opened into a small clearing. The water was clear and the bottom laced with fern roots, together with decomposed vegetation and silt. Water volume was measured to be 10.65 liters. The depth was 13 cm, the width 24 cm, and the length 65 cm. Puddle 2 was on a

moderate incline in a roadside ditch at the foot of a bank and experienced occasional washouts during the study. It measured 4.5 cm in depth and 47.5 cm in average diameter. The edges were gradually sloped, the water clear and the bottom and borders consisted of silt, stones and sticks. Caddis fly larvae, dytiscid beetles and leeches were seen at both sites.

Data for the total number of toads, the number of new males, of resighted males and of females observed in each puddle are given in Table 1. A total of 555 males were seen at least once in Puddle 1, while only 29 females were recorded. In Puddle 2, 210 males were seen and 10 females. In this puddle, 34.6% of the males were observed but once (Fig. 5). Males averaged 32.5 hours from first to last appearance and averaged 2.1 appearances. One individual in Puddle 1 was observed sporadically over 156 hours. The data thus show that the majority of males abandon a puddle one or more times during courtship activity, one male in Puddle 1 having registered six absences between reappearances. Spot observation of other puddles revealed the presence of two marked toads approximately 30 m from their original puddle. Therefore absences of males from a study puddle do not imply their sexual inactivity.

The numbers of new males and of females at Puddle 1 were compared with a Spearman Rank Correlation test, giving a value of $r = 0.65$, supporting but not proving the idea that males and females respond to the same stimuli to migrate to the site. In both puddles, however, a considerable number of males arrived before the first females were observed, and unsurprisingly, lingered on after the last females were seen.

In Fig. 4 the total number of male toads, of repeat males and of first males for Puddle 1 are plotted together with the accumulated rainfall. A sharp increase in rainfall on April 22 elicited no positive response in toad numbers (in either puddle), but rather these continued to decline.

Occasional night checks revealed undiminished activity in the puddles, and there is no evidence suggesting that numbers of toads are related to time of day.

COURTSHIP BEHAVIOR: The amplexing impulse is very intense and males were frequently observed attempting to amplex sticks, stones and other items. On various occasions, numbers of male toads were observed amplexing individuals of *Rana vibicaria* or *Atelopus senex*, killing these in all instances by suffocation. Male *B. holdridgei* respond to the release vibrations of their own species and encounters between males although frequent are brief. Females, most of which succeed in reaching the breeding puddles prior to amplexus, are immediately besieged by males and thus have no opportunity to respond to these voluntarily. Never fewer than two, and as many as eight males would amplex a female forming a compact ball of toads similar to those described by GISLEN and KAURI (8) for *Bufo bufo bufo* in Sweden.

Two females were timed to remain in the water 9 and 28 minutes

TABLE 1

*Chronology of presence of B. holdridgei in two selected puddles
(April, 1975)*

Date	Hour	New*	Puddle 1		Total	Hour	New*	Puddle 2		Total
		♂ ♂	♂ ♂	♀ ♀			♂ ♂	♂ ♂	♀ ♀	
April 17, 1975	0930	0	0	0	0					
19	0930	44	0	0	44					
19	1530	84	8	4	96					
20	0930	90	31	3	124	0800	68	0	0	68
20	1045	58	15	3	76					
20	1600	41	41	1	83	1500	46	45	5	96
21	0805	16	37	2	55	0945	38	64	3	105
21	1430	66	51	7	124	1615	16	44	1	61
22	0815	38	42	2	82	0940	14	28	0	42
22	1500	39	39	4	82	1630	9	16	1	26
23	0820	39	37	0	76	0915	8	13	0	21
23	1430	20	56	3	79	1530	7	12	0	19
24	0745	16	40	0	56	0730	1	3	0	4
24	1415	4	28	0	32	1355	3	1	0	4
25	0800	0	6	0	6	0730	0	0	0	0
26	0950		0	0	0	0930	0	0	0	0
		555	431	29	1015		210	226	10	446

* "New" designates toads observed for the first time. No female was observed more than once.

respectively, beginning to lay eggs shortly after entering and abandoning the puddle as soon as oviposition was completed, leaving the trailing jelly ends of the egg strings attached to the bank. Isolated pairs of toads in captivity succeeded in ovipositing after prolonged amplexus of one or two days. Whether this delay was due to the inefficiency of single males or to the state of gravidness of the females was indeterminable. Amplexus in such cases was inguinal, contrary to pectoral (axillary) which is characteristic for the genus (25).

EGGS AND DEVELOPMENT

Oviposition was induced in the laboratory in four pairs and obtained in the field with 35 captive pairs. Ovarian egg counts were made on five preserved females. The numbers obtained through each method were comparable and the results treated as a single sample. The average number of eggs from the 44 observations was 74.6 (range: 45-137; SD = 21.4; SE = 3.3). Numbers of eggs and snout-vent lengths of 27 toads gave a Spearman Rank Correlation value for r of -0.21 indicating absence of correlation. Egg diameter, determined from measurements of 39 viable eggs in a freshly laid single strand averaged 2.76 mm (range: 2.66-3.08; SD = 0.07; SE = 0.01). Egg size of 42 eggs freshly preserved in five percent formalin and measured several days later gave significantly different results with an average of 3.26 mm (range: 2.93-3.51; SD = 0.16; SE = 0.02).

The eggs are deposited in two strings. The animal pole is black and the vegetal pole cream. Within the outer envelope of preserved strings there is a second crenated cylindrical envelope which encloses the eggs collectively. Within this, and closely applied to it is a third envelope surrounding each individual egg (Fig. 6). Living eggs show only minor proportional differences from the drawing.

A freshly laid egg sample maintained in a jar at the study location at an average temperature of about 15 C reached stage 19 about 289 hours after deposition, and stage 22 by the 20th day (430 hours). *Bufo valliceps* at 25 C reaches stage 32 at 289 hours, and stage 39 at 430 hours, taking 58.5 hours to reach stage 22 and 38 hours to reach stage 19 (12).

DESCRIPTION OF TADPOLE

On May 11, puddles were found packed with eggs and larvae in a wide range of developmental states. Stage 36, averaging 16.7 mm snout-tail-tip, was used for the following description and Figs. 8, 9 and 10.

The tadpole is typical of the genus as exemplified by *B. valliceps* (12) or *B. periglenes* (17) and conforms to the definition of Mexican species as given by ALTIG and BRANDON (1). Since six of the 10 species of *Bufo* known from Costa Rica remain undescribed a differential diagnosis is premature.

The anus is medial; oral disk not emarginate; papillary border of oral disk with both dorsal and ventral gaps, the former being the width of the

disk, and the latter approximately the width of the beak. There are four dorsolateral and three ventrolateral papillae on the border of each side; tooth row formula $\frac{2}{3}$; beak minutely serrated, the upper being slightly concave dorsally in some specimens. Spiracle sinistral with median wall attached to body along the longitudinal axis. Tail tip pointed, caudal fin rounded. The dorsal surface and flanks of the body, and the epaxial region of tail and dorsal lobe of tail fin are heavily pigmented, chestnut brown according to the Ridgeway nomenclature.

Tadpoles of *B. holdridgei* are superficially similar to those of *B. periglenes*. Noteworthy differences are the presence of supernumerary papillae medial to the lateral rows as illustrated (but contradicted in the description) for *B. periglenes*. This species is illustrated as having a convex upper beak, whereas that of *B. holdridgei* is moderately concave in some specimens. The three inferior tooth rows of *B. periglenes* are shown as subequal, but are increasingly shorter from the dorsal to the ventral row in the present species. *Bufo periglenes* has a larger number of lateral papillae, uninterrupted into dorsal and ventral portions, while in *B. holdridgei* there are but four dorsal and three ventral papillae per side, all of these relatively large. The pre-oral region of *B. periglenes* is somewhat hooded, as opposed to the rounded unpronounced snout of *B. holdridgei*. In the latter species, the anal tube is less than one third the length of the hind leg, being subequal in *B. periglenes*. In *B. holdridgei* the nostril is high on the head and about an eye width from the eye, whereas in *B. periglenes*, it is shown as situated about midway between the eye and snout tip. Sizes of the two species are comparable at this stage.

GROWTH AND SECONDARY SEX CHARACTERS

Sizes based on known adults of both sexes are shown in Fig. 7. Average snout-vent length of 29 males is 41.5 mm and of 32 females, 45.7 mm, both figures substantially higher than those given by SAVAGE (18). A giant male specimen (Museo de Zoología, Universidad de Costa Rica; UCR -5858), excluded from these calculations, measured 51.7 mm, well above two standard deviations from the mean of either sex. Dr. Pedro León examined the chromosomes of this individual from testicular material and found them normal. Somatic tissue was not examined. (Fig 12).

Fig. 11 shows the distribution of sizes throughout the year as based on museum specimens and recently collected material. Toads in excess of 37.5 mm (two standard deviations below the mean size of known adults of both sexes) were excluded from the chart. It is evident that adult size is attained well after two years, but the available data is insufficient to calculate this period with any confidence. Metamorphosing toadlets on June 29 averaged 7 mm in snout-vent length.

Hypertrophied testes of six adult males averaged 28.8% of the snout-vent length. An immature specimen measuring 31.2 mm had testes 16% of the body length and another, only 21.1 mm long, 18.4%.

Sexually active male toads have enlarged forearms. There is no evidence of sexual dichromatism as occurs in *B. perigrines*, although a wide range of colors, some of them suggestive of males of the latter species, is found. The male release vibration, although devoid of sound, is a reliable way to determine sex in this species during the reproductive season.

The sex ratio, based on observed toads, is 39 females to 767 males. Using the available information on female turnover rate in the puddles, a "corrected" figure was obtained of 910 females as compared to 555 males. We consider this second figure unrealistic. Subjective observations on the number of eggs laid in the puddles suggest a much lower number of females.

ECOLOGY

Adult *B. holdridgei* are decidedly fossorial. During the non-reproductive season they are seldom if ever seen abroad. They can be found under mossy banks, often as much as a meter above the ground. There is a tendency towards gregariousness. Sixteen toads of both sexes were excavated from a vertical bank several square meters in surface area. Other apparently suitable banks produced no toads. Specimens have occasionally been found along the stream edges. Juvenile toads are active in the leaf litter during the day and several have been taken from fallen bromeliads. Again there is a tendency towards gregariousness of juveniles on the forest floor, but this may be an artifact of proximity to reproductive sites.

Species of anurans found with adult *B. holdridgei* include *Hyla angustilineata*, *Aietopus varius*, *A. senex*, and species of *Eleutherodactylus* of the *rugulosus* complex. Juvenile toads have been found in common association with *Eleutherodactylus podiciferus* and *A. senex*. Various other species of *Hyla* and *Eleutherodactylus* occur at the locality but do not enter into contact with *B. holdridgei*.

At the breeding sites of *B. holdridgei*, competition is encountered with *Rana pipiens*, *R. vibicaria* and *H. angustilineata*, and the pools are occasionally entered by *A. senex*. As pointed out in COURTSHIP BEHAVIOR, these individuals are invariably killed if they remain in the water. *Hyla angustilineata*, *R. pipiens* and *R. vibicaria* are synchronous breeders with *B. holdridgei*. Only well advanced *R. vibicaria* tadpoles have been observed to share ditches with *B. holdridgei*.

Specimens of the toad are frequently mutilated, lacking part or all of a limb, suggesting the presence of undiscovered predators. The only snakes we have observed are the diminutive species of *Geophis* and *Rhadinaea calligaster*, which might pose threats to juveniles, but hardly to adults. Mutilations may be inflicted in juvenile stages however. The role of birds is unknown. Possible relationship between the high mutilation rate and unpalatability has not been investigated. Glossiphonid leeches of the genus *Placobdella* abound in the reproductive sites and have been found on the toads and egg masses. They are presumably opportunistic parasites. Nematodes were commonly found in the

celoms of the toads, and in one instance, the liver was completely riddled with these.

Coloration of adult *B. holdridgei* ranges from dark brown to decided orangish tints, providing excellent camouflage against the substrate. Juveniles are monochromatic dark brown and similar amongst themselves. They are very difficult to sight on the forest floor, especially since, like the adults, they tend to walk rather than hop.

Food items encountered in the stomachs include a wide diversity of mainly cursorial arthropods. Spiders, larval Lepidoptera, Diptera and Coleoptera, adult Coleoptera, Dermaptera, Hymenoptera, and Acarina were the principal items ingested. Adult Hemiptera, Homoptera, Diptera, Collembola and Lepidoptera were found in the Berleze funnel samples but not utilized as prey items.

It is interesting that *B. holdridgei* has only been observed to use artificial reproductive sites. It appears that the toads have adapted to the very drainage ditches which have presumably eliminated the natural standing water that they must have used before man's intervention.

DISCUSSION

Anurans lacking voice and auditory structures would be expected to have complementary behavioral adaptations compensating for these deficiencies. Most anurans which to some degree share these traits with *B. holdridgei* are high altitude forms having, at least in tropical regions, slight evolutionary potential due to their limited populations, distributions and abilities to disperse (10). It is not clear why these morphological adaptations occur in montane species, nor is it evident whether the behavioral adaptations precede or follow the morphological ones. As we learn more about the comparative behavior of these species, it may be possible to determine the sequence.

In Table 2, we present data on body size, egg number, and egg diameter for a selected variety of species of *Bufo*. Body size is of the female wherever possible, and ranges were averaged to obtain the other values. Two distinct populations of *B. biporcatus* are included in the table. The smallest species, *B. melanopleura*, has the smallest egg complement, 33. The largest eggs reported are for *Bufo rosei*, these being 3 mm in diameter. These authors (15) report an estimated 7,000 eggs per female, which a little mathematics will demonstrate to be a physical impossibility for a toad of 37 mm. WAGER (30) however, gives egg diameter as 2.6 mm and doesn't mention numbers. *Bufo holdridgei* ranks beside these species in having an egg diameter of 2.76 mm and a complement of 74.5 eggs. The three latter species are from relatively high altitudes and lack a tympanum (variable in *B. rosei*). *Bufo quercicus*, another diminutive species but otherwise normal and from low elevations lays some 610 eggs, 1.0 mm in diameter, being the smallest in the list. By contrast, *B. canorus*, from an altitude between 8,000 and 10,000 feet lays eggs with a diameter of 2.1 mm, and an estimated average of 1,750. The species is vocal and large specimens measure three inches (76 mm) (11, 24). Large eggs are apparently related to high altitudes, regardless of the vocal or au-

ditory condition of the species. Egg number shows positive correlation (Spearman $r = 0.85$) with body size (*B. rosei* excluded). Egg diameter shows no correlation with body size or with clutch size. It is our contention that the strategy of producing small numbers of large eggs is an adaptation to exceedingly crowded reproductive sites which, moreover, are transitory and provide little food. We have succeeded in raising eggs of *B. holdridgei* through toadlets in filtered water, although the metamorphosing toads were smaller than the size observed in the field.

TABLE 2

Size of adult (snout-vent), average clutch size, egg diameter and references for selected species of Bufo

Species	Size		Egg diameter	
	(mm)	Clutch size	(mm)	Reference
<i>marinus</i>	200	30,000	1.78	9
<i>alvarius</i>	177	7,750	1.4	24
<i>woodhousei</i>	127	25,650	1.25	24
<i>regularis</i>	114	24,476	—	16
<i>bufo bufo</i>	112	5,850	1.75	9
<i>americanus</i>	94	6,000	1.2	31
<i>cognatus</i>	94	20,000	1.2	24
<i>biporcatus</i>	85	2,000	1.48	9
<i>viridis viridis</i>	85	11,000	1.25	9
<i>boreas</i>	84.5	16,500	1.70	24
<i>calamita</i>	80	3,500	1.5	23
<i>melanostictus</i>	80	2,000	1.38	9
<i>canorus</i>	75	1,750	2.1	11
<i>vulgaris</i>	72	3,642	1.75	7
<i>woodhousei fowleri</i>	69	6,000	1.2	31
<i>biporcatus</i>	66	2,000	1.4	9
<i>terrestris</i>	56.7	2,888	1.4	30
<i>periglenes</i>	51.4	200	—	17
<i>holdridgei</i>	45.7	74.5	2.76	—
<i>rosei</i>	37	7,000	3.0	15
<i>quercicus</i>	25.3	610	1.0	31
<i>melanopleura</i>	22.4	33	1.9	22

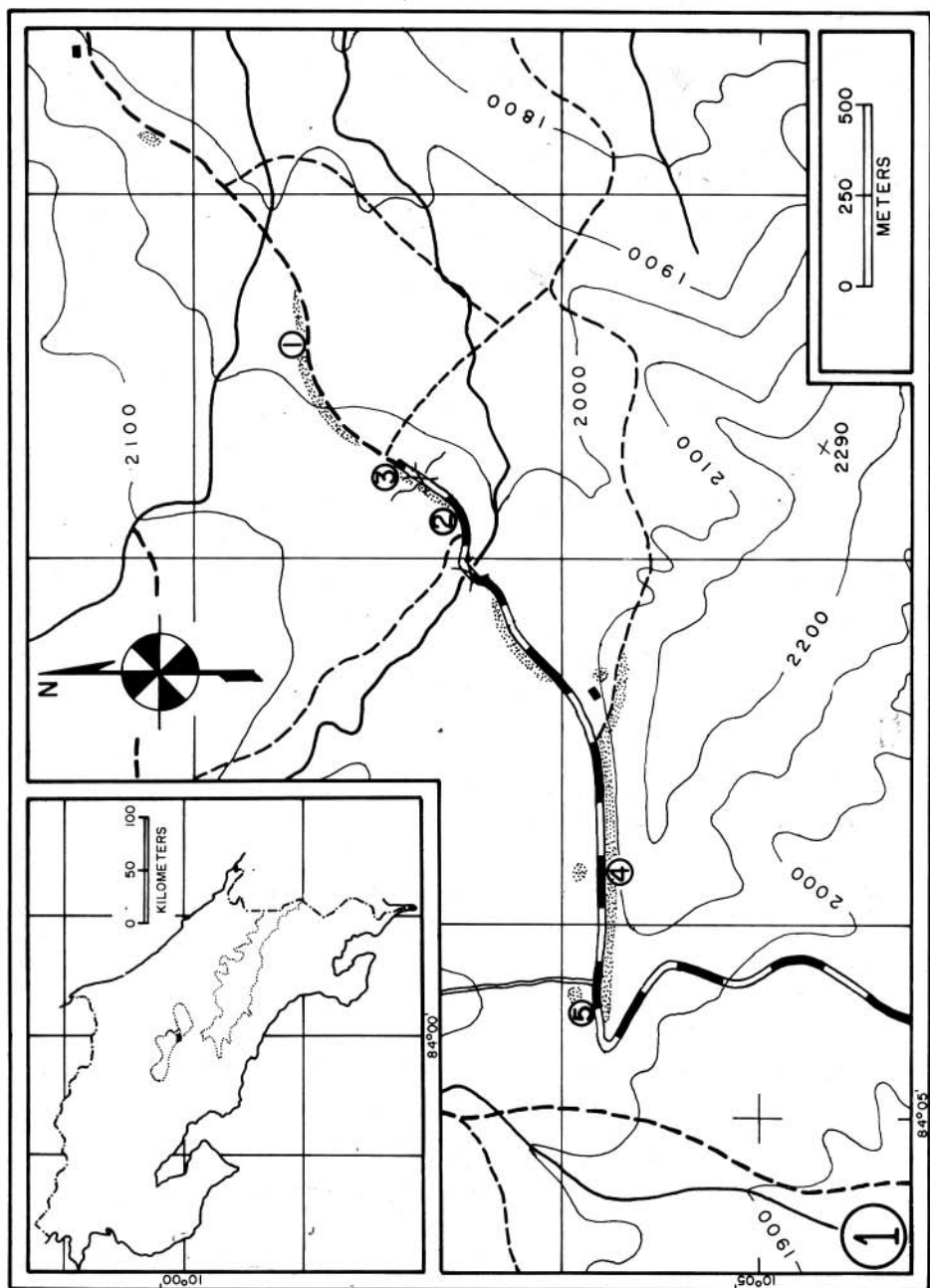
The most recent recorded volcanic activity of Barva was in 1867 when there was an explosion but no devastation. Prior to that, activity was recorded in 1770 (personal communication, Dr. Harmen W. Van der Bilt, Escuela Centroamericana de Geología). If the toads are really limited in distribution to the slopes of Volcán Barva, it seems probable that there has been no violent volcanic activity since the last Pleistocene glacial period. Current unpredictable volcanism in Costa Rica poses a serious peril for this species.

The available types of evidence on *B. holdridgei* and other montane genera and species do not permit satisfactory interpretation of the interrelationships, if any. *Rhombophryne* and *Atelopus* are Neotropical bufonids which produce relatively few, large, unpigmented eggs, while *Crepidophryne* has numerous small unpigmented eggs (29). It appears that the reproductive strategy of *Crepidophryne* is quite different from that of *B. holdridgei*. We consider relationship more likely between *B. holdridgei* and *B. periglenes*, where the differences could be attributed to reinforced interspecific isolating mechanisms evolved through repeated contacts during Pleistocene climatic fluctuations with concomitant vertical migrations of the two. *B. periglenes* has retained a voice and has carried sexual dichromatism to an extreme. *Bufo holdridgei* has no voice, and although presenting occasional "sports" suggestive of male *B. periglenes*, displays no sexual dichromatism. Sexual aggressiveness of *B. holdridgei*, and the hypothesized substance inhibiting development of the eggs of other species may have evolved as isolating mechanisms between these two species. *Crepidophryne epioticus*, *B. holdridgei*, and *B. periglenes* are illustrated in Figure 12. Our adult specimen of *Crepidophryne* was a dingy brown in life, rather than brightly colored as some authors have found for immature individuals.

RESUMEN

Bufo holdridgei es un sapo pequeño limitado en su distribución a las faldas del Volcán Barva, Costa Rica. Igual a muchas otras especies de altura, carece de tímpano y estructuras vocales, hecho que aparentemente da como resultado un comportamiento reproductivo masivo en sitios pequeños y de corta duración en un sitio específico. Se describe la cronología de llegadas y salidas de machos y hembras en dos charcos típicos por la duración de la actividad reproductiva en estos. El número promedio de huevos depositados es 74.5 de 2.76 mm de diámetro. Solamente un sapo conocido posee un complemento menor, mientras otro único tiene huevos de tamaño similar, ambos de estos son de las alturas africanas. Se postula que este es una adaptación a condiciones muy apiñadas de las larvas. Se describe los huevos y larvas, las dimensiones de adultos, y algunas observaciones ecológicas. Con base en el tamaño más pequeño de sus huevos y su falta de pigmentación, se concluye que *Crepidophryne* es más alejado de *B. holdridgei* de lo que se sospechaba anteriormente.

Fig. 1. Map of study locality, Alto del Roble, slopes of Volcán Barva, Costa Rica. Stippled areas denote reproductive sites. Numbers 1 and 2 are the corresponding study puddles, 3 a puddle studied earlier, and 4 and 5, the sites of Figures 2 and 3, respectively.



ACKNOWLEDGMENTS

The coauthors express their gratitude to the following persons and institutions, without whose aid this study could not have been realized: Roberto Ruiz, Roger Sáenz, Daniel Stein and Robert Wilkinson helped in the field work. Luis J. Poveda, Jorge Gómez, Luis Diego Gómez and Rafael Lucas Rodríguez identified plant specimens, Mario Vargas the leeches, and Luis Fernando Jirón, the stomach contents. William Bussing prepared the photograph of the toads and Federico Valverde, the graphs and map. Walter and Jerry James provided first hand information on *Bufo periglenes*. We are particularly indebted to Manuel Antonio González L., caretaker of the finca at the study site, for sharing his house with Novak, and to the Organization for Tropical Studies and the Associated Colleges of the Midwest for loan of equipment and logistical support, respectively.

LITERATURE CITED

1. ALTIG, RONALD, & RONALD A. BRANDON
1971. Generic key and synopses for free-living larvae and tadpoles of Mexican amphibians. *Tulane Stud. Zool. Bot.*, 17: 10-15.
2. BLAIR, W. FRANK (ed.)
1972. *Evolution in the genus Bufo*. Univ. Texas Press, Austin, viii + 459 pp.
3. BLAIR, W. FRANK
1972. *Bufo* of North and Central America, p. 93-101. In W. Frank Blair (ed.), *Evolution in the genus Bufo*. Univ. Texas Press, Austin.
4. BLAIR, W. FRANK
1972. Evidence from hybridization, p. 196-232. In W. Frank Blair (ed.), *Evolution in the genus Bufo*. Univ. Texas Press, Austin.
5. BLAIR, W. FRANK
1972. Characteristics of the testes, p. 324-328. In W. Frank Blair (ed.), *Evolution in the genus Bufo*. Univ. Texas Press, Austin.
6. BOGART, JAMES P.
1972. Karyotypes, p. 171-195. In W. Frank Blair (ed.), *Evolution in the genus Bufo*. Univ. Texas Press, Austin.

Fig. 2. Typical breeding site (4 on map) of *B. holdridgei*. The fern to the left is *Blechnum* sp., the orchids in the mid-background, *Elleanthus hymenophorus*, and the large-leaved dicot, *Gunnera insignis*, a common invader of disturbed habitat at high elevations.

Fig. 3. Another typical breeding site (5 on map) of *B. holdridgei*. This puddle filled at the end of the breeding season, and toad reproductive activity was correspondingly delayed.



2

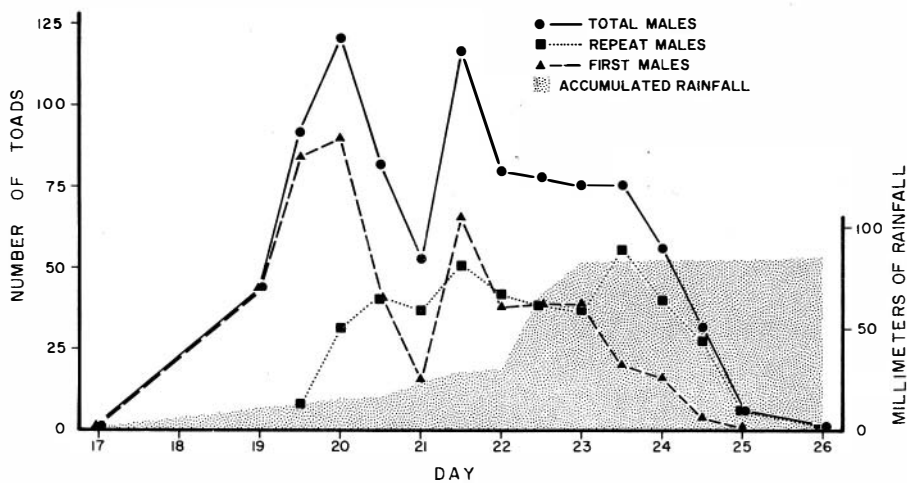


3

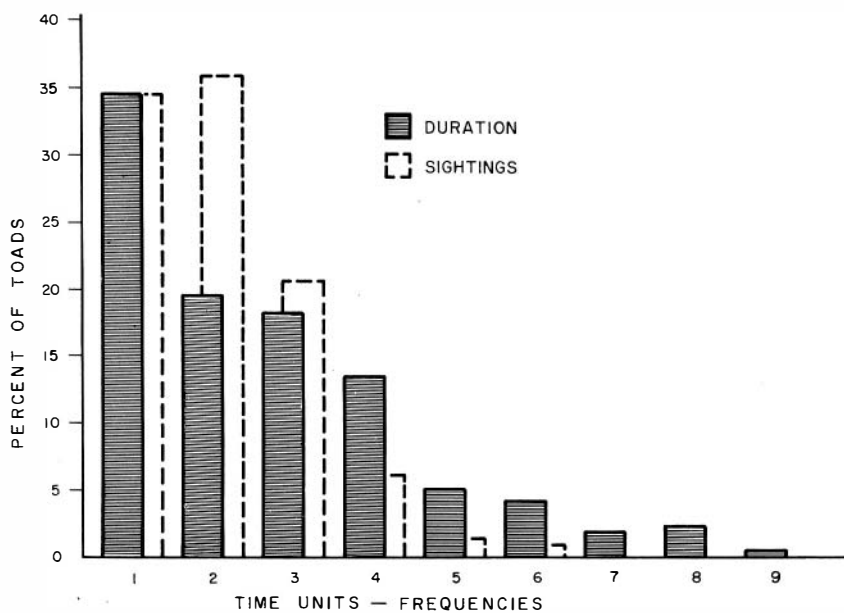
7. BOULENGER, G. A.
1896-1897. *The tailless batrachians of Europe*. Ray Society, London 2 v.
8. GISLÉN, TORSTEN, & HANS KAURI
1959. Zoogeography of the Swedish amphibians and reptiles with notes on their growth and ecology. *Acta Vert.*, 1: 193-397.
9. INGER, ROBERT F.
1954. Systematics and zoogeography of Philippine Amphibia. *Fieldiana Zool.*, 33: 181-531.
10. JANZEN, DANIEL H.
1967. Why mountain passes are higher in the tropics. *Amer. Natur.*, 101: 233-249.
11. KARLSTROM, E. L. & R. L. LIVEZEY
1955. The eggs and larvae of the Yosemite toad *Bufo canorus*. *Herpetologica*, 11: 221-227.
12. LIMBAUGH, BEVERLY A., & E. PETER VOLPE
1957. Early development of the gulf coast toad, *Bufo valliceps* Wiegmann. *Amer. Mus. Novitates*, 1842: 1-32.
13. MARTIN, ROBERT F.
1972. Evidence from osteology, p. 37-70. In W. Frank Blair (ed.), *Evolution in the genus Bufo*. Univ. Texas Press, Austin.
14. MARTIN, WILLIAM F.
1972. Evolution of vocalization in the genus *Bufo*, p. 279-309. In W. Frank Blair (ed.), *Evolution in the genus Bufo*. Univ. Texas Press, Austin.
15. POWER, H. J., & W. ROSE
1929. Notes on the habits and life-histories of some Cape Peninsula Anura, *Trans. Roy Soc. S. Afr.*, 17: 109-115.
16. ROSE, WALTER
1950. *The reptiles and amphibians of Southern Africa*. Maskew Miller Ltd., Cape Town, xxv + 378 pp.
17. SAVAGE, JAY M.
1967. An extraordinary new toad (*Bufo*) from Costa Rica. *Rev. Biol. Trop.*, 14: 153-167.
18. SAVAGE, JAY M.
1972. The systematic status of *Bufo simus* O. Schmidt with description of a new toad from western Panama. *J. Herpetol.*, 6: 25-33.

Fig. 4. Numbers of first observed males, repeat mates and total males of *B. boldridgei* observed in puddle 1 during April, 1975, together with the accumulated rainfall for the period at the locality.

Fig. 5. Histograms comparing frequency of sightings with over-all time span of presence of male *B. boldridgei*, expressed in percentages for puddle 2. Time units are approximately 12 hours. The histograms reveal frequent absences of toads from the puddle.



4

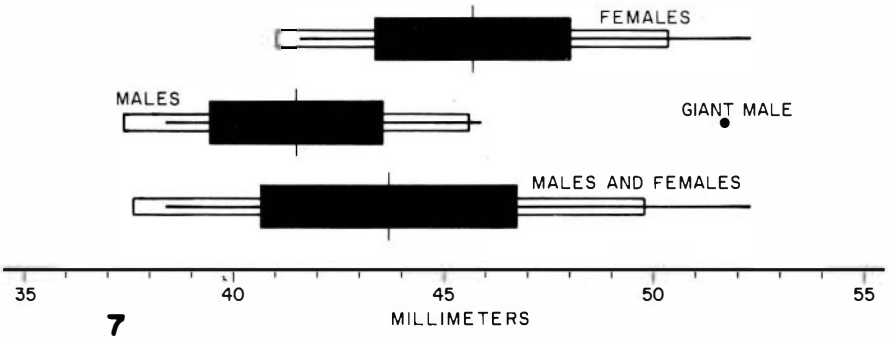
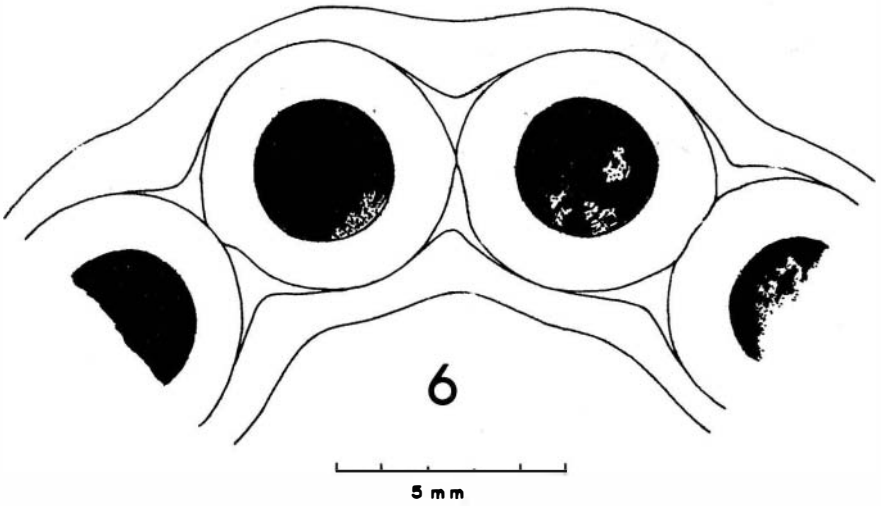


5

19. SAVAGE, JAY M.
1972. The harlequin frogs, genus *Atelopus*, of Costa Rica and western Panama. *Herpetologica*, 28: 77-93.
20. SAVAGE, JAY M.
1974. Type localities for species of amphibians and reptiles described from Costa Rica. *Rev. Biol. Trop.*, 22: 71-122.
21. SAVAGE, JAY M., & ARNOLD G. KLUGE
1961. Rediscovery of the strange Costa Rica toad, *Crepidius epioticus* Cope. *Rev. Biol. Trop.*, 9: 39-51.
22. SCHMIDT, KARL P., & ROBERT F. INGER
1959. *Amphibians exclusive of the genera Afrixalus and Hyperolius*. Exploration du Parc National de l'Upemba. Fasc. 56: 264 pp., 9 pls., map.
23. SMITH, MALCOLM
1954. *The British amphibians & reptiles*. Collins Clear-Type Press, London, xiv + 322 pp.
24. STEBBINS, ROBERT C.
1951. *Amphibians of western North America*. Univ. of Calif. Press, Berkeley, xvii + 539 pp.
25. STEBBINS, ROBERT C.
1966. *A field guide to western reptiles and amphibians*. Houghton Mifflin Co., Boston, xvi + 279 pp.
26. TAYLOR, EDWARD H.
1952. A review of the frogs and toads of Costa Rica. *Univ. Kans. Sci. Bull.*, 35: 577-942.
27. TAYLOR, EDWARD H.
1958. Additions to the known herpetological fauna of Costa Rica with comments on other species. III. *Univ. Kans. Sci. Bull.*, 39: 3-40.
28. TOSI, JR., J. A.
1969. *República de Costa Rica. Mapa Ecológico*. Trop. Science Center, San José, Costa Rica.
29. TRUEB, LINDA
1971. Phylogenetic relationships of certain Neotropical toads with the description of a new genus (Anura: Bufonidae). *Bull. Los Angeles County Mus. Natur. Hist. (Sci.)*, 216: 1-40.
30. WAGER, VINCENT A.
1965. *The frogs of South Africa*. Purnell & Sons. (S. A.) Pty., Ltd., Cape Town. 242 pp.
31. WRIGHT, ALBERT HAZEN
1932. *Life-histories of the frogs of Okefinokee Swamp, Georgia*. Macmillan Co., New York, xv + 479 pp.

Fig. 6. Drawing of preserved eggs of *B. holdridgei*.

Fig. 7. Snout-vent measurements of *B. holdridgei*. The horizontal line is the observed range, the vertical line the mean, the solid box one, and the open box, two standard deviations from the mean. N = 29 for males and 32 for females. The single giant male, excluded from the calculations, is represented by a dot.

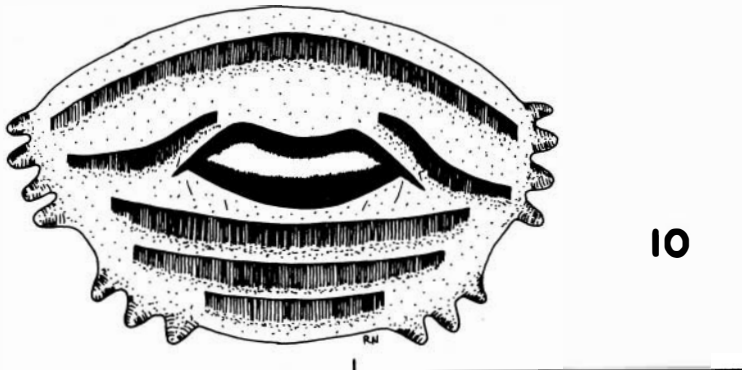
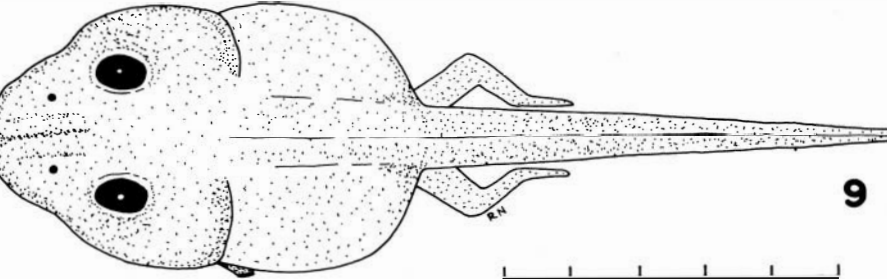
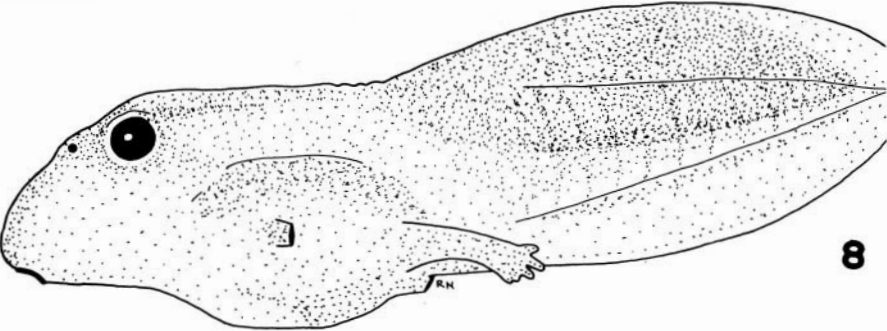


7

Fig. 8. Lateral view of tadpole of *B. holdridgei* at stage 36.

Fig. 9. Dorsal view of tadpole of *B. holdridgei* at stage 36.

Fig. 10. Mouthparts of tadpole of *B. holdridgei* at stage 36.



- Fig. 11. Distribution of immature *B. holdridgei* throughout the year, based on museum specimens collected over various years. The smallest toads shown for June 29 represent metamorphosing individuals for 1975. Toads in excess of 37.5 mm snout-vent are excluded from the graph. At least three size groups can be distinguished for the June through August range.

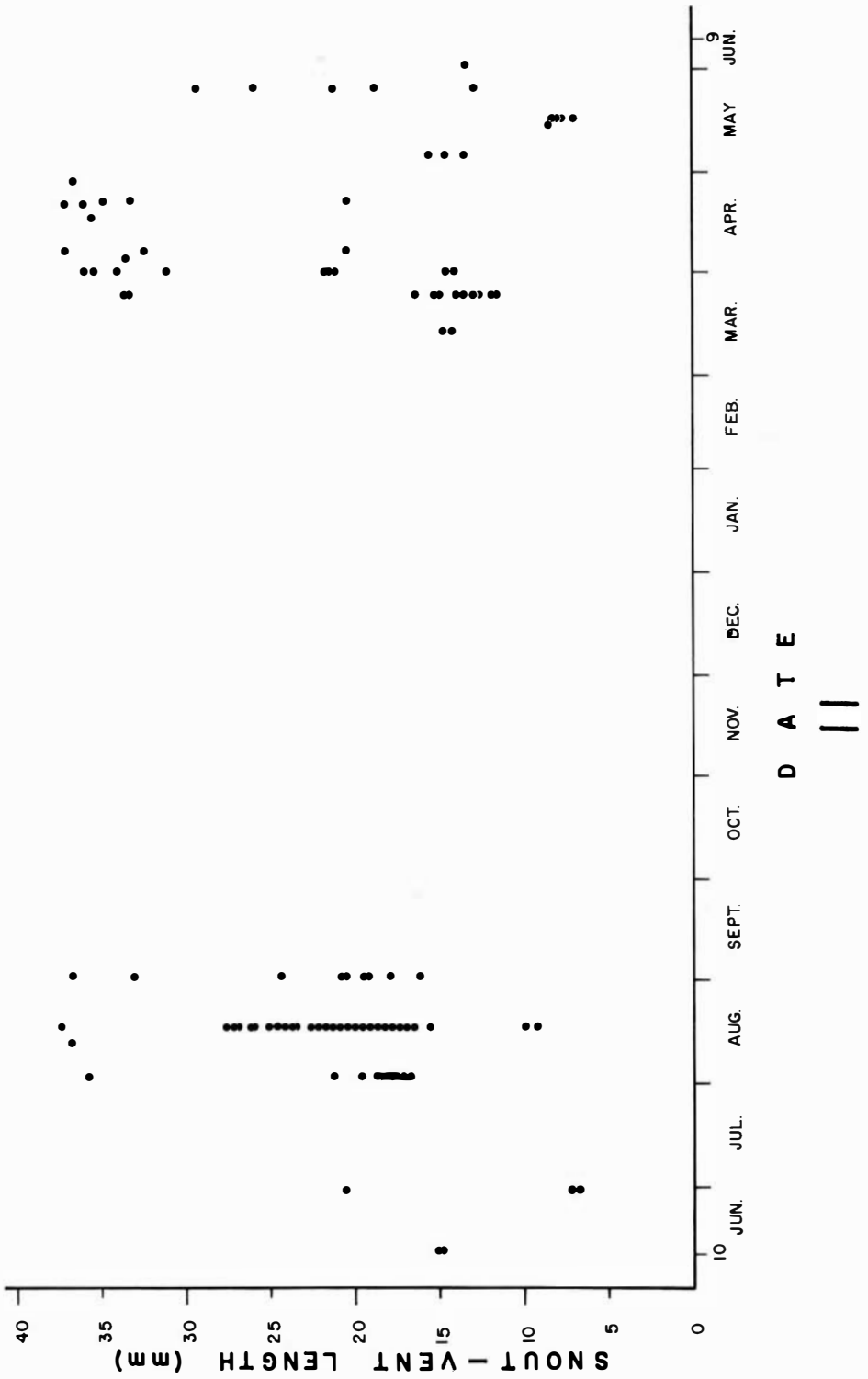


Fig. 12. Upper left: normal male *B. holdridgei*, snout-vent 39.7 mm. Upper right: giant male *B. holdridgei*, snout-vent 51.7 mm. Lower left: adult female *C. epioticus*, snout-vent 37.4 mm. Lower right: adult female *B. periglenes*, snout-vent 48.6 mm. All four specimens in the collection of the Museo de Zoología, Universidad de Costa Rica.

