

## SYSTEMATIC STUDIES OF THE COSTA RICAN MOSS SALAMANDERS, GENUS *NOTOTRITON*, WITH DESCRIPTIONS OF THREE NEW SPECIES

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**ABSTRACT:** Study of allozyme variation, external morphology, and osteology reveals that there are more species of moss salamanders (genus *Nototriton*) in Costa Rica than the two currently recognized. The three species for which names are available are valid, and new diagnoses are presented for them; three additional species are described. The phylogenetic relationships and biogeography of the six species are investigated. The radiation of *Nototriton* in present-day Costa Rica has involved miniaturization accompanied by both morphological and ecological specialization. Costa Rican species inhabit moss-mats and leaf-litter; most of the remaining species in the genus are bromeliad-dwellers. The revised genus *Nototriton* includes two Mexican, one Guatemalan (another, detected in the present study, remains undescribed), two Honduran, and six Costa Rican species. The six Costa Rican species appear to form a monophyletic group, but the phylogenetic relationships of the two northern species groups to each other and to the southern group remain uncertain.

**Key words:** Salamanders; Plethodontidae; *Nototriton*; Costa Rica; Allozymes; Morphometrics; Systematics; New species

SALAMANDERS of the genus *Nototriton* (commonly known as moss salamanders) are inconspicuous components of cloud forest faunas from Oaxaca, Mexico, to central Costa Rica. Most of the species occur in moss mats hanging in trees or bushes, or in moss covering dirt banks, large boulders, or stumps. Others inhabit bromeliads. In a few places (such as on the northeastern slopes of the Cordillera Central in Costa

Rica), they can be found easily, but characteristically they are uncommon. Even species that have been known taxonomically for more than 40 years (e.g., *N. richardi*) are represented by fewer than 25 specimens in the museums of the world. Typically, species of *Nototriton* are small; none exceeds 40 mm in snout-vent length and several species are not known to exceed 30 mm. These salamanders have slender bodies, narrow heads, and long, tapering tails that exceed their snout-vent length. Their eyes are small and oriented anteriorly, and several of the species have

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enlarged nostrils. Their limbs are slender and short, and the feet in some species are greatly reduced and have become syndactylous. Although the species are colorful when living specimens are viewed under a dissecting microscope, they are so small that their color patterns are obscure. Species of *Nototriton* are taxonomically difficult because there is so little perceptible external variation in the genus. The genus itself is of uncertain status, mainly because diagnostic characters are subject to homoplasy, a phenomenon that has plagued phylogenetic analyses of tropical salamanders. Because of these problems, the genus and its members have had a confused taxonomic history; this history is reviewed below in order to provide appropriate background for the taxonomic changes we propose.

#### HISTORICAL BACKGROUND

Stejneger (1911) described *Spelerpes picadoi* from the "valley of the Orosi" in the northern part of the Cordillera de Talamanca in eastern Costa Rica. His description was based on a single small salamander collected in a bromeliad at La Estrella by the famous Costa Rican biologist Claudio Picado (Picado, 1913). Dunn (1926) discussed the anatomy of some specimens from La Palma, Costa Rica, which he assigned to *Oedipus picadoi*, and asserted that some of the type material of the species now known as *Bolitoglossa subpalmata* (type locality La Palma) also was assignable to *O. picadoi*. In a generic revision of the tropical salamanders, Taylor (1944), at that time unfamiliar with Costa Rican species, simply listed Stejneger's taxon as "*Pseudoeurycea? picadoi*". Taylor (1949) continued to refer to the species in that manner, but by then he had begun his field work in Costa Rica and had discovered some diminutive salamanders in the volcanic Cordillera Central which he described as *Chiropterotriton abscondens* (Taylor, 1948) and *Parvimolge richardi* (Taylor, 1949) (both of these genera had been described by Taylor, 1944). Of *C. abscondens*, Taylor stated: "two other diminutive species with which this species

may be confused are '*Oedipus? picadoi* Stejneger and *Parvimolge richardi*, both of which occur in Costa Rica. The first species may be differentiated easily by the enlarged nostril of the young and adults; and *Parvimolge* by the structure of hands and feet, especially the enlarged elongate middle digit" (Taylor, 1948:179). Later, Taylor (1952) placed *picadoi* in the genus *Chiropterotriton*, and erroneously stated that this taxonomic assignment had been made by Taylor (1949); he considered *abscondens* and *picadoi* to be close relatives, distinguished chiefly by nostril size (large in *picadoi*, small in *abscondens*). Taylor (1952) published detailed drawings of the only known specimen of the enigmatic taxon *richardi*, a form that he compared only to *Parvimolge townsendi* of Mexico. The holotype of *richardi* was collected under the bark of a stump in local sympatry with *abscondens*, but Taylor (1954) reported finding *abscondens* to be abundant in moss mats. This was the first report of a species of neotropical salamander that appeared to specialize on moss.

Wake and Lynch (1976) concluded that *picadoi* and *abscondens* were conspecific, and assigned them to an informal "beta group" of the genus *Chiropterotriton*. They were uncertain of the generic status of *richardi*, which they tentatively listed as *Parvimolge*. In retrospect, the reasons for combining *abscondens* and *picadoi* are evident, for we now believe that until recently only one specimen of *picadoi*, the holotype, had ever made its way into any of the major herpetological collections (including that of the University of Costa Rica). Taylor (1952) accepted Dunn's (1926) assignment of La Palma specimens to *picadoi*, but specimens from this locality in the Cordillera Central cannot be separated morphologically from *abscondens*. It was this fact that led Wake and Lynch to recognize only one taxon. We interpret Taylor's (1948) statement concerning the large nostril size in young *picadoi* to be based on his examination of specimens from La Palma, for to our knowledge no juveniles of "true" *picadoi* were collected until recently, when we discovered a pop-

ulation assignable to *picadoi* in the Talamancan region near the type locality. This discovery enabled us to determine that *picadoi* is specifically distinct from the populations in the Cordillera Central that previously had been assigned to this taxon (see below).

Revisionary studies of Guatemalan and Mexican species of the beta group of *Chiropterotriton* revealed that three apparently monophyletic assemblages existed (Lynch and Wake, 1975, 1978; Wake and Elias, 1983). All species north of the Isthmus of Tehuantepec remained in *Chiropterotriton*, and two new genera were described (*Dendrotriton* and *Nototriton*); *picadoi* (*sensu lato*) and *richardi* were placed in *Nototriton* (the latter with reservation) (Wake and Elias, 1983). In addition, the poorly known Talamancan species *Bolitoglossa diminuta* (Robinson, 1976) was tentatively included in *Nototriton* (Wake and Elias, 1983); subsequently the species was returned to *Bolitoglossa* (Bolaños et al., 1987; Papenfuss and Wake, 1987; Wake, 1987).

The unsettled taxonomy of the Costa Rican *Nototriton* is related to the superficial morphological similarity of these tiny salamanders, as well as their relative rarity. Information concerning habitat, distribution, behavior, and morphology has accumulated slowly (e.g., Wake, 1987). The same difficulties have been encountered in Mexico, where Hanken (1983) failed to detect that a single specimen among the hundreds collected for his study of *Thorius* represented an undescribed species of *Nototriton* (Papenfuss and Wake, 1987), which is the only species of the genus found north of the Isthmus of Tehuantepec (where it occurs in local sympatry not only with *Thorius* but also with an undescribed species of *Chiropterotriton*).

Several synapomorphies support an hypothesis of monophyly for *Nototriton* + *Oedipina* (Wake and Elias, 1983; Sessions and Kezer, 1991). However, a persistent problem has been the possibility that some species presently assigned to *Nototriton* (e.g., *N. richardi*) might be a sister taxon of the remaining *Nototriton* plus *Oedi-*

*pina*; that is, *Nototriton* might be paraphyletic. Until recently, *N. richardi* has been too rare to examine this proposition seriously. The *nasalis* group is well supported by osteological synapomorphies (Lynch and Wake, 1978; Papenfuss and Wake, 1987), but this has not been true of the Costa Rican species. The recently described species *N. adelos* from Oaxaca, Mexico, placed in its own species group (Papenfuss and Wake, 1987), remains too poorly known to be considered further.

This paper represents the results of field and laboratory research conducted over a period of several years to clarify systematic, biogeographic, and local ecological problems involving Costa Rican salamanders (e.g., Wake, 1987). In this revision of Costa Rican *Nototriton*, we first outline the criteria we have used in recognizing taxa. We then describe new species and present redescriptions of previously recognized taxa. A discussion of evidence for our taxonomic decisions then follows. This order of presentation is chosen so that names are available for clarity in the discussion of morphological and biochemical data.

#### CRITERIA FOR SPECIES RECOGNITION

Our general goal is to discern genetically cohesive units that are evolutionarily independent entities, and to recognize them taxonomically as species. Two extreme approaches to assigning specific status can be taken. One can assume that a group of geographically separated populations are conspecific unless direct evidence (e.g., sympatry, or relatively great differentiation related to reproductive biology) exists for evolutionary independence. Alternatively, one can assume that each population constitutes a separate species unless evidence is presented for conspecific status. We prefer to take neither conspecific nor heterospecific status as a null hypothesis, but to proceed in a more empirical manner. In some instances, evidence of genetic independence is available. In others, evidence can favor an hypothesis of genetic cohesion. External (e.g., historical, geological, and ecological) fac-

tors also can be examined to estimate the likelihood of genetic interchange among populations, and hence to assess the possibility that the populations are independently evolving entities.

By definition, allopatric populations are evolutionarily independent at the present time. Such populations may have been geographically isolated for only a very short time, and were they to come into contact in the near future, they would probably resume interbreeding. However, since knowledge of future events is impossible, inference about past events must suffice. Our underlying assumption is as follows: the longer two populations have been isolated, and the more differences that have evolved between them in morphology, ecology, behavior, or biochemistry, the more likely it is that they will remain reproductively independent on recontact. Our approach has been to examine interpopulation differences in broad perspective and to then estimate the probability of evolutionary (and hence phylogenetic) independence. Thus, we have tried to avoid two polar extremes: a) excessive use of the polytypic species concept, which characterizes attempts to make the biological species concept operational, and b) the species-level recognition of large numbers of minimally differentiated groups, which some phylogenetic species concepts, based on the presence of *any* consistent differences, would demand (e.g., Cracraft, 1989).

Many authors have struggled with species concepts in relation to taxonomy in recent years (e.g., Chandler and Gromko, 1989; de Queiroz and Donoghue, 1988; Frost and Hillis, 1990; Highton, 1990; McKittrick and Zink, 1988; papers cited in Otte and Endler, 1989). We espouse the evolutionary species concept outlined by Frost and Hillis (1990), which in turn derives from Wiley's (1978, 1980) important modification of Simpson's (1961) evolutionary species concept. Their goal is a taxonomy that reports on the recovered history of inferred supraorganismal entities. The difficulty is both in recovering the history and determining the limits of the entities. We agree with Frost and Hillis (1990) that it is better to overestimate bi-

ological diversity in taxonomy than to underestimate it. Such an approach is especially important for parts of the world, such as the New World tropics, where decisions about which habitats should be conserved are made on the basis of their biological diversity (see Wilson, 1988). This is not a political species concept, but rather an explicit recognition that species are the fundamental elements of biodiversity and that to underestimate them is a disservice to our understanding of biodiversity.

We have attempted to make taxonomic decisions that will be robust. Therefore, the most important criterion is our ability to recognize and diagnose the taxa. In the present instance, all of the species are diagnosed using morphological criteria, but we also present biochemical evidence that is diagnostic. Likelihood of monophyly is also important; we certainly avoid polyphyletic species, but it is impossible to avoid paraphyletic species (since existing species could have given rise to other species). Genetic cohesion, now or in the recent past, is also a general criterion.

#### TAXONOMY

In the following section, we describe three new species of *Nototriton* from Costa Rica. In addition, we present evidence for the distinctiveness of the three previously described taxa of Costa Rican *Nototriton*. All of the species are diagnosed using morphological characters. In later sections of this paper, we present the results of an analysis of variation in proteins that supports our taxonomic decisions.

#### DESCRIPTIONS OF NEW SPECIES

##### *Nototriton tapanti*, new species

Tapanti Moss Salamander

Fig. 1

*Holotype*.—Museum of Vertebrate Zoology (MVZ) 203746, an adult female collected at Rio Quirí (elevation ca. 1300 m), 0.3 km northeast of the junction of the Tapantí Road and the Tausito Road, near Tapantí, Prov. Cartago, Costa Rica, on May 25, 1986, by A. Collazo and D. A. Good. The species is known only from the holotype.

*Diagnosis.*—A diminutive species (the holotype is 23.5 mm SL, standard length, i.e., distance from snout to posterior angle of vent) of *Nototriton* that closely resembles *N. richardi* in most morphological features, but is distinguished from that taxon by having more maxillary teeth (the holotype has 31 total, versus 24 maximum in *N. richardi*), narrower feet (4.1% of SL, vs. 4.4–5.0% in other Costa Rican species), shorter toes (3rd toe 0.9% SL, vs. 0.9–1.7%), a longer trunk (60.7% of SL, vs. 49.3–56.8%), and a lighter ground color. It is the most attenuate member of the genus, and has the most reduced manus and pes found in any plethodontid salamander; its hands and feet are syndactylous, with four fingers and five toes, but the outermost toe is only outlined externally by a groove. The free digital tips are pointed rather than rounded. It has a very large nostril (head width is 4.0 times nostril diameter), comparable in size to that of *N. richardi*, but much larger than in other Costa Rican members of the genus.

*Description.*—An extraordinarily small and slender species, with short limbs, greatly reduced hands and feet, and very weakly differentiated digits. The head has a pointed snout and small eyes that do not protrude beyond the margins of the jaw. The nostrils are of moderate size, and slightly oval. The small bicuspid teeth are relatively numerous: 4 premaxillary, 31 maxillary, and 14 anterior vomerine teeth are present in the holotype. The paratoid glands are relatively prominent, 0.5 by 1.5 mm in the holotype, and lie behind the head in front of the upper end of the gular groove. The head is a little broader than the anterior part of the trunk, but there is not a prominent neck. The trunk is very slender. The tail is long and tapers progressively to a very narrow tip. The short, slender limbs leave six costal folds uncovered when adpressed to the side of the trunk. The hands and feet are exceptionally small, and the digits are poorly demarcated from each other. Only the tips of the longest digits are free, and the inner and outer digits are extremely short. The free tips are pointed and lack distinct subdigital pads. The fingers, in order of de-



FIG. 1.—Holotype of *Nototriton tapanti*, new species, MVZ 203746, taken in life by D. C. Cannatella.

creasing length, are 3-2-4-1; the toes are 3-4-2-1-5.

*Measurements of Holotype (in mm).*—Head width 2.9; head depth 1.7; eyelid length 1.4; eyelid width 0.6; anterior rim of orbit to snout 1.0; interorbital distance 1.3; snout to forelimb 6.6; nostril diameter 0.7; distance between external nares 0.6; projection of snout beyond mandible 0.4; snout to gular fold 4.3; snout to posterior angle of vent (SL) 23.5; snout to anterior angle of vent 21.9; axilla to groin 13.6; tail length 27.0; tail depth at base 1.7; tail width at base 1.5; forelimb length 3.3; width of hand 0.7; hind limb length 3.9; width of foot 0.9; length of longest (third) toe 0.13; length of fifth toe 0.

*Coloration (in alcohol).*—Generally light brown, with a vague impression of a dorsal stripe produced by a pair of fine, dark lines that extend dorsolaterally along the trunk. Just anterior to the pelvis, a dorsal stripe becomes evident that is light tan and sharply demarcated from the dark blackish-brown lateral margins of the tail. The lateral flanks of the trunk have a light cream color. Ventral surfaces have less dense and hence somewhat lighter pigmentation. Small (0.2–0.3 mm in diameter), obscure white spots are abundant on the ventral surfaces, especially on the gular area (which contains 25–30 spots). Upper and lower jaw margins are covered with large, whitish spots. The tip of snout is white, and the light coloration extends onto the top of the snout. The paratoid glands are distinctly lighter than the surrounding ground color.

*Coloration (from diapositive).*—Light brown dorsally, with brown becoming brighter and with a grayish caste posteriorly; lightness of dorsum contrasts to darker color along sides. Series of obscure light spots behind costal folds on either side of

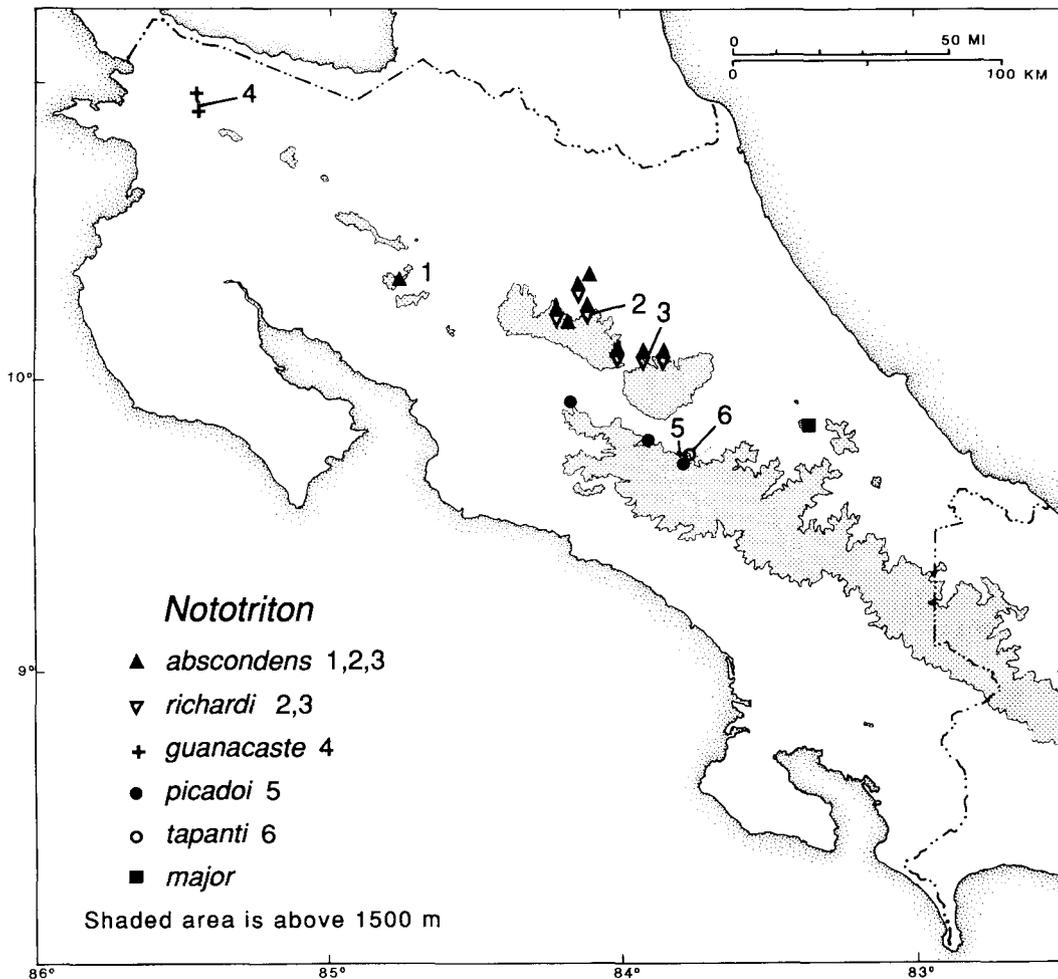


FIG. 2.—Map of Costa Rica, showing the distribution of the six species of *Nototriton* known to occur in that country. Numbers refer to samples used in allozyme study (see Table 1).

midline along trunk, terminating near tail base. Irregular band of fine white marks along dorsolateral margins of tail. Pair of reddish brown marks behind eye on back of head. Paratoid glands lighter than surrounding skin. Snout dark with scattered white marks.

*Habitat*.—The holotype was collected near the type locality of *Bolitoglossa gracilis* (Bolaños et al., 1987), in Lower Montane Rain Forest (Tosi, 1969). The specimen was collected by raking through leaf litter on the forest floor along a slope shaded by second-growth forest. The salamander displayed no escape behavior when captured. The type locality is unprotected

and has been highly modified by recent agricultural activity, but we suspect that the taxon occurs in the nearby Reserva Tapantí.

*Distribution*.—The species is known only from the holotype, collected in the Orosi River valley at the north end of the Cordillera de Talamanca (Fig. 2).

*Etymology*.—The species name is derived from the place name of a small settlement that is used to denote the general region (including a large biological reserve) at the lower end of the narrow upper valley of the Río Orosi.

*Comment*.—The unique holotype, which had only recently been collected

when the paper by Bolaños et al (1987) went to press, was referred by them to *N. richardi*.

*Nototriton major*, new species  
Moravia Moss Salamander  
Fig. 3

**Holotype.**—University of Costa Rica (UCR) 6756, an adult male collected from along Quebrada Platanillo, approximately 6 km east of Moravia de Chirripó, Prov. Cartago, Costa Rica, by members of a herpetology class from the University of Costa Rica on 5 October, 1975. Elevation ca. 1200 m. The species is known only from the holotype.

**Diagnosis.**—The largest Costa Rican member of the genus (the holotype is 37.9 mm SL; other Costa Rican species rarely exceed 30 mm) and possibly the largest species in the genus. In addition to its large size, the only known specimen of the species is distinguished from other Costa Rican *Nototriton* by its short, narrow head (snout-gular length 17.7% and head width 11.3% SL; all other species are 18.0–22.4% and 12.1–15.7% in these characters). It is distinguished from *N. picadoi*, *N. richardi*, *N. tapanti* and the Monteverde population of *N. abscondens* by its smaller nostrils (0.3% SL, vs. 1.0–1.6%). It is further distinguished from particular species of *Nototriton* from Costa Rica as follows: from *N. richardi* and *N. tapanti* by its longer limbs (forelimb 14.8% and hind limb 19.3% of SL, vs. 14.0–14.7% and 17.4–18.7%) and wider feet (5.9% SL, vs. 4.1–5.0%) with well defined, free digits; from *N. picadoi* by its more slender habitus (trunk width 9.5% SL, vs. 10.0–10.8%) and shorter forelimbs (14.8% SL, vs. 16.9–18.2%); from *N. gaunacaste* by its shorter forelimbs (14.8% SL, vs. 17.0–17.9%), longer tail (144.1% SL, vs. 121.0–133.7%), and more slender habitus (trunk width 9.5% SL, vs. 11.0–12.2%); and from *N. abscondens* by its longer tail (144.1% SL, vs. 101.3–136.5%). In addition, *N. major* differs from the Monteverde population of *N. abscondens* in hind limb length (19.3% SL, vs. 15.7–17.3%). The free digital tips are rounded, with discrete subterminal pads.

**Description.**—A very large species for



FIG. 3.—Holotype of *Nototriton major*, new species, UCR 6756, from east of Moravia de Chirripó, Costa Rica.

this genus with small nostrils and a very long tail. The snout is relatively short and broadly rounded. The head is distinctly demarcated from the narrower neck region. The eyes are of moderate size and are moderately protuberant, barely extending laterally beyond the margins of the jaw. The teeth are small and moderate (in relation to body size) in numbers; there are 5 premaxillary, 33 maxillary, and 18 vomerine teeth. The paratoid glands (0.7 by 1.9 mm in the holotype) are evident but not particularly swollen; they lie behind the head and in front of the upper end of the gular groove. The mental gland is inconspicuous and flat, with some dark pigmentation. The very long tail tapers progressively to a narrow tip. The limbs are moderately robust and long; 5 costal folds are left uncovered when they are adpressed to the side of the trunk. The hands and feet are well developed, with distinct, relatively long digits. The tips of the digits are expanded and rounded, and have distinct subdigital pads. The fingers, in order of decreasing length, are 3-2-4-1; the toes are 3-4-2-5-1.

**Measurements of Holotype (in mm).**—Head width 4.3; head depth 3.1; eyelid length 1.9; eyelid width 0.9; anterior rim of orbit to snout 1.5; interorbital distance 2.2; snout to forelimb 9.6; nostril diameter 0.07; distance between external nares 1.3; projection of snout beyond mandible 0.4; mental gland width 0.6; mental gland length 0.8; snout to gular fold 6.7; snout to posterior angle of vent 37.9; snout to anterior angle of vent 35.4; axilla to groin 21.8; tail length 54.6; tail depth at base 3.2; tail width at base 3.1; forelimb length 5.6; width of hand 1.7; hind limb length 7.3; foot width 2.0; length of longest (third) toe 1.3; length of fifth toe 0.7.

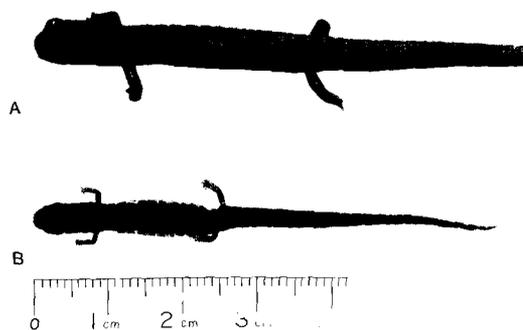


FIG. 4.—*Nototriton guanacaste*, new species, from Volcán Cacao, Costa Rica. Above, specimen photographed in life by D. C. Cannatella, MVZ 207106. Below, preserved holotype, MVZ 207111.

*Coloration of the holotype (in alcohol).*—Generally rich dark brown with no evident color pattern. There are a few rather obscure whitish spots on the lateral flanks of the trunk, and there are some very obscure light chevrons on the dorsum. The venter is gray-brown, with less dense pigment than on the dorsum; all ventral surfaces have densely distributed tiny whitish spots. The limbs are colored like the adjoining trunk, but the dorsal surfaces have some white spots. There is some light coloration on the lateral surfaces of the tail base region. The paratoid glands are very slightly, but distinctly, lighter than the adjacent ground color.

*Habitat.*—No details of the conditions of collection of the unique holotype are known. Quebrada Platanillo is a rapidly flowing, clear-water stream with a rocky bed. The vicinity of the stream is shaded, but there is little primary forest remaining in the vicinity of the type locality. The natural vegetation in the region is classified as Premontane Rain Forest (Tosi, 1969).

*Distribution.*—Known only from the type locality in the mountains east of Moravia de Chirripó, eastern Costa Rica (Fig. 2).

*Etymology.*—The species name refers to its large size. The holotype is a male, and since females are the larger sex in bolitoglossine salamanders it is likely that the species exceeds 40 mm SL.

*Nototriton guanacaste*, new species  
Guanacaste Moss Salamander  
Fig. 4

*Holotype.*—MVZ 207111, an adult female from the upper slopes of Volcán Cacao (elevation 1580 m), Guanacaste National Park, Prov. de Guanacaste, Costa Rica, collected on August 24, 1987, by David C. Cannatella and David A. Good.

*Paratypes.*—MVZ 207106–10, 207112–14, same data as holotype, collected on different days in August, 1987; UCR 8956–66 (collectors and dates unrecorded), Volcán Orosí, Prov. de Guanacaste, Costa Rica.

*Diagnosis.*—A moderately large (maximum SL = 29.7 mm), robust species of *Nototriton*, distinguished from all other Costa Rican species by its long (21.6–22.4% SL, vs. 17.7–21.6% for all other species), broad head. It is distinguished from *N. picadoi*, *N. richardi*, *N. tapanti*, and the Monteverde population of *N. abscondens* by its smaller nostrils (0.4–0.9% SL, vs. 1.0–1.6%). It is further differentiated from *N. richardi* and *N. tapanti* by its more fully elaborated hands and feet (e.g., fifth toe length 1.1–1.7% SL, vs. 0.0–0.2%). It is distinguished from *N. major* by its smaller size and more robust habitus (see *N. major*, above), from *N. picadoi* by its robust habitus (trunk width 11.0–12.2% SL, vs. 10.2–10.8%), and from *N. abscondens* by its more prominent paratoid glands. The free digital tips are rounded with discrete sub-terminal pads.

*Description.*—A species of moderate size for the genus, with a relatively stout body, limbs of moderate length, and well developed hands and feet with fully differentiated digits. The head is relatively large, both long and broad, and is well demarcated from the trunk. The eyes are of moderate size and are moderately protuberant, extending slightly beyond the margins of the jaw. The small teeth are moderate to high in numbers; in the five adults, maxillary teeth range from 25–49, mean 32; vomerine teeth, 10–18, mean 13. Paratoid glands are well developed and prominent. One adult male has a rather flat and inconspicuous mental gland that is 1.3 by 1.3

mm in dimensions. The long tail (up to 1.3 times SL) tapers to a pointed tip. Limbs are of moderate length. Hands and feet have well defined digits that are separated from each other distally; the free portion of the third toe is about twice, or more, the length of the fifth toe. The tips of the longest digits are distinctly rounded, with subdigital pads. The fingers, in order of decreasing length, are 3-2-4-1; the toes 3-4-2-5-1.

*Measurements of Holotype (in mm).—*Head width 4.5; head depth 2.5; eyelid length 1.6; eyelid width 0.9; anterior rim of orbit to snout 1.3; interorbital distance 1.9; snout to forelimb 8.4; nostril diameter 0.07; distance between external nares 1.1; projection of snout beyond mandible 0.1; paratoid gland 1.8 by 0.7; snout to gular fold 6.0; snout to posterior angle of vent 29.7; snout to anterior angle of vent 27.7; axilla to groin 15.8; tail length 35.1; tail depth at base 2.7; tail width at base 2.6; forelimb length 5.1; width of hand 1.3; hind limb length 5.4; width of foot 2.1; length of longest (third) toe 0.5; length of fifth toe 0.4.

*Coloration (in alcohol).—*The holotype is generally medium brown mottled with yellow highlights and small, irregularly shaped, dark brown markings. The flanks are yellow dorsally, becoming dark brown ventrally. The venter is generally dark brown, but somewhat lighter in the gular region and on the tail. The venter has widely scattered small white spots which are most prominent on the ventral surfaces of the limb. The paratoid glands are bright yellow along their dorsal medial borders. The tip of the snout and the margins of the jaws are covered with relatively large cream to yellow spots. There are some yellow markings behind the hind limbs above the cloacal region. The tail is dark, with lighter lateral blotching.

Other specimens vary in the density of the ground color, and in the brightness of the light coloration. All animals have bright lateral coloration, and all of the animals have brightly colored paratoid glands (less so in the smaller specimens). Small white

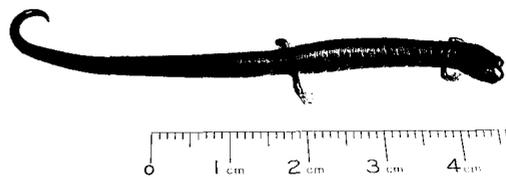


FIG. 5.—A specimen of *Nototriton abscondens*, MVZ 190889, from Cascajal de las Nubes, Costa Rica, photographed in life by Scientific Photographic Laboratory, University of California at Berkeley.

spots are present on ventral surfaces, but they are reduced in numbers in some specimens. A small specimen (MVZ 207114; 18.2 mm SL) has a faint dorsal stripe in a chevron pattern. All of the specimens have relatively rugose skin that appears slightly granular in life. This is especially evident in diapositives of specimens photographed in the field.

*Habitat.*—*Nototriton guanacaste* is similar to *N. abscondens* and *N. picadoi* in occurring primarily in moss growing on the trunks and branches of cloud forest trees.

*Distribution.*—The species is known only from Volcán Orosí and Volcán Cacao, the northwestern-most mountains of the Cordillera de Guanacaste (Fig. 2). The two peaks are joined by a semi-plateau at about 1000 m. These species appears to be restricted to the low-stature, moss-laden forests near the tops of these two mountains. The habitat is very restricted, but fully protected from deforestation in the new Guanacaste National Park.

*Etymology.*—The specific name is chosen in celebration of the outstanding foresight of the Costa Rican people in establishing Guanacaste National Park, to which this species is restricted.

DIAGNOSES OF PREVIOUSLY DESCRIBED  
SPECIES FROM COSTA RICA

*Nototriton abscondens* (Taylor, 1948)

Cordilleran Moss Salamander

Fig. 5

*Chiropterotriton abscondens* Taylor, 1948:  
177 [Holotype RCT—Richard C. Tay-

lor—1414; now FMNH 178285, from “Isla Bonita (American Cinchona Plantation), elev. 5500 ft., Volcán Poás, Caribbean drainage”, Costa Rica].

*Diagnosis.*—A moderately large species of *Nototriton* (to about 33 mm SL; mean SL of 10 males, 27.5 mm; of 10 females, 28.0 mm), distinguished as follows from other Costa Rican species: from *N. richardi* and *N. tapanti* by its more fully elaborated hands and feet (e.g., fifth toe length 0.8–1.4% SL, vs. 0–0.2%); from *N. major* by its smaller size, longer and broader head, and shorter tail (see *N. major*, above); from *N. guanacaste* by its shorter, narrower head (snout–gular length 18.0–21.2% and head width 12.1–14.3% SL, vs. 21.6–22.4% and 14.5–15.7%); and from *N. picadoi* by its relatively slender habitus (trunk width 8.1–10.3% SL, vs. 10.0–10.8%). The free tips of the digits are rounded, with discrete subterminal pads.

*Comments.*—All specimens of Costa Rican *Nototriton* in museums throughout the world are assignable to this taxon, except for a very few specimens of *N. richardi*, the holotype of *N. picadoi*, and the specimens of these and other species listed in this paper. This is a relatively widespread species in the Cordillera Central (Fig. 2), and it displays some geographic variation (see below). The species is most commonly encountered in moss mats on road banks, covering downed logs and on stumps, and on the trunks and branches of trees. It has not been found very far above ground, but is expected to go well up into trees; it has occasionally been taken in bromeliads, especially in the Monteverde region (Hayes et al., 1988). In general, the species occurs in cloud forest between 1400 and 2000 m on the Atlantic slopes, but before the extensive deforestation of the Meseta Central the species occurred on the upper Pacific slopes above Heredia and San José as well, and does so to this day in the Monteverde region.

The Monteverde population differs from those in the Cordillera Central in having larger nostrils and shorter hind limbs, but otherwise the populations are similar. Although they are diagnosable on the basis

of nostril size alone, the populations differ only slightly in protein variants, and we believe that they might form a genetically cohesive unit with other *N. abscondens* and hence that they are not an independent entity. We call attention to the morphological distinctiveness of the Monteverde population, but retain it in *N. abscondens* for the present.

*Nototriton picadoi* (Stejneger, 1911)

Picado's Moss Salamander

Fig. 6

*Spelerpes picadoi* Stejneger, 1911:285 [Holotype USNM 48280 from “La Estrella, southeast of Cartago, Costa Rica”].

*Diagnosis.*—A moderately large species of *Nototriton* (the male holotype, the largest known specimen of the species, is about 32 mm SL), distinguished as follows from other Costa Rican species: from *N. richardi* and *N. tapanti* by its more elaborated hands and feet (e.g., fifth toe length 1.0–1.6% SL, vs. 0–0.2%), longer forelimbs (16.9–18.2% SL, vs. 14.0–14.7%), and by more robust habitus (trunk width 10.0–10.8% SL, vs. 8.8–9.6%); from *N. major* by its smaller size, longer and broader head, larger nostrils, and more robust habitus (see *N. major*, above); from *N. guanacaste* by its shorter, narrower head, larger nostrils, and less robust habitus (see *N. guanacaste*, above); and from *N. abscondens* by its more robust habitus. It is distinguishable from all but *N. richardi*, *N. tapanti*, and the Monteverde population of *N. abscondens* by its larger nostril size (1.0–1.6% SL, vs. 0.3–0.8%). The free tips of the digits are rounded, with discrete subterminal pads.

*Comments.*—Until the recent rediscovery of this species it was known only from the holotype. Specimens from the Cordillera Central in museums and in the literature are all assignable to *N. abscondens*. An exception may be a specimen reported by Dunn (1937), who stated that Prof. Manuel Valerio had collected a single *picadoi* in a bromeliad at 2200 m “on Escazu”. This locality is southwest of San José on a spur of the Cordillera de Talamanca. We have been unable to locate this specimen



FIG. 6.—A specimen of *Nototriton picadoi* from Reserva Tapantí, Costa Rica, photographed in life by D. C. Cannatella, MVZ 207115.

in a museum, but we suspect that it is correctly assigned based on habitat and locality. All of the specimens we have collected have been from moss hanging from tree limbs and on tree trunks; a few have been found in balls of moss, some of which were found as high as 8 m above ground level. The recent collections of this species, deposited in the collections of the University of Costa Rica and the Museum of Vertebrate Zoology, total 13 specimens plus one clutch of eggs. This species is known only from the northern end of the Cordillera de Talamanca (Fig. 2), in Lower Montane Rain Forest (Tosi, 1969). The common name has been applied to salamanders from Monteverde (e.g., Hayes et al., 1988), but this population is assignable to *N. abscondens*. We believe that it is appropriate to restrict the common name to the species as defined in this paper.

*Nototriton richardi* (Taylor, 1949)  
Cordilleran Litter Salamander  
Fig. 7

*Parvimolge richardi* Taylor, 1949:248 [RCT 1436; now FMNH 178295 from "Isla Bonita (American Cinchona Plantation), Atlantic slope of Volcán Poás at an elevation of about 6500 feet", Costa Rica].

*Diagnosis.*—A diminutive species of *Nototriton* (to about 24 mm SL; 4 adult males average 22.4 mm, 4 adult females 22.5 mm SL), distinguished as follows from

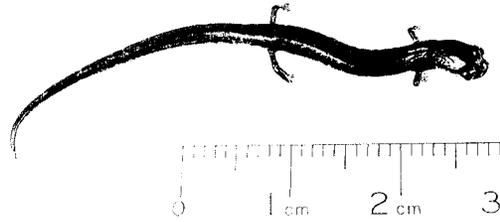


FIG. 7.—A specimen of *Nototriton richardi*, MVZ 194886, from Cascajal de las Nubes, Costa Rica, photographed in life by Scientific Photographic Laboratory, University of California at Berkeley.

other Costa Rican species: more slender, with a shorter head and legs and larger nostrils (head width is  $4.1 \pm 0.5$  times nostril diameter), than *N. abscondens*, *N. guanacaste*, and *N. major*; much slenderer, with a shorter head and legs, than *N. picadoi*; longer toes, wider feet, and a shorter trunk than *N. tapanti*. The free digital tips are pointed, but the hands and feet are essentially syndactylous and digits one and five on the foot are only outlined by grooves externally.

*Comments.*—This is an uncommon species, and it has been misidentified either as a juvenile of *N. abscondens* or *Oedipina uniformis* (the latter because it is very slender and has a long tail) in museum collections. It is probably an inhabitant of leaf litter on the forest floor, for this is where it has been found exclusively in undisturbed situations in the Zona Protectora La Selva (on the northern slopes of Volcán Barva), but it also is found in moss covering tree trunks and stumps, and road banks. It is restricted to the Atlantic slopes of the Cordillera Central (Fig. 2), mainly in Lower Montane Rain Forest (Tosi, 1969), at elevations between 1200 and 1700 m.

#### ANALYSIS OF GENETIC AND MORPHOLOGICAL VARIATION

##### *Analysis of Proteins*

*Materials and methods.*—Eight populations of Costa Rican *Nototriton* (Table 1, Fig. 2), representing five of the species discussed above, were examined for variation in 23 proteins (Table 2). In addition, two populations of Guatemalan *Nototri-*

TABLE 1.—Samples of *Nototriton* and collecting localities in Costa Rica used for protein study. MVZ = Museum of Vertebrate Zoology; ZP = Field numbers from joint field expedition to Zona Protectora La Selva, to be deposited in collections of the University of Costa Rica (UCR) and the CRE collection, currently at the University of Miami.

<i>N. abscondens</i> —MVZ 207120–22, 207124 ( $n = 4$ ), Monteverde (Fig. 2, 1); MVZ 206385–87, 206393, ZP 197 (CRE), 371 (UCR), 422 (UCR), 440 (UCR), 484 (CRE), 491 (UCR), 568 (CRE) ( $n = 11$ ), Zona Protectora La Selva (Fig. 2, 2); MVZ 172174, 181352–53, 190889–95 ( $n = 10$ ), Las Nubes de Coronado (Fig. 2, 3).
<i>N. guanacaste</i> —MVZ 207107–08, 207111–12 ( $n = 4$ ), Type Locality (Fig. 2, 4).
<i>N. picadoi</i> —MVZ 203739, 207115–17, 207119 ( $n = 5$ ), Refugio Tapantí (Fig. 2, 5).
<i>N. richardi</i> —MVZ 194885, 194886 ( $n = 2$ ), Las Nubes de Coronado (Fig. 2, 3); ZP 356–57 (UCR), MVZ 206395 ( $n = 3$ ), Zona Protectora La Selva (Fig. 2, 2).
<i>N. tapanti</i> —MVZ 203746 ( $n = 1$ ), Type Locality (Fig. 2, 6).

*ton* and 21 populations of *Oedipina* (*sensu lato*) representing 11 species (Wake and Good, in preparation, present full data for these taxa) were included as outgroups (based on the arguments for sister group relationships presented in Lynch and

Wake, 1978; Wake and Elias, 1983; Sessions and Kezer, 1991). Liver and intestine samples were collected from freshly killed specimens and stored at  $-76\text{ C}$  until used.

Tissues were combined and homogenized in approximately equal parts tissue and deionized water, and then subjected to horizontal starch gel electrophoresis using standard techniques (Selander et al., 1971; Harris and Hopkinson, 1976). The allele patterns (Table 3) resulting from this analysis were subjected to a variety of phenetic and phylogenetic analyses using the BIOSYS (Swofford and Selander, 1981) and PAUP (Swofford, 1985) computer packages (DOS versions).

In the phylogenetic analyses that follow, a conservative approach to character state transformation designation was taken. All alleles present in the polymorphic state anywhere in the analysis were combined into a single character state (Buth, 1984). If, in a hypothetical case, the allele combinations A, AB, B, and BC occurred in four taxa, all four would be considered to show the same character state; an allele D in a fifth taxon would be considered to be a separate state if there are no taxa with the allele combinations AD, BD, or CD. This approach ignores potentially useful

TABLE 2.—Buffer systems and loci scored in the analysis of relationships among the species of *Nototriton*. Buffer type abbreviations are as follows: A = Poulik, (pH 8.7); B = PGI Phosphate (pH 6.7); C = LiOH (pH 8.2); D = Tris-Citrate II (pH 8.0); E = Tris-Citrate III (pH 7.0).

Enzyme	Enzyme commission number	Locus	Buffer system
3-hydroxyacyl CoA dehydrogenase	1.1.1.35	Hadh	B
Aconitase (2 loci)	4.2.1.3	Acon-1,2	D
Adenosine diaminase	3.5.4.4	Ada	E
Alcohol dehydrogenase	1.1.1.1	Adh	B
Aspartate aminotransferase	2.6.1.1	Aat	A
Esterase (2 loci)	3.1.1.1	Est-1,2	C
Glucose dehydrogenase	1.1.1.47	Gdh	B
Glycosephosphate isomerase	5.3.1.9	Gpi	B,E
Glucose-6-phosphate dehydrogenase	1.1.1.49	Gd	D
Isocitrate dehydrogenase (2 loci)	1.1.1.42	Icdh-1,2	D
L-lactate dehydrogenase	1.1.1.27	Ldh	C
Malate dehydrogenase (2 loci)	1.1.1.37	Mdh-1,2	E
Mannose-6-phosphate isomerase	5.3.1.8	Mpi	B
Nicotinamide adenine dinucleotide dehydrogenase <sup>1</sup>	1.6.99.3	Nadh-dh	C
Peptidase (2 loci)	3.4.13.9	Pep-1,2	A
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh	D
Phosphoglucomutase	2.7.5.1	Pgm	B,E
Pyruvate kinase	2.7.1.40	Pk	B,E

<sup>1</sup> Reduced form.

TABLE 3.—The distribution of alleles among the eight populations of Costa Rican *Nototriton* analyzed. gua = *N. guanacaste*, abm = *N. abscondens* from Monteverde, abz = *N. abscondens* from the Zona Protectora, abn = *N. abscondens* from Las Nubes, pic = *N. picadoi*, riz = *N. richardi* from the Zona Protectora, rin = *N. richardi* from Las Nubes, tap = *N. tapanti*, and out = outgroup. Only alleles present among Costa Rican *Nototriton* are included in the outgroup column; outgroup alleles that are uninformative with regard to Costa Rican *Nototriton* are not included. Gdh, Gpi, Hadh, Mdh-1, and Pgm were monomorphic among all Costa Rican populations studied.

Locus	gua	abm	abz	abn	pic	riz	rin	tap	out
Acon-1	a	a	a(.73) b(.27)	a	a	c	c	a	—
Acon-2	a	a	a	a	a	b	b	a	a
Aat	b(0.90) c(0.10)	a(0.12) b(0.88)	b	b	b	a	a	a	—
Ada	a	a	a	a	a	b	b	c	a
Adh	b	a	a(0.59) b(0.41)	a	b	c	c	a	—
Est-1	b	a	a	a	a	c(0.67) d(0.33)	c	c	—
Est-2	b	b	b	b	a	c	c	d	a
Gd	b	b	b	a(0.05) b(0.95)	b	a	a	a	a/b
Icdh-1	a	a	a	a(0.85) b(0.15)	c	b	b	b	—
Icdh-2	d	d	a(0.09) d(0.91)	d	c	b	b	b	—
Ldh	a	a	a	a	a	b	b	b	—
Mdh-2	a	a	a	a	a	b	b	b	b
Mpi	a(0.10) b(0.90)	a	b	b	b	a	a	a	a/b
Nadh-dh	b	b	b	b	a	b	b	a	b
Pep-1	b	d	d	d	c	a	e	a	—
Pep-2	a	a	a	a	a	a	a	b	—
Pgdh	a(0.10) b(0.90)	b(0.88) c(0.12)	a(0.91) b(0.09)	b	b	c	c	c	c
Pk	a	a	a	a	a	a	a	b	a

information present in transformations (e.g., A—AB—B—BC, in the example above), but we believe that this disadvantage is outweighed by the fact that the obscuring effects of small sample sizes are minimized. As predicted from our expectations regarding the problems of small sample size, a phylogenetic analysis incorporating information from polymorphisms yielded a consensus cladogram that was unresolved, except for the sister group status of *N. richardi* and *N. tapanti*.

*Results.*—Allele distributions in the eight *Nototriton* populations and outgroups are summarized in Table 3. Only those outgroup states that are pertinent to the analysis of variation among Costa Rican *Nototriton* are included. We are forced to make the assumption that the Costa Rican species form a monophyletic group. Be-

cause all potential outgroups are so differentiated and thus offer limited opportunity for cladistic analysis, the strongest evidence of monophyly at this time is the relatively low genetic distances among the Costa Rican species, and the great distances from all of the Costa Rican species to potential outgroups.

The single population of *Nototriton guanacaste* is characterized by three unique alleles (Aat-c, Est-1-b, and Pep-1-b), the *N. picadoi* population by four (Est-2-a, Icdh-1-c, Icdh-2-c, and Pep-1-c), the *N. tapanti* population by four (Ada-c, Est-2-d, Pep-2-b, and Pk-b), the Zona Protectora population of *N. abscondens* by one (Acon-1-b), and the Zona Protectora and Las Nubes populations of *N. richardi* each by one (Est-1-d and Pep-1-e, respectively). The Monteverde and Las Nubes popula-

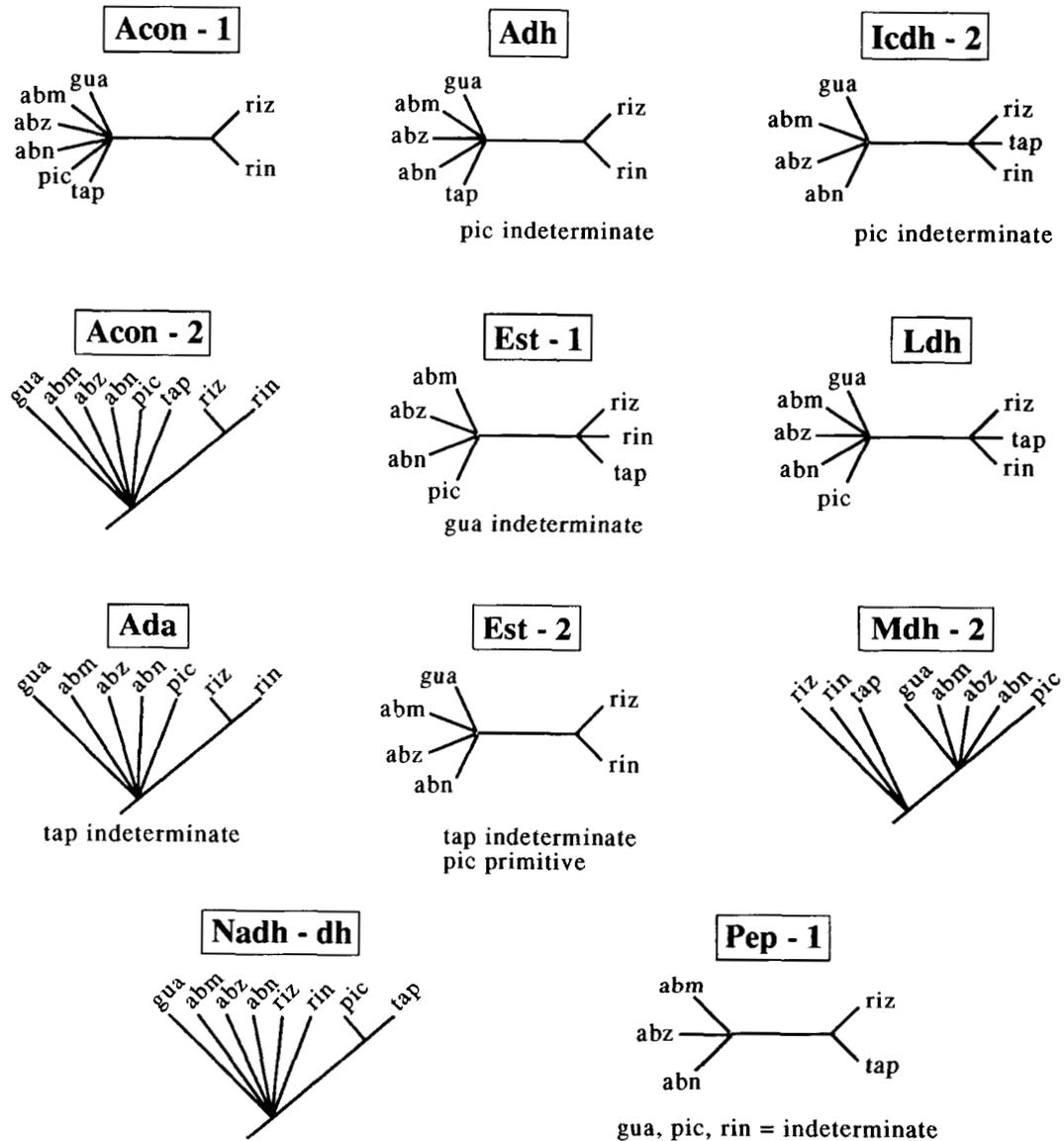


FIG. 8.—Phylogenetic hypotheses for populations sampled based on each of the allozymic characters that are phylogenetically informative. Loci are used as characters and alleles as states. Abbreviation: abn = *N. abscondens*, Las Nubes sample; abm = *N. abscondens*, Monteverde sample; abz = *N. abscondens*, Zona Protectora La Selva sample; gua = *N. guanacaste*; pic = *N. picadoi*; rin = *N. richardi*, Las Nubes sample; riz = *N. richardi*, Zona Protectora La Selva sample; tap = *N. tapanti*.

tions of *N. abscondens* exhibit no unique alleles.

A phylogenetic approach was attempted in order to discern genealogical relationships among the eight populations of Costa Rican *Nototriton*. Table 3 lists the allele distributions among the populations and

the outgroup states where appropriate. These alleles were used as character states (loci as characters, cf. Buth, 1984) in a cladistic analysis (*sensu* Hennig, 1966). Of the 23 proteins surveyed, five (Gdh, Gpi, Hadh, Mdh-1, and Pgm) were monomorphic among the Costa Rican *Nototriton*;

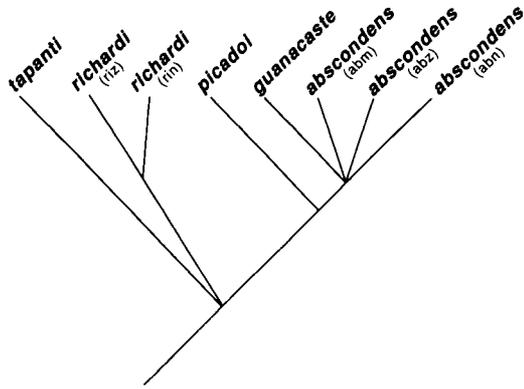


FIG. 9.—Consensus phylogenetic hypothesis, based on the allozymic characters treated separately in Fig. 8. Abbreviations as in Fig. 8.

two others (Pep-2 and Pk) were phylogenetically uninformative because they represent autapomorphies in single populations. Five other variable loci (Aat, Gd, Icdh-1, Mpi, and Pdgh) contained no useful information when allele combinations were grouped into character states as discussed in the methods section above. The phylogenetic patterns suggested by each of the remaining 11 loci that were variable among the Costa Rican *Nototriton* are illustrated in Fig. 8. An analysis of these characters using the PAUP computer package (Swofford, 1985) with Farris optimization and using the branch-and-bound procedure (insuring that the most parsimonious trees were found) yielded several trees, which combine into the strict consensus tree in Fig. 9.

Acon-2 unequivocally suggests sister group status for the two populations of *N. richardi* and Mdh-2 unequivocally suggests that *N. picadoi*, *N. guanacaste*, and *N. abscondens* form a clade (the “*picadoi* group”). Once it is accepted that these patterns exist, Est-2 provides a synapomorphy of *N. guanacaste* and the three populations of *N. abscondens*. All other loci can be mapped onto a variety of phylogenetic hypotheses equally parsimoniously. Mapping them onto the hypothesis in Fig. 9, Acon-1 and ADH could provide either further synapomorphies for the two *N. richardi* populations or they could be synapomorphies linking *N. tapanti* with the

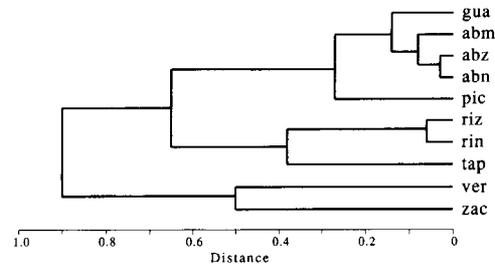


FIG. 10.—Phenogram (UPGMA) based on Cavalli-Sforza arc genetic distance for species of *Nototriton* in allozyme study. For abbreviations, see Fig. 8.

*picadoi* group. Est-1, Icdh-2, and Ldh could provide either synapomorphies of the *picadoi* group or synapomorphies allying *N. tapanti* with *N. richardi*. Because of the presence of autapomorphies in *N. guanacaste* and *N. picadoi*, no further phylogenetic information is provided by Pep-1. Only Nadh-dh, which suggests a synapomorphy for *N. tapanti* and *N. picadoi*, conflicts with the hypothesis generated by Acon-2, Mdh-2, and Est-2 (Fig. 9); this character also conflicts with Est-1 and Ldh.

A phenetic approach also was used to examine patterns of variation among the populations. The numbers of fixed allozymic differences detected between populations are indicated in Table 4. Nei (1978), Rogers (1972), Cavalli-Sforza and Edwards (1967) arc distances, and Cavalli-Sforza and Edwards (1967) chord distances (Tables 5, 6) were calculated using the BIOSYS computer program (Swofford and Selander, 1981), with slight modification. The standard approach to analyzing genetic distance values phenetically is to use the UPGMA technique (Sokal and Mich-

TABLE 4.—Fixed differences in proteins surveyed between populations. Abbreviations as in Table 3.

	gua	abm	abz	abn	pic	riz	rin	tap
gua	—							
abm	3	—						
abz	2	1	—					
abn	3	1	0	—				
pic	6	7	5	6	—			
riz	14	12	15	13	15	—		
rin	14	12	15	13	15	1	—	
tap	15	12	15	13	15	7	8	—

TABLE 5.—Nei (1978, above diagonal) and Rogers (1972, below) genetic differences. Abbreviations as in Table 3.

	gua	abm	abz	abn	pic	riz	rin	ap
gua	—	0.184	0.115	0.196	0.307	1.019	1.030	1.162
abm	0.180	—	0.057	0.094	0.368	0.889	0.900	0.900
abz	0.138	0.087	—	0.058	0.271	1.016	1.028	1.117
abn	0.196	0.107	0.090	—	0.297	1.012	1.023	1.023
pic	0.274	0.315	0.257	0.260	—	1.178	1.190	1.056
riz	0.639	0.593	0.633	0.638	0.691	—	0.048	0.438
rin	0.644	0.598	0.638	0.643	0.696	0.508	—	0.496
tap	0.687	0.598	0.678	0.643	0.652	0.362	0.391	—

ener, 1958). Hedges (1986) discussed the applicability of various genetic distance measures to a number of dendrogram-building algorithms, and he concluded that the best measure for use with UPGMA is the Cavalli-Sforza arc distance. Use of this measure yields the UPGMA phenogram illustrated in Fig. 10. All other distance measures yield an identical topology.

An alternative approach to the analysis of patterns of genetic distance among populations is to examine genetic distances in relation to the geographic distribution of the populations (Good and Wake, 1992). Populations in genetic contact (showing ongoing or recent gene flow) are expected to show a positive correlation of genetic distance with geographic distance, so that the closer two populations are geographically, the nearer their genetic distance will approach zero. Genetically independent populations would not be expected to show this relationship a priori, although they might if genetic independence has occurred very recently. This is because genetic distance is expected to be independent of geographic distance for independently evolving entities. We have used

Nei (1978) distance ( $D_N$ ), which is both upwardly unbounded (as is geographic distance, within gross limits) and strongly correlated with geographic distance under some models of gene flow (Nei, 1972). We present an analysis here for its heuristic value in giving us insight into the genetic data and its interpretation; we emphasize that the recognition of the species is based on morphological traits as well as the presence of unique alleles.

The relation of genetic distance to geographic distance for the eight populations we studied is illustrated in Figs. 11 and 12. The genetic distances between *N. tapanti* and the two populations of *N. richardi* are 0.44 and 0.50, while the two populations of *N. richardi* are much more similar to each other (0.05). The distances from both *N. tapanti* and *N. richardi* to all populations of *N. guanacaste*, *N. abscondens*, and *N. picadoi* are great, and approximately equivalent (0.89–1.19); the distances to the Monteverde sample of *N. abscondens* are consistently a bit lower (0.89–0.90) than the distances to the other populations (1.01–1.19). The distances from *N. picadoi* to each of the populations of *N. guanacaste*

TABLE 6.—Cavalli-Sforza and Edwards arc (below diagonal) and chord (above) genetic distances. Abbreviations as in Table 3.

	gua	abm	abz	abn	pic	riz	rin	tap
gua	—	0.371	0.306	0.339	0.466	0.719	0.719	0.743
abm	0.170	—	0.237	0.208	0.501	0.684	0.684	0.684
abz	0.116	0.069	—	0.142	0.442	0.727	0.727	0.727
abn	0.142	0.054	0.025	—	0.461	0.712	0.712	0.712
pic	0.268	0.310	0.241	0.262	—	0.751	0.751	0.727
riz	0.638	0.578	0.652	0.626	0.696	—	0.204	0.537
rin	0.638	0.578	0.652	0.626	0.696	0.051	—	0.563
tap	0.682	0.578	0.669	0.626	0.652	0.356	0.391	—

and *N. abscondens* are 0.27–0.37, with the distance to the Monteverde population being higher (0.37) than the distances to the other three populations (0.27–0.31). None of these patterns of genetic variation with geographic distance are consistent with an interpretation of ongoing genetic interchange between the units.

Among the populations of *N. guanacaste* and *N. abscondens* that were analyzed, patterns are more complex. These populations fall in a more or less linear series along the Cordillera Central of northwestern Costa Rica. The geographically closest pair of populations, Zona Protectora and Las Nubes (*N. abscondens*), are genetically very similar ( $D_N = 0.01$ ), and probably are, or have recently been, experiencing gene flow. The Monteverde population of *N. abscondens*, at a considerably greater geographic distance, is more problematic, although the genetic distances among the Monteverde, Zona Protectora, and Las Nubes populations conform fairly closely to what is expected of conspecific populations separated by distance (Fig. 12). The genetic distances between the *N. guanacaste* population and either the Zona Protectora or Las Nubes populations of *N. abscondens* ( $D_N = 0.12$ – $0.14$ ) are about what would be expected for the geographic distance, all else being equal. However, the genetic distance between *N. guanacaste* and the Monteverde sample of *N. abscondens* is higher ( $D_N = 0.18$ ) than would be expected, and does not appear to fit the geographic model of isolation by distance, although this conclusion must be regarded as tentative because we have insufficient numbers of samples for statistical testing.

*Discussion of allozyme study.*—Two models are possible for the patterns of allozyme variation observed among the Costa Rican *Nototriton*: genetic independence of the populations or genetic interchange among them. Except between the Zona Protectora and Las Nubes populations of *N. abscondens*, there are fixed allele differences between all pairs of populations, suggesting some degree of genetic isolation (some differences might be the result of sampling error). Our data suggest that there

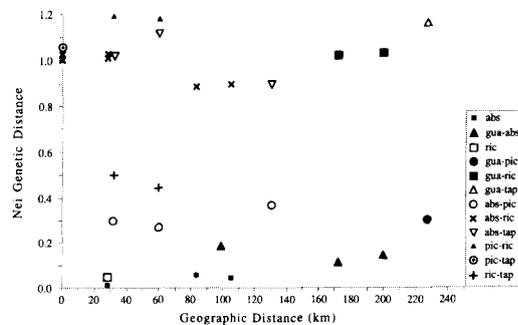


FIG. 11.—Relationship between genetic and geographic distance for Costa Rican populations sampled in allozyme study. For abbreviations, see Fig. 8.

is no gene flow among these populations now, and that there has not been any in the recent past, i.e., they are genetically independent. Intervening populations not yet discovered might show intermediate states, and we believe that such might prove to be the case for the Monteverde population. However, we hypothesize genetic independence for the other populations.

Among *N. guanacaste* and the three populations of *N. abscondens*, patterns are the least clear. If there has been recent genetic contact among all four of these populations, the lack of concordance of the *N. guanacaste*–Monteverde *N. abscondens* comparison with the genetic-geographic distance comparisons among all of the other pairs of populations needs to be explained. Either an increase in rate of allozyme evolution in the Monteverde population or a decrease in Zona Protectora and Las Nubes populations could explain this pattern. The fact that *N. guanacaste* and the Zona Protectora and Las Nubes populations are approximately equally distant from the nearest outgroup (*N. picadoi*), while the Monteverde sample is at a greater genetic distance, suggests that an acceleration in the rate of molecular evolution has occurred in the Monteverde population. Accepting this interpretation for the moment, we can roughly “correct” for the rate change by shortening the branch to this population sufficiently to make the distance from the outgroups (in this case *N. picadoi*, *N. richardi*, and *N. tapanti*) equivalent to the distances from these outgroups to the other popu-

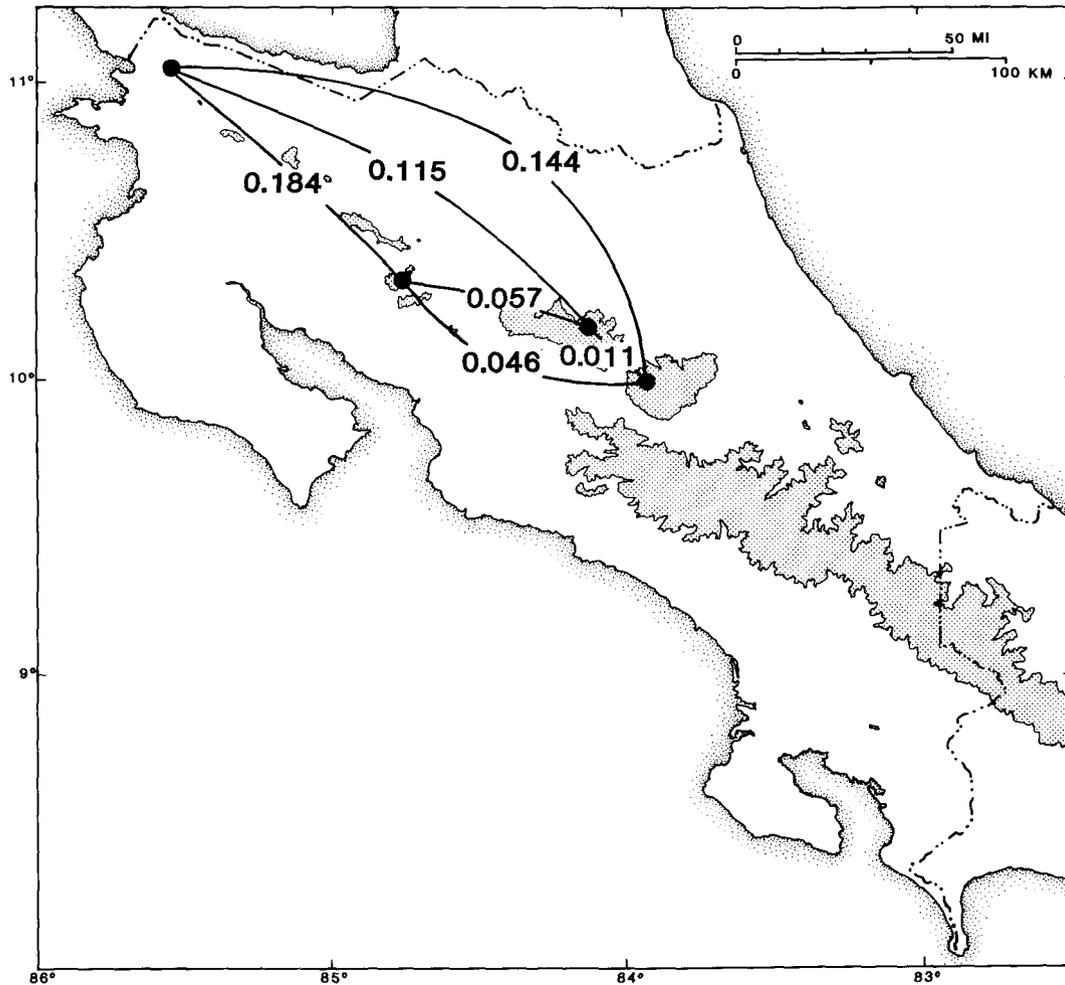


FIG. 12.—Map showing Nei (1978) genetic distances between all populations sampled of *N. abscondens* and *N. guanacaste*.

lations of *N. abscondens*. When this is done, the distance between *N. abscondens* from Monteverde and *N. guanacaste* decreases somewhat, but the pattern still supports genetic independence of the two. We expect that populations of *Nototriton* will be discovered on other isolated peaks in the Cordillera de Guanacaste, and we predict that these will be more similar in morphology and genetics to *N. guanacaste* than to *N. abscondens*, based on the absence of recent contact with those populations. We suspect that *N. guanacaste* was established as a result of a vicariant event which separated it from the main range of *N. abscondens*. If we are correct in this inter-

pretation, even if *N. abscondens* should prove to be paraphyletic, it seems unlikely that there was a previous close association of *N. guanacaste* with the Monteverde population. An appropriate taxonomic solution in such a case would be to recognize the Monteverde population as a distinct taxon.

Within *N. abscondens*, genetic distances are small and habitat, until historical times at least, has been continuous. We suggest that gene flow is or has been present until the last few decades. There is no evidence for genetic independence of these populations, but there is indirect evidence that there is genetic contact, so despite some

TABLE 7.—Morphometric dimensions of Costa Rican *Nototriton* proportional to snout-vent length (SL).

	<i>N. picadoi</i>	<i>N. richardi</i>	<i>N. tapanti</i>	<i>N. major</i>	<i>N. guanacaste</i>	<i>N. abscondens</i> (Monteverde)	<i>N. abscondens</i> (El Angel)
SG	0.180–0.216	0.188–0.205	0.192	0.177	0.216–0.224	0.194–0.212	0.180–0.209
AG	0.544–0.625	0.493–0.568	0.607	0.575	0.545–0.563	0.517–0.570	0.525–0.613
TL	1.203–1.424	1.072–1.482	1.205	1.441	1.210–1.337	1.080–1.244	1.013–1.365
HW	0.123–0.144	0.126–0.150	0.129	0.113	0.145–0.157	0.131–0.141	0.121–0.143
FLL	0.169–0.182	0.140–0.146	0.147	0.148	0.170–0.179	0.148–0.161	0.148–0.183
HLL	0.180–0.189	0.174–0.187	0.174	0.193	0.185–0.201	0.157–0.173	0.175–0.206
TW	0.100–0.108	0.088–0.096	0.094	0.095	0.110–0.122	0.088–0.100	0.081–0.103
TO3	0.22–0.034	0.009–0.017	0.009	0.023	0.028–0.031	0.021–0.028	0.021–0.032
TO5	0.010–0.016	0–0.002	0	0.017	0.011–0.017	0.008–0.013	0.008–0.014
FW	0.060–0.070	0.044–0.050	0.041	0.059	0.066–0.072	0.058–0.062	0.060–0.071
ND	0.010–0.016	0.012–0.016	0.013	0.003	0.004–0.009	0.011–0.014	0.003–0.008
ES	0.050–0.057	0.048–0.056	0.052	0.055	0.051–0.066	0.052–0.058	0.048–0.064

morphological and genetic characters that would permit diagnosis of the Monteverde population, we choose to recognize it, the Zona Protectora, and Las Nubes populations as members of *N. abscondens*.

#### Morphometrics

*Materials and methods.*—Seven populations of Costa Rican *Nototriton* were analyzed for variation in body proportions, as follows: *N. picadoi* ( $n = 7$ ), *N. tapanti* ( $n = 1$ ), *N. richardi* ( $n = 7$ ), *N. major* ( $n = 1$ ), *N. guanacaste* ( $n = 5$ ), and populations of *N. abscondens* from Monteverde and El Angel ( $n = 4$  and  $20$ , respectively). All but one of these samples were taken from the same populations as those sampled for the allozyme survey discussed above (often the same specimens). The El Angel locality is close to the Zona Protectora sample used in the biochemical analysis, and we consider the two to represent a single population.

Only adult specimens of identifiable sex were used in the analysis. These 45 specimens were measured for the following 13 morphometric characters: snout-vent length (SL), axilla-groin length (AG), trunk width (TW), tail length (TL), snout-gular length (SG), head width (HW), nostril diameter (ND), eye-snout length (ES), forelimb length (FLL), hind limb length (HLL), foot width (FW), third toe length (TO3), and fifth toe length (TO5). We realize that the unavoidably small sample size for several species makes it impossible to make statistically valid statements con-

cerning some of the patterns reported below. In such cases, the analysis is presented in the spirit of making predictions concerning the kinds of morphological differentiation that might be found when additional specimens become available.

*Results.*—Table 7 lists each of the 12 morphometric characters as a proportion of snout-vent length. Five of these characters stand out in having distinctive patterns of variation among the seven populations in that there is no overlap between groups of populations. Three species have large nostrils: *N. richardi*, *N. tapanti*, and *N. picadoi* (10–16% SL). The Monteverde population of *N. abscondens* agrees with these species in nostril size. The nostrils of *N. guanacaste*, *N. major*, and the El Angel population of *N. abscondens* are smaller (3.0–9.0% SL). The extreme reduction in size of the feet in *N. tapanti* and *N. richardi* (discussed below) is evident here in the dichotomy of the lengths of the third and fifth toe and in foot width between *N. richardi* and *N. tapanti*, on the one hand, and the other five populations of *Nototriton* on the other. The toes of the former species are not sufficiently free from syndactyly to be measured in most instances, so the difference is absolute. Snout-gular length is greater in *N. guanacaste* than in any other species.

All 13 characters were input both as raw measurements and as residuals (each variable was regressed against SL in an attempt to reduce variation due to size alone) into SAS (SAS Institute, 1985) and sub-

TABLE 8.—Significant comparisons among the seven morphometric characters that show overlapping distributions. "Monteverde" and "El Angel" are populations of *N. abscondens*.

	HW	FLL	HLL	TW
<i>N. picadoi</i> vs. <i>N. richardi</i>		X		
<i>N. picadoi</i> vs. <i>N. guanacaste</i>	X			
<i>N. picadoi</i> vs. Monteverde		X		
<i>N. picadoi</i> vs. El Angel				X
<i>N. richardi</i> vs. <i>N. guanacaste</i>		X		X
<i>N. richardi</i> vs. El Angel		X		
<i>N. major</i> vs. <i>N. guanacaste</i>	X			
<i>N. guanacaste</i> vs. Monteverde		X	X	X
<i>N. guanacaste</i> vs. El Angel	X			X
Monteverde vs. El Angel			X	

jected to principle components (the PRINCOMP procedure) and canonical discriminant function (CANDISC) analyses. These procedures showed varying degrees of separation among the populations, but clusters of points overlapped and both procedures were hampered by unmeasurably short fifth toes in many specimens of *N. richardi* and *N. tapanti*.

Variation in the remaining 7 characters was less useful in discriminating taxa. Although constrained by very small sample sizes for most of the populations, our analyses suggest patterns that might prove to differentiate populations.

Because ratios are not necessarily distributed normally, a Bartlett's test for equality of variances was conducted on all of the ratios resulting from the division by SL of the seven morphometric characters discussed above as being of questionable usefulness. None deviated significantly from normality ( $P < 0.05$ ); accordingly, a parametric ANOVA was conducted in order to determine if there was significant heterogeneity among populations for any character.

Significant heterogeneity was observed in all seven characters. However, when a posthoc analysis of pairwise comparisons was conducted (Scheffé's test), no significant differences ( $P < 0.05$ ) were seen in tail length, axilla-groin length, or eye-nostril distance. The significant comparisons among the remaining four characters are listed in Table 8.

*Discussion.*—Although some of the

comparisons are not significant, a comparison of Tables 7 and 8 suggest the following distributions for the nine morphometric characters that show significant variation among the seven populations of *Nototriton* (i.e., excluding axilla-groin length, tail length, and eye-snout distance). Character states are designated as primitive or derived on the basis of the phylogeny determined by allozyme analysis.

1) Snout-gular length. A long head is apparently an autapomorphy of *N. guanacaste*.

2) Head width. The primitive condition appears to be an intermediate width, with a narrow head being an autapomorphy of *N. major* and a wide head being an autapomorphy of *N. guanacaste*.

3) Arm length. Short arms appear to be characteristic of *N. tapanti*, *N. richardi*, and *N. major*, while long arms are characteristic of *N. picadoi* and *N. guanacaste*. The Monteverde population of *N. abscondens* is intermediate between these two states while the El Angel population of *N. abscondens* ranges from having intermediate to long arms. Substantial homoplasy is evident.

4) Leg length. Short legs are apparently an autapomorphy of the Monteverde population of *N. abscondens*.

5) Trunk width. A large trunk width (signifying a "robust" habitus) is encountered in *N. picadoi* and, to a greater degree, in *N. guanacaste*. This distribution requires either convergence in these two taxa or a reversal to the less robust condition in the *major/abscondens* lineage.

6–8) Toe length and foot width. Reduced feet and toes are synapomorphies of *N. richardi* and *N. tapanti*.

9) Nostril diameter. Nostril diameter is primitively large. It is reduced in the lineage containing *N. major*, *N. guanacaste*, and *N. abscondens*, but there is a reversal to large size in some *N. abscondens* (Fig. 13).

#### *General Morphology and Osteology*

The osteology of *Nototriton* has been discussed by Lynch and Wake (1978), Wake and Elias (1983), and Papenfuss and

Wake (1987). The most recent treatment of the genus recognized four informal species groups, with the two Costa Rican species then recognized [*N. picadoi* (*N. abscondens* of this paper) and *N. richardi*] being placed into their own species groups (Papenfuss and Wake, 1987). This division was forced by osteological information (see Lynch and Wake, 1978), which, while incomplete for *N. richardi*, found several differences between the two species. While we can add some important information on osteology, we are unable to present any useful data for the unique holotypes of *N. major* and *N. tapanti*. Accordingly, our analysis must be considered to be preliminary.

All information reported here was obtained from specimens that were cleared and stained either with alizarin red (for mineralized tissues) alone, or with alcian blue as well (for cartilage). We have examined a single specimen each of *N. picadoi* (MVZ 210419) and *N. guanacaste* (MVZ 207106), but have studied 22 specimens of *N. abscondens* (MVZ 97905, 97922, 203642–58, 203660–61, 203663). The single specimen of *N. richardi* (MVZ 99516) from which a hind limb and a forelimb were removed by Lynch and Wake (1978) was fully cleared and stained for use in this study; additional information is available from the partial skeleton examined by Wake (1966). Some information was derived from x-ray radiographs of the holotype of *N. major*. Figures 14 and 15 present osteological information.

In order to facilitate comparison with the cladogram presented by Lynch and Wake (1978) and the analysis of Wake and Elias (1983), we present a coding of characters used by Lynch and Wake for each of the four Costa Rican species investigated here (capital letters are plesiomorphic and lower case letters are apomorphic states): *N. abscondens* A, b, c, d, E, f, g, H; *N. guanacaste* A, b, c, d, E, f, g, H; *N. picadoi* a, b, c, d, E, f, g, H; *N. richardi* A, b, C, d, E, f, g, H. We have been conservative in coding these characters. When a state is polymorphic, and the plesiomorphic state is present, we have coded the species as being plesiomorphic. We em-

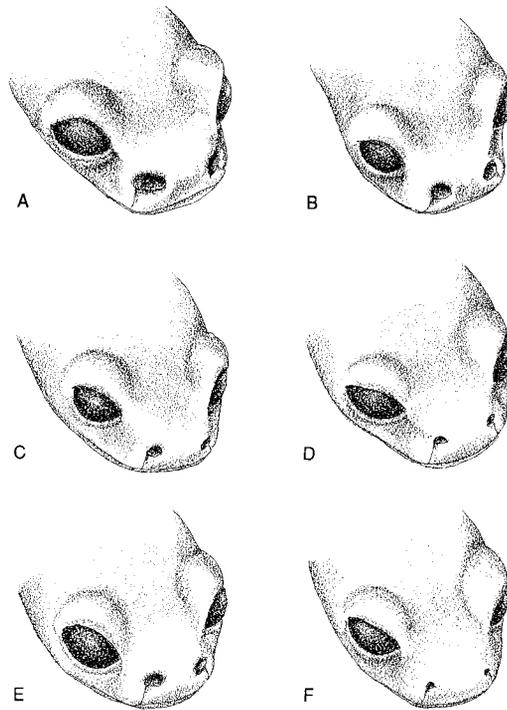


FIG. 13.—Anterior views of the snouts of Costa Rican species of *Nototriton*, showing differences in the shape and size of external nostrils and the head. In life, the nostrils of *N. tapanti* and *N. richardi* are more evidently enlarged than those of the other species, while *N. major* has the smallest nostrils. The head of *N. tapanti* is noticeably flatter than that of *N. richardi* or the other species. A. *N. tapanti*, holotype (MVZ 203746); B. *N. richardi* (MVZ 210406); C. *N. abscondens* (MVZ 207123); D. *N. guanacaste* (MVZ 207113); E. *N. picadoi* (MVZ 203746); F. *N. major* (UCR 6756).

phasize that while there is little information of phylogenetic interest for the Costa Rican species, these characters are of importance in phylogenetic analysis within *Nototriton* and of *Nototriton* with other taxa. In Lynch and Wake (1978), states b, c, d, f and g were synapomorphies of *Nototriton* (excluding *N. richardi*, which was incompletely known).

The only differences between the taxa relate to characters A and C. Character A is the presence of a dual bony link between the frontal and the nasal (see Fig. 14), formed by the prefrontal posteriorly and the nasal anteriorly. In *N. picadoi*, there is no prefrontal bone, and only the nasal

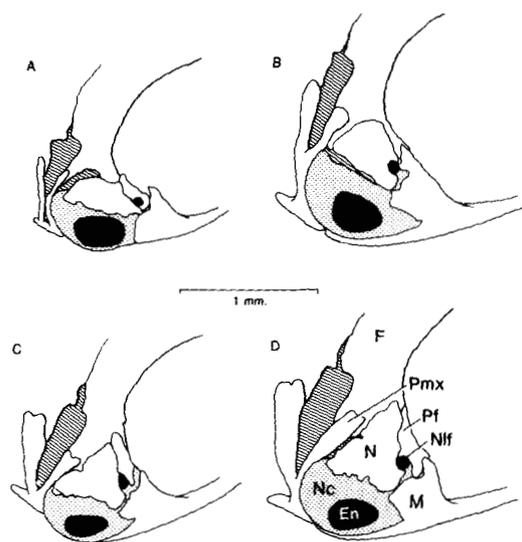


FIG. 14.—Views of the anterior cranial elements of Costa Rican species of *Nototriton*, illustrating the same features figured by Lynch and Wake (1975, 1978). Stipple is cartilage, lined area is space between skeletal elements (internasal fontanelle, plus some space between nasal and olfactory capsule), solid black is external naris (En) and nasolacrimal foramen (Nlf). Other Abbreviations: F, frontal; M, maxilla; N, nasal; Nc, nasal capsule; Pf, prefrontal; Pmx, premaxilla. A. *N. richardi* (MVZ 99516); B. *N. picadoi* (MVZ 210419); C. *N. guanacaste* (MVZ 207100); D. *N. abscondens* (MVZ 203647).

is involved in the link. This is a pattern not before recorded in the genus, but the loss of the prefrontal is a common homoplasy in bolitoglossine salamanders (Wake, 1966; Wake and Elias, 1983). Character C refers to the frontal processes of the nasal, which in the plesiomorphic condition arise and remain separated for their entire lengths. State c is the basal fusion of the processes. In one of the two *N. richardi* for which information for this character is available, the processes arise and remain separated; in the other the very slender processes arise separately, but come into contact shortly above the pars dentalis and appear to fuse, before again separating and remaining separated. Thus, the distinction between these two states is less discrete than previously thought.

One additional character requires comment. Character G, the columellar process of the operculum, is never well developed in bolitoglossines, so the distinction be-

tween presence and absence is sometimes marginal. Such is the case for *N. richardi*, in which a rudimentary process is present; no sign of the process is seen in the other species.

Skull structure easily distinguishes *N. picadoi* and *N. richardi* from the other two species studied (Fig. 14). The skull of *N. picadoi* is remarkably compact and well developed, and there is particularly tight articulation of the frontal and parietal bones, with the effect of reducing the dorsal fontanelle to a very small opening. This fontanelle is relatively large in nearly all of the *N. abscondens*, and is always present to some degree. The fontanelle in *N. guanacaste* is moderately large, but it is almost obliterated in *N. richardi*, as in *N. picadoi*. Prefrontals are well developed in all of the species except *N. picadoi* (Fig. 14), and because this character did not vary in our large series of skeletons of *N. abscondens*, the absence of the bone in *N. picadoi* can be considered to be a diagnostic autapomorphy of the species. The very large frontal lobe of the maxilla is elongated posteriorly and articulates directly and firmly with the frontal behind the moderate-sized nasal of *N. picadoi*. Another distinctive feature of this region in *N. picadoi* is the incision of the pathway of the nasolacrimal duct into the posterolateral margin of the nasal; in other species the duct passes through a niche separating the prefrontal and the nasal (*N. guanacaste*, *N. abscondens*), or the pathway is incised into the anterolateral margin of the prefrontal (*N. richardi*) (Fig. 14).

The skull of *N. richardi* is well formed despite its miniaturization. It differs from the skulls of the other species in that the facial portion of the skull is greatly reduced (Fig. 14). The snout region is much shorter and blunter than in the other species. There is an unusual reduction in the dorsal and anterior parts of the cartilaginous nasal capsule, and the opening of the external naris is much larger than is evident from the only moderately enlarged opening in the skin.

The structure of the digits and phalanges differs among the species. Those of *N. guanacaste* and *N. abscondens* are the most

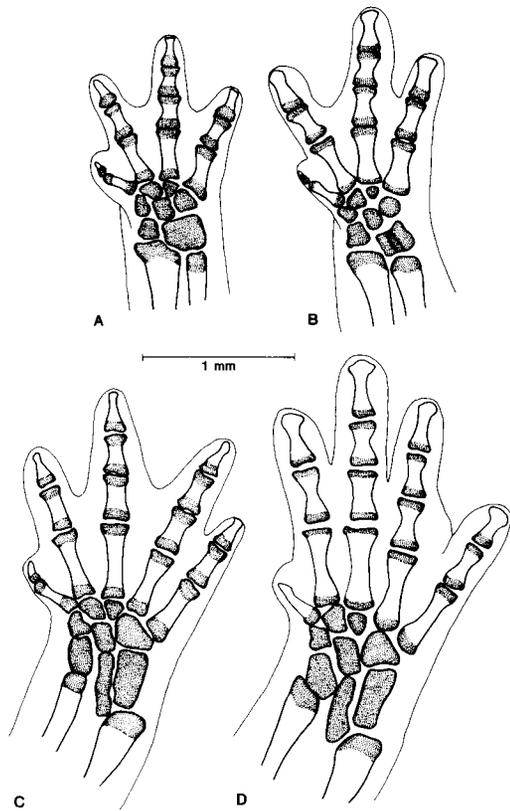


FIG. 15.—Dorsal views of the skeletons of the right forelimbs and hind limbs of *Nototriton guanacaste* (A and C, MVZ 207106) and *N. picadoi* (B and D, MVZ 210419).

similar and generalized [Fig. 15; and Fig. 3g (labeled “*C. picadoi*”) of Lynch and Wake, 1978]. The terminal phalanges are tapered or only slightly expanded within the expanded cutaneous digital tip. A relatively large proportion of each phalanx and metapodial element remains cartilaginous. The digits are well separated from each other. In *N. picadoi*, the digits are especially distinct from one another and the terminal phalanges are expanded into rounded or spatulate-shaped tips (Fig. 15). In contrast, the hands and feet of *N. richardi* (and of *N. tapanti*) are syndactylous, with only the pointed tips being free (see Fig. 3e,f of Lynch and Wake, 1978) (Fig. 16). All of the species share two mesopodial synapomorphies: distal tarsals 4 and 5 are fused (character d in the list presented above), and the intermedium



FIG. 16.—Dorsal views of the anterior part of the bodies of two sympatric species of *Nototriton* to show the diagnostic differences in external hand morphology. Left, *N. abscondens* (MVZ 194869, from region of Cascajal de las Nubes, Prov. San José, Costa Rica) and right, *N. richardi* (194886, from the same site).

and ulnare are fused in the carpus (character f in the list presented above).

None of the species has a septomaxillary bone (a synapomorphy of *Nototriton* within the Bolitoglossini), but they all have preorbital processes of the vomers that bear teeth (a plesiomorphic character, E in the list presented above, that sets them apart from the other more northern members of the genus). All four species have a similar hyobranchial apparatus, but the short radii are very distinct in all except *N. richardi*, in which they barely can be discerned from the somewhat expanded tip of the basi-branchial. In none of the species is the dorsal fontanelle (between the frontal and parietals) enlarged, although that of *N. guanacaste* and *N. abscondens* is clearly larger than that of the remaining species. All of the species have 14 trunk vertebrae and 2 caudosacral vertebrae. As expected, the numbers of caudal vertebrae vary, generally with higher numbers in larger specimens. The highest number recorded is 36, in the holotype of *N. major* (which, coincidentally, is by far the largest specimen of the genus ever encountered in Costa Rica). However, numbers of vertebrae are large for salamanders of this size, and the tails are unusually long in the entire group.

Character state c is a potential synapomorphy of *N. abscondens*, *N. guanacaste*, and *N. picadoi*, but this is a state shared also with Guatemalan and Honduran species and it may be a synapomorphy for the

whole genus. The appearance of the ancestral state as one of two morphs in *N. richardi* might then be a reversal. Character *a* is a distinctive autapomorphy of *N. picadoi*, and while it is not informative in phylogenetic analysis it does constitute additional evidence for the recognition of this taxon as distinct from *N. abscondens*. We have had inadequate material to find any osteological synapomorphy for *N. richardi* and *N. tapanti*, but given the great similarity in external shape of the limb it is likely that some features of the hands and feet will constitute a synapomorphy. The relatively greatly enlarged nostrils of these two species also constitutes a morphological synapomorphy. Accordingly, taking into account the morphological and the allozymic data, *N. richardi* and *N. tapanti* are most likely sister taxa. Although we lack osteological synapomorphies that would support the allozymic data in recognizing the remaining species as a distinct clade, it may be that a *N. richardi*-*N. tapanti* clade is a sister group of a clade including the remaining Costa Rican species.

#### *Habitat and General Ecology*

None of the Costa Rican species of *Nototriton* are sufficiently common to permit detailed ecological study. However, we can present some general information concerning the habitat and ecology of the species, based on our collections. The species occur in cloud forests (Wake, 1987), which in many parts of Costa Rica have been reduced to isolated fragments by volcanic activity and natural climate change, and lately by human disturbance. The habitat of *N. guanacaste* appears to be a natural cloud forest isolate within an extensive area of dry tropical forest on the Pacific slope and wet lowland forest on the Atlantic slope. Within relatively undisturbed cloud forest, *Nototriton* is difficult to find. Salamanders have most commonly been encountered in lightly disturbed areas along roads and trails, where they can be locally abundant in clumps of moss (Taylor, 1954). Along the Atlantic-facing slopes of the Cordillera Central, *N. abscondens* and *N. richardi* occur in local sympatry, but the former is more than ten times as common

as the latter in roadside habitats. Survey work in undisturbed habitat within the Zona Protectora La Selva by Good and colleagues revealed a previously unappreciated microhabitat difference between the two species. The vertical distribution of *N. abscondens* in this region is from 960 m–2050 m in undisturbed forest; the species is consistently found off the ground in moss that is attached to tree trunks, and in clumps of moss hanging mainly from vertical but occasionally horizontal limbs. One specimen was collected under a piece of loose bark. In contrast, *N. richardi* was found between 1520 m and 1800 m, and occurred consistently on the ground. This species usually was found in leaf litter, but once was encountered under moss. In undisturbed portions of the Monteverde Cloud Forest Preserve, *N. abscondens* also is found most frequently in moss masses hanging from trees, but in disturbed zones it occurs in road banks under moss mats and underlying rock rubble. The only species of *Nototriton* we have collected in microsympatry are *N. abscondens* and *N. richardi*, which occur together on the north-facing slopes of Volcáns Poás, Barva, and Irazú. The two are easily confused, and it is especially difficult to distinguish juvenile *N. abscondens* from subadult *N. richardi* (the latter has a larger nostril). The most diagnostic features are the hands and feet, which in *N. abscondens* are larger and have relatively distinctive digits that are free from one another and rounded at the tips, but in *N. richardi* are smaller and have indistinct digits that are nearly fused to one another and have more pointed tips (Fig. 16).

We expect that *N. tapanti* and *N. picadoi* will be found in microsympatry, for they have been taken within less than 5 km in similar habitat in the valley of the Río Grande de Orosi.

We have found *N. guanacaste* most commonly in moss clumps on tree trunks and limbs (to about 4 m above ground) in undisturbed areas at elevations between 1420 and 1580 m (activity temperatures 17.1–18.1 C).

Knowledge of the occurrence of *N. picadoi* in bromeliads is based on the report by Picado (1913). Picado may have col-

lected only a single specimen (the holotype of the species), for no other specimens from the period of his work are found in collections. A specimen from "Escazu" (Dunn, 1937), possibly assignable to this species, also has been reported from bromeliads. We have found the species in lightly disturbed habitat in Refugio Tapantí at elevations slightly above 1300 m, and exclusively under moss on tree trunks and limbs or in large clumps of moss hanging from trees. One specimen has recently been collected on vegetation at night (F. Bolaños, personal communication). The species occurs as high in trees as we have been able to reach (about 4 m). Activity temperatures at this elevation range from 16.2–19.8 C.

The single specimen of *N. tapanti* was collected under moss on the ground in secondary forest. No information is available concerning the habitat of *N. major*.

In summary, available information on habitat suggests that *N. richardi* and *N. tapanti* may be mainly ground-dwelling, while *N. abscondens*, *N. guanacaste*, and *N. picadoi* occur off the ground in undisturbed habitat.

Because so little is known concerning the life history of *Nototriton*, we record what little information we have obtained. A clutch of 7 eggs of *N. guanacaste* was collected on 22 August 1987. These were in a relatively advanced stage of development, and one hatched on 26 August 1987. A second clutch of 4 eggs also was collected on 22 August 1987, in a relatively early stage of development. A clutch of 10 eggs of *N. picadoi*, very near hatching, was collected on 7 August 1990. Two embryos removed from the egg have small amounts of yolk remaining and gills with three distinct rami; they measure approximately 7.0 mm snout-vent length, and the tail is much shorter than snout-vent length. All of the eggs were found in and under moss in habitats typical of adults. The most remarkable observation about these clutches is that none were attended by an adult. McCranie and Wilson (1992) recently reported that the Honduran species *Nototriton barbouri* also abandons its clutches. The expected ancestral condition for terrestrial salamanders is for the nest to be

guarded (Nussbaum, 1985), and this is typical for other bolitoglossines (although *Batrachoseps* may engage in group nesting). To our knowledge, only two clutches of eggs of *Oedipina* have been found in the field, and in both instances the clutches were unattended by females (eggs in MVZ collection). Recently, a specimen of *O. parvipes* laid eggs in the laboratory and it did not attend the eggs. Perhaps abandonment of the eggs is a behavioral synapomorphy of *Oedipina* and *Nototriton*.

#### GENERAL DISCUSSION

Our genetic and morphological data indicate that there is more differentiation of the genus *Nototriton* in Costa Rica than had previously been recognized in terms of numbers of taxa. However, we believe that the six recognized species constitute a monophyletic group relative to other *Nototriton*, and this is a modification of earlier views which envisioned *N. richardi* as a more remote ancestor of other *Nototriton* than we believe to be the case (see below). Members of the genus are restricted to perennially moist and cool habitats and they appear to have restricted elevational limits (roughly between 1000 and 2000 m). These habitats are fragmented geographically, and there has been opportunity for geographic differentiation and presumably for allopatric speciation, a mode that appears to dominate among caudate amphibians (Larson, 1984). Salamanders of this genus are very small, often locally uncommon, and they are found only through diligent search; accordingly, it is likely that more populations remain to be discovered with additional field work, especially along the Atlantic coastal slopes of the Cordillera de Talamanca. Unfortunately, habitat destruction is proceeding at such a rapid rate that the likelihood of extinction is greater than that of discovery at present. Given the stringent habitat and microhabitat requirements of *Nototriton*, we expect that these salamanders will be especially subject to extinction as habitat modification proceeds.

On the basis of our results, we hypothesize that the Costa Rican *Nototriton* form a monophyletic group in relation to other members of the genus and to the related

genus *Oedipina*. There are ecological, morphological, behavioral and biochemical similarities, and we believe that many of them will be shown to be synapomorphies when more data are available.

Among the characters that are potential synapomorphies of the Costa Rican *Nototriton* are the presence of a discrete paratoid gland dorsolaterally just behind the head, a feature that is found in a few other plethodontids but is rare in tropical salamanders. Histological sections disclose that this gland is little more than a local concentration of dermal glands, with no special characteristics, other than the fact that it can be distinguished from the surrounding skin. These small glands usually are lightly pigmented.

We used samples of *Nototriton veraepacis* and a newly discovered undescribed species of *Nototriton*, both from Guatemala, together with many species of *Oedipina*, as outgroups for our biochemical study (based on phylogenetic arguments of Wake and Elias, 1983, and Sessions and Kezer, 1991). The greatest genetic distance found within the Costa Rican group (about  $D_N = 1.0$ ) is substantially less than that measured between Costa Rican species and the two Guatemalan species of *Nototriton* used in the present study. Furthermore, all Costa Rican species have about the same genetic distances (well in excess of 1.0) to the other *Nototriton* studied and to *Oedipina*. The amount of differentiation between the Costa Rican species and the outgroups is so great that cladistic analysis is precluded, since almost no protein variants are shared. This implies a very long history of separation. We expect that a number of protein variants are synapomorphies for a Costa Rican clade, but this cannot be tested at present. Our biochemical results support a closer relationship between *N. richardi* and the other Costa Rican species than had been postulated by Lynch and Wake (1978) or Papenfuss and Wake (1987).

The extraordinarily diminutive *N. richardi* and *N. tapanti* (neither is known to exceed 24 mm in standard length) are specialized in morphology and ecology, and on the basis of the derived nature of the snout, hands and feet and of their ecol-

ogy, all of which are synapomorphies based on outgroup comparisons, we postulate that they are sister taxa. Lynch and Wake (1978) thought that the former retains some osteological traits that appear to be ancestral (Lynch and Wake, 1978). The polymorphic nature of one trait formerly thought to be a plesiomorphy of the species (separated premaxillary processes) removes one of the points raised by Lynch and Wake (1978) when they reserved judgement about the relationships of the species. We hypothesize that a *N. richardi*-*N. tapanti* clade is the sister taxon of the remaining Costa Rican species (see above).

We recommend that the Costa Rican species of *Nototriton* continue to be placed in two species groups: *richardi* (*N. richardi*, *N. tapanti*) and *picadoi* (*N. abscondens*, *N. guanacaste*, *N. major*, *N. picadoi*). Although the *richardi* group has a very restricted distribution, both it and the *picadoi* group occur in two distinct biogeographic regions in Costa Rica. One species of each group, *N. tapanti* and *N. picadoi*, occurs in the northern end of the Talamancan mountain range, while the other species occur in the volcanic arc that runs across the northern part of the country. These two regions are joined by a low ridge (ca. 1500 m elevation) between San José and Cartago which constitutes the continental divide. The Cordillera Central is a volcanically active zone, while the Talamancan range is the ancient montane core of Costa Rica-western Panama. The existence of two species pairs in these two areas leads us to predict that vicariant events associated with the dynamic Cordillera Central were responsible for the separation of once wide-ranging populations, and that the isolated populations that resulted gave rise to the present-day species pairs. *Nototriton tapanti* and *N. picadoi* are the southern-most *Nototriton* known; they are each the most specialized in morphology of their respective species groups.

#### RESUMEN

El estudio de la variación aloenzimática, de la morfología externa y de la osteología de las "salamandras del musgo" del género

*Nototriton*, pone de manifiesto que en Costa Rica hay más especies de las dos que habitualmente se aceptan.

Las tres especies para las que previamente se poseían nombres disponibles resultaron ser válidas y aquí se presentan nuevas diagnósticos para ellas; además se describen tres especies nuevas.

*Nototriton abscondens* es la más extendida y mejor conocida de las especies previamente descritas. Se distribuye a lo largo de las faldas de la Cordillera Central y también en las proximidades de Monteverde. *N. richardi* se distribuye en simpatría con *N. abscondens* en la Cordillera Central. *N. picadoi* se conoce únicamente de las porciones septentrionales de la Cordillera de Talamanca, especialmente en la región de Tapantí. Las especies nuevas son: *N. guanacaste*, una especie relacionada con *N. abscondens*, localizada en los volcanes Cacao y Orosí; *N. major*, la especie de mayor tamaño del género en Costa Rica, que se encuentra en los alrededores de Moravia de Chirripó; y *N. tapanti*, una especie cercana a *N. richardi* localizada cerca de Tapantí. En este trabajo se investigan las relaciones filogenéticas y la biogeografía de las seis especies costarricenses del género.

La radiación del género *Nototriton* en la Costa Rica actual incluye procesos de miniaturización acompañados por especializaciones morfológicas y ecológicas. Las diferentes especies del género en Costa Rica viven en masas de musgos y entre la hojarasca, mientras que la mayor parte de las especies restantes viven en bromeliáceas. El género *Nototriton*, tras esta revisión, comprende dos especies en México, una en Guatemala (y otra más detectada en este trabajo, todavía sin describir), dos en Honduras y seis en Costa Rica. Las seis especies costarricenses parecen constituir un grupo monofilético, pero las relaciones filogenéticas de los dos grupos de especies septentrionales entre sí y de éstos respecto al grupo meridional no han sido resueltas todavía.

*Acknowledgments.*—We thank F. Bolaños and the late Douglas C. Robinson for access to the UCR collections, and both Robinson and J. Savage for discussion. We have been aided in field work by F.

Bolaños, D. Cannatella, A. Collazo, C. d'Orgeix, C. Guyer, P. León, F. Muñoz, K. Nishikawa, M. Santana, S. Sessions, N. Staub, and T. Wake, and we thank F. Bolaños for information and assistance in the UCR collections. Curators of collections in the United States have provided information and access to specimens. The manuscript was improved by comments from reviewers, especially D. C. Cannatella, J. F. Lynch and J. M. Savage. Kodachromes taken by David C. Cannatella aided in our analysis of color and provided prints for three of the species photographed in the field. Studio photographs were taken by the staff of the Scientific Photographic Laboratory of the University of California at Berkeley. Illustrations were prepared by Karen Klitz. We thank Mario García-París for writing the Spanish summary. In addition, we thank Pedro León and the Center for Cell and Molecular Biology of the University of Costa Rica for hospitality and logistic support, the Organization for Tropical Studies and D. Janzen for logistic support, and the government of Costa Rica for collecting permits. This research was supported by grants from the National Science Foundation (current grant BSR 90-19810) and the National Geographic Society.

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Accepted: 28 February 1993  
Associate Editor: David Cannatella

## APPENDIX I

*Specimens Examined or Assigned*

*Nototriton abscondens* FMNH 178285 (holotype of *Chiropterotriton abscondens*); BMNH 95.7.13.41-43, 96.10.8.85-94 (syntypes of *Spelerpes subpalmaris*) Prov. San José, La Palma. Prov. Alajuela: KUMNH 29971 Volcán Poás, Isla Bonita; KUMNH 34928-47, 36694-95, 37104-125 (Cinchona; KUMNH 37085-103, 3.5 mi W Cinchona; KUMNH 104335, 203190-204, 203207 Montaña Azul; MVZ 97898-942, 172182-90, 181350-51, 183694-695, 183699, 193604, 203740-42, 201236 Caida El Angel; MVZ 207122 Carril Bosque Eterno at Pantanosa Trail, Monteverde Reserve; MVZ 207120-21, 207123 Peñas Blancas trail below continental divide, Monteverde Reserve. Prov. Heredia: CRE ZP 197 trail from 1000 m to 1500 m camp, Zona Protectora La Selva; CRE ZP 371 vicinity of 1500 m camp, Zona Protectora La Selva; CRE ZP 568 2050 camp, Zona Protectora La Selva; MVZ 206389 1500 m camp, Zona Protectora La Selva; MVZ 206392 2050 m camp, Zona Protectora La Selva; MVZ 206393 roadhead for 2050 m camp, Zona Protectora La Selva; MVZ 206390-91 trail from 1000 m to 1050 m camp, Zona Protectora La Selva; MVZ 206385-88 vicinity 1500 m camp, Zona Protectora La Selva; MVZ 203743 1.5 km N Poásito Jet.; UCR ZP 422 vicinity of 1500 m camp, Zona Protectora La Selva; UCR ZP 484, 491 1800 m cabin, trail from 1500 m camp to 2050 m camp, Zona Protectora La Selva; UMMZ 142822 (7 specimens) El Angel Waterfall. Prov. Alajuela-Heredia border region: UCR 738-43, 1301-02, 1711-12, 2378-79, 4826, 6879, 7853-54, vicinity of Vara Blanca and Caida El Angel. Prov. Puntarenas: MVZ 207124 Pantanosa trail, Monteverde Reserve; UCR 4396, 6200 Monteverde Reserve.

Prov. San Jose: MVZ 99515, 172170-74, 181243-45, 181352-53; 190889-95, 193170-72, 194867-70, 210409-17 vicinity of Cascajal de Las Nubes; UCR 2051, 2995 Bajo La Hondura; UCR 3002 Finca Echandi. Prov. Alejuela or San Jose: MVZ 203642-63 either El Angel Waterfall or Cascajal de Las Nubes [received after use in lab by James Kezer].

*Nototriton guanacaste* MVZ 207111 (holotype). Prov. Guanacaste: MVZ 207097-105 (clutch of eggs and hatchlings), 207106-10, 207112-14 trail to top of Volcán Cacao; UCR 8956-66 Volcán Orosí.

*Nototriton major* UCR 6756 (holotype).

*Nototriton richardi* FMNH 178295 (holotype of *Parvimolge richardi*). Prov. Alajuela: KUMNH 34906-07, 37026 Cinchona [not examined]; KUMNH 5294748, 203205 2 mi. W Cinchona [not examined]; MVZ 195870 El Angel Waterfall; UCR 744-745, 1009 Caida El Angel; UMMZ 119512, Volcán Poás, 14.1 mi. N Vara Blanca; UMMZ 129830, 136988, 142822 Prov. Alajuela, region of El Angel Waterfall. Prov. Heredia: MVZ 206394 vicinity 1500 m camp, Zona Protectora La Selva; MVZ 206395 1800 m cabin on trail from 1500 m camp to 2050 m camp, Zona Protectora La Selva; UCR ZP 356-57 vicinity 1500 m camp, Zona Protectora La Selva. Prov. San José: MVZ 99515, 194885-87, 210406-08 vicinity of Cascajal de Las Nubes; UCR 2996 Bajo La Hondura; UCR 3003, 3037 Finca Echandi.

*Nototriton picadoi* USNM 48280 (holotype of *Spelerpes picadoi*). Prov. Cartago: MVZ 203739, 203744-45, 207094-96, 207115-119, 210419 above Rio Grande-Rio Dos Amigos Bridge, W side Rio Grande, Refugio Nacional Tapantí, UCR11191 Sendero Orpendola, Refugio Nacional Tapantí.

*Nototriton tapanti* MVZ 203746 (holotype).