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Phylogenetic and taxonomic implications of protein variation in the Mesoamerican salamander genus *Oedipina* (Caudata: Plethodontidae)

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Abstract: An analysis of protein variation was conducted among 28 population samples of salamanders of the genus *Oedipina*, collected in Guatemala, Honduras, Costa Rica, and Panama, in order to assess the validity of currently recognized species and to evaluate their phylogenetic relationships. A set of eight species of the related genus *Nototriton* served as an outgroup. There are four major clades, with Nei genetic distances between clades ranging from 0.46 to 2.67. Patterns of geographic variation within *O. uniformis* as currently recognized indicate that populations within it should be placed into four species: *O. alfaroi* is a valid species, and *O. gracilis* and *O. pacificensis* are resurrected from the synonymy of *O. uniformis*, while *O. bonitaensis*, *O. inusitata*, *O. longissima*, and *O. syndactyla* remain in synonymy. Geographic variation also is apparent in *O. cyclocauda*, *O. poelzi*, and *O. pseudouniformis*, but no taxonomic changes are warranted at this time. The species status of *O. altura* and *O. grandis* is supported. The four samples representing the *parvipes* group are the most distantly related to the other samples, and genetic variation within the group is sufficient to warrant resurrecting the Costa Rican *O. alleni* from the synonymy of *O. parvipes* of Panama and Colombia. All morphologically differentiated samples are also genetically differentiated. Our results demonstrate that 12 species were sampled (*O. alfaroi*, *O. alleni*, *O. altura*, *O. cyclocauda*, *O. gracilis*, *O. grandis*, *O. ignea*, *O. pacificensis*, *O. parvipes*, *O. poelzi*, *O. pseudouniformis*, and *O. uniformis*). no samples were available for *O. carablanca*, *O. collaris*, *O. complex*, *O. elongata*, *O. gephyra*, *O. paucidentata*, *O. stenopodia*, *O. stuarti*, or *O. taylori*, but all are recognizable as distinct species on morphological grounds.

Key words: Caudata, *Oedipina*, Allozymes, Proteins, Variation, Phylogeny, Taxonomy

The great radiation of plethodontid salamanders in the New World tropics has produced a large number of species that are diverse in both morphology and ecology. These species, all members of the monophyletic supergenus *Bolitoglossa*, comprise about 45% of the living species of caudate amphibians (Wake 1987). Members of this supergenus are unique in that the vast majority have a fixed number (14) of trunk vertebrae, regardless of whether the body is elongated or not. Most other clades of salamanders display both inter- and intrapopulational variation in this character. Among the tropical species in the supergenus, only the elongated, attenuate salamanders in the genus *Oedipina* have increased vertebral numbers, and hence *Oedipina* constitutes one of the best supported monophyletic groups of tropical salamanders. Although a few species of *Bolitoglossa* occur farther south, *Oedipina* has the most southerly overall distribution of any salamander genus. The species are ecological specialists for the most part, having fossorial habits even in the lowlands where other

salamanders typically are arboreal (Wake and Lynch 1976).

Many of the species of *Oedipina* are confusingly similar in structure and coloration. Most of the 18 species currently recognized are dark gray to brown, although some species have lightly pigmented dorsal patterns, especially on the head. They all have very long tails (sometimes 3 times the snout-vent length), short legs, and reduced hands and feet. This high degree of similarity has led to much confusion with regard to the identification and systematics of species in the genus. The most difficult complex is the taxon currently known as *O. uniformis*, which includes seven described entities (Brame 1968. *O. bonitaensis*, *O. gracilis*, *O. inusitata*, *O. longissima*, *O. pacificensis*, *O. syndactyla*, and *O. uniformis*). It ranges from sea level to over 2000 m elevation, resulting in its occurrence in a broader range of hábitats than any other salamander.

Two workers, Taylor (1948, 1949, 1952, 1954) and Brame (1960, 1963, 1968, Brame and Duellman 1970), have been responsible for most of the species descriptions and current taxonomy in *Oedipina*. In the most recent and comprehensive revision, Brame (1968) recognized 15 species in two species groups. He described five new species and reduced nine names to synonymy. Brame and Duellman (1970), Brodie and Campbell (1993), and McCranie *et al.* (1993) each added an additional species.

In the course of field work in Central America over the last 15 years (see Wake 1987, Wake *et al.* 1992), an attempt has been made to obtain samples of these elusive animals for morphological and biochemical studies. Recent work has concentrated on Costa Rica, where the majority of species occurs. This report summarizes a re-examination of the species status of several populations of *Oedipina* using biochemical information unavailable to previous workers.

In recent papers we have discussed our species concept and our operational taxonomic procedures (Good and Wake 1992, 1993). Our views are generally concordant with those of Frost and Hillis (1990), and we accept their main recommendations. Briefly, we consider species to be genetically cohesive units that are evolutionarily independent of other such units. In the case of allopatric populations, we are concerned with historical processes that have led to the allopatry, and with estimates of the likelihood of future genetic interchange. These estimates are based on ecology and geography, as well as on measured differences in morphological and biochemical traits. We attempt to estimate relative length of separation on the basis of these characters (primarily morphology and biochemistry). We assume that populations that have been in isolation sufficiently long to permit substantial morphological and biochemical differentiation are more likely to be reproductively incompatible than are more recently isolated populations in which little differentiation has occurred. This approach is necessarily subjective, but it avoids both the acceptance of extremely polytypic species on the one hand and the acceptance of species status for all undifferentiated (or poorly differentiated) geographic isolates on the other. in cases in which tests of sympatry are impossible, our species concept involves an inference regarding the probability of reproductive isolation. Sympatry tests probably are rare among relatively young species, but when they are available the degree of differentiation among closely related sympatric species can guide decisions concerning allopatric populations. In general, we believe that it is better to err in the direction of too many rather than too few species, and when evidence for differentiation is present and we have sufficient material to make a reasonable judgement, we choose to recognize taxa as full species.

MATERIALS AND METHODS

A total of 26 populations (Table 1) representing 11 species (see discussion below) were analyzed for variation at all of the 22

protein loci listed in Table 2. The distribution of alleles at these loci is listed in Table 3, as is the outgroup state, where appropriate, based on the alleles present in eight species of *Nototriton*, a close relative of *Oedipina* (Wake and Elias 1983). In addition, one individual of *O. cyclocauda* from Honduras and five *O. grandis* from Costa Rica were analyzed (Table 1), but only a subset of the loci were scorable. data for these specimens are presented in the text only.

Liver and intestine samples were collected from freshly killed specimens and stored at -76°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, Richardson *et al.* 1986, Hillis and Moritz 1990). The allele patterns resulting from this analysis were subjected to a variety of phenetic and phylogenetic analyses both by hand and using the BIOSYS (Swofford and Selander 1981) and PAUP (Swofford 1985) computer packages. Voucher specimens were deposited in the Museum of Vertebrate Zoology, University of California (MVZ), the University of Miami (CRE), and the University of Costa Rica (UCR).

In the phylogenetic analyses that follow, a conservative approach to character state transformation designation is taken in that all alleles present together in a polymorphic state in any taxon are considered to represent a single character state (Buth 1984). In this way, if, in a hypothetical case, the allele combinations A, AB, B, and BC occur in four taxa, all four are considered to show the same character state. an allele D in a fifth taxon is considered to be a separate state if there are no taxa with the allele combinations AD, BD, or CD. This approach misses potentially useful information in transformation like A-AB-B-BC in the case above (see Mabee and Humphries 1993, Murphy 1993), but the disadvantage is outweighed by the advantage of also missing potentially obscuring patterns resulting from small sample sizes. Small samples were unavoidable in this analysis because many of the species are very rare (e.g., our specimen of *O. altura* was only the second specimen found since the species was first collected in 1964).

All genetic distances reported in this paper are calculated according to Nei (1978).

Full details of synonym for the taxa discussed in this paper are provided by Brame (1968).

RESULTS

We found 129 alleles distributed among the 22 proteins examined in the 26 samples of *Oedipina* (Table 3). No proteins are monomorphic among all samples, although Icdh1 and Icdh2 vary only in the presence of polymorphisms involving unique alleles in single populations. Alleles per protein range from two (Icdh2 and Pk) to ten (Ldh and Pep1). The maximum number of alleles per protein in a single sample is three (Pep1 in sample 20. see Table 1).

There are eight distinct morphotypes (Figs. 1 and 2) among the populations of *Oedipina* surveyed for all loci in this analysis, and these were considered by Brame (1968) to represent eight species (*O. alfaroi*, *O. altura*, *O. cyclocauda*, *O. ignea*, *O. parvipes*, *O. poelzi*, *O. pseudouniformis*, and *O. uniformis*). There are complex patterns of sympatry among these morphotypes, which taken together represent a minimum estimate of species diversity. *Oedipina alfaroi*, *O. altura*, and *O. ignea* were represented in this analysis by single samples and genetic distances within the *O. pseudouniformis* morphotype are slight (0.01-0.02. Table 4). Within the other morphotypes, however, there is considerable protein diversity. Genetic distances within the *O. cyclocauda* morphotype range up to 0.31, in the *O. poelzi* morphotype to 0.39, in the *O. uniformis* morphotype to 0.70, and in the *O. parvipes* morphotype to 0.83.

In order to determine the degree of genetic cohesion within morphotypes, the relation of genetic distance to geographic distance was

examined. Under an isolation-by-distance model, if populations are in genetic contact (i.e., gene flow occurs) and population genetic characters (mutation, migration, etc.) are in equilibrium and more-or-less uniform over geography, points representing pairs of populations on a plot of genetic distance against geographic distance are expected to fall on a straight line intersecting the origin, because genetic divergence between any two populations should be directly proportional to their geographic separation (Nei 1972). If there is no genetic interchange among populations, there is no reason to expect this intersection with the origin (unless genetic contact only recently has been lost), because genetic divergence should be independent of geographic proximity. Fig. 3 is a plot of Nei genetic distance against geographic distance for the populations of the *O. uniformis* morphotype. The Corcovado and Quepos populations (samples 18 and 19. Table 1) are almost identical to each other (Table 4). The El Angel, Capelladas, Monteverde, Las Nubes, Tapanti, Turrialba, and Juan Viñas populations (samples 20-26. Table 1) form a regression line suggesting genetic interchange. These populations, however, do not show a similar kind of relationship with the La Selva (sample 17) or Corcovado/Quepos populations, nor do the La Selva and Corcovado/Quepos populations show a pattern suggestive of genetic contact with each other. There are four fixed allele differences between the El Angel, etc., cluster and Corcovado/Quepos, seven between the El Angel, etc., cluster and La Selva, and 11 between Corcovado/Quepos and La Selva. The corresponding genetic distances are 0.32-0.48, 0.51-0.63, and 0.53. The genetic distances from *O. alfaroi* (representing the *O. alfaroi* morphotype) to the El Angel, etc., cluster (0.36-0.48) are lower than many of the distances within the *O. uniformis* morphotype.

Our interpretation of the above result is that the *O. uniformis* morphotype consists of at least three genetically independent units. These units have been described in the past (Taylor 1952) as separate species, but Brame (1968) synonymized them with *O. uniformis*. The relatively high elevation populations (the El Angel, etc., cluster) retain the name *O. uniformis*. The Atlantic lowland form (La Selva) is *O. gracilis*, and the Pacific lowland form (Corcovado and Quepos) is *O. pacificensis*. *Oedipina alfaroi* is as similar to *O. uniformis* as is *O. pacificensis*, and more similar to *O. uniformis* than is *O. gracilis* (but see discussion of evolutionary rates below).

The plot of comparisons within the *O. poelzi* morphotype (Fig. 4) shows similarities to the analysis of the *O. uniformis* morphotype. The Volcan Barba, Monteverde, and Las Nubes populations (samples 10, 11, and 13) have been contiguous in terms of gene flow, at least until recently, but the Moravia de Chirripo population (sample 12. Table 1) is genetically distinct and may be genetically independent. While the degree of differentiation is such that species recognition of the Moravia population may be warranted, our genetic analysis is inconclusive due to limited samples and we have inadequate preserved material to properly categorize and diagnose the putative undescribed species. Therefore we defer further discussion of this topic to a later time.

Genetic distances between the morphologically distinct Costa Rican and Panamanian populations of the *O. parvipes* morphotype range from 0.61 to 0.83, and we consider the Costa Rican populations (samples 6-7) to represent a distinct species, for which the name *O. alleni* is available (Taylor 1954). The populations from Panama (samples 8-9) are retained as *O. parvipes* for the present, but they, too, are probably specifically distinct (based on morphology. see Brame 1968) from the South American populations (the source of the holotype). again we defer further discussion to another time when more material is available.

The above discussion suggests that there are at least 11 genetically independent units (species) among the samples analyzed for all 22 proteins. These will be discussed hereafter as the distinct OTU's *O. alfaroi*, *O. alleni*, *O. altura*, *O. cyclocauda*, *O. gracilis*, *O. ignea*, *O.*

pacificensis, *O. parvipes*, *O. poelzi*, *O. pseudouniformis*, and *O. uniformis*. *Oedipina poelzi* might be further divisible into two species, but for the purposes of this analysis we retain it as a single unit. This has no effect on the phylogenetic analysis presented below because the two *O. poelzi* species, if they exist, form a monophyletic unit, as demonstrated by morphological synapomorphies.

Phylogenetic relationships among the 11 species of *Oedipina* were analyzed using PAUP (Swofford 1985) with Wagner optimization and the branch-and-bound option to insure finding the most parsimonious tree(s). After combining alleles into character states as described above, two loci were invariant across all taxa (Icdh1 and Icdh2). Nine more loci were variable, but could be mapped onto any possible tree topology with equal parsimony (Acon1, Acon2, Ada, Adh, Gd, Ldh, Nadhdh, Pep1, and Pgm). The character states provided by the remaining 11 loci are listed in Table 5. all of these characters are unordered, and four of them (Hadh, Mdh1, Mpi, and Pk) are unpolarized. Analysis of these characters yielded several dendrograms, which combine into the two alternative phylogenetic hypotheses in Fig. 5. Almost the same tree topology is gained when the characters are coded with polymorphisms taken into account (rather than being combined as described in the Materials and Methods section above) or if *O. parvipes* and *O. alleni* are used as closer, and perhaps more appropriate (because of the great distance to *Nototriton*), outgroups to the remaining taxa. Both analyses place *O. ignea* as the sister taxon to the clade containing *O. alfaroi*, *O. altura*, *O. cyclocauda*, *O. gracilis*, *O. pacificensis*, *O. poelzi*, *O. pseudouniformis*, and *O. uniformis*.

The position of the Guatemalan species*O. ignea* is unclear from these analyses, but among the other taxa, four major clades can be discerned. These will be referred to hereafter as the *parvipes*, *cyclocauda*, *poelzi*, and *uniformis* clades. Our *uniformis* clade does not correspond to the *uniformis* group of Brame (1968), which includes also the *cyclocauda* and *poelzi* clades. On the other hand, Brame's *parvipes* group is identical to our *parvipes* clade. *Oedipina alleni* and *O. parvipes* together (the *parvipes* clade) appear to form the outgroup to all other populations, as suggested by the presence in the latter of the derived characters Aat-1/2 and perhaps Pk-0 (Table 5), although Pk may instead provide a synapomorphy (Pk-1) for the *parvipes* clade. The monophyly of the *parvipes* clade also is suggested by the presence in that clade of Est1-4, Est2-3/4, Gpi-1, Mdh2-3/4, and Pep2-2. The remaining populations are divided into two lineages. The first, containing *O. altura*, *O. cyclocauda*, and *O. pseudouniformis* (the *cyclocauda* clade) is diagnosed by the presence of Mdh1-1. the second, containing all populations with the *O. alfaroi*, *O. poelzi*, and *O. uniformis* morphotypes, is diagnosed by the presence of Mpi-0 and perhaps Hadh-0/4/5. Alternatively, Hadh might provide one synapomorphy (Hadh-4) for the *O. poelzi* morphotype (the *poelzi* clade) and another (Hadh-0/5) for populations of the *O. alfaroi* and *O. uniformis* morphotypes (the *uniformis* clade). This latter relationship is suggested also by the presence of Aat-1 and Gdh-1. Mdh2-1 suggests that *O. alfaroi* is more closely related to *O. gracilis* than the latter is to any other population of the *O. uniformis* group. The distribution of Mdh2-2 suggests sister group status for *O. cyclocauda* and *O. pseudouniformis*.

Largely owing to the presence of numerous autapomorphies in the biochemical data set, there is no homoplasy in the phylogenetic hypothesis above if *O. ignea* is ignored. The presence of Aat-1 in *O. ignea* suggests that it is related to the *uniformis* clade. Alternatively, Est-1 suggests that *O. ignea* might instead be allied with the *cyclocauda* clade. Either position is equally parsimonious. For all other proteins, *O. ignea* either has the ancestral character state or it is autapomorphic.

Genetic distances among the populations of *Oedipina* are listed in Table 4. Given the phylogeny in Fig. 5, and ignoring *O. ignea* for the moment because of its unclear phylogenetic position, some observations concerning evolutionary rates can be made. All of the

genetic distances from *O. alfaroi*, *O. gracilis*, *O. pacificensis*, and *O. uniformis* to any other *Oedipina* are expected to be roughly equal if evolutionary rates are approximately equivalent along all lineages within the clade containing those taxa. This is not the case: *O. gracilis* is consistently more distant from each of the other *Oedipina* (except the *parvipes* clade) than are any of the other three species. Similarly, *O. uniformis* is consistently closer to each of the other *Oedipina* (again excluding the *parvipes* clade) than are *O. pacificensis* or *O. alfaroi*. This suggests that there has been a rate increase of protein evolution in *O. gracilis* and a decrease in *O. uniformis*. On the other hand, if such rate changes occurred, a similar pattern of relative rate comparisons should be expected when comparing these populations to *O. alleni* and *O. parvipes*. This was not seen. However, the magnitude of the distances involved (D_N=1.84-2.67) casts doubt on their utility. differences between Nei distances less than 1.0 are much more meaningful than differences between distances above 1.5 because the greater the difference, the greater the probability that observed similarities are due to chance. Because the rate differences discussed above are suggested by comparisons in the meaningful range for allozmes (0.46-1.66), they are likely to be genuine.

The distances from each of the species in the *cyclocauda* clade to *O. poelzi* on the one hand and to *O. alfaroi* and *O. pacificensis* (the species of the *uniformis* clade that have not undergone apparent rate changes) on the other are expected to be equal if rates are constant according to the above argument. Distances from *O. poelzi* to *O. altura*, *O. cyclocauda*, and *O. pseudouniformis* (0.49-0.89) are consistently lower than the distances from *O. pacificensis* and *O. alfaroi* to the same three species (0.89-1.17), suggesting either that there has been a rate increase in the common ancestor of the *uniformis* clade or a rate decrease in *O. poelzi*. Distances are greater from the *parvipes* clade to *O. alfaroi* and *O. pacificensis* (1.84-2.67) than to either *O. poelzi* (1.07-1.95) or the *cyclocauda* clade (1.14-1.99), suggesting that increased protein evolution in the ancestor of the *uniformis* clade is the more likely explanation (but recall the comments above concerning the unreliability of large Nei distances). Distances from *O. altura* are consistently higher than distances from *O. cyclocauda* or *O. pseudouniformis* to each of the other *Oedipina* species, suggesting an increase in rate of protein evolution in *O. altura*.

In summary, examination of genetic distances suggests that there have been rate increases in the common ancestor of the *uniformis* clade, in *O. altura*, and in *O. gracilis*, and a rate decrease in *O. uniformis*. If this argument is accepted, the genetic distances involving *O. ignea* can be used to estimate its phylogenetic position. These distances are: 1.66-1.96 to the *parvipes* clade, 1.29-1.47 to the *cyclocauda* clade, 1.04-1.13 to the *poelzi* clade, and 1.13-1.18 to the *uniformis* clade species that have not undergone rate changes. The distance between *O. ignea* and the *parvipes* clade are within the range of distances from the *parvipes* clade to either the *cyclocauda* clade or the lineage including the *poelzi* and *uniformis* clades, and accordingly it is not possible to use these data for phylogenetic purposes. However, *O. ignea* is approximately equidistant from both the *poelzi* and *uniformis* clades. If it were anywhere outside of the clade including the *poelzi* and *uniformis* clades, the genetic distance to the species of the *uniformis* clade would be expected to be higher than the distance to the *poelzi* clade because of the hypothesized rate increase in the branch defining the *uniformis* clade. Therefore, the most parsimonious placement for *O. ignea* is as the sister taxon to the *uniformis* clade, as suggested by Aat-1. A potential confounding factor for this hypothesis is that the genetic distances between the *poelzi* and *uniformis* clades are consistently lower than the distances between *O. ignea* and the *uniformis* clade, even though *O. ignea* is hypothesized to be more closely related to the *uniformis* clade than to the *poelzi* clade. We hypothesize an increase in the evolutionary rate in *O. ignea*, which also accounts for the higher

genetic distances from the *cyclocauda* clade to *O. ignea* than from the *cyclocauda* clade to the *poelzi* clade or to the *uniformis* clade.

Two other populations of *Oedipina* were studied, but were excluded from the discussions above because the individuals available were unscorable for several proteins. One of these consisted of a single individual of the *O. cyclocauda* morphotype from Honduras. At 13 of the 18 proteins scorable this individual was identical to the Costa Rican *O. cyclocauda*. At the other five (Icdh1, Ldh, Mdh1, Mdh2, and Pep1), it was fixed for a different allele than that found in the Costa Rican *O. cyclocauda*. In two of these five cases (Icdh1 and Ldh), the Honduran population had an allele that was unique among the *Oedipina* sampled. For two other proteins (Mdh1 and Mdh2) it had the postulated ancestral allele for the genus. At Pep1, the Honduran population shared an allele with *O. altura* but at the same locus the Costa Rican *O. cyclocauda* possessed a unique allele and *O. pseudouniformis* had alleles among the ancestral set. On the basis of allele distribution, therefore, the Honduran population appears to be the sister taxon to the Costa Rican *O. cyclocauda*. The genetic distance between the two of 0.31 is a level of genetic divergence similar to that between the main body of *O. poelzi* and the Moravia de Chirripo population (a possible distinct species. see above), but the geographic distance between the latter units is only about 40 km while that between the Honduran population and Costa Rican *O. cyclocauda* is greater than 640 km. Isolation by distance within a single species could account for this level of divergence. it is far less than would be predicted for populations at a similar geographic distance within either *O. poelzi* or *O. uniformis* (see Figs. 3 and 4).

Following completion of the main laboratory work for this study we obtained specimens from a single population of *O. grandis*. Unfortunately, four proteins were unscorable (Ada, Gdh, Hadh, and Pk). Of the proteins that remained, three (Ldh, Pep1, and Pep2) showed a unique allele in *O. grandis*. The alleles present at the remaining 15 proteins were (compare with Table 3): Aat-c, Acon1-a, Acon2-a, Adh-a, Est1-a, Est2-b, Gd-a, Gpi-a, Icdh1-a(0.875)/d(0.125), Icdh2-a, Mdh1-b, Mdh2-a, Mpi-c, Nadhdh-c, and Pgm-d. Of these, three present phylogenetic information: Aat places *O. grandis* on the lineage containing the *cyclocauda*, *poelzi*, and *uniformis* clades, Mdh1 places it in the *cyclocauda* clade, and Mdh2 suggests that it falls outside of the lineage containing *O. cyclocauda* and *O. pseudouniformis* (Fig. 6). The species is similar in coloration and proportions to *O. altura*.

TABLE 1

Voucher specimens for population samples used in the analysis of allozyme variation in Oedipina. *Locality abbreviations in parentheses correspond to those used in Tables 3 and 4.*

***O. alfaroi* morphotype:**

1. *O. alfaroi* (n=1): MVZ 181228, Moravia de Chirripo region, moss bank on Rd. 232, 2-3 km W of junction with road to El Seis, Prov. Cartago, Costa Rica, 850 m elev., 9°49'N, 83°15'W.

***O. altura* morphotype:**

2. *O. altura* (n=1): MVZ 190849, 0.8 km W El Empalme, Prov. San José, Costa Rica, 2320 m elev., 9°43'N, 83°57'W.

***O. cyclocauda* morphotype:**

3. *O. cyclocauda* (LAS) (n=2): MVZ 203747-48, Estación Biología La Selva, Puerto Viejo de Sarapiquí, Prov. Heredia, Costa Rica, 100 m elev., 10° 26'N, 84° 01'W.
4. *O. cyclocauda* (RIO) (n=2): MVZ 210397, 210399, S Río Frío, 11.4 km (rd.) N San José-Guapiles Rd. at Río Sucio, Prov. Heredia, Costa Rica, 140 m elev., 10°19'N, 83°57'W.

***O. ignea* morphotype:**

5. *O. ignea* (n=5): MVZ 138918, 143947, 160917, 163648-49, Finca Santa Julia, 1.25 km E & 0.75 km S San Rafael Pie de la Cuesta, Depto. San Marcos, Guatemala, 1100 m elev., 14°56'N, 91°54'W.

***O. parvipes* morphotype:**

6. *O. alleni* (COR) (n=2): MVZ 190856-57, Sirena, Corcovado National Park, Prov. Puntarenas, Costa Rica, 8 m elev., 8°29'N, 83°34'W.
7. *O. alleni* (QUE) (n=3): MVZ 210401-03, 1.7 km (rd.) NE Parrita-Quepos Hwy. at point 3.7 km NW Damas, Prov. Puntarenas, Costa Rica, 50 m elev., 9°30'N, 84°13'W.
8. *O. parvipes* (CZ) (n=1): MVZ 210405, Near Río Frijoles, W side Pipeline Rd., 4.8 km (air) NW Gamboa, Canal Zone, Panama, 50 m elev., 9°09'N, 79°44'W.
9. *O. parvipes* (NUS) (n=1): MVZ 210404, Nusagundi, Kuna Yala, Prov. San Blas, Panama, 280 m elev., 9°16'N, 78°58'W.

***O. poelzi* morphotype:**

10. *O. poelzi* (BAR) (n=9): CRE 7432, 7441, MVZ 206396-99, UCR ZP-566, ZP-616, 2050 m camp, Braulio Carillo National Park, Volcán Barba, Prov. Heredia, Costa Rica, 2050 m elev.10°08'N, 84°06'W.
11. *O. poelzi* (MON) (n=5): MVZ 207127, Peñas Blancas Trail below (E) continental divide, Monteverde Reserve, Prov. Alajuela, Costa Rica, 1535 m elev., 10°19'N, 84°48'W; MVZ 207128, Carril Bosque Eterno, N of Pantanosa Trail, Monteverde Reserve, Prov. Alajuela, Costa Rica, 1595 m elev., 10°19'N, 84°48'W; MVZ 207129-30, 207132, La Ventana, Monteverde Reserve, Prov. Alajuela, Costa Rica, 1560 m elev., 10°19'N, 84°48'W.
12. *O. poelzi* (MOR) (n=1): MVZ 194873, Quebrada Platanillo, 5.7 km E Moravia de Chirripo, Prov. Cartago, Costa Rica, 1200 m elev., 9°49'N, 83°24'W.
13. *O. poelzi* (NUB) (n=10): MVZ 163702, Las Nubes, pastures near Castillo's farm, Prov. San José, Costa Rica, 10°02'N, 83°56'W; MVZ 181347, Castillo's farm, Las Nubes area, 12.6 km ENE San Isidro de Coronado on Rd. 216, Prov. San José, Costa Rica, 10°02'N, 83°56'W; MVZ 190831-36, 190896-97, Las Nubes de Coronado, ca. 0-2 km NE Río Cascajal, Prov. San José, Costa Rica, 1700 m elev., 10°02'N, 83°56'W.

***O. pseudouniformis* morphotype:**

14. *O. pseudouniformis* (MOR) (n=1): MVZ 181229, Moravia de Chirripo region, moss bank on Rd. 232, 2-3 km W of junction with road to El Seis, Prov. Cartago, Costa Rica, 850 m elev., 9°49'N, 83°27'W.
15. *O. pseudouniformis* (TUR) (n=1): MVZ 203749, Los Espaveles, above Río Reventazon, C.A.T.I.E., ca. 2 km ESE Turrialba, Prov. Cartago, Costa Rica, 9°53'N, 83°39'W.
16. *O. pseudouniformis* (VIN) (n=4): MVZ 181230, Colorado Swamp, 6.9 km W Turrialba (centro), Prov. Cartago, Costa Rica, 1140 m elev., 9°54'N, 83°43'W; MVZ 190850-52, La Cienega de Colorado, ca. 3 km ENE Juan Viñas, Prov. Cartago, Costa Rica, 1035 m elev., 9°54'N, 83°43'W.

***O. uniformis* morphotype:**

17. *O. gracilis* (n=3): MVZ 203752-54, Estacion Biologia La Selva, Puerto Viejo de Sarapiqui, Prov. Heredia, Costa Rica, 100 m elev., 10° 26'N, 84° 01'W.
18. *O. pacificensis* (COR) (n=2): MVZ 190858-59, Sirena, Corcovado National Park, Prov. Puntarenas, Costa Rica, 8 m elev., 8°29'N, 83°34'W.
19. *O. pacificensis* (QUE) (n=1): MVZ 210396, 1.7 km (rd.) NE Parrita-Quepos Hwy. at point 3.7 km NW Damas, Prov. Puntarenas, Costa Rica, 50 m elev., 9°30'N, 84°13'W.
20. *O. uniformis* (ANG) (n=5): MVZ 181247, Varablanca region, ca. 4 km E national Hwy. 9 on regional Hwy. 120, Prov. Alajuela, Costa Rica, 1950 m elev., 10°10'N, 83°45'W; MVZ 190854-55, Salto El Angel, Prov. Alajuela, Costa Rica, 1340 m elev. 10°10'N, 83°45'W; MVZ 207133, 2.4 km WSW Poasito junction on Hwy. 120, Prov. Alajuela, Costa Rica, 2100 m elev. 10°10'N, 83°45'W; MVZ 207139, 0.6 km W Varablanca junction on Hwy. 120, Prov. Alajuela, Costa Rica, 1925 m elev. 10°10'N, 83°45'W.
21. *O. uniformis* (CAP) (n=2): MVZ 194871-72, Río Turrialba, 5.1 km NE Capelladas, Prov. Cartago, Costa Rica, 1640 m elev., 9°47'N, 83°46'W.
22. *O. uniformis* (MON) (n=5): MVZ 207134, 207136-37, Peñas Blancas Trail below (E) continental divide, Monteverde Reserve, Prov. Alajuela, Costa Rica, 1535 m elev., 10°19'N, 84°48'W; MVZ 207138, Trail from Peñas Blancas Rd. to Ventana, Monteverde Reserve, Prov. Alajuela, Costa Rica, 1560 m elev.; MVZ 207140, Pantanosa Trail, Monteverde Reserve, Prov. Alajuela, Costa Rica, 1590 m elev., 10°19'N, 84°48'W.
23. *O. uniformis* (NUB) (n=10): MVZ 190837-46, Las Nubes de Coronado, 0-2 km NE Río Cascajal, Prov. Cartago, Costa Rica, 1700 m elev. 10°02'N, 83°56'W.
24. *O. uniformis* (TAP) (n=1): MVZ 203750, Río Quiri, 0.3 mi NE junction Tapanti Rd. and Tausito Rd., Prov. Cartago, Costa Rica, 1300 m elev., 9°46'N, 83°47'W.
25. *O. uniformis* (TUR) (n=5): MVZ 194861-65, Volcán Turrialba, 5.0-5.5 km above Pastora, Prov. Cartago, Costa Rica, 2150 m elev., 10°01'N, 83°45'W.

26. *O. uniformis* (VIN) (n=1): MVZ 190893, La Cienega de Colorado, 3 km ENE Juan Viñas, Prov. Cartago, Costa Rica, 1035 m elev., 9°54'N, 83°43'W.

Additional populations (see text):

27. *O. cyclocauda* (n=1): MVZ 167772, 32.0 km (rd.) W Yoro on rd. to Morazan, Depto. Yoro, Honduras, 1000 m elev., 15°16'N, 87°38'W.
28. *O. grandis* (n=5): MVZ 219593, 4 uncatalogued MVZ specimens, Woods beside Río Coton below "La Casita," ca. 2 cm ESE Las Tablas, Prov. Puntarenas, Costa Rica, 1880-1910 m elev., 8°56'N, 82°45'W.

TABLE 2

Buffer systems and loci scored in the analysis of relationships among the species of Oedipina. *Buffer type abbreviations are as follows: A = Poulilk (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 deaminase	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
Glycosephoshate isomerase	5.3.1.9	Gpi	B, E
Glocose-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 (reduced)	12.6.99.3	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

Table 3. Allele distributions at the 22 loci examined in the analysis of variation in Oedipina . Locality abbreviations are explained in Table 1.

		alfaroi morpho-type	altura morpho-type	cyclocauda morphotype		ignea morpho-type	parvipes morphotype				poelzi morphotype			
Locus	outgroup	alfaroi	altura	cyclocauda		ignea	alleni		parvipes		poelzi			
				LAS	RIO		COR	QUE	COR	QUE	BAR	MON	MOR	NUB
Aat	a	b	c	c	c	b (0.70) d (0.30)	a	a	a	a	c	c	c	c (0.95) e (0.05)
Acon1	---	a	a	a	a	a	b	b	c	c	a (0.89) d (0.11)	d	a	a
Acon2	---	a	b	a	a	c	d	d	a (0.50) d (0.50)	a	b	a	a	b
Ada	---	a	b	b	b	c	c	c (0.50) d (0.50)	c	c	b	b	b	b
Adh	---	a	a	a	a	b	c	c	d	d	a	a	a	a
Est1	a	a	b	c	c	d	e	e	e	f	a	a	a	a
Est2	a	a	b	c	c	b	d	d	e	e	f	a	f	f
Gd	a/b	c	d	a	a	e	a (0.50) b (0.50)	b	b	a	a (0.78) b(0.22)	a (0.50) b (0.50)	b	a (0.05) b (0.95)
Gdh	a	b	a	a	a	c	a	a	a	a	a	a	a	a
Gpi	a	a	a	a	a	a	b (0.75) c (0.25)	b	b (0.50) d (0.50)	b	a	a	a	a
Hadh	---	a	b	c	c	d	c	c	c	c	e	e	e	e
Icdh1	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Icdh2	---	a	a	a	a	a	a	a	a	a	a	a	a	a
Ldh	---	a	b	c	c	d	e	e	f	f	g	g	h	g
Mdh1	---	a	b	b	b	a	a	a	c	c	a	a	d	a
Mdh2	a/b	c	a	d	d	b	e	e	f	f	b (0.56) g (0.44)	b	b	b (0.25) g (0.75)
Mpi	---	a	b	c	c	d	e	e	c	c	a	a	a	a
Nadhdh	a	b	c	c	c	d	a	a	e	f	a	a	g	a (0.95) c (0.05)
Pep1	a	b	c	d	d	e	a	a	f	f	a (0.28) b (0.28)	a (0.50) b (0.50)	g	b (0.65) g (0.35)
Pep2	a	a	a	a	a	b	c	c	c	c	a	a	d	a (0.95) e (0.05)
Pgm	---	a	b	c	c	d	e	e	e	e	d	d	d	d
Pk	---	a	a	a	a	a	b	b	b	b	a	a	a	a

Table 3. Continued.

	pseudouniformis morphotype				uniformis morphotype								
Locus	pseudouniformis			gracilis	pacificensis		uniformis						
	MOR	TUR	VIN		COR	QUE	ANG	CAP	MON	NUB	TAP	TUR	VIN
Aat	c	c	c	f	f	f	f	b (0.50) g (0.50)	f	f (0.90) g (0.10)	g	f (0.13) g (0.87)	g
Acon-1	a	a	a	e	a	a	a	a	a	a	a	a	a
Acon-2	a	a	a	e	a	a	a	a (0.50) b (0.50)	a	a	a	a (0.37) b (0.63)	b
Ada	b	b	b	b (0.17) d (0.83)	e	e	b	b	b	b	b	b	b
Adh	e	e	e	a	a	a	a	a	a	a	a	a	a
Est1	a	a	a	a	g	g	a	a	a	a	a	a	a
Est2	g	g	g	h	a	a	a	a	i	a	a	a	a
Gd	a	a	a	c	c	c	c	a (0.50) c (0.50)	c	c	c	c	c
Gdh 1	a	a	a	b	b	b	b (0.70) d (0.30)	b	b (0.90) d (0.10)	b	b	b	b
Gpi	a	a	a	e	a	a	a	a	a	a	a	a	a
Hadh	c	c	c	a	f	f	a	a	a	a	a	a	a
Icdh1	a	a	a	a	a	a	a	a	a (0.70) b (0.30)	a	a	a	a (0.50) c (0.50)
Icdh2	a	a	a	a	a (0.75) b (0.25)	a	a	a	a	a	a	a	a
Ldh	i	i	i	i	j	j	i	i	i (0.40) j (0.60)	i	i	i	i
Mdh1	b	b	b	a	a	a	a	a	a	a	a	a	a
Mdh2	d	d	d	c	b	b	b	b	b	b	b	b	b
Mpi	f	f	f	a	a	a	a	a	a	a	a	a	a
Nadhdh	a	a	a (0.87) c (0.13)	h	c	c	i	i	i	i	i	i	i
Pep1	b	b	b (0.50) h (0.50)	i	i	i	b (0.20) h (0.60) j (0.20)	j	h	h (0.05) j (0.95)	i	j	j
Pep2	a	a	a	f	a	a	a	a	a	a	a	a	a
Pgm	d (0.50) e (0.50)	e	d (0.12) e (0.88)	c	c	c	c (0.90) f (0.10)	c	d (0.60) f (0.40)	c	c	c	c
PK	a	a	a	a	a	a	a	a	a	a	a	a	a

Table 4. Nei (1978) genetic distances among 26 populations of Oedipina. Locality abbreviations are explained in Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1. O. alfaroi	-----	1.15	1.01	1.01	1.18	1.97	1.98	2.15	1.99	0.69	0.62	0.89	0.70	0.89	0.89	0.93	0.69	0.53	0.53	0.36	0.37	0.44	0.37	0.38	0.41	0.48
2. O. altura	1.15	-----	0.61	0.61	1.29	1.97	1.98	1.97	1.99	0.67	0.87	0.89	0.67	0.78	0.79	0.75	1.66	1.03	1.01	0.98	0.90	1.01	1.01	1.01	0.92	0.94
3. O. cyclocauda LAS	1.01	0.61	-----	0.00	1.47	1.56	1.69	1.36	1.15	0.69	0.71	0.79	0.76	.044	0.45	0.42	1.44	0.80	0.79	0.77	0.74	0.89	0.78	0.79	0.84	0.94
4. O. cyclocauda RIO	1.01	0.61	0.00	-----	1.47	1.56	1.69	1.36	1.15	0.69	0.71	0.79	0.76	0.44	0.45	0.42	1.44	0.80	0.79	0.77	0.74	0.89	0.78	0.79	0.84	0.94
5. O. ignea	1.18	1.29	1.47	1.47	-----	1.67	1.82	1.96	1.98	1.04	1.11	1.13	1.07	1.36	1.47	1.42	1.69	1.16	1.13	1.10	1.04	1.05	1.13	1.13	1.12	1.20
6. O. alleni COR	1.97	1.97	1.56	1.56	1.67	-----	0.02	0.61	0.74	1.27	1.25	1.81	1.34	1.26	1.19	1.21	1.96	2.04	1.97	1.94	1.84	2.03	1.96	1.97	1.95	2.14
7. O. alleni QUE	1.98	1.98	1.69	1.69	1.82	0.02	-----	0.61	0.83	1.34	1.26	1.69	1.28	1.36	1.29	1.31	1.84	2.06	1.98	1.95	1.93	2.05	1.97	1.98	1.96	2.15
8. O. parvipes CZ	2.15	1.97	1.36	1.36	1.96	0.61	0.61	-----	0.18	1.86	1.66	1.56	1.66	1.45	1.36	1.36	2.37	2.25	2.15	2.12	2.21	2.24	2.15	2.15	2.27	2.65
9. O. parvipes NUS	1.99	1.99	1.15	1.15	1.98	0.74	0.83	0.18	-----	1.73	1.56	1.71	1.95	1.21	1.15	1.14	2.39	2.07	1.99	1.96	1.95	2.06	1.99	1.99	2.21	2.67
10. O. poelzi BAR	0.69	0.67	0.69	0.69	1.04	1.27	1.34	1.86	1.73	-----	0.16	0.37	0.03	0.49	0.54	0.54	1.08	0.83	0.81	0.58	0.50	0.55	0.61	0.62	0.55	0.56
11. O. poelzi MON	0.62	0.87	0.71	0.71	1.11	1.25	1.26	1.66	1.56	0.16	-----	0.39	0.21	0.53	0.58	0.58	1.09	0.68	0.67	0.46	0.47	0.51	0.49	0.50	0.53	0.61
12. O. poelzi MOR	0.89	0.89	0.79	0.79	1.13	1.81	1.69	1.56	1.71	0.37	0.39	-----	0.32	0.73	0.79	0.75	1.26	0.91	0.89	0.66	0.69	0.63	0.69	0.69	0.74	0.83
13. O. poelzi NUB	0.70	0.67	0.76	0.76	1.07	1.34	1.28	1.66	1.95	0.03	0.21	0.32	-----	0.56	0.62	0.62	1.08	0.85	0.84	0.60	0.55	0.58	0.64	0.65	0.57	0.59
14. O. pseudouniformis MOR	0.89	0.78	0.44	0.44	1.36	1.26	1.36	1.45	1.21	0.49	0.53	0.73	0.56	-----	0.01	0.02	1.43	1.16	1.13	0.73	0.73	0.80	0.77	0.78	0.83	0.93
15. O. pseudouniformis TUR	0.89	0.79	0.45	0.45	1.47	1.19	1.29	1.36	1.15	0.54	0.58	0.79	0.62	0.01	-----	0.01	1.44	1.17	1.15	0.74	0.74	0.84	0.78	0.79	0.84	0.94
16. O. pseudouniformis VIN	0.93	0.75	0.42	0.42	1.42	1.21	1.31	1.36	1.14	0.54	0.58	0.75	0.62	0.02	0.01	-----	1.42	1.13	1.10	0.69	0.72	0.76	0.75	0.76	0.81	0.92
17. O. gracilis	0.69	1.66	1.44	1.44	1.69	1.96	1.84	2.37	2.39	1.08	1.09	1.26	1.08	1.43	1.44	1.42	-----	0.70	0.69	0.51	0.58	0.63	0.51	0.51	0.56	0.62
18. O. pacificensis COR	0.53	1.03	0.80	0.80	1.16	2.04	2.06	2.25	2.07	0.83	0.68	0.91	0.85	1.16	1.17	1.13	0.70	-----	0.00	0.32	0.41	0.40	0.32	0.32	0.41	0.48
19. O. pacificensis QUE	0.53	1.01	0.79	0.79	1.13	1.97	1.98	2.15	1.99	0.81	0.67	0.89	0.84	1.13	1.15	1.10	0.69	0.00	-----	0.31	0.40	0.40	0.32	0.32	0.40	0.48
20. O. uniformis ANG	0.36	0.98	0.77	0.77	1.10	1.94	1.95	2.12	1.96	0.58	0.46	0.66	0.60	0.73	0.74	0.69	0.51	0.32	0.31	-----	0.08	0.11	0.02	0.09	0.08	0.14
21. O. uniformis CAP	0.37	0.90	0.74	0.74	1.04	1.84	1.93	2.21	1.95	0.50	0.47	0.69	0.55	0.73	0.74	0.72	0.58	0.41	0.40	0.08	-----	0.23	0.04	0.07	0.01	0.04
22. O. uniformis MON	0.44	1.10	0.89	0.89	1.05	2.03	2.05	2.24	2.06	0.55	0.51	0.63	0.58	0.80	0.84	0.76	0.63	0.40	0.40	0.11	0.23	-----	0.16	0.22	0.23	0.29
23. O. uniformis NUB	0.37	1.01	0.78	0.78	1.13	1.96	1.97	2.15	1.99	0.61	0.49	0.69	0.64	0.77	0.78	0.75	0.51	0.32	0.32	0.02	0.04	0.16	-----	0.08	0.05	0.10
24. O. uniformis TAP	0.38	1.01	0.79	0.79	1.13	1.97	1.98	2.15	1.99	0.62	0.50	0.69	0.65	0.78	0.79	0.76	0.51	0.32	0.32	0.09	0.31	0.22	0.08	-----	0.07	0.11
25. O. uniformis TUR	0.41	0.92	0.84	0.84	1.12	1.95	1.96	2.27	2.21	0.55	0.53	0.74	0.57	0.83	0.84	0.81	0.56	0.41	0.40	0.08	0.01	0.23	0.05	0.07	-----	0.02
26. O. uniformis VIN	0.48	0.94	0.94	0.94	1.20	2.14	2.15	2.65	2.67	0.56	0.61	0.83	0.59	0.93	0.94	0.92	0.62	0.48	0.48	0.14	0.04	0.29	0.10	0.11	0.02	-----

TABLE 5

Character states at the 11 potentially phylogenetically informative loci in Oedipina. *The species are designated by the first three letters of their species names; out = outgroup.*

Locus	out	alf	alt	cyc	ign	all	par	poe	pse	gra	pac	uni
Aat	0	1	2	2	1	0	0	2	2	1	1	1
Est1	0	0	1	2	3	4	4	0	0	0	5	0
Est2	0	0	1	2	1	3	4	0	6	7	0	0
Gdh	0	1	0	0	2	0	0	0	0	1	1	1
Gpi	0	0	0	0	0	1	1	0	0	2	0	0
Hadh	---	0	1	2	3	2	2	4	2	0	5	0
Mdh1	---	0	1	1	0	0	2	0	1	0	0	0
Mdh2	0	1	0	2	0	3	4	0	2	1	0	0
Mpi	---	0	1	2	3	4	2	0	5	0	0	0
Pep2	0	0	0	0	1	2	2	0	0	3	0	0
Pk	---	0	0	0	0	1	1	0	0	0	0	0

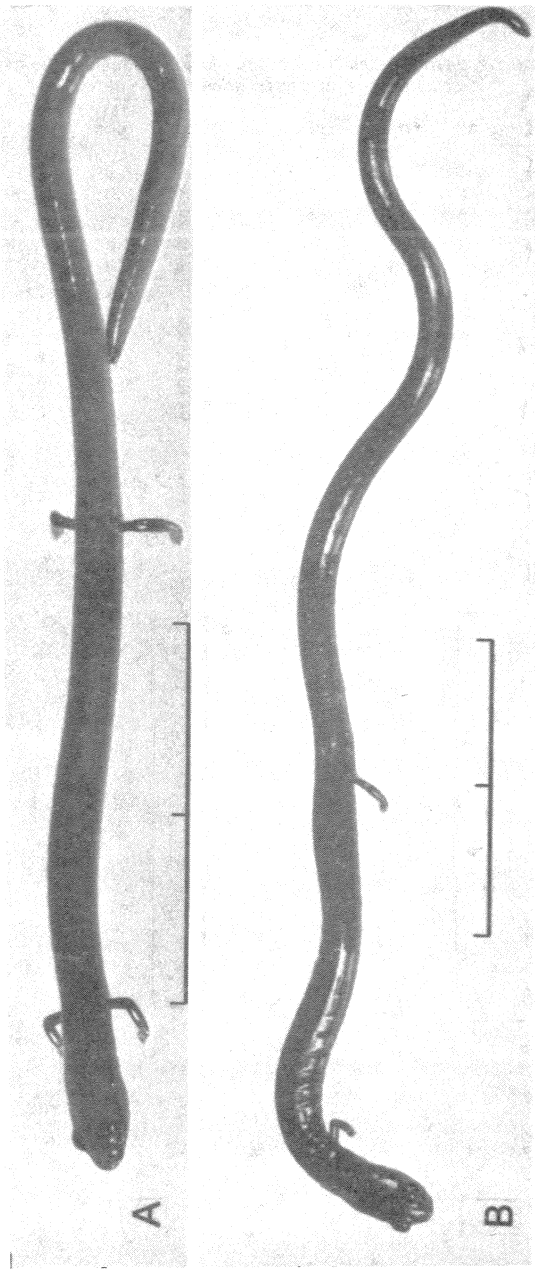
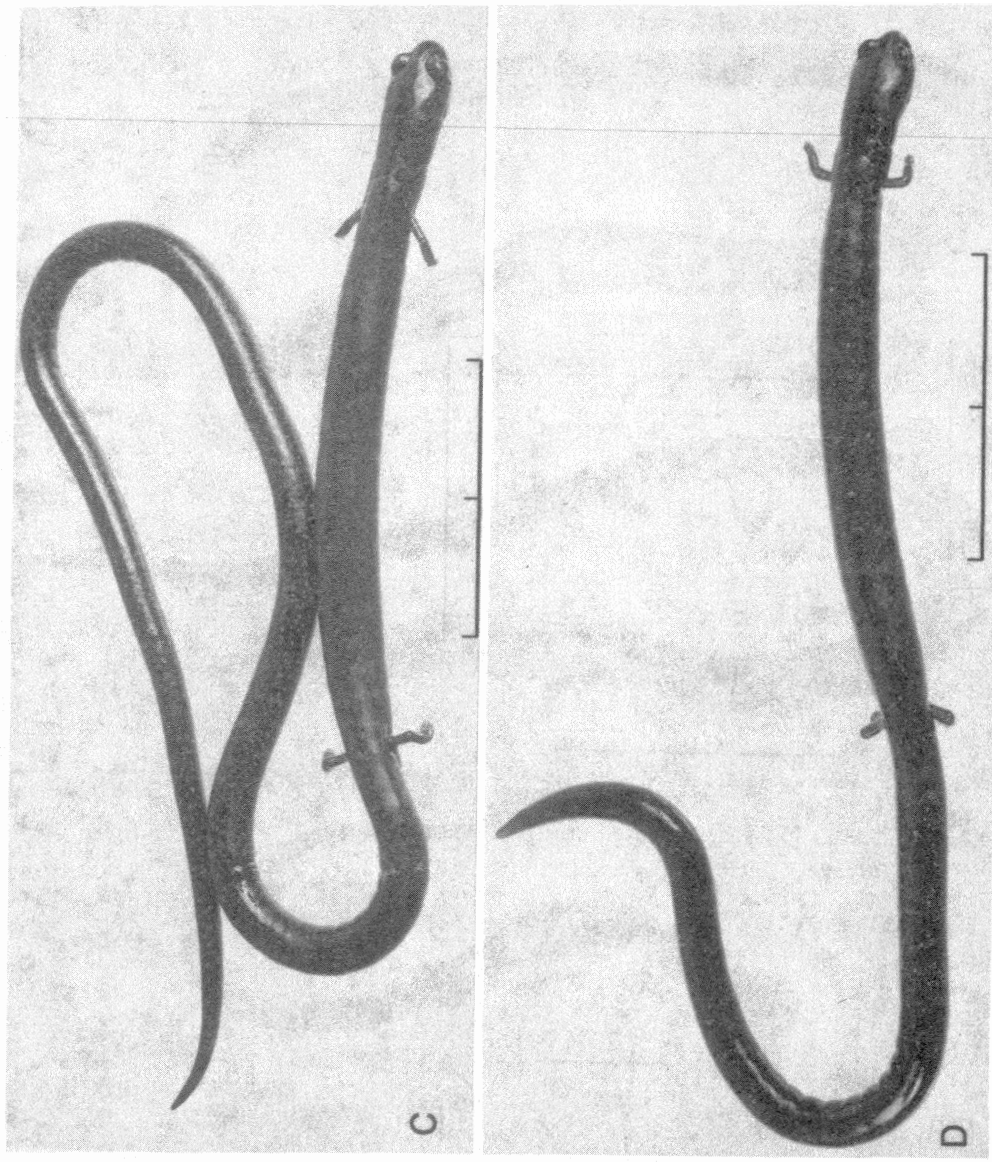


Fig. 1. Species of *Oedipina* displaying different morphotypes. A. The *alfaroi* morphotype: *O. alfaroi*. The remaining specimens represent the *uniformis* morphotype: B. *O. gracilis*; C. *O. uniformis*; D. *O. pacificensis*. The scale is 2 cm long.


C
D

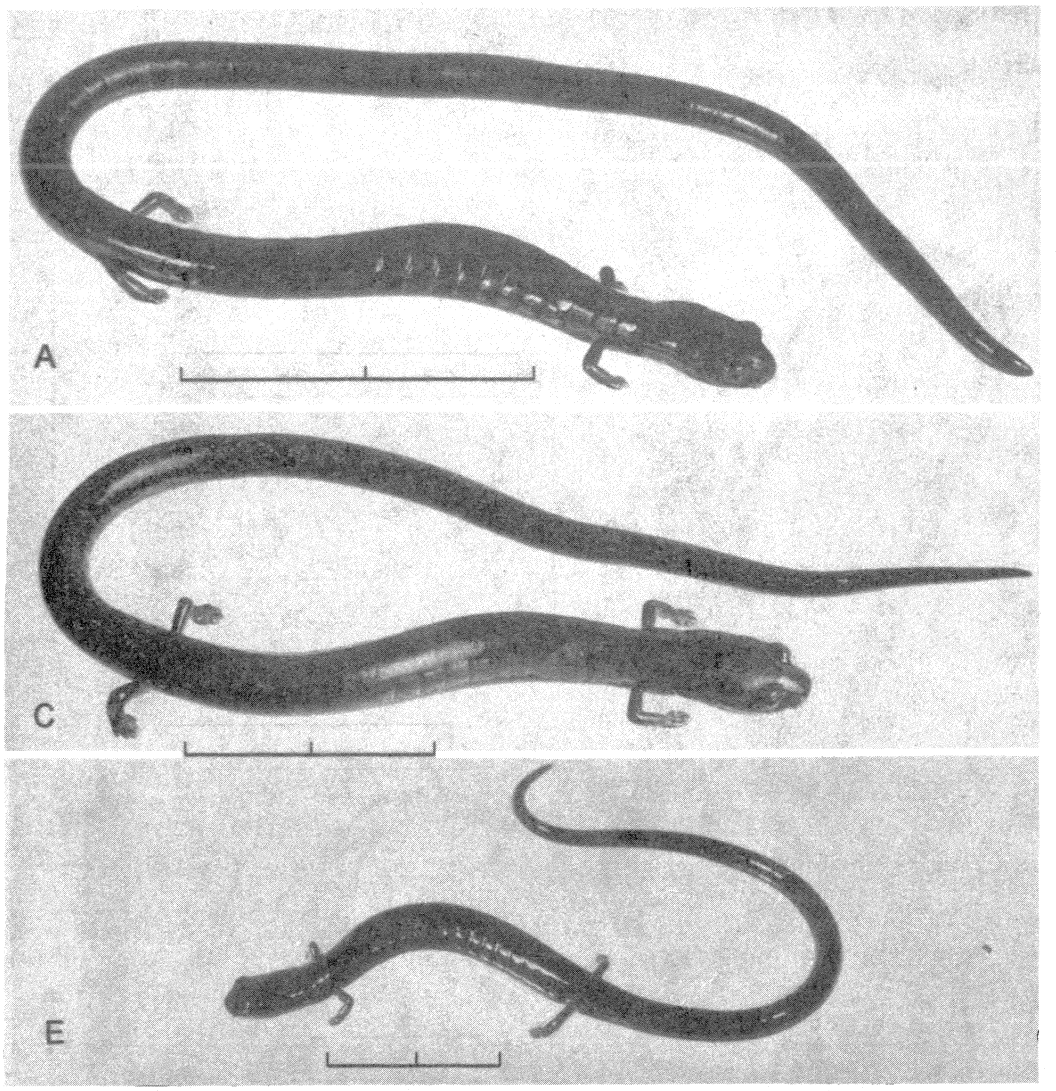
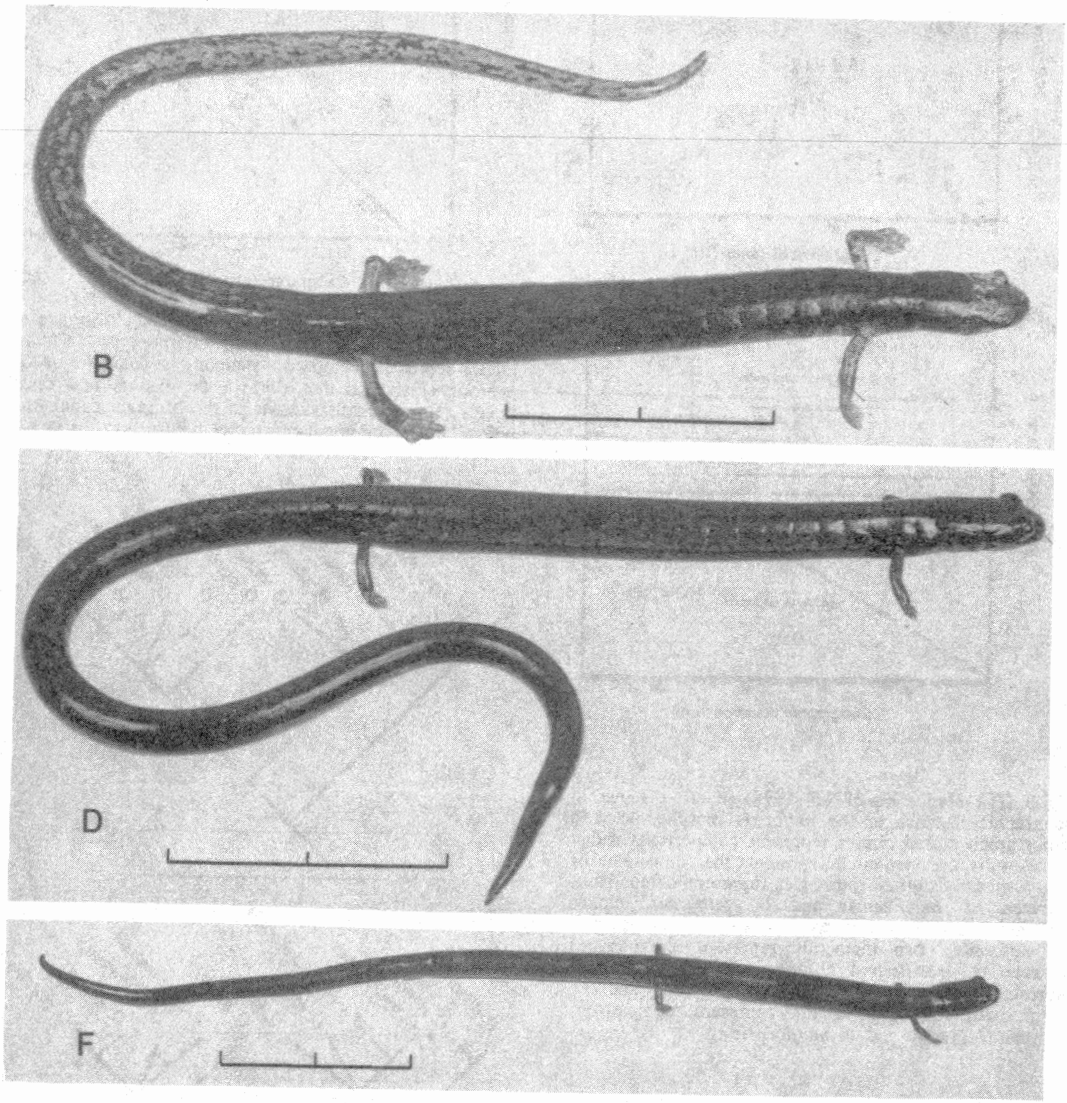


Fig. 2. Species of *Oedipina* displaying different morphotypes. A. The *ignea* morphotype: *O. ignea.* B. The *parvipes* morphotype: *O. alleni.* C. The *poelzi* morphotype: *O. poelzi.* D. The *altura* morphotype: *O. altura.* E. The *pseudouniformis* morphotype: *O. pseudouniformis.* F. The *cyclocauda* morphotype: *O. cyclocauda.* The scale is 2 cm long.


B
D
F

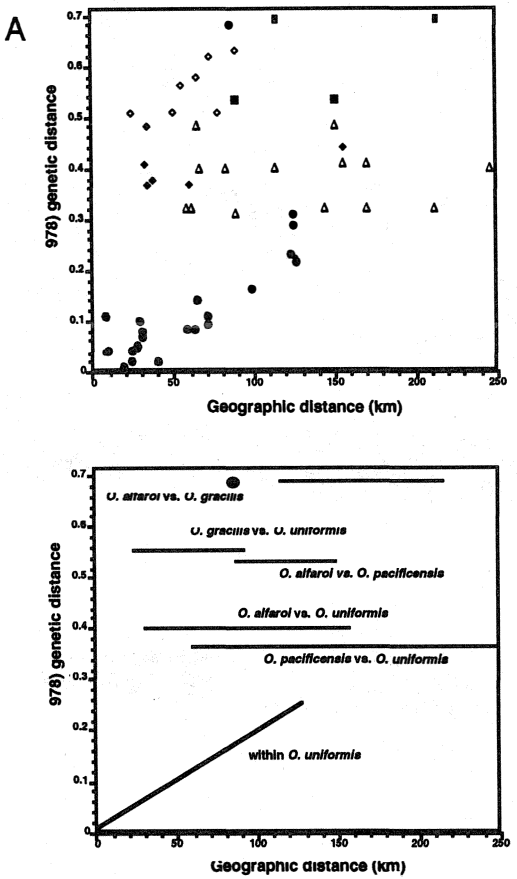


Fig. 3. The relationship of Nei (1978) genetic distance to geographic distance in the *uniformis* morphotype. In upper graph closed circles represent comparisons within *O. unformis*, the open circle represents the comparison of *O. alfaroi* and *O. gracilis*, triangles represent comparisons between *O. pacificensis* and *O. uniformis*, closed diamonds represent comparisons between *O. alfaroi* and *O. uniformis*, open diamonds represent comparisons between *O. gracilis* and *O. uniformis*, closed squared represent comparisons between *O. alfaroi* and *O. pacificensis*, and open squares represent comparisons between *O.* gracilis and *O. pacificensis*

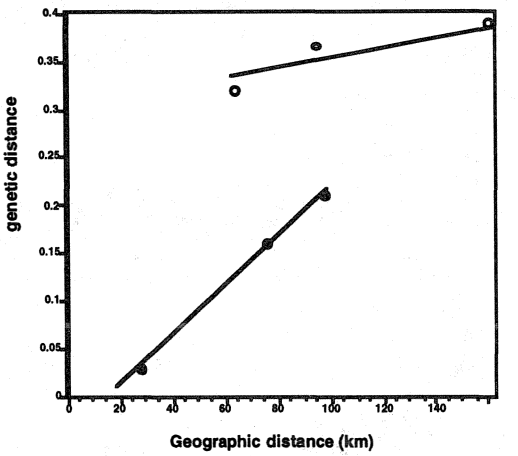


Fig. 4. The relationship of Nei (1978) genetic distance to geographic distance in the *poelzi* morphotype. Closed circles represent comparisons among populations of *O. poelzi* exclusive of the Moravia population, and open circles represent comparisons of the Moravia population with all remaining populations of *O. poelzi*.

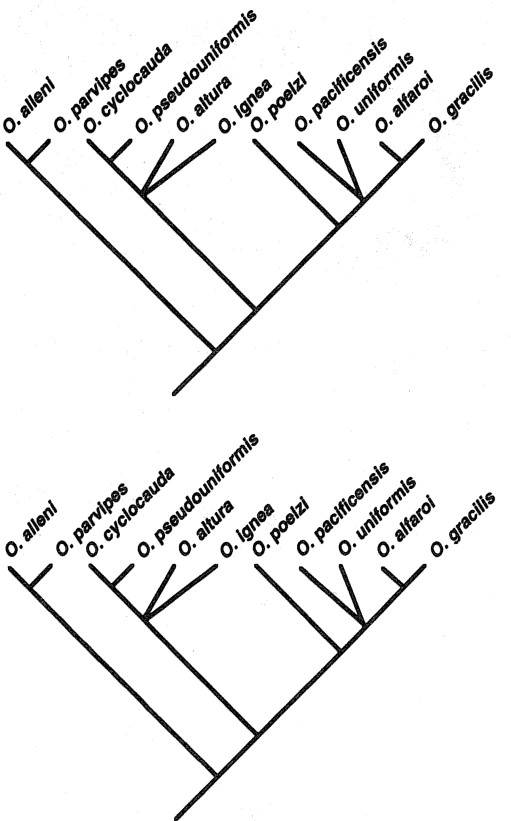


Fig. 5. The two most parsimonious phylogenetic hypotheses of the 11 species of *Oedipina* for which all loci were scored. Consistency index (CI) = 0.974, rescaled consistency index (RC) = 0.925, retention index (RI) = 0.950.

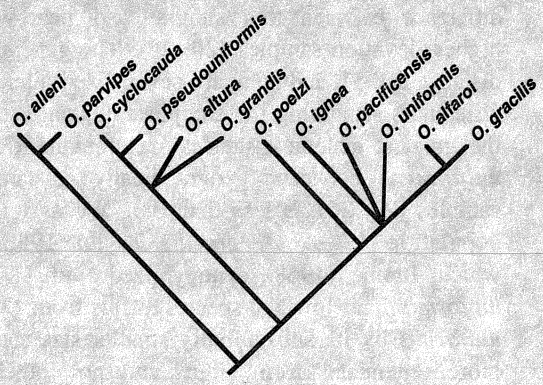


Fig. 6. The most likely phylogenetic hypothesis of *Oedipina*, including *O. grandis*.

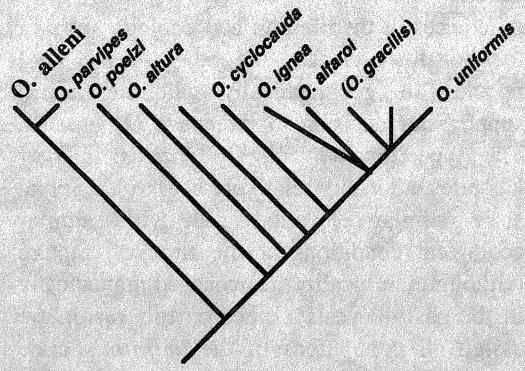


Fig. 7. The phylogenetic hypothesis of Brame (1968, Fig. 29) for the species of *Oedipina* inculded in the present analysis. Taxa in parentheses were not accepted by Brame, but their proposed phylogenetic positions are inferred from Brame's synonymies. *Oedipina grandis* is not included in this figure because it was unknown to Brame.

DISCUSSION

Our operational approach to species recognition requires explicit reasons for considering populations to be either conspecific or specifically distinct. Close morphological and genetic resemblance and similarity in ecological geography suggest conspecificity, and the arguments become compelling when evidence of geographic clines in allele frequencies is found. Such clines suggest that lineage independence has not been attained--that there is no "genetic closure" (Larson 1984). Two cases in point in our data concern the Costa Rican taxa known prior to the present analysis as *O. poelzi* and *O. uniformis*. We have samples from the type locality of the former. the type locality of the latter is simply "Costa Rica", probably at relatively high elevation (see Brame 1968, p. 34). In both taxa a clinal pattern of genetic variation is detected. geographically proximate populations are more similar in proteins than are geographically more distant ones. This type of pattern was used as evidence for the inclusion of populations in *Nototriton abscondens* (Good and Wake 1993) and in four species of *Rhyacotriton* (Good and Wake 1992). *Nototriton abscondens* is extensively sympatric with both *O. poelzi* and *O. uniformis*, and there are localities in common among the three taxa in our samples. Furthermore, all three occur primarily in the volcanic Cordillera Central and nearby localities, and in the western Cordillera de Guanacaste. Allopatric speciation appears to be the dominant mode in salamanders, so concordant patterns are highly relevant.

As we work out from the "core" distributional area of these taxa in the Cordillera Central, we encounter suspect populations that are candidates for species status. In the case of *O. uniformis* there are three. The first of these (population 1) is from an area near Moravia de Chirripo, a somewhat isolated upland mass at the northern end of the Cordillera de Talamanca but in line with the southeastern end of the Cordillera Central. This population contains four unique alleles, but it shares sufficient derived alleles with other members of the *uniformis* clade to justify association with it. This sample differs in morphology from *O. uniformis sensu stricto* in being brown rather than gray-black, in having a more sharply pointed snout, and in having no maxillary teeth. in these features it matches Brame's (1968) redescription of *O. alfaroi* Dunn 1921. Although Brame's samples were collected at lower elevation, our sample occurs in the same general geographic region. we therefore assign it to *O. alfaroi*.

The Moravia de Chirripo region also Fig.s in the case of *O. poelzi*, in which genetic differentiation from populations in the Cordillera Central is of similar magnitude to that found in the *uniformis* clade. However, morphological differentiation is not as great as in the case of *O. alfaroi*. The population from the region of Moravia de Chirripo may warrant species status, but we refrain from describing the species at this time (see above).

The situation in *Nototriton* also is relevant to the discussion of the Moravia de Chirripo isolate. *Nototriton* is more ecologically specialized and less vagile than *Oedipina*. In the case of the *N. abscondens* complex, the sample from the Moravia de Chirripo region is morphologically distinct and was described as a new species, *N. major*, by Good and Wake (1993). Another instructive comparison between *Nototriton* and *Oedipina* involves samples from the Cordillera Central and from the valley of the Río Orosi, at the extreme northern end of the Cordillera de Talamanca, Costa Rica. In this case *O. uniformis* samples in the two areas are only slightly differentiated. The genetic distance between relatively nearby samples from the Cordillera de Talamanca (sample 24) and the Cordillera Central (sample 23) is only 0.084. In contrast, there are two instances of species-level differences in *Nototriton* between the same localities, and genetic distances are 0.30 and 0.50 (Good and Wake 1993). These examples suggest that past vicariant events have had varying effects on different táxones, depending on their vagility and dispersal propensity. In the case of Moravia de Chirripo, *Nototriton major* and *O. alfaroi* clearly have diverged from their "stem" lineages, while such divergence is less apparent in *O. poelzi*. this contrast between táxones is even more apparent when comparing the Cordillera Central and Talamancan populations of *Nototriton* and *O. uniformis*.

We identified two additional cases of allopatric differentiation relating to the *uniformis* group. One involves sample 17, from the Organization for Tropical Studies' La Selva field station in the Atlantic lowlands of Costa Rica. There is substantial genetic difference between this sample and nearby, higher elevation samples (20, 22). The genetic distance is 0.51, and there are seven fixed allele differences separating the samples. In contrast, the greatest genetic distances in montane *O. uniformis*, between geographically distant samples (22, 26), is less than 0.3. We assign population 17 to *O. gracilis* Taylor 1952, which Brame (1968) synonymized with *O. uniformis*. This lowland species differs from *O. uniformis* by its substantially smaller size and more attenuate form, but in no other morphological details that we can discern (see also Brame, p. 39-40). The combination of genetic and size differences together with differences in distribution and ecology (one in the hot lowlands and the other in the cool uplands in geographically adjoining areas) support our decision to recognize *O. gracilis*. The type locality for *O. gracilis* is near Guapiles, a locality relatively near to La Selva, at a similar elevation and with similar ecological conditions at the time of capture (although the locality has changed dramatically in the past 40 years). Based on this result, we assign all populations of the *uniformis* clade from the Atlantic lowlands of Costa Rica (except *O. alfaroi*) to *O. gracilis*. Curiously, the *uniformis* clade has not yet been recorded in the Atlantic lowlands of Nicaragua or Panama. Sympatry is unknown among species in the clade, but it is to be expected between *O. uniformis* and *O. gracilis* along the northern slopes of the Cordillera Central in the Provincia de Heredia, Costa Rica.

The final case involving the *uniformis* group relates to two samples (18, 19) that also occur in lowland sites, but in the Pacific lowlands rather than the Atlantic lowlands. These samples are differentiated from upland *O. uniformis* by genetic distances of 0.32-0.48 (four fixed differences) and from *O. gracilis* by distances of 0.69-0.70 (11 fixed differences). Because of the combination of substantial genetic distance, geographic distinctiveness, and (relative to *O. uniformis*) differences in elevational distribution and ecology, we assign

these Pacific lowland populations to *O. pacificensis* Taylor 1952, a taxon that was reduced to synonoymy with *O. uniformis* by Brame (1968). The type locality is near San Isidro del General, at 646 m elevation, and the species may extend upward to about 1000 m. in this respect the distribution is similar to that of *O. alfaroi* of the Atlantic slope. The species characteristically has whitish to tannish postocular stripes on the head, and the head occasionally appears almost white. This feature is not unique to the taxon, however, and the color pattern may at times be obscure. We are unaware of any other morphologically distinctive features. the species apparently is somewhat larger than *O. gracilis* but not as large as *O. uniformis* (see also Brame 1968, p. 40). We assign all populations of the *uniformis* group from the Pacific lowlands and adjacent middle-elevation areas (as in the area around San Isidro del General) of Costa Rica and Panama to *O. pacificensis*.

In contrast to *O. uniformis*, we find no reasons for separating the populations of *O. pseudouniformis* from each other. Our samples were taken from relatively nearby areas, and we have no evidence of range disruption, as we do for the *O. uniformis* complex. Furthermore, *O. pseudouniformis* appears to have a broader distribution than do either of the other two táxones, perhaps relating to higher vagility.

No more than two species in either the *parvipes* or *uniformis* group (the latter including the *cyclocauda*, *poelzi*, and *uniformis* clades) have been taken in sympatry, although Brame (1968) listed *O. alfaroi*, *O. cyclocauda*, *O. gracilis*, and *O. pseudouniformis* as occurring in close proximity. These species occur in various combinations of pairwise sympatry in Limón Province in the eastern lowlands of Costa Rica (see also Wake 1987, Fig. 15). Our results suggest that *O. gracilis* and *O. alfaroi* are the most genetically similar species of *Oedipina* that are sympatric. Hábitats at low elevation in eastern Costa Rica have been changed severely by deforestation, and any salamanders now are difficult to find there. At somewhat higher elevations along the Atlantic slopes of the Cordillera de Talamanca, four other *uniformis* group species, *O. collaris*, *O. poelzi*, *O. pseudouniformis*, and *O. uniformis*, occur in general proximity to each other, but all are now uncommon (*O. collaris* is known only from a single specimen in this area). In the *parvipes* group, *O. parvipes* and *O. compex*, both uncommon, occur together on Barro Colorado Island, Panama. Species in the *parvipes* group occur in sympatry with other species of *Oedipina* in both the Atlantic and Pacific lowlands of Costa Rica (*O. carablanca* with *O. cyclocauda* and *O. gracilis* at Guapiles. and *O. alleni* with *O. pacificensis* from the vicinity of Parrita to the Osa Peninsula on the Pacific coast).

Specimens of *O. cyclocauda* and *O. pseudouniformis* are difficult to separate on morphological grounds, although *O. cyclocauda* has slightly shorter legs, and the two have not been taken in sympatry, so we have some question concerning correct allocation of preserved specimens of these genetically very distinct táxones.

Despite the relatively weak morphological differentiation of the many species in Brame's (1968) *uniformis* group (including our *cyclocauda*, *poelzi*, and *uniformis* clades), our demonstration of deep genetic divergence suggests that it is an old lineage that has been differentiated for millions of years. Members of the *uniformis* group are widespread in the uplands and lowlands, especially in southern Mesoamerica, and they can be locally abundant. Accordingly, several species in the group are relatively well known. On the other hand, animals in Brame's *parvipes* group (identical to our *parvipes* clade) are restricted to relatively low elevations and are difficult to collect (Brame had fewer than 100 preserved specimens of all species for his revision), and as a whole this group is relatively poorly known. Brame discussed the possibility of raising his *parvipes* and *uniformis* groups to the rank of genera, but decided against it because he wanted to emphasize the close relationship between them. Our results corroborate a sister group relationship of the two groups.

We analyzed only two species of Brame's *parvipes* group, and thus cannot elucidate phylogenetic relationships within that group. Within Brame's *uniformis* group, however, we had ten of the 16 species available for analysis. Brame provided the only previous discussion of relationships among the species in his *uniformis* group, and his hypothesis is illustrated in Fig. 7. His results are similar to ours in that, of the species available to us, he grouped *O. uniformis* (including *O. gracilis* and *O. pacificensis*), *O. alfaroi*, and *O. ignea* into a monophyletic unit. This corresponds exactly to our *uniformis* clade. His hypothesis differs from ours, however, in two ways. He did not recognize our *cyclocauda* clade, but instead suggested that *O. cyclocauda*, *O. pseudouniformis*, and *O. altura* form sequential outgroups to the *uniformis* clade. He also suggested that *O. poelzi* is more distantly related to the *uniformis* clade than are the members of our *cyclocauda* clade. This conflicts with our hypothesis that *O. poelzi* is the sister taxon to the *uniformis* clade. Brame's evidence for the "stair-step" pattern of relationships of *O. poelzi*, *O. altura*, *O. pseudouniformis*, *O. cyclocauda*, and the *uniformis* clade was that each represents a sequential step in an apparent morphocline from the long-limbed, broad-footed, broad-headed *parvipes* group species to the short-limbed, narrow-footed, narrow-headed *uniformis* clade species. *Oedipina poelzi* does, in fact, approach the *parvipes* group more closely than the other species in leg length and foot width, but Brame's Fig. 25 (p. 53) does not corroborate his statement about intermediacy in head width. In this character, the *cyclocauda* clade appears to most closely approach the *parvipes* group, while *O. poelzi* and the *uniformis* clade have become derived in opposite directions (wider heads in *O. poelzi* and narrower in the *uniformis* clade). Our phylogeny would require either a reversal in *O. poelzi* to more robust limbs or a parallel reduction of the limbs in the *cyclocauda* and *uniformis* clades.

Cytological studies of two species of *Oedipina* (*O. poelzi* and *O. uniformis*) have disclosed that they have unusual meiosis and strongly heteromorphic X/Y sex chromosomes (Kezer *et al.* 1989). They share this heteromorphic system with *Nototriton*, and to some degree with *Thorius* and *Dendrotriton* (Sessions and Kezer 1991), but cytological studies of the *parvipes* group have not yet been undertaken. It is critical that cytological information be obtained, because at present the X/Y chromosome system constitutes an important synapomorphy that may be significant in sorting out the patterns of phylogenetic relationships in the supergenus *Bolitoglossa*.

Members of the genus *Oedipina* are, with some *Bolitoglossa*, the most "tropical" of salamanders. Only members of the *parvipes* group in *Oedipina* and species in the alpha group of *Bolitoglossa* (Wake and Lynch 1976), of all salamanders, reach South America. The majority of species of *Oedipina* occur in what Wake and Lynch referred to as Talamancan Central America, and only one species (*O. elongata*) extends northward as far as Mexico. All species of the *parvipes* group occur in the tropical lowlands (areas below 500 m elevation), but at least some of these species occur as high as 1250 m. The species of the *parvipes* group have a number of extreme specializations, notably in head shape and in the shape of the hands and feet. Species of the *uniformis* group also occur in tropical lowlands, from Guatemala at least to western Panama. Presumably, it is the successful invasion of the tropical lowlands that has facilitated dispersal of salamanders into South America and into Nuclear Central America. Some species of the *uniformis* group occur exclusively in the uplands, where they live at least as high as 2300 m.

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RESUMEN

Se analizó la variación proteica entre muestras de 28 poblaciones de salamandras del género *Oedipina*, de Guatemala, Honduras, Costa Rica, y Panama, para evaluar la validez de las especies actualmente reconocidas así como sus relaciones filogenéticas. Se usó como grupo de referencia ocho especies del género emparentado *Nototriton* . Hay cuatro clades principales con distancias genéticas de Nei de 0.46 a 2.67. Los patrones de variación geográfica dentro de la *O. uniformis* as actualmente reconocida indican que en realidad contiene poblaciones que deberían ubicarse en cuatro especies: *O. alfaroi* es una especie válida, y *O. gracilis* y *O. pacificensis* se reviven de la sinomnimia de *O. uniformis*, mientras *O. bonitaensis*, *O. inusitata*, *O. longissima*, y *O. syndactyla* continúan en sinonimia. La variación geográfica también es aparente en *O. cyclocauda*, *O. poelzi*, y *O. pseudouniformis*, pero no se justifican cambios taxonómicos en este momento. Se apoya el estado específico de *O. altura* y *O. grandis* . Las cuatro muestras que representan al grupo *parvipes* group son las más lejanamente relacionadas con el resto de las muestras, y la variación genética dentro del grupo es suficiente para justificar la reinstalación de las *O. alleni* costarricenses de la sinonimia con *O. parvipes* de Panamá y Colombia. Todas las muestras morfológicamente diferenciadas también son diferentes genéticamente. Nuestros resultados indican que se muestrearon 12 especies (*O. alfaroi*, *O. alleni*, *O. altura*, *O. cyclocauda*, *O. gracilis*, *O. grandis*, *O. ignea*, *O. pacificensis*, *O. parvipes*, *O. poelzi*, *O. pseudouniformis*, y *O. uniformis*). no dispusimos de muestras de *O. carablanca*, *O. collaris*, *O. complex*, *O. elongata*, *O. gephyra*, *O. paucidentata*, *O. stenopodia*, *O. stuarti*, u *O. taylori*, pero todas son reconocibles como especies diferentes según la morfología.

REFERENCES

Brame, A. H., Jr. 1960. The salamander genus *Oedipina* in northern Central America. Bull. So. California Acad. Sci. 59: 53-162.

Brame, A. H., Jr. 1963. A new Costa Rican salamander (genus *Oedipina*) with a re-examination of *O. collaris* and *O. serpens*. Contrib. Sci. Nat. Hist. Mus. Los Angeles Co. 65: 3-12.

Brame, A. H., Jr. 1968. Systematics and evolution of the Mesoamerican salamander genus *Oedipina*. J. Herpetol. 2: 1-64.

Brame, A. H., Jr. , & W. E. Duellman. 1970. A new salamander (genus *Oedipina*) of the *uniformis* group from western Panama. Contrib. Sci. Nat. Hist. Mus. Los Angeles Co. 201: 1-8.

Brodie, E. D., Jr., & J. A. Campbell. 1993. A new salamander of the genus *Oedipina* (Caudata: Plethodontidae) from the Pacific versant of Guatemala. Herpetologica 49: 259-265.

Buth, D. G. 1984. The application of electrophoretic data in systematic studies. Ann. Rev. Ecol. Syst. 15: 501-522.

Dunn, E. R. 1921. Two new Central American salamanders. Proc. Biol. Soc. Washington 34: 143-146.

Frost, D. R., & D. M. Hillis. 1990. Species in concept and practice: applications in herpetology. Herpetologica 46: 87-104.

Good, D. A., & D. B. Wake. 1992. Geographic variation and speciation in the torrent salamanders of the genus *Rhyacotriton* (Caudata: Rhyacotritonidae). Univ. California Publ. Zool. 126: 1-91.

Good, D. A., & D. B. Wake. 1993. Systematic studies of the diminutive salamanders of the genus *Nototriton* from Costa Rica, with descriptions of three new species. Herp. Monogr. 7: 131-159.

Harris, H., & D. A. Hopkinson. 1976. Handbook of Enzyme Electrophoresis and Human Genetics. North-Holland Publ. Co., Amsterdam. Unpaginated.

Hillis, D. M., & C. Moritz. 1990. Molecular Systematics. Sinauer Assoc., Sunderland, MA. 588 p.

Kezer, J., S. K. Sessions, & P. Leon. 1989. The meiotic structure and behavior of the strongly heteromorphic X/Y sex chromosomes of neotropical plethodontid salamanders of the genus *Oedipina*. Chromosoma 98: 433-442.

Larson, A. 1984. Neontological inferences of evolutionary pattern and process in the salamanders of the family Plethodontidae. Evol. Biol. 17: 119-217.

Mabee, P. M., & J. Humphries. 1993. Coding polymorphic data: examples from allozymes and ontogeny. Syst. Biol. 42: 166-181.

McCranie, J. R., L. D. Wilson, & K. L. Williams. 1993. A new species of *Oedipina* (Amphibia: Caudata: Plethodontidae) from northern Honduras. Proc. Biol. Soc. Washington 106: 385-389.

Murphy, R. W. 1993. The phylogenetic analysis of allozyme data: invalidity of coding alleles by presence/absence and recommended procedures. Biochem. Syst. Ecol. 21: 25-38.

Nei, M. 1972. Genetic distance between populations. Am. Natur. 106: 283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Richardson, B. J., P. R. Baverstock, & M. Adams. 1986. Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Structure. Academic Press, Sydney. 410 p.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, & J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud. Genet. VI. Univ. Texas Publ. 7103: 49-90.

Sessions, S. K., & J. Kezer. 1991. Evolutionary cytogenetics of bolitoglossine salamanders (family Plethodontidae). P. 89-130. *In* D. M. Green and S. K. Sessions (eds.). Amphibian Cytogenetics and Evolution. Academic Press, Nueva York.

Swofford, D. L. 1985. PAUP, Phylogenetic Analysis Using Parsimony. User's Manual. Illinois Natrual History Survey, Champaign. Unpaginated.

Swofford, D. L., & R. K. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 281-283.

Taylor, E. H. 1948. New Costa Rican salamanders. Proc. Biol. Soc. Washington 61: 177-180.

Taylor, E. H. 1949. New salamanders from Costa Rica. Univ. Kansas Sci. Bull. 33: 279-288.

Taylor, E. H. 1952. The salamanders and caecilians of Costa Rica. Univ. Kansas Sci. Bull. 34, pt. 2: 695-791.

Taylor, E. H. 1954. Additions to the known herpetological fauna of Costa Rica with comments on other species. No. 1. Univ. Kansas Sci. Bull. 36, pt. 1: 597-639.

Wake, D. B. 1987. Adaptive radiation of salamanders in Middle American cloud forests. Ann. Missouri Bot. Gard. 74: 242-264.

Wake, D. B., & P. Elias. 1983. New genera and a new species of Central American salamanders, with a review of the tropical genera (Amphibia, Caudata, Plethodontidae). Contrib. Sci. Nat. Hist. Mus. Los Angeles Co. 345: 1-19.

Wake, D. B., & J. F. Lynch. 1976. The distribution, ecology, and evolutionary history of plethodontid salamanders in tropical America. Sci. Bull. Nat. Hist. Mus. Los Angeles Co. 25: 1-65.

Wake, D. B., T. J. Papenfuss, & J. F. Lynch. 1992. Distribution of salamanders along elevational transects in Mexico and Guatemala. Tulane Stud. Zool. Bot., Suppl. Publ. 1: 303-319.