Syst. Zool., 34(2):109-126, 1985

EVOLUTIONARY GENETICS OF THE ANDEAN LIZARD GENUS *PHOLIDOBOLUS* (SAURIA: GYMNOPHTHALMIDAE): PHYLOGENY, BIOGEOGRAPHY, AND A COMPARISON OF TREE CONSTRUCTION TECHNIQUES

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Abstract.—Electrophoretic examination of the products of 29 gene loci in the five species of *Pholidobolus* and three species of *Proctoporus* largely substantiates the taxonomic arrangement that was based on earlier morphological work. The electrophoretic data are used to construct a phylogeny of the species and the morphological data are reanalyzed for comparison; these data sets are largely consistent. The hypothesized phylogeny places *Ph. affinis* as the sister species to the other *Pholidobolus*, *Ph. macbrydei* as the sister species to the remaining taxa, and the two populations referred to *Ph. prefrontalis* as the sister species to *Ph. montium* plus *Ph. annectens*. Because of the small size of the genus *Pholidobolus* and the concomitant small number of possible phylogenetic hypotheses, comparisons among various methods of constructing phylogenetic trees from electrophoretic data are facilitated. All 105 possible bifurcating unrooted trees were examined for each of 13 goodness-of-fit statistics; three similar trees were consistently favored by all criteria. If outgroup rooting is used, the most favored of these trees is identical to the most parsimonious cladogram. However, with midpoint rooting, almost all phylogenetic information is lost and the resulting tree is identical to the UPGMA phenogram. The midpoint-rooting option of distance trees reinvokes an assumption (equal rates of change) that the distance methods are supposed to avoid. This assumption is clearly not met for the genus *Pholidobolus*.

Biogeographic analysis of the species of *Pholidobolus* with respect to the hypothesized phylogeny of the genus reveals that no contact zones of this parapatric complex involve two sister species. This emphasizes the need for phylogenetic reconstruction as the first step in the examination of speciation mechanisms. This finding also suggests that competing species, rather than geographic barriers, may at times serve to isolate differentiating populations. [Evolutionary genetics; phylogeny; biogeography; speciation; genetic distances; network rooting; *Pholidobolus*; *Proctoporus*.]

The species in the gymnophthalmid lizard genus *Pholidobolus* are restricted to elevations of 1,800 to 4,000 m in the Andes of Ecuador. These lizards are distributed parapatrically, with only three known points of sympatry between pairs of species (Fig. 1). The nominal genus was in a chaotic systematic state until it was revised by Montanucci (1973). Of the five species in *Pholidobolus*, two had been placed in other genera and two were undescribed until Montanucci's (1973) monograph. These systematic problems were caused in large part by the morphological similarity of the taxa.

The techniques of allozyme electrophoresis have been very useful in the study of the phylogenies of closely related groups of species. These techniques are ideal for examining the evolution of *Pholidobolus*, as well as for testing the taxonomic decisions of Montanucci (1973). Because of the parapatric distribution of these species, the relatively recent geologic uplift of the Andes to the heights at which the species of *Pholidobolus* occur (Simpson, 1979), and the morphological similarity of the species, it is to be expected that these taxa have diverged recently—probably within the last two to three million years. This recent divergence, together with the identification of appropriate outgroups (Presch, 1980) and the small size of the genus, makes *Pholidobolus* an ideal group for electrophoretic study.

Recent systematic studies involving electrophoresis have been divided be-

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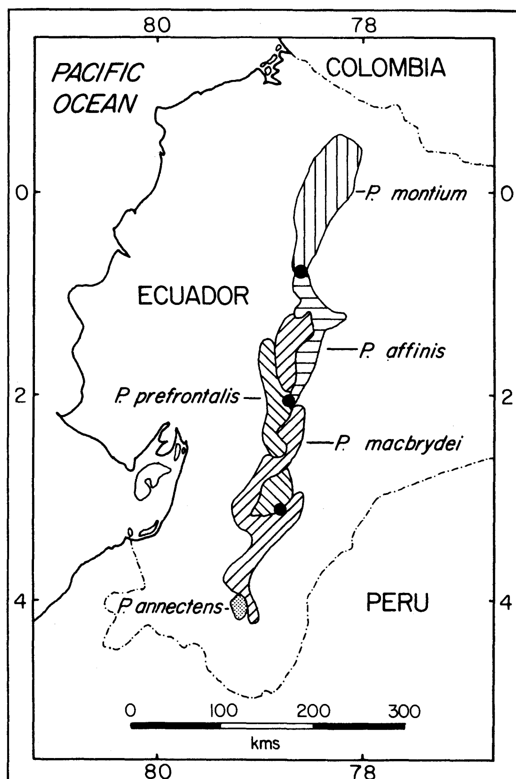

FIG. 1. Distributions of the species of *Pholidobolus*. Black dots indicate reported points of sympatry (adapted from Montanucci, 1973).

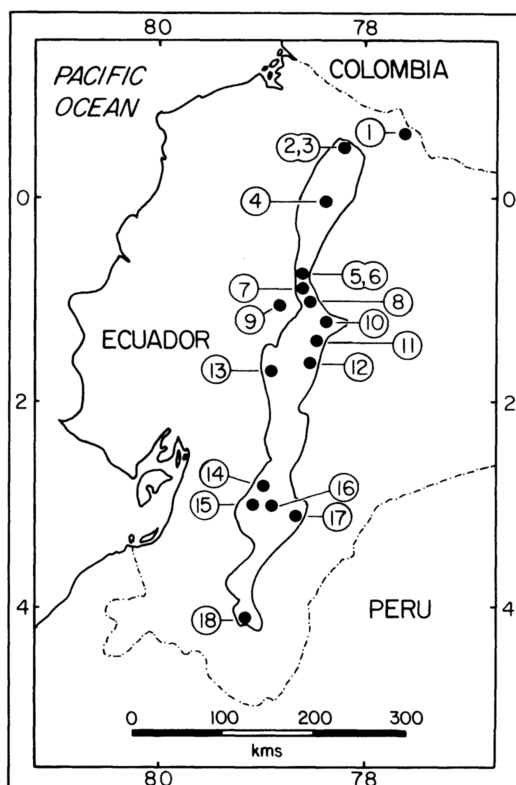

FIG. 2. Localities sampled for specimens of *Pholidobolus* and *Proctoporus* that were examined electrophoretically. Localities are superimposed over the collective distribution of the species of *Pholidobolus*. Numbers refer to the locality list in the Appendix.

tween qualitative cladistic evaluations of electrophoretic data (e.g., Wake et al., 1978; Avise et al., 1980a; Honeycutt et al., 1981; Patton et al., 1981; Hillis et al., 1983; Miyamoto, 1983a, b; Murphy et al., 1983; Sites et al., 1984) and quantitative evaluations that employ various measures of genetic distance and tree construction techniques. The latter group of studies are divided further into studies that employ UPGMA trees that assume constant rates of divergence for all taxa (e.g., Larson and Highton, 1978; Sites et al., 1981, 1984) versus those that employ other tree construction techniques that are designed to avoid this assumption (see Swofford, 1981). Finally, this latter group of studies is subdivided still further into studies that employ methods of outgroup rooting (e.g., Simon, 1979; Berlocher and Bush, 1982) in contrast to those that rely entirely on ingroup genetic distances to build trees that are interpreted as reflecting phylogeny (e.g., Avise et al., 1980b; Seidel and Lucchino, 1981; Hanken, 1983; Larson, 1983; Zink and Johnson, 1984). Comparisons among these various approaches using real data sets are needed in order to interpret the findings of studies using a particular approach. Because of the small size of the genus *Pholidobolus* and the concomitant small number of possible phylogenetic hypotheses, it was possible to compare trees derived by each of these various methods and to examine the reasons for differences.

MATERIALS AND METHODS

An attempt was made to obtain samples from throughout the collective range of

the species of *Pholidobolus* (Fig. 2). The gene products of 57 specimens were examined electrophoretically, divided as follows: 6 *Proctoporus* (3 species) for use as the outgroup; 20 *Ph. montium* from five localities; 6 *Ph. affinis* from four localities; 10 *Ph. prefrontalis* from two localities; 14 *Ph. macbrydei* from three localities; and a single specimen of the geographically restricted *Ph. annectens*. The three species of *Proctoporus* included one undetermined species from Amazonian-slope cloud forest, one high-Andean species (*Pr. unicolor*), and one species from the Pacific cloud forest (*Pr. hypostictus*). The genus *Proctoporus* was used as the outgroup because of the relatively close relationship of the genus to *Pholidobolus* (Presch, 1980) and because of its availability. Localities and museum numbers for all specimens examined are listed in the Appendix.

Livers were removed from lizards in the field and frozen immediately in liquid nitrogen for transport to the laboratory. The vouchers were then preserved and deposited in the herpetological collection of the Museum of Natural History, The University of Kansas. In the laboratory, tissues were maintained at −80°C until use.

Livers were ground with a teflon homogenizer and diluted 1:1 with 0.01 M tris-0.001 M EDTA-0.001 M β-mercaptoethanol, pH 7.5. Homogenates were then centrifuged at 15,000 rpm for 5 min, and the supernatants were refrozen at −80°C prior to use.

Standard procedures of horizontal starch gel electrophoresis were employed (see Selander et al., 1971). The following buffer systems were used (see Table 1): **1.** TC 6.7. Electrode: 0.223 M tris-0.086 M citric acid, pH 6.3. Gel: 0.008 M tris-0.003 M citric acid, pH 6.7. **2.** TC-NADP 6.7. Same as TC 6.7, except 8 mg NADP added to gel and 10 mg NADP added to cathodal electrode tray. **3.** TC 8.0. Electrode: 0.687 M tris-0.157 M citric acid, pH 8.0. Gel: 22.89 mM tris-5.22 mM citric acid, pH 8.0. **4.** TME 7.4. Electrode: 0.10 M tris-0.10 M maleic acid-0.01 M EDTA-0.01 M magnesium chloride, pH 7.4. Gel: 1:9 dilution of electrode buffer. **5.** TBE 8.0. Electrode: 0.50 M tris-0.65 M boric acid-0.02 M EDTA, pH 8.0. Gel: 1:9 dilution of electrode buffer. **6.** TBE 9.1. Electrode and gel: 175.0 mM tris-17.5 mM boric acid-2.75 mM EDTA, pH 9.1. **7.** TBE-NAD 9.1. Same as TBE 9.1, except 100 mg NAD added to gel and 60 mg NAD added to cathodal electrode tray. **8.** TBE-NADP 9.1. Same as TBE 9.1, except 8 mg NADP added to gel and 10 mg NADP added to cathodal electrode tray. **9.** Poulik 8.7. Electrode: 0.30 M boric acid, pH 8.2. Gel: 0.076 M tris-0.005 M citric acid, pH 8.7. **10.** LiOH 8.4. Electrode: 0.03 M lithium hydroxide-0.19 M boric acid, pH 8.1. Gel: 0.003 M lithium hydroxide-0.019 M boric acid-0.045 M tris-0.0072 M citric acid, pH 8.4.

Gels were prepared from 50% Connaught starch (lot 370-1) and 50% Otto Hiller electrostarch (lot 392). Gels were 12% starch for all buffer systems. Two drops of β-mercaptoethanol were added to the gel buffer mixture after boiling and degassing. Gels were electrophoresed under the following conditions: buffer systems 1 and 2—6.25 V/cm for 10 hr; buffer system 3—5.0 V/cm for 11 hr; buffer system 4—5.0 V/cm for 14.5 hr; buffer system 5—8.0 V/cm for 9 hr; buffer systems 6, 7, and 8—12.5 V/cm for 11 hr; buffer system 9—7.5 V/cm for 9 hr; and buffer system 10—12.5 V/cm for 10 hr. All gels were maintained at 4°C during electrophoresis.

Each gel was sliced into 1-mm-thick slabs for staining. The loci examined and buffer conditions used are listed in Table 1. The procedures for staining were largely those described by Harris and Hopkinson (1976), Siciliano and Shaw (1976), and Selander et al. (1971). Phosphorescent stains (acid phosphatase and β-glucosidase) were viewed and photographed under 375-nm UV light.

Multiple loci were numbered from cathode to anode. Electromorphs were assigned letters according to their mobility, again beginning with the electromorph closest to the cathode. Peptidase loci were given the letter designations of Harris and Hopkinson (1976) corresponding to their substrate specificities. DL-leucyl-D-alanine, DL-leucylglycine, DL-leucylglycylglycine, and L-leucyl-L-proline were used as peptidase substrates.

TABLE 1. Loci examined, abbreviations used, Enzymes Commission (E.C.) numbers (Commission on Biochemical Nomenclature, 1979), and associated buffer systems.

Locus	No. loci scored	Abbreviation	E.C. no.	Buffer system
Acid phosphatase	1	AP	3.1.3.2	TC 6.7
Aconitase	1	ACO	4.2.1.3	TBE-NADP 9.1
Alcohol dehydrogenase	1	ADH	1.1.1.1	TBE-NAD 9.1
Esterase	1	EST	3.1.1.1	LiOH 8.4
Glucose phosphate isomerase	1	GPI	5.3.1.9	TBE 8.0
β-Glucosidase	1	β-GSD	3.2.1.21	TC-NADP 6.7
Glyceraldehyde-3-phosphate dehydrogenase	1	G3PD	1.2.1.12	TBE-NAD 9.1
Glutamate-oxaloacetate transaminase	2	GOT	2.6.1.1	TME 7.4
α-Glycerophosphate dehydrogenase	1	α-GPD	1.1.1.8	TBE-NAD 9.1
3-Hydroxyisobutyrate dehydrogenase	1	HDH	1.1.1.31	TBE 9.1
Isocitrate dehydrogenase	1	IDH	1.1.1.42	TC 6.7
Lactate dehydrogenase	2	LDH	1.1.1.27	TBE-NAD 9.1
Malate dehydrogenase	2	MDH	1.1.1.37	TC 6.7
Mannosephosphate isomerase	1	MPI	5.3.1.8	TC 8.0
Nucleoside phosphorylase	1	NP	2.4.2.1	TME 7.4
6-Phosphogluconate dehydrogenase	1	6PGD	1.1.1.44	TC-NADP 6.7
Phosphoglucomutase	2	PGM	2.7.5.1	TC 6.7
Peptidase (A, S, C)	3	PEP	3.4.11, 13	LiOH 8.4
Peptidase (D)	1	PEP	3.4.13.9	LiOH 8.4
Sorbitol dehydrogenase	1	SDH	1.1.1.14	TBE 8.0
Superoxide dismutase	2	SOD	1.15.1.1	Poulik 8.7, TBE 9.1
Triosephosphate isomerase	1	TPI	5.3.1.1	Poulik 8.7

The genetic distance measures described by Nei (1972), Rogers (1972), and Hillis (1984) were calculated for all pairwise combinations of populations of *Pholidobolus* and *Proctoporus*. These distances were used to construct UPGMA phenograms (Sneath and Sokal, 1973). Genetic distances were recalculated after pooling all populations of each species, and UPGMA phenograms were reconstructed. In addition, the WAGPROC program of David L. Swofford (version 3.3) was used to construct distance Wagner trees (Farris, 1972; Swofford, 1981) from these restricted genetic distances, using both outgroup and midpoint rooting. These trees were optimized using linear programming (D. L. Swofford's NETOPT program; see Waterman et al., 1977). All possible ingroup trees were optimized and ranked for 13 proposed goodness-of-fit criteria: percent standard deviation, with and without the restriction of no negative branch lengths (Fitch and Margoliash, 1967); Farris' f-statistic, with and without the restriction of no negative branch lengths, and with and without the restriction that path distances should meet or exceed observed distances (Farris, 1972); a weighted version of Farris' f-statistic, with and without the above restrictions (Farris, 1981); total tree length (Waterman et al., 1977); two types of neighborliness values, N_1 and N_2 (Fitch, 1981); and the S_0 measure of Tateno et al. (1982), with and without the restriction of no negative branch lengths. These linear programming optimizations (Waterman et al., 1977) and rankings were conducted using a program supplied by D. L. Swofford.

The most parsimonious cladogram was constructed by standard phylogenetic methods (Wiley, 1981). Electromorphs found in both the ingroup and the outgroup were considered primitive. Electromorphs were ordered into transformation series following the taxonomic outgroup and functional outgroup criteria of Watrous and Wheeler (1981), as expanded by Farris (1982).

RESULTS

Among the products of the 29 loci studied, 132 electromorphs were identified within the nine species examined (Table 2). All of the taxa showed relatively low levels of genetic polymorphism at the loci

studied. Average number of alleles per locus ranged from 1.0 for the undetermined species of *Proctoporus* in the outgroup to 1.41 for combined populations of *Ph. prefrontalis.* As this study was conducted primarily to determine the phylogeny of the species of *Pholidobolus,* highly variable loci (e.g., most esterase loci) were eliminated from consideration during initial screening. Because of this bias, average heterozygosity values for the populations shown in Table 3 should be interpreted with caution, for they may be misleading.

The genetic identities and distances (Nei, 1972; Rogers, 1972; Hillis, 1984) between each pair of populations are presented in Table 3. These distances were used to construct UPGMA trees; the tree for modified Nei's distance (Hillis, 1984) is shown in Figure 3 (the trees for unmodified Nei's distance and Rogers' distance had similar topologies). The identities and distances for combined populations of species are presented in Table 4. The construction of distance Wagner trees is discussed below.

Sixteen alleles were identified as primitive based on their presence in both the ingroup (*Pholidobolus*) and the outgroup (*Proctoporus*). Changes in 13 loci were identified along the branch between *Pholidobolus* and *Proctoporus;* without additional information, the synapomorphies of *Pholidobolus* cannot be sorted from the synapomorphies of *Proctoporus* (if *Proctoporus* is monophyletic). Within *Pholidobolus,* 12 electrophoretic synapomorphies were identified that fully resolve the relationships within the genus (Fig. 4). One electromorph (6-PGD^{a}) found in one heterozygous individual of *Ph. prefrontalis* and in one of the species of *Proctoporus* was considered to be a convergence; other interpretations would require additional homoplasy. One other electromorph was found to be convergent—PGM-1^{a}, found in relatively low frequencies in single populations of both *Ph. prefrontalis* and *Ph. macbrydei.* Alternatively, this could be a primitive allele retained in low frequency, although the choice of interpretations has no effect on the supported phylogeny.

DISCUSSION

Validity of the taxa.—As seen in the UPGMA phenogram of all of the study populations (Fig. 3), virtually all populations within a species are genetically very similar. This substantiates the species designations of Montanucci (1973). The single exception is *Ph. prefrontalis,* which consists of two geographically separated populations—one on the Pacific slopes of the Andes, and the other in the Cuenca Basin (Fig. 1). Although the two populations of *Ph. prefrontalis* are closer to each other than to any other populations, they are electrophoretically considerably more distinct than are populations within any of the other species. Montanucci (1973) also presented morphological data for the two populations of *Ph. prefrontalis* showing them to be largely distinct for several pattern characteristics. Therefore, for the remainder of the analysis, the two populations of *Ph. prefrontalis* are treated separately, as they may be best considered distinct species.

Phylogeny.—The most parsimonious cladogram is presented in Figure 4. All character-state changes are indicated on the respective branches of the tree. There are only two homoplasies in the cladogram depicted in Figure 4; these two convergences (i.e., PGM-1^{a} and 6-PGD^{a}) are not congruent. Because of the congruence of several synapomorphies along each of the principal branch lengths, this hypothesized phylogeny appears to be well substantiated. The only branch without multiple synapomorphies is the branch that connects the two populations of *Ph. prefrontalis.*

The rate of electrophoretic evolution among the species of *Pholidobolus* has been very uneven (Fig. 4). The geographically restricted *Ph. annectens* is electrophoretically the most autapomorphic taxon, whereas *Ph. affinis* has differentiated little from the ancestral genotype of *Pholidobolus.* The groupings of the UPGMA phenogram (Fig. 3) reflect these differences in rates, grouping *Ph. affinis, Ph. macbrydei,* and *Ph. prefrontalis* based on patristic similarity (mostly shared, primitive characters). In

TABLE 2. Frequencies of electromorphs observed among 18 populations of *Pholidobolus* and *Proctoporus*. Populations within a species are numbered from north to south (see Appendix for localities).

Locus	*Proctoporus*			*Ph. affinis*				*Ph. prefrontalis*	
	A	*unicolor*	*hypostictus*	1	2	3	4	1	2
AP	a (1.0)	e (0.75) f (0.25)	b (1.0)	e (1.0)	e (1.0)	d (1.0)	d (0.50) e (0.50)	e (1.0)	e (1.0)
ACO	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
ADH	a (1.0)	a (1.0)	c (0.17) d (0.83)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
EST	d (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)
GPI	e (1.0)	d (1.0)	d (0.17) g (0.83)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	c (0.11) d (0.89)	d (1.0)
β-GSD	f (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	b (0.78) e (0.22)	b (1.0)
G3PD	a (1.0)	a (1.0)	b (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
GOT-1	b (1.0)	a (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (0.94) c (0.06)	b (1.0)
GOT-2	a (1.0)	e (1.0)	b (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (0.28) f (0.72)	g (1.0)
α-GPD	c (1.0)	c (1.0)	c (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	b (1.0)	b (1.0)
HDH	a (1.0)	a (1.0)	c (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)
IDH	b (1.0)	b (1.0)	b (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	a (0.50) c (0.50)
LDH-1	a (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	d (1.0)	c (1.0)
LDH-2	b (1.0)	a (1.0)	c (1.0)	b (0.50) e (0.50)	e (1.0)	e (1.0)	e (1.0)	b (1.0)	f (1.0)
MPI	a (1.0)	a (1.0)	d (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)
MDH-1	a (1.0)	a (0.75) b (0.25)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
MDH-2	a (1.0)	b (1.0)	c (1.0)	f (0.50) g (0.50)	f (1.0)	g (1.0)	g (1.0)	e (1.0)	e (1.0)
NP	b (1.0)	c (1.0)	a (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)
6PGD	b (1.0)	a (1.0)	f (1.0)	c (0.50) e (0.50)	c (0.50) e (0.50)	c (1.0)	c (1.0)	c (1.0)	a (0.50) c (0.50)
PGM-1	f (1.0)	c (1.0)	g (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	a (0.22) b (0.56) c (0.22)	b (1.0)
PGM-2	c (1.0)	c (1.0)	a (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)
PEP-A	b (1.0)	c (0.75) g (0.25)	a (1.0)	f (1.0)	f (1.0)	f (1.0)	f (1.0)	f (1.0)	f (1.0)
PEP-S	a (1.0)	b (1.0)	c (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	e (1.0)	e (1.0)
PEP-C	a (1.0)	a (1.0)	b (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)
PEP-D	a (1.0)	a (1.0)	e (1.0)	f (1.0)	f (1.0)	f (1.0)	f (1.0)	d (1.0)	f (1.0)
SDH	c (1.0)	c (1.0)	d (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)
SOD-1	g (1.0)	f (1.0)	c (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	a (1.0)	a (1.0)
SOD-2	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
TPI	a (1.0)	a (1.0)	b (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)

TABLE 2. Continued.

Ph. macbrydei			*Ph. montium*					*Ph. annectens*
1	2	3	1	2	3	4	5	1
e (1.0)	e (1.0)	e (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	e (1.0)
a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
a (1.0)	a (0.75) b (0.25)	a (0.86) b (0.14)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
b (1.0)	b (1.0)	c (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
d (1.0)	d (1.0)	d (1.0)	b (1.0)	d (0.83) f (0.17)	b (0.30) d (0.70)	b (0.10) d (0.90)	d (1.0)	a (1.0)
a (1.0)	c (1.0)	a (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	d (1.0)
a (1.0)	a (1.0)	a (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	a (1.0)
b (1.0)	b (1.0)	b (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)
d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	c (1.0)
a (1.0)	a (1.0)	a (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)
b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)
c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)
b (1.0)	b (0.75) c (0.25)	b (0.29) c (0.71)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)
b (1.0)	b (1.0)	b (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)
e (1.0)	e (1.0)	c (0.07) e (0.93)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)
a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	d (1.0)
d (1.0)	d (1.0)	d (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)
c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (0.30) d (0.70)	c (0.75) d (0.25)	c (0.50) d (0.50)	d (1.0)
c (1.0)	a (0.62) c (0.38)	c (0.93) d (0.07)	b (1.0)	b (0.50) c (0.50)	b (0.80) c (0.20)	b (0.90) c (0.10)	b (1.0)	e (1.0)
c (1.0)	c (1.0)	c (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (0.50) c (0.50)	b (0.50) c (0.50)
f (1.0)	f (1.0)	e (0.07) f (0.93)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	f (1.0)
e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)	e (1.0)
d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	d (1.0)	c (1.0)
f (1.0)	f (1.0)	f (1.0)	f (1.0)	b (1.0)	b (0.60) f (0.40)	f (1.0)	f (1.0)	c (1.0)
b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	b (1.0)	a (1.0)
c (1.0)	c (1.0)	c (0.93) e (0.07)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	c (1.0)	b (1.0)
a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)
a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)	a (1.0)

TABLE 3. Genetic distances and identities among 18 populations of *Proctoporus* and *Pholidobolus*. The first row of each triplet corresponds to the measure of Nei (1972), the second to the measure of Hillis (1984), and the third to the measure of Rogers (1972). Distances are above the diagonal; identities are below. Average heterozygosities are on diagonal.

	Proctoporus			*Ph. affinis*				*Ph. prefrontalis*	
Taxa	A	*unicolor*	*hypostictus*	1	2	3	4	1	2
Proctoporus		0.726	1.566	1.201	1.279	1.288	1.279	1.148	1.270
A	0.000	0.732	1.576	1.203	1.288	1.288	1.288	1.170	1.288
		0.519	0.788	0.698	0.720	0.724	0.720	0.678	0.715
Proctoporus	0.484		1.268	0.795	0.813	0.884	0.844	1.056	1.270
unicolor	0.481	0.039	1.269	0.810	0.810	0.887	0.832	1.042	0.916
	0.481		0.718	0.552	0.561	0.588	0.572	0.655	0.613
Proctoprous	0.209	0.281		1.361	1.379	1.388	1.379	1.701	1.520
hypostictus	0.207	0.281	0.019	1.393	1.393	1.393	1.393	1.719	1.543
	0.212	0.282		0.736	0.746	0.750	0.746	0.807	0.775
Pholidobolus	0.301	0.451	0.256		0.018	0.064	0.036	0.350	0.340
affinis (1)	0.300	0.445	0.248	0.052	0.020	0.067	0.041	0.366	0.361
	0.302	0.448	0.264		0.034	0.086	0.069	0.323	0.301
Pholidobolus	0.278	0.443	0.252	0.982		0.081	0.054	0.394	0.358
affinis (2)	0.276	0.445	0.248	0.980	0.017	0.082	0.056	0.402	0.361
	0.280	0.439	0.254	0.966		0.086	0.069	0.345	0.310
Pholidobolus	0.276	0.413	0.250	0.938	0.922		0.009	0.430	0.405
affinis (3)	0.276	0.412	0.248	0.935	0.921	0.000	0.010	0.439	0.401
	0.276	0.412	0.250	0.914	0.914		0.017	0.362	0.345
Pholidobolus	0.278	0.430	0.252	0.964	0.947	0.991		0.394	0.371
affinis (4)	0.276	0.435	0.248	0.960	0.945	0.990	0.017	0.402	0.366
	0.280	0.428	0.254	0.931	0.931	0.983		0.345	0.328
Pholidobolus	0.317	0.348	0.183	0.705	0.674	0.651	0.674		0.178
prefrontalis	0.310	0.353	0.179	0.693	0.669	0.645	0.669	0.057	0.179
(1—Pacific)	0.322	0.345	0.193	0.677	0.655	0.638	0.655		0.195
Pholidobolus	0.281	0.394	0.219	0.712	0.699	0.667	0.690	0.837	
prefrontalis	0.276	0.400	0.214	0.697	0.697	0.669	0.694	0.836	0.034
(2—Cuenca)	0.285	0.387	0.235	0.699	0.690	0.655	0.672	0.805	
Pholidobolus	0.310	0.440	0.285	0.850	0.817	0.793	0.817	0.757	0.737
macbrydei (1)	0.310	0.445	0.283	0.842	0.817	0.793	0.817	0.748	0.738
	0.310	0.434	0.284	0.832	0.810	0.793	0.810	0.741	0.724
Pholidobolus	0.308	0.355	0.228	0.774	0.742	0.718	0.742	0.765	0.717
macbrydei (2)	0.309	0.368	0.225	0.766	0.741	0.717	0.741	0.751	0.713
	0.309	0.344	0.232	0.742	0.720	0.703	0.720	0.743	0.694
Pholidobolus	0.312	0.395	0.242	0.811	0.779	0.754	0.779	0.729	0.699
macbrydei (3)	0.310	0.407	0.246	0.804	0.780	0.755	0.780	0.713	0.701
	0.314	0.386	0.243	0.780	0.758	0.741	0.758	0.707	0.676
Pholidobolus	0.172	0.202	0.174	0.478	0.470	0.483	0.487	0.555	0.597
montium (1)	0.172	0.205	0.172	0.473	0.473	0.483	0.483	0.559	0.600
	0.172	0.205	0.177	0.475	0.466	0.483	0.487	0.543	0.586
Pholidobolus	0.175	0.252	0.181	0.496	0.488	0.501	0.505	0.583	0.581
montium (2)	0.172	0.263	0.179	0.496	0.496	0.506	0.506	0.592	0.590
	0.180	0.251	0.188	0.486	0.477	0.494	0.499	0.575	0.563
Pholidobolus	0.178	0.241	0.184	0.490	0.482	0.483	0.487	0.566	0.598
montium (3)	0.172	0.245	0.179	0.483	0.483	0.487	0.487	0.571	0.601
	0.187	0.244	0.195	0.481	0.472	0.469	0.474	0.553	0.578
Pholidobolus	0.175	0.240	0.182	0.516	0.506	0.515	0.520	0.580	0.628
montium (4)	0.172	0.243	0.179	0.509	0.509	0.519	0.519	0.592	0.633
	0.179	0.243	0.187	0.512	0.502	0.509	0.513	0.567	0.616
Pholidobolus	0.193	0.260	0.183	0.532	0.522	0.526	0.531	0.597	0.652
montium (5)	0.197	0.264	0.179	0.524	0.524	0.532	0.532	0.607	0.652
	0.194	0.261	0.190	0.526	0.517	0.517	0.522	0.572	0.638
Pholidobolus	0.226	0.284	0.141	0.446	0.439	0.400	0.421	0.483	0.496
annectens (1)	0.231	0.297	0.138	0.438	0.438	0.404	0.428	0.473	0.497
	0.224	0.279	0.148	0.445	0.436	0.397	0.414	0.481	0.487

TABLE 3. Continued.

Ph. macbrydei			*Ph. montium*					*Ph. annectens*
1	2	3	1	2	3	4	5	1
1.170	1.177	1.165	1.758	1.744	1.729	1.745	1.645	1.487
1.170	1.176	1.172	1.758	1.758	1.758	1.758	1.626	1.464
0.690	0.691	0.686	0.828	0.820	0.813	0.821	0.806	0.776
0.822	1.035	0.929	1.598	1.377	1.424	1.425	1.349	1.259
0.810	0.998	0.899	1.584	1.334	1.406	1.414	1.332	1.215
0.566	0.656	0.614	0.795	0.749	0.756	0.757	0.739	0.721
1.257	1.477	1.418	1.748	1.707	1.695	1.705	1.697	1.962
1.263	1.493	1.404	1.758	1.719	1.722	1.719	1.719	1.981
0.716	0.757	0.768	0.823	0.812	0.805	0.813	0.810	0.852
0.163	0.257	0.210	0.738	0.700	0.713	0.662	0.632	0.806
0.172	0.267	0.218	0.749	0.700	0.729	0.674	0.646	0.825
0.168	0.258	0.220	0.525	0.514	0.519	0.488	0.474	0.555
0.202	0.298	0.250	0.756	0.718	0.731	0.680	0.650	0.824
0.202	0.299	0.249	0.749	0.700	0.729	0.674	0.646	0.825
0.190	0.280	0.242	0.534	0.523	0.528	0.498	0.483	0.564
0.232	0.282	0.331	0.728	0.691	0.728	0.663	0.642	0.916
0.232	0.333	0.280	0.728	0.680	0.720	0.656	0.632	0.907
0.207	0.297	0.259	0.517	0.506	0.531	0.491	0.483	0.603
0.202	0.298	0.250	0.720	0.683	0.719	0.655	0.633	0.865
0.202	0.299	0.249	0.728	0.680	0.720	0.656	0.632	0.848
0.190	0.280	0.242	0.513	0.501	0.526	0.487	0.478	0.586
0.278	0.268	0.317	0.590	0.540	0.570	0.544	0.517	0.727
0.290	0.287	0.338	0.582	0.525	0.560	0.524	0.499	0.749
0.259	0.257	0.293	0.457	0.425	0.447	0.433	0.428	0.519
0.305	0.333	0.359	0.517	0.543	0.514	0.465	0.428	0.702
0.303	0.338	0.355	0.510	0.528	0.509	0.457	0.427	0.699
0.276	0.306	0.324	0.414	0.437	0.422	0.384	0.362	0.513
	0.073	0.040	0.595	0.561	0.591	0.536	0.517	0.833
0.000	0.079	0.039	0.595	0.553	0.588	0.531	0.510	0.825
	0.090	0.059	0.448	0.437	0.462	0.422	0.414	0.569
0.929		0.098	0.638	0.623	0.644	0.580	0.556	0.895
0.924	0.042	0.103	0.642	0.619	0.642	0.578	0.553	0.885
0.910		0.116	0.478	0.474	0.494	0.453	0.444	0.599
0.961	0.907		0.606	0.573	0.604	0.546	0.526	0.853
0.962	0.902	0.040	0.600	0.558	0.593	0.536	0.515	0.832
0.941	0.884		0.462	0.451	0.476	0.436	0.428	0.583
0.552	0.528	0.546		0.076	0.050	0.031	0.054	0.618
0.552	0.526	0.549	0.000	0.082	0.060	0.033	0.056	0.613
0.552	0.522	0.538		0.084	0.076	0.043	0.069	0.466
0.571	0.536	0.564	0.926		0.029	0.045	0.065	0.604
0.575	0.538	0.572	0.921	0.027	0.036	0.046	0.068	0.613
0.563	0.549	0.526	0.916		0.057	0.062	0.092	0.458
0.554	0.525	0.547	0.951	0.971		0.022	0.029	0.544
0.556	0.526	0.552	0.942	0.965	0.057	0.029	0.032	0.556
0.538	0.506	0.524	0.924	0.943		0.047	0.062	0.430
0.585	0.560	0.579	0.969	0.956	0.978		0.012	0.589
0.588	0.561	0.585	0.967	0.955	0.971	0.025	0.014	0.593
0.578	0.547	0.564	0.957	0.938	0.953		0.033	0.454
0.597	0.574	0.591	0.948	0.937	0.972	0.988		0.568
0.600	0.575	0.597	0.945	0.935	0.968	0.986	0.034	0.551
0.586	0.556	0.572	0.931	0.908	0.938	0.967		0.431
0.435	0.409	0.426	0.539	0.547	0.580	0.555	0.566	
0.438	0.413	0.435	0.542	0.542	0.573	0.553	0.576	0.017
0.431	0.401	0.417	0.534	0.542	0.570	0.546	0.569	

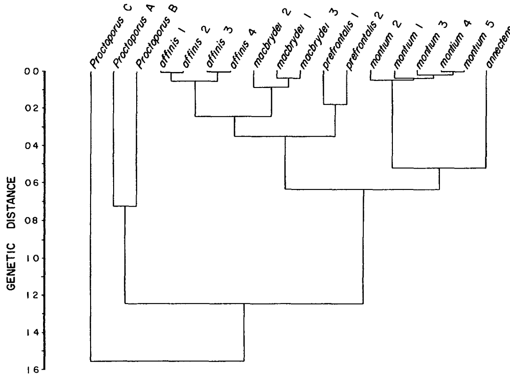

FIG. 3. UPGMA phenogram of all study populations of *Pholidobolus* and *Proctoporus*. Scale is in modified Nei's distance units (Hillis, 1984). This is not the hypothesized phylogeny.

order for a UPGMA phenogram to reflect phylogeny, rates of divergence of all taxa must be equal; this assumption has been made for electrophoretic data by many authors (for a review, see Thorpe, 1982). However, the molecular clock hypothesis is clearly not applicable in the case of this electrophoretic data set for *Pholidobolus* (thus the differences between the phenogram of Fig. 3 and the cladogram of Fig. 4). The lack of clocklike behavior in this data set could be a result of unmet requirements of the molecular clock hypothesis, or it could be that a molecular clock does not exist for allozymes (at least in this case). Whether or not a molecular clock exists, the existence of electrophoretic data sets that demonstrate unequal rates of change (such as the *Pholidobolus* data presented here) suggests that a molecular clock

TABLE 4. Genetic distances and identities among three species of *Proctoporus* and pooled samples of six clades of *Pholidobolus*. The first row of each triplet corresponds to the measure of Nei (1972), the second to the measure of Hillis (1984), and the third to the measure of Rogers (1972). Distances are above the diagonal; identities are below.

Taxon	*Proctoporus*			*Pholidobolus*					
	A	*unicolor*	*hypostictus*	*affinis*	*macbrydei*	*prefrontalis* (Cuenca)	*prefrontalis* (Pacific)	*montium*	*annectens*
Proctoporus A		0.726 0.732 0.519	1.566 1.576 0.788	1.240 1.263 0.707	1.146 1.172 0.676	1.270 1.288 0.715	1.148 1.170 0.678	1.727 1.754 0.813	1.487 1.464 0.776
Proctoporus unicolor	0.484 0.481 0.481		1.268 1.269 0.718	0.814 0.818 0.557	0.930 0.875 0.622	0.932 0.916 0.613	1.056 1.042 0.655	1.411 1.399 0.752	1.259 1.215 0.721
Proctoporus hypostictus	0.209 0.207 0.212	0.281 0.281 0.282		1.360 1.393 0.736	1.405 1.361 0.757	1.520 1.543 0.775	1.701 1.719 0.807	1.694 1.719 0.806	1.962 1.981 0.852
Pholidobolus affinis	0.290 0.283 0.293	0.443 0.441 0.443	0.257 0.248 0.264		0.217 0.221 0.241	0.350 0.358 0.313	0.371 0.385 0.338	0.659 0.660 0.492	0.832 0.833 0.570
Pholidobolus macbrydei	0.318 0.310 0.324	0.395 0.417 0.378	0.245 0.256 0.243	0.805 0.801 0.759		0.327 0.337 0.311	0.271 0.301 0.267	0.554 0.557 0.448	0.855 0.858 0.582
Pholidobolus prefrontalis (Cuenca)	0.281 0.276 0.285	0.394 0.400 0.387	0.219 0.214 0.225	0.705 0.699 0.687	0.721 0.714 0.689		0.178 0.179 0.195	0.479 0.464 0.400	0.702 0.699 0.513
Pholidobolus prefrontalis (Pacific)	0.317 0.310 0.322	0.348 0.353 0.345	0.183 0.179 0.193	0.690 0.681 0.662	0.763 0.740 0.733	0.837 0.836 0.805		0.540 0.526 0.431	0.727 0.749 0.519
Pholidobolus montium	0.178 0.173 0.187	0.244 0.247 0.248	0.184 0.179 0.194	0.517 0.517 0.508	0.575 0.573 0.552	0.619 0.629 0.600	0.582 0.591 0.569		0.570 0.586 0.444
Pholidobolus annectens	0.226 0.231 0.224	0.284 0.297 0.279	0.141 0.138 0.148	0.435 0.435 0.430	0.425 0.424 0.418	0.496 0.497 0.487	0.483 0.473 0.481	0.565 0.557 0.556	

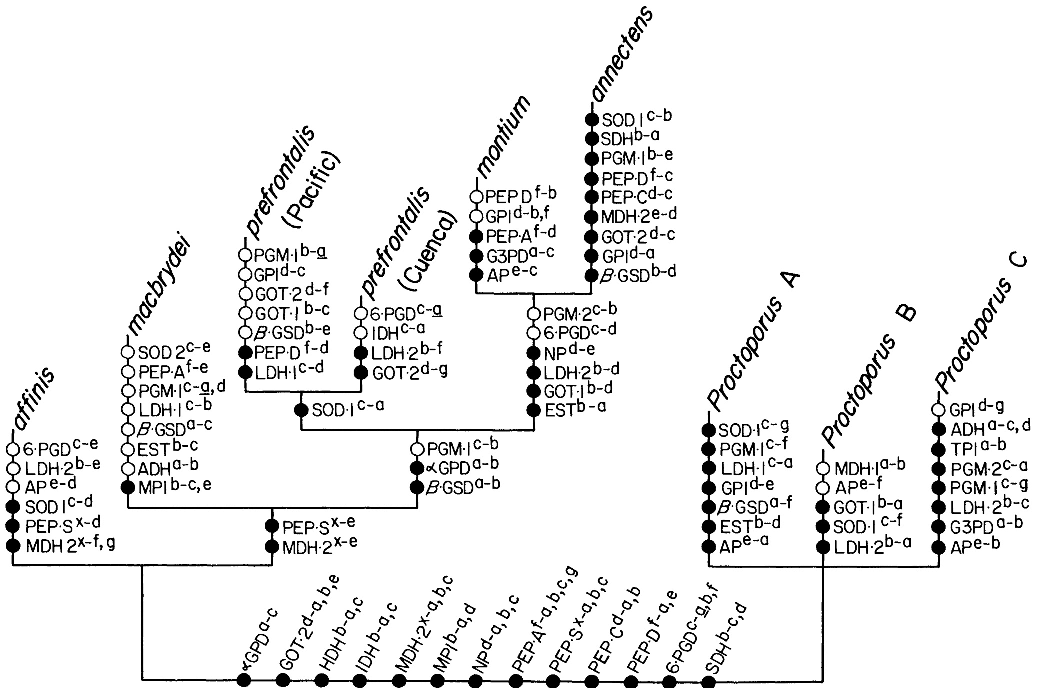

FIG. 4. Cladogram of *Pholidobolus*. Changes in allelic states are indicated along each stem (e.g., SOD-$1^{c\text{-}b}$ indicates a change from electromorph *c* to electromorph *b* at locus SOD-1). Retention of a primitive allele at some frequency is indicated by an open circle; solid circles indicate complete loss of the primitive allele. Changes along the base of the cladogram (between *Pholidobolus* and *Proctoporus*) are non-directional. An *x* indicates that the primitive electromorph is unknown. Homoplasies are indicated by underlining. Primitive alleles (i.e., those found in both *Pholidobolus* and *Proctoporus*) are: AP^e, ACO^a, ADH^a, EST^b, β-GSD^a, G-3-PD^a, GOT-1^b, GPI^d, LDH-1^c, LDH-2^b, PGM-1^c, PGM-2^c, MDH-1^a, SOD-1^c, SOD-2^a, and TPI^a.

should not be an a priori assumption in electrophoretic studies.

Although many of the morphological characters studied by Montanucci (1973) were treated in a quantitative manner, he did code seven characters into primitive and derived states based on outgroup comparisons (Table 5). The distribution of these characters among the species of *Pholidobolus* as determined from Montanucci (1973) is shown in Table 6, and the congruence of morphological synapomorphies with the hypothesized phylogeny based upon the electrophoretic data is shown in Figure 5. Although these morphological characters resolve only a part of the phylogeny, they are mostly consistent with the cladogram derived from electrophoretic data. However, a convergence is required of one of the morphological characters; *Ph. montium* and *Ph. annectens* lack prefrontals (derived condition), as do some individuals of *Ph. macbrydei*. The hypothesis of a clade uniting *Ph. montium*, *Ph. annectens*, and *Ph. macbrydei* based on this character would require the convergence of several congruent electrophoretic characters (Fig. 4); therefore, this hypothesis is rejected. The electrophoretic data mirror the morphological data in the highly derived condition of *Ph. annectens* versus the largely primitive condition of *Ph. affinis*. In fact, the electrophoretic "clock" is not much better than the morphological "clock" for these species.

Because the assumptions of equal rates of change for all taxa are not met for *Pholidobolus*, a UPGMA phenogram based on genetic distances does not accurately reflect the phylogeny of this group. However, other procedures of constructing trees from genetic distances do not require

TABLE 5. Morphological characters of *Pholidobolus* as polarized by Montanucci (1973).

Character	Primitive state	Derived state
A Eyelid	Opaque, 3–6 scales	Transparent, single scale
B Lateral granules	Present	Absent
C Prefrontals	Present	Absent
D Supraoculars	Three	Two
E Femoral pores	Present	Absent
F Lateral body fold	Present	Absent
G Body and limb scutellation	High number of scales	Reduced number of scales

equal rates of change for all taxa. These include distance Wagner trees (Farris, 1972) and Fitch-Margoliash trees (Fitch and Margoliash, 1967). It is of interest to examine the ability of these methods to reflect the most parsimonious cladogram.

Most electrophoretic studies have used either the distance measure of Nei (1972) or that of Rogers (1972) to construct trees. Hillis (1984) presented a modification of Nei's distance that corrects some of the problems of these measures. One method of testing these three measures is to compare among distances between populations of the same two species. Because there is evidence of monophyly for each of the species of *Pholidobolus* (Fig. 4), estimates of time-since-divergence should be similar for all distances between any populations of two given species. Although Nei's distance (and its modification) and Rogers' distance are scaled differently, the corresponding identities are all on the same scale (0–1), thus permitting comparisons. The ranges of these three identity measures among population-by-population comparisons between species are presented in Table 7. In 8 of the 10 cases, modified Nei's identities have the smallest range, whereas Rogers' identities have the smallest range in the other two cases. However, the differences among the three measures are small; this is a result of the relatively few highly polymorphic loci included in this study, as discussed earlier. As more polymorphic loci are included, the range of modified Nei's distances remains small whereas Nei's distances and Rogers' distances begin to vary widely (see Hillis, 1984). For this reason, modified Nei's distances were used in the distance Wagner analyses.

There are 105 possible unrooted bifurcating trees involving the six *Pholidobolus* clades (the five species of *Pholidobolus*, with

TABLE 6. Distribution of the morphological character states presented in Table 5 among the five species of *Pholidobolus* as reported by Montanucci (1973). Primitive character states are represented by 0s and derived states by 1s. Polymorphism within a species is indicated by 0/1.

Character	*affinis*	*macbrydei*	*prefrontalis*	*montium*	*annectens*
A	0	0	0	0	1
B	0	0/1	0/1	0/1	1
C	0	0/1	0	1	1
D	0	1	1	1	1
E	0/1	0/1	1	1	0/1
F	0	0	0	0	1
G	0	1	1	1	1

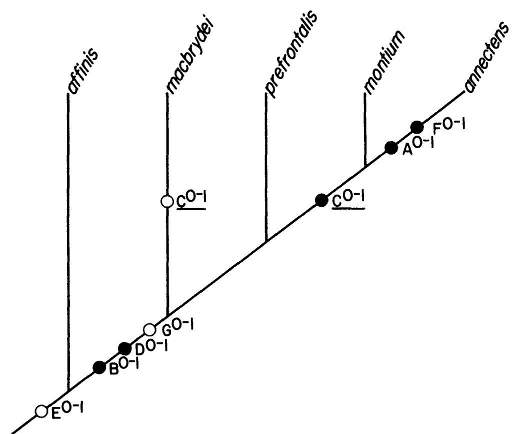

FIG. 5. Phylogenetic tree of Figure 4 with morphological character changes superimposed (see Tables 5 and 6). Open and closed circles have the same meanings as in Figure 4. Underlining indicates homoplasy.

TABLE 7. Ranges of genetic identities among all interpopulational comparisons between species. The first line of each triplet is the range of Nei's identity (Nei, 1972), the second is the range of modified Nei's identity (Hillis, 1984), and the third is the range of Rogers' similarity (Rogers, 1972).

Taxon	*affinis*	*prefrontalis*	*macbrydei*	*montium*
prefrontalis	0.651–0.712 0.645–0.697[a] 0.638–0.699			
macbrydei	0.718–0.850 0.717–0.842[a] 0.703–0.832	0.699–0.765 0.701–0.751[a] 0.676–0.743		
montium	0.470–0.532 0.473–0.532[a] 0.466–0.526	0.555–0.652 0.559–0.652[a] 0.543–0.638	0.525–0.597 0.526–0.600 0.522–0.586[a]	
annectens	0.400–0.446 0.404–0.438[a] 0.397–0.445	0.483–0.496 0.473–0.497 0.481–0.487[a]	0.409–0.435 0.413–0.438[a] 0.401–0.431	0.539–0.580 0.542–0.576[a] 0.534–0.570

[a] Range with smallest span.

Ph. prefrontalis represented by its two vicariant populations). For the *Pholidobolus* data, three of these trees ranked as best by all 13 of the criteria discussed earlier (Fig. 6). Rankings of all trees for Farris' f (1972) and weighted f (1981), percent standard deviation (Fitch and Margoliash, 1967), total tree length (Waterman et al., 1977), Fitch's N_1 (1981), and S_0 (Tateno et al., 1982) are shown in Figure 7. The three best trees differ only in the placement of the two populations of *Ph. prefrontalis*, whether as monophyletic (6A) or paraphyletic (6B and 6C). The tree that places the two populations of *Ph. prefrontalis* as closest relatives after rooting (as supported by the cladistic analysis) is ranked as the best tree by the majority of the criteria, although one of the other two trees is ranked as best by several criteria (Table 8). The branch connecting the two populations of *Ph. prefrontalis* is the only branch on the cladogram that is supported by only one synapomorphy (Fig. 4). The unrooted tree of Figure 6A is the same topology as the ingroup unrooted cladogram (Fig. 4).

Although the above procedures result in the same unrooted ingroup tree topology as the cladogram using many of the goodness-of-fit criteria, rooting procedures fail in the absence of an outgroup. Without an outgroup, the midpoint rooting procedure (a return to the equal rates of change assumption) roots the tree to create a topology equivalent to the UPGMA phenogram (Fig. 8); this destroys all phylogenetic information except for the *Ph. montium–Ph. annectens* pair, again as a result of the failure of the molecular clock hypothesis for

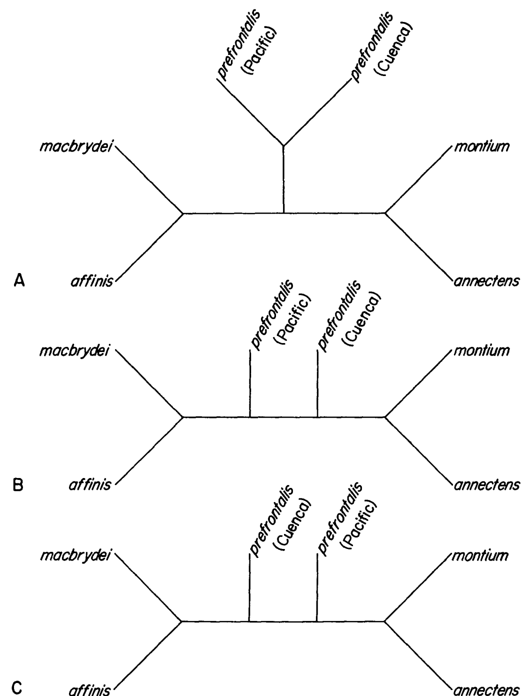

FIG. 6. Topologies of the three unrooted distance Wagner trees that were ranked as best by the 13 criteria discussed in the text.

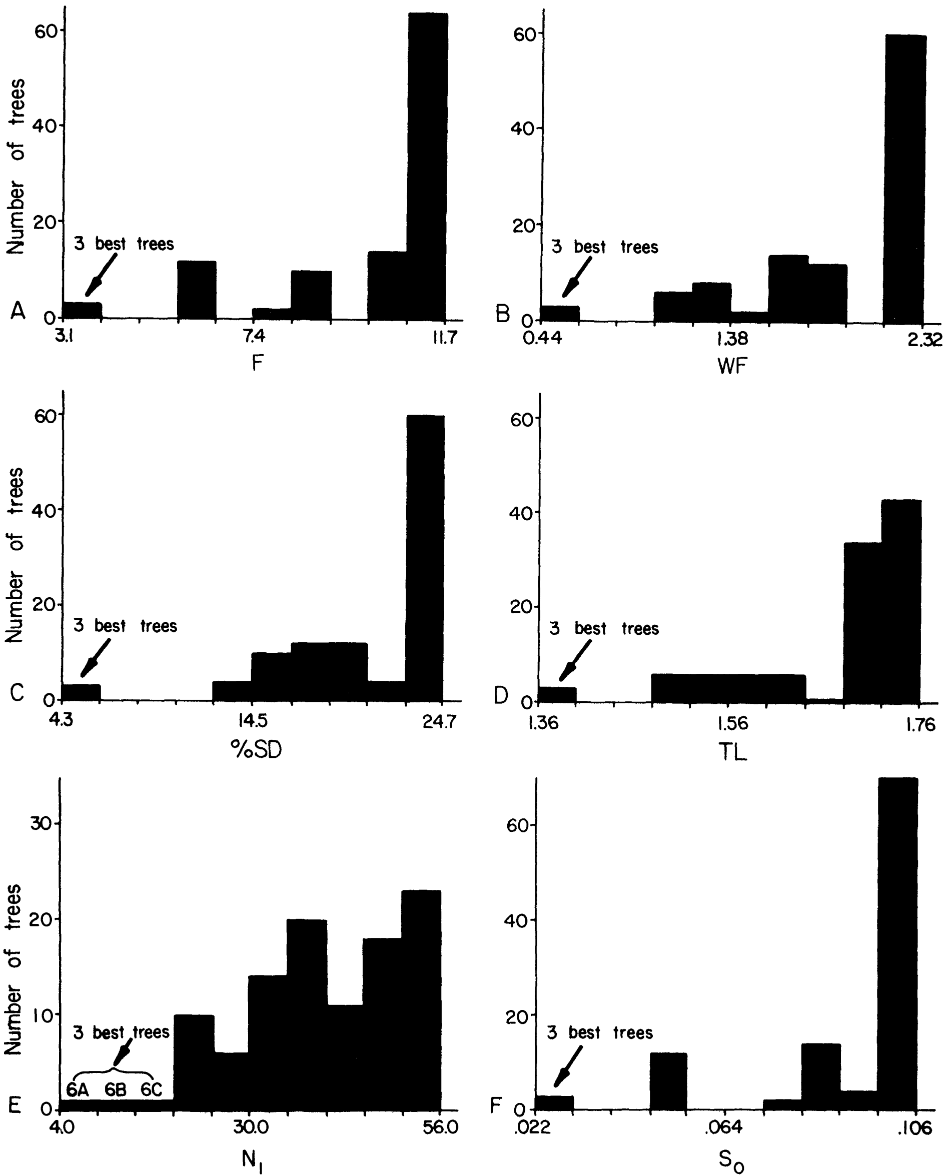

FIG. 7. Rankings of all possible unrooted distance Wagner trees of *Pholidobolus* for six criteria: (A) *f*-statistic (Farris, 1972); (B) weighted *f*-statistic (Farris, 1981); (C) percent standard deviation (Fitch and Margoliash, 1967); (D) total length (Waterman et al., 1977); (E) Fitch neighborliness statistic (Fitch, 1981); (F) S_0 value (Tateno et al., 1982). No negative branch lengths permitted in any of these cases. The three best trees in each case are those shown in Figure 6.

these electrophoretic data. However, if the outgroup is included in the distance Wagner analysis, the topology of the cladogram is reconstructed (Fig. 9).

The above example demonstrates that, at least for the *Pholidobolus* data, the distance procedures provide a good approximation of the most parsimonious cladogram, but only when outgroup rooting is used. Without using outgroup rooting or

TABLE 8. Rankings and scores (in parentheses) of 13 goodness-of-fit statistics for the three tree topologies shown in Figure 6.

Criterion[a]	6A	6B	6C
SD	2 (4.31)	3 (4.96)	1 (3.49)
SD:NN	1 (4.31)	2 (4.96)	3 (5.01)
F	3 (3.23)	2 (3.06)	1 (2.63)
F:NN	2 (3.23)	1 (3.06)	3 (3.36)
F:C	2 (3.33)	1 (3.27)	3 (3.92)
WF	2 (0.44)	3 (0.48)	1 (0.36)
WF:NN	1 (0.44)	2 (0.48)	2 (0.48)
WF:C	1 (0.53)	2 (0.70)	3 (0.78)
TL	1 (1.37)	2 (1.38)	3 (1.39)
N_1	1 (4)	2 (12)	3 (16)
N_2	1 (4)	2 (12)	3 (16)
S_0	2 (0.022)	3 (0.023)	1 (0.019)
S_0:NN	1 (0.022)	2 (0.023)	3 (0.024)

[a] Abbreviations are as follows: SD, percent standard deviation (Fitch and Margoliash, 1967); SD:NN, percent standard deviation, no negative branch lengths allowed; F, *f*-statistic (Farris, 1972); F:NN, *f*-statistic, no negative branch lengths allowed; F:C, *f*-statistic, path lengths constrained so as to equal or exceed observed distances; WF, WF:NN, and WF:C, as for the *f*-statistic, but weighted $(1 - p/d)$ as described by Farris (1981); TL, total length (Waterman et al., 1977); N_1 and N_2, two methods of calculating neighborliness, as described by Fitch (1981); S_0, goodness-of-fit statistic of Teteno et al. (1982); S_0:NN, S_0, with no negative branch lengths allowed.

additional information, it becomes necessary to return to the assumption (equal rates of change) that these methods are supposed to avoid. This study emphasizes the caution with which distance trees constructed without the aid of an outgroup should be interpreted. If no outgroup is available for biochemical analysis, then often other data sets (usually morphological) may be helpful in rooting the trees. In the case of *Pholidobolus,* had an outgroup not been available for study, then the evidence provided by Montanucci (1973) that *Ph. affinis* is the sister species to the rest of the genus (Fig. 5) could have been used to root the undirected distance Wagner trees correctly.

It appears that the ability of these distance methods to approximate a cladogram is largely dependent on the degree of support of each of the branches. For instance, the only branch not always resolved by the distance procedures was the branch that unites the two populations of *Ph. prefrontalis* (Fig. 6), which is the only branch that is supported by just one synapomorphy (Fig. 4). The danger also exists of overresolving a tree with the distance

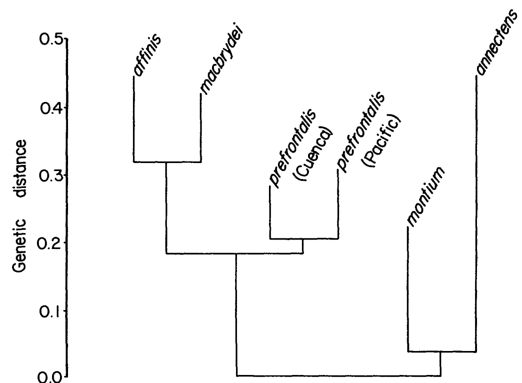

FIG. 8. Distance Wagner tree with midpoint rooting, optimized for minimum tree length. This is not the hypothesized phylogeny.

methods as a dichotomous solution is always reached, whether or not synapomorphies exist to justify the solution. The electrophoretic data in this example provide no phylogenetic justification for the resolution of the phylogeny of the three species of *Proctoporus* (Fig. 4), although a resolution is given by the distance methods.

Biogeography.—It is surprising that the two most closely related species of *Pholidobolus* (*Ph. montium* and *Ph. annectens*) are geographically the most distantly separated (Fig. 1). It is probable that changes in the distributions of the species of *Pholidobolus* since their speciation have resulted in this unusual pattern. That rapid change in distributions of *Pholidobolus* is possible

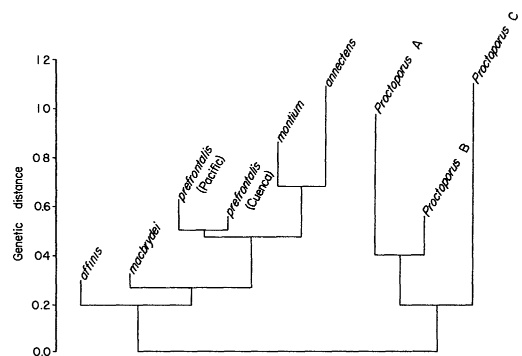

FIG. 9. Distance Wagner tree with outgroup rooting, optimized for minimum tree length.

in the case of rapid environmental change is evidenced by the change in the distributions of *Ph. affinis* and *Ph. montium* over just a decade (Hillis and Simmons, 1985). Rapid changes brought about by irrigation have allowed *Ph. montium* to quickly increase its distribution into areas formerly occupied by *Ph. affinis*. The ancestral ranges of *Ph. affinis, Ph. macbrydei,* and *Ph. prefrontalis* may have been more limited during a past period of environmental fluctuation, and the ancestral *Ph. montium–Ph. annectens* clade more or less continuous through some part of the Ecuadorian Andes. Subsequent expansion of the ranges of the former three species would then have isolated *Ph. montium* from *Ph. annectens,* assuming that the parapatric distributions of the species must be maintained. Again, there is evidence in support of this assumption, as the range of *Ph. affinis* contracted as the range of *Ph. montium* expanded in the case reported by Hillis and Simmons (1985), so that a parapatric front was maintained. This raises the possibility that competing species, rather than geographic barriers, may serve to isolate conspecific populations resulting in their eventual speciation. The separation of *Ph. prefrontalis* populations by the distribution of *Ph. macbrydei* (Fig. 1) may have initiated the apparent ongoing differentiation of the two populations of the former species (Fig. 4).

Only the more distantly related species co-occur at points of sympatry. Two of the three areas of sympatry involve *Ph. affinis,* the sister taxon to the rest of the species (Fig. 4). The third case is not actually an area of syntopy; the two species involved were not collected at the same locality, but merely in close proximity. This case involves *Ph. macbrydei* and *Ph. prefrontalis,* again relatively distantly related species.

Whatever the explanation for the current distributions of *Pholidobolus,* it is clear that if the hypothesized phylogeny of the genus (Fig. 4) is correct, then the distributions of the species must have changed considerably over time. Studies of speciation mechanisms often have centered on groups of parapatrically distributed organisms such as *Pholidobolus* (for example, see the studies cited by White, 1978). Usually the assumption is made that adjacent species in a parapatric complex of species are each other's closest relative, and mechanisms of speciation are examined at such junctures. Although this is probably often the case, the phylogeny of *Pholidobolus* demonstrates the possible fallacy of such a priori assumptions, and emphasizes the necessity of phylogeny reconstruction as the first step in any investigation of speciation mechanisms.

ACKNOWLEDGMENTS

William E. Duellman, John E. Simmons, and Patricia A. Burrowes aided in the collection of specimens for this study and tolerated my company in the field. Steve Reilly assisted in some of the laboratory work. David L. Swofford was of considerable help in making his computer programs available for my use. William E. Duellman, Darrel Frost, John S. Frost, Arnold G. Kluge, Carl S. Lieb, Richard L. Mayden, Richard Montanucci, Jack W. Sites, Jr., Alan R. Templeton, David B. Wake, and E. O. Wiley have read the manuscript and have offered many useful comments. This study was supported by National Science Foundation grants BSR 83-07002 and DEB 82-19388, by the Center for Biomedical Research at The University of Kansas, and by a National Science Foundation fellowship. Finally, I thank Sergio Figueroa of the Programa Nacional Forestal, Ministerio de Agricultura y Ganadería of Ecuador for providing the necessary permits to collect the specimens for this study, and Eugenia del Pino and the Cristóbal Galarza family for assistance and hospitality in Ecuador.

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Received 19 September 1984; accepted 8 November 1984.

APPENDIX

Localities and Museum Numbers of Specimens Examined Electrophoretically (Sample Numbers Correspond to Fig. 2)

1. *Proctoporus* sp. (A). Ecuador: Napo: 12 km E Santa Barbara; 2,550 m. KU 196393. **2.** *Pholidobolus montium* (population 1). Ecuador: Imbabura: Lago Cuicocha; 3,070 m. KU 196344. **3.** *Proctoporus unicolor* (B). Ecuador: Imbabura: Lago Cuicocha; 3,070 m. KU 196391–92. **4.** *Pholidobolus montium* (population 2). Ecuador: Pichincha: Quito, Parque La Carolina. KU 196341–43. **5.** *Pholidobolus montium* (population 3). Ecuador: Cotopaxi: 3.5 km W Mulaló; 2,730 m. KU 196361–65. **6.** *Pholidobolus montium* (population 4). Ecuador: Cotopaxi: 2.5 km W Mulaló; 2,730 m. KU 196345–50; four additional specimens in the collection of Universidad Central de Ecuador (WED 53806–09). **7.** *Pholidobolus montium* (population 5). Ecuador: Cotopaxi: 7 km N Latacunga; 2,800 m. KU 196351. **8.** *Pholidobolus affinis* (population 1). Ecuador: Cotopaxi: 3.3 km S San Miguel de Salcedo; 2,450 m. KU 196315–16. **9.** *Proctoporus hypostictus* (C). Ecuador: Cotopaxi: Pilaló; 2,320 m. KU 196386–88. **10.** *Pholidobolus affinis* (population 2). Ecuador: Tungurahua: 1.1 km SW Pelileo; 2,520 m. KU 196320. **11.** *Pholidobolus affinis* (population 3). Ecuador: Chimborazo: 2.6 km S Puela; 2,420 m. KU 196319. **12.** *Pholidobolus affinis* (population 4). Ecuador: Chimborazo: 6.7 km E Riobamba; 2,550 m. KU 196317–18. **13.** *Pholidobolus prefrontalis* (population 1). Ecuador: Bolivar: 8.3 km S Guaranda; 2,390 m. KU 196377–85. **14.** *Pholidobolus macbrydei* (population 1). Ecuador: Azuay: 42.8 km NW Cuenca; 3,820 m. KU 196338–40. **15.** *Pholidobolus macbrydei* (population 2). Ecuador: Azuay: Laguna de Zurucuchu; 3,200 m. KU 196322–25. **16.** *Pholidobolus prefrontalis* (population 2). Ecuador: Azuay: Cuenca; 2,600 m. KU 196376. **17.** *Pholidobolus macbrydei* (population 3). Ecuador: Azuay: 17.6 km SE Gualaceo; 3,150 m. KU 196326–32. **18.** *Pholidobolus annectens*. Loja: 8.5 km S Loja; 2,300 m. KU 196321.