INTEGRATING PHYLOGENETICS AND ENVIRONMENTAL NICHE MODELS TO EXPLORE SPECIATION MECHANISMS IN DENDROBATID FROGS

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Abstract.—We developed an approach that combines distribution data, environmental geographic information system layers, environmental niche models, and phylogenetic information to investigate speciation processes. We used Ecuadorian frogs of the family Dendrobatidae to illustrate our methodology. For dendrobatids there are several cases for which there is significant environmental divergence for allopatric and parapatric lineages. The consistent pattern that many related taxa or nodes exist in distinct environmental space reinforces Lynch and Duellman's hypothesis that differential selection likely played an important role in species differentiation of frogs in the Andes. There is also some evidence that the Río Esmeraldas basin is a geographic barrier to species distributed in low to middle elevations on the western side of the Andes. Another useful aspect of this approach is that it can point to common environmental parameters that correlate with speciation. For dendrobatids, sister clades generally segregate along temperature/elevational and/or seasonality axes. The joint analysis of environmental and geographic data for this group of dendrobatid frogs has identified potentially important speciation mechanisms and specific sister lineages that warrant intensive study to test hypotheses generated in this investigation. Further, the method outlined in this paper will be increasingly useful as knowledge of distribution and phylogeny of tropical species increases.

Key words.—Dendrobatidae, Ecuador, geographic information systems, modes of speciation, niche modeling, phylogeography.

Research integrating phylogenetic hypotheses with geographic (Lynch 1989; Chesser and Zink 1994; Barraclough et al. 1998; Schneider and Moritz 1998; Schneider et al. 1999) and ecological (Anderson et al. 2002a,b; Johnson and Cicero 2002) data has revealed new insights on the factors that influence the evolution and distribution of species. For example, methods have been developed that combine species-level phylogenies with geographic range data to explore alternative geographic modes of speciation (Lynch 1989; Barraclough et al. 1998; Barraclough and Vogler 2000; Mattern and McLennan 2000; but see Losos and Glor 2003). Likewise, the combination of phylogenetic hypotheses and climate-based modeling of species' ranges has been used to infer historical distributions (Hugall et al. 2002) and whether speciation is correlated with ecological shifts (Peterson et al. 1999).

Geographic range maps, environmental information associated with a given species' range (an estimate of the environmental niche), and niche-based predictive distribution models provide different and complementary information on the distribution of a given species. Geographic range maps are generally created by hand using locality data (usually from museum specimens) and expert opinion to portray the geographic range limits of a species. A species' environmental niche is all the environmental conditions and resources that are necessary for an organism to maintain a viable population (MacArthur 1972). The environmental niche can be estimated by combining known localities of a given spe-

cies with spatial surfaces of meaningful niche dimensions to determine suitable environmental parameters for that species. Species distribution models map this information spatially to predict the geographic distribution of a species in relation to these niche dimensions (Austin 1985; Peterson 2001). Although significant niche dimensions may be omitted, we believe that species distribution models are a useful starting point to study factors potentially influencing species' distributions. Assuming adequate sampling, model overprediction indicates that suitable habitat (as measured by a suite of environmental characteristics) exists in areas where a given species is not present. Therefore, other factors, such as history, dispersal barriers, extinction, or competition may have limited the observed range of a given species (Anderson et al. 2002b).

Range maps have been used in combination with phylogenetic hypotheses to infer geographic modes of speciation (Lynch 1989; Chesser and Zink 1994; Barraclough and Vogler 2000). In general, if the ranges of sister taxa consistently do not overlap, the mode of speciation is hypothesized to be allopatric, whereas if sister species are primarily syntopic, sympatric speciation is inferred to be prevalent. Patterns of geographic ranges have been examined for sister species and at deeper nodes in the phylogeny (Barraclough and Vogler 2000; Johnson and Cicero 2002). To date, most attempts to infer speciation modes have compared geographic ranges in relation to phylogenies without considering the environmental space occupied. Inclusion of an environmental axis in these analyses can permit us to assess whether ecologically mediated divergent selection is consistently associated with speciation. If allopatric sister species consistently segregate

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in environmental space, then ecologically mediated selection may have a role in speciation. Alternatively, if allopatric sister species are consistently in identical or nearly identical environmental space, then ecological divergence (in relation to the parameters examined) is not a major factor, suggesting alternatives such as incidental divergence in isolation (Peterson et al. 1999).

When environmental information is combined with current geographic ranges of species, six general scenarios are of interest (Fig. 1). These six categories represent segments along a continuum of variation but serve as a useful heuristic. Cases A–C suggest ecologically mediated divergent selection associated with speciation occurring in sympatry (case A), parapatry (case B), or allopatry (case C). Where there is no divergence in environmental parameters between sister taxa, either the crucial ecological dimension was not included among the variables measured (especially case D) or speciation has occurred in the absence of ecological divergence (plausible for cases E and F). Given the possibility of shifts in geographic or environmental range after speciation, this correlational approach is more compelling when applied to a large number of species (e.g., Lynch 1989; Peterson et al. 1999; but see Losos and Glor 2003). Alternatively, it can be used as an exploratory analysis to focus subsequent analyses of distribution, ecology, and phenotype for specific species pairs or clades.

In tropical systems, numerous mechanisms of speciation have been proposed to explain high species richness (Moritz et al. 2000), and we expect that the approach described here can be used to explore general trends and to target more intensive study of particular systems. The Andes represent a topographically and environmentally complex tropical system with extraordinarily high species richness, especially for anurans (Ron and Merino 2000). Lynch and Duellman (1997) put forth a speciation model for Andean anurans based on vicariance events resulting from isolation associated with the Andes uplift and ecologically driven climatic depression of vegetation zones (also see Lynch 1986). They suggest that speciation was caused by a combination of isolation and divergent selection in relation to differences in habitats occupied. This model predicts that sister taxa should occupy distinct geographic and environmental space (Fig. 1C).

The Ecuadorian frogs of the family Dendrobatidae represent a promising group for a case study of these methods for several reasons. First, Santos et al. (2003) recently developed a well-sampled phylogeny of the Dendrobatidae. Second, there have been recent and extensive reviews of the systematics of members of this family (Coloma 1995; Clough and Summers 2000) as well as of other Ecuadorian frogs, such as *Eleutherodactylus* (Lynch and Duellman 1980; Lynch and Duellman 1997) and *Gastrotheca* (Duellman and Hillis 1987). These studies generally show that sister species have allopatric or parapatric distributions and replace each other either altitudinally or latitudinally. For allopatric distributions, it is not clear to what degree the environmental spaces associated with sister taxa are distinct.

Here, we develop a methodological framework to examine simultaneously geographic ranges of species and their environmental envelopes in a phylogenetic context as a way to explore factors that may have influenced speciation. We then use selected Ecuadorian clades of the family Dendrobatidae to illustrate the approach. Although inferences from this case study are limited by sparse distribution records and a small number of clearly identified sister taxa, as is common in tropical radiations, the analyses do illustrate the potential of this approach for exploring the role of environmental niche shift in speciation.

MATERIALS AND METHODS

Environmental Layers

To build high-resolution (400-m) climate layers from existing weather records, we used a thin plate smoothing spline (ANUSPLIN; Hutchinson 1999) to interpolate monthly climate surfaces for precipitation and temperature. Thin plate smoothing splines are a generalization of standard multivariate linear regression, in which the parametric model is replaced by a suitable smooth nonparametric function (Hutchinson 1995). We included three independent variables: longitude, latitude, and elevation. Elevation was incorporated because temperature, and often rainfall, depends on elevation and inclusion of elevation in the model is known to reduce statistical error (Hutchinson 1995). We used the 400-m digital elevation model produced by Sierra et al. (1999). We used only weather stations that had at least nine years of data. This resulted in 264 rainfall points spread across Ecuador. The standard predictive errors for precipitation surfaces ranged from 11.3% to 16.5%, which are similar to errors in other topographically complex areas (e.g. Faith et al. 2001). The number of weather stations sampled for temperature was smaller than rainfall, but the strong dependence of temperature on elevation enabled us to create accurate surfaces. We used data from 163 climate stations to create maximum and minimum monthly temperature surfaces. The standard predictive errors varied from 2.6% to 3.1% and 2.8% to 3.3% for maximum and minimum temperatures, respectively. Small errors such as these are typical for temperature surfaces (Hutchinson 1995; Faith et al. 2001). We used ANUCLIM (ver. 5.1; Houlder et al. 2001) to create 16 bioclimatic parameters that are biologically meaningful combinations of monthly climate variables (Nix 1991). We also resampled (from 200-m to 400-m resolution) a water budget (number of months for which potential evapotranspiration surpasses precipitation) layer created by Sierra et al. (1999).

Selection of Species

A phylogenetic tree for dendrobatid frogs was obtained from Santos (2002) and Santos et al. (2003). Of the 47 species in Ecuador, 53% are included in this tree (Santos et al. 2003). Recent investigation indicates that inclusion of an additional population of *Epipedobates tricolor* in the phylogenetic analyses provides different results than those from previous systematic reviews of the *E. tricolor* clade, therefore we present a new phylogenetic hypothesis for this clade (see Results). We used sequences from *Colostethus machalilla* (AY364551; Ecuador: Manabi, Ayampe River), *Epipedobates* sp. f (AY364575; Ecuador: Pichincha, Mindo), *E. anthonyi* (AY364576; Ecuador: El Oro, El Progreso-Pasaje) and *E. tricolor* (AY395961; Ecuador: Bolivar, Moraspungo). We
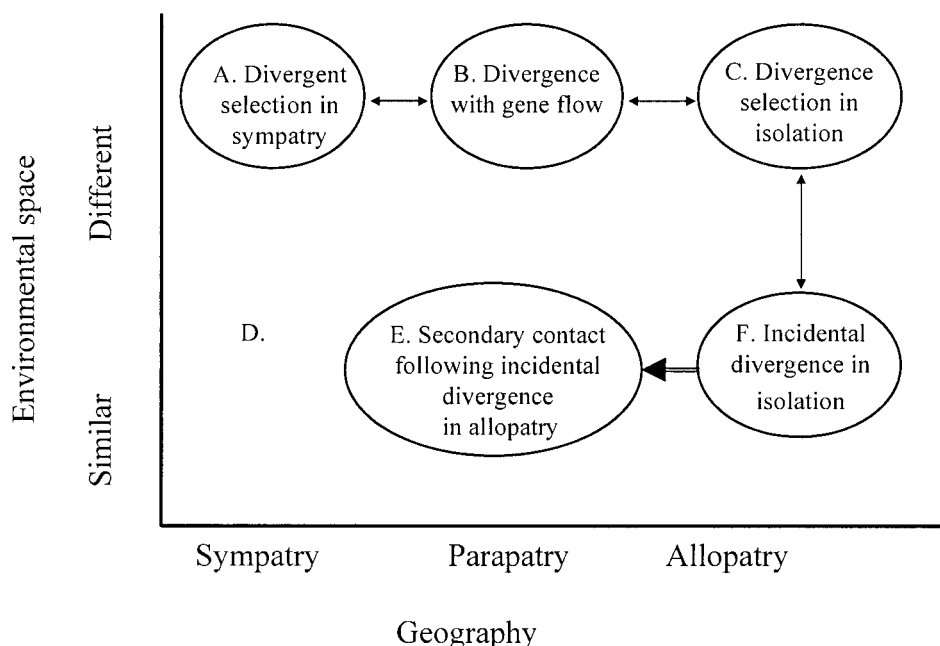
FIG. 1. Speciation mechanisms predicted from combined information on geographic and environmental space use by sister lineages.

used three outgroups (*C. fugax* [AY364547; Ecuador: Morona Santiago, Santiago], *E. hahneli* [AY364573; Ecuador: Francisco de Orellana, Estación Científica Yasuní Universidad Católica], and *E. parvulus* [AY364574; Ecuador: Morona Santiago, Mendez]). The outgroups are members of the *E. parvulus* clade because this group was shown to be the sister clade of the *E. tricolor* clade (Santos et al. 2003). The sequences matrix used for this analysis included 2453 characters from the mitochondrial genes 12S rDNA, tRNA-valine, and almost the entire 16S rDNA. We aligned the sequences initially using ClustalX (Thompson et al. 1997) and adjusted them by hand. We excluded from the analysis only 14 characters from the sequence corresponding to the 16S rDNA gene. We analyzed this configuration under parsimony by using PAUP* (Swofford 2002) by an exhaustive search. The GRT + G + I model of sequence evolution was selected as the best fitting model by a hierarchical test of models and was used to estimate the sequence parameters (Posada and Crandall 1998). Pairwise distances between members of *E. tricolor* clade and outgroups were determined by estimating sequence divergence with the uncorrected p-distance. Of the total 2.4 kb of sequence analyzed, 1958 characters were constant, 208 were parsimony-uninformative, and 287 were parsimony informative. Three most-parsimonious trees were found with a length of 646, a consistency index of 0.9, and a rescaled consistency index of 0.77. One of these trees was also the maximum likelihood tree and is used in the current analyses (Fig. 2).

From these phylogenies, we selected a subset of clades based on four criteria. First, we selected groups within which we were confident that taxonomic sampling was complete. However, in such a diverse fauna the possibility of missing species (either extinct or nonsampled) cannot be ruled out. One selected clade includes three species of *Epipedobates* and one species of *Colostethus* showing that *Epipedobates* is

paraphyletic with respect to *Colostethus*. Such paraphyly is pervasive in several clades within Dendrobatidae (Santos et al. 2003). Second, our fine-scale environmental data were limited to Ecuador (the finest global resolution dataset is 10 km²; New et al. 1999), so we only considered clades including species with most or all of their distribution ranges in Ecuador. The only species not restricted to Ecuador in our analyses are *E. boulengeri* (distributed marginally in southern Colombia) and *E. anthonyi* (northern Peru). To ensure that localities outside of Ecuador did not provide different information than those within Ecuador, we extracted environmental data associated with point locality data for *E. anthonyi* from Peru (Colombian point localities for *E. boulengeri* were not available from major U.S. collections). We determined that the environmental space extracted from these localities was within that described based on the Ecuadorian localities. Therefore, excluding these non-Ecuadorian localities would not influence our results. Third, to properly quantify environmental space use, we only considered clades containing species with at least 10 (with one exception) known localities. Fourth, we considered only highland species or those west of the Andes because of the poor quality of environmental layers for the lowlands east of the Andes where there are few climate stations.

On the basis of these criteria, we were able to evaluate the following three clades where the numbers in parentheses following species names indicate the number of unique locality records for each species: (1) *Colostethus pulchellus* (31) and *C. vertebralis* (54); (2) *Epipedobates boulengeri* (23), *E.* sp. f (11), *C. machalilla* (20), *E. anthonyi* (23), and *E. tricolor* (6); and (3) *C. infraguttatus* (24), *C. toachi* (19), and *C. awa* (32). Point locality data were gathered from three major herpetological collections: the Natural History Museum at the University of Kansas (KU); the Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ); and Museo de
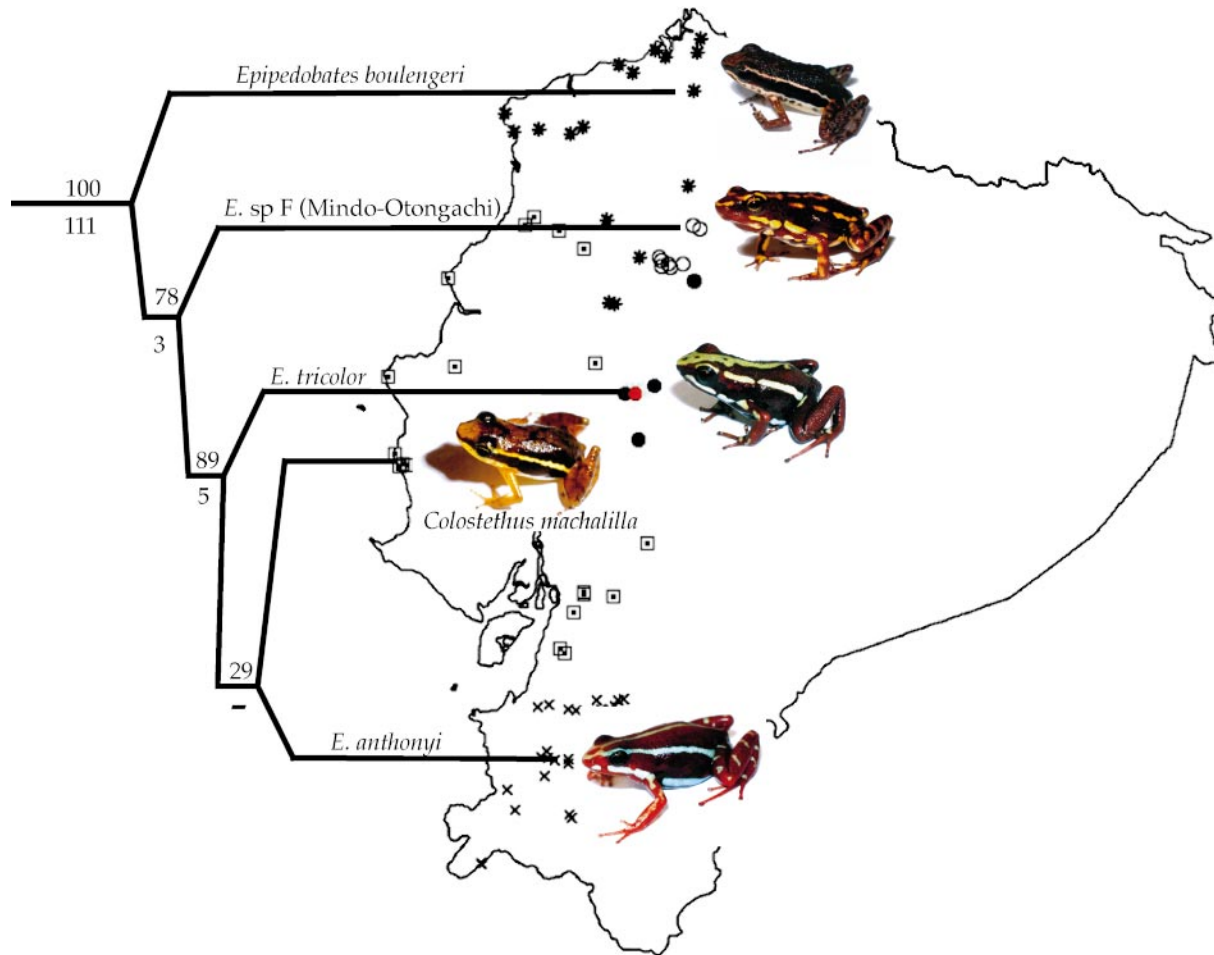
FIG. 2. Phylogenetic hypothesis and geographic distribution for *Epipedobates tricolor* clade. Red point is the type locality of *E. tricolor*.

Historia Natural Gustavo Orcés, Escuela Politécnica del Nacional Ecuador (EPN). We verified the taxonomy of all museum specimens, except those from KU. We also used published records for the genus *Colostethus*, all of which have been verified with respect to correct identification (Coloma 1995). Before modeling, we plotted all the points for a given species to check for outliers in both geographic and environmental space. Outlying points in either geographic or environmental space were identified based on expert opinion and either verified or removed.

Predicted Species Distributional Models

Study taxa

To determine which variables to include in the modeling exercise, we identified redundant environmental layers via correlations. Correlations were run with the environmental values of 1000 randomly selected points from Ecuador. Subsequently, we used the following seven environmental variables in the models: water budget, elevation, mean annual precipitation, coefficient of variation of annual precipitation, precipitation of the driest quarter, mean annual temperature, and coefficient of variation of mean annual temperature.

Various methods exist to create species distribution models

from presence-only data but there have been few rigorous comparisons among methods (but see Elith 2000). We explored two methods to create models: BIOCLIM (Nix 1986) and GARP (Stockwell and Nobel 1992). Each of these methods performs well with presence-only data. Both BIOCLIM and GARP produced similar results such that the qualitative conclusions reached in this paper would be robust regardless of the method employed. We chose the BIOCLIM method because it was the most straightforward to apply to both current distributional models and those inferred for deeper nodes in the phylogeny. The BIOCLIM method extracts the value of the environmental data layer at each point locality for a given species. The model matches the bioclimatic profile with climate estimates at other sites on a grid to determine other locations with similar values, thereby producing a map of the predicted potential distribution of a given species.

Ancestral nodes

To allow analysis of species in clades with unbalanced topology, we compared environmental parameters of the focal species with estimates from adjacent ancestral nodes. Notwithstanding errors associated with estimation of ancestral states (see below), we expected that this approach would

allow more powerful inference than taking the mean range or summing the ranges of multiple sister taxa. We determined the maximum and minimum value for each environmental parameter for each extant taxon and estimated the ancestral value using both the maximum likelihood method proposed by Schluter et al. (1997) and the generalized least squares method of Martins and Hansen (1997). We used branch length information from the maximum likelihood trees in both analyses. These methods yielded very similar results and both provided error estimates associated with predicted ancestral state values. Here we present only the maximum likelihood estimates. Errors associated with estimating ancestral states are generally high and increase with node depth (Schluter et al. 1997; Martins 1999). Further, strong directionality during character evolution may cause bias in character reconstruction (Pagel 1999). We tested for directionality in the five-taxon clade using Pagel's program Continuous and determined that the variables conformed to the standard constant-variance random walk model.

Ancestral values (maximum and minimum for each climate parameter) were used to predict the ancestral environmental envelope. This envelope is analogous to the BIOCLIM model described above. To evaluate the sensitivity of our results to errors in ancestral node reconstruction, we created additional BIOCLIM models using the most conservative error bounds generated by the maximum likelihood analysis, that is, for each environmental parameter, the lower bounds of the minimum estimate and upper bounds of the maximum estimate.

Analysis of Overlap in Environmental Space

We conducted a principal components analysis (PCA) with all of the extant members of each clade to examine the overall level of divergence in environmental space among the extant taxa. We then quantified how pairs of sister taxa overlapped in environmental space using both overlap of the environmental variables at point localities themselves, and overlap of the distribution models, which are a representation of these environmental variables in geographic space (realized potential niche). To assess overlap of environmental variables, we used PCA for each pair of sister taxa or sister lineages. These analyses allowed us to evaluate whether species groups were separating along certain environmental gradients. We also used PCA to evaluate the overlap in environmental space between deeper nodes in the phylogeny and corresponding sister taxa (i.e., the ancestral node of *C. toachi* and *C. awa* compared to *C. infraguttatus*). Since we did not have point locality information from the sister ancestral node, we used the environmental information from 200 points taken randomly from within the predicted ancestral environmental envelope. To determine whether separation in environmental space was statistically significant, we followed each PCA with a multivariate analysis of variance (MANOVA) in which species was the fixed factor and PCA axis scores were dependent variables. We present an overall statistic as well as tests of between-subject effects to determine which PCA axis accounts for significance in the overall test. PCA and MANOVA analyses were done with values from both the best-estimate BIOCLIM models (for both species and ancestors)

and the error BIOCLIM models, which used the most conservative error bounds.

We calculated the overlap of the BIOCLIM models between sister species (ancestral node and terminal) as the area of overlap divided by the total area occupied by both species. We refer to this as total overlap. In cases where overlap was asymmetrical, we also estimated overlap relative to the range of each species (or ancestral node).

RESULTS

For each clade we describe (1) the phylogeny, (2) the geographic distribution, (3) overlap of the distributional models, and (4) overlap in terms of the environmental variables themselves (PCA). The qualitative patterns of difference among extant species and among extant species and ancestors in both PCAs and MANOVAs were robust considering the typically large confidence limits around maximum likelihood estimates of ancestral states.

Clade 1: Colostethus pulchellus and C. vertebralis

Colostethus pulchellus is common on the wet slopes of the Andes in northern Ecuador. *Colostethus vertebralis* is allopatric to *C. pulchellus* and exists in high inter-Andean valleys in south-central Ecuador. There is only 5.6% total overlap in the BIOCLIM models for these species (Fig. 3a). The x-axis of the PCA (45% of the variation explained) depicts a gradient from localities with consistent year-round rainfall to drier, more seasonal localities (Fig. 3b). The y-axis (31% variation explained) represents a gradient associated with elevation; cooler sites all loaded positively on this axis and warmer sites loaded negatively. Statistically significant separation among species occurs along both PCA axes (overall: $F_{2,91} = 124.2$, $P < 0.001$; x-axis: $F_{2,91} = 13.9$, $P < 0.001$; y-axis: $F_{2,91} = 207.3$, $P < 0.001$).

Clade 2: Epipedobates boulengeri, E. sp. f, E. tricolor, Colostethus machalilla and E. anthonyi

The molecular phylogeny identified a clade within which the sister taxa *E. anthonyi* and ''*C.*'' *machalilla*; are sister to *E. tricolor*, the sister taxon to this three species clade is *Epipedobates* sp. f, and *E. boulengeri* is sister to all four of these species (Fig. 2). For the most part, these species are parapatrically distributed and there is both latitudinal and elevational replacement. The five taxa separate relatively evenly in environmental space along both axes (Fig. 4). The PCA x-axis (42.6% variation explained) describes seasonality with variables describing consistent wet climates (i.e., high annual rainfall, dry quarter rainfall, and water budget) loading positively and variables describing seasonality loading negatively (i.e., high coefficient of variation [CV] of rainfall and temperature). The y-axis (36.0% variation described) describes a temperature gradient with annual temperature loading positively and elevation loading negatively. *Epipedobates tricolor* and *E.* sp. f exist in wetter, less seasonal areas and separate from *E. anthonyi* and to a lesser extent *C. machalilla* along the x-axis. *Epipedobates boulengeri* spans the entire seasonality axis. *Epipedobates boulengeri* and *C. machalilla* exist in warmer regions and separate from *E.* sp. f along the
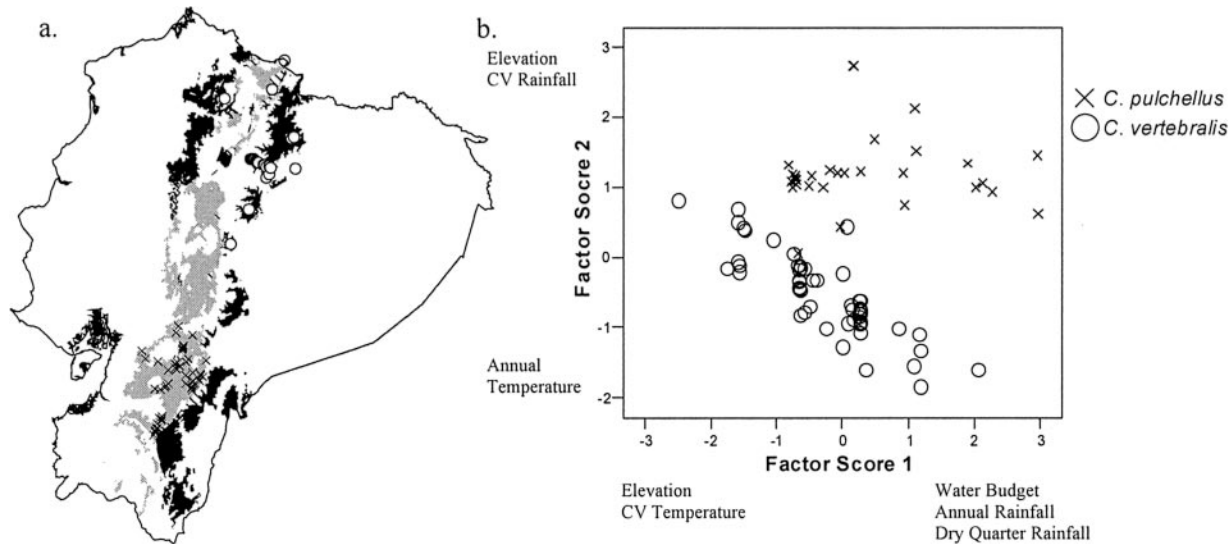
FIG. 3. Environmental analyses for *Colostethus vertebralis* versus *C. pulchellus*; (a) niche model in which black is *C. vertebralis* and gray is *C. pulchellus*; (b) principal components analysis (PCA); variation explained by x-axis = 45% and y-axis = 31%.

y-axis. *Epipedobates anthonyi* and *E. tricolor* span the temperature gradient.

The sister taxa *C. machalilla* and *E. anthonyi* are each relatively widespread in low to middle elevations and their ranges meet at a narrow stretch of lowland, east from Golfo de Guayaquil. However, the BIOCLIM models predict that each of these species could exist on either side of this stretch (Fig. 5a). The total model overlap in these taxa is about 43% and there is little asymmetry in the overlap of BIOCLIM models in these two species (the proportion of the BIOCLIM model of *C. machalilla* included in the model of *E. anthonyi* is 54% and the proportion of the model for *E. anthonyi* included in *C. machalilla* is 69%). The PCA x-axis is dominated by temperature and elevation with some effect of seasonality. Temperature and the coefficients of variation of rainfall and temperature load positively on this axis whereas elevation and the rainfall in the dry quarter load negatively (55% of the variation explained). The y-axis is predominantly a seasonality axis with water budget and total rainfall loading positively and the coefficients of rainfall and temperature loading negatively (30% of the variation explained; Fig. 5b). Overall separation in environmental space was statistically significant between *E. anthonyi* and *C. machalilla* ($F_{2,40}$ = 15.0, $P < 0.001$); however, post-hoc analyses indicated that only the x-axis was significant ($F_{1,41}$ = 30.8, $P < 0.001$; y-axis: $F_{1,41}$ = 0.1, $P > 0.05$).

Epipedobates tricolor has a limited high-elevation distribution, and is parapatric to *C. machalilla* and *E.* sp. f. There is no overlap in the BIOCLIM models between *E. tricolor* and the ancestral node to *C. machalilla* and *E. anthonyi* (Fig. 5c). These lineages also separate in ordination space. The x-axis of the PCA is a temperature axis with sister node predicted to be in lower, warmer areas than *E. tricolor* (38% of the variation explained). The y-axis, which achieves greater separation, is dominated by seasonality with *E. tricolor* in wetter less seasonal areas than the range predicted for its sister node (27% of the variation explained; Fig. 5d). Significant separation occurs along both axes with *E.* sp. f in

higher, wetter and less seasonal habitats and its sister ancestral node in lower and more seasonal habitats (overall: $F_{2,203}$ 91.8, $P < 0.001$; x-axis: $F_{1,204}$ = 93.2, $P < 0.001$; y-axis: $F_{1,204}$ = 35.3, $P < 0.001$).

Epipedobates sp. f exists to the north of *E. tricolor* and again has a relatively restricted distribution at a high elevation. The BIOCLIM model predicted for the ancestral node of *E. anthonyi*, *C. machalilla*, and *E. tricolor* does not overlap with that for *E.* sp. f (Fig. 5e). The PCA for this comparison has the x-axis dominated by elevation and temperature and the y-axis by seasonality (42% and 28% of the variation explained, respectively). Significant separation occurs along both axes with *E.* sp. f in higher, wetter, and less seasonal habitats and its sister ancestral node in lower and more seasonal habitats (Fig. 5f; overall: $F_{2,208}$ = 134.2; $P < 0.001$; x-axis: $F_{1,209}$ = 115.7, $P < 0.001$; y-axis: $F_{1,209}$ = 68.3, $P < 0.001$).

Epipedobates boulengeri extends northwards from the rest of the extant taxa in the clade and is parapatric to *C. machalilla* and *E.* sp. f. The BIOCLIM model for the ancestral node of *E. tricolor*, *C. machalilla*, *E. anthonyi*, and *E.* sp. f has a relatively small area, 59% of which is contained within the western limits of the range of *E. boulengeri* (Fig. 5g). The sister lineages are separated in environmental PCA space along both the x-axis, which is dominated by elevation and temperature, and less so on the y-axis, which is dominated by seasonality (31% and 30% of the variation explained, respectively; Fig. 5h). *Epipedobates boulengeri* in general inhabits lower, warmer areas with lower annual rainfall than its predicted ancestral node. Overall separation was statistically significant ($F_{2,220}$ = 11.7; $P < 0.001$); however, post-hoc tests indicated that only the y-axis, the seasonality axis, was significant (x-axis: $F_{1,221}$ = 0.1; $P > 0.05$; y-axis: $F_{2,210}$ = 23.5; $P < 0.001$).

Clade 3: Colostethus infraguttatus, C. toachi, and C. awa

Colostethus toachi and *C. awa* are sister taxa, with *C. infraguttatus* as their sister (Fig. 6a). In geographic space, *C.*
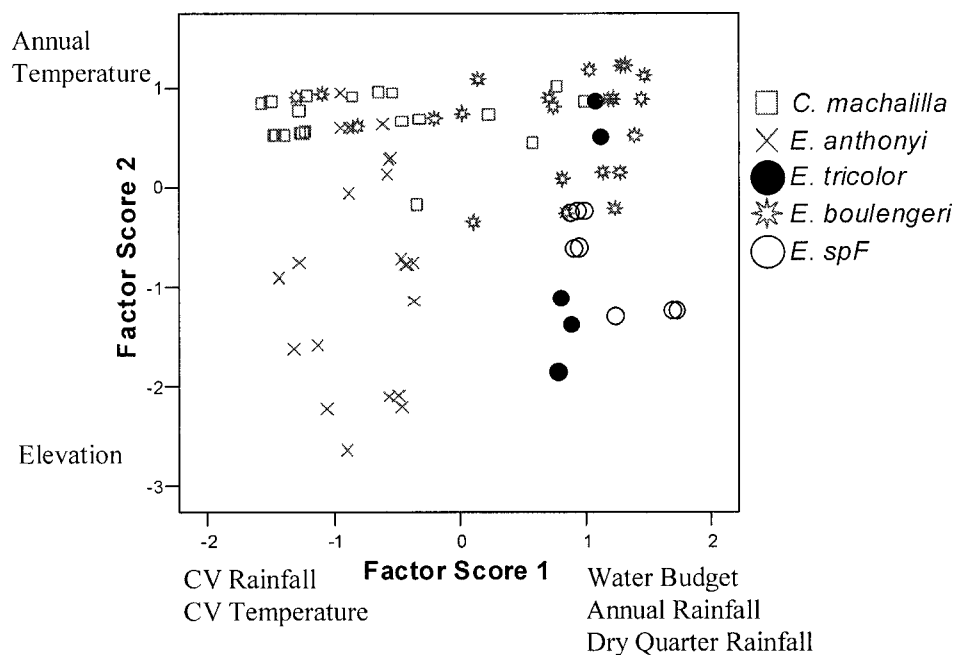
FIG. 4. Principal components analysis for all extant taxa in the *Epipedobates tricolor* clade; variation explained by x-axis = 43% and y-axis = 36%.

toachi and *C. awa* are broadly sympatric, with *C. toachi* contained completely in a portion of *C. awa*'s range. The PCA x-axis for this clade is mostly a seasonality axis with little separation of species (50% of variation explained). The y-axis, which explains 36% of the variation, is a temperature axis with *C. infraguttatus* in low warm regions and *C. toachi* and *C. awa* generally in higher regions (Fig. 6b).

The BIOCLIM models for these species mirror this pattern, wherein the model for *C. awa* predicts 100% of the area predicted by the model of *C. toachi* (Fig. 6c), whereas the model for *C. toachi* only predicts 16% of that for *C. awa*. The PCA x-axis for *C. toachi* and *C. awa* is a temperature/elevation axis with *C. toachi* restricted to higher elevations and cooler areas (46% of the variation explained). The y-axis is based on seasonality with *C. toachi* inhabiting more aseasonal (i.e., low coefficients of variation for temperature and rainfall) Andean slopes and *C. awa* being spread out along the seasonality gradient (33% of the variation explained; Fig. 6d). Separation was statistically significant overall and along each axis (overall $F_{2,42} = 9.7$; $P < 0.001$; x-axis: $F_{1,43} = 11.7$; $P < 0.001$; y-axis: $F_{1,43} = 4.3$; $P < 0.05$).

Colostethus infraguttatus is parapatric to *C. awa*; likewise, the BIOCLIM model for the node ancestral to *C. toachi* and *C. awa* is parapatric to that for *C. infraguttatus* with only 1.7% overlap (Fig. 6e). In the PCA, the x-axis predominantly describes seasonality and the y-axis is based on elevation and temperature (37% and 25% of the variation explained, respectively; Fig. 6f). Separation occurs mostly along the y-axis with the ancestral node mostly in wetter areas that are climatically more stable and *C. infraguttatus* in more seasonal environments. Overall, separation was statistically significant ($F_{2,226} = 125.4$; $P < 0.001$), however, post-hoc tests indicated that only the y-axis was significant (x-axis: $F_{1,43} = 0.4$; $P > 0.05$; y-axis: $F_{1,43} = 250.9$; $P < 0.001$).

In general, there are two combinations of environmental variables that explain most of the variation in the ordination analyses and that are potentially important in differentiating environmental space occupied by sister lineages. These are a temperature/elevation axis representing the gradient from cooler high-elevation areas to warmer low-elevation areas, and a seasonality axis, which spans the range from constant climate (high rainfall and water budget) to seasonal climates (high CV temperature and CV rainfall).

Review of the systematics of Epipedobates tricolor

Given the inconsistencies in the taxonomic treatment of *E. tricolor*, we briefly review its systematics. *Epipedobates tricolor* (Boulenger, 1899) was described from specimens collected at El Porvenir (Provincia de Bolívar, Ecuador; Fig. 2) and six localities are known from the western Andean slopes in central Ecuador (Provincia de Bolívar). *Epipedobates anthonyi* (Noble 1921) was described from specimens collected at Salvia (Provincia de El Oro, southwestern Ecuador) and 23 localities are known from the lowlands and western Andean slopes in southern Ecuador (Provincias de El Oro Azuay and Loja) and northern Peru (Departamentos Piura and Túmbes). The smallest distance between these disjunct regions is approximately 200 km. The external morphology of both taxa is similar (Fig. 2). Adults of most populations have aposematic coloration and are characterized by the presence of mid-dorsal and complete oblique-lateral stripes. Duellman and Wild (1993) considered *E. anthonyi* to be a junior synonym of *E. tricolor* based on comparisons made by Silverstone (1976) and analysis of specimens from southwestern Ecuador. Based on morphological characters, Schulte (1999) recognized both *E. tricolor* and *E. anthonyi* within this same region. The phylogeny of Clough and Summers (2000) in-
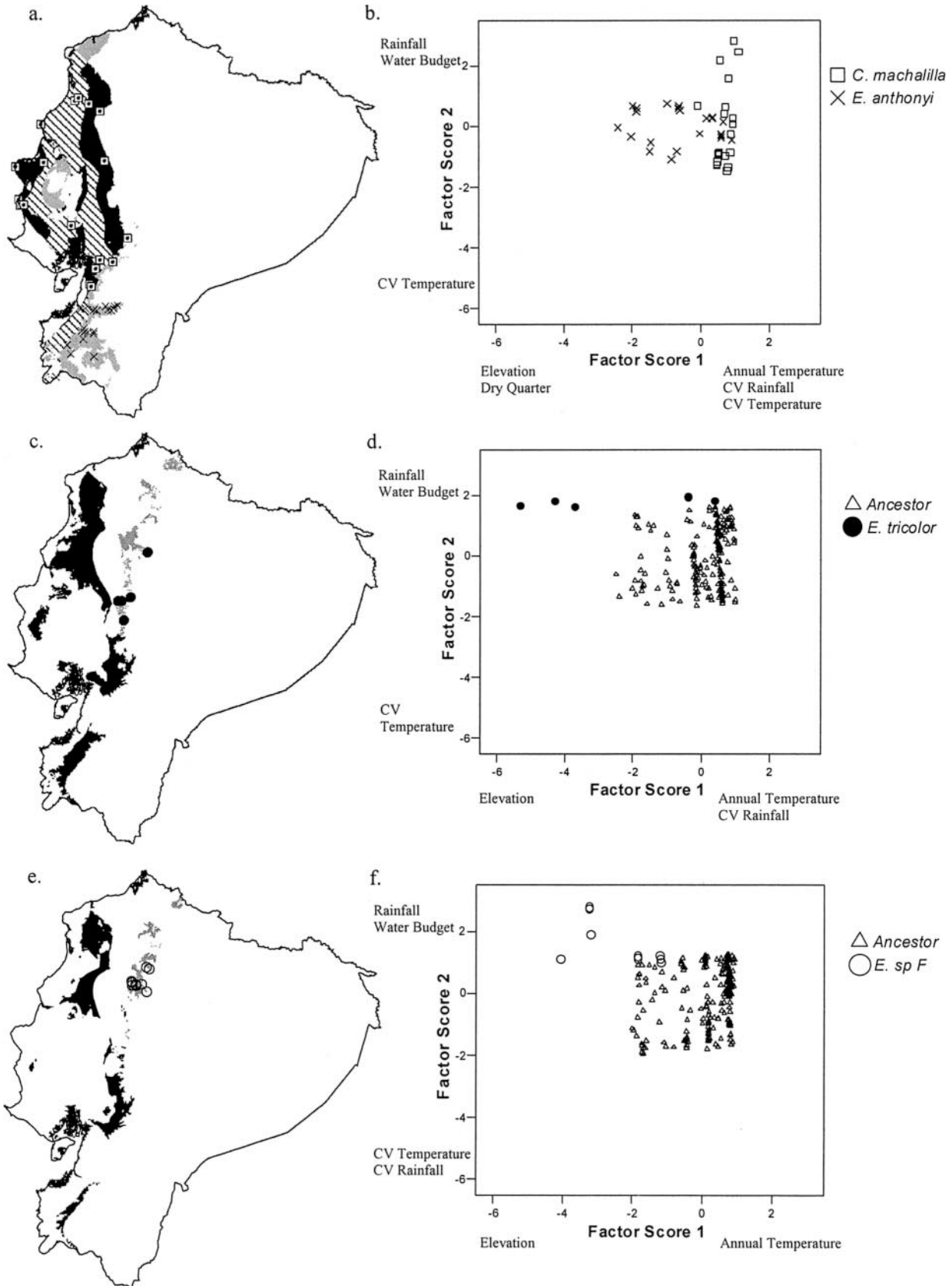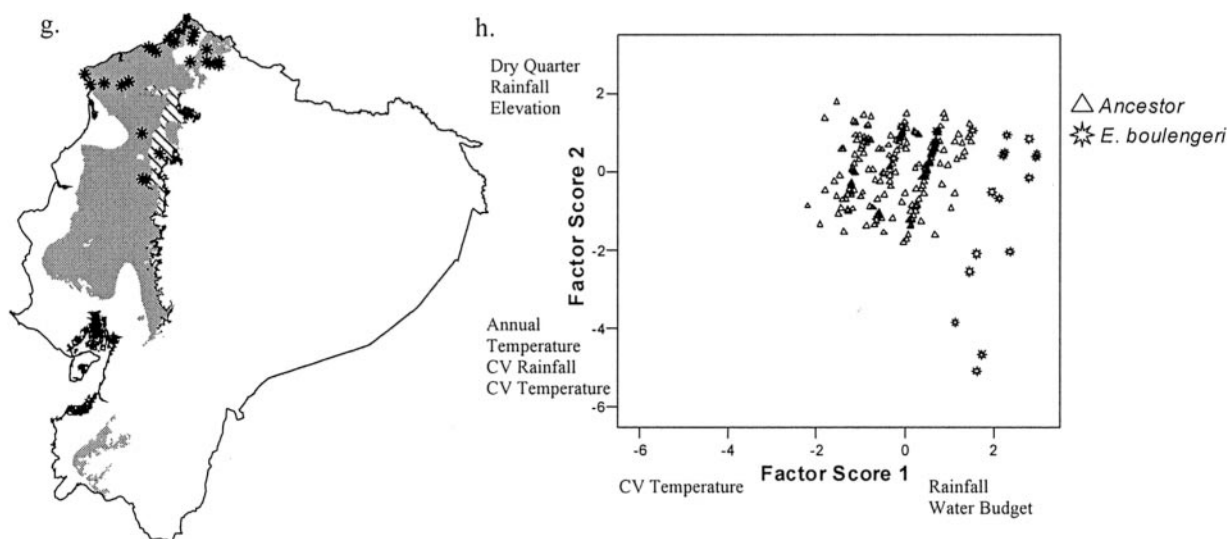
FIG. 5. Continued. Environmental analyses for the clade that includes *Epipedobates boulengeri*, *E.* sp. f, *E. tricolor*, *Colostethus machalilla*, and *E. anthonyi*; (a) niche model for *C. machalilla* versus *E. anthonyi*, where black is *C. machalilla*, gray is *E. anthonyi*, and diagonal shading is the overlap region (diagonal shading indicates overlap throughout figure); (b) principal components analysis (PCA) for *E. anthonyi* versus *C. machalilla*; variation explained by x-axis = 55% and y-axis = 30%; (c) niche model *E. tricolor* and the ancestral node to *E. anthonyi* and *C. machalilla*, where gray is *E. tricolor* and black is the ancestor; (d) PCA for *E. tricolor* and its ancestral node; variation explained by x-axis = 38% and y-axis = 27%; (e) environmental niche model for *E.* sp. f and the ancestral node to *E. anthonyi*, *C. machalilla*, and *E. tricolor*, where gray is *E.* sp. f and black is the ancestor; (f) PCA for *E.* sp. f and its ancestral node; variation explained by x-axis = 42% and y-axis = 28%; (g) environmental niche model *E. boulengeri* and the ancestral node to *E. anthonyi*, *C. machalilla*, *E. tricolor*, and *E.* sp. f, where gray is *E. boulengeri* and black is the ancestor; and (h) PCA for *E. boulengeri* and its ancestral node; variation explained by x-axis = 31% and y-axis = 30%.
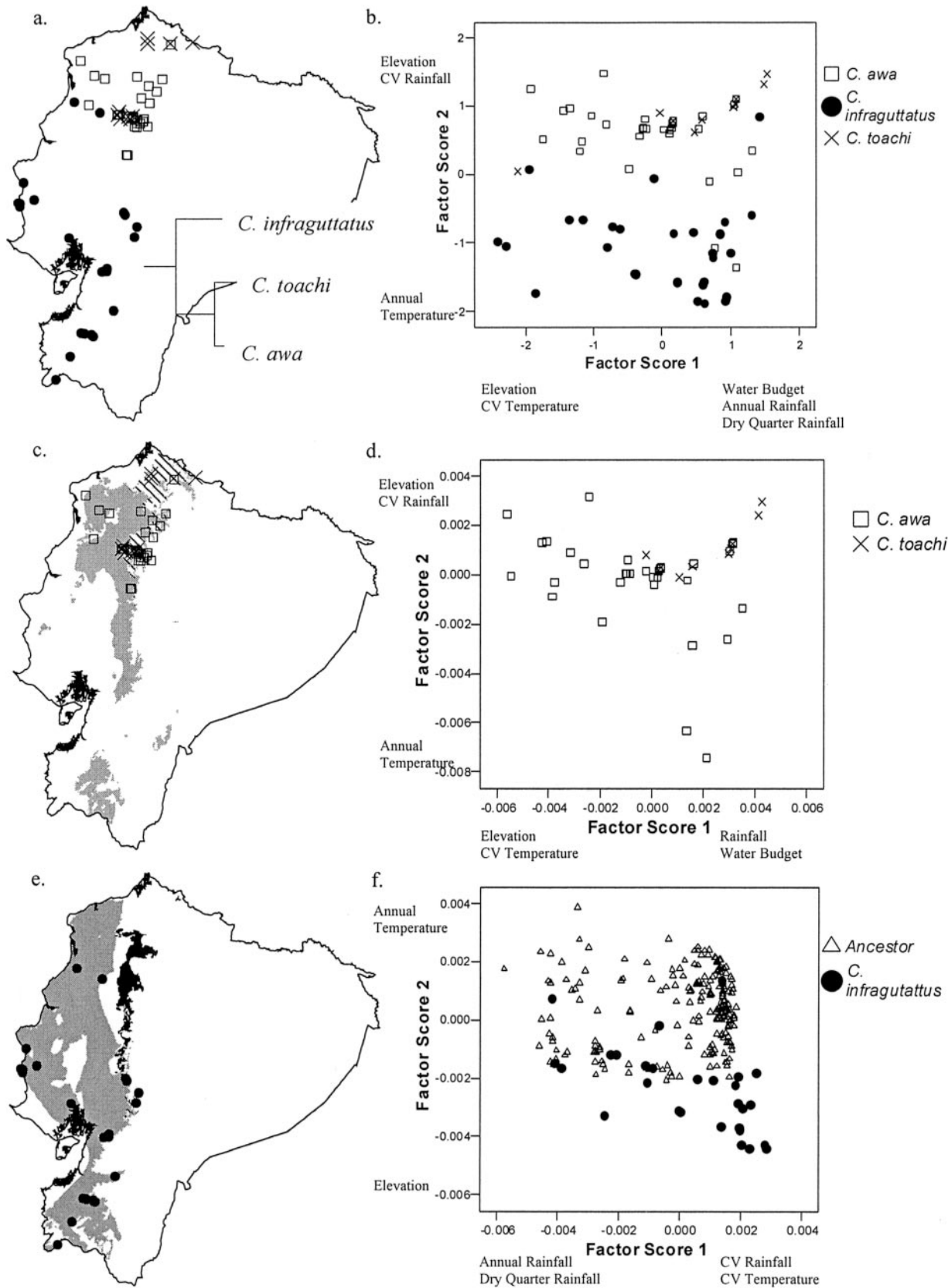
cluded three populations from southwestern Ecuador (two of them near to those compared by Duellman and Wild 1993). They recognized three species from their samples: (1) *E. anthonyi* (Piñas, Río Puyango basin), (2) *E. tricolor* (Santa Isabel, Río Jubones basin), and (3) *E.* sp. (Pasaje, Provincia de El Oro, Río Jubones basin). Santos et al. (2003) followed Duellman and Wild (1993) and assigned their two samples from southwestern Ecuador to *E. tricolor*.

Our phylogeny is the first to include a sample from the western Andean slopes in central Ecuador. The phylogeny shows that the lineages from southern and central Ecuador are not sister species and therefore populations in these two regions may represent different species. Because the type locality is from central Ecuador, the binomial *E. tricolor* should only be applied to populations in central Ecuador. In terms of southern populations, Santos et al. (2003) demonstrated that samples from Girón, Santa Isabel, Río Jubones basin and Zapotillo, Loja, Río Puyango basin showed low genetic differentiation. These results indicate that there are insufficient data to determine whether more than one species should be recognized within the southern part of the range. Although the existence of more than one species is plausible given the high degree of morphological variation among populations within the southern range, none of them is *E. tricolor*. We suggest that all of them be treated as *E. anthonyi* until more evidence of their taxonomic status is available.

DISCUSSION

In this paper we illustrate the benefits of considering both geographic and environmental space in relation to a phylog-

eny to infer general correlates of divergence and speciation. Results such as those presented here could be conclusive in their own right, especially for a large and comprehensively sampled phylogeny, or more likely, can help generate hypotheses for specific clades that can be tested through subsequent ecological and population genetic analyses (see Losos and Glor 2003). We observed a general trend of environmental divergence among sister groups, irrespective of geography. Conversely, there are no cases suggesting incidental divergence in isolation. By incidental divergence, we mean speciation not associated with ecologically mediated divergent selection. Among the seven comparisons possible here, four show clear separation of sister lineages in environmental space, of which two are geographically allopatric lineages (Fig. 1, case C) and two are parapatric lineages (Fig. 1, case B). Even lineages with broad geographic overlap are divergent in some portion of environmental space, especially when geographic range size is highly asymmetric (e.g., *C. toachi* and *C. awa*). There was substantial and symmetrical overlap in environmental range of the geographically parapatric lineages *E. anthonyi* versus *C. machalilla* and *E. boulengeri* versus its sister node (Fig. 1, case E); nevertheless, all pairs of allopatric sister taxa showed some level of divergence in their environmental niche. Observed differences in environmental space could reflect shifts in the fundamental niche, an evolutionary response, or differences in the realized niche due to competitive displacement within the fundamental niche. Whether the latter represents an adaptive, rather than purely ecological response, can only be determined by quantitative genetic analyses (e.g., common garden experi-

ments) of relevant physiological traits. Overall, these results suggest a considerable role of environmentally mediated divergence in speciation in geographic settings ranging from allopatry to parapatry. This contrasts strongly with the only previous analysis of this type in which Peterson et al. (1999) observed that most allopatric putative sister lineages of Mexican birds had limited environmental divergence.

Patterns of avian sympatry and parapatry in the same general area (northern Andes) as examined here for anurans are often explained by refuges/stable areas that formed repeatedly during glaciations and subsequent range expansions from these areas (Fjeldsa 1994; Roy et al. 1997; Chesser 2002). Likewise, comparisons of geographic ranges in relation to phylogeny have tended to support allopatric speciation rather than divergence-with-gene-flow models in both birds and mammals (Patton and Smith 1992; Arctander and Fjeldsa 1994; Roy et al. 1999), though environmental ranges of sister taxa have not been analyzed. Although evidence suggests that allopatry may be an important speciation mode for these Andean birds and mammals, frogs generally have lower gene flow than birds and mammals and, therefore, are more likely to diverge across a given environmental gradient. For example, for both *E. tricolor* and its sister node and *E.* sp. f and its sister node, the former taxon is distributed at higher elevations and replaced at lower elevations by the predicted range of the sister node. This pattern is similar to that found in frogs of the genus *Eleutherodactylus*, which show altitudinal replacement of parapatric sister species in western Ecuador (Lynch and Duellman 1997). Hence, the present analysis of Andean frogs supports Lynch and Duellman's model of divergent selection in association with climate or isolation resulting from the Andean uplift, but also reveals several cases in which the divergence-with-gene-flow model could apply. It may well be that taxa with low vagility (and hence predicted low gene flow), such as anurans, may respond more strongly to environmental gradients on the spatial scale we examined than do relatively high-vagility birds or mammals.

Vanzolini and Williams (1981) proposed a speciation model that should result in sympatric or parapatric lineages existing in unique environmental space; an outcome represented in our data. In their vanishing refuge hypotheses, a given species is subdivided into a series of refuges, as in classical refuge models, but as refuges decrease in size due to environmental forcing, some populations adapt to and persist in the novel habitat. The patterns we observe of parapatric and sympatric species existing in unique environmental space could have formed by such a mechanism, but fundamentally this mechanism is one of divergence driven by natural selection and adaptation across a habitat gradient, as in parapatric divergence.

Another useful result of our analysis is that it can point to common environmental parameters that may correlate with speciation. For the cases examined here, sister lineages generally separate along either a temperature/elevational or seasonality axis, or both. Neotropical frogs are often sensitive to changes in temperature, total annual rainfall, and the seasonality of both rainfall and temperature, which indicates that climatic conditions are likely important in limiting distributions (Lynch and Duellman 1997). The temperature/elevation axis generally describes species replacements along an elevation gradient on a given slope. The seasonality axis is often related to the latitudinal climate gradient that exists on the west side of the Ecuadorian Andes with moist, aseasonal climates in the north, which become drier and more seasonal (especially for rainfall) in the south.

Cases that warrant further investigation through more detailed analysis of ecological phenotypic and phylogeographical evidence include those that suggest divergence with gene flow and those that have secondary contact along what may have been a historical barrier. The two cases that most strongly suggest divergence with gene flow are *E. tricolor* and its sister node (in this case the extant species *E. anthonyi* and *C. machalilla*) and *C. infraguttatus* and its sister node (the extant species *C. toachi* and *C. awa*). Two allopatric cases, *E. anthonyi* and *C. machalilla*, and *E. elachyhistus* and its sister node (extant taxa *C. toachi*, *C. awa*, and *C. infraguttatus*), show substantial prediction by the niche models of a given lineage into the range of its sister lineage. In these cases, ''over'' prediction occurs on either side of the Río Esmeraldas, suggesting it may be a historical biogeographic barrier. This barrier has been identified for other taxa such as butterflies and plants (Whitmore and Prance 1987); however, the nature and influence of this potential barrier across different taxonomic groups warrant further research. Our method fails to give insight into the speciation mode for the sister taxa *C. toachi* and *C. awa*. These species have asymmetrical geographic distributions in both geographic and environmental space such that one taxa exists in a subset of the environmental space of its sister (Fig. 1). Two factors could explain this pattern. These sister taxa may have differentiated via incidental divergence in allopatry and then come into sympatry, in which case there should be evidence for range expansion by one or both taxa. Alternatively, sympatric speciation may have occurred along an ecological dimension not included in the present analyses; for example, perhaps some factor at a finer environmental resolution (micro-habitat) was important.

Limitations and Prospects of Methods

The methodology outlined in this paper will be more informative when applied comprehensively to diverse genera

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FIG. 6. Environmental analyses for the clade that includes *Colostethus infraguttatus*, *C. toachi*, and *C. awa*, (a) geographic distribution and phylogenetic hypothesis, (b) principal components analysis (PCA) for all extant taxa; variation explained by x-axis = 50% and y-axis = 36%; (c) environmental niche model for *C. toachi* and *C. awa*, where gray is *E. toachi*, *C. awa* is completely contained within *C. toachi*, and diagonal shading is the overlap region; (d) PCA for *C. awa* and *C. toachi*; variation explained by x-axis = 46% and y-axis = 33%; (e) environmental niche model for *C. infraguttatus* and the ancestral node to *C. awa* and *C. toachi*, where gray is *C. infraguttatus*, black is the ancestor, and diagonal shading is the overlap region; (f) PCA for *C. infraguttatus* and its ancestral node; variation explained by x-axis = 37% and y-axis = 25%.

with more extensive information on distribution and relationships. First, point data should be sufficiently dense to accurately estimate the niche dimension of a given species. In our case, there are several species that are poorly represented in museums. Second, the method should use complete and extensively sampled phylogenies; with dendrobatid frogs and most other taxa (especially species-rich tropical taxa); however, complete phylogenies are not yet available. Finally, we are unlikely to be measuring all the important environmental variables that encompass a species' niche suggesting our niche analyses may not be an accurate evaluation of the complete environmental space used by a given organism. When species overlap extensively in environmental space, it is important to also consider the possibility of ecological differentiation in unexamined dimensions. Nonetheless, because amphibians are physiologically sensitive to dry conditions, precipitation and temperature are likely to be significant environmental variables (Lynch and Duellman 1997).

A potential problem in most studies involving the geography of speciation is that species ranges may not be stable over time as a result of range shifts or expansions (Losos and Glor 2003). This is a valid argument against methods that simply combine species ranges with phylogenetic information (Lynch 1989; Barraclough et al. 1998; Barraclough and Vogler 2000). Nonetheless, assuming that analogous environmental gradients existed under historic climates and that environmental limitations on species distributions were consistent over time, distribution patterns of species in relation to each other (i.e., parapatry) may be maintained over time. Our initial results suggest a consistent pattern of environmental divergence among sister lineages. Whether the divergence was associated with, and perhaps caused by speciation, or occurred post speciation cannot be determined on a case-by-case basis. However, if the paucity of cases consistent with incidental divergence in allopatry (case F in Fig. 1) is maintained in larger datasets, then our tentative association of ecological divergence with speciation would be more secure.

Predicting distributions at ancestral nodes in the phylogeny also has limitations. First, the assumptions of directionality and rate of evolution are difficult to test. Second, there are typically large confidence limits around estimates of parameters at ancestral nodes. However, although incorporation of these errors increased parameter space occupied by ancestral nodes, there was no qualitative change to the conclusions drawn for the dendrobatid clades examined here. Third, the predicted ancestral distributions may be somewhat problematic because, ideally, the ancestral range would be created under the climatic regime at the time of the speciation event. However, again, if we assume that similar environmental gradients existed under historic climates, then it may be valid to extrapolate nodal distributions on current climate patterns.

Although limited by uncertainty of inferences obtained from unbalanced trees, joint analysis of environmental and geographic data for this group of dendrobatid frogs has identified potentially important speciation mechanisms and particular sister lineages that warrant more intensive ecological and genetic study. More generally, museums are undertaking significant georeferencing projects to produce connected databases (Krishtalka and Humphrey 2000), environmental data

are being created at finer resolutions with less error, and phylogenies are being completed with extensive (or complete) taxon sampling for a variety of groups. Given these developments, the methods outlined in this paper will be increasingly relevant.

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