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Genetic divergence and speciation in lowland and montane peruvian poison frogs

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Abstract

Amazonia is famous for high biodiversity, and the highlands of the transition zone between the Andes and the lowlands of the Amazon basin show particularly high species diversity. Hypotheses proposed to explain the high levels of diversity in the highlands include repeated parapatric speciation across ecological gradients spanning the transition zone, repeated allopatric speciation across geographic barriers between the highlands and lowlands, divergence across geographic barriers within the transition zone, and simple lineage accumulation over long periods of time. In this study, we investigated patterns of divergence in frogs of the genus *Epipedobates* (family Dendrobatidae) using phylogenetic and biogeographic analyses of divergence in mitochondrial DNA (1778 aligned positions from genes encoding cyt *b*, 12S and 16S rRNA for 60 *Epipedobates* and 11 outgroup specimens) and coloration (measured for 18 specimens representing nine species in *Epipedobates*). The majority of phenotypic and species diversity in the poison frog genus *Epipedobates* occurs in the transition zone, although two morphologically conserved members of the genus are distributed across the lowlands of the Amazon basin. Phylogenetic analysis reveals that there is a single highland clade derived from an ancestral colonization event in northern Peru by a population of lowland ancestry. *Epipedobates trivittatus*, a widespread Amazonian species, is a member of the highland clade that reinvaded the lowlands. Comparative analyses of divergence in coloration and mtDNA reveals that divergence in coloration among populations and species in the highlands has been accelerated relative to the lowlands. This suggests a role for selection in the divergence of coloration among populations and species.

Keywords: Poison frogs; Epipedobates; Amazonia; Transition zone; Divergence-systematics

1. Introduction

The Amazon basin is a region of unparalleled biodiversity, the origin of which has been a subject of continuing debate. Empirically, one notable pattern is that species diversity appears to be high along the slopes that mark the transition zone between the peripheral mountain ranges (particularly the Andes) and the Amazonian lowlands. In frogs, for example, Duellman (1982) noted that regions of high species diversity are characterized by areas where humid forests exist continuously from the lowlands to highland elevations above 1000 meters. Species richness and endemism in these slope regions have also been noted for birds, small mammals, insects and plants (e.g., Fjeldsa, 1994; Fjeldsa and Rahbek, 2006; Hall, 2005; Kessler et al., 2001; Lomolino, 2001). Highland regions encompass only 15.3% of South America in area but harbor more than half of the continent's amphibian species, over 90% of which are endemic (Duellman, 1999).

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In Peru, the transition zone encompasses a large region from 50 to 250 km wide linking the east Andes versant and lowland Amazonia. This region includes a series of interrupted, smaller mountain ranges, or cordilleras, which were generated from secondary orogenies and subsequent erosion post dating the formation of the Andes (Sauer, 1971). Within the cordilleras, the elevation rarely exceeds 2000 m, and upper regions tend to consist of moist premontane cloud forest and cloud forest. The ranges further west progressively increase in altitude and decrease in rainfall, likely a rain-shadow effect from the eastern ranges adjacent to lowland Amazonia. Elevations of the transition zone at times overlap those of more montane regions. The fall line, an area where an upland region and low-elevation plains region meet, is used to demarcate the lower threshold of the transition zone and defines the separation between lowland and highland regions. The elevation of the fall line within this study averages 300 m, and highland and transition zone regions both are defined as areas exceeding 300 m in elevation. The transition zone and highland regions are ecologically distinct from lowland regions, which exist at elevations below 300 m.

Various theories have been proposed to explain the high diversity in the transition zone, with studies conducted to date yielding conflicting results. These theories may be divided along several different axes of explanation: allopatric versus parapatric divergence and speciation, multiple versus single origins of the transition-zone taxa, divergence caused by selection versus genetic drift, and species diversity driven by increased rates of diversification or simply by the amount of time available for species to accumulate.

A variety of hypotheses addressing Amazonian biodiversity emphasize geographic barriers to gene flow as a mechanism for allopatric speciation (e.g., Sick, 1967; Rasanen et al., 1990; Patton and da Silva, 1998; Haffer, 1997). Several researchers have suggested that allopatric divergence and speciation occurred across the transition zone between the Andes and the lowlands, through repeated bouts of separation and introgression (e.g., Bush, 1994; Colinvaux, 1993).

In contrast, other researchers have hypothesized that selection drives divergence and speciation across ecological gradients, affecting populations parapatrically distributed across the transition zone and into the lowlands (e.g., Endler, 1982; Smith et al., 2001; T.B. Smith et al., 2005 for similar situations in Africa and Australia). These studies have emphasized the importance of natural selection in generating divergence and reproductive isolation, despite continuing gene flow across a gradient of differing habitats, in the absence of geographic barriers. Recent theoretical work supports the plausibility of this type of parapatric speciation (Gavrilets, 2000; Gavrilets et al., 2000; Doebeli and Dieckmann, 2003), and recent tests comparing morphological and genetic divergence across parapatric and allopatric populations support some of the predictions of this hypothesis for African birds and Australian lizards (Smith et al., 1997, 2001; T.B. Smith et al., 2005).

Both the allopatric and parapatric models emphasize multiple invasions of the highlands by populations of widespread lowland taxa to explain highland diversity. Alternatively, it is possible that species and populations in the transition zone could derive from only one or a few colonizations, followed by dispersal and diversification of populations within the transition zone (e.g., Cracraft, 1985). In frogs, Lynch and Duellman (1997) emphasized the role of allopatric isolation in generating divergence and speciation among populations due to repeated bouts of geological uplift and vicariance in the transition zone.

The mechanism of parapatric speciation described above represents a form of ecological speciation (Schluter, 2000). Ecological speciation is driven by divergent forces of natural selection acting on populations (Rundle and Nosil, 2005). Other forms of selection can also drive divergence and speciation. For example, sexual selection may frequently be an important factor driving divergence and speciation, but may not always be considered ecological selection (Kirkpatrick and Ravigne', 2002). Divergence and speciation may also be caused by genetic drift (e.g., Roy, 1997). The emphasis on selection versus drift (Rundle and Nosil, 2005) is another axis along which previous hypotheses of Amazonian diversification have been divided (e.g., Endler, 1982 versus Haffer, 1997).

Selection could drive divergence and speciation across ecological gradients spanning the transition zone to the lowlands, but it could also act to drive divergence among populations solely within the transition zone. Several authors have argued that geographic heterogeneity among regions in the transition zone has been a key force enhancing rates of diversification, either through habitat heterogeneity (ecological speciation), increased isolation (allopatric speciation), or a combination of both (e.g., Fjeldsa, 1994; Fjeldsa and Rahbek, 2006).

A number of researchers have argued that high levels of species diversity in the transition zone have arisen through an increased rate of speciation in those regions (e.g., Fjeldsa, 1994; Lynch and Duellman, 1997; Garcia-Moreno and Fjeldsa, 2000; Fjeldsa and Rahbek, 2006). Conversely, it is possible that the transition zone has been inhabited for a longer period of time than the lowlands, and hence a higher number of species exist there simply due to lineage accumulation (e.g., Stebbins, 1974). This "time-for-speciation" hypothesis appears to explain key patterns of species diversity in emydid turtles (Stephens and Wiens, 2003) and possibly in hylid frogs (Wiens et al., 2003; S.A. Smith et al., 2005). This hypothesis predicts that species in the region colonized more recently (e.g., the lowlands) are derived from clades that already existed in the region of higher diversity (e.g., the transition zone).

Despite the recent emphasis on examining diversification patterns both phenotypically and genetically to distinguish influences of isolation and ecology on speciation (e.g., Smith et al., 2001; T.B. Smith et al., 2005), few studies have examined these hypotheses in the transition zone of the Andean slopes. Anuran amphibians are excellent candidates for

disclosing patterns of divergent selection associated with isolation and climatic change resulting from Andean uplift (Graham et al., 2004). Their low vagility makes adaptation to local conditions likely, and populations of many species are found both in the transition zone and in the lowlands. In this paper, we address some of the contrasting predictions of the hypotheses described above with phylogenetic, biogeographic and comparative analyses of a genus of South American poison frogs (family Dendrobatidae).

The poison frogs are well known for their bright, aposematic coloration (e.g., Myers and Daly, 1983). They are both widespread and divergent throughout Amazonia, making them appropriate taxa for studies of speciation patterns and processes. The species-rich genus *Epipedobates* is distributed from western Ecuador to southern Peru and Bolivia, and across the Amazon basin to eastern Brazil and French Guiana. Of the 32 recognized species in the genus *Epipedobates*, 40% occur in the lowlands at elevations less than 300 m, though the ranges of some species extend into higher elevation areas. The other 60% of species occupy restricted ranges along the Andean front of western Amazonia, in the transition zone and highland regions of elevations higher than 300 m (Myers et al., 1998). Most of the lowland *Epipedobates* are morphologically conservative, whereas the highland endemics exhibit significant color variation among and (in the case of *E. bassleri*) within species (Fig. 1). Species density per square kilometer is nearly six times greater in the transition zone and highland regions than in the lowland regions (lowland regions comprise 78% and highland regions comprise 22% of hospitable land in the Amazon basin; Duellman, 1999). Further, more than two species of *Epipedobates* rarely occur sympatrically in the lowlands, but in some regions as many as five species of *Epipedobates* occur sympatrically in the highlands (Schulte, 1999).

Given their distribution and highland-centered diversity, the frogs of the genus *Epipedobates* represent an excellent system in which to investigate the role of the transition zone in the diversification of Amazonian species. Their readily observable differences in phenotype (coloration) can easily be compared to genetic divergence in neutral markers (base substitutions in mitochondrial DNA) to help differentiate factors driving regional diversification and speciation (Smith et al., 2001; T.B. Smith et al., 2005). Coloration is associated with selection in the context of aposematism across the poison-frog family (Summers and Clough, 2001; Santos et al., 2003). Coloration is also associated with mate choice preferences (and hence potentially with divergence and reproductive isolation) in one species of dendrobatid frog, *Dendrobates pumilio*, in the Bocas del Toro Archipelago of Panama (Summers et al., 1999; Siddiqi et al., 2004).

In this paper, we use phylogenetic analyses to clarify the relationships within and among transition zone and lowland populations and species. We also use biogeographic analyses to investigate historical patterns of dispersal and colonization, and we use comparative phenotypic analyses combined with ancestral-state reconstructions to investigate the evolution of coloration in these frogs. We use these analyses to address a series of predictions derived from the hypotheses described above.

First, a number of authors have suggested that the distribution of populations across ecological gradients spanning the transition zone and into the lowlands results in repeated parapatric speciation (Endler, 1982; Moritz et al., 2000; Smith et al., 2001; T.B. Smith et al., 2005). With respect to poison frogs of the genus *Epipedobates*, this hypothesis predicts multiple origins of transition-zone species from lowland ancestry, with each transition-zone endemic having a sister-taxon relationship with a lowland population of one of the widespread lowland species. Other researchers have proposed a similar scenario of multiple instances of invasion and speciation, but with allopatric rather than parapatric speciation as the key force driving speciation (e.g., Bush, 1994; Colinvaux, 1993). These hypotheses also predict multiple origins of highland taxa from lowland ancestry as an explanation for transition-zone diversity. The alternative is that only one or a few colonizations occurred, followed by widespread dispersal and diversification within the transition zone.

Second, the hypothesis that divergent selection among populations within the transition zone has been the major force generating new species (e.g., Fjeldsa and Rahbek, 2006) predicts that phenotypic divergence will be more pronounced among transition zone populations and species than phenotypic divergence among lowland populations and species showing similar levels of genetic divergence in neutral molecular markers. This kind of test assumes that the phenotypic characters measured are relevant to reproductive isolation (T.B. Smith et al., 2005).

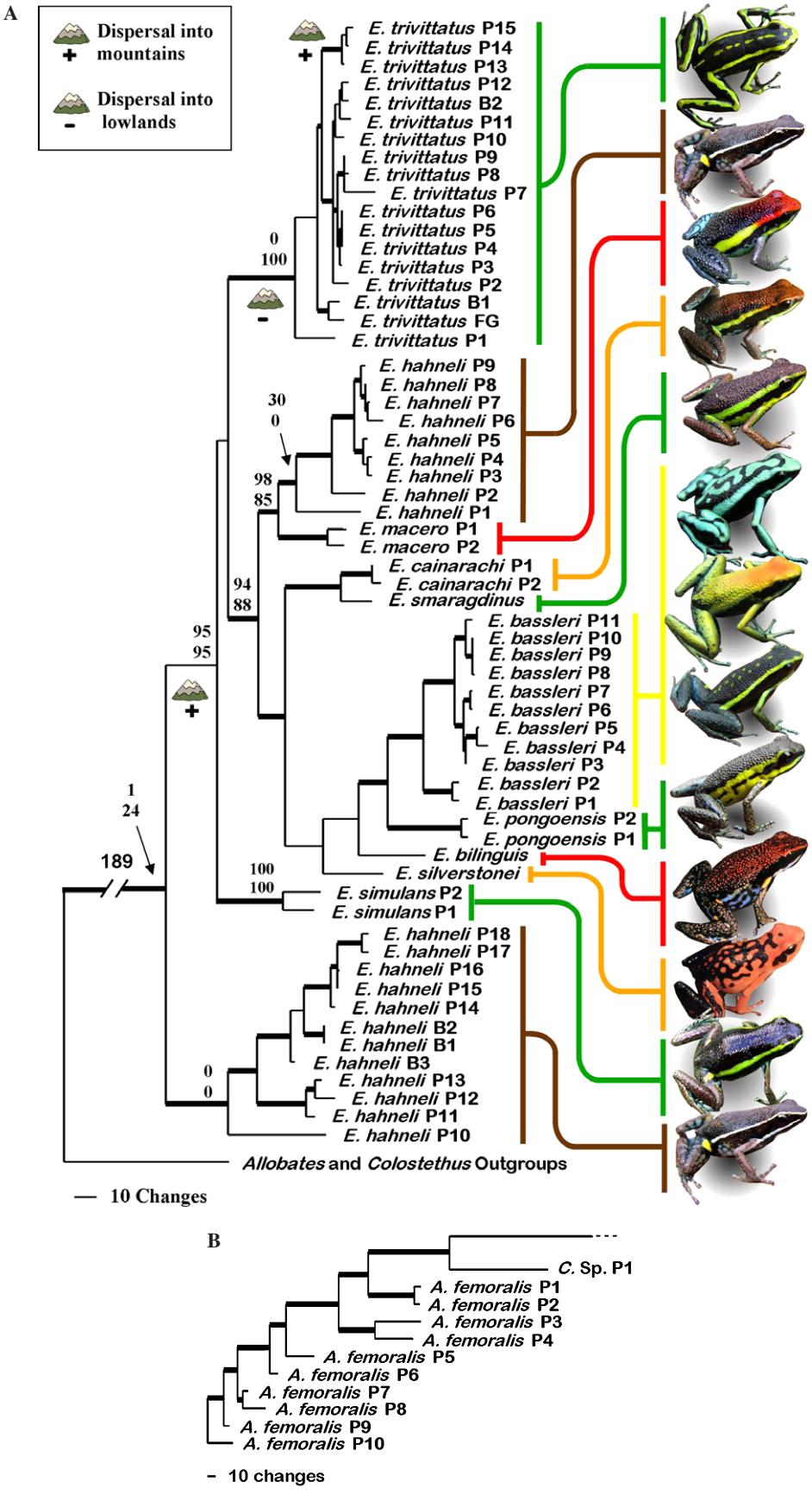
Finally, the time-for-speciation hypothesis (Stephens and Wiens, 2003; S.A. Smith et al., 2005) predicts that the transition-zone serves as a "museum" of relatively ancient lineages, which gave rise to more recent (and hence less diverse) clades in the lowlands. This opposes most other hypotheses concerning the diversity of transition-zone species, which predict that highland clades are derived from lowland ancestry (e.g., Fjeldsa, 1994; Fjeldsa and Rahbek, 2006).

In addition to evaluating predictions from these hypotheses, we also discuss the relevance of our results to specific issues concerning the systematics and biogeography of the genus *Epipedobates* in Peru and surrounding areas.

2. Methods

2.1. Tissue collection

Tissue samples from Peru were collected during June and July of 2003 by clipping a toe from an adult frog or by clipping a piece of tail from a tadpole; tissues were preserved in a buffer solution of 20% DMSO saturated with sodium chloride and EDTA. Several toes were collected from a population when possible. Voucher specimens representing each species were also collected and deposited in the University of San Marcos Museum of Natural History in Lima, Peru. Collecting and export permits were obtained


A
Dispersal into mountains
Dispersal into lowlands
E. trivittatus P15
E. trivittatus P14
E. trivittatus P13
E. trivittatus P12
E. trivittatus B2
E. trivittatus P11
E. trivittatus P10
E. trivittatus P9
E. trivittatus P8
E. trivittatus P7
E. trivittatus P6
E. trivittatus P5
E. trivittatus P4
E. trivittatus P3
E. trivittatus P2
E. trivittatus B1
E. trivittatus FG
E. trivittatus P1
E. hahneli P9
E. hahneli P8
E. hahneli P7
E. hahneli P6
E. hahneli P5
E. hahneli P4
E. hahneli P3
E. hahneli P2
E. hahneli P1
E. macero P1
E. macero P2
E. cainarachi P1
E. cainarachi P2
E. smaragdinus
E. bassleri P11
E. bassleri P10
E. bassleri P9
E. bassleri P8
E. bassleri P7
E. bassleri P6
E. bassleri P5
E. bassleri P4
E. bassleri P3
E. bassleri P2
E. bassleri P1
E. pongoensis P2
E. pongoensis P1
E. bilinguis
E. silverstonei
E. simulans P2
E. simulans P1
E. hahneli P18
E. hahneli P17
E. hahneli P16
E. hahneli P15
E. hahneli P14
E. hahneli B2
E. hahneli B1
E. hahneli B3
E. hahneli P13
E. hahneli P12
E. hahneli P11
E. hahneli P10
Allobates and Colostethus Outgroups
10 Changes
B
C. Sp. P1
A. femoralis P1
A. femoralis P2
A. femoralis P3
A. femoralis P4
A. femoralis P5
A. femoralis P6
A. femoralis P7
A. femoralis P8
A. femoralis P9
A. femoralis P10
10 changes

from the Ministry of *Natural Resources* (INRENA) in Lima, Peru (Authorization No. 061-2003-INRENA-IFFS-DCB, Permit No. 002765-AG-INRENA and CITES Permit No. 4326). Brazilian samples were collected by J.P. Caldwell and were obtained via a tissue grant to K. Summers from the Louisiana State University Museum of Natural Sciences Collection of Genetic Resources. Tissues obtained by J.P. Caldwell were collected during expeditions funded by the National Science Foundation (DEB-9200779 and DEB-9505518 to L.J. Vitt and J.P. Caldwell). Samples from French Guiana were collected by B. Noonan.

Recent phylogenetic studies have not included multiple individuals of species of *Epipedobates* sampled widely from the eastern Andean front and the Amazon basin (i.e., Santos et al., 2003; Vences et al., 2003). When possible, attempts were made to collect tissue samples from multiple individuals across species ranges (where applicable). The species included in this analysis represent approximately two-thirds of Peruvian *Epipedobates* (Frost, 2004). Species with broad distributions (i.e., *E. hahneli* and *E. trivittatus*, which occur in both the lowlands and the transition zone) have been sampled across their ranges as widely as possible. Species identifications, localities, and GenBank accession numbers for each sample are listed in Tables 1 and 2.

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from tissue samples using the Qiagen DNeasy Tissue Kit. The cytochrome *b* (cyt*b*), 12S and 16S mitochondrial gene regions were amplified using the following primer sets for a total of 1778 bp: cyt*b*: CB1-L, CB2-H (Kocher et al., 1989); KSCYB1(A)-L, KSCYB1-H (Clough and Summers, 2000); KSCB1L1 (GCCAATG GCGCTTCATTTTTCT), KSCBARH1 (GGGGTAAAAT TGTCTGGGTCT) (designed for this study); Cytb18R-L, CytbAR-H (Goebel et al., 1999); 12S: 12SA-L (Palumbi et al., 1991), 12SK-H (Goebel et al., 1999); 16S: LGL 286, LGL 381 (Palumbi et al., 1991). PCR amplifications were purified with the Qiagen QIAquick PCR Purification Kit or ExoSAP-IT (USB Corporation, Cleveland, OH, USA). Products were sequenced using Applied Biosystems' (ABI) Prism Sequencing Kit (Perkin–Elmer Corporation, Foster City, CA, USA). Samples were then prepared for sequencing as in Clough and Summers (2000) and were electrophoresed on the ABI 377 Automated DNA Sequencer for analysis.

2.3. Sequence analysis

Each sample was sequenced in both directions and complementary sequences were aligned using Autoassembler version 1.4.0 (Applied Biosystems, Inc., 1995). Consensus sequences were transferred to Gene Jockey (Taylor, 1990) for alignment with a sequence of the same region from a different individual. All cyt*b* sequences were translated to confirm proper reading frame and absence of stop codons. DNA sequences were aligned using the Probabilistic Alignment Kit (PRANK; Löytynoja and Goldman, 2005; http://www.ebi.ac.uk/goldman/prank). Because PRANK keeps track of gaps introduced into a multiple sequence alignment, rather than automatically penalizing them, it is expected to resolve indel events more effectively than do other methods (Higgins et al., 2005; Löytynoja and Goldman, 2005).

2.4. Phylogenetic analysis

Allobates femoralis and one species of *Colostethus* were used as outgroups for a phylogenetic analysis using Bayesian inference (Huelsenbeck and Ronquist, 2001). The dataset was partitioned into codon-position specific sets of nucleotides (1st, 2nd and 3rd positions for cyt *b*, with a separate, single partition for 12S and 16S), and MrModeltest version 2.0 (Nylander, 2004) was used to identify a substitution model, nucleotide frequencies and optimal priors for the gamma parameter and the proportion of invariant sites for each partition. Sequence data may better be explained by partitioning a dataset than by applying an average model across genes and codon positions, as indicated by higher model-likelihood scores in partitioned analyses (Nylander et al., 2004). MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was run for 8 million generations, using the models indicated by MrModeltest. −Ln scores were used to identify the burn-in phase after all trees were summed. The Bayesian analysis was repeated to ensure consistency of the resulting tree topology.

Shimodaira and Hasegawa (1999) tests were conducted to assess the validity of certain relationships among taxa by comparing the log-likelihood of the tree topology to that of alternative topologies.

2.5. Biogeographic analysis

The historical distributions of Peruvian *Epipedobates* were analyzed using dispersal–vicariance analysis (DIVA; Ronquist, 1996). Geographic locations of all the samples were divided into the following ten groups and coded as letters: Southern Peru Lowland, Central Peru Lowland, Northern Peru Lowland, Southern Peru Mountains, Central Peru Mountains, Northern Peru Mountains, Ecuador,

Fig. 1. (A) Bayesian Phylogram. Thickened branches indicate nodes supported by greater than 75% Bayesian posterior probabilities. Numbers above branches denote ancestral Bayesian posterior probabilities calculated in SIMMAP. The top number reflects the probability of a montane ancestor, and the bottom number reflects the probability of a brightly colored ancestor. The mountain symbols represent ancestral dispersal events into or out of the mountains (a plus sign indicates a dispersal into the mountains and a minus sign indicates a dispersal into the lowlands, as calculated using DIVA). (B) Phylogenetic relationships of outgroup species from Bayesian analysis.

Table 1

Species	Location	Region/Country	Cyt *b*	12S	16S
A. femoralis	Boca Manu	Cuzco, Peru	DQ523139	DQ522998	DQ523069
A. femoralis	Mazuko	Madre de Dios, Peru	DQ523125	DQ522984	DQ523055
A. femoralis	Rio Manati	Iquitos, Loreto, Peru	DQ523110	DQ522969	DQ523040
A. femoralis	Rio Sucusari	Iquitos, Loreto, Peru	DQ523093	DQ522952	DQ523023
A. femoralis	Saposoa	Tarapoto, San Martin, Peru	DQ523152	DQ523011	DQ523082
A. femoralis	Shucshuyacu	Yurimaguas, Loreto, Peru	DQ523142	DQ523001	DQ523072
A. femoralis	Itaya River	Iquitos, Loreto, Peru	DQ523132	DQ522991	DQ523062
A. femoralis	Nauta Road	Iquitos, Loreto, Peru	DQ523129	DQ522988	DQ523059
A. femoralis	Tahuayo	Iquitos, Loreto, Peru	DQ523095	DQ522954	DQ523025
Colostethus sp.	Near Bonilla	Tarapoto, San Martin, Peru	DQ523118	DQ522977	DQ523048
Epipedobates bassleri	Altoshima	Tarapoto, San Martin, Peru	DQ523153	DQ523012	DQ523083
E. bassleri	Road to Yurimaguas	Tarapoto, San Martin, Peru	DQ523115	DQ522974	DQ523045
E. bassleri	Road to Yurimaguas	Tarapoto, San Martin, Peru	DQ523150	DQ523009	DQ523080
E. bassleri	Road to Sisa	Tarapoto, San Martin, Peru	DQ523140	DQ522999	DQ523070
E. bassleri	Road to Sisa	Tarapoto, San Martin, Peru	DQ523143	DQ523002	DQ523073
E. bassleri	Road to Sisa	Tarapoto, San Martin, Peru	DQ523155	DQ523014	DQ523085
E. bassleri	Rd to Moyobamba	Tarapoto, San Martin, Peru	DQ523113	DQ522972	DQ523043
E. bassleri	Near Chazuta	Tarapoto, San Martin, Peru	DQ523157	DQ523016	DQ523087
E. bassleri	Saposoa	Tarapoto, San Martin, Peru	DQ523158	DQ523017	DQ523088
E. bassleri	Sauce	Tarapoto, San Martin, Peru	DQ523130	DQ522989	DQ523060
E. bassleri	Huallaga Canyon	Tarapoto, San Martin, Peru	DQ523127	DQ522986	DQ523057
E. bilinguis	Primavera	Napo, Ecuador	DQ523144	DQ523003	DQ523074
E. cainarachi	Cainarachi Valley	Tarapoto, San Martin, Peru	DQ523123	DQ522982	DQ523053
E. cainarachi	Road to Yurimaguas	Tarapoto, San Martin, Peru	DQ523094	DQ522953	DQ523024
E. hahneli	Aguamo-Muyuma	Tarapoto, San Martin, Peru	DQ523107	DQ522966	DQ523037
E. hahneli	Amazonas	Amazonas, Brazil	DQ523133	DQ522992	DQ523063
E. hahneli	Amazonas	Amazonas, Brazil	DQ523137	DQ522996	DQ523067
E. hahneli	Boca Manu	Cuzco, Peru	DQ523097	DQ522956	DQ523027
E. hahneli	Convento	Tarapoto, San Martin, Peru	DQ523102	DQ522961	DQ523032
E. hahneli	Itaya River	Iquitos, Loreto, Peru	DQ523103	DQ522962	DQ523033
E. hahneli	Ivochote	Cuzco, Peru	DQ523108	DQ522967	DQ523038
E. hahneli	Road to Sisa	Tarapoto, San Martin, Peru	DQ523149	DQ523008	DQ523079
E. hahneli	Near Chazuta	Tarapoto, San Martin, Peru	DQ523121	DQ522980	DQ523051
E. hahneli	Porto Walter	Acre, Brazil	DQ523134	DQ522993	DQ523064
E. hahneli	Rio Amigos	Madre de Dios, Peru	DQ523126	DQ522985	DQ523056
E. hahneli	Rio Manati	Iquitos, Loreto, Peru	DQ523145	DQ523004	DQ523075
E. hahneli	Saposoa	Tarapoto, San Martin, Peru	DQ523156	DQ523015	DQ523086
E. hahneli	Itaya River	Iquitos, Loreto, Peru	DQ523131	DQ522990	DQ523061
E. hahneli	Alto Purus River	Ucayali, Peru	DQ523104	DQ522963	DQ523034
E. hahneli	Alto Purus River	Ucayali, Peru	DQ523111	DQ522970	DQ523041
E. hahneli	Alto Purus River	Ucayali, Peru	DQ523151	DQ523010	DQ523081
E. hahneli	Cachiyacu Road	Tarapoto, San Martin, Peru	DQ523092	DQ522951	DQ523022
E. hahneli	Cachiyacu Road	Tarapoto, San Martin, Peru	DQ523096	DQ522955	DQ523026
E. hahneli	Cachiyacu Road	Tarapoto, San Martin, Peru	DQ523148	DQ523007	DQ523078
E. hahneli	Valle San Antonio	Tarapoto, San Martin, Peru	DQ523119	DQ522978	DQ523049
E. macero	Ivochote	Cuzco, Peru	DQ523109	DQ522968	DQ523039
E. macero	Alto Purus River	Ucayali, Peru		DQ523018	DQ523089
E. pongoensis	Convento	Tarapoto, San Martin, Peru	DQ523114	DQ522973	DQ523044
E. pongoensis	Huallaga Canyon	Tarapoto, San Martin, Peru	DQ523146	DQ523005	DQ523076
E. silverstonei	Cordillera Azul	Huanuco, Peru	DQ523154	DQ523013	DQ523084
E. simulans	Mazuko	Madre de Dios, Peru	DQ523159	DQ523019	
E. simulans	Quincemille	Cuzco, Peru	DQ523160	DQ523020	DQ523090
E. smaragdinus	Iscozazin	Pasco, Peru	DQ523112	DQ522971	DQ523042
E. trivittatus	Amazonas	Amazonas, Brazil	DQ523135	DQ522994	DQ523065
E. trivittatus	Alto Purus River	Ucayali, Peru	DQ523098	DQ522957	DQ523028
E. trivittatus	Road to Barranquita	Tarapoto, San Martin, Peru	DQ523128	DQ522987	DQ523058
E. trivittatus	Near Bonilla	Tarapoto, San Martin, Peru	DQ523124	DQ522983	DQ523054
E. trivittatus	Near Chazuta	Tarapoto, San Martin, Peru	DQ523141	DQ523000	DQ523071
E. trivittatus	Chumilla	San Martin, Peru	DQ523100	DQ522959	DQ523030
E. trivittatus		French Guiana	DQ523147	DQ523006	DQ523077
E. trivittatus	Iscozazin	Pasco, Peru	DQ523116	DQ522975	DQ523046
E. trivittatus	Cordillera Azul	San Martin, Peru	DQ523120	DQ522979	DQ523050
E. trivittatus	Porto Walter	Acre, Brazil	DQ523099	DQ522958	DQ523029
E. trivittatus	Rio Manati	Iquitos, Loreto, Peru	DQ523101	DQ522960	DQ523031
E. trivittatus	Rio Sucusari	Iquitos, Loreto, Peru	DQ523106	DQ522965	DQ523036

Table 1 (*continued*)

Species	Location	Region/Country	Cyt *b*	12S	16S
E. trivittatus	Cordillera Oriental	Amazonas, Peru	DQ523138	DQ522997	DQ523068
E. trivittatus	Santa Rosa	Huanuco, Peru	DQ523117	DQ522976	DQ523047
E. trivittatus	Tahuayo River	Iquitos, Loreto, Peru	DQ523105	DQ522964	DQ523035
E. trivittatus	Tahuayo River	Iquitos, Loreto, Peru	DQ523122	DQ522981	DQ523052
E. trivittatus	Tahuayo River	Iquitos, Loreto, Peru	DQ523136	DQ522995	DQ523066
E. trivittatus	Shilcayo Valley	Tarapoto, San Martin, Peru	DQ523091	DQ522950	DQ523021

South western Brazil, North Central Brazil and French Guiana. Each taxon was placed into one of the 10 groups. To simplify the analysis, clades composed of many individuals of the same species and same geographic group were reduced to a single individual. This reduced the dataset to 28 individuals; each of which represented either a single species or a single population from one of the ten geographic groups. The Bayesian tree was pruned in MacClade to reflect the reduced dataset. The dataset was then divided into four groups: the *trivitattus* group (which contained 12 individuals from seven geographic groups), the lowland *hahneli* group (which contained 12 individuals from seven geographic groups), the highland group (which contained nine species, for a total of 10 individuals from seven geographic groups) and an outgroup (which contained *Allobates femoralis* from 9 geographic locations: ancestral distributions were coded for all known locations of outgroup species). A second analysis was performed with each taxon placed into one of two groups: high elevation or low elevation. The first analysis was designed to estimate distributions of the ancestors of the *E. trivittatus*, *E. hahneli* and highland clades, particularly the geographic location of the ancestor to the highland clade. The second analysis was designed to estimate the number of radiations between high and low elevations.

2.6. Comparative analysis of coloration

Photographs of 18 individuals representing 9 species (four populations of *Epipedobates bassleri*, seven populations of *E. hahneli*, and one individual each of *E. simulans*, *E. smaragdinus*, *E. pongoensis*, *E. macero*, *E. cainarachi*, *E. bilinguis* and *E. silverstonei*) were loaded into Adobe Photoshop version CS. Photos of all species were taken with a digital camera (either a Fuji Finepix s5100, Nikon CoolPix or Nikon D1). In an effort to minimize differences in white balance among pictures, coloration was standardized by adjusting the RGB histograms separately so that the start of each histogram peak represented 0 and the end represented 255. A flash was used for each photo. Color values were selected using the Color Picker in Adobe Photoshop CS with a 5×5 pixel average. RGB values were recorded for two regions: the dorsum and lateral line. In each region, five predetermined points were measured and averaged to yield a single value for R, G and B in that region. The resulting dataset contained six numbers per individual (three RGB values for each of two regions). A pairwise "coefficient of color variation" was calculated among individuals in two sample groups: lowland (six individuals), and highland (12 individuals). These values were loaded into a comparison matrix, similar to a genetic-distance matrix. The coefficient of color variation (CCV) was calculated by summing the absolute value of the difference between each of the six RGB color measurements of two individuals. If the coloration of two individuals was very similar in both regions, the differences in all 6 RGB values was very small; however, if two individuals differed in coloration, differences in one or more of the RGB values would be greater.

Two corresponding genetic-distance matrices were generated in PAUP* v. 4.0b (Swofford, 2004) using Kimura 2-parameter models. The individuals included in the matrices corresponded to those from the photographs, grouped into lowland and highland, as described above. Values from the CCV and genetic-distance matrices for each group were arranged into a single table, with each row containing a CCV and a genetic-distance value comparing two individuals in a group. Because we were interested in comparing color divergence among individuals with similar levels of genetic divergence, all samples that exceeded 7% genetic divergence (the maximum amount of divergence observed within the lowland *E. hahneli* group) were removed from the analysis. The resulting dataset retained 33 of 78 values from the highland group, and allowed a comparison of color divergence within a subset of highland species that reflect levels of genetic divergence similar to those found in the lowland group, so as to remove potentially confounding effects of genetic distance.

Comparisons of CCV means were performed using Monte Carlo methods in PopTools 2.6.9 (Hood, 2005). A total of 100,000 randomizations of the data were performed. At each iteration, differences in the test statistic between the randomized datasets were compared to the observed differences. The frequency at which differences in the randomized test statistic were greater than the observed differences was used to estimate a *p*-value.

2.7. Ancestral-state reconstruction

Ancestral-state reconstruction was performed with SIMMAP 1.0, using the last 30,000 stored tree files from the Bayesian analysis. A character matrix was generated for all taxa by coding two different binary characters: highland vs. lowland collection locality, and dull vs. brightly colored. The highland vs. lowland character was coded based on

Table 2

Species/Code	Location	Elevation	Coordinates
Allobates femoralis **P1**	Itaya, Loreto, Peru	100 m	S 4.45′ W 73.57′
A. femoralis **P2**	Itaya, Loreto, Peru	100 m	S 4.45′ W 73.57′
A. femoralis **P3**	Loreto, Peru	Lowland	NA
A. femoralis **P4**	Boca Manu, Cuzco, Peru	250 m	S12.25′ W 70.9′
A. femoralis **P5**	Saposoa, San Martin, Peru	850 m	S 6.77107′, W 76.94120′
A. femoralis **P6**	Loreto, Peru	Lowland	NA
A. femoralis **P7**	Rio Sucusari, Loreto, Peru	100 m	S 3.24073′ W 72.92835′
A. femoralis **P8**	Tahuayo, Loreto, Peru	140 m	S 4.18707′ W 73.10457′
A. femoralis **P9**	Loreto, Peru	Lowland	NA
A. femoralis **P10**	Rio Manova Loreto, Peru	110 m	S 3.65201′ W 72.20045′
Colostethus sp.	Bonilla, San Martin, Peru	200 m	S 6.21007′ W 76.27226′
Epipedobates bassleri **P1**	Saposoa, San Martin, Peru	850 m	S 6.77107′, W 76.94120′
E. bassleri **P2**	Altoshima, San Martin, Peru	670 m	available upon request
E. bassleri **P3**	Chazuta, San Martin, Peru	560 m	available upon request
E. bassleri **P4**	Sauce, San Martin, Peru	720 m	available upon request
E. bassleri **P5**	Huallaga, San Martin, Peru	390 m	available upon request
E. bassleri **P6**	Cainarachi, San Martin, Peru	920 m	S 6.48726′ W 76.31520′
E. bassleri **P7**	Tarapoto, San Martin, Peru	760 m	S 6.47152′ W 76.30297′
E. bassleri **P8**	Sisa, San Martin, Peru	550 m	available upon request
E. bassleri **P9**	Sisa, San Martin, Peru	550 m	available upon request
E. bassleri **P10**	Tarapoto, San Martin, Peru	780 m	S 6.43307′ W 76.30383′
E. bassleri **P11**	Moyobamba, San Martin, Peru	320 m	S 6.32393′ W 76.73437′
E. bilinguis	Primavera, Napo, Ecuador	300 m	S 0.23544′ W 76.96819′
E. cainarachi **P1**	Cainarachi, San Martin, Peru	350 m	S 6.45043′ W 76.31758′
E. cainarachi **P2**	Tarapoto, San Martin, Peru	770m	S 6.43536′ W 76.35011′
E. hahneli **B1**	Amazonas, Brazil	50 m	NA
E. hahneli **B2**	Amazonas, Brazil	50 m	NA
E. hahneli **P1**	Ivochote, Cuzco, Peru	620 m	S12.47086′ W 72.99389′
E. hahneli **P2**	Sisa, San Martin, Peru	650 m	S 6.58299′ W 76.50974′
E. hahneli **P2**	Tarapoto, San Martin, Peru	500 m	NA
E. hahneli **P3**	Tarapoto, San Martin, Peru	500 m	NA
E. hahneli **P4**	Tarapoto, San Martin, Peru	600 m	S 6.47777′ W 76.32274′
E. hahneli **P6**	Saposoa, San Martin, Peru	850 m	S 6.77107′ W 76.94120′
E. hahneli **P7**	Chazuta, San Martin, Peru	560 m	S 6.52818′ W 76.13942′
E. hahneli **P8**	Tarapoto, San Martin, Peru	500 m	NA
E. hahneli **P9**	Tarapoto San Martin, Peru	600 m	S 6.47777′ W 76.32274′
E. hahneli **P11**	Itaya, Loreto, Peru	100 m	S 4.45′ W 73.57′
E. hahneli **P10**	Rio Manova Loreto, Peru	110 m	S 3.65201′ W 72.20045′
E. hahneli **P12**	Itaya, Loreto, Peru	100 m	S 4.45′ W 73.57′
E. hahneli **P13**	Convento, San Martin, Peru	200 m	S 6.25107′ W 76.31459′
E. hahneli **P14**	Alto Purus, Ucayali, Peru	300 m	S10.90′ W 73.17′
E. hahneli **P15**	Alto Purus, Ucayali, Peru	300 m	S10.90′ W 73.17′
E. hahneli **P16**	Alto Purus, Ucayali, Peru	300 m	S10.90′ W 73.17′
E. hahneli **P I7**	Allpahuayo, Loreto, Peru	130 m	S 3.87′ W 73.57′
E. hahneli **P18**	Boca Manu, Cuzco, Peru	250 m	S12.25′ W 70.9′
E. macero **P1**	Ivochote, Cuzco, Peru	620 m	S 12.47086′ W 72.99385′
E. macero **P2**	Alto Purus, Ucayali, Peru	300 m	S10.90′ W 73.17′
E. pongoensis **P1**	Convento, San Martin, Peru	200 m	S 6.25107′ W 76.31459′
E. pongoensis **P2**	Huallaga, San Martin, Peru	390 m	S 6.54870′ W 75.96169′
E. silverstonei	Tingo Maria, Huanuco, Peru	1000 m	S 9.31918′ W 75.97697′
E. simulans **P1**	Madre de Dios, Peru	380 m	S12.97040′ W 70.34107′
E. simulans **P2**	Quincemille, Cuzco, Peru	490 m	S13.18396′ W 70.63308′
E. smaragdinus	Iscozazin, Pasco, Peru	350 m	S10.18879′ W 75.16052′
E. trivittatus **B1**	Amazonas, Brazil	50 m	NA
E. trivittatus **B2**	Porto Walter, Acre, Brazil	200 m	S8.25′ W 72.74′
E. trivittatus **FG**	French Guiana	200 m	S 3.9′ W 53.0′
E. trivittatus **P1**	Rio Sucusari, Loreto, Peru	100 m	S 3.24073′ W 72.92835′
E. trivittatus **P2**	Iscozazin, Pasco, Peru	350 m	S 10.18879′ W 75.16052′
E. trivittatus **P3**	Panasa, San Martin, Peru	Lowland	NA
E. trivittatus **P4**	Tahuayo, Loreto, Peru	140 m	S 4.18707′ W 73.10457′
E. trivittatus **P5**	Tahuayo, Loreto, Peru	140 m	S 4.18707′ W 73.10457′
E. trivittatus **P6**	Santa Rosa, Huanuco, Peru	Lowland	NA
E. trivittatus **P7**	Chazuta, San Martin, Peru	260 m	S 6.57434′ W 76.14362′
E. trivittatus **P8**	Barranquita, San Martin, Peru	220 m	S 6.28910′ W 76.22865′
E. trivittatus **P9**	Chumilla, San Martin, Peru	Lowland	NA

Table 2 (*continued*)

Species/Code	Location	Elevation	Coordinates
E. trivittatus **P11**	Loreto, Peru	Lowland	NA
E. trivittatus **P12**	Alto Purus, Ucayali, Peru	300 m	S10.90′ W 73.17′
E. trivittatus **P13**	Bonilla, San Martin, Peru	200 m	S 6.21007′ W 76.27226′
E. trivittatus **P14**	Tarapoto, San Martin, Peru	540 m	S 6.43066′ W 76.29034′
E. trivittatus **P15**	Boca Manu, Cuzco, Peru	300 m	S 12.25′ W 70.90′

elevation as described above. The dull vs. brightly colored character was coded by designating brightly colored frogs as those with more than 20% of their body displaying a color other than brown, gray or black.

2.8. Test of neutrality

To investigate the hypothesis of neutral evolution we used Tajima's *D* test (Tajima, 1989), implemented in the program MEGA2 (Kumar et al., 2001). This method compares the number of segregating nucleotide sites to the nucleotide diversity. Under the neutral model, there should be no difference between these two quantities. This statistic is influenced by both recent population-level dynamics, as well as long-term mutational patterns (Garrigan and Hedrick, 2003). We used two different intraspecific datasets for these analyses: *E. bassleri* sequences from the transition zone and *E. hahneli* sequences from the lowlands. We focused on variation in the cytochrome *b* gene region, the largest gene region analyzed in our study.

3. Results

3.1. Phylogenetic analysis

The final dataset included 1778 bp (800 from cyt*b*, 479 from 12S and 497 from 16S), of which 535 were parsimony informative. The topology that resulted from the Bayesian phylogenetic analysis is shown in Fig. 1.

Populations of *E. hahneli* formed two distinct clades (Fig. 1): one clade mainly comprising individuals from lowland populations throughout the Amazon basin (see maps, Figs. 2A–C), forms the sister group to the rest of *Epipedobates*. Within this lowland clade, a division occurs between northern (near the Itaya River) and southern (Brazilian and southeastern Peruvian) populations. The individual from Convento, which is geographically adjacent to the localities for the highland *E. hahneli* clade, falls within the northern lowland clade. Thus, the Convento population may be the result of an expansion from the lowlands to the base of the highlands, independent of the one that led to the radiation of the highland clade in this analysis.

The other clade of *E. hahneli*, consisting largely of populations from the highland, transition-zone region of central cordilleras in San Martin, Peru, appears to be the sister taxon to a morphologically diverse clade of highland endemic species. *Epipedobates macero*, a highland endemic species from southeastern Peru, is the sister taxon to this highland *E. hahneli* clade. The large highland clade, including the highland *E. hahneli* and all of the montane endemic species except *E. simulans*, is separated from lowland *E. hahneli* clade by the clade comprising *E. trivittatus* and *E. simulans*. Within the highland *E. hahneli* clade, an individual from Ivochote, in southeastern Peru, represents the sister lineage of a group of individuals from the central cordilleras near Tarapoto.

The average genetic distance within the highland *hahneli* clade, calculated using a Kimura 2-parameter model in MEGA version 2.1 (Kumar et al., 2001), was 2.9%. Similarly, the average genetic distance within the lowland clade was 3.2%. The mean distance between the two clades, calculated by designating each one as a separate group in MEGA, was 7.1%. This distance is comparable to those between species of *Epipedobates* (e.g., 7.9% between *E. bassleri* and the highland *E. hahneli* clade, or 8.0% between *E. trivittatus* and the lowland *E. hahneli* clade).

Epipedobates trivittatus, the other widespread, lowland species included in this analysis, was supported as a monophyletic group by high Bayesian posterior probabilities (Fig. 1). This species appears to be the result of a recent expansion, as the geographic structure seen in the lowland clade of *E. hahneli* is not seen in *E. trivittatus*. Generally, species with limited phylogeographic structure have had some fluidity of geographic movement over recent evolutionary time, and lineage sorting has not yet led to geographic partitioning of phylogenetic branches (Avise, 2000). Branch lengths within the clade of *E. trivittatus* were generally very short (Fig. 1), and Kimura 2-parameter genetic distances revealed an average divergence of 1.6%. Morphologically, *E. trivittatus* is relatively conserved across its range, although there is some variation in color (yellow to green) and number (two to three) of stripes (Silverstone, 1976). Genetic distances between the members of the highland clade indicate that the highland species may not be very closely related to one another. With the exception of *E. cainarachi* and *E. smaragdinus*, which were most closely related to one another by genetic distances of 2.6–3.0%, the shortest genetic distance from each of the highland species was to an individual of *E. hahneli* from the road to Yurimaguas from Tarapoto. Distances ranged from 3.0 to 5.5% from this individual to the various highland species, whereas distances between highland taxa on adjacent branches ranged from 6.6 to 9.0%.

3.2. Biogeographic analysis

DIVA analysis revealed complex patterns of ancestral dispersal for many species of *Epipedobates* (Figs. 3–5). The ancestors of *E. trivittatus* appear to have radiated north and south along the transition zone and lowland regions, as well as eastward across the Amazon basin (Fig. 5, map a). The highland populations of *E. trivittatus* appear to derive from a reinvasion of the highlands by this group. Similar patterns appeared in the lowland *E. hahneli* group (Fig. 5, map d). The ancestor of the highland *E. hahneli* clade appears to have originated in southern Peru and radiated northward along the transition zone (Fig. 5, map b), whereas the highland *Epipedobates* clade appears to have

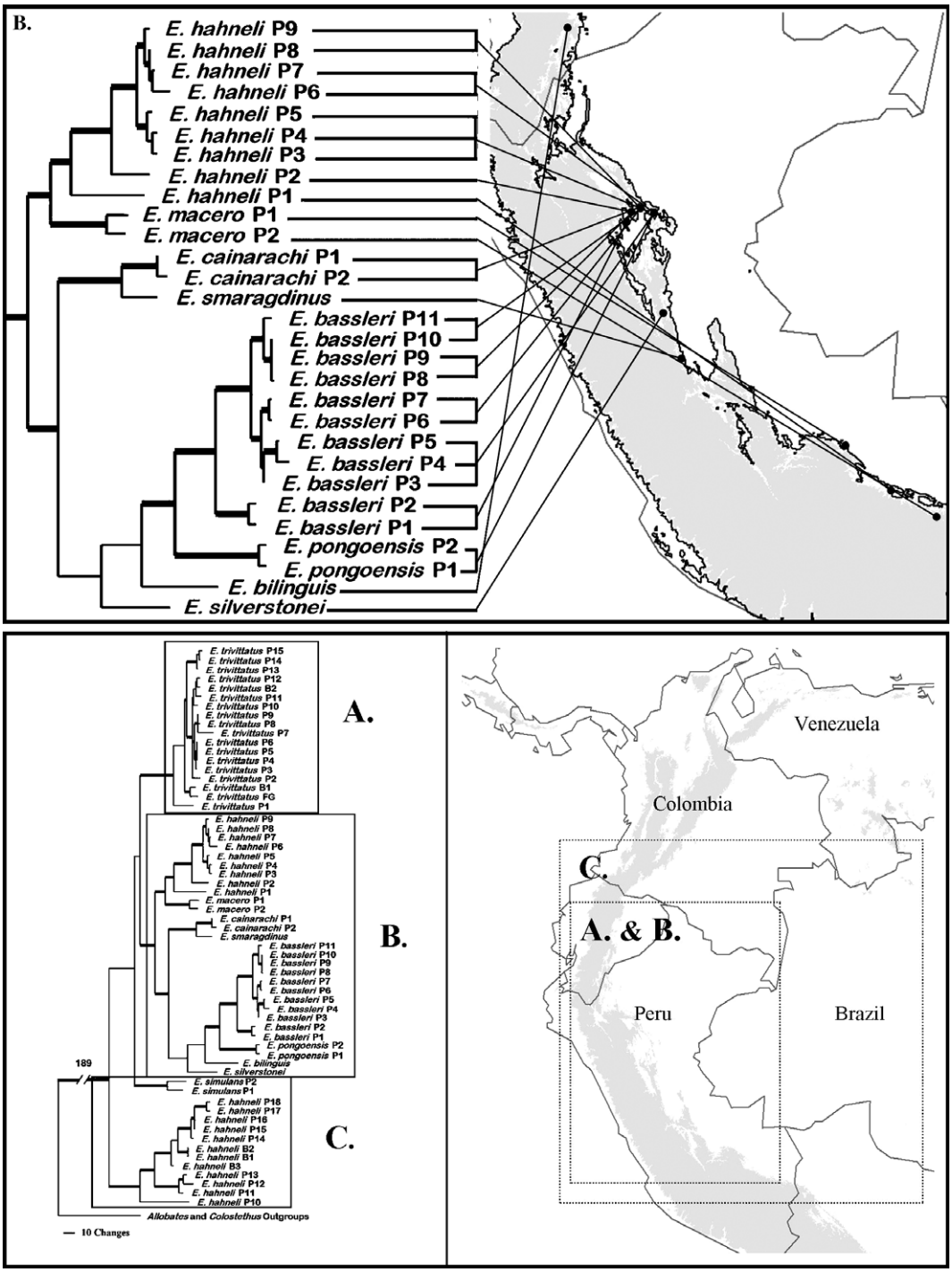


Fig. 2. Sample distributions. Tree indicates which taxa are represented on each distribution map; overview of the scope of each detailed distribution map is shown. On all maps, shaded areas represent regions above 1000 m. On detailed distribution maps, a black line depicts the Andean fall line, average elevation 300 m, representing the division between lowland and highland species. Detail map A depicts sample distribution of the *E. trivittatus* clade. Detail map B depicts sample distribution of the montane clade. Detail map C depicts sample distribution of the lowland *E. hahneli* clade.

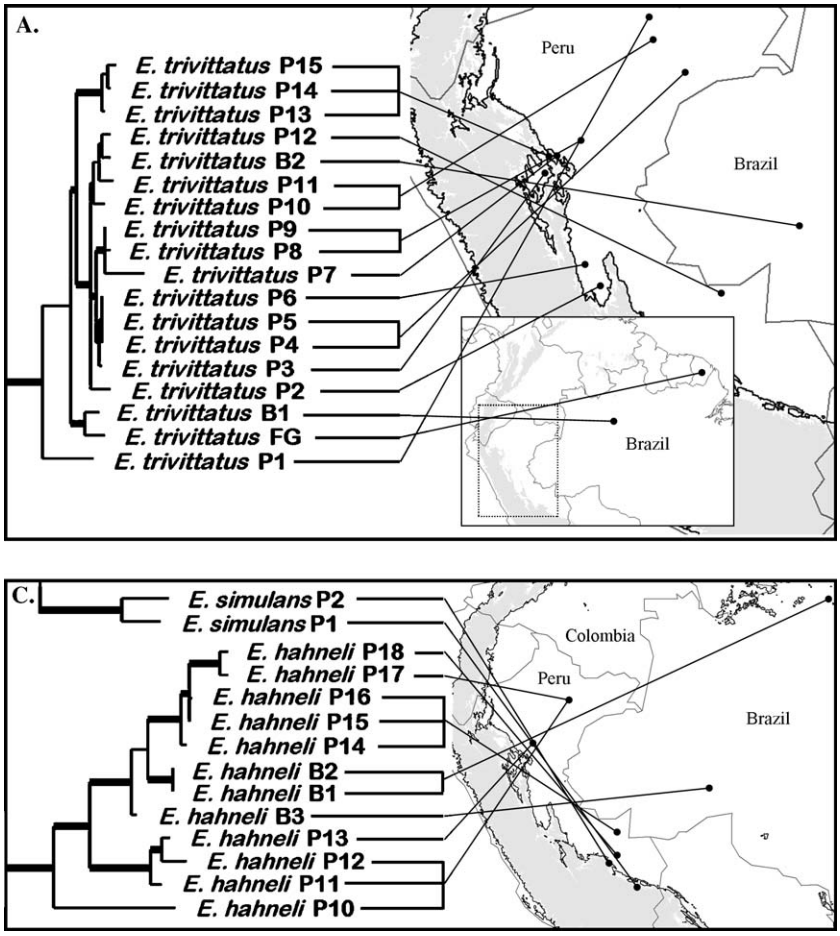


Fig. 2 (*continued*)

radiated northward and southward from north central Peru (Fig. 5, map c). Analysis of deeper nodes indicates one dispersal into the highlands, followed by subsequent reinvasion of the lowlands.

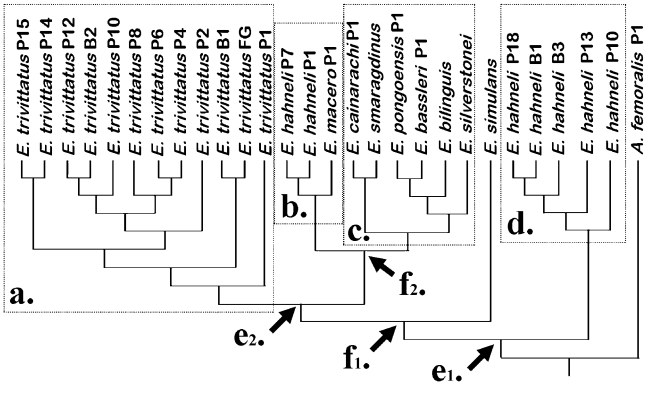


Fig. 3. Pruned tree from reduced dataset used in DIVA analysis. Each taxon reflects a unique species or population from each of ten different geographic groups (see Section 2: Biogeographic Analysis). Letters a, b, c and d represent groups analyzed for ancestral-distribution and depicted in Fig. 5 (a, *E. trivittatus* group; b, lowland *E. hahneli* group; c, montane group; d, lowland *E. hahneli* group). Letters e_1, e_2 and f_1, f_2 represent nodes examined in the highland vs. lowland ancestral distribution analysis (e_1, e_2: low and f_1, f_2: high: both distributions are listed under e and f (respectively) in Fig. 5).

3.3. Comparative analysis of coloration

While there was no significant difference in genetic variation between the highland group and the lowland *E. hahneli* group, analysis of color variation revealed a significant difference in coloration between the two groups ($p < 0.0001$). Despite comparable genetic distances, the

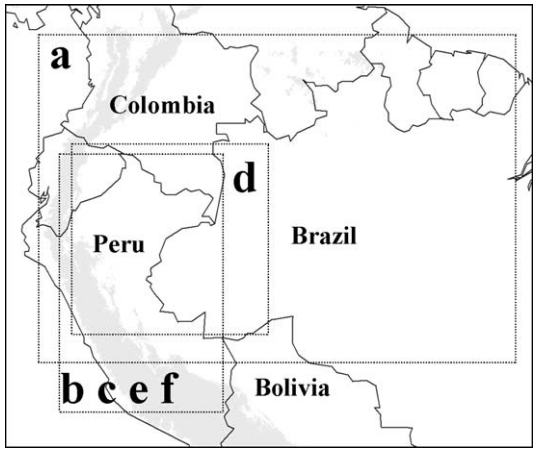


Fig. 4. Scope of maps from DIVA analysis. Letters correspond to tree in Fig. 3 and to detailed distribution maps in Fig. 5.

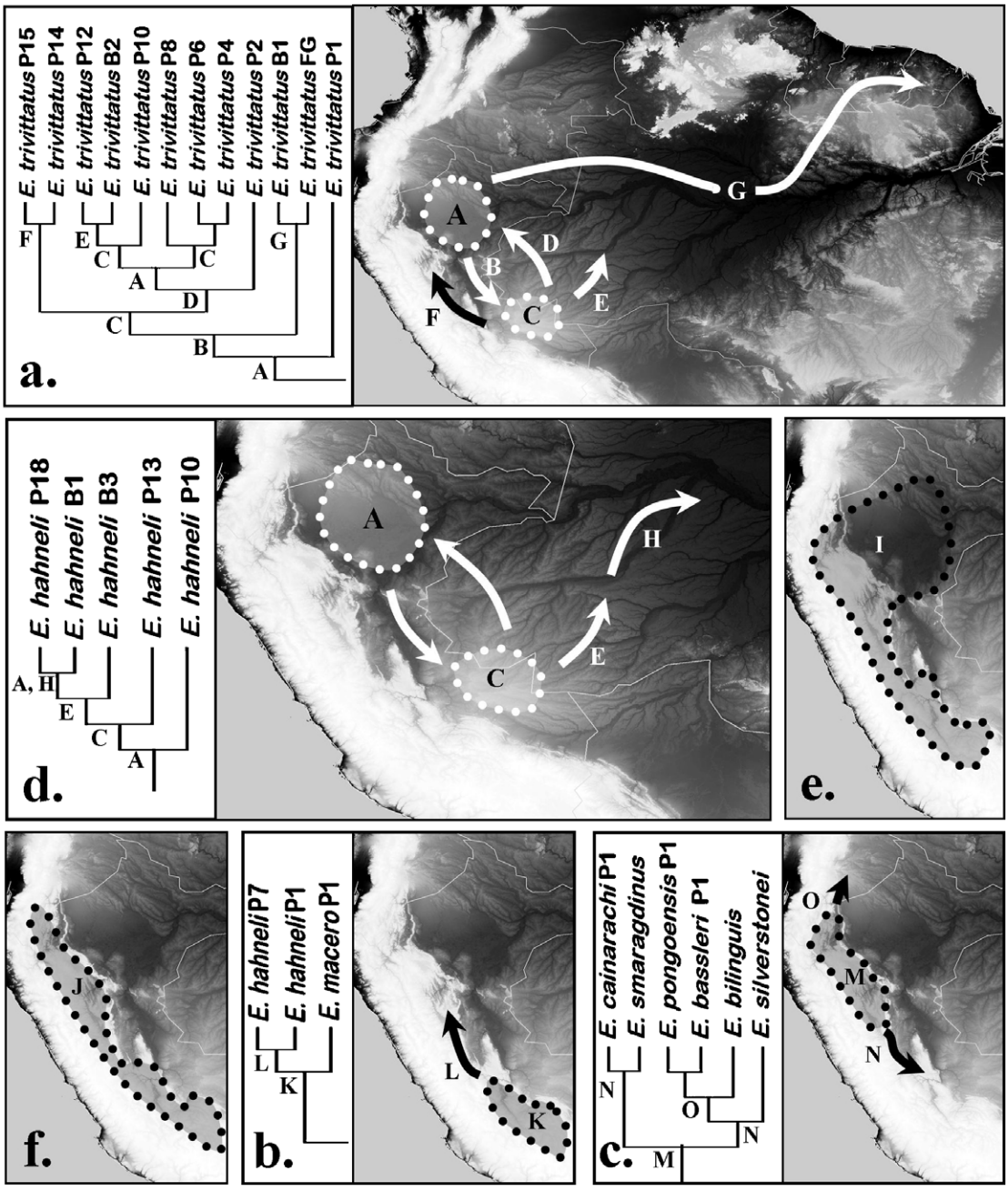


Fig. 5. Detailed maps from DIVA ancestral distribution analysis. Lighter areas represent higher elevations. Lowercase letters correspond to those on the tree in Fig. 3 (the region of the tree containing taxa depicted on each map is shown next to each map) and on the overview map (Fig. 4). Uppercase letters correspond to ancestor distributions, as depicted on each map, and arrows illustrate the general directions of ancestor dispersal. Map a: Biogeography of *E. trivittatus* clade. Map b: Biogeography of montane *E. hahneli* clade. Map c: Biogeography of montane clade. Map d: Biogeography of lowland *E. hahneli* clade. Maps e and f: distributions predicted for ancestors at deeper nodes (see Fig. 3).

highland species exhibit significantly greater color variation than the lowland *E. hahneli* group (Fig. 6).

3.4. Ancestral state reconstruction

The SIMMAP analysis indicated that the ancestor of *Epipedobates* likely was dully colored and of lowland origin, and that a transition both to the highlands and bright coloration occurred in the ancestor to the highland *E. hahneli*/*E. trivittatus* clade (Fig. 1). A transition back to dull coloration occurred in the lineage leading to the highland *E. hahneli* clade; the ancestor to that clade has a 30% probability of being of highland origin, though the ancestor of the highland *E. hahneli* and *E. macero* clade appears to have been both brightly colored and highland (Fig. 1). The lineage leading to *E. trivittatus* appears to represent a secondary radiation into the lowlands, as the ancestor of the *E. trivittatus* clade was recovered as being of lowland origin.

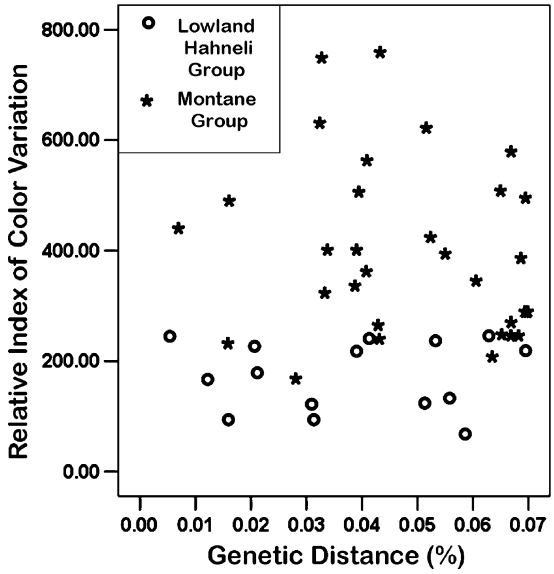


Fig. 6. Scatterplot comparing color variation, calculated using the coefficient of color variation (CCV; see Section 2), and genetic variation in the lowland *E. hahneli* group and the montane group. Monte Carlo comparison of means showed a significant difference in coloration between these groups ($p < 0.0001$), but not in genetic distance ($p < 0.0971$).

3.5. Test of neutrality

Tajima's *D* tests indicated no significant departures from neutrality in either species: *E. bassleri*: *N* sequences = 11, *N* sites = 729, pS (segregating sites per site) = 0.076, π (nucleotide diversity) = 0.028, Tajima's *D* = 0.386; *E. hahneli*: *N* sequences = 9, *N* sites = 700.

4. Discussion

We begin by discussing the systematics and biogeography of the Peruvian *Epipedobates* and related taxa. We then discuss the relevance of our results to predictions from the hypotheses for transition zone diversification and speciation discussed Section 1.

4.1. Systematics and biogeography of peruvian epipedobates

The phylogenetic relationships within Peruvian *Epipedobates* derived from our analysis are not entirely those predicted by previously established species designations. In particular, *E. hahneli* is recovered as a paraphyletic taxon with respect to *E. trivittatus* and the highland endemic members of the genus. *Epipedobates hahneli* is a morphologically conservative species that occurs across the Amazonian lowlands and into the Andean slopes on the western front of the basin (Haddad and Martins, 1994). Each of the two clades recovered for this species was supported by high Bayesian posterior probabilities (Fig. 1). The paraphyly of *E. hahneli* was tested using a Shimodaira and Hasegawa (1999) test that compared the recovered tree topology to a topology that constrained *E. hahneli* to be monophyletic. The likelihood score for a monophyletic *E. hahneli* was significantly different from the score for the topology recovered by ML ($p < 0.001$), which supports the hypothesis of paraphyly.

Species-level paraphyly may result from several causes, including inadequate phylogenetic information, unrecognized parology, incomplete lineage sorting, interspecific hybridization or imperfect taxonomy (Funk and Omland, 2003). Imperfect taxonomy provides the most likely explanation for the paraphyly of *E. hahneli*; it comprises a nonmonophyletic grouping of lineages that have retained an ancestral morphology and whose evolutionary distinctness was not detected by earlier morphological studies. Such "cryptic species" may reflect the retention ancestral morphology or the convergent evolution of similar morphologies (Funk and Omland, 2003).

The lack of phylogenetic structure within *E. trivittatus* was also unexpected. Other taxa of frogs that are widespread across Amazonia show deep phylogenetic divergence and high genetic distances (e.g., *Allobates femoralis*: Lougheed et al., 1999), but this is not the case for *E. trivittatus*. Instead, this species seems to have undergone a recent rapid radiation across Amazonia, apparently after colonizing the lowlands from the transition-zone.

4.2. Single versus multiple origins of transition zone taxa

The results of the dispersal–vicariance analysis using DIVA and the reconstruction of ancestral states using SIMMAP both support a single origin of transition-zone taxa from lowland ancestry (with the exception of one recolonization event in *E. trivittatus*), with later dispersal and vicariance within the south-to-north corridor formed by the transition zone (Figs. 1 and 5). Hence, our results stand in contrast to the predictions of hypotheses of repeated parapatric speciation across ecological gradients (e.g., Endler, 1982) or repeated allopatric speciation across barriers to dispersal imposed by changes in elevation in combination with climatic fluctuations (e.g., Bush, 1994). Instead, our results support a single origin of transition-zone species from a colonization event in the northern part of Peru. The ancestors of these colonists dispersed in a complex manner throughout the transition zone and gave rise to the high species diversity seen in the region today. This pattern has been seen in other taxa found in the transition zone of the eastern Andean versant (e.g., Cracraft, 1985; Burns and Naoki, 2004; Pérez-Emán, 2005). In some cases, sister-species pairs are allopatric (e.g., *E. smaragdinus* and *E. cainarachi*), whereas in others they are partially sympatric (e.g., *E. pongoensis* and *E. bassleri*) (Fig. 1). Hence, there is no clear pattern suggesting that allopatric or parapatric/sympatric speciation is a more common mode of speciation in these frogs. However, a more detailed analysis of genetic and phenotypic divergence within a single species (*E. bassleri*) suggests that geographic isolation is associated with both genetic and phenotypic divergence among populations, suggesting a role for allopatry in divergence and speciation in these frogs (Roberts et al., in press).

4.3. Selection on coloration versus genetic drift

The analyses of coloration and genetic distance indicate that divergence among populations and species in coloration has been accelerated relative to genetic divergence in neutral markers (Fig. 6). In turn this supports the hypothesis that selection on morphology across heterogeneous habitats or social environments in the transition zone has driven divergence, rather than divergence in color being a simple consequence of increased genetic drift in the presence of more effective barriers to gene flow in the transition zone. Hence, our results support the general hypothesis that selection drove divergence among populations in the transition zone. Note that we infer some effect of geographic isolation on phenotypic divergence from a general association between divergence and geographic separation among *E. bassleri* populations (see above), but we infer an additional effect of selection from the accelerated divergence in coloration among highland populations and species, relative to divergence among lowland populations with similar levels of genetic divergence.

The type of selection is not known, but selection on coloration may be associated with toxicity and aposematism. Comparative analyses have indicated a role for selection in the evolution of aposematic coloration across the poison-frog family (Summers and Clough, 2001; Santos et al., 2003), and recent research indicates selection on coloration in the context of mimicry in several species of the genus *Epipedobates* (Darst and Cummings, 2006). Our study indicates that highland species are more brightly colored than their lowland ancestors (Fig. 1). Why this is the case is not known, but it could be associated with changes in diet (Darst et al., 2005). Assuming that the highland habitat is conducive to brighter coloration in the context of aposematism, different populations may have evolved different versions of aposematic coloration. If the populations were allopatrically isolated due to the various processes that may accelerate isolation in the highlands (e.g., Lynch and Duellman, 1997; Fjeldsa and Rahbek, 2006), then selection to maintain similarity in coloration between populations should have been weak or non-existant. Hence, each isolated population would have been free to evolve its own specific brighter, more contrasting coloration in response to the local predation regime. Note that the effects of selection are likely to be enhanced in allopatry (Schluter, 2000). Hybrids produced if and when populations came into contact again would then be disfavored by selection against individuals without the normal aposematic phenotype (e.g., Jiggins et al., 2001).

Alternatively, divergence in coloration among populations of poison frogs could be influenced by sexual selection in the context of divergent female preferences (Summers et al., 1999; Siddiqi et al., 2004). In a more detailed study of genetic and morphological variation among populations in one transition-zone species (*E. bassleri*), Roberts et al. (in press) found rapid divergence in coloration among populations, and also found that divergence in coloration is accelerated relative to both genetic divergence and general morphological divergence, compared to populations of *E. hahneli* in the lowlands. Sexual selection is a possible explanation for this kind of pattern (Summers et al., 1999).

These selective scenarios are speculative, but are amenable to testing. For example, the sexual-selection hypothesis predicts that females will prefer males of their own, local color morph (controlling for other factors) in cases where populations show divergent coloration.

4.4. Highland versus lowland origin of the major clades

Several recent studies of birds have provided evidence consistent with the hypothesis that lowland clades are derived from highland clades (e.g., Garcia-Moreno et al., 1999; Voelker, 1999; Burns and Naoki, 2004). In contrast, our results do not support this prediction of the time-for-speciation hypothesis. Reconstruction of ancestral distributions using dispersal–vicariance analysis as implemented in DIVA indicates that highland taxa were derived from lowland taxa rather than the reverse. Reconstruction of ancestral states using SIMMAP to map posterior probabilities indicates that the ancestral state basal to the highland clade was lowland, and that (with the exception of *E. trivittatus*), the lowland clades are not derived from highland clades. Hence, our results indicate dispersal from the lowlands into the highlands, rather than the reverse, for the origin of the major highland clade. In turn, our results are consistent with an elevated rate of speciation in the highlands relative to the lowlands, as opposed to a longer period of time for lineage accumulation to occur.

The time-for-speciation hypothesis can be considered a "null model" for the occurrence of high species diversity. Other null models for high species diversity based on ecological considerations have been proposed (notably the "mid-domain effect" (Colwell et al., 2004)). However, the mid-domain effect is likely to be confounded by patterns of phylogenetic lineage accumulation (Davies et al., 2005). Hence, we consider the time-for-speciation hypothesis a more appropriate null model.

5. Conclusions

We have used our results to test predictions from several contrasting sets of hypotheses concerning the high levels of population and species diversity in the transition zone of the Andes versant. Dispersal–vicariance analysis and ancestral-state reconstruction indicate a single colonization of the highlands (with the exception of a single reinvasion of the transition zone by *E. trivittatus*). This result contrasts with the predictions of several proposed models for the generation of highland diversity, including repeated divergence across ecological gradients between parapatrically distributed populations, or repeated parallel bouts of allopatric divergence and speciation between

the lowlands and highlands. Our results are consistent with models that focus on divergence among populations after colonization by a single ancestral population that dispersed along the Andes versant. Our comparative analyses of divergence in coloration and mtDNA suggest that selection, rather than genetic drift, has been the main factor driving divergence in coloration. The dispersal-vicariance analysis and the ancestral-state reconstructions both indicate that the highland taxa arose from lowland ancestry, rather than the reverse. This result is counters a major prediction of the time-for-speciation hypothesis, but confirms the predictions of a variety of other hypotheses explaining the generation of highland diversity. Hence, overall, our results support a scenario in which a single ancestral population of an "*E. hahneli* like" population from the lowlands invaded the highlands and dispersed in a complex manner (including reinvasion of the lowlands). These dispersed populations then diverged (probably in allopatry) due to divergent selection regimes.

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