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The role of tropical dry forest as a long-term barrier to dispersal: a comparative phylogeographical analysis of dry forest tolerant and intolerant frogs

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Abstract

We used a comparative phylogeographical approach to investigate the origins of the disjunct wet forest biota of the Golfo Dulce region along the Pacific slope of Costa Rica. This region is isolated by Pacific dry forests north and south and isolated from Caribbean wet forests by mountains. We studied three sympatric lowland frog species in the *Craugastor fitzingeri* species group that prefer wet forest but differ in their response to dry habitats. In dry forest, *C. fitzingeri* can survive along streams while *C. crassidigitus* and *C. talamancae* are entirely absent. We collected samples from across the ranges of all three species, and obtained mitochondrial DNA sequence data from the COI and cytochrome *b* genes. We observed significant phylogeographical structure in *C. crassidigitus* and *C. talamancae*, but much less in *C. fitzingeri*, demonstrating that mountain barriers and dry forest habitat have reduced mitochondrial gene flow in the strictly wet-forest species. Additionally, we discovered that the Golfo Dulce and Central Panama populations of *C. crassidigitus* appear to have diverged in the Pliocene or earlier, suggesting that the dry forest separating these populations is old. Our phylogenetic analysis of 12 of approximately 16 species of the *C. fitzingeri* species group suggests that the three lowland species are each other's closest relatives. Because of this shared phylogenetic history, we attribute the striking differences in phylogeographical structure to the different ecologies of the frogs. In summary, we find that what appear to be minor differences in the natural history of these three closely related species may profoundly impact the potential for dispersal, range size, and cladogenesis.

Keywords: Central America, dispersal, phylogeography, SOWH test, tropical dry forest, tropical wet forest

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Introduction

The primary goal of biogeographical studies is to characterize the distribution patterns of organisms and determine the evolutionary forces, environmental conditions, and historical events that have shaped these patterns (Lomolino *et al*. 2004). Comparing species ranges with habitat heterogeneity may allow one to evaluate the influence of environmental conditions on the distribution of species (Graham *et al*. 2004). However, our ability to infer process from pattern is limited because environments also change and past conditions are often unknown. Furthermore, species may be able to acclimate and survive harsh conditions long enough to traverse apparent environmental barriers. Thus, we would like to know which environmental barriers have influenced organismal distributions over the long term and which are only recent or ephemeral phenomena (e.g. Wüster *et al*. 2005). Here, we use the genealogical information provided by comparative phylogeographical analysis (e.g. Bermingham & Avise 1986; Avise 2000; Schäuble & Moritz 2001; Campbell *et al*. 2006) to investigate the relative roles of mountains and tropical dry forest habitat in shaping the geographical distributions of related species of predominantly wet forest leaf-litter frogs on the Pacific and Caribbean versants of lower Central America (Fig. 1).

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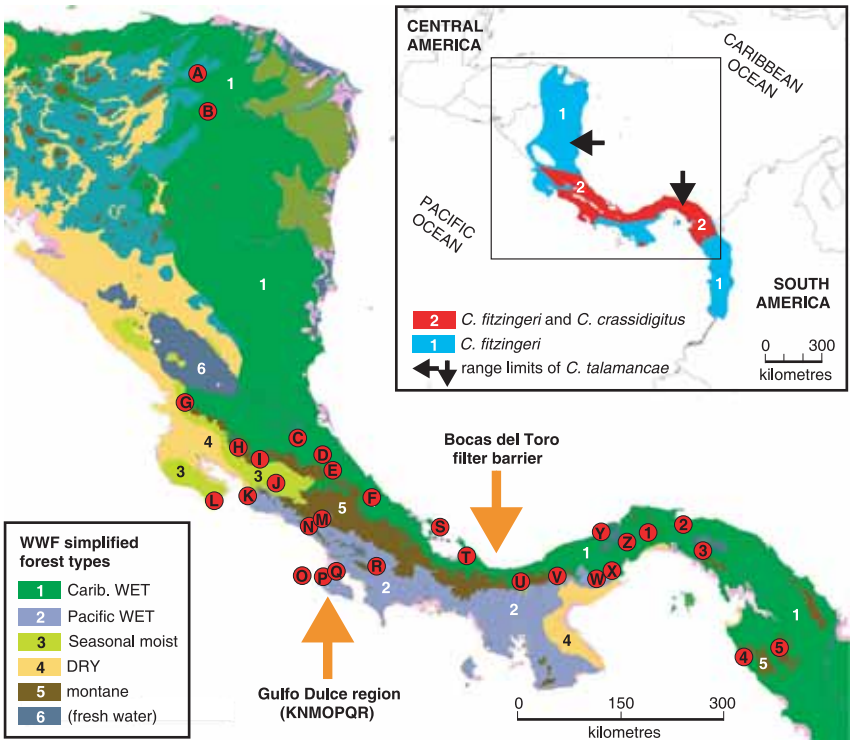


Fig. 1 Map of Isthmian Central America showing sampling sites for *Craugastor fitzingeri* group samples used in this study. Letters (A–Z) and numbers (1–5) in red circles denote areas where at least one of the following species was sampled: *C. crassidigitus*, *C. fitzingeri*, or *C. talamancae*. Red dots may combine neighbouring collecting localities into a single 'area', e.g. area 'W' covers collecting localities 'Altos del María' and 'El Valle de Anton' (see Table 1). Inset map shows the geographical distributions of *C. crassidigitus*, *C. fitzingeri*, and *C. talamancae* on a political map of lower Central America, according to the Global Amphibian Assessment database (IUCN *et al.* 2004). Note, the geographical isolation of Golfo Dulce *C. crassidigitus* may be seen more clearly in Savage (2002). The geographical distribution of *C. talamancae* is limited to the Caribbean versant, with northern and eastern range limits indicated by grey arrows. Coloured regions in the main map represent simplified World Wildlife Fund ecoregions ('Caribbean WET' combines three WWF ecoregions: Central American Atlantic moist, Isthmian-Atlantic moist, and Chocó-Darién moist. 'DRY' combines two ecoregions: Central American and Panamanian dry forests. 'Montane' combines three WWF ecoregions: Central American, Talamancan and Eastern Panamanian montane forests). Note, the distributions of wet- vs. dry-forest amphibians in the GAA database would suggest that the eastern half of the 'Pacific Wet' WWF ecoregion indicated here may be more akin to dry forest. The Golfo Dulce region and the approximate location of the Bocas del Toro filter barrier (see Discussion) are indicated by orange arrows.

While most phylogeographical and biogeographical studies of lower Central America have focused on the biotic interchange between North and South America (e.g. Simpson 1940; Vanzolini & Heyer 1985; Webb 1985; Bermingham & Martin 1998; Zeh *et al*. 2003a, b), a smaller but perhaps more enigmatic puzzle has been largely ignored. Many of the plant and animal species found in the tropical moist and wet forests (Holdridge 1967) of the Caribbean lowlands of lower Central America also have discontinuous populations in the wet forest on the Pacific versant in the Golfo Dulce region of southwest Costa Rica (McDiarmid & Savage 2005). Geographical distributions are generally well characterized for vertebrates, and the disjunct pattern of the Golfo Dulce fauna has been documented for birds, snakes, and frogs. Specific examples from birds of various families include black-faced antthrush (*Formicarius analis*, Formicariidae), buff-throated foliage-gleaner (*Automolus ochrolaemus*, Furnariidae), bicoloured antbird (*Gymnopithys leucaspis*, Thamnophilidae), and wedge-billed woodcreeper (*Glyphorynchus spirurus*, Dendrocolaptidae) (Ridgely *et al*. 2003). The Golfo Dulce disjunction pattern is rare among lizards but common among snakes, including the eyelash viper (*Bothrops schlegelii*, Viperidae) and various colubrids (e.g. *Erythrolamprus mimus*, *Rhadinaea decorata*, *Tantilla supracincta*, and *Urotheca guentheri*) (Köhler 2003). Frog species that show this disjunct pattern include *Bufo haematiticus* (Bufonidae), *Dendropsophus ebraccatus* and *Smilisca phaeota* (Hylidae), as well as *Craugastor crassidigitus* (Brachycephalidae) (Savage 2002).

Wet-forest populations in the Pacific Golfo Dulce region are isolated from conspecific populations elsewhere by dry forests and mountains. On the Pacific slope, the Golfo

Dulce is bounded on the northwest and southeast sides by the tropical dry forests of the Guanacaste Province of Costa Rica and the Azuero Peninsula of Panama, respectively (Holdridge 1967; Tosi 1969). Caribbean populations are separated from the Golfo Dulce by the 2000-m high continental divide of the Talamanca Mountains (Savage 1982; McDiarmid & Savage 2005). The Pacific Coast dry forests of Guanacaste and the Azuero Peninsula presumably result from rain shadows caused by the Tilarán and Tabasará mountains, respectively, which block the moist Caribbean trade winds from the northeast. The Golfo Dulce region, in contrast, hosts a wet forest habitat because the Talamanca Mountains paralleling this area are high enough that a zone of negative pressure is created over the landscape as the high winds pass over the mountains. Moist air from the Pacific Ocean is drawn into this low-pressure zone and supplies the region with rain (Coen 1983).

We use a comparative phylogeographical analysis of three closely related and widely sympatric frog species to investigate the origins of the Golfo Dulce fauna, and ascertain the role of dry forests and mountains in shaping the wet-forest communities of lowland Central America. By studying species with overlapping distributions, we aim to infer the geological and environmental processes that influenced the patterns of dispersal and isolation responsible for the ecological assemblages we observe today (Bermingham & Avise 1986; Hickerson & Cunningham 2005; Kerdelhué *et al*. 2006). Our focal species are Fitzinger's leaf-litter frog *Craugastor fitzingeri*, the slender-toed leaf-litter frog, *C. crassidigitus*, and the Almirante robber frog, *C. talamancae* (Anura: Brachycephalidae). All three are members of the *C. fitzingeri* species group (Miyamoto 1986; Savage *et al*. 2004; Crawford & Smith 2005). The geographical distribution of *C. talamancae* is almost entirely nested within that of *C. crassidigitus*, whose distribution in turn is nested entirely within that of *C. fitzingeri* (Fig. 1, inset). Because *C. crassidigitus* is absent from the Pacific Coast dry forests, the Golfo Dulce populations are well isolated (Savage 2002). *Craugastor fitzingeri* covers the range of *C. crassidigitus* plus it extends northward to eastern Honduras and southward into western Colombia, and is found in dry forest habitats of northern Pacific Costa Rica and southern Panama (Lynch & Myers 1983; Ibáñez *et al*. 2001; Ruíz-Carranza *et al*. 1996; Köhler 2001; McCranie & Wilson 2002; Savage 2002; Lynch & Suárez-Mayorga 2004). *Craugastor talamancae* occurs from 15 to 646-m elevation in Costa Rica (Savage 2002), but we have found this frog as high as 895 m in Central Panama (Table 1). *Craugastor fitzingeri* occurs from near sea level to 1520 m (Savage 2002), while *C. crassidigitus* has been recorded from near sea level to a recorded high of 2000 m (Lynch & Myers 1983).

History and ecology both contribute to the evolution of species ranges and the assembly of communities (Ricklefs & Schluter 1993; Schneider *et al*. 1998). The evolutionary interaction of the *C. fitzingeri* group with the geography and environments of lower Central America provides an opportunity to analyse the relative contributions of biogeographical history and ecological preference in the distribution of wet-forest amphibians found in the Golfo Dulce region of Pacific Costa Rica. We use phylogenetic analyses of mitochondrial DNA (mtDNA) sequence data to distinguish among three *a priori* biogeographical hypotheses regarding the origin of the Golfo Dulce populations of *C. crassidigitus* and *C. fitzingeri*. As illustrated in Fig. 2, these populations may have been connected historically to Caribbean populations (Northern Route), to Central Panama (Eastern Route) or may have been isolated by the rise of the central Talamanca Mountains (vicariance). In turn, we compare the phylogeographical structure of the three species in the *C. fitzingeri* group to determine if geographical range and habitat preference correlate with the degree of population subdivision and historical isolation in these frogs.

Materials and methods

Sampling

Twenty-nine *Craugastor crassidigitus*, 31 *C. fitzingeri* and 10 *C. talamancae* samples were collected from across Costa Rica (CR), Panama (PA) and Honduras (HN). Five of the *C. fitzingeri* samples were collected from four localities beyond the range of *C. crassidigitus*: marked localities A, B, J, and L in Fig. 1. To place our three focal species in their phylogenetic context, we also sampled six additional named species and three undescribed species of the 13-member *C. fitzingeri* group (Savage *et al*. 2004). Of these nine additional *C. fitzingeri* group species, one is a lowland species restricted to eastern Panama and Colombia (*C. raniformis*) and eight are montane frogs restricted to Costa Rica and Panama west of the Canal. The montane species included in this analysis were *C. andi*, *C. cuaquero*, *C. longirostris*, *C. melanostictus*, *C. tabasarae*, *C.* sp. cf. *crassidigitus* (Fortuna, PA), *C.* sp. cf. *longirostris* (near El Copé, PA), and *C.* sp. 'pn' (central and eastern PA). Outgroups for phylogenetic analyses were chosen based on a broad-scale study of *Craugastor* (Crawford & Smith 2005) and included *C. ranoides* and *C. fleischmanni* (*C. rugulosus* species group) plus *C. megacephalus* (*C. biporcatus* species group).

Frogs were collected in the field, photographed, and euthanized with dilute chloretone $(CH_3)COHCCl_3$. Fresh liver samples were stored in an NaCl-saturated buffer containing 0.25 m EDTA and 20% dimethyl sulphoxide (DMSO, Seutin *et al*. 1991). Corresponding voucher specimens were fixed in 10% formalin, stored in 70% ethanol (Pisani 1973), and deposited in biodiversity collections at public research institutions. Additional samples were generously provided by research museums. Detailed sample information is provided in Table 1.

Table 1 Sample codes, institutional voucher numbers, field tag numbers, locality information, and GenBank Accession nos for all *Craugastor* frog samples used in this study. Sample codes correspond to those presented in the gene tree (Fig. 3) and in the text. For each sample code, the letter (A–Z) or number (1–5) preceding the period (.) refers to an area illustrated in Fig. 1, while the numbers following the period distinguish samples within areas. Sample codes with lower case letters (a–d) are from areas where no *C. crassidigitus*, *C. fitzingeri*, or *C. talamancae* were sampled and are not illustrated in Fig. 1. The * symbol denotes a species identification that may be regarded as provisional, pending further investigations. GenBank numbers referring to COI sequences are listed above those referring to cyt *b* sequences

Sample code	Species	Institutional voucher no.†	Field collection no.‡	Collection locality§	Geographical coordinates	GenBank nos
G.1	*andi**	MVZ 207255	DAG 3258	Vulcán Cacao, Guanacaste, CR. 1315 m.	−85.4500, 10.9333	No COI EF629473
E.1	*crassidigitus*	UCR 16390	AJC 0420	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	DQ350166 DQ350209
E.2	*crassidigitus*	UCR 16387	AJC 0407	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	EF629404 EF629445
E.3	*crassidigitus*	UCR 16388	AJC 0417	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	EF629405 EF629446
E.4	*crassidigitus*	UCR 16389	AJC 0419	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	EF629406 EF629447
E.5	*crassidigitus*	UCR 16391	AJC 0421	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	EF629407 EF629448
E.6	*crassidigitus*	UCR 16393	AJC 0424	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	EF629408 EF629449
E.7	*crassidigitus*	UCR 16900	FB 2611	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	EF629401 EF629465
E.8	*crassidigitus*	UCR 16901	FB 2612	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	EF629402 EF629466
E.9	*crassidigitus*	UCR 16899	FB 2610	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	EF629400 No cyt *b*
E.10	*crassidigitus*	UCR 16386	AJC 0402	Santa Marta, Guayacán, Limón, CR. 520 m.	−83.53555, 10.00523	EF629403 EF629444
G.2	*crassidigitus*	MVZ 207250	DAG 3243	PN Volcán Cacao, Guanacaste, CR. 1250 m.	−85.45972, 10.93167	DQ350176 No cyt *b*
H.1	*crassidigitus*	MVZ 207249	DAG 3213	Monteverde, Puntarenas, CR. 1480 m.	−84.70, 10.30	EF629415 EF629471
I.1	*crassidigitus*	UCR 16398	AJC 0567	La Peña, Alfaro Ruíz, Alajuela, CR. 1240 m.	−84.44028, 10.21889	EF629409 EF629451
M.1	*crassidigitus*	UCR 16396	AJC 0556	RB Quebradas, San Isidro, San José, CR. 1315 m.	−83.68966, 09.44005	DQ350167 DQ350210
N.1	*crassidigitus*	UCR 16385	AJC 0385	Tinamaste, Fila Costeña, San José, CR. 500 m.	−83.76663, 09.29505	EF629412 No cyt *b*
N.2	*crassidigitus*	UCR 16384	AJC 0386	Tinamaste, Fila Costeña, San José, CR. 500 m.	−83.76663, 09.29505	EF629413 EF629443
P.1	*crassidigitus*	UCR 16395	AJC 0539	Drake, Osa, Puntarenas, CR. 200 m.	−83.62476, 08.64850	DQ350168 DQ350211
Q.1	*crassidigitus*	UCR 16394	AJC 0538	Rincón de Osa, Puntarenas, CR. 125 m.	−83.52403, 08.70552	DQ350169 DQ350212
R.1	*crassidigitus*	FMNH 257730	AJC 0085	RB Las Cruces, San Vito, Puntarenas, CR. 1000 m.	−82.97582, 08.78333	DQ350170 DQ350213
U.1	*crassidigitus*	FMNH 257606	AJC 0275	PN Santa Fe, Veraguas, PA. 800 m.	−81.05000, 08.61700	DQ350171 DQ350214
V.1	*crassidigitus*	FMNH 257615	AJC 0283	PN Omar Torrijos H., El Copé, Coclé, PA. 750 m.	−80.59167, 08.66667	DQ350172 DQ350215
V.2	*crassidigitus*	MVUP 1853	AJC 0570	PN Omar Torrijos H., El Copé, Coclé, PA. 750 m.	−80.59167, 08.66667	No COI EF629452
W.1	*crassidigitus*	MVUP 2016	AJC 1130	Altos del María, Panamá, PA. 990 m.	−80.07830, 08.63337	No COI EF629463
W.2	*crassidigitus*	MVUP 2017	AJC 1131	Altos del María, Panamá, PA. 980 m.	−80.07830, 08.63337	EF629414 EF629464
X.1	*crassidigitus*	FMNH 257551	AJC 0215	PN Altos de Campana, Panamá, PA. 900 m.	−79.95000, 08.70000	DQ350173 DQ350216
X.2	*crassidigitus*	FMNH 257835	AJC 0338	PN Altos de Campana, Panamá, PA. 900 m.	−79.95000, 08.70000	EF629411 EF629442
1.1	*crassidigitus*	USNM 563031	AJC 0970	Cerro Jefe, Panamá, PA. 600 m.	−79.40327, 09.22175	DQ350174 DQ350217
2.1	*crassidigitus*	FMNH 257695	AJC 0209	Nusagandi, Kuna Yala, PA. 400 m.	−78.98330, 09.31670	DQ350175 DQ350218
3.1	*crassidigitus*	USNM 563032	AJC 1055	Río Urtí, Majé, Panamá, PA. 130 m.	−78.74873, 09.00343	EF629410 EF629460
a.1	*E.* sp. cf. *crassidigitus*	FMNH 257676	AJC 0189	Jaguar Trail, Fortuna, Chiriquí, PA. 1000 m.	−82.217, 08.750	No COI EF635372
H.2	*cuaquero**	MVZ 207253	DAG 3209	RB Monteverde, Puntarenas, CR. 1630 m.	−84.7500, 10.3333	EF629438 EF629472
H.3	*cuaquero**	MVZ 207254	DAG 3238	RB Monteverde, Puntarenas, CR. 1535 m.	−84.78333, 10.30000	No COI DQ350220
b.1	*fleischmanni**	MVZ 149791	HBS 834	El Empalme, San José, CR. 2000 m.	−83.95000, 9.73333	EF629424 EF629470
A.1	*fitzingeri*	USNM 534183	LDW 11568	Quebrada Machin, Colón, HN. 540 m.	−85.29167, 15.31944	No COI EF629469
B.1	*fitzingeri*	USNM 538604	LDW 11846	Río Kosmaco, Olancho, HN. 140 m.	−85.16667, 14.73333	DQ350178 DQ350221

Table 1 *Continued*

Sample code	Species	Institutional voucher no.†	Field collection no.‡	Collection locality§	Geographical coordinates	GenBank nos
B.2	*fitzingeri*	USNM 538606	LDW 11879	Río Kosmaco, Olancho, HN. 140 m.	−85.16667, 14.73333	DQ350179 DQ350222
D.1	*fitzingeri*	UCR 16256	AJC 0001	Cruce de Jimenez, Guácimo, Limón, CR. 200 m.	−83.7184, 10.2140	EF629418 No cyt *b*
D.2	*fitzingeri*	UCR 16257	FB 2563	EARTH, Pocora, Guácimo, Limón, CR. 80 m.	−83.56737, 10.23570	DQ350180 DQ350223
E.11	*fitzingeri*	UCR 16373	AJC 0425	Amphibian RC, Guayacán, Limón, CR. 550 m.	−83.54863, 10.04328	DQ350181 DQ350224
E.12	*fitzingeri*	UCR 16371	AJC 0399	Santa Marta, Guayacán, Limón, CR. 520 m.	−83.53555, 10.00523	EF629417 No cyt *b*
I.2	*fitzingeri*	UCR 16370	AJC 0393	MHN La Paz, Alajuela, CR. 1230 m.	−84.55855, 10.18223	DQ350182 DQ350225
J.1	*fitzingeri*	UCR 16366	AJC 0355	Río Jaris, Universidad de la Paz, San José, CR. 550 m.	−84.28333, 09.88333	DQ350183 DQ350226
K.1	*fitzingeri*	UCR 16374	AJC 0471	Punta Leone, Garabito, Puntarenas, CR. 100 m.	−84.65, 09.70	EF629416 No cyt *b*
L.1	*fitzingeri*	UCR 16375	AJC 0497	Montezuma, Nicoya, Puntarenas, CR. 100 m.	−85.09167, 09.65000	DQ350184 DQ350227
N.3	*fitzingeri*	UCR 16368	AJC 0374	F Los Arboles, Platanillo, Puntarenas, CR. 110 m.	−83.85650, 09.31763	DQ350185 DQ350228
N.4	*fitzingeri*	UCR 16369	AJC 0383	Alfombra, Fila Costeña, San José, CR. 915 m.	−83.77203, 09.31228	DQ350186 DQ350229
O.1	*fitzingeri*	UCR 16380	AJC 0509	Isla del Caño, Osa, Puntarenas, CR. 120 m.	−83.88717, 08.70600	DQ350187 DQ350230
P.2	*fitzingeri*	UCR 16377	AJC 0504	Aguitas, Drake, Osa, Puntarenas, CR. 100 m.	−83.66333, 08.67833	DQ350188 DQ350231
P.3	*fitzingeri*	UCR 16382	AJC 0541	Drake, Osa, Puntarenas, CR. 200 m.	−83.62475, 08.64850	DQ350189 DQ350232
Q.2	*fitzingeri*	UCR 16381	AJC 0536	Rincón de Osa, Puntarenas, CR. 125 m.	−83.52403, 08.70552	DQ350190 DQ350233
R.2	*fitzingeri*	FMNH 257745	AJC 0102	RB Las Cruces, San Vito, Puntarenas, CR. 1000 m.	−82.97500, 08.78333	DQ350191 DQ350234
T.1	*fitzingeri*	None yet.	FS 76	Peninsula Valiente, Ngöbe Buglé, PA. 50 m.	−81.75000, 09.00000	DQ350192 DQ350235
V.3	*fitzingeri*	SIUC H-7010	KRL 0693	PN Omar Torrijos H., El Copé, Coclé, PA. 750 m.	−80.59167, 08.66667	DQ350193 DQ350236
W.3	*fitzingeri*	QCAZ 30666	AJC 0857	El Valle de Anton, Coclé, PA. 612 m.	−80.14375, 08.60183	EF635371 No cyt *b*
Y.1	*fitzingeri*	MVUP 2006	AJC 1103	Fort Sherman, Gatún, Colón, PA. 160 m.	−79.97542, 09.28022	EF629423 EF629462
Z.1	*fitzingeri*	NAUMV 0762	AJC 0345	Gamboa, Colón, PA. 24 m.	−79.70000, 09.11700	DQ350194 DQ350237
Z.2	*fitzingeri*	USNM 563033	AJC 0349	Gamboa, Colón, PA. 30 m.	−79.70000, 09.11670	DQ350195 DQ350238
1.2	*fitzingeri*	QCAZ 30661	AJC 0969	Cerro Jefe, Panamá, PA. 600 m.	−79.40327, 09.22175	DQ350196 DQ350239
2.2	*fitzingeri*	FMNH 257614	AJC 0208	Nusagandi, Kuna Yala, PA. 400 m.	−78.98330, 09.31670	DQ350197 DQ350240
3.2	*fitzingeri*	USNM 563037	AJC 0990	Río Tigre, Lago Bayano, Panamá, PA. 74 m.	−78.79568, 09.07933	EF629421 EF629458
3.3	*fitzingeri*	USNM 563038	AJC 0997	Río Urtí, Majé, Panamá, PA. 130 m.	−78.74873, 09.00343	EF629422 EF629459
4.1	*fitzingeri*	USNM 563034	AJC 0904	Río Piña, Bahía Piña, Darién, PA. 85 m.	−78.18583, 07.63333	EF629420 EF629455
4.2	*fitzingeri*	USNM 563035	AJC 0914	Río Piña, Bahía Piña, Darién, PA. 85 m.	−78.18583, 07.63333	DQ350198 DQ350241
5.1	*fitzingeri*	QCAZ 30668	AJC 0579	Cana, PN Darién, Darién, PA. 500 m.	−77.68406, 07.75607	EF629419 EF629453
5.2	*longirostris*	CH 4735	None.	Cana, PN Darién, Darién, PA. 550 m.	−77.68406, 07.75607	DQ350199 DQ350242
5.3	*longirostris*	None yet.	AJC 0590	Cana, PN Darién, Darién, PA. 1500 m.	−77.72217, 07.76367	No COI EF635373
V.4	sp. cf. *longirostris*	None yet.	KRL 1438	Río Blanco, Peña Blanca, El Copé, Coclé, PA. ~1000 m.	−80.60, 08.67	EF629439 EF629468
W.4	sp. nov. 'pn'	MVUP 2019	AJC 1136	Altos del María, Panamá, PA. 930 m.	−80.07667, 08.63312	EF629440 No cyt *b*
5.4	sp. nov. 'pn'	MVUP 1863	AJC 0592	Cana, PN Darién, Darién, PA. 1550 m.	−77.73028, 07.77111	EF629425 No cyt *b*
C.1	*megacephalus*	FMNH 257714	AJC 0072	EB La Selva, Sarapiquí, Heredia, CR. 76 m.	−84.0070, 10.4303	No COI EF635374
c.1	*melanostictus*	MVZ 203856	DAG T 251	RN Tapantí—Tres de Junio, Cartago, CR. 2570 m.	−83.78616, 9.61400	EF629437 No cyt *b*
5.5	*raniformis*	USNM 563041	AJC 0571	Cana, PN Darién, Darién, PA. 500 m.	−77.68405, 07.75607	DQ350200 DQ350243
5.6	*raniformis*	QCAZ 30662	AJC 0576	Cana, PN Darién, Darién, PA. 500 m.	−77.68405, 07.75607	DQ350201 DQ350244
3.4	*raniformis*	MVUP 1898	AJC 1057	Río Urtí, Majé, Panamá, PA. 130 m.	−78.74873, 09.00343	EF629426 EF629461
4.3	*raniformis*	QCAZ 30663	AJC 0915	Río Piña, Bahía Piña, Darién, PA. 85 m.	−78.18569, 07.63344	DQ350202 DQ350245
d.1	*ranoides*	UCR 18073	FB 4342	Peninsula Santa Elena, Guanacaste, CR. 50 m.	−85.79170, 10.91670	DQ350203 DQ350246

Table 1 *Continued*

Sample code	Species	Institutional voucher no.†	Field collection no.‡	Collection locality§	Geographical coordinates	GenBank nos
V.5	*tabasarae*	None yet.	KRL 1387	PN Omar Torrijos H., El Copé, Coclé, PA. 750 m.	−80.59167, 08.66667	EF629428 EF629467
W.5	*tabasarae*	MVUP 2039	AJC 1214	Río María, Altos del María, Panamá, PA. 895 m.	−80.07282, 08.64183	EF629427 No cyt *b*
C.2	*talamancae*	UCR 16401	AJC 0521	EB La Selva, Sarapiquí, Heredia, CR. 76 m.	−84.0070, 10.4303	EF629429 EF629450
D.3	*talamancae*	UCR 16402	AJC 0002	EARTH, Pocora, Guácimo, Limón, CR. 80 m.	−83.56737, 10.23570	EF629435 No cyt *b*
F.1	*talamancae*	UCR 18058	AJC 0959	RB Hitoy Cerere, Estrella, Limón, CR. 150 m.	−83.02412, 09.67323	EF629432 EF629456
S.1	*talamancae*	USNM 563047	AJC 0864	Isla Popa, Bocas del Toro, PA. 20 m.	−82.11008, 09.22344	EF629430 No cyt *b*
S.2	*talamancae*	MVUP 1872	AJC 0865	Isla Popa, Bocas del Toro, PA. 20 m.	−82.11008, 09.22344	EF629431 EF629454
V.5	*talamancae*	MVUP 1780	KRL 0684	PN Omar Torrijos H., El Copé, Coclé, PA. 800 m.	−80.59167, 08.66667	DQ350207 DQ350250
W.6	*talamancae*	MVUP 2037	AJC 1212	Río María, Altos del María, Panamá, PA. 895 m.	−80.07282, 08.64183	EF629434 No cyt *b*
1.3	*talamancae*	MVUP 2040	AJC 0968	Cerro Jefe, Panamá, PA. 600 m.	−79.40327, 09.22175	DQ350208 DQ350251
2.3	*talamancae*	FMNH 257694	AJC 0207	Nusagandi, Kuna Yala, PA. 400 m.	−78.98330, 09.31670	EF629436 EF629441
2.4	*talamancae*	USNM 563046	AJC 0978	Burbayar, Llano-Cartí Road, Panamá, PA. 300 m.	−79.00000, 09.31278	EF629433 EF629457

†CH, Círculo Herpetológico de Panamá, Panama City, Panama; FMNH, Field Museum of Natural History, Chicago, USA; MVUP, Museo de Vertebrados de la Universidad de Panamá, Panama City, Panama; MVZ, Museum of Vertebrate Zoology, University of California at Berkeley, USA; NAUMV, Northern Arizona University Museum of Vertebrates, Flagstaff, USA; QCAZ, Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador; SIUC, Southern Illinois University at Carbondale, USA; USNM, National Museum of Natural History, Washington, DC, USA.

‡AJC, Andrew J. Crawford; DAG, David A. Good; FB, Federico Bolaños V. (Robert Puschendorf, collector); FS, Frank Solís; HBS, H. Bradley Shaffer; KRL, Karen R. Lips; LDW, Larry David Wilson (James 'Randy' McCranie, collector).

§EARTH, Universidad 'Escuela de Agricultura de la Región Tropical Húmeda'; EB, Estación Biológica; MHN, Monumento Histórico Natural; RB, Reserva Biológica; RC, Research Center; PN, Parque Nacional; RN, Refugio Nacional; F, Finca; PA, Republic of Panama; CR, Costa Rica; HN, Honduras. Final numbers indicate elevation in metres (m).

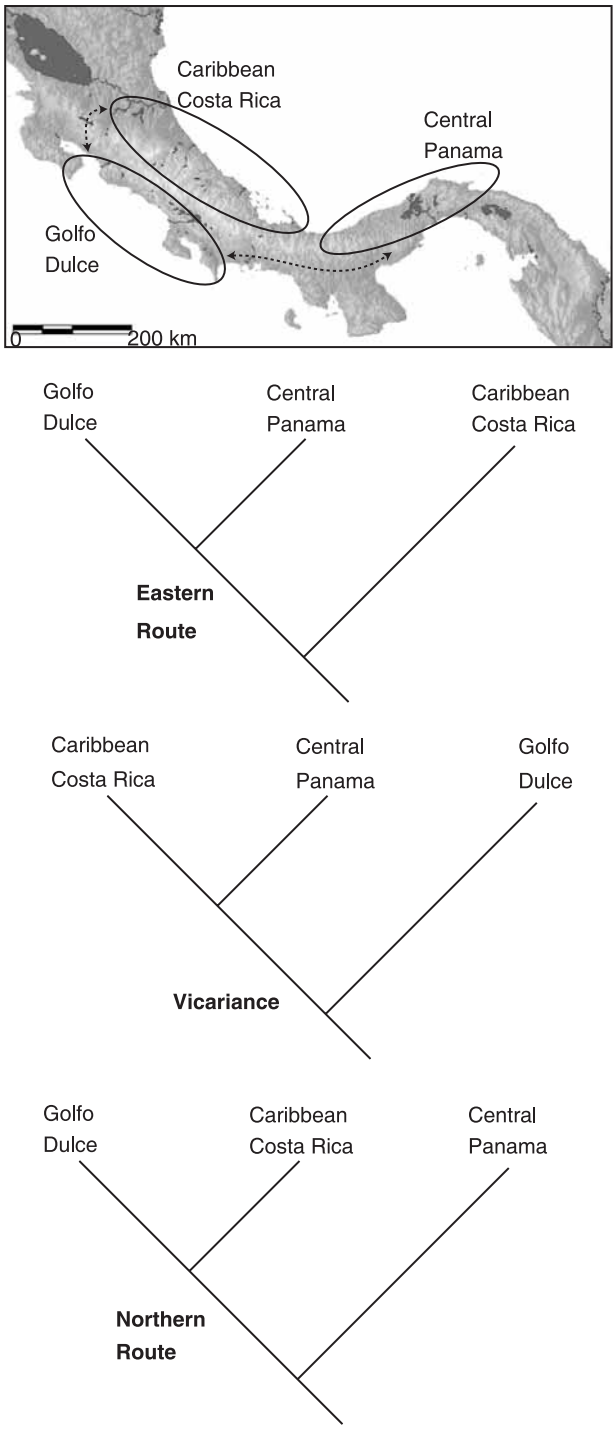


Fig. 2 Upper panel shows a map of Costa Rica and Panama with three labelled geographical regions. Double-headed dashed arrows represent possible dispersal routes that may have connected the Golfo Dulce region genealogically to the other two regions. The longer arrow illustrates a possible Eastern Route, the shorter arrow represents a possible Northern Route. The three cladograms represent phylogenetic predictions based on three hypotheses tested here concerning the biogeographical origin of the Golfo Dulce *Craugastor* fauna: two possible dispersal routes, Eastern and Northern, plus a possible vicariance origin resulting from the rise of the Talamanca Mountains.

Laboratory protocols

Genomic DNA was extracted using a standard phenol–chloroform protocol. A 717-bp fragment of the cytochrome *b* (cyt *b*) gene was amplified using primers CB1 (5′-CCATCCAACATCTCAGCATGATGAAA-3′) and CB3 (5′-GGCGAATAGGAAGTATCATTC-3′) with an annealing temperature of 42 °C (aka primers 144 and 164, respectively, from Table 3 in Goebel *et al*. 1999). A 639-bp fragment of the COI gene was amplified using primers COIf (5′-CCTGCAGGAGGAGGAGAYCC-3′) and COIa (5′-AGTATAAGCGTCTGGGTAGTC-3′) with an annealing temperature of 57 °C (aka primers 109 and 124, respectively, from Table 3 in Goebel *et al*. 1999). Amplified fragments were sequenced using polymerase chain reaction (PCR) primers and BigDye 3.1 terminator reaction chemistry and analysed on either an ABI PRISM 377 or 3100 automated sequencer (Applied Biosystems Inc.). DNA sequences were aligned with sequencher 4.2 (Gene Codes Corporation) and checked by eye. We inferred the amino acid sequences for all DNA sequences to check for the presence of premature stop codons or other nonsense mutations.

Phylogenetic analysis

To evaluate potential phylogenetic heterogeneity between the cyt *b* and COI data sets, we used the incongruence length difference (ILD) permutation test (Farris *et al*. 1995) with 500 random partitions of the combined data set as implemented in paup* 4.0b10 (Swofford 1998). This test may be used to investigate the possibility of incomplete linkage among vertebrate mitochondrial genes (Ballard & Whitlock 2004) or to detect contamination among samples. The ILD test is conservative (Sullivan 1996) and a significance level of $\alpha = 0.01$ or 0.001 may be most appropriate (Cunningham 1997).

We tested the data for significant departure from this assumption of stationarity using a chi-squared test implemented in paup*. Fifty-six models of DNA sequence evolution were then evaluated using the Bayesian information criterion, as implemented in dt-modsel (version: 13-Aug-02) by Minin *et al*. (2003). This method uses decision theory and incorporates branch length error as an additional consideration in choosing the most appropriate model. Maximum-likelihood (ML) models were evaluated and parameters calculated from neighbour-joining (NJ) trees (Saitou & Nei 1987) obtained from a matrix of LogDet distances (e.g. Lockhart *et al*. 1994). Using this method, we sought the single best-likelihood model of DNA sequence evolution applicable to all sites, as well as the most appropriate models for each codon position alone.

ML and most parsimonious (MP) trees were inferred using paup* by heuristic searches. We conducted ML searches using fixed parameter values which were estimated iteratively:

the chosen model and preliminary parameter values were used to infer an ML tree, and this new tree topology was used to re-estimate parameter values for the final ML tree searches. MP searches were conducted six times, each analysis used 5000 random addition sequence replicates. Confidence limits on MP trees were inferred using the nonparametric bootstrap (Felsenstein 1985) in paup* and involved 2000 bootstrap pseudoreplicates each of which was analysed via 30 replicate searches. For both MP and bootstrap analyses, MaxTrees was set to 100 000.

In conducting a Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analysis (Rannala & Yang 1996; Yang & Rannala 1997) using the computer program, mrbayes version 3.1.2 (Ronquist & Huelsenbeck 2003), one has the option of applying independent models to different partitions of the data, thereby creating far more complex models than the 10-parameter general time reversible model (Tavaré 1986). Huelsenbeck & Rannala (2004) showed that phylogenetic accuracy is more vulnerable to model underparameterization than over-parameterization. While Bayesian MCMC deals efficiently with highly complex models, striking the balance between phylogenetic accuracy and parameter identifiability is a nontrivial problem (Castoe *et al*. 2004; Nylander *et al*. 2004). Therefore, we increased model complexity by partitioning the data by the three codon positions, while avoiding over-parameterization by selecting the appropriate model for each partition using the decision theory method, which tends to choose less complex models (Minin *et al*. 2003). We conducted two parallel runs of the MCMC algorithm for 6 million generations each, sampled one tree per 1000 generations, discarded the first 1001 saved trees as burn-in, and estimated the posterior probability distribution of topologies, branch lengths and parameter values from the combined 10 000 samples collected. All runs employed four chains with Metropolis-coupled MCMC heating. For comparison, we also conducted a nonpartitioned Bayesian MCMC analysis.

Divergence time estimation

We employed a likelihood-ratio test (LRT) to evaluate whether the combined data set was significantly unlikely under the assumption of rate constancy of molecular evolution (Felsenstein 1981). To ensure the two models (with and without clock) were nested hierarchically, the topology we inferred from the unconstrained ML tree search was used as a constraint tree during the searches under the enforced-clock model while branch lengths and parameter values were re-estimated.

To estimate divergence times within and among our three focal species of frogs, we applied one molecular-clock method (utilizing only the genetic distance information) and one relaxed-clock approach (relying upon the phylogenetic tree). First, we applied a 1.91% rate of total divergence per million years for model-corrected amphibian mtDNA sequence divergence (Crawford 2003a, b). This rate was obtained by applying a model-based correction to the original mtDNA data used in Macey *et al*. (1998) to estimate a molecular clock in toads based on a 10-million-year-old calibration point (see also Macey *et al*. 2001 for a comparison of rates among vertebrates). This re-calibrated rate was then applied to our model-corrected genetic distance in Table 2 (plus/minus two standard errors), because uncorrected genetic distances may bias divergence time estimates towards the calibration point (Arbogast *et al*. 2002). Second, we used nonparametric rate smoothing (NPRS) (Sanderson 1997) to create an ultrametric tree, which we then calibrated by assuming that the divergence of the most recent common ancestor (MRCA) of the *C. fitzingeri* species group occurred between 20 and 37 million years ago (Ma), as estimated by Crawford & Smith (2005). These authors used a Bayesian MCMC method (Thorne *et al*. 1998; Thorne & Kishino 2002) and a biogeographical hypothesis for the origin of the genus *Craugastor* to estimate divergence times of all species groups within *Craugastor* based on mitochondrial (ND2

Table 2 Means and standard errors of pairwise genetic distances within and among three geographical regions (Fig. 2) for three species of *Craugastor*. Within each cell, the top value indicates *C. crassidigitus*, the middle value *C. talamancae*, and the bottom value indicates *C. fitzingeri*. Genetic diversity within regions based on average pairwise distances is shown along the diagonal. Below the diagonal are values for between-region total genetic distance. Values are Tamura-Nei+Γ model-corrected genetic distances (see text) based on samples with data for both genes (COI and cyt *b*). CR, Costa Rica. Note: *C. talamancae* does not occur in the Golfo Dulce region

	Golfo Dulce	Central Panama	Caribbean CR
Golfo Dulce	0.0313 (0.0045)		*C. crassidigitus*
	n/a		*C. talamancae*
	0.0082 (0.0017)		*C. fitzingeri*
Central Panama	0.1388 (0.0154)	0.0658 (0.0076)	
	n/a	0.1115 (0.0156)	
	0.0258 (0.0042)	0.0114 (0.0024)	
Caribbean CR	0.2620 (0.0310)	0.3120 (0.0340)	0.0743 (0.0109)
	n/a	0.2444 (0.0272)	0.0597 (0.0080)
	0.0662 (0.0098)	0.0662 (0.0094)	0.0140 (0.0026)

plus five tRNA genes) and nuclear (c-*myc*) gene sequences. (This same highly parametric Bayesian MCMC method is not appropriate for the present data set because it would confound polymorphism with divergence.)

Hypothesis testing

To evaluate statistical support for our three *a priori* biogeographical hypotheses for the origin of the Golfo Dulce populations, we compared the scores of optimal trees without vs. with topological constraints enforced. We enforced the three topological constraints on *C. crassidigitus* and *C. fitzingeri* separately, for a total of six predictions based on our three biogeographical scenarios (Fig. 2). We tested five additional null hypotheses concerning the evolution of the *C. fitzingeri* species group. We tested the monophyly of our three focal species (*C. crassidigitus*, *C. fitzingeri*, and *C. talamancae*), the monophyly of four lowland species (the previous three species plus *C. raniformis*), and the monophyly of all five lowland species (the previous four plus *C. longirostris*). We also tested two hypotheses of Savage *et al*. (2004): the sister-relationship between *C. fitzingeri* and *C. raniformis* (two species with yellow spots on the posterior of the thighs) and the monophyly of Talamancan highland species. In total, we evaluated 11 hypotheses of relationships.

For testing the relative support of alternative topologies, we used five different statistical tests. First, we calculated the marginal posterior probability of the monophyletic group predicted by each of our 11 null hypotheses. This method should provide a straightforward estimate of the posterior probability of the clade or combination of clades in question, but its validity depends on using the correct model of evolution (Huelsenbeck & Rannala 2004).

Next, we performed one likelihood-based nonparametric test, the paired-sites test (aka, SH test) of Shimodaira & Hasegawa (1999), as implemented in paup*. This test uses bootstrap resampling and corrects critical values for multiple comparisons, and is known to be conservative (Buckley 2002; Shimodaira 2002). The significance of the difference in the sum of sitewise log-likelihoods for all trees was evaluated by bootstrap sampling of site scores (RELL sampling) with 2000 replicates (Kishino & Hasegawa 1989) and calculating how far the observed differences are from the mean of the bootstrap replicates. The accuracy of this test is increased with the inclusion of all reasonable trees, in addition to the ML tree (H_1) and the constrained tree (H_0) (Shimodaira & Hasegawa 1999). Therefore, we included 12 arbitrarily chosen MP trees as well, and then obtained the one-tailed *P* values for each H_0 tree relative to H_1 and the 12 MP trees.

We performed two parsimony-based paired-sites tests of topology. We used the Wilcoxon signed-ranks test, aka, the Templeton test (Templeton 1983) and the winning-sites test (Prager & Wilson 1988), both implemented in paup*. As with the SH test, these tests compare, on a per-site basis, the support provided by the data, but trees are compared in a pairwise fashion, ignoring the fact that one of the two trees may be the optimal tree (Felsenstein 2004).

Finally, we performed a parametric bootstrap analysis of each of the 11 null hypotheses via a SOWH-like test (Swofford *et al*. 1996; Goldman *et al*. 2000). In the SOWH test, the probability of the observed difference, δ, in tree scores between the optimal tree (H_1) and the constrained tree (H_0) is evaluated by comparison against a null distribution of differences in tree scores obtained from simulated data sets that are generated using the ML model of evolution and the null hypothesis topology (Huelsenbeck *et al*. 1996; Goldman *et al*. 2000). The SOWH test is more powerful than the SH test and performs better than all other topological tests (Shi *et al*. 2005), but may be sensitive to model misspecification (Buckley 2002; Felsenstein 2004). Full or even partial ML optimization of simulated data sets was computationally prohibitive for the present data set. Therefore, we evaluated the difference between optimal ML and constrained ML topologies using the MP criterion, making it a 'SOWH-like' test, but one commonly used (e.g. Pauly *et al*. 2004). This modified test is potentially more conservative than the standard likelihood method because the ML and MP trees may differ. When the ML tree topology is evaluated under the parsimony criterion, the ML tree could well be longer than the most-parsimonious (MP) tree, in which case δ may be reduced and the null hypothesis harder to reject.

Under the MP criterion, we calculated the length of each of our 11 H_0 trees and the H_1 tree. Subtracting the tree length (TL) of H_1 from the TL of each H_0 gave us our 11 test statistics (δ). Next, 100 simulated data sets were generated using seq-gen version 1.3.2 (Rambaut & Grassly 1997). For each of the simulated data sets, paired unconstrained and constrained MP trees were inferred by 300 replicate MP searches. The probability of rejecting a given null hypothesis was obtained by comparing the observed δ-value to the distribution of δ obtained from the 100 simulated data sets.

We compared levels of mtDNA sequence polymorphism and divergence within three species (*C. crassidigitus*, *C. fitzingeri* and *C. talamancae*) by calculating model-corrected levels of genetic variation within and among the three geographical regions in question (Fig. 2). Means and standard errors (based on 2000 bootstrap replicates) were calculated using mega version 3.0 (Kumar *et al*. 2004).

Results

We obtained DNA sequence from both the COI and cyt *b* genes from 67 frogs of the total sample of 89 *Craugastor*, including 26 *C. fitzingeri*, 24 *C. crassidigitus*, 7 *C. talamancae*, and 8 samples representing five additional species in the *C.*

fitzingeri species group (Savage *et al*. 2004), as well as two outgroup taxa, *C. fleischmanni* and *C. ranoides* (Crawford & Smith 2005; Puschendorf *et al*. 2005). The alignment was unambiguous, and the inferred amino acid sequence contained no stop codons. We obtained 639 bp of the COI gene and 714 bp of the cyt *b* gene, for a total of 1353 bp in the final data set. Among ingroup taxa with data for both genes, we counted 528 parsimony-informative sites plus 75 singletons.

The 22 samples from which we obtained DNA sequence from just one gene included an additional 5 *C. fitzingeri* samples, 5 *C. crassidigitus*, 3 *C. talamancae*, 1 sample each of *C. cuaquero*, *C. longirostris*, and *C. tabasarae*, plus 5 samples representing four additional species not in the two-gene data set: *C. andi*, *C. melanostictus*, *C.* sp. cf. *crassidigitus*, *C.* sp. nov. 'pn', and an additional outgroup taxon (*C. megacephalus*). These 22 additional samples were used in phylogenetic reconstructions but were not used in model selection or hypothesis testing. See Table 1.

Evolutionary models and phylogenetic results

Using a chi-squared test, we detected no departure from homogeneity of nucleotide frequencies among all samples, including the outgroup taxa ($P = 0.9853$). The ILD failed to reject the null hypothesis of homogeneity between the COI and cyt *b* data sets for the ingroup ($P = 0.350$) but repeating the test with the outgroup *C. fleischmanni* rejected homogeneity of the data partitions ($P = 0.005$).

The most appropriate ML model for the combined data set was the Tamura & Nei (1993) model with the distribution of rates of evolution among sites described by the gamma density shape parameter, α (Yang 1994), and a proportion of invariable sites, *I* (Hasegawa *et al*. 1987) or TrN93+Γ+*I* model. When using mega to calculate average pairwise distances within and among the three geographical regions of interest (Fig. 2), we adopted the TrN93+Γ model because mega does not implement the *I* parameter. To compensate, we re-estimated α for this model (0.227184) and used this value in mega.

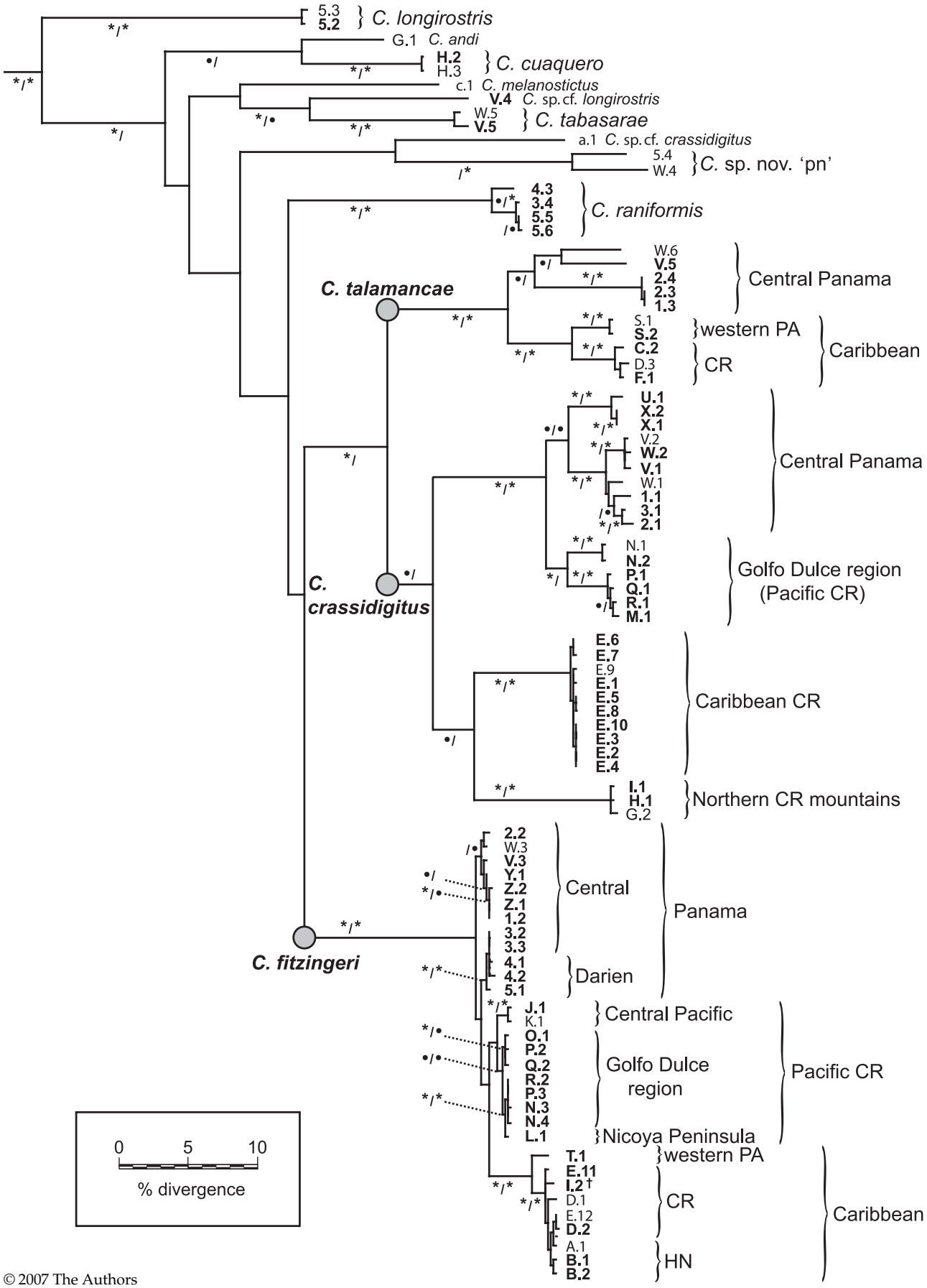
When the data were partitioned by codon position, the following models were recommended. For first position sites the Kimura 2-parameter or K2P (Kimura 1980) +Γ+*I* model was selected. For second position sites, the HKY+Γ+*I* model (Hasegawa *et al*. 1985) was selected. For third position sites, the TIM+Γ model was selected. In mrbayes, the K2P was implemented by fixing the priors on all base frequencies at the constant value 0.25, while the TIM model was replaced by a full six-rate model.

Parameter values for the nonpartitioned TrN93+Γ+*I* model were estimated iteratively with final values as follows: frequencies of nucleotides A, C, G and T were 0.262952, 0.350508, 0.114824 and 0.271716, respectively; the A–G transition rate was 20.63020 and the C–T transition rate was 7.64818, relative to the transversion rate of 1.0; α was 1.118286 with an *I* parameter value of 0.489922. Using this model and fixed parameter values, the ML tree inferred from the 69 frogs with complete two-gene data showed a support value of –12157.65888 log-likelihood units in repeated searches. The first MP search recovered 864 shortest trees of 2476 steps. After repeating the MP search five more times, we recovered no new shortest trees.

We also conducted MP, bootstrap and Bayesian analyses on the complete data set of 89 samples. The results of our tri-partitioned Bayesian analysis are presented in Fig. 3. No appreciable differences were observed between the tri-partitioned and nonpartitioned Bayesian consensus trees (unlike in Castoe *et al*. 2004). We observed no significant conflict between Bayesian and MP bootstrap support (bss), with one exception: while Bayesian analyses placed Caribbean and Pacific CR samples of *C. fitzingeri* as sisters with a marginal posterior probability (mpp) = 0.85 (Fig. 3), the MP bootstrap analysis placed Pacific CR with Central PA samples with a significant bss = 98.

Our sampling provided the following insights into interspecific relationships of the *C. fitzingeri* group. *Craugastor fleischmanni* and other outgroup samples appeared far removed from the rest of the ingroup samples (result not shown), while *C. longirostris* from eastern Panama formed the sister lineage to all other *C. fitzingeri* group samples (Fig. 3). *Craugastor talamancae* and *C. crassidigitus* are sister species with mpp = 1.0 and bss = 59. Some support was found for a monophyletic group formed by our four lowland species: *C. crassidigitus*, *C. fitzingeri*, *C. talamancae*, and *C. raniformis*. These four species formed a clade with a nonsignificant mpp = 0.90, but this result was supported by the

Fig. 3 Phylogeny of all *Craugastor fitzingeri* group samples (Table 1) inferred from a Bayesian phylogenetic analysis of tri-partitioned mtDNA sequence data. Scale bar reflects the more conservative genetic distances estimated from the unpartitioned ML model. Outgroups and the distantly related *C. fleischmanni* are not shown. Statistical support for each node indicated by Bayesian marginal posterior probability (mpp) before the slash, and MP bootstrap scores (bss) following the slash. An asterisk (*) indicates maximal statistical support: a mpp value of 1.0 or a bss value of ≥ 95%. A dot (•) indicates still significant support: a mpp of 0.99–0.95 or a bss value of 94–80%. Each mtDNA sample is represented by its sample code given in Table 1. For each sample code, the letter (A–Z) or number (1–5) preceding the period (.) refers to an area illustrated in Fig. 1, while the numbers following the period distinguish different frogs sampled within named areas. Sample codes with lower case letters (a–d) are not illustrated in Fig. 1, but are listed in Table 1. Sample codes in bold represent individuals from which data for both genes were obtained. The dagger (†) indicates that sample I.2 (Table 1) actually comes from a collecting locality on the Pacific versant, 2 km from the continental divide (Fig. 1).



ML and strict consensus MP trees based on the two-gene data set (not shown). The recently described *C. tabasarae* (Savage *et al*. 2004) was the sister species of an undescribed taxon, *C.* sp. cf. *longirostris* with strong statistical support (Fig. 3). *Craugastor cuaquero* appeared to be the sister to a taxon tentatively identified as *C. andi* (Fig. 3). Most other basal relationships among members of the *C. fitzingeri* group were poorly resolved. Of principal importance to this study, mtDNA haplotypes representing conspecific individuals of *C. crassidigitus*, *C. fitzingeri*, and *C. talamancae* were recovered as monophyletic with very high mpp and bss values, although *C. crassidigitus* monophyly received a marginal bss = 69 (Fig. 3).

Intraspecific relationships

Within *C. crassidigitus*, C. *fitzingeri*, and *C. talamancae,* we uncovered striking differences among species in branch lengths and in degree of support for intraspecific relationships. Although *C. crassidigitus* and *C. talamancae* have the more restricted distributions, their mtDNA haplotypes showed larger genetic distances among geographical regions and well-resolved basal relationships (Fig. 3). Golfo Dulce and Central Panama samples of *C. crassidigitus* were reciprocally monophyletic and together formed the sister lineage to the Caribbean CR + northern mountain CR samples (Fig. 3). Within regions, we observed long branch-lengths and significant phylogenetic structure in both *C. crassidigitus* and *C. talamancae* (Table 2).

The phylogeography of *C. fitzingeri* differed from *C. crassidigitus* and *C. talamancae* most notably in the low intraspecific genetic diversity (Table 2) and poorly supported relationships at basal nodes (Fig. 3). Genetic diversity within any of the three geographical regions was 3.8-fold to 9.8-fold lower in *C. fitzingeri* than in the other two species, while between any given pair of regions, *C. fitzingeri* showed 3.7-fold to 5.4-fold lower total genetic divergence (Table 2). *Craugastor fitzingeri* samples from outside the range of both of the other two species (areas A, B, J, and L in Fig. 1) represented very little additional genetic divergence (haplotypes A.1, B.1, B.2, J.1 and L.1 in Fig. 3) relative to other haplotypes.

The phylogeographical data from *C. crassidigitus*, *C. fitzingeri*, and *C. talamancae* shared some features in common. None of these three species rejected our initial assumption that Central Panama samples represent a single clade, despite being drawn from two coasts and two mountain ranges (Fig. 2). In *C. fitzingeri,* basal relationships are unclear (Fig. 3). In both *C. crassidigitus* and *C. fitzingeri*, Caribbean CR appeared genetically isolated relative to the other two regions (Table 2 and Fig. 3). In both *C. crassidigitus* and *C. talamancae,* the deep intraspecific root node clearly separated Caribbean CR from Central Panama samples, despite the continuous lowland wet forest habitat connecting these two regions along the Caribbean coast.

Divergence time estimation

We applied the LRT to the subset of 67 samples with data for both genes and rejected the molecular clock ($P < 0.001$). We applied the NPRS (relaxed clock) method to the phylogeny based on the complete data set (Fig. 3) and obtained the following six divergence time intervals of particular interest (for comparison, the molecular clock results are given in parentheses). Comparing the Golfo Dulce vs. Central Panama populations, we estimated that in *C. fitzingeri,* these populations diverged in the Pliocene, 2.6–4.7 Ma (0.9–1.8 Ma). In *C. crassidigitus,* the Golfo Dulce and Central Panama populations last shared a common ancestor as long ago as the Late Miocene, 4.2–8.1 Ma (5.8–9.3 Ma). *Craugastor fitzingeri* samples last shared a common ancestor in the Late Miocene, 5.3–9.8 Ma (2.6–4.5 Ma). The MRCA of *C. talmanacae* diverged in the Late Miocene, 5.5–11 Ma (10–17 Ma). The MRCA of *C. crassidigitus* diverged in the Mid- to Late Miocene, 8.0–15 Ma (13–20 Ma). All three species shared a common ancestor in the Early to Mid-Miocene, 12–23 Ma (19–36 Ma).

Phylogenetic hypothesis testing

All five topology tests rejected the 'northern route' and 'vicariance' hypotheses for *C. crassidigitus* (Fig. 2). All tests failed to reject any biogeographical hypothesis for *C. fitzingeri* (Table 3). Regarding our statistical tests of interspecific relationships, the three nonparametric tests (SH and parsimony-based) failed to reject any hypothesis. All tests failed to reject the monophyly of our three focal lowland species (*C. crassidigitus*, *C. fitzingeri*, *C. talamancae*), the monophyly of the preceding three + *C. raniformis* (also lowland), or the monophyly of *C. fitzingeri* + *C. raniformis*. However, Bayesian mpp and the SOWH test rejected the monophyly of *C. longirostris* + the other four lowland species, while the SOWH test also rejected the monophyly of highland taxa (Table 3).

Discussion

Comparative phylogeography permits us to separate historical processes that may have influenced whole communities of organisms from ecological or demographic forces acting on single evolutionary lineages or species (Bermingham & Avise 1986; Campbell *et al*. 2006). In the present study, we are interested in the age and degree of geographical isolation of the Golfo Dulce community of *Craugastor* frogs, which we place in a broader geographical context in order to also analyse the influence of ecology on the phylogeography of these frogs. Our focal species form a monophyletic clade (Fig. 3), thus, minimizing differences that may owe purely to phylogeny (e.g. grossly different mechanics of nucleotide substitution), and are broadly

Table 3 Results of eleven statistical tests of topology predicted by three biogeographical hypotheses for the origin of the Golfo Dulce populations (above the line, two species) and five phylogenetic hypotheses for the *Craugastor fitzingeri* species group (below the line). Tests included only the 69 frogs with data for both genes. Tests significant at $P < 0.05$ are shown in bold font. H_0 represents the null or constrained tree that a given test evaluates relative to H_1, the optimal tree. Templeton and winning sites tests indicate a range of P values due to the recovery of multiple MP trees under a given null constraint. For the unconstrained topologies, the MP tree had a length of 2476 steps and the ML tree (with fixed parameter values) received a score of 12157.65888 negative log-likelihood units. Negative values in the penultimate column imply that the ML tree (H_1) was sometimes longer than a given constrained tree (H_0) when re-analyzed under the parsimony criterion. TL, tree length. Dagger (†) denotes > 1 ML tree was inferred. Asterisk (*) denotes 300 (instead of 100) parametric bootstraps were performed

		Parsimony-based nonparametric results			SH test (likelihood-based nonparametric results)			SOWH-like test: parametric test with MP evaluation of ML trees). (Note: TL of ML tree = 2497)		
Null hypothesis	Bayesian mpp	Tree length (TL)	Templeton test	Winning sites	–ln L	–ln L(H_1) – –ln L(H_0)	Prob.	TL of H_0	ΔTL = $H_1 - H_0$	Prob. (ΔTL | H_0)
C. fitzingeri Eastern Route	0.0884	2476	1.0000	1.0000	12160.01894	2.36006	0.7420	2488	–9	1.00
C. crassidigitus Eastern Route	1.0	2476	1.0000	1.0000	12157.65888	0.00000	1.000	2497	0	1.00
C. fitzingeri vicariance	0.0548	2484	0.0736–0.2382	0.1153–0.3020	12160.18062	2.52174	0.7265	2493	–4	1.00
C. crassidigitus vicariance	**< 0.0001**	2506	**< 0.0028**	**< 0.0055**	12204.68107	47.02219	**0.0075**	2520	23	**< 0.01**
C. fitzingeri Northern Route	0.7852	2483	> 0.2622	> 0.3366	12157.65888†	0.00000	> 0.8324	2497	0	1.00
C. crassidigitus Northern Route	**< 0.0001**	2506	**< 0.0028**	**<0.0055**	12204.68107	47.02219	**0.0075**	2521	24	**< 0.01**
monophyly (*C. crassidigitus*, *C. fitzingeri*, *C. talamancae*)	0.3329	2476	1.0000	1.0000	12158.83110†	1.17222	0.8085	2500	3	0.28.
monophyly (lowland species)	0.9372	2476	1.0000	1.0000	12157.65888	0.00000	1.000	2497	0	1.00
monophyly (highland species)	0.1171	2480	> 0.4141	> 0.5412	12158.36728†	0.70840	0.8020	2504	7	**0.043***
monophyly (*C. fitzingeri* + *C. raniformis*)	0.0593	2478	> 0.6697	> 0.8317	12159.32589	1.66701	0.7665	2498	1	0.62
monophyly (lowland species including *C. longirostris*)	**< 0.0001**	2490	> 0.0989	> 0.1201	12166.89089	9.23201	0.4150	2512	15	**0.01**

sympatric (Fig. 1) thus minimizing differences in population structure resulting from different environmental histories.

The present study highlights two serious difficulties facing all researchers interested in the evolution of amphibian communities. First, many amphibian species remain undiscovered or undescribed, and many named species harbour lineages that have apparently been separated for significant periods of time (e.g. Ron *et al*. 2006; Stuart *et al*. 2006). This study alone included three undescribed highland species and revealed substantial cryptic phylogeographical diversity in two named lowland species. Second, amphibians are declining at an alarming rate (Stuart *et al*. 2004). One of our three undescribed highland taxa, *Craugastor* sp. cf. *longirostris*, may well be extinct already (see Lips *et al*. 2006). Some of the known highland species may be gone as well. *Craugastor cuaquero* is known only from the type locality, the site of drastic amphibian decline (Pounds & Crump 1994; Pounds *et al*. 1997) while *Craugastor andi* and *C. fleischmanni* are both listed as 'Critically Endangered' and 'possibly Extinct' by the Global Amphibian Assessment (IUCN *et al*. 2004). If the Museum of Vertebrate Zoology had not preserved these samples before the amphibian declines, we would not have had these highland forms for our phylogenetic analyses and our conclusions regarding the monophyly of our focal species would have been called into question.

Notwithstanding the differences among the phylogeographical patterns of our three focal frog species, the species share two striking phylogeographical traits with one another and with a variety of other species that have been studied in Central America. First, populations of *Craugastor crassidigitus*, *C. talamancae*, and to a lesser extent *C. fitzingeri*, are genetically discontinuous across the narrow regional landscape, thus providing additional evidence that many conspecific populations inhabiting lower Central America have been evolutionarily independent for long periods of time. Similar patterns of regional phylogeographical structure have been documented for primary freshwater fishes (Bermingham & Martin 1998; Perdices *et al*. 2002; Reeves & Bermingham 2006), birds (Brumfield & Braun 2001; González *et al*. 2003), frogs (Crawford 2003a; Weigt *et al*. 2005) and a widespread tree species (Dick *et al*. 2003). Second, *C. crassidigitus* and *C. talamancae* certainly occupied much of their current geographical range relatively early in their evolutionary history, suggesting that the physical ability to disperse, in and of itself, is not limiting contemporary gene flow between populations. Reeves & Bermingham (2006), commenting on a similar pattern among freshwater fish in the region, hypothesized that dispersal across a landscape devoid of competitors vs. one populated with conspecifics could leave significantly different mtDNA traces. In the first case, suitable environment may be all that is needed to yield a very high probability of successful colonization by an immigrant, whereas in the second case the immigrant would have only a 1/N probability of replacing a conspecific resident's mtDNA type in the population.

In all three species, the deepest phylogeographical split separates northwestern from southeastern populations along the Caribbean versant. While the species' ranges appear to correspond well with habitat heterogeneity (or perhaps with lack of collecting effort in the case of *C. talamancae* in eastern Panama), this Caribbean phylogeographical break is somewhat surprising given the existence of a continuous belt of wet forest linking Costa Rica with Central Panama along the Caribbean coast (Fig. 1). This regional phylogeographical discontinuity has been observed in a number of freshwater fish (Bermingham & Martin 1998; Perdices *et al*. 2002; Reeves & Bermingham 2006), manakins (Brumfield & Braun 2001), dirt frogs (Crawford 2003a) and the tree, *Symphonia globulifera* (Dick *et al*. 2003). Across species, the phylogeographical breaks correspond to the Bocas del Toro Province near the border of Panama and Costa Rica, but do not match perfectly in location. Taken together, the multispecies data establish the existence of a filter barrier in the Bocas del Toro region (Fig. 1). A filter barrier reduces but does not eliminate the probability of dispersal between geographical areas (Remington 1968).

The effect of a filter barrier on different types of organisms should vary with the dispersal ability of the species (e.g. Schäuble & Moritz 2001). In the case of *C. fitzingeri*, *C. crassidigitus*, and *C. talamancae*, mean intraspecific genetic distances across the Bocas del Toro barrier varied threefold; uncorrected genetic distances were 5%, 14%, and 13%, respectively, suggesting that the barrier has impeded *C. fitzingeri* less than its congeners, or that *C. fitzingeri* expanded its geographical range more recently. The intraspecific divergences observed for *Craugastor* species across the Bocas del Toro filter barrier are similar to interspecific divergences estimated for other Neotropical frog genera. Although these studies are not based on the same combination of mitochondrial genes, we note that related but nonsister *Epipedobates* species (Dendrobatidae) from Peru are only 7–8% diverged in mtDNA sequence (Roberts *et al*. 2006), while Guiana *Atelopus* (Bufonidae) are 7–9% different from their closest relatives found in Peru and Brazil (Noonan & Gaucher 2005).

Although the Bocas del Toro region is geologically very dynamic (Coates *et al*. 2003), the geological aspect apparent today that potentially accounts for the filter barrier is simply the narrowness of the lowland wet forest. In the short south-to-north distance from the Talamanca Mountains to the Caribbean Ocean, the elevation drops off sharply, leaving a very narrow corridor for dispersal along the Caribbean versant (Fig. 1). There is no evidence to suggest that this region previously lacked continuous wet forest habitat (Piperno & Pearsall 1998).

Craugastor crassidigitus, *C. talamancae*, and *C. fitzingeri* all share sufficient aspects of their evolutionary history to warrant consideration of their ecology in any attempt to reconcile the observed phylogeographical differences among species. These three focal species prefer wet forest habitats, but *C. fitzingeri* appears to have a higher tolerance for dry forests. It persists in dry habitat along sheltered riparian gallery forests (Savage 2002) and has been recorded from drier sites along the Pacific Coast (Lynch & Myers 1983; Köhler 2001; Savage 2002). All three species overlap extensively in elevation, but *C. talamancae* does not extend as high as the other two. All three are found in both forest edges and forest interiors, but *C. fitzingeri* appears to predominate in marginal habitats. Lynch & Myers (1983, p. 534) report that *C. fitzingeri* is often abundant 'in disturbed or edge situations.' *Craugastor crassidigitus* occurs 'in both mature and second-growth forest, and also in forest-edge situations' but 'it generally is more abundant in upland forest' (Lynch & Myers 1983, pp. 526–527).

The modest differences in habitat association among species would favour dispersal by *C. fitzingeri* relative to the other two species. The phylogeography of *C. fitzingeri* demonstrates that this species has either higher levels of contemporary gene flow or has recently expanded across the landscape. Because our three focal species share a common environmental distribution and common phylogenetic history until their separation in the Miocene, we conclude that the slight differences in habitat preference provides *C. fitzingeri* with a greater dispersal potential which in turn explains the significantly reduced phylogeographical structuring relative to *C. crassidigitus* and *C. talamancae*.

The greater dispersal potential of *C. fitzingeri* is also supported by the phylogeographical pattern of the samples collected from the extreme ends of the species' geographical range, beyond the regions of sympatry. The *C. fitzingeri* samples from beyond the geographical range of the other two species (areas A, B, J, and L in Fig. 1) were not genetically distinct (haplotypes A.1, B.1, B.2, J.1, and L.1 in Fig. 3), demonstrating they have been recently connected by migration with more central localities. This result could not have been predicted *a priori*, because more widespread species sometimes show the greater phylogeographical structure (e.g. Roberts 2006). Thus, we find that *Craugastor* ecology predicts both the degree of phylogeographical structuring and the extent of species' ranges.

The origin of the Golfo Dulce populations

Only the phylogenetic data representing *C. crassidigitus* permitted us to distinguish among our three *a priori* hypotheses (Fig. 2) for the origin of the Golfo Dulce populations of *C. fitzingeri* and *C. crassidigitus*. The mtDNA data for *C. fitzingeri* were unable to reject any of the three *a priori* hypotheses, whereas 'Eastern Route' is the only hypothesis compatible with the data from *C. crassidigitus* (Table 3). Our analyses indicate that Panama's Pacific slope served as the most recent link between the Golfo Dulce and the rest of *C. crassidigitus*. Independent of the direction of this hypothesized dispersal event, two scenarios may explain these results. The Golfo Dulce *C. crassidigitus* may have arisen by vicariance with the Caribbean CR populations (8.0–15 Ma), with subsequent dispersal out of the Golfo Dulce and into Central Panama via the Pacific Coast (4.2–8.1 Ma). Alternatively, the first divergence event may have separated Caribbean CR from Central Panama at the Bocas del Toro barrier, with subsequent dispersal into the Golfo Dulce along the Pacific Coast.

Under either of the above scenarios, we may draw two conclusions regarding the history of the region and environmental influences on *C. crassidigitus* diversification: (i) the dry forests of the Pacific Coast of Panama have been less of a barrier than the dry forests or mountains of northern CR (Fig. 1), but (ii) the Pacific Panama dry forest have served as a barrier to *C. crassidigitus* since 4.2 Ma or prior (genetic divergence was 14%; Table 2). Using comparative phylogeography, we argue that the dry forest specifically is what separates Golfo Dulce and Central Panama populations of *C. crassidigitus*. Other historical or geological factors would have affected the intraspecific divergence within *C. crassidigitus* and *C. fitzingeri* similarly, while in fact we found that the Golfo Dulce haplotypes from *C. fitzingeri* showed only 2.6% mean genetic distance rather than the 14% observed in *C. crassidigitus*.

Craugastor *as a model for the origin of the Golfo Dulce fauna*

The 'Eastern Route' connecting Golfo Dulce with Central Panama via the dry Pacific versant seemed *a priori* the least likely of the three possible results because (i) Central Panama is connected with Caribbean CR by the continuous wet forest of the Caribbean versant (across the Bocas del Toro filter barrier), (ii) the 'Northern Route' required only traversing the very short distance between Caribbean and Pacific CR (Fig. 2), and (iii) there is no evidence to suggest that the dry forests of Isthmian Central America were recently wet. The Pacific dry forests of today were savannah habitat during Pleistocene glacial maxima (Piperno & Pearsall 1998) and would have been even more inhospitable to forest-dwelling frogs during these climatic drying and cooling events. However, our biogeographical conclusions correspond well with the environmental history of the region. Dry forest habitat in Panama substantially predates the Pleistocene, going back > 4 million years (Graham & Dilcher 1995), a date that agrees well with our minimum divergence time estimate (4.2 Ma) for *C. crassidigitus* populations separated by this dry forest. Thus, the palaeontological, geological, and phylogeographical data together support the old age of the spatial pattern of wet and dry forests of lower Central America.

While our genealogical information alone does not demonstrate the direction of dispersal, we hypothesize that *C. crassidigitus* colonized the Golfo Dulce from the Pacific based on three observations: (i) this species potentially predated the Golfo Dulce wet forest, (ii) the Caribbean coast hosts all three lowland species, whereas the Pacific Coast lacks *C. talamancae* (Fig. 1), and (iii) the Golfo Dulce shows less within-region genetic diversity than the other two regions (Table 2). If so, colonization of Golfo Dulce would have taken place from the east, via Central Panama (Table 3). This pattern of colonization was also suggested for *Phyllobates* (Maxson & Myers 1985), a genus of frogs of likely South American origin.

As a further test of the age of wet forest habitat, we can compare phylogeographical data from open-habitat species found on either side of the wet forest (e.g. Wüster *et al*. 2005). If the Golfo Dulce wet forest has been isolated by dry forest or savannah since > 4 (Ma), as we contend, then any dry-forest or open-habitat species occurring on either side of the Golfo Dulce should show a complementary genetic break of similar age. This prediction was borne out in a recent analysis of the túngara frog, which is found in the open and dry forest habitats on either side of the Golfo Dulce region. This Golfo Dulce 'break' was dated to 4–16 Ma using either of two South American calibration points and a clock-independent temporal analysis (Weigt *et al*. 2005).

In summary, the phylogeographical data presented here, coupled with the palaeogeography of lower Central America and previously published phylogeographical studies, suggest that the environmental mosaic of wet forest and dry forest habitats seen today in the region has been in place since the early Pliocene or late Miocene. Our estimated age for the drying of the Pacific slope of western Panama is notably older than the interval 1–2 Ma suggested by McDiarmid & Savage (2005). While there may have been brief intervals during the Pleistocene in which wet forest might have become established and *C. crassidigitus* simply failed to move through the region, the historical data and current environmental reconstructions do not support this hypothesis (Piperno & Pearsall 1998). We argue here that the regional-scale environmental heterogeneity along the Pacific versant both predated and withstood the climatic fluctuations of the Pleistocene. The lasting historical imprint left on the community assembly of the local fauna is revealed through comparative phylogeographical analysis of closely related and sympatric species. Such lineages provide our best opportunities to tease apart the relative influences of environment, history, and ecology on the zoogeography of species.

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References

Arbogast BS, Edwards SV, Wakeley J, Beerli P, Slowinski JB (2002) Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annual Review of Ecology and Systematics*, **33**, 707–740.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts.

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. *Molecular Ecology*, **13**, 729–744.

Bermingham E, Avise J (1986) Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics*, **113**, 939–965.

Bermingham E, Martin AP (1998) Comparative mtDNA phylogeography of Neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology*, **7**, 499–517.

Brumfield RT, Braun MJ (2001) Phylogenetic relationships in bearded manakins (Pipridae: *Manacus*) indicate that male plumage colour is a misleading taxonomic marker. *The Condor*, **103**, 248–258.

Buckley TR (2002) Model misspecification and probabilistic tests of topology: evidence from empirical data sets. *Systematic Biology*, **51**, 509–523.

Campbell P, Schneider CJ, Adnan AM, Zubaid A, Kunz TH (2006) Comparative population structure of *Cynopterus* fruit bats in peninsular Malaysia and southern Thailand. *Molecular Ecology*, **15**, 29–47.

Castoe TA, Doan TM, Parkinson CL (2004) Data partitions and complex models in Bayesian analaysis: the phylogeny of gymnphthalmid lizards. *Systematic Biology*, **53**, 1–22.

Coates AG, Aubry M-P, Berggren WA, Collins LS, Kunk M (2003) Early Neogene history of the Central American arc from Bocas del Toro, western Panama. *GSA Bulletin*, **115**, 271–287.

Coen E (1983) Climate. In: *Costa Rican Natural History* (ed. Janzen DH), pp. 35–46. University of Chicago Press, Chicago, Illinois.

Crawford AJ (2003a) Huge populations and old species of Costa Rican and Panamanian dirt frogs inferred from mitochondrial and nuclear gene sequences. *Molecular Ecology*, **12**, 2525–2540.

Crawford AJ (2003b) Relative rates of nucleotide substitution in frogs. *Journal of Molecular Evolution*, **57**, 636–641.

Crawford AJ, Smith EN (2005) Cenozoic biogeography and evolution in direct-developing frogs of Central America (Leptodactylidae: *Eleutherodactylus*) as inferred from a phylogenetic analysis of nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution*, **35**, 536–555.

Cunningham CW (1997) Can the incongruence test predict when data should be combined? *Molecular Biology and Evolution*, **14**, 733–740.

Dick CW, Abdul-Salim K, Bermingham E (2003) Molecular systematic analysis reveals cryptic tertiary diversification of a widespread tropical rain forest tree. *American Naturalist*, **162**, 691–703.

Farris JS, Källersjö M, Kluge AG, Bult C (1995) Testing significance of incongruence. *Cladistics*, **10**, 315–319.

Felsenstein J (1981) Evolutionary trees from DNA sequences—a maximum-likelihood approach. *Journal of Molecular Evolution*, **17**, 368–376.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Felsenstein J (2004) *Inferring Phylogenies*. Sinauer & Associates Inc., Sunderland, Massachusetts.

Goebel AM, Donnelly JM, Atz ME (1999) PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome *b* in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. *Molecular Phylogenetics and Evolution*, **11**, 163–199.

Goldman N, Anderson JP, Rodrigo AG (2000) Likelihood-based tests of topologies in phylogenetics. *Systematic Biology*, **49**, 652–670.

González MA, Eberhard JR, Lovette IJ, Olson SL, Bermingham E (2003) Mitochondrial DNA phylogeography of the Bay Wren (Troglodytidae: *Thryothorus nigricapillus*) complex. *The Condor*, **105**, 228–238.

Graham A, Dilcher D (1995) The Cenozoic record of tropical dry forest in northern Latin America and the southern United States. In: *Seasonally Dry Tropical Forests* (eds Bullock SH, Mooney HA, Medina E), pp. 124–145. Cambridge University Press, Cambridge, England, UK.

Graham CH, Ron SR, Santos JC, Schneider CJ, Moritz C (2004) Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution*, **58**, 1781–1793.

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160–174.

Hasegawa M, Kishino H, Yano T (1987) Man's place in Hominoidea as inferred from molecular clocks of DNA. *Journal of Molecular Evolution*, **26**, 132–147.

Hickerson MJ, Cunningham CW (2005) Contrasting Quaternary histories in an ecologically divergent sister pair of low-dispersing intertidal fish (*Xiphister*) revealed by multilocus DNA data. *Evolution*, **59**, 344–360.

Holdridge LR (1967) *Life Zone Ecology*. Tropical Science Center, San José, Costa Rica.

Huelsenbeck JP, Rannala B (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology*, **53**, 904–913.

Huelsenbeck JP, Hillis DM, Jones R (1996) Parametric bootstrapping in molecular phylogenetics: applications and performance. In: *Molecular Zoology: Advances, Strategies, and Protocols* (eds Ferraris JD, Palumbi SR), pp. 19–45. John Wiley and Sons, Inc, New York.

Ibáñez R, Solís FA, Jaramillo CA, Rand AS (2001) An overview of the herpetology of Panama. In: *Mesoamerican Herpetology: Systematics, Zoogeography, and Conservation* (eds Johnson JD, Webb RG, Flores-Villela O), pp. 159–170. Centenial Museum, Special Publ. No. 1, University of Texas at El Paso, El Paso, Texas.

IUCN, Conservation International, and NatureServe (2004) *Global Amphibian Assessment*. Available from URL: www.globalamphibians.org [accessed on 16 May 2005].

Kerdelhué C, Magnoux E, Lieutier F, Roques A, Rousselet J (2006) Comparative population genetic study of two oligophagous insects associated with the same hosts. *Heredity*, **97**, 38–45.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111–120.

Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution*, **29**, 170–179.

Köhler G (2001) *Anfibios y Reptiles de Nicaragua*. Herpeton Verlag, Offenbach, Germany.

Köhler G (2003) *Reptiles of Central America*. Herpeton Verlag, Offenbach, Germany.

Kumar S, Tamura K, Nei M (2004) mega3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.

Lips KR, Brem F, Brenes R *et al*. (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences, USA*, **103**, 3165–3170.

Lockhart PJ, Steel MA, Hendy MD, Penny D (1994) Recovering evolutionary trees under a more realistic model of sequence evolution. *Molecular Biology and Evolution*, **11**, 605–612.

Lomolino MV, Sax DF, Brown JH (2004) *Foundations of Biogeography Classic Papers with Commentaries*. University of Chicago Press, Chicago, Illinois.

Lynch JD, Myers CW (1983) Frogs of the *fitzingeri* group of *Eleutherodactylus* in eastern Panama and Chocoan South America (Leptodactylidae). *Bulletin of the American Museum of Natural History*, **175**, 484–568.

Lynch JD, Suárez-Mayorga ÁM (2004) Anfibios en el Chocó biogeográfico. In: *Colombia Diversidad Biótica IV, El Chocó biogeográfico/Costa Pacífica* (ed. Rangel-Ch JO), pp. 633–667. Universidad Nacional de Colombia, Bogotá, Colombia.

Macey JR, Schulte JA II, Larson A *et al*. (1998) Phylogenetic relationships of toads in the *Bufo bufo* species group from the eastern escarpment of the Tibetan Plateau: a case of vicariance and dispersal. *Molecular Phylogenetics and Evolution*, **9**, 80–87.

Macey JR, Strasburg JL, Brisson JA, Vredenburg VT, Jennings M, Larson A (2001) Molecular phylogenetics of western North American frogs of the *Rana boylii* species group. *Molecular Phylogenetics and Evolution*, **19**, 131–143.

Maxson LR, Myers CW (1985) Alburnin evolution in tropical

poison frogs (Dendrobatidae): a preliminary report. *Biotropica*, **17**, 50–56.

McCranie JR, Wilson LD (2002) The amphibians of Honduras. *Contributions to Herpetology*, **19**, 1–625.

McDiarmid RW, Savage JM (2005) The herpetofauna of the Rincón area, Península de Osa, Costa Rica, a Central American lowland evergreen forest site. In: *Ecology and Evolution in the Tropics: A Herpetological Perspective* (eds Donnelly MA, Crother BI, Guyer C, Wake MH, White ME), pp. 366–427. University of Chicago Press, Chicago, Illinois.

Minin V, Abdo Z, Joyce P, Sullivan J (2003) Performance-based selection of likelihood models for phylogeny estimation. *Systematic Biology*, **52**, 674–683.

Miyamoto MM (1986) Phylogenetic relationships and systematics of the *Eleutherodactylus fitzingeri* group (Anura, Leptodactylidae). *Copeia*, **1986**, 503–511.

Noonan BP, Gaucher P (2005) Phylogeography and demography of Guianan harlequin toads (*Atelopus*): diversification within a refuge. *Molecular Ecology*, **14**, 3017–3031.

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL (2004) Bayesian phylogenetic analysis of combined data. *Systematic Biology*, **53**, 47–67.

Pauly GB, Hillis DM, Cannatella DC (2004) The history of a Nearctic colonization: molecular phylogenetics and biogeography of the Nearctic toads (*Bufo*). *Evolution*, **58**, 2517–2535.

Perdices A, Bermingham E, Montill A, Doadriob I (2002) Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America. *Molecular Phylogenetics and Evolution*, **25**, 172–189.

Piperno DR, Pearsall DM (1998) *The Origins of Agriculture in the Lowland Neotropics*. Academic Press, San Diego, California.

Pisani GR (1973) *A Guide to Preservation Techniques for Amphibians and Reptiles. Herpetological Circular 1*. Society for the Study of Amphibians and Reptiles, St. Louis, Missouri.

Pounds JA, Crump ML (1994) Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. *Conservation Biology*, **8**, 72–78.

Pounds JA, Fogden MPL, Savage JM, Gorman GC (1997) Tests of null models for amphibian declines on a tropical mountain. *Conservation Biology*, **11**, 1307–1322.

Prager EM, Wilson AC (1988) Ancient origin of lactalbumin from lysozyme: analysis of DNA and amino acid sequences. *Journal of Molecular Evolution*, **27**, 326–335.

Puschendorf R, Chaves G, Crawford AJ, Brooks DR (2005) *Eleutherodactylus ranoides* (NCN), dry forest population, refuge from decline? *Herpetological Review*, **36**, 53.

Rambaut A, Grassly NC (1997) seq-general: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Computational and Applied Biosciences*, **13**, 235–238.

Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution*, **43**, 304–311.

Reeves RG, Bermingham E (2006) Colonization, population expansion, and lineage turnover: phylogeography of Mesoamerican characiform fish. *Biological Journal of the Linnean Society*, **88**, 235–255.

Remington CL (1968) Suture–zones of hybrid interaction between recently joined biotas. *Evolutionary Biology*, **2**, 321–428.

Ricklefs RE, Schluter D (1993) Species diversity, regional and historical influences. In: *Species Diversity in Ecological Communities* (eds Ricklefs RE, Schluter D), pp. 350–363. University Chicago Press, Chicago, Illinois.

Ridgely RS, Allnutt TF, Brooks T *et al*. (2003) *Digital Distribution Maps of the Birds of the Western Hemisphere, Version 1.0*. NatureServe, Arlington, Virginia. Available from URL: http://www.natureserve.org/getData/birdMaps.jsp.

Roberts TE (2006) History, ocean channels, and distance determine phylogeographic patterns in three widespread Philippine fruit bats (Pteropodidae). *Molecular Ecology*, **15**, 2183–2199.

Roberts JL, Brown JL, von May R, Arizabald W, Schulte R, Summers K (2006) Genetic divergence and speciation in lowland and montane peruvian [sic.] poison frogs. *Molecular Phylogenetics and Evolution*, **41**, 149–164.

Ron SR, Santos JC, Cannatella DC (2006) Phylogeny of the túngara frog genus *Engystomops* (= *Physalaemus pustulosus* species group; Anura: Leptodactylidae). *Molecular Phylogenetics and Evolution*, **39**, 392–403.

Ronquist F, Huelsenbeck JP (2003) mrbayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Ruíz-Carranza PM, Ardila-Robayo MC, Lynch JD (1996) Lista actualizada de la fauna de Amphibia de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Fisicas y Naturales*, **20**, 365–415.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Sanderson MJ (1997) A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution*, **14**, 1218–1231.

Savage JM (1982) The enigma of the Central American herpetofauna: dispersal or vicariance? *Annals of the Missouri Botanical Garden*, **69**, 464–547.

Savage JM (2002) *The Amphibians and Reptiles of Costa Rica: A Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois.

Savage JM, Bradford DH, Lips KR, Jaslow AP (2004) A new species of rainfrog (genus *Eleutherodactylus*) from the Serranía de Tabasará, west-central Panama and reanalysis of the *fitzingeri* species group. *Herpetologica*, **60**, 519–529.

Schäuble CS, Moritz C (2001) Comparative phylogeography of two open forest frogs from eastern Australia. *Biological Journal of the Linnean Society*, **74**, 157–170.

Schneider CJ, Cunningham M, Moritz C (1998) Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. *Molecular Ecology*, **7**, 487–498.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, **69**, 82–90.

Shi X, Gu H, Susko E, Field C (2005) The comparison of the confidence regions in phylogeny. *Molecular Biology and Evolution*, **22**, 2285–2296.

Shimodaira H (2002) An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, **51**, 492–508.

Shimodaira H, Hasegawa M (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, **16**, 1114–1116.

Simpson GG (1940) Mammals and land bridges. *Journal of the Washington Academy of Sciences*, **30**, 137–163.

Stuart SN, Chanson JS, Cox NA *et al*. (2004) Status and trends of

amphibian declines and extinctions worldwide. *Science*, **306**, 1783–1786.

Stuart BL, Inger RF, Voris HK (2006) High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. *Biology Letters*, **2**, 470–474.

Sullivan J (1996) Combining data with different distributions of among-site rate variation. *Systematic Biology*, **45**, 375–380.

Swofford DL (1998) *PAUP*. Phylogenetic Analysis Using Parsimony *and Other Methods*, Version 4b10. Sinauer & Associates, Sunderland, Massachusetts.

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM (1996) Phylogenetic inference. In: *Molecular Systematics* (eds Hillis DM, Moritz C, Mable BK), pp. 407–514. Sinauer & Associates, Sunderland, Massachusetts.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Tavaré S (1986) Some probabilistic and statistical problems on the analysis of DNA sequences. *Lectures in Mathematics and the Life Sciences*, **17**, 57–86.

Templeton AR (1983) Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution*, **37**, 221–244.

Thorne JL, Kishino H (2002) Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, **51**, 689–702.

Thorne JL, Kishino H, Painter IS (1998) Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution*, **15**, 1647–1657.

Tosi JA (1969) *Ecological Map of Costa Rica*. Tropical Science Center, San José, Costa Rica.

Vanzolini PE, Heyer WR (1985) The American herpetofauna and the interchange. In: *The Great American Biotic Interchange* (eds Stehli FG, Webb SD), pp. 475–487. Plenum Press, New York.

Webb SD (1985) Late Cenozoic mammal dispersals between the Americas. In: *The Great American Biotic Interchange* (eds Stehli FG, Webb SD), pp. 357–386. Plenum Press, New York.

Weigt LA, Crawford AJ, Rand AS, Ryan MJ (2005) Biogeography of the túngara frog, *Physalaemus pustulosus*: a molecular perspective. *Molecular Ecology*, **14**, 3857–3876.

Wüster W, Ferguson JE, Quijada-Mascareñas JA, Pook CE, Da Graça Salomão M, Thorpe RS (2005) Tracing an invasion: landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). *Molecular Ecology*, **14**, 1095–1108.

Yang Z (1994) Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *Journal of Molecular Evolution*, **39**, 306–324.

Yang Z, Rannala B (1997) Bayesian phylogenetic inference using DNA sequences: a Markov chain Monte Carlo method. *Molecular Biology and Evolution*, **14**, 717–724.

Zeh JA, Zeh DW, Bonilla MM (2003a) Phylogeography of the harlequin beetle-riding pseudoscorpion and the rise of the Isthmus of Panamá. *Molecular Ecology*, **12**, 2759–2769.

Zeh DA, Zeh JA, Bonilla MM (2003b) Phylogeography of the giant harlequin beetle (*Acrocinus longimanus*). *Journal of Biogeography*, **30**, 747–753.

This research forms part of a series of molecule-based investigations of the evolutionary history of the flora and fauna of Central America carried forward by members of the Bermingham Laboratory. Andrew Crawford was sponsored at STRI by NSF International Programs and Smithsonian Institution Molecular Evolution postdoctoral fellowships. During his time in Panama, he has focused on the population genetics, systematics and biogeography of amphibians in Central America. Carolina Polanía came to STRI as a research intern, and then worked as a staff researcher at La Planada Nature Reserve in Colombia. She is currently looking to start a graduate program.
