Geographic distribution, colour variation and molecular diversity of miniature frogs of the *Eleutherodactylus limbatus* group from Cuba

Ariel Rodríguez¹, Roberto Alonso², José Antonio Rodríguez³ & Miguel Vences⁴

¹⁾ Instituto de Ecología y Sistemática, Carr. de Varona, Km 3¹/₂, Capdevila, Boyeros, AP 8029, CP 10800, La Habana, Cuba
 ²⁾ Museo de Historia Natural 'Felipe Poey', Facultad de Biología, Universidad de La Habana, La Habana, Cuba
 ³⁾ Unidad de Servicios Ambientales PN Alejandro de Humboldt, Sector Baracoa, Guantánamo, Cuba
 ⁴⁾ Zoologisches Institut, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany

Corresponding author: ARIEL RODRÍGUEZ, e-mail: eauriculatus@yahoo.es

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Abstract. The endemic Cuban *Eleutherodactylus limbatus* group contains five species of miniature species of frogs (*E. cubanus, E. iberia, E. jaumei, E. limbatus, E. orientalis*), and one larger and more generalized species (*E. etheridgei*). Several of the miniature species have contrasting colour patterns with bright yellow or white stripes on a dark dorsum, and two of these species are known to sequester skin alkaloids. Based on a review of literature, museum data and numerous own, unpublished field records we provide an updated list of georeferenced locality records of all species of the group that confirms their strict allopatric distribution pattern despite the close geographic proximity of some species. A phylogenetic tree based on newly analysed partial DNA sequences of the mitochondrial cytochrome *b* gene (566 bp) placed the dull-coloured species *E. etheridgei* and *E. cubanus* in a basal position, followed by a well-differentiated *E. orientalis*, and a highly supported but poorly differentiated clade containing *E. iberia, E. jaumei* and *E. limbatus*. In addition to these three forms, this clade also included various subclades with a similar degree of differentiation, which rendered paraphyletic the formally described species, indicating the need for a taxonomic revision. The evolution of contrasting dorsal colour patterns (dorsolateral stripes on a dark brown, light brown or yellow dorsum) apparently was characterized by homoplasy. The highest diversity of this group is concentrated in small areas in the eastern mountains, and the population of *E. limbatus* sampled from western Cuba was genetically similar to an eastern Cuban population, suggesting that only one relatively shallow evolutionary lineage might have succeeded in expanding its range into the west of the island.

Key words. Amphibia, Anura, Terrarana, Eleutherodactylidae, *Eleutherodactylus cubanus, E. iberia, E. jaumei, E. limbatus, E. orientalis*, cytochrome *b*, molecular phylogeny, geographic distribution, colour evolution, taxonomy.

Introduction

Aposematism theory predicts that warning colour and pattern will be constant within geographic units, driven by selective pressures of predators (e.g., GUILFORD & DAWKINS 1993): if patterns are constant, predators having once encountered a noxious specimen should be more likely to avoid a second specimen that is phenotypically similar to the first one. Nevertheless, the geographic scale on which such processes act can be small, depending on the sedentariness of predators. Several groups of poison frogs are known to be highly polymorphic in colour, and individual populations of the same species can be highly dissimilar in their colouration. Such phenomena are especially known in alkaloid-containing species such as dendrobatids (Lör-TERS et al. 2007, ROBERTS et al. 2007) and Mantella (CHIARI et al. 2004, RABEMANANJARA et al. 2007). In some species of these groups, the situation is further complicated by different colour morphs co-occurring in the same population (e.g., BATISTA & KÖHLER 2008, CHIARI et al. 2004, LÖT-TERS et al. 2007, WANG & SHAFFER 2008), Müllerian mimicry (BROWN et al. 2011, SCHAEFER et al. 2002, SYMULA et al. 2001), and sexual selection possibly impacting on colour evolution (BROWN et al. 2010, MAAN & CUMMINGS 2009, REYNOLDS & FITZPATRICK 2007). The complex interaction among these mechanisms are far from satisfyingly understood, but the large intraspecific variation of poison frog colouration is a fact to be taken into account whenever colour is used as a taxonomically relevant character to identify and diagnose such species.

Typically, amphibians derive their toxins, mostly peptides, from their own biosynthesis (DALY 1995). However, a few clades of anurans are known to sequester lipophilic alkaloids into their skin, which appear to be assimilated mainly from their food (SAPORITO et al. 2004, 2007, 2011). These are the Neotropical dendrobatids (numerous

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genera in the family Dendrobatidae), the South American *Melanophryniscus* toadlets (Bufonidae), the Australian *Pseudophryne* toadlets (Myobatrachidae), and the genus *Mantella* (Mantellidae) from Madagascar (DALY et al. 2005). Recently, RODRÍGUEZ et al. (2011) provided evidence for a fifth alkaloid-sequestering clade by reporting on skin alkaloids in two species of miniature *Eleutherodactylus* of the *E. limbatus* group from Cuba (*E. iberia* and *E. orientalis;* Eleutherodactylidae).

The Eleutherodactylidae is one of five families in the Terrarana clade of New World direct-developing frogs as defined by HEDGES et al. (2008) and HEINICKE et al. (2009). The genus Eleutherodactylus contains five subgenera and 187 species primarily distributed in the Caribbean (AmphibiaWeb 2012). In Cuba, 52 of the 62 native amphibians belong to this genus (DÍAZ & CÁDIZ 2008). Among these, the E. limbatus group is an endemic Cuban clade containing, at present, six species: E. cubanus, E. etheridgei, E. iberia, E. jaumei, E. limbatus, and E. orientalis (HEDGES et al. 2008). Except the more generalized E. etheridgei, of up to 27.5 mm in snout-vent length (SVL), all species of this group are minute and reach maximum adult sizes of 17 mm SVL, and Eleutherodactylus iberia is considered one of the smallest species of frogs on earth with no more than 11 mm in adult SVL at its type locality (ESTRADA & HEDGES 1996). The taxonomy of this group of dwarf frogs is based almost entirely on differences in colour pattern, which can be interpreted as a trend towards stronger pattern contrast: from the dull-coloured E. etheridgei and E. cubanus, to yellowish brown dorsally with light dorsolateral bands and a whitish venter (E. jaumei, E. limbatus, E. orientalis), and to uniform dark brown to blackish with bright yellow dorsolateral stripes (E. iberia). Advertisement calls, which often are highly informative for the differentiation of closely related species (e.g., RODRÍGUEZ [2010] for Cuban Eleutherodactylus), appear to be rather similar among different members of this group (ALONSO et al. 2007, ESTRADA & HEDGES 1996), which are, as far as is known, distributed in strict allopatry. While one species, E. limbatus, is considered to be widespread in Cuba, all other species are restricted to very small areas in the mountainous regions of easternmost Cuba (DíAZ & CÁDIZ 2008), a diversity pattern similar to that found in other groups of Eleutherodactylus (RODRÍGUEZ et al. 2010) and probably typical of the Cuban batrachofauna in general.

Eleutherodactylus limbatus was described by COPE (1862) from an unspecified locality in eastern Cuba, which later was restricted to the Guantánamo region by BAR-BOUR (1914), who also collected a series of specimens from the surroundings of Guabairo, Cienfuegos Province in the lowlands of central Cuba. Subsequent herpetological explorations have steadily increased its known geographic distribution, and the species is now considered to occur island-wide (ALONSO et al. 2007, DÍAZ & CÁDIZ 2008, SCHWARTZ & HENDERSON 1991, VALDES 1989). BARBOUR & SHREVE (1937) described *Eleutherodactylus orientalis* as a subspecies of *Sminthillus* (*=Eleutherodactylus) limbatus* from a series of specimens collected at El Yunque de

Baracoa, Guantánamo Province. This subspecies was later raised to species rank by ESTRADA & HEDGES (1996) on the basis of colouration and morphological differences. Other than its type locality, the only other record of Eleutherodactylus orientalis is from a specimen collected by L. F. De Armas in 1993 from Rio Cuzco, Jagueyón, Guantánamo, and deposited in the Zoological Collection of the Institute of Ecology and Systematics (CZACC 14.3776). This record has not been considered by several researchers who have characterized E. orientalis as restricted to El Yunque de Baracoa (Díaz & Cádiz 2008, Estrada & Hedges 1996, ESTRADA & ALONSO 1997), but it is important to note that DIAZ & CÁDIZ (2008), in their distribution map for E. limbatus, placed a question mark in a locality that could represent this specimen (CZACC 14.3776). Many doubts surround the taxonomic status of this population as no living specimens have been recorded from this locality since the initial collection (L. M. Díaz pers. comm.).

Since its description in 1937, Eleutherodactylus cubanus was known only from its type locality at Cueva del Aura, Pico Turquino, Santiago de Cuba, but after its rediscovery in 1995, its known distribution has been expanded to cover almost all the area above 800 m a.s.l. in the Sierra Maestra mountains (Alonso et al. 2007, Díaz & Cádiz 2008). In the same manner, E. iberia was known from a single locality, Arroyo Sucio, Monte Iberia, Holguín (600 m a.s.l.) (ESTRADA & HEDGES 1996), but shortly after, in 1998, the senior author also found it on the coast of Bahia de Taco, Guantánamo (ALONSO & RODRÍGUEZ 1999) and in 2003 recorded the call of a male at Yamanigüey, Holguín (ALONSO et al. 2007). FONG (2000) recorded the presence of the species at a higher altitude on the El Toldo Plateau, and FONG et al. (2005) noted the close proximity of El Toldo localities to another where they observed E. limbatus on the southern slope of the plateau (between 800-300 m a.s.l.). Unfortunately, these latter authors did not list voucher specimens nor did they provide exact data for the locations of their findings. Recently, in their compilation work, DíAZ & CÁDIZ (2008) added two new localities for the species; Tetas de Julia, Guantánamo and Mina Mercedita, Holguín (the latter one located on the slope of the El Toldo Plateau and probably very close to the record of E. limbatus of FONG et al. (2005)). DÍAZ & CÁDIZ (2008) did not comment on the findings of FONG et al. (2005), but placed several dots in their distribution map of *E. limbatus* (p. 62) within the distribution of E. iberia (p. 60). The last addition to the group was E. jaumei, described from Río La Nigua, Peladeros, Santiago de Cuba by ESTRADA & ALONSO (1997). Since the original description, the only other published record of the species is one by DÍAZ & CÁDIZ (2007) from El Naranjal, on the ascent to Barrio Nuevo, Sierra Maestra, Santiago de Cuba.

Overall, there has been a progressive increase in the known distribution of all species in the *Eleutherodactylus limbatus* group, probably derived from an increase in field efforts. In spite of this, not a single case of strict sympatry has been recorded among species in the group. However, several records lack exact locality data, and the taxonomic reliability of some records is questionable. The most recent taxonomic review of the amphibians of Cuba by DÍAZ & CÁDIZ (2008) constituted a big step forward as it included comprehensive distribution maps for all species and thus updated those provided by SCHWARTZ & HENDER-SON (1991), but lists of voucher specimens and geographical coordinates for most of the distribution records have not yet been published (RODRÍGUEZ 2009). Such detailed geographic distribution accounts for every species are crucial for more in-depth studies of the taxonomy, ecology, evolution, and conservation in the group, and to focus fieldwork on as yet unexplored localities that could yield additional records of these species.

The phylogenetic relationships of these miniature species have only been analysed in the context of a molecular systematic revision of the whole Terrarana clade (HEDGES et al. 2008, HEINICKE et al. 2007), with single representatives of each species included. The subtree of the *Eleutherodactylus limbatus* group, reproduced by RODRIGUEZ et al. (2011), suggested monophyly of the group, but did not provide relevant support values for the basal relationships among its members. *Eleutherodactylus cubanus, E. etheridgei*, and *E. orientalis* were placed in an unresolved basal position while *E. iberia, E. jaumei*, and *E. limbatus* formed a highly supported clade in which *E. jaumei* and *E. limbatus* were sister species with similarly high support.

Here we provide an overview of colour pattern variation in representative populations of the Eleutherodactylus limbatus group, along with molecular phylogenetic data for 58 individuals of all species of the group, as a basis for forthcoming detailed analyses of their phylogeography, evolution of alkaloid sequestering, and systematics. We infer from the molecular tree that, similar to other poison frogs, homoplasy in colour patterns is frequent in these frogs. We also provide detailed geographic distribution accounts for all the species in this group, updating many historical records and including new localities for several species. Geographically, our data lead us to formulate the hypothesis that eastern Cuba harbours most of the genetic and chromatic variation of this group of frogs and that the correlation between environmental heterogeneity and biotic diversity in this area might be an interesting subject of further research.

Materials and methods

Fieldwork was performed largely in 2009 and 2010, although some samples from previously collected and preserved material were used as well. Our ingroup samples came from 18 distinct localities (Fig. 1) with the goal of including at least two localities per species as of current taxonomy. Geographic coordinates and altitude of each locality visited were recorded in the field using different GPS receivers with WGS84 projection. Most of the specimens were captured during the day by intensive searches in the leaf litter, typically in areas where choruses of the respective species were heard. The only exceptions were *Eleutherodactylus etheridgei* specimens, which were collected among rocks during the night due to the nocturnal habits of this species (ALONSO et al. 2007). In most of the localities visited, we took photographs of live specimens on their natural substrate. Specimens were euthanized by immersion in chlorobutanol solution and tissue samples (toe clips or thigh muscle) taken and preserved in pure ethanol. Ultimately, the specimens were fixed and preserved in 70% ethanol, and deposited in the Zoological Collection of the Institute of Ecology and Systematics of Cuba (CZACC) in Havana. Locality data and voucher specimen information is summarized in Table 1.

We also gathered our own distribution data for these species, obtained from 1999 to 2011 during several field expeditions to the distribution ranges of each of the limbatus group species. Our list includes localities at which the presence of these small species was visually confirmed, often guided by their high-pitched advertisement calls, unmistakable among the ones of the other Cuban Eleutherodactylus (ALONSO et al. 2007). Each point locality was georeferenced in the field using Garmin GPS receivers (Etrex and Oregon). Additionally, we retrieved information from publications and collection databases, including CZACC and non-Cuban institutions available on HerpNet (http://www. herpnet.org) and GBIF (http://www.gbif.org) data providers. Geographic coordinates of these additional records were obtained from topographic maps, on a 1:50,000 scale, and guided by the original information provided by the authors. To maintain the accuracy of the database, we discarded all cases where the published information did not allow the pinpointing of a geographic location with ± 1 km spatial error. Altitudinal data for every locality, with 90 m spatial resolution, was obtained from the SRTM (Shuttle Radar Topographic Mission) database (http://srtm.csi.cgiar.org/).

For the 18 localities from which DNA sequences were obtained (and where species identity could thus be ascertained), we performed an exploratory analysis of climatic niche divergence between populations. Using ArcGis 9.2 software (ESRI, Redlands, CA) we compiled for each locality the corresponding data of 19 bioclimatic variables obtained from a global interpolation of meteorological data for the period 1950–2000 at 1 km resolution, as available from the WorldClim database (HIJMANS et al. 2005). The obtained data matrix was subjected to a Principal Components Analysis with Statistica 6.1 software (StatSoft, Inc., Tulsa, OK).

Total genomic DNA was extracted from tissue samples using proteinase K (10mg/ml) digestion followed by a standard salt-extraction protocol (BRUFORD et al. 1992). Standard polymerase chain reactions were performed in a final volume of 10 µl and using 0.3 µl each of 10 µM primer, 0,25 µl of total dNTP (10 mM), 0.08 µl of 5 u/µl GoTaq and 2.5 µl 5x GoTaq Reaction Buffer (Promega). We PCR-amplified a fragment of the mitochondrial cytochrome *b* (cob) gene using either primers Cytb-a (CCA TGA GGA CAA ATA TCA TTY TGR GG) and Cytb-c (CTA CTG GTT GTC CTC CGA TTC ATG T) of BOSSUYT & MILINKO-VITCH (2000), or the newly designed primers Cytb-limbatus-L2 (GCA CAR ACC TAG TTC AAT G) and Cytb-lim



Figure 1. (A) Geographic distribution of the Cuban *Eleutherodactylus limbatus* group, based on field data, HerpNet and GBIF records, published localities, and specimens in the CZACC collection (only those records with accurate geographic data (coordinates/descriptions) were considered). See Appendix for a list of localities and coordinates. (B) Sampling sites of species and populations used in the study of molecular and chromatic variation; colours and symbols correspond to species and lineages identified by molecular analysis and are the same as in Figs. 7 and 8. (C, D) Distribution of dorsal and ventral colour patterns in sampled populations. Symbols refer to the colour patterns defined in Fig. 6.

Table 1. Vouchers, localities, and Genbank accession numbers of specimens included in the molecular phylogenetic analyses. Locality names and the corresponding municipality and province names are provided. Sample codes are as used in the phylogenetic tree; voucher numbers refer to the Zoological Collection, Institute of Ecology and Systematics (CZACC), the Felipe Poey Museum, University of Havana (MFP), or the original field numbers of the senior author (AR), n.a. = not available.

Sample code / Voucher	Locality	Latitude	Longitude	Altitude (m a.s.l.)	Genbank accession #
E. atkinsi					
AR549 / n.a.	Balcón del Caribe Hotel, Santiago de Cuba, Santiago de Cuba	19.96606	-75.86351	59	JQ614290
AR550 / n.a.	Balcón del Caribe Hotel, Santiago de Cuba, Santiago de Cuba	19.96606	-75.86351	59	JQ614291
AR551 / n.a.	Balcón del Caribe Hotel, Santiago de Cuba, Santiago de Cuba	19.96606	-75.86351	59	JQ614292
AR617 / CZACC14.13253	Trail to Yunque de Baracoa, Baracoa, Guantánamo	20.34449	-74.56769	181	JQ614293
AR618 / CZACC14.13254	Trail to Yunque de Baracoa, Baracoa, Guantánamo	20.34449	-74.56769	181	JQ614294
AR619 / CZACC14.13255	Trail to Yunque de Baracoa, Baracoa, Guantánamo	20.34449	-74.56769	181	JQ614295
E. cubanus					
ARTS360 / CZACC14.14005	Barrio Nuevo, Buey Arriba, Granma	20.02677	-76.69599	1319	JQ614296
ARTS361 / CZACC14.14006	Barrio Nuevo, Buey Arriba, Granma	20.02677	-76.69599	1319	JQ614297
ARTS362 / CZACC14.14007	Barrio Nuevo, Buey Arriba, Granma	20.02677	-76.69599	1319	JQ614298
Cub7 / CZACC14.13166	La Nueve, La Bayamesa, Buey Arriba, Granma	20.05501	-76.603643	1321	JQ614299
Cub8 / CZACC14.13167	La Nueve, La Bayamesa, Buey Arriba, Granma	20.05501	-76.603643	1321	JQ614300
Cub9 / CZACC14.13168	La Nueve, La Bayamesa, Buey Arriba, Granma	20.05501	-76.603643	1321	JQ614301
E. etheridgei					
ARTS882 / CZACC14.13241	Siboney, Santiago de Cuba, Santiago de Cuba	19.96125	-75.71517	60	JQ614302
ARTS883 / CZACC14.13242	Siboney, Santiago de Cuba, Santiago de Cuba	19.96125	-75.71517	60	JQ614303
ARTS884 / CZACC14.13243	Siboney, Santiago de Cuba, Santiago de Cuba	19.96125	-75.71517	60	JQ614304
E. iberia					
ARTS700 / CZACC14.14138	El Toldo Plateau, Moa, Holguín	20.452463	-74.868904	831	IO614305
ARTS701 / CZACC14.14139	El Toldo Plateau, Moa, Holguín	20.452463	-74.868904	831	JQ614306
ARTS702 / CZACC14.14140	El Toldo Plateau, Moa, Holguín	20.452463	-74.868904	831	JQ614307
ARTS683 / CZACC14.14121	Mina Mercedita, Moa, Holguín	20.430549	-74.839666	288	JQ614308
ARTS684 / CZACC14.14122	Mina Mercedita, Moa, Holguín	20.430549	-74.839666	288	JQ614309
ARTS685 / CZACC14.14123	Mina Mercedita, Moa, Holguín	20.430549	-74.839666	288	JQ614310
ARTS666 / CZACC14.14104	Headwaters of Arroyo Sucio, Moa, Holguín	20.469429	-74.794987	414	JQ614311
ARTS667 / CZACC14.14105	Headwaters of Arroyo Sucio, Moa, Holguín	20.469429	-74.794987	414	JQ614312
ARTS668 / CZACC14.14106	Headwaters of Arroyo Sucio, Moa, Holguín	20.469429	-74.794987	414	JQ614313
AR674 / n.a.	Balcón de Iberia trail, Baracoa, Guantánamo	20.52392	-74.69184	153	JQ614314
AR675 / CZACC14.14009	Balcón de Iberia trail, Baracoa, Guantánamo	20.52392	-74.69184	153	JQ614315
AR676 / CZACC14.14010	Balcón de Iberia trail, Baracoa, Guantánamo	20.52392	-74.69184	153	JQ614316
AR707 / CZACC14.14036	Bahía de Taco, Baracoa, Guantánamo	20.51213	-74.66508	70	JQ614317
AR708 / CZACC14.14038	Bahía de Taco, Baracoa, Guantánamo	20.51213	-74.66508	70	JQ614318
AR709 / CZACC14.14039	Bahía de Taco, Baracoa, Guantánamo	20.51213	-74.66508	70	JQ614319
ARTS25 / CZACC14.14046	Surroundings of Silla de Báez, Baracoa, Guantánamo	20.41656	-74.60355	356	JQ614320
ARTS26 / CZACC14.14047	Surroundings of Silla de Báez, Baracoa, Guantánamo	20.41656	-74.60355	356	JQ614321
ARTS27 / CZACC14.14048	Surroundings of Silla de Báez, Baracoa, Guantánamo	20.41656	-74.60355	356	JQ614322
ARTS642 / CZACC14.14089	Joa, Baracoa, Guantánamo	20.329075	-74.504669	46	JQ614323
ARTS643 / CZACC14.14090	Joa, Baracoa, Guantánamo	20.329075	-74.504669	46	JQ614324
ARTS644 / CZACC14.14091	Joa, Baracoa, Guantánamo	20.329075	-74.504669	46	JQ614325
IBE5 / CZACC14.13239	Monte Iberia, Baracoa, Guantánamo	20.47539	-74.72933	572	JQ614326

Sample code / Voucher	Locality	Latitude	Longitude	Altitude	Genbank
				(111 a.s.1.)	accession #
E. jaumei					
ARTS315 / AR979	Arroyón, Peladeros, Sierra Maestra, Guamá, Santiago de Cuba	19.98978	-76.70001	514	JQ614327
ARTS317 / AR981	Arroyón, Peladeros, Sierra Maestra, Guamá, Santiago de Cuba	19.98978	-76.70001	514	JQ614328
ARTS318 / n.a.	Arroyón, Peladeros, Sierra Maestra, Guamá, Santiago de Cuba	19.98978	-76.70001	514	JQ614329
ARTS373 / CZACC14.14062	El Copal, Peladeros, Sierra Maestra, Guamá, Santiago de Cuba	20.00106	-76.68453	897	JQ614330
ARTS374 / CZACC14.14063	El Copal, Peladeros, Sierra Maestra, Guamá, Santiago de Cuba	20.00106	-76.68453	897	JQ614331
ARTS375 / CZACC14.14064	El Copal, Peladeros, Sierra Maestra, Guamá, Santiago de Cuba	20.00106	-76.68453	897	JQ614332
ARTS370 / CZACC14.14067	Arroyo La Nigua, Sierra Maestra, Guamá, Santiago de Cuba	20.01162	-76.68269	923	JQ614333
ARTS371 / CZACC14.14068	Arroyo La Nigua, Sierra Maestra, Guamá, Santiago de Cuba	20.01162	-76.68269	923	JQ614334
E. limbatus					
ARTS529 / CZACC14.14080	Arrovo la Llavita. El Moncada, Viñales, Pinar del Río	22.538583	-83.834849	272	IO614335
ARTS530 / CZACC14 14081	Arroyo la Llavita, El Moncada, Viñales, Pinar del Río	22.538583	-83 834849	272	IO614336
ARTS532 / CZACC14 14083	Arroyo la Llavita, El Moncada, Viñales, Pinar del Río	22.550505	-83 834849	272	IO614337
ARTS109 / MFP11534	Trail to Loma El Gato, Santiago de Cuba, Santiago de Cuba	20.01555	-76.04531	791	JQ614338
ARTS111 / MFP11535	Trail to Loma El Gato, Santiago de Cuba, Santiago de Cuba	20.01555	-76.04531	791	JQ614339
ARTS151 / CZACC14.14074	Trail to Loma El Gato, Santiago de Cuba, Santiago de Cuba	20.01555	-76.04531	791	JQ614340
AR553 / n.a.	Gran Piedra, Research Station, Santiago de Cuba, Santiago de Cuba	20.01053	-75.63686	1087	JQ614341
AR554 / CZACC14.14054	Gran Piedra, Research Station, Santiago de Cuba, Santiago de Cuba	20.01053	-75.63686	1087	JQ614342
AR556 / CZACC14.14055	Gran Piedra, Research Station, Santiago de Cuba, Santiago de Cuba	20.01053	-75.63686	1087	JQ614343
ARTS676 / CZACC14.14114	Arroyo Bueno, Moa, Holguín	20.445447	-74.805927	185	JQ614344
ARTS677 / CZACC14.14115	Arroyo Bueno, Moa, Holguín	20.445447	-74.805927	185	JQ614345
ARTS678 / CZACC14.14116	Arroyo Bueno, Moa, Holguín	20.445447	-74.805927	185	JQ614346
ARTS641 / CZACC14.14088	Los Naranjos, Vista Alegre, Maisí, Guantánamo	20.160834	-74.279967	567	JQ614347
E orientalia	, ,				
	Suma of Día Currea Januarán El Salvador Cuantánama	20 201166	75 206990	447	10614240
AR15500 / AR1140	Surge of Río Cuzco, Jagueyón, El Salvador, Guantánamo	20.391100	-75.500000	447	JQ014540
ART\$588 / C7ACC14 14005	Surge of Río Cuzco, Jagueyon, El Salvador, Guantánamo	20.371100	-75 306000	447	IO614250
AD667 / n a	Trail to Vunque de Paraços Paraços Cuestínemo	20.371100	-75.500080	++++/	10614251
ARUU/ / II.a.	Trail to Yungue de Daracoa, Daracoa, Guantánamo	20.34499	-/4.30/93	101	JQ014331
ARU00 / 11.a.	Trail to Tunque de Daracoa, Baracoa, Guantanamo	20.34499	-/4.30/93	181	JQ014352
Акобу / п.а.	iran to funque de Baracoa, Baracoa, Guantanamo	20.34499	-/4.56/93	181	JQ014353

batus-R2 (TTA GTT ATT GGT CGG AAG GTT). The successfully amplified products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (SAP) or Antarctic Phosphatase (AP) according to the manufacturer's instructions (NEB). Purified PCR templates were sequenced with the respective L-primer using dye-labelled dideoxy terminator cycle sequencing on an ABI 3130 automated DNA sequencer. Chromatograms were checked and sequences were corrected by hand, where necessary, using Codon-Code Aligner (v. 3.5.6, Codon Code Coorporation). The newly analysed sequences were submitted to GenBank (accession numbers: JQ614290–JQ614353).

We conducted Bayesian inference (BI) searches for phylogeny reconstruction. The model of sequence evolution (a General Time-Reversible GTR+I+G model) was determined by the Akaike Information Criterion (AIC) in Mr-Modeltest (NYLANDER 2008). Searches were run in Mr-Bayes 3.1.2 (RONQUIST & HUELSENBECK 2003) using default conditions (one cold and three heated chains); posterior probabilities were obtained from the majority rule consensus tree. The analysis was run for 50 million generations, with every 1000th tree being sampled and 50% of them discarded as a conservative burn-in after empirically assessing that chains had converged and log-likelihood values had stabilized. Runs were repeated 2 times with 1 million generations each, and the trees obtained were congruent in all relevant aspects of the topology. We calculated pairwise uncorrected distances between the main clades detected in the phylogenetic analysis and estimated its standard deviations with 2000 bootstrap replicates using MEGA4 software (TAMURA et al. 2007).

Results

Our geographic distribution database contains a total of 100 localities of the E. limbatus group (Appendix 1), but some of these are very close to each other and are therefore not represented separately in the map (Fig. 1). The majority of these localities were confirmed by us (mainly by AR, RA, and JAR) in the field. Of the recorded sites, 11 are of E. cubanus, 8 of E. etheridgei, 20 are a-priori assigned to E. iberia (based on its supposedly diagnostic colour pattern; but see the Discussion below), 10 of E. jaumei, 46 are a-priori assigned to E. limbatus, and 5 to E. orientalis (of which 4 are in the surroundings of the type locality Yunque de Baracoa, and 1 is the Río Cuzco locality which is characterized by a divergent haplotype and does probably not belong to *E. orientalis*; see below). Most species of the group are restricted to small ranges in eastern Cuba, with a single species, E. limbatus, having a more extended range. It occurs in eastern Cuba and, probably rather discontinuously, in suitable habitats in central (Sierra del Escambray, Sierra de Banao, and Sierra de Najasa) and western Cuba (numerous records from Pinar del Rio, Artemisa, and some from Mayabeque and Matanzas Provinces). Besides providing detailed coordinates for sites, our data extend slightly the known distribution area of some species, when compared to the previous summary of distributional data (DÍAZ & CADIZ 2008): E. limbatus 48 km westwards, into the Viñales region in Pinar del Río Province (localities El Moncada and surroundings of Constantino Cave (see Appendix)); E. iberia 27 km to the southeast, from its previous easternmost record in Bahía de Taco, to Joa, the new locality in the surroundings of Baracoa; E. jaumei 2 km northwards, from its previous record at Peladeros River to the new locality in Arroyo La Nigua (924 m a.s.l.), representing also an altitudinal extension of 464 m from the previously highest locality (El Naranjal 460 m a.s.l.); and E. etheridgei, 4 km westward from the previous record from Siboney to the new locality Juticí. Overall, our geographic distribution accounts can be considered a refinement of previous results and broadly agree with the distribution maps of DIAZ & CADIZ (2008). In the present study, we sampled a subset of populations for tissue and an assessment of colour in life. Our data are largely in accordance with the schematic representation of colour pattern variability, e.g., of E. cubanus and E. iberia given by DíAZ & CÁDIZ (2008). Figures 2-5 show the various colour patterns encountered in this study: (i) dull-coloured species with a predominance of brown and without strongly contrasting and well-delimited white or yellow stripes (E. cubanus, E. etheridgei, and E. atkinsi which was here included as outgroup and is probably closely related to the *E. limbatus* group (HEDGES et al. 2008)) (Fig. 2), (ii) populations with broad, sharply delimited dorsolateral stripes that are typically yellow anteriorly and white over most of their length; dorsal surface of head typically yellowish, dorsum light brown and often with some darker markings; flanks black and venter whitish yellowish; this pattern is found in E. jaumei, E. orientalis, and in a population from Rio Cuzco (Fig. 3); (iii) specimens with the pattern typical of E. limbatus, i.e., uniform light brown dorsum, dark brown to black flanks, narrow and sharply delimited dorsolateral stripes that are yellow anteriorly and white posteriorly; venter whitish yellow (Fig. 4); (iv) populations with the pattern typical of E. iberia, i.e., dark brown to black dorsum and flanks, narrow and sharply delimited dorsolateral stripes that are predominantly yellow, venter entirely or partially black (Fig. 5). For further analysis, we separated these colour patterns into a dorsal and ventral component and defined a number of different patterns, easily identifiable in preserved specimens (Fig. 6), which could now be assigned to populations, lineages and species.

The majority-rule consensus tree derived from BI analysis of cytochrome b DNA sequences from 64 specimens (Fig. 7) unsurprisingly agrees in general with the phylogeny of HEDGES et al. (2008) who also used mitochondrial sequences albeit from a much lower number of specimens of the Eleutherodactylus limbatus group. The basal relationships among the major clades within the group yielded poor BI bootstrap values. Eleutherodactylus etheridgei is placed basally, followed by E. cubanus and E. orientalis. The samples from three species, E. iberia, E. jaumei and E. lim*batus*, form a highly supported clade, which is structured in subclades of relatively low genetic divergence (0.7-7.6 % uncorrected p distance; see Tab. 2) that do not agree with current taxonomy, however. The six samples from two localities of *E. jaumei* form a monophyletic group that is sister to a clade with E. limbatus specimens from far western (El Moncada) and eastern Cuba (Loma El Gato). Specimens of E. limbatus from another nearby site in eastern Cuba (Gran Piedra) form a separate clade that is sister to a clade containing all other samples, and which we here divide into four major subclades, all apparently restricted to far eastern Cuba: (i) populations of E. iberia from coastal localities in an area northwest of the town of Baracoa, including the locality of Monte Iberia at quite high altitude,

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Figure 2. Photographs of adult individuals of species in the *Eleutherodactylus limbatus* group and related taxa. (A) *E. cubanus*, Barrio Nuevo, Guisa, Granma; (B) *E. cubanus*, Barrio Nuevo, Guisa, Granma; (C) *E. cubanus*, Barrio Nuevo, Guisa, Granma (ventral view); (D) *E. etheridgei*, Siboney, Santiago de Cuba; (E) *E. atkinsi*, Balcón del Caribe, Santiago de Cuba; (F) *E. atkinsi*, El Yunque, Baracoa, Guantánamo.

Diversity of miniature frogs from Cuba



Figure 3. Photographs of adult individuals of species in the *Eleutherodactylus limbatus* group. (A) *E. jaumei*, El Copal, Guamá, Santiago de Cuba; (B) *E. jaumei*, Arroyón, Guamá, Santiago de Cuba; (C) *E. orientalis*, Río Cuzco, El Salvador, Guantánamo; (D) *E. orientalis*, El Yunque, Baracoa, Guantánamo; (E) *E. jaumei*, El Copal, Guamá, Santiago de Cuba (ventral view); (F) *E. orientalis*, El Yunque, Baracoa, Guantánamo (ventral view).

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Figure 4. Photographs of adult individuals of species in the *Eleutherodactylus limbatus* group. (A) *E. limbatus*, El Moncada, Viñales, Pinar del Río; (B) *E. limbatus*, Loma El Gato, Guamá, Santiago de Cuba; (C) *E. limbatus*, Arroyo Bueno, Moa, Holguín; (D) *E. limbatus*, La Gran Piedra, Santiago de Cuba; (E) *E. limbatus*, Vista Alegre, Maisí, Guantánamo; (F) *E. limbatus*, La Gran Piedra, Santiago de Cuba; Cuba (ventral view).

Diversity of miniature frogs from Cuba



Figure 5. Photographs of adult individuals of species in the *Eleutherodactylus limbatus* group. (A) *E. iberia*, Arroyo Sucio, Moa, Holguín; (B) *E. iberia*, Bahía de Taco, Baracoa, Guantánamo; (C) *E. iberia*, Balcón de Iberia, Baracoa, Guantánamo; (D) *E. iberia*, Silla de Báez, Baracoa, Guantánamo; (E) *E. iberia*, El Toldo, Moa, Holguín; (F) *E. iberia*, El Toldo, Moa, Holguín (ventral view).

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Figure 6. The four basic dorsal and three ventral colour patterns found in the diminutive frogs of the *Eleutherodactylus limbatus* group in Cuba. Upper row, dorsal colouration: (A) dark dorsum without distinct and sharply delimited light dorsolateral stripes (dorsum either uniform, with indistinct markings, or with broad and diffuse, poorly contrasting dorsolateral stripes and dark flanks, as in the photo (colour pattern observed in *E. cubanus*); (B) light dorsum with dark spots and distinct white dorsolateral stripes; (C) brown dorsum (of lighter colour than on the flanks) and distinct narrow white dorsolateral lines; (D) black dorsum (not differing from flank colour) with strongly contrasting narrow white dorsolateral lines. Lower row, ventral colouration: (F) light venter; (E, G) intermediate pattern with predominantly black venter with extensive light spotting and/or striping; (H) largely uniform black venter. Symbols as used in Figs. 1 and 7. All photographs are taken of preserved specimens. Note that the ventral side of one side of the thigh is damaged from tissue extraction in some specimens. Silvery white band on the tibia (in A, C, E) is a section of the field tag string. In some cases, parts of black needles used to fix the specimen are visible. Scale bars = 2 mm.

(ii) populations of *E. iberia* from various highland populations, including the type locality, and the lowland populations of Joa and Vista Alegre, (iii) the Toldo population of *E. iberia* that exhibited a deviant haplotype despite being in close geographic proximity to populations belonging to the *E. iberia* highland clade, (iv) the Rio Cuzco population, here named *E.* sp. Rio Cuzco, which by its colour pattern bears a strong resemblance of *E. orientalis* but does not seem to be phylogenetically related to that species.

A PCA of bioclimatic data revealed a large overlap along the most informative first two factors of most species and intraspecific lineages (Fig. 8). PC-1 mostly reflected the contribution of annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, and the mean temperature of the driest, wettest, coldest and warmest quarter, while PC-2 mostly reflected the contribution of the mean diel range, isothermality, annual precipitation, precipitation of the wettest and driest months, and precipitation of the wettest, driest, and coldest quarter (Tab. 3). Furthermore, most lineages were spread rather widely over bioclimatic space. *E. cuba-nus* and the *E. limbatus* lineage from Gran Piedra clustered narrowly together as the sampled populations were from rather high altitudes. The genetically divergent Rio Cuzco population differed from all other species and lineages except *E. etheridgei* by its rather low values of PC-2. A wide bioclimatic niche overlap occurs among the "central" and "northeastern" lineages of *E. iberia*, reflecting that the "central" haplotypes were also found in several low-altitude populations.



Figure 7. Phylogenetic relationships among samples of the *Eleutherodactylus limbatus* group. The tree is a majority rule consensus tree from a BI analysis, based on a 566 bp matrix of partial sequences of the cytochrome *b* gene. For each locality, colour patterns are identified by symbols as defined in Fig. 2. Bayesian posterior probabilities are coded as follows: * = 0.94-0.98, ** = 0.99-1.0 (not shown if below 0.94).

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Table 2. Pairwise uncorrected genetic distances (p) (lower diagonal) and within-group standard deviation (upper diagonal) between the main clades detected within the *limbatus* group (N denominates the number of specimens included in each clade). Data from an alignment of mtDNA sequences of a 566 bp fragment of the cytochrome *b* gene obtained from 64 specimens; standard deviations were estimated by 2000 bootstrap replicates.

	Species (N)	1	2	3	4	5	6	7	8	9	10	11
1	E. atkinsi (6)		0.014	0.015	0.015	0.016	0.015	0.015	0.017	0.017	0.017	0.015
2	E. etheridgei (3)	0.167		0.133	0.015	0.014	0.015	0.014	0.016	0.164	0.169	0.170
3	E. cubanus (6)	0.165	0.013		0.013	0.013	0.013	0.013	0.016	0.150	0.155	0.147
4	E. orientalis (3)	0.178	0.175	0.130		0.145	0.145	0.132	0.140	0.151	0.151	0.147
5	E. limbatus (6)	0.187	0.150	0.134	0.014		0.041	0.009	0.010	0.071	0.068	0.066
6	E. jaumei (6)	0.182	0.157	0.136	0.014	0.007		0.009	0.012	0.079	0.075	0.067
7	<i>E. limbatus</i> Gran Piedra (3)	0.179	0.145	0.139	0.013	0.055	0.058		0.009	0.057	0.057	0.059
8	E. sp. Río Cuzco (3)	0.192	0.159	0.152	0.016	0.062	0.076	0.044		0.030	0.031	0.032
9	<i>E. iberia</i> Toldo (3)	0.193	0.017	0.016	0.016	0.011	0.012	0.010	0.008		0.010	0.008
10	E. iberia Central (13)	0.192	0.017	0.016	0.015	0.011	0.012	0.010	0.008	0.045		0.006
11	E. iberia North-east (10)	0.183	0.015	0.014	0.014	0.010	0.009	0.009	0.007	0.041	0.026	

Discussion

With the exception of *E. etheridgei*, Cuban frogs of the *Eleutherodactylus limbatus* group exhibit diurnality and small size. *Eleutherodactylus orientalis*, *E. iberia*, *E. limbatus*, and *E. jaumei* show, additionally, a contrasting bright colouration for which an aposematic function can be hypothesized because lipophilic alkaloids have been isolated in the first two species (RODRÍGUEZ et al. 2011). As in other species of aposematic frogs (e.g., WANG & SHAFFER 2008),

colouration appears to be extraordinarily labile in species of the *Eleutherodactylus limbatus* group and diverse colour variants could have evolved by independent processes, under different predatory pressures. The phylogenetic data presented herein provide evidence for several instances of homoplasy in colour pattern in the *Eleutherodactylus limbatus* group. To underline this statement, we here assume that the mitochondrial tree correctly represents the species tree. Additional preliminary analysis of data including longer mitochondrial DNA sequences and DNA sequences



Figure 8. Scores of the first two principal components derived from a principal component analysis of climatic data from the 20 localities included in the phylogenetic analysis of the *Eleutherodactylus limbatus* group. Colours and symbols of species and subclades are as in Figs. 1 and 7.

Variable	Description	PC-1	PC-2	PC-3
BIO1	Annual mean temperature	0.98	0.03	0.07
BIO2	Mean diel range (mean of monthly range (max. temp min. temp.))	0.38	-0.77	0.17
BIO3	Isothermality (P2/P7) (* 100)	-0.01	-0.72	-0.52
BIO4	Temperature seasonality (standard deviation *100)	0.29	-0.06	0.79
BIO5	Maximum temperature of warmest month	0.98	-0.06	0.11
BIO6	Minimum temperature of coldest month	0.97	0.12	-0.02
BIO7	Temperature annual range	0.49	-0.61	0.48
BIO8	Mean temperature of wettest quarter	0.93	-0.20	0.23
BIO9	Mean temperature of driest quarter	0.98	0.08	-0.05
BIO10	Mean temperature of warmest quarter	0.98	0.02	0.11
BIO11	Mean temperature of coldest quarter	0.98	0.05	0.00
BIO12	Annual precipitation	0.04	0.96	0.10
BIO13	Precipitation of wettest month	-0.19	0.90	0.00
BIO14	Precipitation of driest month	0.53	0.79	-0.19
BIO15	Precipitation seasonality (coefficient of variation)	-0.58	-0.51	0.37
BIO16	Precipitation of wettest quarter	-0.05	0.90	0.27
BIO17	Precipitation of driest quarter	0.52	0.78	-0.21
BIO18	Precipitation of warmest quarter	-0.58	0.39	0.63
BIO19	Precipitation of coldest quarter	0.56	0.76	-0.22
	Explained variance	8.66	6.30	1.98
	% Total variance	46.29	32.47	10.37
	Eigenvalue	8.80	6.17	1.97

Table 3. Eigenvalues and factor loadings from a Principal Component Analysis of bioclimatic variables at sampling sites of *Eleutherodactylus limbatus* group representatives. Principal Components (PC) loadings > 0.7 (positive or negative) are marked in bold.

from nuclear genes, for a larger set of populations, corroborate this assumption (A. RODRÍGUEZ & M. VENCES work in progress). It therefore can be inferred that a partially or entirely dark venter evolved in parallel in *E. cubanus* and *E.* iberia, and the trend towards a generally more contrasting colouration eventually resulted in a uniformly dark brown to black ventral side in most populations of the latter species. However, some populations genetically assigned to E. iberia (Joa), and E. sp. Rio Cuzco, which is nested within E. iberia, most probably reversed this state towards a whitish venter. An alternative explanation will be to consider the dark venter as a primitive (plesiomorphic) condition that was later lost in E. orientalis, E. limbatus, E. jaumei, E. sp Rio Cuzco, and some populations of *E. iberia*, but given that the two basal species (Eleutherodactylus atkinsi and E. etheridgei) exhibit a light ventral colouration, this evolutionary hypothesis seems less likely.

Regarding dorsal colour, the morph with broad dorsolateral stripes and a partly yellowish anterodorsal colouration is found in three phylogenetically distant populations, i.e., *Eleutherodactylus orientalis, E. jaumei*, and *E.* sp. Rio Cuzco. This pattern might have characterized the ancestor of the well-supported *iberia/jaumei/limbatus* clade, and independently evolved several times into morphs with narrower dorsolateral stripes and increased dark colour on the dorsum and flanks: twice towards the *limbatus* morph in which the dorsolateral stripes simply became narrower, and once into the *iberia* morph with very narrow dorsolateral stripes and its dark brown to black colouration on flanks and dorsum. This latter pattern might have reversed in several populations (Mina Mercedita, Arroyo Bueno, and Vista Alegre) into a less dark morph reminiscent of *E. limbatus*.

Alternative but equally parsimonious scenarios of colour evolution are obviously possible, involving additional parallel instances of the evolution of black ventral colour rather than reversals, or narrow dorsolateral stripes being ancestral with subsequent reversals to broader stripes. We here refrain from an explicit reconstruction of ancestral states because the support in our tree is rather low for basal nodes, and our molecular sampling of populations especially of the widespread Eleutherodactylus limbatus is incomplete. Although, as was stated above, the general topology of our tree agrees also with preliminary unpublished trees based on nuclear DNA, a careful analysis of these of data is necessary to exclude the possibility that the inferred patterns of colour evolution are masked by phenomena of occasional hybridization with mitochondrial introgression. However, independent from the precise evolutionary scenario that might be suggested by forthcoming studies, it seems to be clear that colour patterns are not stable within species in the *E. limbatus* group and that the evolutionary history of dorsal and ventral colours has been complex.

All species of the *Eleutherodactylus limbatus* group occur in eastern Cuba, and according to current taxonomy, all but one (*E. limbatus*) are endemic to this region in an area that roughly makes up one-quarter of the total island area. The single population of *E. limbatus* from western Cuba (El Moncada) included in this study did not differ in colour pattern, and its cytochrome *b* sequence was not strongly differentiated, from an eastern conspecific population (Loma El Gato). Although we cannot exclude that other populations of this species group of high genetic divergence might occur in western or central Cuba, none of the known western or central populations are characterized by a deviant colour pattern (DíAZ & CÁDIZ 2008, A. RODRÍGUEZ & R. ALONSO unpubl. observations).

Although several species and intraspecific lineages of the *Eleutherodactylus limbatus* group live in very close geographical proximity, no instance of sympatry has been recorded so far. For instance, both *E. cubanus* and *E. jaumei* appear to be microendemic to the Sierra Maestra massif, but here are segregated by elevation: *E. cubanus* occurring above ca. 900 m a.s.l., and *E. jaumei* below ca. 900 m a.s.l., with a very short linear distance (2 km) separating localities of these two species in the region of Barrio Nuevo. Likewise, in the area around the city of Baracoa, *E. orientalis* is restricted to the Yunque de Baracoa mountain whereas at similar altitudes to the east and west, and at distances of less than 10 km, populations of the "central" and "northeastern" clades of *E. iberia* occur (Joa and Silla de Baez, respectively).

Despite the close geographic proximity of lineages and the high degree of homoplasy in colour patterns, the available data do not indicate that the evolution of colour patterns in the Eleutherodactylus limbatus group is influenced by Müllerian mimicry phenomena. This might be a consequence of the allopatric distribution pattern of the species of the E. limbatus group, since in dendrobatid and mantellid poison frogs, hypothesized instances of Müllerian mimicry typically are found in cases of sympatric and even syntopic occurrence of two species (BROWN et al. 2011, SCHAEFER et al. 2002, SYMULA et al. 2001). Regardless of these differences, the general evolutionary plasticity of colouration is a common theme in all of these probably aposematic frogs, which often include conspecific populations of highly dissimilar colour pattern, as it seems to also be the case in the E. limbatus group.

The high diversity of the *Eleutherodactylus limbatus* group in eastern Cuba might be linked to the environmental heterogeneity of this region. In fact, this region is characterized by numerous mountain massifs of divergent geologic history intersected by lowlands, and characterized by a high bioclimatic variation among individual sites (HERNÁNDEZ-SANTANA et al. 1989). Although this has not yet been analysed using explicit spatial models, available distributional data leave little doubt that the overall highest species diversity and also the highest degree of local endemism of Cuban frogs is concentrated in this region (DÍAZ & CÁDIZ 2008, HEDGES 1999). Like in other groups of frogs, e.g., in Madagascar (KAFFENBERGER et al. 2012, WOLLENBERG et al. 2008), such areas of high environmental heterogeneity might act as centres of speciation from

latter scenario, in Cuba, might apply to a species named *Eleutherodactylus* sp. 4 cf. *auriculatus* (see RODRÍGUEZ et al. 2010), and to *E. limbatus*. With more expansive taxonomic sampling it will be possible to test this hypothesis in other frog clades, such as the *E. varians* group, which also contains three species in the east and only one species (although with various subspecies) in central and western Cuba (DíAZ & CÁDIZ 2008). By increasing the geographic density of sampling, it

where some species then disperse into other areas. This

also will become possible to test how strictly, within lineages, the genetic landscape agrees with the topographic landscape in eastern Cuba (as in LIN et al. 2012). Environmental niche comparisons provided herein are based on very low sample sizes because only a limited number of all the known populations (Fig. 1A) have so far been reliably assigned to lineages based on DNA sequences (Fig. 1B, Fig. 7). The poor bioclimatic niche separation among most of the closely related species and lineages (except possibly the two E. limbatus lineages, and E. cubanus/E. jaumei) and the rather wide niches of most lineages rather suggest that non-adaptive speciation processes such as diversification in mountain or forest refugia primarily drive the diversification of this group, as has been suggested for other clades of West Indian Eleutherodactylus (BARKER et al. 2011, RODRÍGUEZ et al. 2010).

As a conclusion, our updated distributional data and molecular phylogeny suggest that (i) the centre of diversity and endemism of the E. limbatus group lies in eastern Cuba, (ii) that the taxonomy of three species (E. iberia, E. *jaumei*, and *E. limbatus*) is in need of revision, and (iii) that the evolution of colour patterns in these dwarf frogs has been complex and characterized by homoplasy. Expanding the molecular phylogeny by additional individuals, populations, and genes, will allow to trace more reliably the evolution of colour patterns, and to correlate these with the presence or absence of skin alkaloids. Also, analysing altitudinal and bioclimatic heterogeneity throughout the island and correlating these variables spatially with species and genetic diversity of Eleutherodactylus frogs and other organisms will allow testing the hypothesis of eastern Cuba being the main centre of speciation in the island.

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Diversity of miniature frogs from Cuba

Appendix

Localities of occurrence of species of the *Eleutherodactylus limbatus* group from Cuba. In each instance, the municipality and province are indicated. Localities identified during our own fieldwork were georeferenced with GPS receivers and are indicated as "This work". Additional localities were included when the original information provided sufficient accuracy for geographic positioning, these include: specimens deposited in the Zoological Collection of the Institute of Ecology and Systematics of Cuba (CZACC), literature data, and online repositories of collection databases (HerpNet and GBIF). In the later case, collections storing the respective voucher specimens are also listed: CAS = California Academy of Sciences; CM = Carnegie Museums; KU = University of Kansas Biodiversity Research Center; MCZ = Museum of Comparative Zoology, Harvard University; USNM = United States National Museum. Note that localities are assigned to species based alone on their colour pattern; as we demonstrate, the taxonomy of several populations assigned to *E. iberia, E. jaumei, E. limbatus*, and *E. orientalis* in this list is in need of revision; see text for details.

Species	Locality	Altitude (m a.s.l.)	Latitude	Longitude	Source
E. cubanus	Minas de Frío, Bartolomé Masó, Granma	998	19.989611	-76.988631	(Díaz 1998)
E. cubanus	Surroundings of la Platica, Bartolomé Masó, Granma	1058	20.008447	-76.882944	This work
E. cubanus	Trail to Turquino Peak, Bartolomé Masó, Granma	1198	20.007833	-76.865611	This work
E. cubanus	Between Alto del Cardero and La Esmajagua, Guamá, Santiago de Cuba	874	19.969200	-76.845056	This work
E. cubanus	Aguada del Joaquin, Bartolomé Masó, Granma	1363	20.014528	-76.839750	This work
E. cubanus	Joaquín Peak, Guamá, Santiago de Cuba	1668	20.013056	-76.833972	This work
E. cubanus	Barrio Nuevo, Buey Arriba, Granma	1319	20.026770	-76.695990	This work
E. cubanus	Spring in Barrio Nuevo, Buey Arriba, Granma	1283	20.027340	-76.694140	This work
E. cubanus	Alto del Zapato, Buey Arriba, Granma	879	20.041389	-76.658611	(Díaz et al. 2005)
E. cubanus	La Nueve, La Bayamesa, Buey Arriba, Granma	1321	20.055007	-76.603643	This work
E. cubanus	La Bayamesa Peak, Guamá, Santiago de Cuba	1727	20.045800	-76.589050	This work
E. etheridgei	Siboney, Santiago de Cuba, Santiago de Cuba	60	19.961253	-75.715167	This work
E. etheridgei	Guantanamo Bay, peak of John Paul Jones Hill, Caimanera, Guantánamo	116	19.904389	-75.130250	GBIF / HerpNet (USNM)
E. etheridgei	1 km W of Siboney, Santiago de Cuba, Santiago de Cuba	22	19.960200	-75.725745	This work
E. etheridgei	La Cantera, Siboney, Santiago de Cuba, Santiago de Cuba	16	19.956547	-75.734005	This work
E. etheridgei	Punta Justicí, Santiago de Cuba, Santiago de Cuba	14	19.951347	-75.749647	This work
E. etheridgei	0.5 km West of Punta Justicí, Santiago de Cuba, Santiago de Cuba	9	19.951001	-75.749208	This work
E. etheridgei	Mouth of Río Jauco, Maisí, Guantánamo	16	20.080234	-74.335978	(Díaz & Cádiz 2007)
E. etheridgei	El Copey cave, Maisí, Maisí, Guantánamo	36	20.250766	-74.168997	This work
E. iberia	El Toldo plateau (1), Moa, Holguín	806	20.452653	-74.899181	This work
E. iberia	El Toldo plateau (2), Moa, Holguín	831	20.455389	-74.883918	This work
E. iberia	El Toldo plateau (3), Moa, Holguín	826	20.452463	-74.868904	This work
E. iberia	Slope of El Toldo, Moa, Holguín	380	20.432573	-74.850872	This work
E. iberia	Alto del Ácana, Moa, Holguín	382	20.433704	-74.829453	This work
E. iberia	Trail to Arroyo Sucio headwaters, Moa, Holguín	498	20.467877	-74.801960	This work
E. iberia	Headwaters of Arroyo Sucio, Moa, Holguín	478	20.469394	-74.800316	This work
E. iberia	La Caoba, Yamaniguey, Moa, Holguín	50	20.568500	-74.761667	This work
E. iberia	Potosí, Yamaniguey, Moa, Holguín	58	20.556167	-74.751417	This work
E. iberia	Nuevo Mundo, Baracoa, Guantánamo	284	20.503639	-74.750639	This work
E. iberia	Monte Iberia, Baracoa, Guantánamo	633	20.483725	-74.731139	This work
E. iberia	Spring at Monte Iberia, Baracoa, Guantánamo	572	20.475394	-74.729333	This work
E. iberia	Balcón de Iberia, Baracoa, Guantánamo	153	20.503750	-74.724361	This work
E. iberia	Trail to Monte Iberia lagoons, Baracoa, Guantánamo	628	20.466700	-74.723600	This work
E. iberia	Monte Iberia lagoons, Baracoa, Guantánamo	611	20.468194	-74.715278	This work
E. iberia	Miramar, between Bahía de Taco and Santa María, Baracoa, Guantánamo	29	20.523917	-74.691833	This work
E. iberia	Bahía de Taco, Baracoa, Guantánamo	70	20.512139	-74.665083	This work
E. iberia	Sierra de la Maguira, Baracoa, Guantánamo	181	20.436770	-74.609490	This work

Species	Locality	Altitude (m a.s.l.)	Latitude	Longitude	Source
E. iberia	Surroundings of Silla de Báez, Baracoa, Guantánamo	356	20.416560	-74.603550	This work
E. iberia	Joa, Baracoa, Baracoa, Guantánamo	46	20.329075	-74.504669	This work
E. jaumei	Arroyón, Guamá, Santiago de Cuba	549	19.989780	-76.700010	This work
E. jaumei	Trail between Arroyón and Pinalito, Guamá, Santiago de Cuba	701	19.991970	-76.690230	This work
E. jaumei	Surroundings of Pinalito, Guamá, Santiago de Cuba	631	19.992644	-76.686944	This work
E. jaumei	Trail between Copal and Pinalito, Guamá, Santiago de Cuba	911	20.003770	-76.685070	This work
E. jaumei	Alto de Pinalito, Guamá, Santiago de Cuba	825	19.996389	-76.685000	This work
E. jaumei	El Copal, Guamá, Santiago de Cuba	855	20.001060	-76.684530	This work
E. jaumei	Headwaters of Arroyo La Nigua, Buey Arriba, Granma	924	20.011620	-76.682690	This work
E. jaumei	Forest near Pinar Quemado, Guamá, Santiago de Cuba	867	20.006750	-76.682090	This work
E. jaumei	Confluence of Arroyos La Nigua and Peladeros, Guamá, Santiago de Cuba	376	20.001400	-76.670700	This work
E. limbatus	Arroyo la Llavita, El Moncada, Viñales, Pinar del Río	272	22.538583	-83.834849	This work
5. limbatus	Surroundings of Constantino Cave, Sierra de Galeras, Viñales, Pinar del Río	144	22.652000	-83.784639	This work
E. limbatus	Surroundings of Los Portales Cave, Sierra de la Guira, La Palma, Pinar del Río	145	22.670400	-83.477500	This work
E. limbatus	Cajálbana, La Palma, Pinar del Río	139	22.776472	-83.440639	This work
E. limbatus	Arroyo el Azufre, Sierra de la Guira, Los Palacios, Pinar del Río	306	22.656750	-83.425778	This work
E. limbatus	San Diego de los Baños, Consolación del Sur, Pinar del Río	99	22.649167	-83.380389	This work
E. limbatus	Ojo de Agua, Sierra del Rosario, Bahía Honda, Artemisa	232	22.808333	-83.157222	CZACC
E. limbatus	La Caridad, Soroa, Candelaria, Artemisa	228	22.807778	-83.022528	This work
E. limbatus	Soroa Hotel, Candelaria, Artemisa	199	22.795583	-83.010306	This work
E. limbatus	Trail to El Mirador, Soroa, Candelaria, Artemisa	198	22.793833	-83.007611	This work
E. limbatus	El Mulo, Sierra del Rosario, Candelaria, Artemisa	203	22.858000	-82.948300	This work
E. limbatus	Sierra del Rosario Research Station, Candelaria, Artemisa	191	22.850806	-82.931556	This work
E. limbatus	El Taburete, Sierra del Rosario, Candelaria, Artemisa	254	22.842361	-82.923889	This work
E. limbatus	Tapaste, San José de las Lajas, Mayabeque	152	23.032778	-82.134167	CZACC
E. limbatus	1 Mile east of Tapaste, San José de las Lajas, Mayabeque	259	23.008000	-82.113700	GBIF / HerpNet (USNM)
E. limbatus	Pan de Matanzas, Matanzas, Matanzas	177	23.033056	-81.683056	CZACC
E. limbatus	Soledad, Palmira, Cienfuegos	52	22.124390	-80.317890	GBIF / HerpNet (MCZ, CM, USNM, KU, CAS)
E. limbatus	Colonia Guabairo, Palmira, Cienfuegos	148	22.169330	-80.288480	GBIF / HerpNet (USNM)
E. limbatus	Alto de Codina, Cumanayagua, Cienfuegos	879	21.904528	-80.050583	This work
E. limbatus	Filo de Caja de Agua, Sierra de Banao, Sancti Spíritus, Sancti Spíritus	769	21.876889	-79.603111	This work
E. limbatus	Pine forest at La Sabina, Sierra de Banao, Sancti Spíritus, Sancti Spíritus	619	21.882056	-79.600222	This work
E. limbatus	Caja de Agua, Sierra de Banao, Sancti Spíritus, Sancti Spíritus	656	21.866806	-79.599333	This work
E. limbatus	Hoyo del Plátano, Sierra de Banao, Sancti Spíritus, Sancti Spíritus	693	21.856430	-79.593030	(Estrada 1994)
E. limbatus	Jarico, Sierra de Banao, Sancti Spíritus, Sancti Spíritus	248	21.857222	-79.574722	This work
E. limbatus	Sierra de Najasa, Najasa, Camagüey	250	21.042620	-77.781240	(Schwartz & Henderson 1988, Valdes 1989)
E. limbatus	Loma La Juana, Sierra del Cobre, Santiago de Cuba, Santiago de Cuba	900	20.018770	-76.054370	This work
E. limbatus	Trail in Loma del Gato, Santiago de Cuba, Santiago de Cuba	791	20.015550	-76.045310	This work

Species	Locality	Altitude (m a.s.l.)	Latitude	Longitude	Source
E. limbatus	Arroyo de San Pedro, Sierra del Cobre, Santiago de Cuba, Santiago de Cuba	884	20.010220	-76.041310	This work
E. limbatus	Surroundings of El Jardín, La Gran Piedra, Santiago de Cuba, Santiago de Cuba	1023	20.015380	-75.637680	This work
E. limbatus	La Siberia, Gran Piedra, Santiago de Cuba, Santiago de Cuba	1028	20.014500	-75.637510	This work
E. limbatus	La Gran Piedra, BIOECO Research Station, Santiago de Cuba, Santiago de Cuba	1087	20.010528	-75.636861	This work
E. limbatus	Trail to La Siberia, Gran Piedra, Santiago de Cuba, Santiago de Cuba	1121	20.012810	-75.634190	This work
E. limbatus	La Reserva, Gran Piedra, Santiago de Cuba, Santiago de Cuba	1081	20.008139	-75.617611	This work
E. limbatus	Trail to San Ramón de las Yeguas, Santiago de Cuba, Santiago de Cuba	1036	20.000500	-75.617200	This work
E. limbatus	Surroundings of El Palenque, Sierra de Cristal, Frank País, Holguín	588	20.566056	-75.448833	This work
E. limbatus	El Desayuno spring, Sierra de Cristal, Segundo Frente, Santiago de Cuba	748	20.532722	-75.436583	This work
E. limbatus	Surroundings of La Tagua, Guantánamo, Guantánamo	769	20.351611	-75.158306	This work
E. limbatus	Farallones de las Comadres, La Tagua, Guantánamo, Guantánamo	772	20.326139	-75.128000	This work
E. limbatus	Monte Cristo, Cupeyal, Yateras, Guantánamo	844	20.325306	-75.073917	CZACC
E. limbatus	Confluence of Yarey and Jaguaní rivers, Moa, Holguín	417	20.436028	-74.912778	This work
E. limbatus	Vázquez, Piedra la Vela, Yateras, Guantánamo	359	20.420528	-74.899444	This work
E. limbatus	Surroundings of Arroyo Bueno, Moa, Holguín	185	20.445447	-74.805927	This work
E. limbatus	El Café, Llanos de Mal Nombre, Yateras, Guantánamo	436	20.354611	-74.763306	This work
E. limbatus	Río Yumurí, Baracoa, Maisí, Guantánamo	218	20.282694	-74.292000	This work
E. limbatus	Vista Alegre, Maisí, Maisí, Guantánamo	567	20.160834	-74.279967	This work
E. limbatus	Río Maya Canyon, Maisí, Guantánamo	224	20.213593	-74.227875	This work
E. orientalis	Resolladero del Río Cuzco, Jagueyones, El Salvador, Guantánamo	290	20.391166	-75.306880	This work
E. orientalis	Yunque de Baracoa, summit, Baracoa, Guantánamo	545	20.346031	-74.569667	This work
E. orientalis	Trail to Yunque de Baracoa, Baracoa, Guantánamo (1)	141	20.342447	-74.560583	This work
E. orientalis	Trail to Yunque de Baracoa, Baracoa, Guantánamo (2)	181	20.342420	-74.562840	This work
E. orientalis	Trail to Yunque de Baracoa, Baracoa, Guantánamo (3)	292	20.344406	-74.565111	This work

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Corrigendum to the paper:

RODRÍGUEZ, A., R. ALONSO, J. A. RODRÍGUEZ & M. VENCES (2012): Geographic distribution, colour variation and molecular diversity of miniature frogs of the *Eleutherodactylus limbatus* group from Cuba. – Salamandra **48**(2): 71–91.

Unfortunately, in Table 2 of this paper, a number of values have been wrongly represented due to a copy-paste error. We here reproduce the table with the corrected values of genetic divergence.

Table 2 (corrected). Pairwise uncorrected genetic distances (p) (lower diagonal) and standard errors (upper diagonal) between the main clades detected within the *limbatus* group (N indicates the number of specimens included on each clade). Data from an alignment of mtDNA sequences of a 566 bp fragment of the cytochrome b gene obtained from 64 specimens; standard errors were estimated by 2000 bootstrap replicates.

	Species (N)	1	2	3	4	5	6	7	8	9	10	11
1	E. atkinsi (6)		0.014	0.015	0.015	0.016	0.015	0.015	0.017	0.017	0.017	0.015
2	E. etheridgei (3)	0.167		0.013	0.015	0.014	0.015	0.014	0.016	0.017	0.017	0.015
3	E. cubanus (6)	0.165	0.133		0.013	0.013	0.013	0.013	0.016	0.016	0.016	0.014
4	E. orientalis (3)	0.178	0.175	0.130		0.014	0.014	0.013	0.016	0.016	0.015	0.014
5	E. limbatus (6)	0.187	0.150	0.134	0.145		0.007	0.009	0.010	0.011	0.011	0.010
6	E. jaumei (6)	0.182	0.157	0.136	0.145	0.041		0.009	0.012	0.012	0.012	0.009
7	E. limbatus Gran Piedra (3)	0.179	0.145	0.139	0.132	0.055	0.058		0.009	0.010	0.010	0.009
8	E. sp. Río Cuzco (3)	0.192	0.159	0.152	0.140	0.062	0.076	0.044		0.008	0.008	0.007
9	<i>E. iberia</i> Toldo (3)	0.193	0.164	0.15	0.151	0.071	0.079	0.057	0.030		0.010	0.008
10	E. iberia Central (13)	0.192	0.169	0.155	0.151	0.068	0.075	0.057	0.031	0.045		0.006
11	E. iberia North-east (10)	0.183	0.170	0.147	0.147	0.066	0.067	0.059	0.032	0.041	0.026	