

DNA barcoding applied to *ex situ* tropical amphibian conservation programme reveals cryptic diversity in captive populations

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Abstract

Amphibians constitute a diverse yet still incompletely characterized clade of vertebrates, in which new species are still being discovered and described at a high rate. Amphibians are also increasingly endangered, due in part to disease-driven threats of extinctions. As an emergency response, conservationists have begun *ex situ* assurance colonies for priority species. The abundance of cryptic amphibian diversity, however, may cause problems for *ex situ* conservation. In this study we used a DNA barcoding approach to survey mitochondrial DNA (mtDNA) variation in captive populations of 10 species of Neotropical amphibians maintained in an *ex situ* assurance programme at El Valle Amphibian Conservation Center (EVACC) in the Republic of Panama. We combined these mtDNA sequences with genetic data from presumably conspecific wild populations sampled from across Panama, and applied genetic distance-based and character-based analyses to identify cryptic lineages. We found that three of ten species harboured substantial cryptic genetic diversity within EVACC, and an additional three species harboured cryptic diversity among wild populations, but not in captivity. *Ex situ* conservation efforts focused on amphibians are therefore vulnerable to an incomplete taxonomy leading to misidentification among cryptic species. DNA barcoding may therefore provide a simple, standardized protocol to identify cryptic diversity readily applicable to any amphibian community.

Keywords: amphibian decline, cryptic species diversity, DNA barcode of life, *ex situ* conservation, species delimitation

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Introduction

Forty-one per cent of amphibian species throughout the world are threatened (Stuart *et al.* 2004; Hoffmann *et al.* 2010; Hof *et al.* 2011). The most insidious and as yet unstoppable agent of amphibian decline is a pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* Longcore *et al.* 1999 that infects epidermal cells of its host and may cause death by inhibition of electrolyte transport (Longcore *et al.* 1999; Voyles *et al.* 2009). In Isthmian Central America, this pathogen seems to be advancing in an easterly-moving wave, whose detrimental effects

are most severe at high elevation sites (Lips 1999; Lips *et al.* 2006). The highland frog faunas of western and central Panama have declined precipitously whereas extreme eastern Panama apparently still supports abundant and diverse faunas (Woodhams *et al.* 2008; Crawford *et al.* 2010a).

As with much of the flora and fauna of the tropical realm, Neotropical amphibian taxonomy remains regrettably incomplete, despite the fact that not only the number but even the rate of new species descriptions per year has been increasing (Glaw & Köhler 1998; Köhler *et al.* 2005). Ironically, the rate of new discoveries and the rate of species declines are increasing simultaneously (Hanken 1999). We are therefore in a race against time to both accurately characterize and conserve amphibian diversity, making current efforts at amphibian conservation in Panama

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and around the world unprecedented in their scope and urgency (Mendelson *et al.* 2006; Wake & Vredenburg 2008).

For critically endangered species, captive breeding offers our most intensive form of intervention and a short-term attempt to prevent extinction (Mendelson *et al.* 2006). Recently, an international consortium of zoos and conservation organizations has spearheaded an effort to begin captive breeding of many species of frogs from Central Panama (Gagliardo *et al.* 2008; Zippel *et al.* 2011). Collecting permits were granted and frogs were collected from various sites in an effort to capture a representative sample of conspecific genetic diversity, assuming that current taxonomy accurately reflected evolutionary diversity. To the extent that our current taxonomy belies the true diversity of independent evolutionary lineages, however, species' endangerment may be underestimated (Bickford *et al.* 2007; Angulo & Icochea 2010), species' geographical ranges may be overestimated (Wynn & Heyer 2001), and management efforts may unknowingly neglect certain species (Daugherty *et al.* 1990). If heterospecific lineages are unknowingly incorporated into an *ex situ* programme, zookeepers risk attempting to cross reproductively isolated species (Howard *et al.* 1989) or, perhaps worse, creating hybrid progeny that may be maladapted to their parents' native environment (cf. Berven 1982). Our ability to preserve these endangered species through captive breeding efforts therefore depends critically on accuracy and precision of our taxonomy.

An emerging consensus among taxonomists and evolutionary biologists affirms the importance of multiple genetic markers and independent sources of data for robust species delimitation and description (Dayrat 2005; Rissler & Apodaca 2007; Fujita *et al.* 2012). Although mitochondrial DNA (mtDNA) is just one of many sources of data that may be applied to problems of species delimitation (Sites & Marshall 2003), its standardized structure, high mutation rate and rapid sorting of ancestral polymorphism make it one of the more efficient sources (Wiens & Penkrot 2002; Avise 2004). DNA barcoding in animals refers to a global campaign to populate a public database of mtDNA and other data from vouchered specimens representing most of the world's diversity and all vertebrate species (Hebert *et al.* 2003). DNA barcoding was originally proposed as a tool for species identification, but it may also be used for rapidly appraising cryptic diversity (Floyd *et al.* 2002; Crawford *et al.* 2010a; April *et al.* 2011). Although mtDNA by itself is of limited use in delimiting or describing species (Brower 2006), it has been readily adopted as a key component of an integrative taxonomic framework (Sites & Marshall 2003; Padial & de la Riva 2007; Vieites *et al.* 2009).

The term 'cryptic species' refers to the presence of multiple distinct species grouped or 'hidden' under a single taxonomic binomen (Bickford *et al.* 2007). Our experience with evolutionary genetic and biogeographical studies of the biota of Isthmian Central America suggests that the potential is high for the existence of cryptic species and the problems they entail for conservation efforts, especially in amphibians. Phylogeographical studies of various frogs have revealed that central Panama is often a centre of cryptic diversity housing a multiplicity of previously unrecognized old lineages that were obscured by our current taxonomy (Crawford *et al.* 2007, 2010a; Wang *et al.* 2008). For example, data from the túngara frog *Engystomops (Physalaemus) pustulosus* (Cope 1864) show that even for species with geographical distributions spread across much of the Neotropics, the genetic divergences just within Panama are among the highest observed anywhere in the species' range (Weigt *et al.* 2005). Even in the absence of any obvious physical barriers to dispersal, divergences within Panama exceed that observed among conspecific populations of *E. pustulosus* separated by the Andean mountains of South America.

Here we used a DNA barcoding approach to assay mtDNA variation in the *ex situ* collection of the El Valle Amphibian Conservation Center (EVACC) located in central Panama and managed as part of the Panama Amphibian Rescue and Conservation (PARC) project, which is a conservation consortium of zoos and institutions including the Houston Zoo and the Smithsonian Tropical Research Institute (for more information see <http://amphibianrescue.org/>). We obtained genetic data from 10 of the 11 species of the highest conservation priority maintained in EVACC, along with data from wild populations of these same species, to assess whether any of the 10 threatened species may harbour cryptic lineages in the wild and whether any cryptic lineages are being housed in EVACC currently (Table 1). This information will provide a basis for subsequent taxonomic studies and possible description of candidate species uncovered here, as well as inform *ex situ* conservation actions.

Materials and methods

Statement of compliance

Animal Care and Use Protocols were approved by the Houston Zoo and Smithsonian Tropical Research Institute. Field samples were collected with the kind permission of the *Autoridad Nacional del Ambiente* of Panama, under permit numbers 20-2000, SE/A-88-05, SE/A-51-06, SE/A-37-07, SE/A-66-07, SC/A-4-08, SE/A-73-08 and SE/A-128-10.

Table 1 Summary by original taxonomy of sampling from *ex situ* and wild populations of Panamanian frogs along with conspecific genetic divergence in captive samples and in all samples. COI and 16S refer to number of mitochondrial DNA sequences of each gene included in the analysis

Family	Genus species	EVACC COI & 16S data (proportion of samples with both genes)	Wild COI and 16S data (proportion of samples with both genes)	π_B MRCA (SE)			
				EVACC COI	EVACC 16S	All samples COI	All samples 16S
Bufonidae	<i>Atelopus limosus</i>	18 & 19 (16 of 21)	4 & 3 (3 of 4)	0.0075 (0.0026)	0.0010 (0.0007)	0.0077 (0.0027)	0.0009 (0.0006)
Centrolenidae	* <i>Centrolene</i> sp.	2 & 3 (1 of 4)	N/A	0.0677 (0.0107)	0.0136 (0.0052)	N/A	N/A
Craugastoridae	[†] <i>Pristimantis museosus</i>	38 & 15 (14 of 39)	36 & 40 (24 of 52)	0.1951 (0.0195)	0.1087 (0.0150)	0.2195 (0.0179)	0.1319 (0.0135)
Craugastoridae	[‡] <i>Craugastor punctariolus</i>	2 & 14 (2 of 14)	18 & 15 (14 of 19)	0.0000 (0.0000)	0.0000 (0.0000)	0.1602 (0.0178)	0.0933 (0.0133)
Craugastoridae	<i>Craugastor tabasarae</i>	2 & 9 (2 of 9)	3 & 5 (3 of 5)	0.0015 (0.0015)	0.0277 (0.0069)	0.0076 (0.0025)	0.0266 (0.0067)
Strabomantidae	<i>Strabomantis bufoniformis</i>	0 & 6 (0 of 6)	26 & 29 (24 of 37)	N/A	0.0000 (0.0000)	0.1138 (0.0128)	0.0492 (0.0081)
Dendrobatidae	<i>Colostethus panamansis</i>	2 & 1 (1 of 2)	35 & 30 (34 of 43)	0.1175 (0.0147)	N/A	0.1129 (0.0130)	0.0432 (0.0078)
Hemiphractidae	<i>Gastrotheca cornuta</i>	34 & 84 (25 of 93)	5 & 5 (5 of 5)	0.0615 (0.0091)	0.0175 (0.0049)	0.0619 (0.0092)	0.0172 (0.0048)
Hemiphractidae	<i>Hemiphractus fasciatus</i>	45 & 38 (26 of 57)	7 & 9 (6 of 10)	0.0928 (0.0115)	0.0333 (0.0059)	0.0932 (0.0114)	0.0370 (0.0065)
Hylidae	<i>Ecnomiohyla</i> spp.	1 & 7 (0 of 8)	2 & 1 (1 of 2)	N/A	0.0000 (0.0000)	0.0086 (0.0033)	0.1205 (0.0155)

N/A, indicates that genetic distances were not calculable, as <2 samples were available.

**Centrolene* sp. samples were identified as belonging to the confamilial taxon, *Rulyrana* cf. *flavopunctata*, by BLAST search to GenBank, and may constitute an unnamed taxon new to Panama. These samples were not compared to wild-caught Panamanian samples as neither of these genera is known from Panama.

[†]*Pristimantis museosus* data from wild populations included the closely related *P. cruentus* and an unnamed candidate species, *P. aff. museosus* (Crawford *et al.* 2010a), whereas EVACC data included a candidate species referred to as *P. aff. latidiscus*. For comparisons of [‡]*Craugastor punctariolus* with wild populations, we included samples belonging to the newly described species, *P. evanesco*, as individuals may have been selected for *ex situ* conservation before the latter species was described, as well as samples from Costa Rica of the closely related *C. ranoides* that also occurs in Panama. π_B refers to the mean divergence between all pairs of haplotypes whose most recent common ancestor (MRCA) is the same as the MRCA of all samples, with standard errors (SE) in parentheses, and is proportional to the expected coalescent time (Hudson 1990; Slatkin 1991).

Sampling

From the EVACC captive facility we obtained 277 genetic samples comprised mostly of skin swabs (Mendoza *et al.* 2012; Prunier *et al.* 2012) along with some frozen samples of liver, toe clips or blood. These samples represented 10 of 11 nominal frog species of special conservation concern (Table 1). To place the genetic data from the captive populations into a wider geographical context, we added 177 additional samples (2–52 samples per species) of conspecific or closely related Panamanian amphibians obtained from frozen tissue collections of the *Círculo Herpetológico de Panamá* (CH), published data from specimens deposited in the National Museum of Natural History's Division of Amphibians and Reptiles (USNM), and other ancillary sources. The numbers of DNA sequences obtained per taxon in EVACC and from the wild are given in Table 1. Specimen numbers, locality information and GenBank accession numbers are provided in Supplementary Table S1. DNA sequences, chromatograms and specimen data are also publicly available on BoLD (Ratnasingham & Hebert 2007) under project codes 'EVACC' and 'EVACW'. A map of collecting sites is provided in Fig. 1. For three EVACC species we included suspected or known heterospecific DNA

barcode data to facilitate identifications. Because *Pristimantis museosus* (Ibáñez *et al.* 1994) and *P. cruentus* (Peters 1873) may be involved in a cryptic species complex (Savage 1981; Miyamoto 1984; Crawford *et al.* 2010b), we included samples of both species along with previously uncovered mtDNA lineages closely allied to these species (Crawford *et al.* 2010a). *Craugastor punctariolus* (Peters 1863) founders were moved to EVACC prior to the description of a close relative, *C. evanesco* Ryan *et al.* 2010; therefore we included samples of both taxa along with another close relative, *C. ranoides* (Cope 1886) from Costa Rica, given that the latter occurs in Panama and these taxa are known to show low genetic and morphological divergence (Miyamoto 1983; Campbell & Savage 2000; Crawford & Smith 2005). EVACC samples included *Ecnomiohyla rabborum* Mendelson *et al.* 2008 and a sample initially labelled *E. sp.* We therefore included previously published data from *E. miliaria* (Cope 1886) for comparison.

Molecular genetic protocols

Prior to genomic DNA (gDNA) extraction, swabs were cut to fit in 1.5 mL microcentrifuge tubes with 180 μ L of lysis buffer and subjected to 1 min of agitation on a Mini-Beadbeater-8 (BioSpec) with no beads added. All samples (swabs and tissues) were then treated with proteinase K and digested overnight. For EVACC samples, gDNA was extracted using a BioSprint 96 (QIAGEN) robotic extractor based on magnetic beads. Field-collected CH and AJC tissues were extracted on an AutoGenprep 965 (AutoGen) robotic extractor that implements a standard phenol-chloroform protocol.

We collected mtDNA sequence data from two genes, the animal Barcode of Life (Hebert *et al.* 2003), also known as the Folmer fragment of cytochrome oxidase I (COI), and a fragment of the 16S ribosomal RNA gene (16S). The COI marker was PCR-amplified using the primer pair dgHCO2198 (5'-TAA ACT TCA GGG TGA CCA AAR AAY CA-3') and dgLCO1490 (5'-GGT CAA CAA ATC ATA AAG AYA TYG G-3') (Folmer *et al.* 1994; Meyer *et al.* 2005) and 0.25 μ g/ μ L of bovine serum albumin. The 16S marker was amplified using 16SB-H (aka, 16Sbr-H) (5'-CCG GTC TGA ACT CAG ATC ACG T-3') and 16SA-L (aka, 16Sar-L) (5'-CGC CTG TTT ATC AAA AAC AT-3') (Kessing *et al.* 2004). For both markers, PCR contained 2.0 mM of Mg²⁺, utilized an annealing temperature of 49 °C, with all additional reaction and cycling conditions standard (Kessing *et al.* 2004). PCR products were cleaned using *ExoI* and *SAP* enzymes (Werle *et al.* 1994), with Sanger sequencing reactions run on ABI 3130 automated sequencers. All enzymatic and sequencing reactions, including trouble-shooting, were performed in a high-throughput 96-well format. Failed

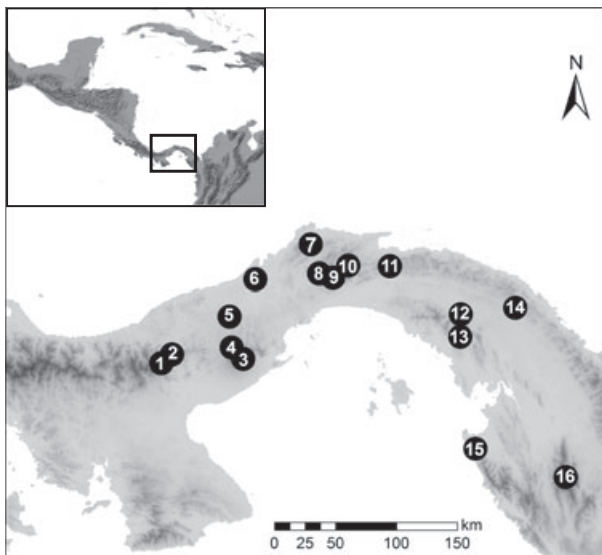


Fig. 1 Map of central and eastern Panama showing sampling locations for genetic samples included in this study. Number localities refer to 1) Río Blanco, 2) El Copé, 3) Altos del María, 4) San Miguel, 5) Río Indio, 6) Fort Sherman, 7) Cerro Bruja, 8) Río Chico, 9) Cerro Azul, 10) Cerro Brewster, 11) Nusagandi, 12) Wacuco (Majé), 13) Cerro Chucantí, 14) Nurra, 15) Cerro Sapo, 16) Cana. The Panama Canal connecting the two oceans lies between localities 6 and 7. Locality details and a list of samples per site are found in Supplementary Table S1.

samples were not submitted to individualized trouble-shooting procedures.

COI sequences were aligned by eye (no length variation was observed) and checked for inferred mis-sense mutations using MacClade (Maddison & Maddison 2005) and the Barcode of Life Database (BoLD) platform (Ratnasingham & Hebert 2007). 16S sequences were aligned using *ClustalX* (Thompson *et al.* 1997) including only congeneric samples to avoid introducing excessive numbers of gaps in alignments. For each gene and for the combined two-gene data set we inferred a neighbour-joining (NJ) tree (Saitou & Nei 1987) using HKY + Γ distances (Hasegawa *et al.* 1985; Yang 1994), i.e., correcting for increased transition rates, unequal nucleotide frequencies and among-site heterogeneity in rates of substitution, all known characteristics of animal mtDNA (Kocher *et al.* 1989). Although most DNA barcoding studies have utilized Kimura 2-parameter (K2P) distances (Kimura 1980), we prefer the HKY model as it accounts for nucleotide frequency bias and performs as well as, or superior to, the K2P model in DNA barcoding analyses (Collins *et al.* 2011; Srivathsan & Meier 2012).

Within each named species and for each gene we calculated the mean divergence between pairs of haplotypes since the most recent common ancestor, that is π_B (Charlesworth 1998) across the root node as inferred from the NJ trees (see above). π_B is proportional to the expected coalescent time (Hudson 1990; Slatkin 1991). Mean HKY distances with standard errors were obtained using MEGA 5 (Tamura *et al.* 2011) that implements the equivalent model under the name Tamura 3-parameter model (Tamura 1992).

To evaluate possible 'unconfirmed candidate species' (Vieites *et al.* 2009) or 'primary species hypotheses' we applied the *Automatic Barcode Gap Discovery* (ABGD) algorithm (Puillandre *et al.* 2011). In a recent comparison of barcoding algorithms, ABGD was found to be efficient as well as robust to variation in sampling design (Paz & Crawford 2012), a potential issue in this study of captive populations. Rather than presume a single threshold of genetic divergence to identify potential candidate species, ABGD evaluates a range of thresholds suggested by the data themselves, and through an iterative refinement procedure may suggest slightly different thresholds for different clades within the same data set. At present, ABGD implements Jukes-Cantor (Jukes & Cantor 1969) and K2P, or accepts distance matrices produced by MEGA or *dnadist* in the PHYLIP package (Felsenstein 2005). To estimate genetic distances we sought to apply evolutionary models that were justifiable by the data, yet no more complex than the HKY + Γ model (Collins *et al.* 2011; Srivathsan & Meier 2012). We estimated genetic distance with the program *dnadist* in the PHYLIP package, in which the most complex model implemented is

the F84 + Γ model (Felsenstein & Churchill 1996), equivalent to HKY + Γ . To select best-fit nucleotide substitution models for a given species data set (Supplemental Table S2), we first used *jModeltest* version 0.1.1 (Posada 2008) and the Bayesian Information Criterion, which may select simpler models than the Akaike Information Criterion depending on sample and effect sizes (Burnham & Anderson 2004). Model and parameter values were used as input for calculations in *dnadist*, with the resulting genetic distance matrix input into ABGD. Two-gene analyses included only samples with data from both genes. Prior maximum intraspecific divergences included 15 thresholds between 1% and 15%, although the exact values are dictated by the data such that thresholds are not distributed uniformly across this interval. ABGD was applied to the combined EVACC plus wild-caught data, as we were looking for candidate species in the named taxon as a whole, not just within EVACC.

For those species or species groups that showed evidence of cryptic diversity (see Results) based on the phenetic or distance-based criterion implemented in ABGD, we also evaluated lineage diversity using a character-based phylogenetic approach (Samadi & Barberousse 2006; De Queiroz 2007). We inferred a maximum likelihood (ML) molecular phylogeny (Felsenstein 1981) using the software GARLI version 2.0.1019 (Zwickl 2006). The character matrix was partitioned by gene (COI vs 16S) and the best-fit nucleotide substitution model (see above) for each gene was applied independently to each partition. As relatively simple models were sufficient (see below) no further partitioning alternatives were pursued. Search parameters employed default values. Clade support was assessed by non-parametric bootstrapping (Felsenstein 1985) involving 200 re-sampling replicates and unpartitioned data and fixed parameter values to speed tree searches and optimization.

DNA barcode gap analyses such as ABGD are based on genetic distances whereas species are diagnosed and described using derived character state changes (Goldstein & DeSalle 2011). As a complement to the ML phylogenetic analyses, we therefore asked whether the COI barcode data provided any potential autapomorphies that could be used to diagnose or describe potential candidate species in the future. Diagnostic nucleotide sites for candidate species were inferred using the Character Attribute Organization System (CAOS) software (Sarkar *et al.* 2008; Bergmann *et al.* 2009). We limited this analysis to the COI data because this marker is more quickly evolving and shows no evidence of length variation among Panamanian amphibians (Crawford *et al.* 2010a), thus providing more variable sites of less ambiguous homology relative to 16S (Xia *et al.* 2012). We report only homogeneous sPu characters, i.e., nucleotide sites showing fixed differences between two lineages, for ease of interpretation.

Results

Success in obtaining DNA sequence data from EVACC samples varied widely between genes and among taxa. COI and 16S data were obtained from 52% and 73% of all EVACC samples respectively. For samples from EVACC, within-taxon genetic divergences (π_B) ranged from zero for *Craugastor punctariolus* (two samples with COI, 14 samples of 16S) and *Strabomantis bufoniformis* (Boulenger 1896; six samples of 16S), up to a maximum of 20% at COI and 11% at 16S for *Pristimantis museosus* (38 samples with COI, 15 samples of 16S; Table 1). Note, however, this latter taxon included EVACC samples preliminarily identified based on morphology as a possible cryptic species, *P. aff. latidiscus* (Fig. 2). Other species with noteworthy 'conspecific' divergence included *Hemiphractus fasciatus* (Peters 1862; Fig. 3) with 9.3% divergence at COI (45 samples) and 3.3% at 16S (38 samples), and *Colostethus panamansis* (Dunn 1933) with 12% divergence at COI (two samples; Table 1). Two other taxa showed modest within-species divergence, *Centrolene* Jiménez de la Espada 1872 sp. and *Gastrotheca cornuta* (Boulenger 1898), whereas two more species, *Atelopus limosus* Ibáñez *et al.* 1995 and *Craugastor tabasarae* (Savage *et al.* 2004), showed nucleotide variability, but very low divergence (Table 1). The final taxon, *E. fimbrimembra*, showed no divergence among the eight EVACC samples at either gene (Table 1), but in point of fact one highly divergent sample identified as *Ecnomiohyla* sp. was found within EVACC. This sample was sequenced successfully only for COI whereas the other samples yielded only 16S. Only by comparison with wild-caught samples could these sequences be compared (see below).

For most species, the level of within-taxon genetic divergence observed among EVACC samples matched that observed for the combined EVACC plus wild-caught data set. For example, *A. limosus* showed little divergence within EVACC or among all samples, and the addition of wild-caught *P. museosus* (Fig. 2) or *H. fasciatus* (Fig. 3) samples to conspecific EVACC data increased divergence only slightly (Table 1). Two exceptions to this trend were presented by *S. bufoniformis* (Fig. 3) and *C. punctariolus* (Fig. 4), which showed no genetic variation within EVACC, but substantial genetic divergence among wild-caught samples. Recall that *C. punctariolus* samples were combined with two additional named species to facilitate identification of EVACC samples (Fig. 4). Combining EVACC plus wild-caught samples across these three nominal taxa revealed 16% divergence at COI (18 samples) and 9.3% at 16S (15 samples). EVACC plus wild-caught samples of *S. bufoniformis* together showed 11% divergence at COI (24 samples) and 4.9% at 16S (29 samples). Samples of *E.*

fimbrimembra from EVACC versus *E. miliaria* from the wild showed a remarkable 12% divergence at the 16S gene (eight samples; Table 1, Supplementary Fig. S1).

Applying the ABGD algorithm to the COI, 16S and two-gene data sets for each of the 10 focal taxa revealed no evidence for cryptic lineages in two cases (*A. limosus* and *Centrolene* sp., although the latter appears to be a new species or new record for the country) and, not surprisingly, strong evidence of additional lineages within the three taxa already known or suspected to contain additional species, *P. museosus*, *C. punctariolus* and *Ecnomiohyla* spp. (Table 2). ABGD identified the 'E. sp.' sample from EVACC as *E. miliaria* (Supplementary Fig. S1). Among the remaining five taxa, the evidence was rather weak for additional species hypotheses within *C. tabasarae*, *S. bufoniformis*, *C. panamansis* and *G. cornuta*, whereas *H. fasciatus* could potentially harbour candidate species (Table 2, Fig. 3). For example, in *S. bufoniformis* a barcode gap threshold of 10% at COI or 2.7% at 16S implied no cryptic species were present, whereas in *H. fasciatus* a barcode gap threshold of 10% at COI supported the presence of three species (i.e. two additional candidate species) and for 16S a threshold of 4.7% supported two species. Results for combined two-gene analyses are not usually reported in the literature, but we provided them here for an additional, perhaps intermediate, perspective (Table 2).

Even though the barcode gap thresholds are higher with COI than with 16S (Vences *et al.* 2005a; Xia *et al.* 2012), ABGD analyses of each species gave largely consistent results among data sets (Table 2), with two exceptions. Samples related to *P. museosus* contained six primary species hypotheses according to the COI data when the prior assumptions of maximum intraspecific divergence ranged as high as 15%. Two of the six groups were comprised of 'singletons,' that is primary species hypothesis consisting of a single specimen (Table 2). Using the combined COI + 16S data including only samples with both genes (Table 1), ABGD identified just four groups among *P. museosus* and related specimens. Two candidate species inferred from COI-only data were missing in the two-gene analyses as one of the COI 'singleton' candidate species was grouped with other samples and a second singleton COI candidate lacked 16S data. Using the 16S data alone, the same four primary species hypotheses were recovered, across prior thresholds of 5% to 10%, although a threshold of 12% collapsed all samples into a single putative taxon (Table 2). The second taxon that appeared to give inconsistent results among data sets was *Ecnomiohyla* spp., with minimal divergence at COI yet high divergence at 16S (Tables 1 and 2). This contrast is a simple artefact caused by the one divergent 16S sample (likely heterospecific to the other specimens) not amplified for COI.

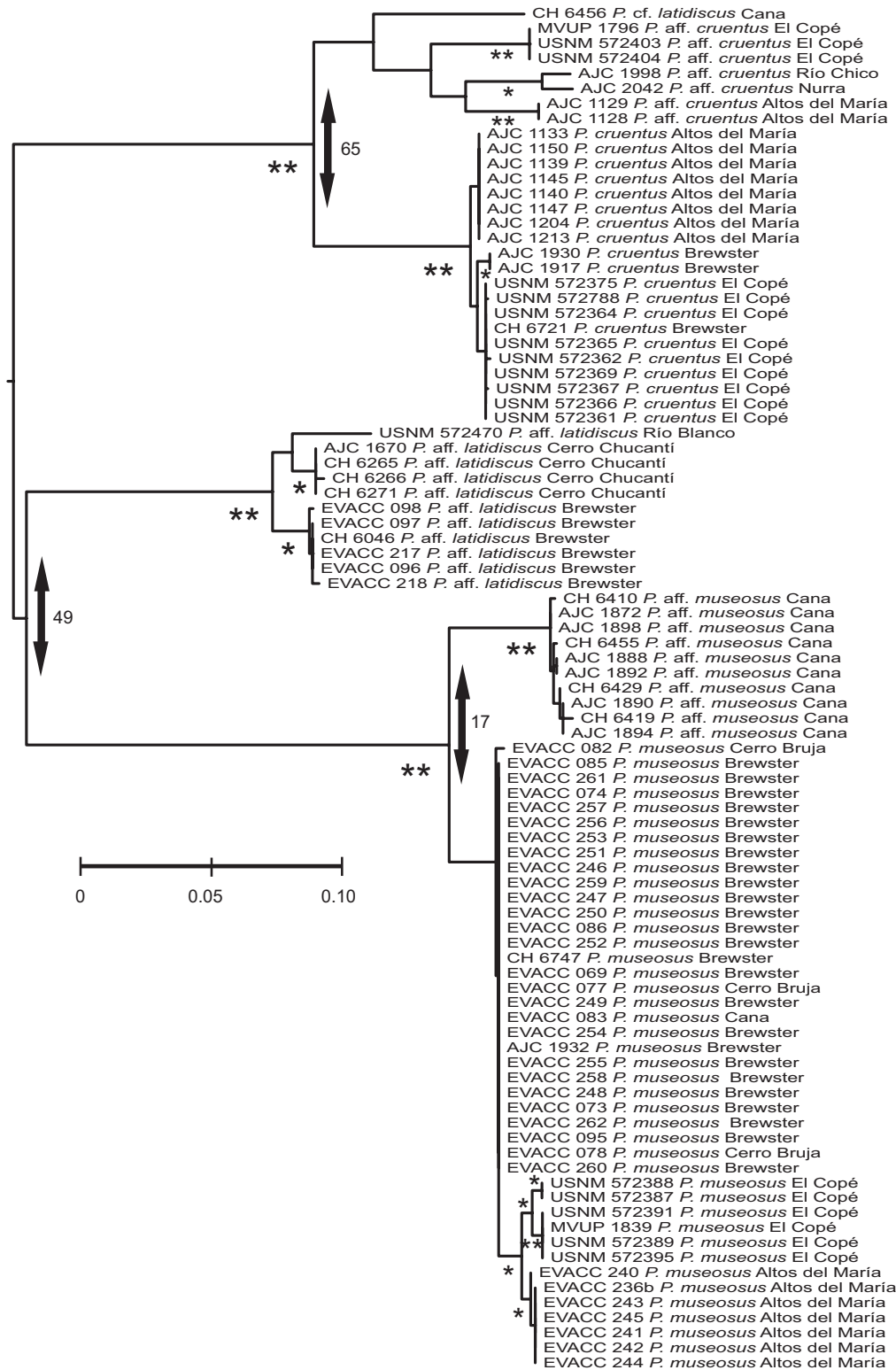


Fig. 2 Maximum likelihood phylogeny based on a partitioned analysis of COI and 16S mitochondrial DNA gene fragments from *Pristimantis museosus* and closely related taxa, inferred using the software *GARLI* 2.0 and rooted at mid-point. Single asterisk (*) indicates nodes with 80% to 94% bootstrap support and double asterisks (**) indicate support $\geq 95\%$. Numbers by double-headed arrows reflect the number of sites at the COI gene showing fixed nucleotide differences between the indicated sister lineages, as inferred from the CAOS analysis. Scale bar indicates inferred patristic distance.

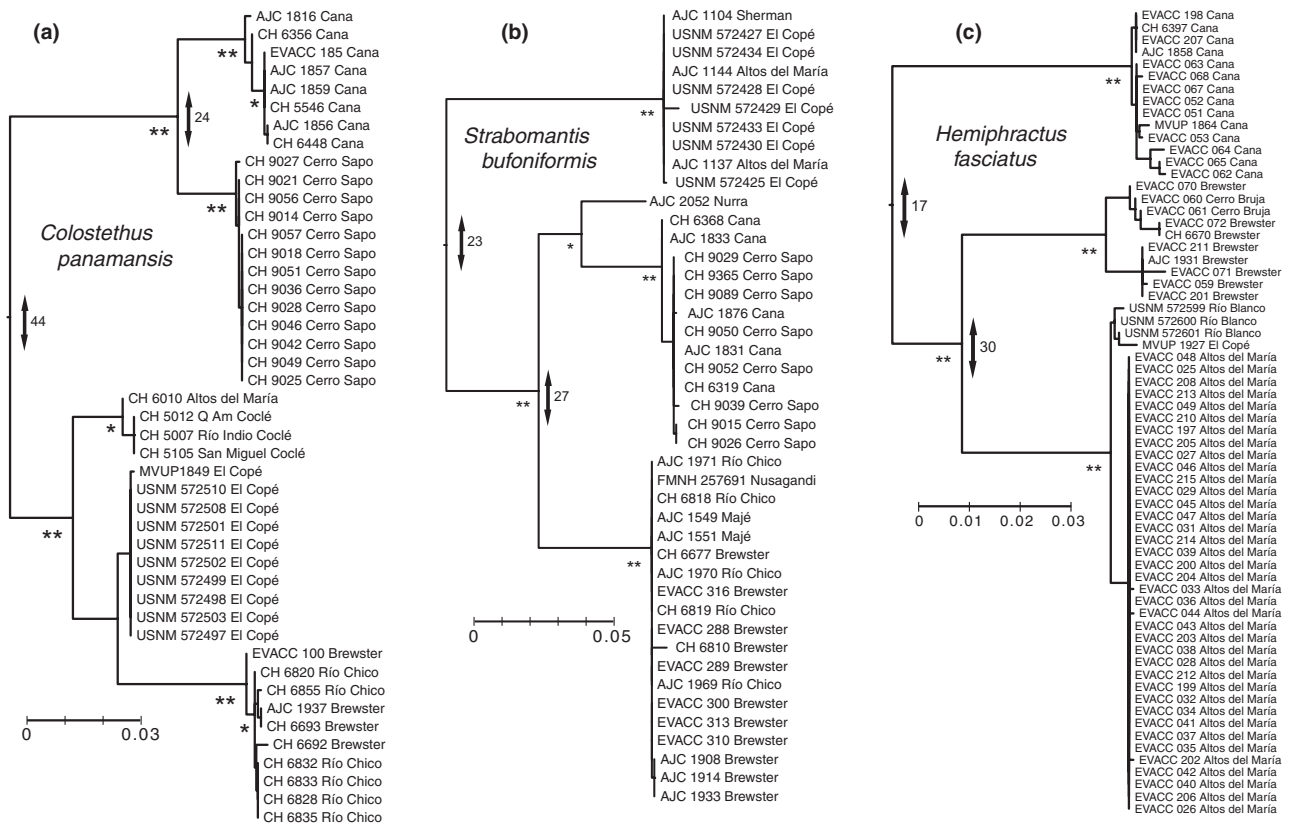


Fig. 3 Three maximum likelihood phylogenies based on a partitioned analysis of COI and 16S mitochondrial DNA gene fragments from three species inferred independently using the software *GARLI* 2.0 and rooted at mid-point. Asterisks and arrows are as in Fig. 2. Scale bars indicate inferred patristic distance separately for each tree. The three species are (a) *Colostethus panamansis*, (b) *Strabomantis bufoniformis*, (c) *Hemiphractus fasciatus*.

Phylogenetic inference using ML confirmed the existence of multiple divergent and statistically supported monophyletic lineages, and revealed that within-species divergence is largely structured geographically. The samples related to *P. museosus* and *P. cruentus* are divided into three major mtDNA lineages and a total of four to six potential species (see *ABGD* results). The locality Brewster (Fig. 1) hosts all three of these principle lineages in sympatry, and other sites such as Altos del María and El Copé host at least two lineages (Fig. 2). Phylogenetic analyses of three additional species with notable conspecific diversity, *C. panamansis*, *S. bufoniformis* and *H. fasciatus*, revealed shared patterns of spatial genetic structure. In each species, specimens from eastern Panama (Cana and Cerro Sapo) formed distinct clades (Fig. 3) relative to all other samples. Specimens from central Panama were further subdivided into two distinctive groups occupying either side of the Panama Canal. In *S. bufoniformis* and *H. fasciatus* the areas east and west of the Canal formed reciprocally monophyletic clades (Figs 1 and 3). *Craugastor ranoides* of Costa Rica grouped with (or within) a closely related *C. evanesco* relative to a deeply diverged *C. punctariolus* (Fig. 4).

The CAOS analysis of COI gene sequences revealed 17–44 fixed nucleotide differences between pairs of clades even for moderately diverged populations (Fig. 3). Character-based DNA barcoding supports the above distance-based inferences, although clades showing greater patristic distances do not necessarily show greater numbers of fixed differences in character states. The COI gene therefore offers a wealth of molecular-based autapomorphies, should taxonomists want to use such information to support species descriptions as well as diagnoses (Goldstein & DeSalle 2011).

Discussion

We argue the parallel races to characterize and conserve amphibian diversity as mutually interdependent: the success of one depends upon the success of the other (Dubois 2003). Conservation planning and action are often based on lists of species and knowledge of their distribution, implying that incomplete taxonomy may be an impediment to achieving the goals of biological conservation (Mace 2004). What is not described cannot be protected (Daugherty *et al.* 1990; May 1990). Genetic

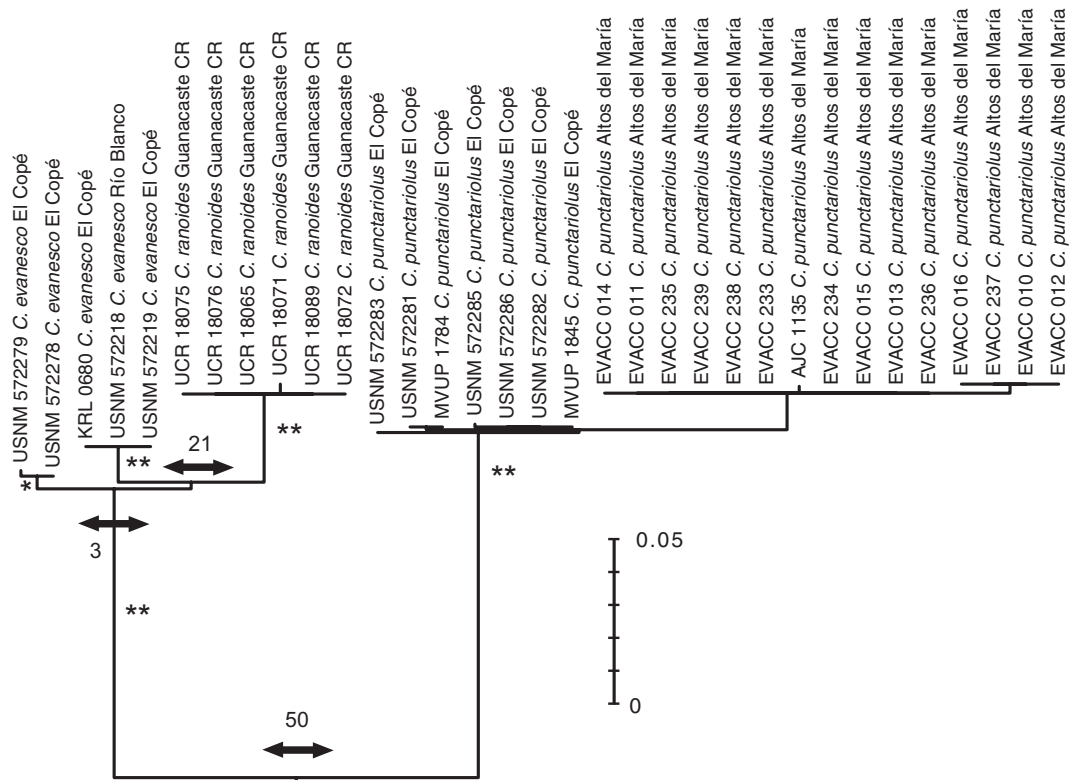


Fig. 4 Maximum likelihood phylogeny based on a partitioned analysis of COI and 16S mitochondrial DNA gene fragments from *Craugastor punctariolus* and two related species, inferred using the software *GARLI* 2.0 and rooted at mid-point. Asterisks and arrows are as in Fig. 2. Scale bar indicates inferred patristic distance.

analyses, therefore, offer information vital to successful intervention and conservation, especially for species representing poorly known taxonomic groups or from biogeographically complex regions (Allendorf & Luikart 2007). Phylogeographical studies and DNA barcoding efforts built around a solid systematic and taxonomic framework can reveal sympatric cryptic species, provide a measure of relatedness between allopatric populations, and provide more accurate estimates of species' ranges (Moritz 1994; Rocha *et al.* 2007). In this study, we used mtDNA data as an assay of previously unrecognized lineage diversity that could hamper captive breeding efforts if ignored.

Using DNA barcoding we have identified three nominal taxa currently being maintained at EVACC that show substantial divergence within the captive population (*P. museosus*, *C. panamansis* and *H. fasciatus*; Figs 2 and 3), along with two other nominal taxa that harbour cryptic diversity among wild populations within Panama that was not captured among our EVACC samples (*S. bufoniformis* and *C. punctariolus*; Figs 3 and 4). This result is alarming for *ex situ* conservation efforts, yet it may not be that surprising given that cryptic diversity may still be the rule rather than the exception among amphibians

(Meegaskumbura *et al.* 2002; Vieites *et al.* 2009; Funk *et al.* 2011). DNA barcoding was also useful in identifying the EVACC sample *Ecnomihyla* sp. as belonging to *E. miliaria* as it matched previously published COI barcodes (Crawford *et al.* 2010a).

Although measuring genetic divergence is relatively straightforward, determining whether divergent lineages represent distinct species is not. We suggest that DNA barcoding provides an excellent 'first pass' assay for cryptic diversity, yet determining specific status of each mtDNA lineage should be accomplished through an integrative approach to taxonomy (Will *et al.* 2005; Padiál *et al.* 2009). Robust species delimitation and description of new species should integrate information from multiple sources, such as morphology, ecology and, in the case of frogs, male advertisement calls when possible (Angulo & Reichle 2008; Jansen *et al.* 2011). Among the species studied here, however, half call very rarely or have no known calls (*e.g.*, *C. tabasarae*, *C. punctariolus*, *S. bufoniformis*, *P. museosus* and *H. fasciatus*). Pending further taxonomic studies, therefore, we can use the mtDNA data in hand to identify what are known as 'unconfirmed candidate species' (Vieites *et al.* 2009; Padiál *et al.* 2010), that is we can flag divergent lineages for

Table 2 Number of primary species hypotheses per taxon inferred by the *Automatic Barcode Gap Discovery* (ABGD) algorithm (Puillandre *et al.* 2011) applied to three mitochondrial DNA sequence data sets: the COI 'Barcode of Life' fragment, the 16S ribosomal gene, and a combination of COI and 16S sequence data. Numbers in parentheses indicate the maximum value of the *a priori* threshold for conspecific divergence that yielded the given number of primary species hypotheses. For each taxon-by-gene combination, the first entry provides the smallest number >1 of primary candidate hypotheses and its corresponding threshold, whereas the second entry provides the minimum threshold that yielded a single inferred taxon (i.e. no candidate species). Cells have only one entry when a threshold of 15% (the maximum value considered) still supports the presence of multiple taxa, or when a threshold <1% is consistent with a single inferred taxon. N/A indicates insufficient number of sequences for ABGD analysis. Note, the *Craugastor punctariolus* data set includes three named taxa, yet the ABGD algorithm recovers >2 (i.e. four) candidate species only with low thresholds of $\leq 2.2\%$ divergence with COI data, $\leq 1.0\%$ with 16S data or $\leq 1.5\%$ with the combined data set

Genus species	COI	16S	Two-gene
<i>Atelopus limosus</i>	1 (0.19%)	1 (0.10%)	1 (0.13%)
* <i>Centrolene</i> sp.	N/A	1 (0.10%)	N/A
† <i>Pristimantis museosus</i>	6 (15%)	4 (10%)	4 (15%)
		1 (12%)	
‡ <i>Craugastor punctariolus</i>	2 (15%)	2 (15%)	2 (15%)
<i>Craugastor tabasarae</i>	1 (0.10%)	2 (1.9%)	1 (0.10%)
		1 (2.7%)	
<i>Strabomantis bufoniformis</i>	3 (8.4%)	10 (2.3%)	3 (5.2%)
	1 (10%) [§]	1 (2.7%)	1 (6.1%) [§]
<i>Colostethus panamansis</i>	3 (5.7%)	5 (2.2%)	3 (4.7%)
	1 (6.9%)	1 (2.6%)	1 (5.7%)
<i>Gastrotheca cornuta</i>	2 (2.6%)	1 (0.23%)	1 (0.72%)
	1 (3.2%)		
<i>Hemiphractus fasciatus</i>	3 (10%)	2 (4.7%)	3 (8.4%)
	1 (12%)	1 (5.7%) [§]	1 (10%) [§]
<i>Ecnomiohyla</i> spp.	1 (0.10%)	2 (10%)	N/A
		1 (12%)	

N/A, indicates that genetic distances were not calculable, as <2 samples were available.

**Centrolene* sp. samples were identified as belonging to the confamilial taxon, *Rulyrana* cf. *flavopunctata*, by BLAST search to GenBank, and may constitute an unnamed taxon new to Panama. These samples were not compared to wild-caught Panamanian samples as neither of these genera is known from Panama.

†*Pristimantis museosus* data from wild populations included the closely related *P. cruentus* and an unnamed candidate species, *P. aff. museosus* (Crawford *et al.* 2010a), while EVACC data included a candidate species referred to as *P. aff. latidiscus*. For comparisons of

‡*Craugastor punctariolus* with wild populations, we included samples belonging to the newly described species, *P. evanesco*, as individuals may have been selected for *ex situ* conservation before the latter species was described, as well as samples from Costa Rica of the closely related *C. ranoides* that also occurs in Panama.

§ Γ -shape parameter (α) increased to 0.5 when maximum likelihood parameter estimates from *jModeltest* yielded very low values (e.g., $\alpha = 0.03$) resulting in genetic distances that were much too high given the data. By increasing α , genetic distance estimates are lower (more conservative).

further study. Thus, mtDNA may serve a practical role in the planning and emergency implementation of captive assurance colonies of tropical amphibians and other endangered yet poorly known animals.

To quantify lineage diversity in the absence of a complete taxonomy, amphibian taxonomists have suggested thresholds of 16S or COI divergence that appear to be associated with specific status in frogs. Vences *et al.* (2005b) assayed genetic divergence at the 16S gene and found that presumably heterospecific lineages could show as little as 2% divergence whereas presumably conspecific populations could show as much as 6% divergence, arguing against a 'one size fits all' threshold for species delimitation. As a first approximation, however, 5% divergence at 16S and 10% divergence at COI were

suggested as thresholds to identify potential candidate species from mtDNA surveys (Vences *et al.* 2005a). Subsequent work on Neotropical frogs suggested a 'more inclusive' threshold of 3% at the 16S marker (Fouquet *et al.* 2007). If we apply these latter thresholds (3% at 16S and 10% at COI) to our data, then in addition to the *P. museosus* and *C. punctariolus* cryptic species, we also find one or two candidate species within *H. fasciatus* (Table 2; Fig. 3). If we focus instead on the raw genetic divergences (Table 1) rather than the barcode gap analysis, and apply the above thresholds, then *S. bufoniformis* and *C. panamansis* also contain candidate species that need to be evaluated with integrated taxonomic data (cf. Fig. 3).

The animal mitochondrial genome does not always reflect genetic diversity or divergence at the nuclear gen-

ome, so conservation planners should bear in mind the limits of inferences based solely on mtDNA (Moritz 1994). Within-population variability at mitochondrial loci may or may not predict polymorphism at nuclear loci (Nabholz *et al.* 2008; Piganeau & Eyre-Walker 2009). Divergence at mtDNA may underestimate nuclear genome divergence due to introgression (e.g. Hailer *et al.* 2012) or may overestimate nuclear divergence due to sex-biased dispersal (e.g. Turmelle *et al.* 2011). Thus, the ideal survey of genetic variation would include data from both genomes. As a tool for standardized initial surveys of genetic divergence, mtDNA does have its advantages, however. Significant conflict between mitochondrial and nuclear markers tends to be rare, although the former may offer a more sensitive indicator of population structure (Zink & Barrowclough 2008) due to higher mutation rates and fourfold smaller effective population size (Avice 2004). Finally, animal mtDNA in general and DNA barcoding in particular offer a standardized, high-throughput methodology that may be applied to nearly all taxa, regardless of previous genetic data (Borisenko *et al.* 2009), and these data can be applied to species discovery and delimitation as well as identification (Padial & de la Riva 2007; Goldstein & DeSalle 2011).

Regardless of whether divergent lineages should or should not be recognized and described as distinct species, even conspecific divergence is important in *ex situ* conservation programmes. With the exceptions of the *P. museosus* and *C. punctariolus* lineages, all other nominally conspecific yet divergent lineages represent allopatric populations (Fig. 3). These populations may have genetic variants that represent incompatibilities (Howard *et al.* 1989) or local adaptations (e.g., Phillimore *et al.* 2010; Lind *et al.* 2011) and perhaps cannot or should not be interbred in captivity. DNA barcoding may provide a rapid and standardized assay of population divergence that could inform *ex situ* planning and implementation for species without prior genetic information, such as tropical frogs in assurance colonies.

As amphibian populations continue to be decimated, conservationists must conduct increasingly ambitious efforts to preserve remnants of biodiversity, often utilizing *ex situ* techniques, with limited resources and on species for which very little basic information is available. We suggest that genetic considerations be an integral part of any amphibian conservation response, especially for those projects that involve captive breeding, translocations and releases of amphibians, and funding for such components be allocated during the project's inception (Zippel *et al.* 2006). As argued, DNA barcoding fulfils this objective well. Confirming the existence of cryptic species or any confounding population substructure within a putative amphibian species that is a conservation target early on in a programme can only increase the

likelihood of a forming a successful captive assurance colony and, ultimately, a re-established wild population.

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P.C., E.G., H.R., E.B. and A.J.C. designed research; A.J.C., E.G., H.R., C.C., A.C.D., R.I. and K.R.L. performed research; A.J.C., C.C. and A.C.D. analysed data; and A.J.C., P.C., K.R.L., E.G., A.C.D. and R.I. wrote the manuscript

Data Accessibility

DNA sequences: GenBank accession numbers are provided in Supplementary Table S1 and DNA sequence data, chromatograms and specimen data are available in two public projects, 'EVACC' and 'EVACW', in the Barcode of Life Database (BoLD).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Maximum likelihood phylogeny based on a partitioned analysis of COI and 16S mitochondrial DNA gene fragments from *Ecnomiohyla* samples, inferred using the software *GARLI* 2.0 and rooted at mid-point. Double asterisks (**) indicate support $\geq 95\%$. Scale bar indicates inferred patristic distance.

Table S1 Taxonomy, sample number, museum voucher numbers (when available), collecting locality and GenBank numbers for all samples used in this study. All localities are in the Republic of Panama. See Fig. 1 in main text for map. AJC = Andrew J. Crawford field number; CH = Círculo Herpetológico de Panamá, Panama City, Republic of Panama; EVACC = El Valle Amphibian Conservation Center sample number, Republic of Panama; FB = Federico Bolaños field number; KRL = Karen R. Lips field number; MVUP = Museo de Vertebrados de la Universidad de Panamá, Republic of Panama; 'swab' = non-vouchered genetic sample from live specimen; UCR = Universidad de Costa Rica, Museo de Zoología, San Pedro, Costa Rica; USNM = Smithsonian Institution's National Museum of Natural History, Division of Amphibians and Reptiles, Washington, D.C., USA.

Table S2 Model of nucleotide substitution best supported by the Bayesian Information Criterion as implemented in *jModeltest* and applied to each mitochondrial DNA data set (EVACC and wild-caught samples combined).

Supplementary Table S1, Table S2 and Figure S1 in support of the manuscript, Crawford *et al.* ‘DNA barcoding applied to *ex situ* tropical amphibian conservation program reveals cryptic diversity in captive populations.’

Supplementary Table S1.

Taxonomy, sample number, museum voucher numbers (when available), collecting locality and GenBank numbers for all samples used in this study. All localities are in the Republic of Panama. See Figure 1 in main text for map. AJC = Andrew J. Crawford field number; CH = Círculo Herpetológico de Panamá, Panama City, Republic of Panama; EVACC = El Valle Amphibian Conservation Center sample number, Republic of Panama; FB = Federico Bolaños field number; KRL = Karen R. Lips field number; MVUP = Museo de Vertebrados de la Universidad de Panamá, Republic of Panama; ‘swab’ = non-vouchered genetic sample from live specimen; UCR = Universidad de Costa Rica, Museo de Zoología, San Pedro, Costa Rica; USNM = Smithsonian Institution’s National Museum of Natural History, Division of Amphibians and Reptiles, Washington, D.C., USA.

Family	Genus species	Collector number	Museum number	Locality	Genbank COI	GenBank 16S
Bufonidae	<i>Atelopus limosus</i>	CH 6694	CH 6694	Brewster, Panamá, 810 m	KC129181	KC129289
Bufonidae	<i>Atelopus limosus</i>	CH 6717	CH 6717	Brewster, Panamá, 810 m	KC129182	KC129290
Bufonidae	<i>Atelopus limosus</i>	CH 6742	CH 6742	Brewster, Panamá, 810 m	KC129183	KC129291
Bufonidae	<i>Atelopus limosus</i>	CH 9180	CH 9180	Brewster, Panamá, 810 m	KC129184	N/A
Bufonidae	<i>Atelopus limosus</i>	EVACC 001	swab	Sierra Llorona, Panamá	KC014624	KC014770
Bufonidae	<i>Atelopus limosus</i>	EVACC 002	swab	Sierra Llorona, Panamá	N/A	KC014771
Bufonidae	<i>Atelopus limosus</i>	EVACC 003	swab	Sierra Llorona, Panamá	KC014625	KC014772
Bufonidae	<i>Atelopus limosus</i>	EVACC 004	swab	Sierra Llorona, Panamá	KC014626	KC014773
Bufonidae	<i>Atelopus limosus</i>	EVACC 005	swab	Sierra Llorona, Panamá	KC014627	KC014774
Bufonidae	<i>Atelopus limosus</i>	EVACC 006	swab	Sierra Llorona, Panamá	KC014628	KC014775
Bufonidae	<i>Atelopus limosus</i>	EVACC 007	swab	Sierra Llorona, Panamá	KC014629	KC014776
Bufonidae	<i>Atelopus limosus</i>	EVACC 008	swab	Sierra Llorona, Panamá	KC014630	KC014777
Bufonidae	<i>Atelopus limosus</i>	EVACC 009	swab	Sierra Llorona, Panamá	KC014631	KC014778
Bufonidae	<i>Atelopus limosus</i>	EVACC 220	swab	Sierra Llorona, Panamá	KC014632	N/A
Bufonidae	<i>Atelopus limosus</i>	EVACC 221	swab	Sierra Llorona, Panamá	KC014633	N/A

Bufonidae	<i>Atelopus limosus</i>	EVACC 222	swab	Sierra Llorona, Panamá	KC014634	KC014779
Bufonidae	<i>Atelopus limosus</i>	EVACC 223	swab	Sierra Llorona, Panamá	KC014635	KC014780
Bufonidae	<i>Atelopus limosus</i>	EVACC 224	swab	Sierra Llorona, Panamá	KC014618	KC014763
Bufonidae	<i>Atelopus limosus</i>	EVACC 226	swab	Sierra Llorona, Panamá	KC014619	KC014764
Bufonidae	<i>Atelopus limosus</i>	EVACC 227	swab	Brewster, Panamá, 810 m	KC014620	KC014765
Bufonidae	<i>Atelopus limosus</i>	EVACC 228	swab	Brewster, Panamá, 810 m	KC014621	KC014766
Bufonidae	<i>Atelopus limosus</i>	EVACC 229	swab	Brewster, Panamá, 810 m	KC014622	KC014767
Bufonidae	<i>Atelopus limosus</i>	EVACC 230	swab	Brewster, Panamá, 810 m	N/A	KC014762
Bufonidae	<i>Atelopus limosus</i>	EVACC 231	swab	Brewster, Panamá, 810 m	KC014623	KC014768
Bufonidae	<i>Atelopus limosus</i>	EVACC 232	swab	Brewster, Panamá, 810 m	N/A	KC014769
Centrolenidae	<i>Centrolene</i> sp.	EVACC 087	swab	Parque Nacional Darién, 800 m	KC014637	N/A
Centrolenidae	<i>Centrolene</i> sp.	EVACC 088	swab	Parque Nacional Darién, 800 m	N/A	KC014783
Centrolenidae	<i>Centrolene</i> sp.	EVACC 089	swab	Parque Nacional Darién, 800 m	N/A	KC014782
Centrolenidae	<i>Centrolene</i> sp.	EVACC 090	swab	Parque Nacional Darién, 800 m	KC014636	KC014781
Craugastoridae	<i>Craugastor evanesco</i>	KRL 0629	USNM 572278	El Copé, Coclé, 700 m	FJ766675	FJ784324
Craugastoridae	<i>Craugastor evanesco</i>	KRL 0630	USNM 572279	El Copé, Coclé, 700 m	FJ766674	FJ784325
Craugastoridae	<i>Craugastor evanesco</i>	KRL 0680	destroyed	El Copé, Coclé, 700 m	FJ766637	FJ784332
Craugastoridae	<i>Craugastor evanesco</i>	KRL 0804	USNM 572218	Río Blanco, Coclé, 1100 m	KC129216	KC129324
Craugastoridae	<i>Craugastor evanesco</i>	KRL 0835	USNM 572219	El Copé, Coclé, 700 m	FJ766636	FJ784393
Craugastoridae	<i>Craugastor punctariolus</i>	AJC 1135	CH 9619	Altos del María, 930 m	KC129217	KC129325
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 010	swab	Altos del María, Panamá, 950 m	N/A	KC014786
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 011	swab	Altos del María, Panamá, 950 m	N/A	KC014787
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 012	swab	Altos del María, Panamá, 950 m	N/A	KC014788
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 013	swab	Altos del María, Panamá, 950 m	N/A	KC014789
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 014	swab	Altos del María, Panamá, 950 m	N/A	KC014790
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 015	swab	Altos del María, Panamá, 950 m	N/A	KC014791
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 016	swab	Altos del María, Panamá, 950 m	N/A	KC014792
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 233	swab	Altos del María, Panamá, 950 m	N/A	KC014793
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 234	swab	Altos del María, Panamá, 950 m	KC014640	KC014794
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 235	swab	Altos del María, Panamá, 950 m	KC014641	KC014795
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 236	swab	Altos del María, Panamá, 950 m	N/A	KC014952
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 237	swab	Altos del María, Panamá, 950 m	N/A	KC014785

Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 238	swab	Altos del María, Panamá, 950 m	N/A	KC014796
Craugastoridae	<i>Craugastor punctariolus</i>	EVACC 239	swab	Altos del María, Panamá, 950 m	N/A	KC014797
Craugastoridae	<i>Craugastor punctariolus</i>	KRL 0682	MVUP 1784	El Copé, Coclé, 700 m	FJ766673	FJ784333
Craugastoridae	<i>Craugastor punctariolus</i>	KRL 0863	USNM 572281	El Copé, Coclé, 700 m	FJ766672	FJ784411
Craugastoridae	<i>Craugastor punctariolus</i>	KRL 0870	USNM 572282	El Copé, Coclé, 700 m	FJ766671	FJ784417
Craugastoridae	<i>Craugastor punctariolus</i>	KRL 0872	USNM 572283	El Copé, Coclé, 700 m	FJ766670	FJ784418
Craugastoridae	<i>Craugastor punctariolus</i>	KRL 0954	MVUP 1845	El Copé, Coclé, 700 m	FJ766669	FJ784448
Craugastoridae	<i>Craugastor punctariolus</i>	KRL 1180	USNM 572285	El Copé, Coclé, 700 m	FJ766668	FJ784483
Craugastoridae	<i>Craugastor punctariolus</i>	KRL 1198	USNM 572286	El Copé, Coclé, 700 m	FJ766667	FJ784488
Craugastoridae	<i>Craugastor ranoides</i>	FB 4340	UCR 18072	Guanacaste, Costa Rica, 50 m	KC129220	KC129329
Craugastoridae	<i>Craugastor ranoides</i>	FB 4341	UCR 18071	Guanacaste, Costa Rica, 50 m	N/A	KC129326
Craugastoridae	<i>Craugastor ranoides</i>	FB 4343	UCR 18089	Guanacaste, Costa Rica, 50 m	KC129218	KC129327
Craugastoridae	<i>Craugastor ranoides</i>	FB 4344	UCR 18065	Guanacaste, Costa Rica, 50 m	KC129219	KC129328
Craugastoridae	<i>Craugastor ranoides</i>	FB 4345	UCR 18075	Guanacaste, Costa Rica, 50 m	KC129221	KC129330
Craugastoridae	<i>Craugastor ranoides</i>	FB 4346	UCR 18076	Guanacaste, Costa Rica, 50 m	KC129222	KC129331
Craugastoridae	<i>Craugastor tabasarae</i>	AJC 1214	MVUP 2039	Altos del María, Panamá, 895 m	N/A	KC129333
Craugastoridae	<i>Craugastor tabasarae</i>	CH 6055	CH 6055	Brewster, Panamá, 810 m	KC129223	KC129332
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 018	swab	Altos del María, Panamá, 950 m	N/A	KC014800
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 019	swab	Altos del María, Panamá, 950 m	N/A	KC014801
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 020	swab	Altos del María, Panamá, 950 m	N/A	KC014802
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 021	swab	Brewster, Panamá, 810 m	N/A	KC014803
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 022	swab	Brewster, Panamá, 810 m	N/A	KC014804
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 023	swab	Brewster, Panamá, 810 m	N/A	KC014805
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 024	swab	Brewster, Panamá, 810 m	N/A	KC014806
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 216	swab	Altos del María, Panamá, 950 m	KC014642	KC014798
Craugastoridae	<i>Craugastor tabasarae</i>	EVACC 219	swab	Altos del María, Panamá, 950 m	KC014643	KC014799
Craugastoridae	<i>Craugastor tabasarae</i>	KRL 0706	USNM 572294	El Copé, Coclé, 700 m	FJ766684	FJ784342
Craugastoridae	<i>Craugastor tabasarae</i>	KRL 1373	USNM 572295	El Copé, Coclé, 700 m	FJ766683	FJ784512
Craugastoridae	<i>Craugastor tabasarae</i>	KRL 1387	USNM 572296	El Copé, Coclé, 700 m	FJ766682	FJ784515
Craugastoridae	<i>Pristimantis</i> aff. <i>cruentus</i>	AJC 1128	CH 9616	Altos del María, 930 m	N/A	JN991444
Craugastoridae	<i>Pristimantis</i> aff. <i>cruentus</i>	AJC 1129	CH 9617	Altos del María, 930 m	KC129234	KC129344
Craugastoridae	<i>Pristimantis</i> aff. <i>cruentus</i>	AJC 1998	CH 9650	Rio Chico, Panamá, 135 m	N/A	KC129346

Craugastoridae	<i>Pristimantis</i> aff. <i>cruentus</i>	AJC 2042	CH 9651	Nurra, Darién, 140 m	N/A	KC129345
Craugastoridae	<i>Pristimantis</i> aff. <i>cruentus</i>	KRL 0683	USNM 572403	El Copé, Coclé, 700 m	FJ766799	FJ784334
Craugastoridae	<i>Pristimantis</i> aff. <i>cruentus</i>	KRL 0739	MVUP 1796	El Copé, Coclé, 700 m	FJ766797	FJ784354
Craugastoridae	<i>Pristimantis</i> aff. <i>cruentus</i>	KRL 0861	USNM 572404	El Copé, Coclé, 700 m	FJ766796	FJ784409
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	AJC 1670	CH 9653	Cerro Chucantí, Darién, 1365 m	KC129238	KC129350
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	CH 6046	CH 6046	Brewster, Panamá, 900 m	KC129240	KC129352
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	CH 6265	CH 6265	Cerro Chucantí, Darién, 1240 m	KC129237	KC129349
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	CH 6266	CH 6266	Cerro Chucantí, Darién, 1240 m	KC129235	KC129347
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	CH 6271	CH 6271	Cerro Chucantí, Darién, 1240 m	KC129236	KC129348
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	EVACC 096	swab	Brewster, Panamá, 810 m	KC014726	KC014937
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	EVACC 097	swab	Brewster, Panamá, 810 m	KC014725	N/A
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	EVACC 098	swab	Brewster, Panamá, 810 m	KC014724	KC014936
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	EVACC 217	swab	Brewster, Panamá, 810 m	N/A	KC014939
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	EVACC 218	swab	Brewster, Panamá, 810 m	KC014727	KC014938
Craugastoridae	<i>Pristimantis</i> aff. <i>latidiscus</i>	KRL 1489	USNM 572470	Río Blanco, Coclé, 1100 m	KC129239	KC129351
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	AJC 1872	CH 9635	Cana, Darién, 1250 m	KC129245	KC129357
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	AJC 1888	MVUP 2389	Cana, Darién, 1250 m	KC129246	KC129358
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	AJC 1890	CH 9637	Cana, Darién, 1250 m	KC129242	KC129354
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	AJC 1892	CH 9638	Cana, Darién, 1250 m	KC129243	KC129355
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	AJC 1894	CH 9639	Cana, Darién, 1250 m	KC129248	KC129360
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	AJC 1898	CH 9640	Cana, Darién, 1246 m	KC129250	KC129362
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	CH 6410	CH 6410	Cana, Darién, 1246 m	KC129244	KC129356
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	CH 6419	CH 6419	Cana, Darién, 1246 m	KC129247	KC129359
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	CH 6429	CH 6429	Cana, Darién, 1246 m	KC129249	KC129361
Craugastoridae	<i>Pristimantis</i> aff. <i>museosus</i>	CH 6455	CH 6455	Cana, Darién, 1246 m	KC129241	KC129353
Craugastoridae	<i>Pristimantis</i> cf. <i>latidiscus</i>	CH 6456	CH 6456	Cana, Darién, 1320 m	KC129251	KC129363
Craugastoridae	<i>Pristimantis</i> <i>cruentus</i>	AJC 1133	CH 9618	Altos del María, 930 m	KC129255	KC129367
Craugastoridae	<i>Pristimantis</i> <i>cruentus</i>	AJC 1139	CH 9621	Altos del María, 950 m	KC129253	KC129365
Craugastoridae	<i>Pristimantis</i> <i>cruentus</i>	AJC 1140	CH 9622	Altos del María, 940 m	KC129260	KC129372
Craugastoridae	<i>Pristimantis</i> <i>cruentus</i>	AJC 1145	CH 9624	Altos del María, 940 m	KC129261	KC129373
Craugastoridae	<i>Pristimantis</i> <i>cruentus</i>	AJC 1147	CH 9625	Altos del María, 940 m	KC129252	KC129364
Craugastoridae	<i>Pristimantis</i> <i>cruentus</i>	AJC 1150	CH 9626	Altos del María, 940 m	KC129259	KC129371

Craugastoridae	<i>Pristimantis cruentus</i>	AJC 1204	MVUP 2029	Altos del María, 900 m	KC129258	KC129370
Craugastoridae	<i>Pristimantis cruentus</i>	AJC 1213	MVUP 2038	Altos del María, 895 m	KC129257	KC129369
Craugastoridae	<i>Pristimantis cruentus</i>	AJC 1917	CH 9641	Brewster, Panamá, 810 m	KC129254	KC129366
Craugastoridae	<i>Pristimantis cruentus</i>	AJC 1930	CH 9643	Brewster, Panamá, 810 m	KC129256	KC129368
Craugastoridae	<i>Pristimantis cruentus</i>	CH 6721	CH 6721	Brewster, Panamá, 810 m	JN991371	JN991442
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 0811	USNM 572788	El Copé, Coclé, 700 m	FJ766787	FJ784380
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 1333	USNM 572361	El Copé, Coclé, 700 m	FJ766784	FJ784502
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 1407	USNM 572375	El Copé, Coclé, 700 m	FJ766783	FJ784520
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 1420	USNM 572362	El Copé, Coclé, 700 m	FJ766782	FJ784525
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 1455	USNM 572364	El Copé, Coclé, 700 m	FJ766781	FJ784531
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 1462	USNM 572365	El Copé, Coclé, 700 m	FJ766780	FJ784535
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 1474	USNM 572366	El Copé, Coclé, 700 m	FJ766779	FJ784538
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 1528	USNM 572367	El Copé, Coclé, 700 m	FJ766778	FJ784548
Craugastoridae	<i>Pristimantis cruentus</i>	KRL 1550	USNM 572369	El Copé, Coclé, 700 m	FJ766777	FJ784557
Craugastoridae	<i>Pristimantis museosus</i>	AJC 1932	CH 9645	Brewster, Panamá, 810 m	KC129266	KC129375
Craugastoridae	<i>Pristimantis museosus</i>	CH 6747	CH 6747	Brewster, Panamá, 810 m	KC129262	KC129374
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 069	swab	Brewster, Panamá, 810 m	KC014729	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 073	swab	Brewster, Panamá, 810 m	KC014728	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 074	swab	Brewster, Panamá, 810 m	KC014735	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 077	swab	Cerro Bruja, Panamá, 750 m	KC014734	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 078	swab	Cerro Bruja, Panamá, 750 m	KC014733	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 082	swab	Cerro Bruja, Panamá, 750 m	KC014732	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 083	swab	Cerro Bruja, Panamá, 750 m	KC014731	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 085	swab	Brewster, Panamá, 810 m	KC014730	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 086	swab	Brewster, Panamá, 810 m	KC014761	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 095	swab	Brewster, Panamá, 810 m	KC014760	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 236b	swab	Altos del María, Panamá, 950 m	KC014759	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 240	swab	Altos del María, Panamá, 950 m	KC014758	KC014951
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 241	swab	Altos del María, Panamá, 950 m	KC014757	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 242	swab	Altos del María, Panamá, 950 m	KC014756	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 243	swab	Altos del María, Panamá, 950 m	KC014755	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 244	swab	Altos del María, Panamá, 950 m	KC014754	N/A

Craugastoridae	<i>Pristimantis museosus</i>	EVACC 245	swab	Altos del María, Panamá, 950 m	KC014753	KC014950
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 246	swab	Brewster, Panamá, 810 m	KC014752	KC014949
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 247	swab	Brewster, Panamá, 810 m	KC014751	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 248	swab	Brewster, Panamá, 810 m	KC014750	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 249	swab	Brewster, Panamá, 810 m	KC014749	KC014948
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 250	swab	Brewster, Panamá, 810 m	KC014748	KC014947
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 251	swab	Brewster, Panamá, 810 m	KC014747	KC014946
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 252	swab	Brewster, Panamá, 810 m	KC014746	KC014945
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 253	swab	Brewster, Panamá, 810 m	KC014745	KC014944
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 254	swab	Brewster, Panamá, 810 m	KC014744	KC014943
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 255	swab	Brewster, Panamá, 810 m	KC014743	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 256	swab	Brewster, Panamá, 810 m	KC014742	KC014942
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 257	swab	Brewster, Panamá, 810 m	KC014741	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 258	swab	Brewster, Panamá, 810 m	KC014740	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 259	swab	Brewster, Panamá, 810 m	KC014739	KC014941
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 260	swab	Brewster, Panamá, 810 m	KC014738	N/A
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 261	swab	Brewster, Panamá, 810 m	KC014737	KC014940
Craugastoridae	<i>Pristimantis museosus</i>	EVACC 262	swab	Brewster, Panamá, 810 m	KC014736	N/A
Craugastoridae	<i>Pristimantis museosus</i>	KRL 0636	USNM 572387	El Copé, Coclé, 700 m	KC129263	N/A
Craugastoridae	<i>Pristimantis museosus</i>	KRL 0637	USNM 572388	El Copé, Coclé, 700 m	KC129264	N/A
Craugastoridae	<i>Pristimantis museosus</i>	KRL 0699	USNM 572389	El Copé, Coclé, 700 m	FJ766798	FJ784340
Craugastoridae	<i>Pristimantis museosus</i>	KRL 0909	MVUP 1839	El Copé, Coclé, 700 m	FJ766795	FJ784430
Craugastoridae	<i>Pristimantis museosus</i>	KRL 0938	USNM 572391	El Copé, Coclé, 700 m	KC129265	N/A
Craugastoridae	<i>Pristimantis museosus</i>	KRL 1554	USNM 572395	El Copé, Coclé, 700 m	FJ766793	FJ784559
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 0204	FMNH 257691	Nusagandi, Kuna Yala, 450 m	KC129286	KC129401
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1104	MVUP 2007	Fort Sherman, Panamá, 200 m	KC129267	KC129376
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1137	CH 9620	Altos del María, 940 m	N/A	KC129398
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1144	CH 9623	Altos del María, 940 m	N/A	KC129383
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1549	MVUP 2410	Majé, Panamá, 120 m	KC129287	KC129403
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1551	CH 9627	Majé, Panamá, 120 m	KC129281	KC129392
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1831	CH 9629	Cana, Darién, 525 m	N/A	KC129400
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1833	CH 9630	Cana, Darién, 525 m	N/A	KC129402

Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1876	CH 9636	Cana, Darién, 1246 m	KC129270	KC129381
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1908	CH 9631	Brewster, Panamá, 810 m	KC129282	KC129393
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1914	CH 9632	Brewster, Panamá, 810 m	KC129288	KC129404
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1933	CH 9646	Brewster, Panamá, 810 m	KC129283	KC129394
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1969	CH 9647	Río Chico, Panamá, 135 m	N/A	KC129397
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1970	CH 9648	Río Chico, Panamá, 135 m	KC129268	KC129379
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 1971	CH 9649	Río Chico, Panamá, 135 m	KC129284	KC129395
Craugastoridae	<i>Strabomantis bufoniformis</i>	AJC 2052	CH 9652	Nurra, Darién, 140 m	N/A	KC129378
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 6319	CH 6319	Cana, Darién, 525 m	N/A	KC129396
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 6368	CH 6368	Cana, Darién, 462 m	KC129272	KC129384
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 6677	CH 6677	Brewster, Panamá, 810 m	KC129285	KC129399
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 6810	CH 6810	Brewster, Panamá, 810 m	N/A	KC129377
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 6818	CH 6818	Río Chico, Panamá, 135 m	KC129280	KC129391
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 6819	CH 6819	Río Chico, Panamá, 135 m	KC129269	KC129380
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 9015	CH 9015	Cerro Sapo, Darién, 233 m	KC129271	KC129382
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 9026	CH 9026	Cerro Sapo, Darién, 233 m	KC129273	KC129386
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 9029	CH 9029	Cerro Sapo, Darién, 683 m	KC129274	KC129387
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 9039	CH 9039	Cerro Sapo, Darién, 683 m	KC129275	KC129388
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 9050	CH 9050	Cerro Sapo, Darién, 683 m	KC129276	KC129389
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 9052	CH 9052	Cerro Sapo, Darién, 683 m	KC129277	KC129390
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 9089	CH 9089	Cerro Sapo, Darién, 233 m	KC129278	N/A
Craugastoridae	<i>Strabomantis bufoniformis</i>	CH 9365	CH 9365	Cerro Sapo, Darién, 645 m	KC129279	N/A
Craugastoridae	<i>Strabomantis bufoniformis</i>	EVACC 288	swab	Brewster, Panamá, 810 m	N/A	KC014957
Craugastoridae	<i>Strabomantis bufoniformis</i>	EVACC 289	swab	Brewster, Panamá, 810 m	N/A	KC014956
Craugastoridae	<i>Strabomantis bufoniformis</i>	EVACC 300	swab	Cerro Bruja, Panamá, 750 m	N/A	KC014955
Craugastoridae	<i>Strabomantis bufoniformis</i>	EVACC 310	swab	Cerro Bruja, Panamá, 750 m	N/A	KC014954
Craugastoridae	<i>Strabomantis bufoniformis</i>	EVACC 313	swab	Brewster, Panamá, 810 m	N/A	KC014953
Craugastoridae	<i>Strabomantis bufoniformis</i>	EVACC 316	swab	Brewster, Panamá, 810 m	N/A	KC014958
Craugastoridae	<i>Strabomantis bufoniformis</i>	KRL 0862	USNM 572425	El Copé, Coclé, 700 m	FJ766635	FJ784410
Craugastoridae	<i>Strabomantis bufoniformis</i>	KRL 0962	USNM 572427	El Copé, Coclé, 700 m	FJ766634	FJ784451
Craugastoridae	<i>Strabomantis bufoniformis</i>	KRL 1078	USNM 572428	El Copé, Coclé, 700 m	N/A	FJ784460
Craugastoridae	<i>Strabomantis bufoniformis</i>	KRL 1168	USNM 572429	El Copé, Coclé, 700 m	N/A	KC129385

Craugastoridae	<i>Strabomantis bufoniformis</i>	KRL 1314	USNM 572430	El Copé, Coclé, 700 m	N/A	FJ784499
Craugastoridae	<i>Strabomantis bufoniformis</i>	KRL 1412	USNM 572433	El Copé, Coclé, 700 m	FJ766633	FJ784522
Craugastoridae	<i>Strabomantis bufoniformis</i>	KRL 1562	USNM 572434	El Copé, Coclé, 700 m	FJ766632	FJ784565
Dendrobatidae	<i>Colostethus</i> aff. <i>panamansis</i>	AJC 1859	CH 9634	Cana, Darién, 1246 m	KC129213	KC129321
Dendrobatidae	<i>Colostethus</i> cf. <i>panamansis</i>	AJC 1856	CH 9654	Cana, Darién, 1246 m	KC129215	KC129323
Dendrobatidae	<i>Colostethus</i> cf. <i>panamansis</i>	CH 6448	CH 6448	Cana, Darién, 1246 m	KC129189	KC129296
Dendrobatidae	<i>Colostethus panamansis</i>	AJC 1816	CH 9628	Cana, Darién, 550 m	KC129188	KC129295
Dendrobatidae	<i>Colostethus panamansis</i>	AJC 1857	CH 9655	Cana, Darién, 1246 m	KC129214	KC129322
Dendrobatidae	<i>Colostethus panamansis</i>	AJC 1937	MVUP 2392	Brewster, Panamá, 800 m	KC129194	KC129301
Dendrobatidae	<i>Colostethus panamansis</i>	CH 5007	CH 5007	Río Indio, Coclé, 650 m	KC129193	KC129300
Dendrobatidae	<i>Colostethus panamansis</i>	CH 5012	CH 5012	Río Indio, Coclé, 650 m	KC129192	KC129299
Dendrobatidae	<i>Colostethus panamansis</i>	CH 5105	CH 5105	San Miguel, Coclé, 400 m	KC129191	KC129298
Dendrobatidae	<i>Colostethus panamansis</i>	CH 5546	CH 5546	Cana, Darién, 1246 m	KC129211	KC129319
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6010	CH 6010	Altos del María, Panamá, 910 m	KC129190	KC129297
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6356	CH 6356	Cana, Darién, 494 m	KC129212	KC129320
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6692	CH 6692	Brewster, Panamá, 790 m	N/A	KC129302
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6693	CH 6693	Brewster, Panamá, 810 m	KC129209	KC129317
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6820	CH 6820	Río Chico, Panamá, 135 m	KC129196	KC129304
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6828	CH 6828	Río Chico, Panamá, 135 m	KC129198	KC129306
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6832	CH 6832	Río Chico, Panamá, 135 m	N/A	KC129307
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6833	CH 6833	Río Chico, Panamá, 135 m	KC129202	KC129310
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6835	CH 6835	Río Chico, Panamá, 135 m	KC129195	KC129303
Dendrobatidae	<i>Colostethus panamansis</i>	CH 6855	CH 6855	Río Chico, Panamá, 135 m	KC129197	KC129305
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9014	CH 9014	Cerro Sapo, Darién, 233 m	KC129210	KC129318
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9018	CH 9018	Cerro Sapo, Darién, 233 m	KC129208	KC129316
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9021	CH 9021	Cerro Sapo, Darién, 233 m	KC129207	KC129315
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9025	CH 9025	Cerro Sapo, Darién, 233 m	KC129206	KC129314
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9027	CH 9027	Cerro Sapo, Darién, 233 m	KC129205	KC129313
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9028	CH 9028	Cerro Sapo, Darién, 683 m	KC129204	KC129312
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9036	CH 9036	Cerro Sapo, Darién, 683 m	KC129203	KC129311
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9042	CH 9042	Cerro Sapo, Darién, 683 m	KC129185	KC129292
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9046	CH 9046	Cerro Sapo, Darién, 683 m	KC129201	N/A

Dendrobatidae	<i>Colostethus panamansis</i>	CH 9049	CH 9049	Cerro Sapo, Darién, 683 m	KC129200	KC129309
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9051	CH 9051	Cerro Sapo, Darién, 683 m	KC129199	KC129308
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9056	CH 9056	Cerro Sapo, Darién, 683 m	KC129187	KC129294
Dendrobatidae	<i>Colostethus panamansis</i>	CH 9057	CH 9057	Cerro Sapo, Darién, 683 m	KC129186	KC129293
Dendrobatidae	<i>Colostethus panamansis</i>	EVACC 100	swab	Brewster, Panamá, 800 m	KC014639	N/A
Dendrobatidae	<i>Colostethus panamansis</i>	EVACC 185	swab	Cana, Darién, 1200 m	KC014638	KC014784
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 0953	MVUP 1849	El Copé, Coclé, 700 m	FJ766619	FJ784447
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1332	USNM 572497	El Copé, Coclé, 700 m	N/A	FJ784501
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1338	USNM 572498	El Copé, Coclé, 700 m	FJ766618	FJ784504
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1339	USNM 572499	El Copé, Coclé, 700 m	FJ766617	FJ784505
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1343	USNM 572501	El Copé, Coclé, 700 m	FJ766616	FJ784507
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1344	USNM 572502	El Copé, Coclé, 700 m	FJ766615	FJ784508
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1359	USNM 572503	El Copé, Coclé, 700 m	FJ766614	FJ784509
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1390	USNM 572508	El Copé, Coclé, 700 m	FJ766613	FJ784516
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1421	USNM 572510	El Copé, Coclé, 700 m	FJ766612	FJ784526
Dendrobatidae	<i>Colostethus panamansis</i>	KRL 1433	USNM 572511	El Copé, Coclé, 700 m	FJ766611	FJ784529
Hemiphractidae	<i>Gastrotheca cornuta</i>	AJC 1929	CH 9642	Brewster, Panamá, 810 m	KC129226	KC129335
Hemiphractidae	<i>Gastrotheca cornuta</i>	CH 6745	CH 6745	Brewster, Panamá, 810 m	KC129225	KC129334
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 102	swab	Cerro Bruja, Panamá, 750 m	N/A	KC014898
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 103	swab	Cerro Bruja, Panamá, 750 m	N/A	KC014897
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 104	swab	Cerro Bruja, Panamá, 750 m	N/A	KC014896
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 105	swab	Brewster, Panamá, 810 m	N/A	KC014895
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 106	swab	Brewster, Panamá, 810 m	N/A	KC014894
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 108	swab	Brewster, Panamá, 810 m	N/A	KC014893
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 109	swab	Brewster, Panamá, 810 m	N/A	KC014892
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 110	swab	Brewster, Panamá, 810 m	N/A	KC014891
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 111	swab	Brewster, Panamá, 810 m	N/A	KC014890
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 112	swab	Brewster, Panamá, 810 m	N/A	KC014889
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 113	swab	Brewster, Panamá, 810 m	N/A	KC014888
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 114	swab	Brewster, Panamá, 810 m	N/A	KC014887
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 115	swab	Brewster, Panamá, 810 m	N/A	KC014886
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 116	swab	Brewster, Panamá, 810 m	N/A	KC014885

Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 117	swab	Brewster, Panamá, 810 m	N/A	KC014884
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 118	swab	Brewster, Panamá, 810 m	N/A	KC014883
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 119	swab	Brewster, Panamá, 810 m	N/A	KC014882
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 120	swab	Brewster, Panamá, 810 m	N/A	KC014881
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 121	swab	Brewster, Panamá, 810 m	N/A	KC014880
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 122	swab	Brewster, Panamá, 810 m	N/A	KC014879
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 125	swab	Brewster, Panamá, 810 m	N/A	KC014878
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 126	swab	Brewster, Panamá, 810 m	N/A	KC014877
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 127	swab	Brewster, Panamá, 810 m	N/A	KC014876
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 128	swab	Brewster, Panamá, 810 m	N/A	KC014875
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 129	swab	Brewster, Panamá, 810 m	KC014678	KC014874
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 130	swab	Brewster, Panamá, 810 m	N/A	KC014873
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 131	swab	Brewster, Panamá, 810 m	N/A	KC014872
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 132	swab	Altos del María, Panamá, 950 m	N/A	KC014871
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 133	swab	Altos del María, Panamá, 950 m	N/A	KC014870
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 134	swab	Altos del María, Panamá, 950 m	KC014677	KC014869
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 135	swab	Altos del María, Panamá, 950 m	N/A	KC014815
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 136	swab	Altos del María, Panamá, 950 m	KC014676	KC014868
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 137	swab	Altos del María, Panamá, 950 m	N/A	KC014867
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 138	swab	Altos del María, Panamá, 950 m	N/A	KC014866
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 139	swab	Brewster, Panamá, 810 m	KC014675	KC014865
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 140	swab	Brewster, Panamá, 810 m	N/A	KC014864
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 141	swab	Altos del María, Panamá, 950 m	N/A	KC014863
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 142	swab	Altos del María, Panamá, 950 m	N/A	KC014862
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 143	swab	Altos del María, Panamá, 950 m	N/A	KC014861
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 144	swab	Brewster, Panamá, 810 m	N/A	KC014860
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 145	swab	Brewster, Panamá, 810 m	N/A	KC014859
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 146	swab	Brewster, Panamá, 810 m	KC014674	KC014858
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 147	swab	Brewster, Panamá, 810 m	N/A	KC014857
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 148	swab	Altos del María, Panamá, 950 m	N/A	KC014856
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 150	swab	Brewster, Panamá, 810 m	N/A	KC014855
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 151	swab	Brewster, Panamá, 810 m	KC014673	N/A

Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 152	swab	Brewster, Panamá, 810 m	KC014672	KC014854
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 153	swab	Brewster, Panamá, 810 m	N/A	KC014853
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 154	swab	Brewster, Panamá, 810 m	N/A	KC014852
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 155	swab	Brewster, Panamá, 810 m	N/A	KC014851
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 156	swab	Brewster, Panamá, 810 m	N/A	KC014850
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 157	swab	Brewster, Panamá, 810 m	N/A	KC014849
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 158	swab	Brewster, Panamá, 810 m	KC014671	N/A
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 159	swab	Brewster, Panamá, 810 m	N/A	KC014848
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 160	swab	Brewster, Panamá, 810 m	N/A	KC014847
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 161	swab	Brewster, Panamá, 810 m	N/A	KC014846
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 162	swab	Altos del María, Panamá, 950 m	KC014670	N/A
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 163	swab	Altos del María, Panamá, 950 m	N/A	KC014845
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 164	swab	Altos del María, Panamá, 950 m	N/A	KC014844
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 165	swab	Altos del María, Panamá, 950 m	N/A	KC014843
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 166	swab	Altos del María, Panamá, 950 m	N/A	KC014842
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 167	swab	Altos del María, Panamá, 950 m	N/A	KC014841
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 168	swab	Altos del María, Panamá, 950 m	KC014669	KC014840
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 169	swab	Altos del María, Panamá, 950 m	KC014668	N/A
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 170	swab	Altos del María, Panamá, 950 m	N/A	KC014839
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 171	swab	Altos del María, Panamá, 950 m	N/A	KC014838
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 172	swab	Altos del María, Panamá, 950 m	N/A	KC014837
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 175	swab	Altos del María, Panamá, 950 m	KC014667	KC014836
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 176	swab	Altos del María, Panamá, 950 m	KC014666	N/A
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 179	swab	Altos del María, Panamá, 950 m	KC014665	KC014835
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 180	swab	Altos del María, Panamá, 950 m	KC014664	N/A
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 181	swab	Altos del María, Panamá, 950 m	KC014663	KC014834
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 182	swab	Brewster, Panamá, 810 m	KC014662	N/A
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 184	swab	Brewster, Panamá, 810 m	N/A	KC014833
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 263	swab	Altos del María, Panamá, 950 m	KC014661	KC014832
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 264	swab	Altos del María, Panamá, 950 m	KC014660	KC014831
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 265	swab	Altos del María, Panamá, 950 m	KC014659	KC014830
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 266	swab	Altos del María, Panamá, 950 m	KC014658	KC014829

Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 267	swab	Altos del María, Panamá, 950 m	KC014657	N/A
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 268	swab	Brewster, Panamá, 810 m	KC014656	KC014828
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 269	swab	Altos del María, Panamá, 950 m	KC014655	KC014827
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 270	swab	Altos del María, Panamá, 950 m	KC014654	KC014826
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 271	swab	Altos del María, Panamá, 950 m	KC014653	KC014825
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 272	swab	Brewster, Panamá, 810 m	KC014652	KC014824
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 273	swab	Altos del María, Panamá, 950 m	KC014651	KC014823
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 274	swab	Brewster, Panamá, 810 m	KC014650	KC014822
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 275	swab	Altos del María, Panamá, 950 m	N/A	KC014821
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 276	swab	Brewster, Panamá, 810 m	KC014649	KC014820
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 277	swab	Altos del María, Panamá, 950 m	KC014648	KC014819
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 278	swab	Brewster, Panamá, 810 m	KC014647	KC014818
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 279	swab	Altos del María, Panamá, 950 m	KC014646	KC014817
Hemiphractidae	<i>Gastrotheca cornuta</i>	EVACC 280	swab	Altos del María, Panamá, 950 m	KC014645	KC014816
Hemiphractidae	<i>Gastrotheca cornuta</i>	KRL 0799	USNM 572472	El Copé, Coclé, 700 m	FJ766706	FJ784373
Hemiphractidae	<i>Gastrotheca cornuta</i>	KRL 1163	USNM 572473	El Copé, Coclé, 700 m	FJ766705	FJ784477
Hemiphractidae	<i>Gastrotheca cornuta</i>	KRL 1429	USNM 572474	El Copé, Coclé, 700 m	FJ766704	FJ784528
Hemiphractidae	<i>Hemiphractus fasciatus</i>	AJC 0850	MVUP 1864	Cana, Darién, 1600 m	KC129233	KC129343
Hemiphractidae	<i>Hemiphractus fasciatus</i>	AJC 1858	CH 9633	Cana, Darién, 1597 m	KC129229	KC129339
Hemiphractidae	<i>Hemiphractus fasciatus</i>	AJC 1931	CH 9644	Brewster, Panamá, 810 m	KC129231	KC129341
Hemiphractidae	<i>Hemiphractus fasciatus</i>	CH 6397	CH 6397	Cana, Darién, 1597 m	N/A	KC129338
Hemiphractidae	<i>Hemiphractus fasciatus</i>	CH 6670	CH 6670	Brewster, Panamá, 810 m	KC129232	KC129342
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 025	swab	Altos del María, Panamá, 950 m	KC014687	KC014909
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 026	swab	Altos del María, Panamá, 950 m	KC014686	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 027	swab	Altos del María, Panamá, 950 m	KC014685	KC014908
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 028	swab	Altos del María, Panamá, 950 m	KC014723	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 029	swab	Altos del María, Panamá, 950 m	KC014722	KC014935
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 031	swab	Altos del María, Panamá, 950 m	KC014721	KC014934
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 032	swab	Altos del María, Panamá, 950 m	N/A	KC014919
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 033	swab	Altos del María, Panamá, 950 m	KC014684	KC014906
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 034	swab	Altos del María, Panamá, 950 m	N/A	KC014907
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 035	swab	Altos del María, Panamá, 950 m	N/A	KC014910

Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 036	swab	Altos del María, Panamá, 950 m	KC014688	KC014911
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 037	swab	Altos del María, Panamá, 950 m	KC014689	KC014912
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 038	swab	Altos del María, Panamá, 950 m	KC014690	KC014913
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 039	swab	Altos del María, Panamá, 950 m	N/A	KC014914
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 040	swab	Altos del María, Panamá, 950 m	KC014691	KC014915
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 041	swab	Altos del María, Panamá, 950 m	KC014692	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 042	swab	Altos del María, Panamá, 950 m	KC014693	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 043	swab	Altos del María, Panamá, 950 m	KC014694	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 044	swab	Altos del María, Panamá, 950 m	KC014695	KC014916
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 045	swab	Altos del María, Panamá, 950 m	KC014696	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 046	swab	Altos del María, Panamá, 950 m	KC014697	KC014917
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 047	swab	Altos del María, Panamá, 950 m	KC014698	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 048	swab	Altos del María, Panamá, 950 m	KC014699	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 049	swab	Altos del María, Panamá, 950 m	KC014700	KC014918
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 051	swab	Cana, Darién, 1400 m	KC014701	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 052	swab	Cana, Darién, 1400 m	KC014702	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 053	swab	Cana, Darién, 1400 m	KC014703	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 059	swab	Brewster, Panamá, 810 m	KC014704	KC014920
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 060	swab	Cerro Bruja, Panamá, 750 m,	KC014705	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 061	swab	Cerro Bruja, Panamá, 750 m,	KC014706	KC014921
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 062	swab	Cana, Darién, 1400 m	KC014707	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 063	swab	Cana, Darién, 1400 m	KC014708	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 064	swab	Cana, Darién, 1400 m	KC014709	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 065	swab	Cana, Darién, 1400 m	KC014710	KC014922
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 067	swab	Cana, Darién, 1400 m	KC014711	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 068	swab	Cana, Darién, 1400 m	KC014712	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 070	swab	Brewster, Panamá, 810 m	KC014713	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 071	swab	Brewster, Panamá, 810 m	KC014714	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 072	swab	Brewster, Panamá, 810 m	N/A	KC014923
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 197	swab	Altos del María, Panamá, 950 m	KC014715	KC014924
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 198	swab	Cana, Darién, 1400 m	N/A	KC014925
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 199	swab	Altos del María, Panamá, 950 m	N/A	KC014926

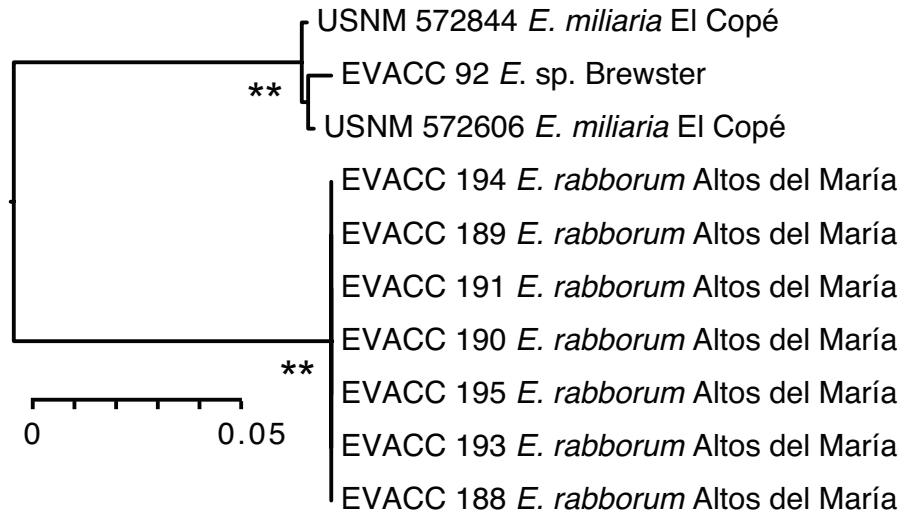
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 200	swab	Altos del María, Panamá, 950 m	KC014716	N/A
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 201	swab	Brewster, Panamá, 810 m	N/A	KC014927
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 202	swab	Altos del María, Panamá, 950 m	KC014717	KC014928
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 203	swab	Altos del María, Panamá, 950 m	N/A	KC014929
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 204	swab	Altos del María, Panamá, 950 m	KC014718	KC014930
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 205	swab	Altos del María, Panamá, 950 m	KC014719	KC014931
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 206	swab	Altos del María, Panamá, 950 m	KC014720	KC014932
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 207	swab	Cana, Darién, 1400 m	N/A	KC014933
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 208	swab	Altos del María, Panamá, 950 m	KC014679	KC014899
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 210	swab	Altos del María, Panamá, 950 m	N/A	KC014900
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 211	swab	Brewster, Panamá, 810 m	N/A	KC014901
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 212	swab	Altos del María, Panamá, 950 m	KC014680	KC014902
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 213	swab	Altos del María, Panamá, 950 m	KC014681	KC014903
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 214	swab	Altos del María, Panamá, 950 m	KC014682	KC014904
Hemiphractidae	<i>Hemiphractus fasciatus</i>	EVACC 215	swab	Altos del María, Panamá, 950 m	KC014683	KC014905
Hemiphractidae	<i>Hemiphractus fasciatus</i>	KRL 1162	MVUP 1927	El Copé, Coclé, 700 m	FJ766707	FJ784476
Hemiphractidae	<i>Hemiphractus fasciatus</i>	KRL 1484	USNM 572599	Río Blanco, Coclé, 1000 m	KC129230	KC129340
Hemiphractidae	<i>Hemiphractus fasciatus</i>	KRL 1490	USNM 572600	Río Blanco, Coclé, 1000 m	KC129227	KC129336
Hemiphractidae	<i>Hemiphractus fasciatus</i>	KRL 1547	USNM 572601	Río Blanco, Coclé, 1000 m	KC129228	KC129337
Hylidae	<i>Ecnomiohyla miliaria</i>	KRL 0758	USNM 572844	El Copé, Coclé, 700 m	FJ766699	FJ784360
Hylidae	<i>Ecnomiohyla miliaria</i>	KRL 0876	USNM 572606	El Copé, Coclé, 700 m	KC129224	N/A
Hylidae	<i>Ecnomiohyla rabborum</i>	EVACC 188	swab	Altos del María, Panamá, 950 m	N/A	KC014810
Hylidae	<i>Ecnomiohyla rabborum</i>	EVACC 189	swab	Altos del María, Panamá, 950 m	N/A	KC014811
Hylidae	<i>Ecnomiohyla rabborum</i>	EVACC 190	swab	Altos del María, Panamá, 950 m	N/A	KC014812
Hylidae	<i>Ecnomiohyla rabborum</i>	EVACC 191	swab	Altos del María, Panamá, 950 m	N/A	KC014813
Hylidae	<i>Ecnomiohyla rabborum</i>	EVACC 193	swab	Altos del María, Panamá, 950 m	N/A	KC014807
Hylidae	<i>Ecnomiohyla rabborum</i>	EVACC 194	swab	Altos del María, Panamá, 950 m	N/A	KC014808
Hylidae	<i>Ecnomiohyla rabborum</i>	EVACC 195	swab	Altos del María, Panamá, 950 m	N/A	KC014809
Hylidae	<i>Ecnomiohyla sp.</i>	EVACC 092	swab	Brewster, Panamá, 810 m	KC014644	KC014814

Supplementary Table S2.

Model of nucleotide substitution best supported by the Bayesian Information Criterion as implemented in *jModeltest* and applied to each mitochondrial DNA dataset (EVACC and wild-caught samples combined). For explanation of model names and characteristics see Posada (2008). Each model is followed by the number of parsimony-informative characters (PICs) for data sets used in phylogenetic analyses. Relative rate of COI show how many times greater was the substitution rate multiple for COI relative to 16S in the partitioned maximum likelihood analysis using the phylogenetic inference software *GARLI* version 2.0.1019 (Zwickl 2006). Four species not subjected to phylogenetic inference are indicated by ‘-’.

Genus species	COI	16S	2-gene	Relative rate of COI
<i>Atelopus limosus</i>	F81	JC	F81	-
* <i>Centrolene</i> sp.	N/A	N/A	N/A	-
** <i>Pristimantis museosus</i>	HKY + Γ (195 PICs)	SYM + Γ (118 PICs)	TVM + Γ	2.39
*** <i>Craugastor punctariolus</i>	HKY + Γ (105 PICs)	HKY + I (56 PICs)	HKY + Γ	1.98
<i>Craugastor tabasarae</i>	HKY	HKY	HKY	-
<i>Strabomantis bufoniformis</i>	HKY + Γ (89 PICs)	K80 (36 PICs)	HKY + Γ	4.64
<i>Colostethus panamansis</i>	HKY + I (95 PICs)	TrNef + I (37 PICs)	HKY + Γ	2.21
<i>Gastrotheca cornuta</i>	HKY + Γ	K80 + Γ	HKY + Γ	-
<i>Hemiphractus fasciatus</i>	HKY + Γ (89 PICs)	K80 + Γ (34 PICs)	HKY + Γ	2.66
<i>Ecnomiohyla</i> spp.	N/A (0 PICs)	K80 (0 PICs)	N/A	1.37

Supplementary Figure S1. Maximum likelihood phylogeny based on a partitioned analysis of COI and 16S mitochondrial DNA gene fragments from *Ecnomiohyla* samples, inferred using the software *GARLI* 2.0 and rooted at mid-point. Double asterisks (**) indicate support $\geq 95\%$. Scale bar indicates inferred patristic distance.



References

- Posada D (2008) *jModelTest*: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253–1256.
- Zwickl DJ (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*, PhD thesis, The University of Texas.