UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

SISTEMÁTICA

SISTEMÁTICA Y BIOGEOGRAFÍA DEL COMPLEJO DE ESPECIES *CRAUGASTOR PODICIFERUS* (ANURA: STRABOMANTIDAE) EN AMÉRICA CENTRAL ÍSTMICA EMPLEANDO ADN MITOCONDRIAL Y NUCLEAR

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

ERICK ARIAS PIEDRA

TUTORA PRINCIPAL DE TESIS: **DRA. GABRIELA PARRA OLEA**
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Cd. Mx. **FEBRERO, 2019**

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MÉXICO, Cd. Mx. **FEBRERO, 2019**

UNAM
POSGRADO
Ciencias Biológicas

COORDINACIÓN

OFICIO CPCB/059/2019

Asunto: Oficio de Jurado para Examen de Grado.

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión del Subcomité por Campo de Conocimiento de Ecología y Manejo Integral de Ecosistemas del Posgrado en Ciencias Biológicas, celebrada el día 17 de septiembre de 2018, se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del alumno **ARIAS PIEDRA ERICK** con número de cuenta **515046843** con la tesis titulada: **"SISTEMÀTICA Y BIOGEOGRAFÌA DEL COMPLEJO DE ESPECIES CRAUGASTOR PODICIFERUS (ANURA: STRABOMANTIDAE) DE AMÉRICA CENTRAL ISTMICA EMPLEANDO ADN MITOCONDRIAL Y NUCLEAR"**, realizada bajo la dirección de la **DRA. GABRIELA PARRA OLEA:**

Presidente:	DR. JUAN JOSÉ MORRONE LUPI
Vocal:	DRA. ELLA GLORIA VÁZQUEZ DOMÍNGUEZ
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Suplente	DRA. CLAUDIA PATRICIA ORNELAS GARCÍA

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 16 de enero de 2019.

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

DR. ADOLFO GERARDO NAVARRO SIGÜENZA
COORDINADOR DEL PROGRAMA

c.c.p. Expediente del (la) interesado (a)

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ÍNDICE

RESUMEN

El grupo de especies *Craugastor podiciferus* (Anura: Craugastoridae) son ranas de desarrollo directo distribuidas desde Honduras hasta el centro de Panamá, y todas las especies descritas confluyen en Costa Rica y el oeste de Panamá –América Central Ístmica. La historia taxonómica del grupo ha sido compleja, especialmente por el alto nivel de polimorfismos fenotípicos a nivel intra- e interpoblacional. Al inicio del presente trabajo se reconocían ocho especies dentro del grupo, aunque se sospechaba la presencia de varias especies no descritas. Algunos trabajos previos validaron la monofilia del grupo de especies *C. podiciferus* y sugirieron que el origen del mismo se remonta a 20 millones de años. En este trabajo se recolectaron especímenes a lo largo del rango de distribución del grupo de especies *C. podiciferus* y se obtuvieron secuencias de ADN mitocondrial (16S y COI) y nuclear (SNPs) para reconstruir sus relaciones filogenéticas. Se obtuvieron hipótesis filogenéticas robustas que apoyan la presencia de varias especies no descritas dentro del grupo de especies *C. podiciferus.* Los análisis de delimitación de especies basados en secuencias de ADN sugieren que el grupo está conformado por al menos 23 linages, muchos de los cuales ameritan revisiones exhaustivas para determinar si deben ser considerados especies distintas. Aunque hubo diferencias entre las topologías obtenidas, los analisis filogenéticos indican que el grupo está representado por cuatro grandes clados. El clado basal, está formado por una especie nueva que se describe en este trabajo. Un segundo clado mayor incluye varios linajes de tierras altas, incluyendo a *C. podiciferus sensu stricto*. Dos especies fueron descritas dentro de este último clado, así como una que estaba como sinónimo de *C. podiciferus* Cope (1875). Los dos clados restantes contienen al menos 12 linajes de tierras bajas e intermedias, y dentro de estos clados una especie fue descrita como *C. gabbi* Arias, Chaves, Crawford, & Parra-Olea 2016. Los resultados sugieren que el grupo de especies *C. podiciferus* se originó y diversificó en las tierras altas de América Central Ístmica (ACI), ya que sus clados más basales se restrigen a las tierras altas del istmo. Además, los 23 linages identificados están distribuidos en Costa Rica y oeste de Panamá. La evidencia reciente sugiere que ACI emergío durante el Mioceno temprano (~23 millones de años antes), lo cual apoya la hipótesis de que el grupo se diversificó en ACI. Las especies que conforman el grupo de especies *C. podiciferus* ameritan claras estrategias de conservación, ya que, a pesar de que son relativamente abundantes sus áreas de distribución son restringidas.

ABSTRACT

The *Craugastor podiciferus* species group (Anura: Craugastoridae) are direct-developing frogs that are distributed from Honduras to central Panama, with all its described species converging in Costa Rica and western Panama – Isthmian Central America. The taxonomic history of the group has been complex, especially due to the high level of intra- and interpopulation phenotypic polymorphisms. Before this study, eight species were recognized within the group, though the presence of several undescribed species was suspected. Previous studies validated the monophyly of the *C. podiciferus* species group and suggested that its origin date back to 20 million years ago. In this study, specimens were collected throughout the range of distribution of the *C. podiciferus* species group and mitochondrial (16S and COI) and nuclear (SNPs) DNA sequences were obtained to infer its phylogenetic relationships. Robust phylogenies were obtained, which support the presence of several unnamed species within of the *C. podiciferus* species group. DNA sequence-based species delimitation analyses suggest that the group consists of at least 23 lineages, many of which need exhaustive reviews to determine whether they should be considered as different species. Although there were differences among the phylogenies, all phylogenetic analyses indicate that the group consists of four large clades. The basal clade is formed by a new species that is described here. A second major clade includes several highland lineages, including *C. podiciferus sensu stricto*. Two species were described within this clade and one more that was under synonymy of *C. podiciferus* Cope (1875). The two remaining clades contain at least 12 lineages of lowlands and midlands; within of these clades one species was described as *C. gabbi* Arias, Chaves, Crawford, & Parra-Olea 2016. The results suggest that the *C. podiciferus* species group originated and diversified in the highlands of Isthmian Central America (ICA), by the fact that the most basal clades are restricted to the highlands of the isthmus. Also, the 23 identified linages are distributed in Costa Rica and western Panama. The recent evidence suggests that ICA emerged during the early Miocene (~ 23 million years ago), supports the hypothesis that the study group diversified ICA. The species within of the *C. podiciferus* species group merit clear conservation strategies, because although they are relatively abundant, their distribution areas are restricted.

INTRODUCCIÓN GENERAL

América Central Ístmica

El Neotrópico es reconocido como una de las regiones más biodiversas del mundo, incluyendo siete de los 25 *hotspots* de biodiversidad a nivel mundial (Myers *et al.* 2000). Desde una perspectiva biogeográfica, América Central es el área comprendida entre el Istmo de Tehuantepec, México y la Cordillera de los Andes en Colombia (Savage 1982; Gutiérrez-García & Vázquez-Domínguez 2013). Esta puede ser dividida en dos unidades biogeográficas independientes: 1) América Central Nuclear, el área entre el Istmo de Tehuantepec y el sur de Nicaragua, que alberga las tierras más antiguas de la región (Weber *et al.* 2007; Martens *et al.* 2010) y han tenido un papel importante en la diversificación temprana de la biota centroamericana (Savage 2002; Heinicke *et al.* 2007; Duellman *et al.* 2016); y 2) América Central Ístmica (ACI), el área que comprende Costa Rica y Panamá e incluye tierras relativamente más jóvenes (Montes *et al.* 2012a,b, 2015) y que han tenido un papel importante en la conformación actual de la biodiversidad regional permitiendo el Gran Intercambio de la Biota Americana (Bacon *et al.* 2015, 2016).

ACI se caracteriza por su biodiversidad y alto nivel de endemismos (Reid & Miller 1989). Esta región contiene a más especies de anfibios, reptiles, aves, insectos y plantas vasculares por unidad de área que casi cualquier otra parte del mundo (Davis *et al.* 1997; Anger & Dean 2010; Garrigues & Dean 2014; AmphibiaWeb 2019). Esta gran biodiversidad se atribuye a dos factores, el cierre del Istmo de Panamá y su historia de actividad volcánica y orogénica. El cierre del Istmo de Panamá, permitió el Gran Intercambio de la Biota Americana (GABI por sus siglas en inglés) entre Norteamerica y Sudamerica, permitiendo la confluencia de

especies del norte y sur en ACI (Savage 1966; Webb 1991, 2006; Pinto-Sánchez *et al.* 2012). La actividad volcánica y orogénica en ACI resultó en una alta heterogeneidad climática y geomorfológica (Montes *et al.* 2012a,b, 2015; Bagley & Johnson 2014). La biodiversidad de ACI se vio reforzada por la diversificación *in situ*, que generó un alto nivel de endemismos asociados a la alta diversidad de hábitats (Campbell 1999; Savage 2002; Kluge & Kessler 2006; Boza-Oviedo *et al.* 2012). Desde una perspectiva geológica, ACI se divide en dos grandes bloques: Chorotega y Chocó. Chorotega, que se extiende desde el escarpe de Hess en el sur de Nicaragua hasta la fractura norte de Panamá. Y Chocó, que se extiende desde la fractura norte de Panamá hasta la zona de subducción entre las placas Nazca y sudamericana en Colombia (Gutiérrez-García & Vázquez-Domínguez 2013).

Los bloques de Chorotega y Chocó comparten una estrecha relación histórica, por otra parte, el conocimiento sobre su formación ha cambiado aceleradamente. La hipótesis tradicional sobre el origen de la región se denomina "modelo de isla", ya que propone un origen para ACI reciente en el Mioceno Medio (~15 Ma) a ~3 Ma (Coates & Obando 1996; Coates *et al.* 2004). Bajo este modelo, en el Mioceno Medio (~15 Ma) ACI estaba conformada por un archipiélago de islas. Este modelo sugiere que el levantamiento regional generó la unión de tierras emergidas, pero desconectadas de Sudamérica, hace ~6.5 Ma y propone el cierre del Istmo de Panamá hace ~3 Ma. Una hipótesis más reciente, denominada "modelo de península", plantea un levantamiento y un cierre mucho más antiguo (Montes *et al.* 2012a,b, 2015). Este modelo sugiere que ACI existe como tierra emergida pero desconectada de Sudamérica desde el Oligoceno Tardío al Mioceno Temprano (~25 Ma) y calcula el cierre del Istmo de Panamá entre 15–13 Ma. La hipótesis de Montes *et al.* (2012a,b, 2015) sobre el cierre temprano del Istmo de Panamá ha sido apoyada por los aportes filogenéticos. Por ejemplo, se conoce que varios grupos se

diversificaron en ACI desde el Mioceno Temprano (~23 Ma), incluyendo ranas (Crawford & Smith 2005; Streicher *et al.* 2009; Duellman *et al.* 2016), salamandras (Rovito *et al.* 2015), serpientes (Castoe *et al.* 2009) y peces dulceacuícolas (Říčan *et al.* 2013).

Aunque los anfibios constituyen uno de los grupos más biodiversos de la región (AmphibiaWeb 2019) y de los mejor estudiados (Taylor 1948, 1952; Savage 1966, 1982, 2002), aún es notable la carencia de estudios biogeográficos sobre su diversificación en ACI, desde la evidencia actual del cierre del Istmo de Panamá durante el Mioceno (Montes *et al.* 2012a,b, 2015). Este es un tema relativamente nuevo, que plantea una serie de interrogantes con respecto al origen y diversificación de los anfibios en ACI. Por ejemplo, este modelo pone a prueba la importancia de las fluctuaciones climáticas durante el Plioceno–Pleistoceno en la diversificación de taxones de tierras altas (Savage 2002), ya que es factible sugerir que la diversificación es anterior al Pleistoceno (Castoe *et al.* 2009; Streicher *et al.* 2009). Las tierras altas de ACI (>750 m) conforman la ecoregión "*Talamanca montane forest*, TMF" (Olson *et al.* 2001), caracterizada por cambios altitudinales significativos, alta variación climática y carencia de conectividad con otras tierras altas de América. Las características de TMF se ven enriquecidas por el alto número de anfibios endémicos a TMF (Campbell 1999; García-París *et al.* 2000; Savage 2002; Boza-Oviedo *et al.* 2012).

El grupo de especies *Craugastor podiciferus*

Uno de los principales retos dentro del conocimiento de los anfibios, es entender las relaciones filogenéticas y la diversidad de las ranas con desarrollo directo (superfamilia Brachycephaloidea, Padial *et al.* 2014). Este grupo es considerado como el menos estudiado de todos los vertebrados (Hedges *et al.* 2008), contiene poco más de 1100 especies descritas,

representando más del 16 % de los anuros descritos en el Mundo (Frost 2019). Por su abundancia relativa, las ranas con desarrollo directo representan componentes importantes dentro de los ecosistemas. Las ranas con desarrollo directo constituyen un grupo importante de depredadores y también gran cantidad de biomasa (Lynch & Duellman 1997; Savage 2002; Pinto-Sánchez *et al.* 2014).

Los vacíos de información dentro de los terraranas (ranas con desarrollo directo) es explicado en parte por la poca cantidad de caracteres morfológicos disponibles para analizar, la plasticidad de los pocos caracteres útiles y el alto nivel de poliformismos morfológicos (Hedges *et al.* 2008). La tasa de descripción de especies nuevas de ranas con desarrollo directo aumentó a partir de la segunda mitad del siglo XX, alcanzando tasas de hasta 15 especies nuevas por año (Hedges *et al.* 2008). Sin embargo, por décadas los taxónomos no han alcanzado un consenso sobre las relaciones filogenéticas y la organización de las especies en categorias menores (familias, géneros o subgéneros) que permitieran una mejor clasificación de su diversidad (Lynch 1986, 1993, 2000; Savage 1987, 2002; Hedges 1989; Lynch & Duellman 1997; Hedges *et al.* 2008).

Con el desarrollo de nuevas tecnologías que facilitaron la obtención de datos moleculares a través de la secuenciación y la reconstrucción filogenética, se generaron diversos estudios que evaluaron las relaciones filogenéticas dentro de las ranas con desarrollo directo (Frost *et al.* 2006; Heinicke *et al.* 2007, 2009, 2018; Hedges *et al.* 2008; Pyron & Wiens 2011; Padial *et al.* 2014; Pie *et al.* 2017). Hedges *et al.* (2008) sugirieron el reconocimiento de diversas familias, géneros, subgéneros y grupos de especies. Pyron & Wiens (2011) y Padial *et al.* (2014) realizaron cambios en la sistemática de las ranas con desarrollo directo. Cinco de estos géneros de ranas con desarrollo directo (*Craugastor, Diasporus, Eleutherodactylus, Pristimantis* y

Strabomantis) habitan ACI, pero *Craugastor* es el más diverso con casi 100 especies en la región. La monofilia del género *Craugastor* (Cope) había sido reconocida desde Lynch (1986) basado en análisis de la musculatura de la mandíbula, y su validez como género ha permanecido estable desde Hedges *et al* (2008). Las relaciones filogenéticas dentro de *Craugastor* permanecen bajo discusión, actualmente se reconocen tres subgéneros y seis grupos de especies (Padial *et al.* 2014).

Las relaciones filogenéticas dentro de los grupos de especies de *Craugastor* son poco entendidas (Padial *et al.* 2014); sin embargo, los estudios empleando taxonomía alfa son de vital importancia, ya que evaluan la diversidad real dentro de un grupo mediante la delimitación de especies. Aunque el género *Craugastor* representa un porcentaje alto de la fauna anfibia de ACI (Frost 2019), la homoplasia entre las especies y el polimorfismo fenotípico han sido un impedimento para entender la taxonomía y la sistemática de las ranas de desarrollo directo en la región (Lynch 2000; Crawford 2003; Hedges *et al.* 2008; Streicher *et al*. 2009). Algunos estudios moleculares con *Craugastor* de ACI, revelaron la existencia de varias especies no descritas (Crawford 2003; Crawford & Smith 2005; Crawford *et al.* 2007; Streicher *et al.* 2009), sugiriendo que la taxonomía de las ranas de desarrollo directo es insuficiente y posiblemente hay una alta proporción de especies crípticas. Sumado al hecho de que los anfibios como grupo contienen mayor proporción de especies crípticas dentro de los vertebrados (Pérez-Ponce de León & Poulin 2016). Que son aquellas especies tratadas bajo un mismo nombre dada su alta similitud morfológica (Bickford *et al.* 2007).

Dentro del género *Craugastor* (Brachycephaloidea: Craugastoridae), el grupo de especies *C. podiciferus* es un ejemplo de estas dificultades taxonómicas. El grupo de especies *C. podiciferus* contiene actualmente nueve especies descritas (Frost 2019). Cope (1875) describió la

primera especie del grupo, *C. podiciferus* y cuatro variedades para ésta. Además, este mismo autor describió otras dos especies, *C. muricinus* y *C. habenatus* con especímenes de la misma localidad. Cope (1875) expuso el fenómeno de polimorfismos dentro del grupo al mencionar "los colores de esta especie varían considerablemente, más de lo que yo haya observado en cualquier otra rana." Cope (1886, 1893) describió posteriormente otras tres especies del grupo, *C. bransfordii* (Cope, 1886), *C. polyptychus* (Cope, 1886) y *C. stejnegerianus* (Cope, 1893), las primeras dos con especímenes de la misma localidad. Tres especies más fueron descritas durante la primera mitad del siglo XX, *C. blairi* (Barbour, 1928), *C. persimilis* (Barbour, 1926) y *C. underwoodi* (Boulenger, 1896). Taylor (1952) realizó la primera revisión exhaustiva para el grupo, describiendo dos especies adicionales, *C. rearki* y *C. costaricensis.* Savage & Emerson (1970) basados en análisis morfológicos, redujeron el número de especies dentro del grupo a solamente dos, *C. bransfordii* y *C. podiciferus.*

Usando datos bioquímicos, Miyamoto (1983) encontró que las poblaciones de *C. bransfordii* en el pacífico de Costa Rica son altamente divergentes de aquellas en la vertiente del caribe, reasignando el nombre *C. stejenegrianus* para las poblaciones del pacífico. Estos hallazgos fueron corroborados por diferencias en cariotipos (Chen 2001, 2005). Savage (2002) reasignó los nombres *C. persimilis, C. polyptychus* y *C. underwoodi,* dejando a *C. rearki* y *C. costaricensis* bajo sinonimia de *C. bransfordii* y *C. muricinus, C. habenatus* y *C. blairi* bajo sinonimia de *C. podiciferus.* Previo a Savage (2002), dos especies adicionales fueron nombradas, *C. jota* (Lynch, 1980) y *C. lauraster* (Savage, McCranie & Espinal, 1996). De esta manera, después de Savage (2002) ocho especies fueron reconocidas como parte del grupo, *C. bransfordii, C. jota, C. lauraster, C. persimilis, C. podiciferus, C. polyptychus, C. stejnegerianus* y *C. underwoodi*.

Estudios filogenéticos basados en secuencias de ADN han apoyado la monofilia del grupo (Hedges *et al.* 2008; Pyron & Wiens 2011; Padial *et al.* 2014), sugiriendo que el grupo contiene especies no nombradas (Crawford 2003; Crawford & Smith 2005; Streicher *et al.* 2009). Crawford (2003) encontró divergencias genéticas altas dentro del grupo, similares a aquellas encontradas entre géneros de ranas australianas (Anura: Myobatrachidae), aunque según el autor, estas poblaciones no presentan diferencias morfológicas. Crawford & Smith (2005) usando 10 muestras del grupo, encontraron dos especies no nombradas (*Craugastor* sp. B y *Craugastor* sp. C) y además demostraron que *C. stjenegrianus* es parafilético. Posteriormente, Streicher *et al.* (2009) realizó un estudio filogeográfico utilizando poblaciones de tierras altas (>1000 m), que históricamente fueron asignadas a la especie *C. podiciferus.* Estos autores encontraron que las poblaciones referidas como *C. podiciferus* representan un complejo de especies con al menos seis especies no nombradas, y además confirmaron la presencia de *Craugastor* sp. B (Streicher *et al.* 2009). Arias *et al.* (2016) emplearon datos mitocondriales y morfológicos, confirmando los resultados previos (Crawford & Smith 2005) al encontrar que las poblaciones de tierras intermedias del Pacífico Sur de Costa Rica y oeste de Panamá que fueron históricamente asignadas a *C. stejnegerianus* en realidad pertenecen a una especie distinta, a la que nombraron como *C. gabbi* Arias, Chaves, Crawford, & Parra-Olea, 2016.

Delimitación de especies

La delimitación de especies es el proceso por el cual se determinan los límites entre las mismas y se describen nuevas especies (Sites & Marshall 2003). Este proceso está estrechamente relacionado con el concepto de especie, por lo tanto, la reciente adopción de un concepto unificado de especie ha generado una revolución en la delimitación (de Queiroz 2007). De

Queiroz (2007) propone separar entre conceptualización de especie y criterios operacionales de especie. El primero, definido como la única propiedad necesaria de la categoría de especie, un concepto teórico, explicado como un linaje que evoluciona independientemente, mientras que los criterios operaciones de especies, son las múltiples líneas de evidencia que son relevantes para la delimitación de estas (de Queiroz 2007). La separación entre la conceptualización de las especies y sus criterios operacionales ha permitido la generación de diversos protocolos que permiten una delimitación reproducible de las especies. De Queiroz (2007) sugiere que las especies deben ser delimitadas utilizando múltiples líneas de evidencia.

Aunque se ha sugerido la presencia de varias especies no nombradas dentro del grupo de especies *C. podiciferus* (Savage 2002; Crawford & Smith 2005; Streicher *et al.* 2009), solamente una especie, *C. gabbi*, fue descrita y nombrada posterior a Savage *et al.* (1996). El descenso en descripción de nuevas especies puede ser explicado por el cambio de paradigma en la sistemática y la necesidad de apoyar las nuevas especies con al menos dos líneas de evidencia, morfológica y molecular (de Queiroz 1998; Dayrat 2005; Will *et al.* 2005; Padial *et al.* 2010). En el pasado, los estudios dentro del género carecían de evidencia integradora; por ejemplo, todos los estudios utilizando evidencia molecular no empleaban análisis morfológicos. Sin embargo, la evidencia sugiere que es posible resolver complejos de especies crípticas utilizando un criterio de taxonomía integradora (Padial *et al.* 2008; Pérez-Ponce de León & Nadler 2010; Arias *et al.* 2016).

Es posible identificar tres clados dentro del grupo de especies *C. podiciferus* con base en los siguientes caracteres morfológicos: 1) el clado *C. bransfordii,* conformado por *C. bransfordii, C. polyptychus* y *C. underwoodi,* reconocido por tener el tubérculo tenar igual o apenas ligeramente más pequeño que el palmar; 2) el clado *C. podiciferus,* conformado por *C. jota* y *C.*

podiciferus, reconocido por tener el vientre liso muy diferente al vientre granular o areolado de las demás especies del grupo; y 3) el clado *C. stejnegerianus*, representado por *C. gabbi, C. lauraster, C. persimilis* y *C. stejnegerianus*, reconocido por tener el tubérculo tenar mucho más pequeño que el palmar. Padial *et al.* (2014) recuperó los tres clados antes mencionados; sin embargo, el alto grado de similitud morfológica entre las especies del grupo continúa siendo una dificultad para delimitar correctamente las especies y para evaluar la diversidad real dentro de cada grupo de especies.

En grupos como *Craugastor*, reconocidos por altos índices de polimorfismos y homoplasia, la delimitación de especies ha sido particularmente compleja (Lynch & Duellman 1997; Lynch 2000; Savage 2002; Hedges *et al.* 2008). Algunos autores se han referido a diversas especies de *Craugastor* como "especies crípticas" (Savage & Emerson 1970; Miyamoto 1983; Savage 2002; Crawford 2003; Streicher *et al.* 2009). Pérez-Ponce de León & Nadler (2010) distinguieron entre especie críptica *sensu stricto* (morfológicamente indistinguibles) *versus* definición funcional de especie críptica, definida por el taxónomo con base en su alto grado de similitud morfológica. Nadler & Pérez-Ponce de León (2011) sugieren que la delimitación de "especies crípticas" se debe basar en prueba de hipótesis desde un enfoque molecular. Si la hipótesis nula de una única especie es rechazada con base en datos moleculares, entonces, un detallado análisis morfológico puede proveer caracteres morfológicos que permitan la separación confiable de las especies y una diagnosis.

La delimitación de especies dentro de grupos morfológicamente conservados, se beneficia de los avances en el acceso a secuencias de ADN. La secuenciación Sanger o tradicional ha permito obtener gran cantidad de secuencias, pero están restringidas a secciones específicas del genoma, en especial aquellas del mitogenoma en animales (Hebert *et al.* 2003).

Estas secuencias mitocondriales han sido de gran utilidad para el reconociento de nuevas especies y las propuestas de hipótesis filogenéticas (Vences *et al.* 2005; Fouquet *et al.* 2007; Smith *et al.* 2008), sin embargo, tiene limitaciones para resolver ciertos complejos de especies. El desarrollo tecnológico en la secuenciación de la nueva generación, ha permitido incrementar el acceso a secuencias genómicas de organismos no modelos, ofreciendo una gran oportunidad de superar las dificultades inherentes al uso de los enfoques de secuenciación tradicional (Herrera & Shank 2016). Una de estas metodologías es la secuenciación del ADN asociado a sitios de restricción (RAD-seq), que combina fragmentación enzimática con secuenciación masiva, para la obtención de gran cantidad de marcadores o SNPs (Baird *et al.* 2008; Peterson *et al.* 2012). RAD-seq ha mostrado gran utilidad para obtener filogenias y delimitación de especies robustas dentro de grupos taxonómicos de difícil tratamiento (Peterson *et al.* 2012; Streicher *et al.* 2014; Leaché *et al.* 2015).

La delimitación molecular de especies es actualmente uno de los campos de mayor desarrollo en sistemática (Sites & Marshall 2003; Flot 2015). Una gran variedad de métodos han sido desarrollados recientemente: ABGD (Puillandre *et al.* 2012), PTP (Zhang *et al.* 2013), mPTP (Kapli *et al.* 2016), BPP (Yang 2015; Yang and Rannala 2010, 2014), GMYC (Pons *et al.* 2006; Fujisawa & Barraclough 2013), Structurama (Huelsenbeck *et al.* 2011) y SpedeSTEM (Ence & Carstens 2011), entre otros. La efectividad de los métodos en identificar especies continúa bajo discusión (Sukumaran & Knowles 2017). Sin embargo, el uso de estos métodos puede ser útil como una primera aproximación para reconocer la diversidad dentro de un grupo determinado, en especial para aquellos grupos morfológicamente conservados y polimórficos. Estos métodos pueden fallar en reconocer las especies, aunque pueden servir para identificar

especies candidatas que posteriormente deberán ser confirmadas con otras líneas de evidencia como morfología, bioacústica, segregación en nicho u otras.

El grupo de especies *C. podiciferus* actualmente está compuesto por nueve especies de terraranas pobremente estudiadas, abundantes en la hojarasca y ampliamente distribuidas (colectivamente) en ACI (Savage 2002; AmphibiaWeb 2019). Los miembros de este grupo son morfológicamente conservados y con alto nivel de polimorfismos, por lo cual se ha sugerido que contiene varias especies no descritas. El origen del grupo *C. podiciferus* se ha calculado en ~20 Ma cuando ACI estaba emergida en su posición actual (Crawford & Smith 2005; Streicher *et al.* 2009). Considerando la distribución y abundancia, el grupo de especies *C. podiciferus* constituye un excelente modelo para evaluar las relaciones filogenéticas de un grupo de terraranas que diversificó en paralelo con la formación de ACI durante el Mioceno. En el presente trabajo de tesis abordé el estudio del complejo de especies *C. podiciferus*, incluyendo sus relaciones filogenéticas, su composición taxonómica y además, se describe la importancia de las tierras altas de ACI en la diversificación del grupo. Para responder estas preguntas obtuve secuencias de los genes mitocondriales 16S (16S) y citocromo oxidasa 1 (COI), obtenidos a través de secuenciación Sanger. Además, empleé una técnica de secuenciación de nueva generación (RAD-seq), lo cual permitió analizar cientos de loci y contar con una hipótesis filogenética robusta.

OBJETIVOS

Objetivo general:

Investigar la sistemática y evolución del grupo de especies *Craugastor podiciferus* con un criterio de taxonomía integradora.

Objetivos específicos:

1. Inferir las relaciones filogenéticas dentro del grupo de especies *C. podiciferus*, usando dos marcadores mitocondriales y secuencias nucleares (SNPs) obtenidas con RAD-seq.
2. Proponer una hipótesis taxonómica para el grupo de especies *C. podiciferus*, integrando la evidencia molecular y morfológica para la delimitación de las especies.
3. Describir morofológicamente las especies que se han delimitado, incluyendo la descripción de especies nuevas.
4. Proponer una hipótesis del papel de las tierras altas de ACI en la diversificación del grupo de especies *C. podiciferus*.

CAPÍTULO I

RELACIONES FILOGENÉTICAS, DELIMITACIÓN Y BIOGEOGRAFÍA

Mitochondrial and nuclear DNA reveal high cryptic diversity in the *Craugastor podiciferus* species group (Anura: Craugastoridae) in Isthmian Central America

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Running Title: Systematic of *Craugastor podiciferus*

Abstract

The *Craugastor podiciferus* species group contains ten species of terraranas from Central America with high levels of polymorphism and possibly containing overlooked species masked under the current names. We performed an extensive geographical sampling of the *C. podiciferus* species group to evaluate its phylogenetic relationships and biogeographic history based on two mitochondrial markers and nuclear ddRAD loci. We also conducted various species delimitation methods to test the presence of unnamed species within the group. The phylogenetic relationships recovered showed that the group contains four major clades, three of them corresponding to previously known clades, and the remaining one recently described. Ours results suggest that the species richness within the group is underestimated; however, the species delimitation was highly discordant between the mitochondrial and nuclear analyses and among methods. We propose that the *C. podiciferus* species group contains at least 23 lineages, all distributed in Costa Rica and western Panama. A biogeographic analysis shows that the group originated and diversified in the highlands of the Talamancan montane forest ecoregion.

Key words: Biogeography, Lagrange, species delimitation, systematic, taxonomy

Introduction

Species biodiversity is not homogeneously distributed over the globe. The Neotropics are one of the regions with higher biodiversity in the world, including seven of the 25-biodiversity hotspots (Myers *et al.* 2000). One of these hotspots, the Mesoamerica biodiversity hotspot, includes the Isthmian Central America (ICA) region, which is located in Costa Rica and Panama and it contains an exceptional biodiversity and endemism. This region hosts more species of amphibians, reptiles, birds, insects, and vascular plants per area unit than almost any other place in the world (Davis *et al.* 1997; Anger & Dean 2010; Garrigues & Dean 2014; AmphibiaWeb

2019). The high biodiversity in ICA is explained by two major factors, the closure of the Isthmus of Panama and its long history of volcanic and orogenic activity. The closure of the Isthmus of Panama allowed the Great American Biotic Interchange (GABI) between North and South America, resulting in the confluence of north American and south American species in ICA (Savage 1966; Webb 1991, 2006; Pinto-Sánchez *et al.* 2012). The long history of volcanic and orogenic activity in ICA resulted in a high geographic and climatic heterogeneity (Bagley & Johnson 2014; Montes *et al*. 2012a,b, 2015). The diversity in ICA was enhanced by *in situ* diversification, generating high levels of endemisms associated with a high diversity of habitats (Campbell 1999; Savage 2002; Kluge 2006; Boza-Oviedo *et al.* 2012).

ICA contains the highest species richness relative to area in the World (AmphibiaWeb 2019; Frost 2019) including some northern and southern species and several micro endemic taxa associated to the highlands. Nevertheless, big areas within ICA remain unexplored, and some particular groups (e.g. *Craugastor*, *Diasporus*, *Pristimantis*) have not been well studied yet. Amphibians contain the highest proportion of cryptic species within metazoans (Pérez-Ponce de León & Poulin 2016). This overlooked diversity masks the real diversity of a group and makes it difficult to make biogeographical inferences for the region.

Previous molecular studies with direct-developing frogs (*Craugastor* and *Pristimantis*) and direct-developing salamanders (*Bolitoglossa*) revealed extremely high levels of genetic diversity both in highlands (García-París *et al.* 2000; Wiens *et al.* 2007; Streicher *et al*. 2009) and lowlands (Crawford 2003; Crawford *et al.* 2007; Wang *et al.* 2008) of the ICA. The *Craugastor podiciferus* species group (Hedges *et al.* 2008) is currently composed of ten described species (Arias *et al.* 2018). This group occurs from eastern Honduras to central Panama, ranging from the sea level to 2700 m a.s.l. in a wide variety of habitats (Savage 2002; AmphibiaWeb 2019). The systematics and taxonomy of the *C. podiciferus* species group has been poorly studied, though previous molecular studies support the presence of several

undescribed species (Savage 2002; Crawford 2003; Crawford & Smith 2005; Streicher *et al.* 2009). Crawford (2003) found high genetic divergences between populations of *C. stejnegerianus* in the ICA lowlands. Streicher *et al.* (2009) used the specimens from the highlands (>1000 m a.s.l.), referred to *C. podiciferus*, and mentioned that the name *C. podiciferus* could mask a species complex formed by up to six distinct taxa, and also supported the existence of an undescribed species (*Craugastor* sp. B) related to *C. podiciferus*. Based on mitochondrial data, Arias *et al.* (2016) recently showed that populations formerly considered part of *C. stejnegerianus* from southwestern Costa Rica and western Panama belong to a different species, which they named *C. gabbi*.

Several studies are concordant with the presence of a great cryptic diversity within amphibians from ICA (García-París *et al.* 2000; Crawford 2003; Crawford & Smith 2005; Crawford *et al*. 2007; Streicher *et al*. 2009; Batista *et al.* 2016). Nevertheless, these studies were limited to few species and restricted to few mitochondrial or nuclear markers. In addition, these studies were restricted only to species from highlands or lowlands. To our knowledge, no molecular studies have extensively evaluated the phylogenetic relationships and the overlooked diversity of an amphibian species group restricted to ICA, including populations both from highlands and lowlands and using mitochondrial markers and an extensive nuclear dataset. Here we use mitochondrial and genome-scale datasets to: 1) infer the phylogenetic relationships of the *C. podiciferus* species group, 2) to test the existence of overlooked species within the current names, and 3) to investigate the center of origin for the group and suggest a possible historical framework for its species diversification.

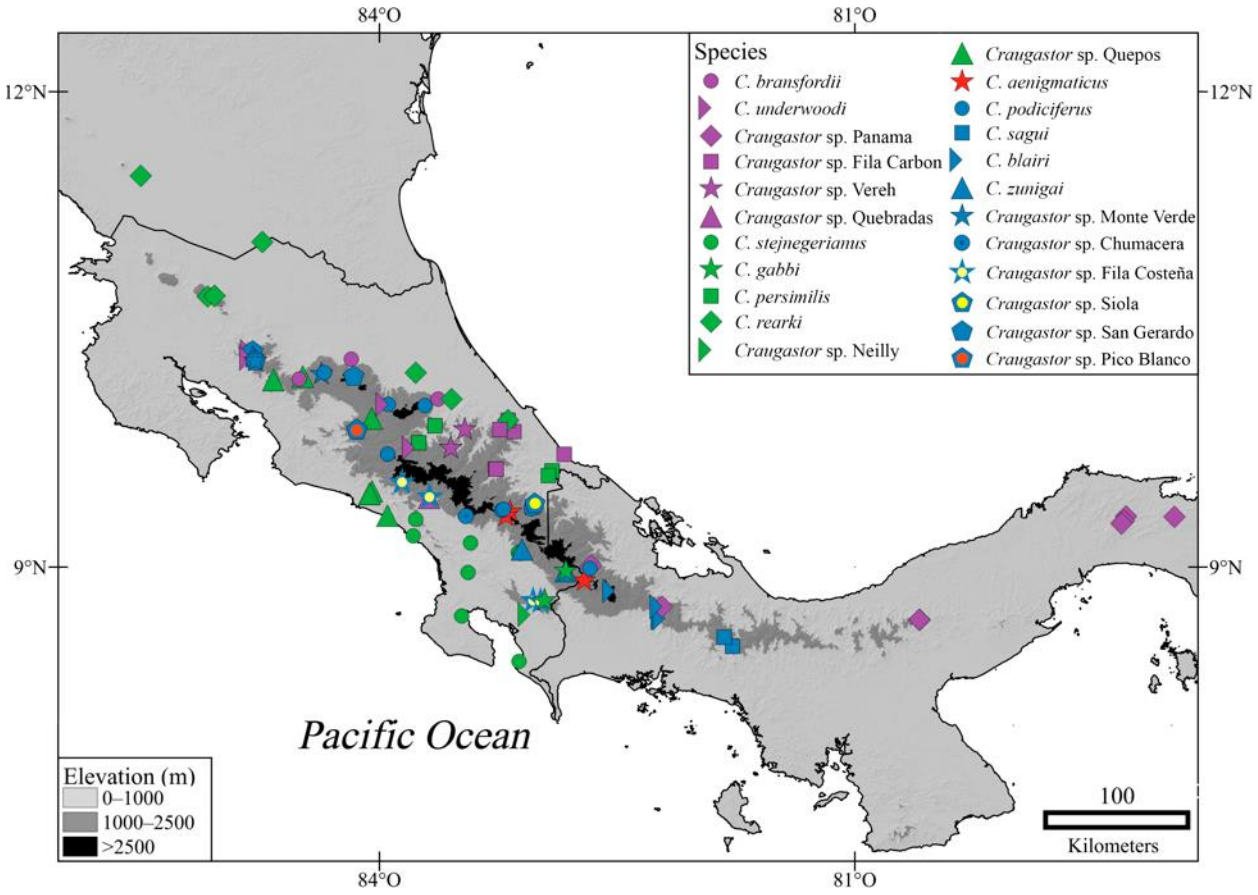

FIGURE 1. Geographic distribution of the *Craugastor podiciferus* species group.

2. Materials and methods

2.1. Taxon sampling

Tissue samples were collected from 103 specimens, including all species of the *C. podiciferus* species group from Honduras, Nicaragua, Costa Rica, and Panama (Fig. 1, Appendix I). The frogs were obtained in the field, euthanized, fixed in a 10% formalin solution and processed over to 70% ethanol for long-term storage. The tissue sample used for the genetic analyses was preserved in 96% ethanol or RNAlater. The vouchers were deposited at the Museo de Zoología, Universidad de Costa Rica (UCR), the Division of Amphibians and Reptiles at the Field Museum of Natural History (FMNH), Circulo Herpetológico, Panama (CH), and Senckenberg Research Institute and Nature Museum, Frankfurt, Germany (SMF). Museum collection

acronyms follow Frost (2019), with the addition of AJC refers to Andrew J. Crawford field numbers, AH refers to Andreas Hertz field numbers, and EAP refers to Erick Arias field numbers.

2.2. Mitochondrial data

2.2.1. Amplification and sequencing

We extracted total genomic DNA from the preserved tissue samples using the Animal Genomic DNA Kit (BioBasic Canada Inc.), the DNeasy Blood & Tissue Kit (Qiagen), or the phenol-chloroform standard protocol (Sambrook & Russell 2006). We amplified the large subunit ribosomal RNA (16S) and cytochrome oxidase subunit I (COI) mitochondrial genes. The primers 16Sar and 16Sbr (Palumbi *et al.* 1991) were used for 16S and dgLCO and dgHCO (Meyer 2003) for COI. Amplifications were performed using a total volume of 15 µL, which contained 1 µL DNA template (*c.* 50 ng μL^{-1}), 0.75 U Taq polymerase (Amplificasa®, Biotecnologias Moleculares), 1 x PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), and 0.3-0.5 µM forward and reverse primers. The PCR conditions were as follow: 16S, an initial cycle of 5 min at 94ºC, followed by 35 cycles of 45 s at 94ºC, 30 s at 50 or 55ºC, 45 or 120 s at 72ºC, plus a final cycle of 3 min at 72ºC; COI, an initial cycle of 2 min at 94ºC, followed by 35 cycles of 30 s at 94ºC, 30 s at 50ºC, 45 s at 72ºC, plus a final cycle of 3 min at 72ºC. PCR products were cleaned with ExoSap-IT (USB Corporation) and sequenced in both directions using the original amplification primers and BigDye termination reaction chemistry (Applied Biosystems). The cycle-sequencing products were column-purified with Sephadex G-50 (GE Healthcare) and run on an ABI 3500xL Genetic Analyzer (Applied Biosystems). Consensus sequences for each individual were constructed using SEQUENCHER 5.3 (Genes Codes Corp.).

2.2.2. Phylogenetic analyses

We generated 16S and COI sequences for members of the *C. podiciferus* species group, and retrieved sequences from GenBank for *C. gollmeri,* which was included as outgroup. A list with the examined material, their localities and DNA voucher and GenBank accession numbers used in this study is provided in Appendix I. Sequence alignments were performed using the MUSCLE 3.7 software (Edgar 2004) with default parameters and trimmed to the point where a majority of the taxa had sequence data. We partitioned the sequence data by gene, and further partitioned COI by codon position. We used PartitionFinder v1.1.1 (Lanfear *et al.* 2012) and the Bayesian Information Criterion (BIC) to select the best partition scheme and the best model of sequence evolution for each partition. We used a single set of branch-lengths across all partitions (*branchlengths=linked*), the search of the best partition scheme was using a heuristic search (*scheme=greedy*). We defined four partitions one for 16S and three for COI according to its codon positions. The partition scheme and the model of sequence evolution for each partition as selected by PartitionFinder (Lanfear *et al.* 2012) were used in the Bayesian methods (see below).

We used maximum likelihood and Bayesian methods to infer a phylogenetic tree from the concatenated loci in the final dataset. We performed the maximum likelihood analysis using RAxML-HPC v8 (Stamatakis 2014) with the GTR + GAMMA model of nucleotide substitution (default model of RAxML) and the –f a option, which searches for the best-scoring tree and performs a rapid bootstraps analysis (*i.e.* 1000 bootstraps) to estimate node support by resampling the data. A partitioned Bayesian phylogenetic analysis was performed using MrBayes 3.2.6 (Ronquist *et al.* 2012) with the partition scheme and the models of sequence evolution that was previously selected. Two separate analyses were run, each consisting of 20 million generations, sampling every 1000 generations and using four chains with default heating parameters. We examined a time-series plot of the likelihood scores of the cold chain to check stationarity using the Tracer 1.6 software (Rambaut *et al*. 2014). We discarded the first 25% of trees as burn-in and used the remaining trees to estimate the consensus tree along with the posterior probabilities for each node and each parameter.

We used the program BEAST v1.8.3 (Drummond *et al*. 2012) to estimate ultrametric phylogenetic tree using an uncorrelated lognormal relaxed clock, a Yule tree prior, and with the partition scheme and the model of sequence evolution for each partition as selected previously. We ran the analysis for 50 million generations, sampling every 1000 generations, and discarding the first 5000 samples as burn-in when estimating a consensus tree.

All phylogenetic analyses were run on the CIPRES portal (Miller *et al.* 2010). Estimates of pairwise evolutionary genetic divergence between groups were computed using MEGA7 (Tamura *et al.* 2013), assuming uncorrected distances based on the Tamura 3-parameter model (Tamura 1992), with rate variation among sites modeled as a gamma distribution with the shape parameter = 4 as the default of the software.

2.3. Nuclear data

2.3.1. ddRADseq data collection

We generated ddRADseq data from 48 samples following the protocol described by Peterson *et al.* (2012) and modified by Leaché *et al*. (2015). High-molecular weight DNA was purified with RNase, examined for quality on agarose gels, and quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific). We used 1000 ng of genomic DNA for each sample, except for three samples whose account of DNA was of 241–630 ng. We double-digested the genomic DNA with 20 units each of a rare cutter SbfI (restriction site 5´-CCTGCAGG-3´) and a common cutter MspI (restriction site 5´-CCGG-3´) in a single reaction with the manufacturer recommended buffer (New England Biolabs) for 2 h at 37 ºC. Fragments post-digestion were purified with Serapure 1.5X and quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific) before ligation of barcoded Illumina adaptors onto the fragments.

The oligonucleotide sequences used for barcoding and adding Illumina indexes during library preparation were those employed in Leaché *et al*. (2015). The barcodes differed by at least two base pairs to reduce the chance of errors caused by inaccurate barcode assignment.

Equimolar amounts of each sample were pooled, with each pool containing up to eight unique barcoded samples, in a 96-well plate format. Each pool was purified with Serapure 1.5X, rehydrated in 50 μL and quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific) before size selection. The pooled libraries were size-selected (~500 pb) on a e-gel (Invitrogen) according to manufacturer instructions. The two external and internal lines (next to leader line) in the e-gel were not used, in the four lines available only two different libraries were run, with a maximum of 500 ng by line. The size-selected libraries were purified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific) and amplified using PCR with the primers designed by Leaché *et al*. (2015) and using the Phire Hot Start II polymerase (Thermo Fisher Scientific). The amplified libraries were purified with Serapure 1.5X, quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific).

The fragment size distribution and concentration of each pool was determined on an Agilent BioAnalyzer and qPCR was performed to determine sequenceable library concentrations before multiplexing equimolar amounts of all 6 pools for sequencing on a single Illumina HiSeq 2000 lane (100 bp, single-end run) at the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley.

2.3.2. ddRADseq bioinformatics

We processed raw Illumina reads with the software pipeline ipyrad v0.5.15 (Eaton 2014), which consists of seven steps. We first demultiplexed the samples using their unique barcode and adapter sequences. Before of the second step, six samples with <50,000 reads passing the quality filter were excluded from further analyses. In the second step, reads were edited and filtered. The 6-bp restriction site overhang and the 5-bp barcode were removed. Sites with accuracy of the base call under 99% (Phred quality score = 20) were changed into ‘‘N” characters, and reads with >9 N’s (~10 %) were discarded.

During the steps 3–6 the reads from each sample were clustered using the program

VSEARCH version 1.11.1 (https://github.com/torognes/vsearch) and aligned with MUSCLE version 3.8.31 (Edgar 2004). The first clustering step establishes homology among reads within samples. We determined the optimal value for the clustering parameter using the clustering threshold series approach described by Ilut *et al*. (2014). This method, which maximizes the account of cluster with two haplotypes, seeks to assemble reads into loci such that false homozygosity (splitting reads from a single locus into two) and false heterozygosity (due to clustering of paralogs) is minimized (i.e., the optimum clustering threshold). We generated a clustering threshold series (sensu Ilut *et al.* 2014) using similarity thresholds ranging from 0.85 to 0.98 for 19 samples. The optimal clustering threshold was 0.9 (Fig. 2a), which is used within and between sample clustering. After the clustering of reads within samples, we estimated the error rate and heterozygosity from the base counts in each site across all clusters, and these values were used to generate consensus sequences for each cluster. Consensus sequences were then clustered across samples and aligned as described above. Within 3–6 steps, we also discarded loci that had >4 undetermined or heterozygous sites (default ipyrad settings), or >2 haplotypes (to filter paralogs), and used a minimum depth of coverage of 6 for genotype calls.

Following Nieto-Montes de Oca *et al*. (2017), we performed multiples replicates of the seventh step to determine the optimal value for the parameters: maximum numbers of SNPs allowed in a locus, maximum number of insertions/deletions allowed in across-sample clusters, and the maximum proportion of samples allowed to share a heterozygous site. The number of retained loci increased linearly with higher numbers of SNPs allowed until it began to plateau at a maximum of 30 SNPs (Fig. 2b). We used this value for the assembly of the final dataset under the rationale that above this value the small number of additional loci that were retained potentially represented paralogs. The number of retained loci increased with higher numbers of indels allowed, until a maximum of 8 indels (default ipyrad settings) (Fig. 2c). Thus, we permit a maximum of 8 insertions/deletions in across-sample clusters for the assembly of the final dataset. The number of loci retained increased until a value of 0.1 (corresponding to 9 samples), which

we again chose for the final value under the rationale that loci exhibiting higher shared heterozygosity potentially represented paralogs (Fig. 2d). We set the minimum number of ingroup samples with data for a given locus to be retained in the final dataset to about 25% of the samples (11). Unless otherwise stated, all phylogenetic analyses were performed with this dataset.

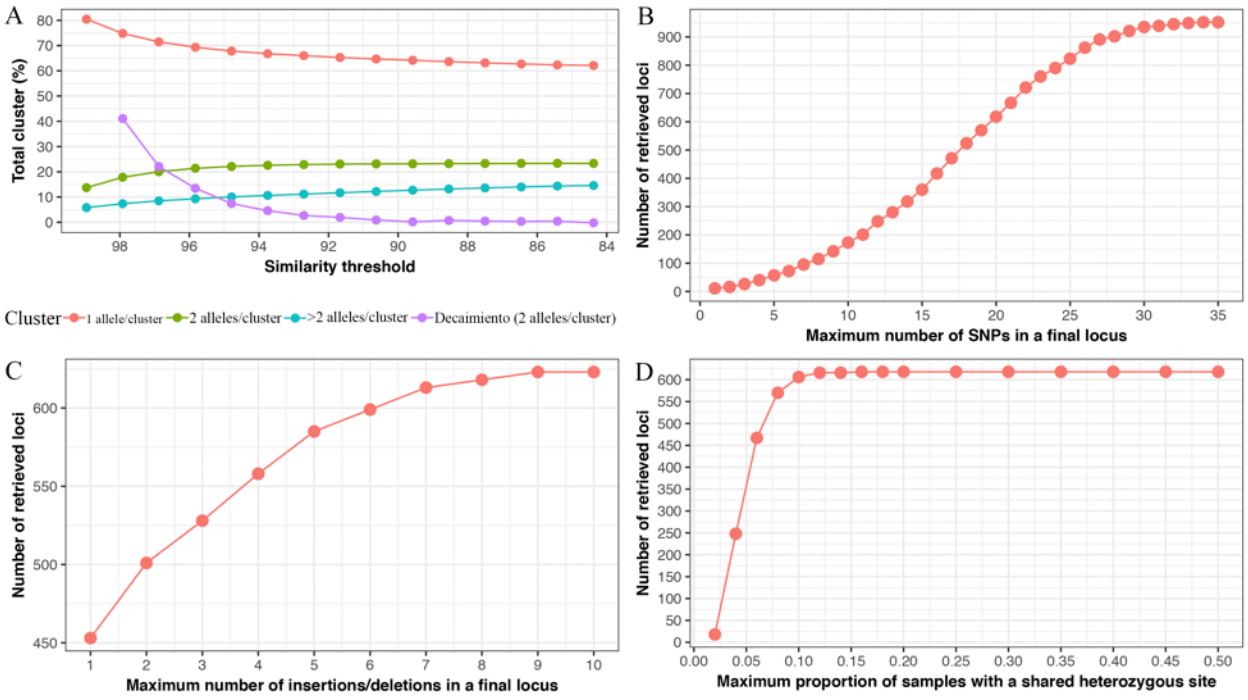

FIGURE 2. a) Variation in the proportion of clusters with 1, 2, and >2 alleles retrieved with different similarity thresholds; **b)** variation in the number of retrieved loci with different maximum numbers of SNPs in a final locus; **c)** variation in the number of retrieved loci with different maximum numbers of insertions/deletions in across-sample clusters (c); **d)** variation in the number of retrieved loci with different maximum proportions of samples with a shared heterozygous site. The different line colors in **a** represent different numbers of alleles/cluster.

2.3.3. Phylogeny reconstruction

We used maximum likelihood and Bayesian methods to reconstruct a phylogenetic tree from the concatenated ddRAD loci in the final dataset. We ran RAxML, MrBayes, and BEAST analyses using the same parameters as the mitochondrial dataset, though only the GTR+I+G model was used for these analyses. We also performed RAxML, MrBayes, and BEAST analyses for each of the three clades within of the *C. podiciferus* species group (*i.e. C. branfordii* clade, *C. podiciferus* clade, and *C. stejnegerianus* clade). To perform the analyses by clade, we replicated the pipeline in ipyrad for each clade maximizing the number of retained loci. We did not attempt to estimate gene trees because the short read lengths cause the individual loci to be minimally phylogenetically informative (Nieto-Montes de Oca *et al*. 2017).

2.4. Species delimitation

We used various methods to address species boundaries in the *C. podiciferus* species group and evaluated the effect of the tree used. The analyses were similar for datasets, mitochondrial and nuclear data. The species delimitation based on nuclear the dataset was ran using both the complete phylogeny and the partial phylogenies (see section 2.3.3), except for the BPP analysis, where only the partial phylogenies were used.

We used three species discovery methods, GMYC, PTP, and mPTP that infer putative species limits on a given phylogenetic tree. The GMYC method (Pons *et al.* 2006; Fujisawa & Barraclough 2013) infers the transition point between inter- and intra-species branching rates on a time-calibrated ultrametric tree. We ran the GMYC analyses on the web server (http://species.h-its.org/gmyc/) under the single threshold and multiple threshold models using the ultrametric trees from the concatenated BEAST analyses (see above).

The PTP method (Zhang *et al.* 2013) uses the number of substitutions to identify significant changes in the rate of branching in a phylogenetic tree (which may not be ultrametric). We ran PTP in the web server (http://species.h-its.org/ptp/) for 500,000 generations, with thinning = 100 and burn-in = 10%. The bPTP web server runs both the maximum likelihood

and Bayesian versions of PTP. Following a conservative approach, we considered as species those clades with a value greater than 0.01 (mitochondrial) or 0.05 (nuclear). The values above the nodes in PTP results represent posterior delimitation probabilities; the posterior probabilities of those taxa form one species. Given that PTP uses any tree completely bifurcated, we used the trees of RAxML, MrBayes, and BEAST to evaluate the effect of the tree as input. All analyses were replicated excluding the out-group.

The third method, the mPTP (Kapli *et al.* 2016), is similar to the PTP because it uses the number of substitutions to identify significant changes in the rate of branching in a phylogenetic tree. However, mPTP incorporates different rates of coalescence within clades, allowing different levels of intraspecific genetic diversity. Similar to PTP, mPTP runs both ML and MCMC analyses. MCMC analyses were run for 100 million generations, sampling every 10,000 and the first 2 million generations were discarded as burn-in. All ML and Bayesian analyses were run both –*single* and –*multi* rates of coalescence among species and used a minimum branch length of 0.0001. Following a conservative approach, for the MCMC analyses we consider as species those clades with a value greater than 0.95 (mitochondrial) or 0.99 (nuclear). As in PTP, we used the trees of RAxML, MrBayes and BEAST to evaluate the effect of the tree as input.

We used a fourth method, the Automatic Barcode Gap Discovery (ABGD; Puillandre *et al.* 2012). Unlike the three tree-based methods, it uses an aligned matrix. This method was used only for the mitochondrial dataset. Analyses were performed separately for 16S and COI. The method seeks to quantify the location of the barcode gap that separates intra- from interspecific distances. We used Pmin (0.01) and Pmax (0.1), JC69 and K80 corrected distances and relative gap width 1.5 (default) and 1.0.

We also used BPP version 3.1 (Yang 2015; Yang & Rannala 2010, 2014) to jointly perform species delimitation and species tree inference under the multispecies coalescent model. We used the method A10 that evaluate the species delimitation from a guide tree, using a

rjMCMC algorithm (Rannala & Yang 2013). The assignment of individuals to putative species is fixed, and the model is to merge different specified groups into a single species, but not to split pre-defined populations into multiple species. We used the topology of BEAST analyses as user-specified guide tree. The analysis was run for a total of 2,500,000 iterations (sampling interval of 5) with a burn-in of 1000. We evaluated the influence of the ancestral population size (θ) and root age (G) considering three different combinations as in Leaché & Fujita (2010). The first combination of priors: $\theta \sim$ G (1, 10) and $\theta \sim$ G (1, 10. The second combination of priors: $\theta \sim$ G (2, 2000) and $\theta \sim$ G (2, 2000). The third combination is a mixture of priors: $\theta \sim$ G (1, 10) and $\theta \sim$ G (2, 2000). All BPP analyses used algorithm 0 with the fine-tuning parameter ε=15 each with 500,000 generations (each fifth sampled) and a burn-in of 10,000 produced consistent results. With the nuclear dataset, the number of generations was 100,000. Each analysis was run at least twice to confirm consistency between runs. Following a conservative approach, only speciation events simultaneously supported by probabilities superior or equal to 0.99 for all three combinations of priors were considered for species delimitation.

We also used the genetic distance for delimitation for the mitochondrial dataset. Although, not considered as a species delimitation method, the genetic distance has been used as indicator of separation interspecific. For amphibians, the 16S gene fragment has been suggested as a DNA barcode marker for amphibian diversity inventories (Vences *et al.* 2005) to complement the standard COI-5' marker used in general for animals (Smith *et al*. 2008). Fouquet *et al.* (2007) suggested a threshold of 3% in 16S gene as significant for to identify possible distinct species. Vences *et al.* (2005) suggested a threshold of 10% in COI for identify candidate species. Estimates of pairwise evolutionary genetic divergence between groups were computed using MEGA6 (Tamura *et al.* 2013), assuming uncorrected distances based on the Tamura 3-parameter model (Tamura 1992), with rate variation among the sites modeled as a gamma distribution with the shape parameter = 4 as the default of the software. We evaluated the species identity with the

anterior methods. If the genetic distance between the clades was lower than the threshold the clades were collapsed.

2.5 Geographic range evolution

To infer routes of dispersal or vicariance events, the distribution range of the *Craugastor podiciferus* species group was divided into five areas based on Terrestrial Ecoregions of the World proposed by Olson *et al*. (2001): (A) Costa Rican seasonal moist forest, (B) Isthmian-Atlantic moist forest, (C) Isthmian-Pacific moist forest, (D) Talamancan montane forest and (E) Choco-Darien moist forest. We used the R package BioGeoBEARS (Matzke 2014) and implemented the LAGRANGE DEC model (Ree & Smith 2008) within a maximum likelihood framework. Furthermore, a founder-event speciation was added to any of these models and estimated as an additional free parameter j. Because no species is distributed over more than four defined areas, we set the maximum number of areas to four.

2.6 Relaxed molecular clock analysis

We used the BEAST calibrated tree to infer the ancestral area probability. The lack of *C. podiciferus* in the fossil record makes dating divergences based on molecular sequence data difficult. We use the dating for the group estimated by Streicher *et al*. (2009) to make a rough estimate of divergence times within the species group. Following Streicher *et al*. (2009) and assuming that ICA has emerged ~25 Mya, we restricted the origin of the *C. podiciferus* species group to 25 Mya. Details for the analyses of BEAST were described above.

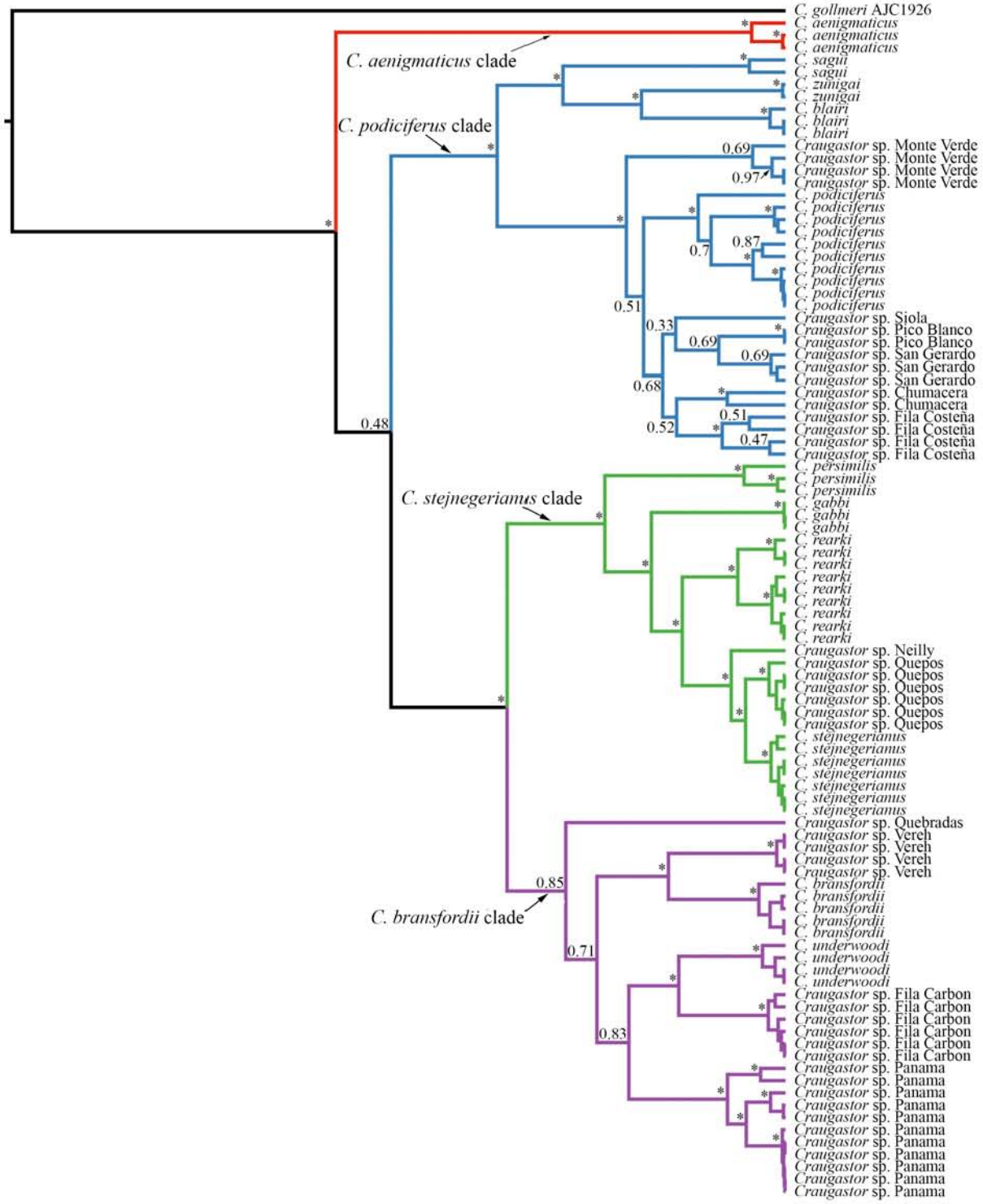

FIGURE 3. Maximum clade credibility tree from the concatenated BEAST analysis of *Craugastor podiciferus* species group based on 16S and COI mitochondrial DNA genes. Posterior probabilities from BEAST are shown. The scale bar refers to the estimated substitutions per site. The asterisks represent support of >95.

3. Results

3.1 Phylogeny of the Craugastor podiciferus species group

3.1.1. Mitochondrial phylogenies.

The resulting mitochondrial data matrix included 96 sequences with a total sequence length of 1222 bp including gaps (565 bp for 16S and 657 bp for COI). The following substitution models were selected: GTR+I+G for 16S, K80+I+G for COI codon position 1, HKY+I for COI codon position 2, and GTR+G for COI codon position 3. The mitochondrial genetic distances are shown in Table 1.

The phylogeny inferred with BEAST (Fig. 3) found the *C. podiciferus* species group to be composed of four major clades. These clades correspond to the three previously recognized morphological complexes (*C. bransfordii, C. podiciferus*, and *C. stejnegerianus*), and an additional basal clade of a group that was described recently. The first basal clade, *C. aenigmaticus*, is composed of samples from the Cordillera de Talamanca. The second major clade, the *C. podiciferus* clade, contains two well-supported subclades. The first includes samples from La Nevera, Alturas, and Fortuna from the highlands of southwestern Costa Rica and western Panama, and the second has samples of the type locality of *C. podiferus* and several populations from the highlands of Costa Rica and western Panama. The *C. stejnegerianus* clade comprises six groups: *C. persimilis, C. gabbi, C. rearki, C. stejnegerianus, Craugastor* sp. Neilly, and *Craugastor* sp. Quepos. This clade ranges from eastern Honduras to western Panama. The fourth major clade, *C. bransfordii* clade is composed of six groups: *C. bransfordii*, *C. underwoodi, Craugastor* sp. Fila Carbon, *Craugastor* sp. Panama, *Craugastor* sp. Quebradas, and *Craugastor* sp. Vereh.

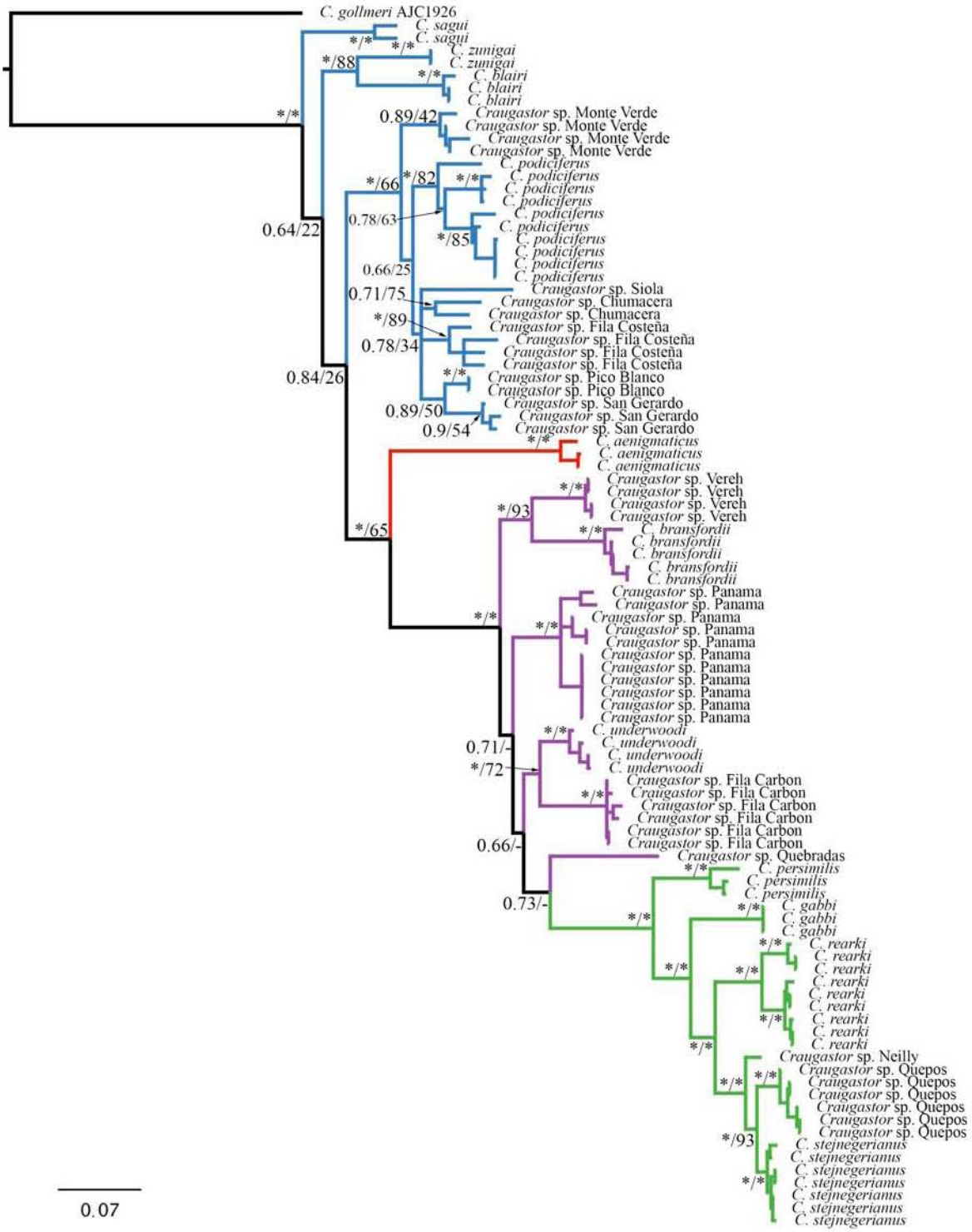

FIGURE 4. Bayesian phylogram derived from MrBayes of the *Craugastor podiciferus* species group based on the 16S and COI mitochondrial DNA gene markers. Posterior probabilities of clades and Bootstraps values are before and after slashes, respectively. The scale bar refers to the estimated substitutions per site. The asterisks represent posterior probability values > 0.95.

Table 1. Mean uncorrected genetic distances among lineages of *Craugastor podiciferus* species group using 16S (above) and COI (below) mitochondrial genes. Genetic distances within the three major clades are highlighted. Values less that the thresholds (3% in 16S and 10% in COI) are shown in red.

ID	16S/COI	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	*Craugastor aenigmaticus*	—	17.5	13.3	15.6	15.0	14.2	14.4	15.1	14.8	14.8	17.3	—	14.2	16.7	16.3	16.2	16.8	18.7	20.3	19.6	18.9	19.6	21.7
2	*Craugastor sagui*	21.8	—	7.9	7.4	7.3	7.1	7.4	9.5	8.2	8.4	9.1	—	11.1	17.5	12.0	11.1	12.1	12.8	16.1	16.2	16.2	16.3	18.0
3	*Craugastor zunigai*	25.0	17.3	—	3.4	7.2	6.2	7.3	9.3	9.4	9.6	8.9	—	12.3	17.0	13.2	13.7	13.1	13.8	16.3	16.4	15.7	16.4	19.4
4	*Craugastor blairi*	20.2	20.3	18.3	—	7.6	7.4	7.6	10.5	8.3	9.9	9.4	—	13.3	17.7	12.0	12.5	13.0	14.1	17.0	17.1	17.0	17.2	21.0
5	*Craugastor* sp. Monte Verde	19.5	16.6	18.7	19.9	—	2.5	2.0	3.7	6.6	4.4	4.5	—	9.1	14.9	9.9	11.1	10.4	11.5	14.6	14.7	14.6	14.8	16.6
6	*C. podiciferus*	22.2	17.8	18.5	19.8	12.0	—	2.7	4.1	6.6	4.6	5.1	—	7.6	13.6	10.1	11.2	9.6	11.5	13.2	13.2	13.1	13.2	15.9
7	*Craugastor* sp. Pico Blanco	20.8	18.4	20.7	17.2	11.3	10.0	—	2.6	6.9	4.7	3.1	—	9.8	14.5	10.4	11.7	9.9	11.8	15.2	15.3	15.4	15.3	14.9
8	*Craugastor* sp. San Gerardo	21.9	18.4	21.4	20.5	12.8	12.4	7.3	—	7.8	5.8	4.5	—	9.8	14.2	10.9	11.7	9.2	13.4	15.3	15.4	15.5	15.4	13.7
9	*Craugastor* sp. Siola	24.0	19.8	22.8	19.6	12.8	12.9	11.4	13.7	—	8.0	9.0	—	11.0	15.8	9.2	10.3	10.5	11.8	13.0	11.5	11.5	11.6	16.1
10	*Craugastor* sp. Chumacera	19.3	19.3	21.3	18.3	12.4	11.8	9.9	12.8	11.6	—	7.4	—	11.2	17.6	11.8	12.1	12.7	14.2	17.8	16.8	16.8	16.9	19.9
11	*Craugastor* sp. Fila Costeña	21.2	17.8	23.4	19.9	12.5	12.3	12.0	14.4	13.8	9.6	—	—	12.0	16.5	11.4	12.7	10.9	12.7	14.2	15.9	15.8	16.0	16.0
12	*Craugastor* sp. Quebradas	25.4	19.2	22.0	24.5	24.2	22.6	24.2	25.3	26.3	22.8	25.2	—	—	—	—	—	—	—	—	—	—	—	—
13	*Craugastor* sp. Vereh	26.9	20.2	23.4	23.9	19.7	22.6	23.8	23.7	26.0	20.8	22.6	16.2	—	5.4	6.0	6.8	5.0	9.5	11.6	10.2	10.2	10.3	10.2
14	*C. bransfordii*	25.3	18.8	21.1	23.0	21.1	19.9	20.6	22.3	24.0	20.8	21.2	16.7	11.7	—	10.4	11.3	9.4	14.4	14.1	14.1	14.1	14.2	13.4
15	*C. underwoodi*	23.5	19.1	21.8	19.9	19.4	19.7	19.7	22.4	22.9	20.9	21.2	15.3	14.5	13.2	—	4.1	5.4	8.5	11.5	10.9	10.9	11.0	12.1
16	*Craugastor* sp. Fila Carbon	22.9	20.7	23.4	22.9	22.4	23.3	23.9	26.3	24.9	22.5	23.0	17.3	14.0	15.6	9.5	—	4.3	7.7	12.2	11.6	11.5	11.6	11.6
17	*Craugastor* sp. Panama	21.3	18.2	22.5	22.2	21.1	21.1	19.8	23.4	24.1	21.5	22.1	17.3	14.3	13.2	11.9	14.0	—	7.6	11.8	10.5	10.4	10.5	8.8
18	*C. persimilis*	24.2	22.4	26.5	24.0	23.0	23.8	24.9	27.4	24.1	26.2	26.4	23.8	20.8	21.8	19.1	19.6	20.9	—	7.6	6.3	7.0	6.5	9.0
19	*C. gabbi*	25.4	26.7	27.6	24.3	24.1	23.5	24.7	26.3	26.7	26.7	26.9	22.0	20.1	23.3	20.6	19.6	21.5	16.7	—	4.2	4.3	4.3	8.0
20	*Craugastor* sp. Neilly	24.9	27.5	27.4	22.6	24.6	27.2	26.9	28.9	29.3	29.8	29.2	24.2	22.4	22.9	20.5	20.4	22.6	16.3	14.1	—	1.1	0.2	6.0
21	*Craugastor* sp. Quepos	23.0	27.3	27.4	22.6	24.6	25.3	26.0	26.2	27.3	28.4	26.2	23.6	23.4	22.7	19.1	20.1	22.2	15.3	15.1	6.9	—	1.1	6.1
22	*C. stejnegerianus*	21.8	27.5	25.2	22.3	25.1	25.5	25.8	27.0	28.7	27.8	27.2	22.6	21.6	21.6	20.9	20.3	21.9	16.2	14.7	5.6	5.5	—	6.0
23	*C. rearki*	22.6	25.4	24.9	23.8	23.6	28.0	27.6	28.6	28.3	27.9	27.7	23.4	20.8	22.0	22.2	20.0	21.1	17.2	15.7	11.9	11.6	13.8	—

The mitochondrial phylogenies inferred with MrBayes (Fig. 4) and RAxML (not shown) were highly discordant with the BEAST phylogeny (Fig 3). Only the *Craugastor aenigmaticus* clade and the *C. stejnegerianus* clade were recovered with the same internal relationships. Within of the *C. podiciferus* clade, the *Craugastor zunigai* + *Craugastor blairi* subclade was recovered and the relationships of the subclade that includes the type locality of *C. podiciferus* was similar to the BEAST topology. Within the *C. bransfordii* clade, only the subclades *C. bransfordii* + *Craugastor* sp. Vereh and *C. underwoodi* + *Craugastor* sp. Fila Carbon were recovered.

3.1.1. Nuclear phylogenies.

The Illumina HiSeq2500 lane generated 66,364,543 reads demultiplexed to 48 samples. The total number of retained loci in the nuclear data matrix was of 7,770 (697,771 characters; 33% of missing data). The phylogenies inferred with RAxML, MrBayes and BEAST had the same topology (Fig. 5). The *C. podiciferus* species group was composed of four major clades, which were similar in composition to the one obtained by the mitochondrial data set and the BEAST analysis, only with differences on some internal relationships. The basal clade, *Craugastor aenigmaticus*, is formed by samples from the montane rainforest of the Cordillera de Talamanca. The *C. podiciferus* clade is composed of two subclades, one contain to *C. blairi, C.* sagui, and *C. zunigai* along the highlands of southwestern Costa Rica and western Panama. A second subclade includes *C. podiciferus sensu stricto* and other populations from the highlands of ICA (1000–2700 m a.s.l.). Several populations phylogenetically close to *C. podiciferus* in the mitochondrial phylogeny were not included in the nuclear analysis.

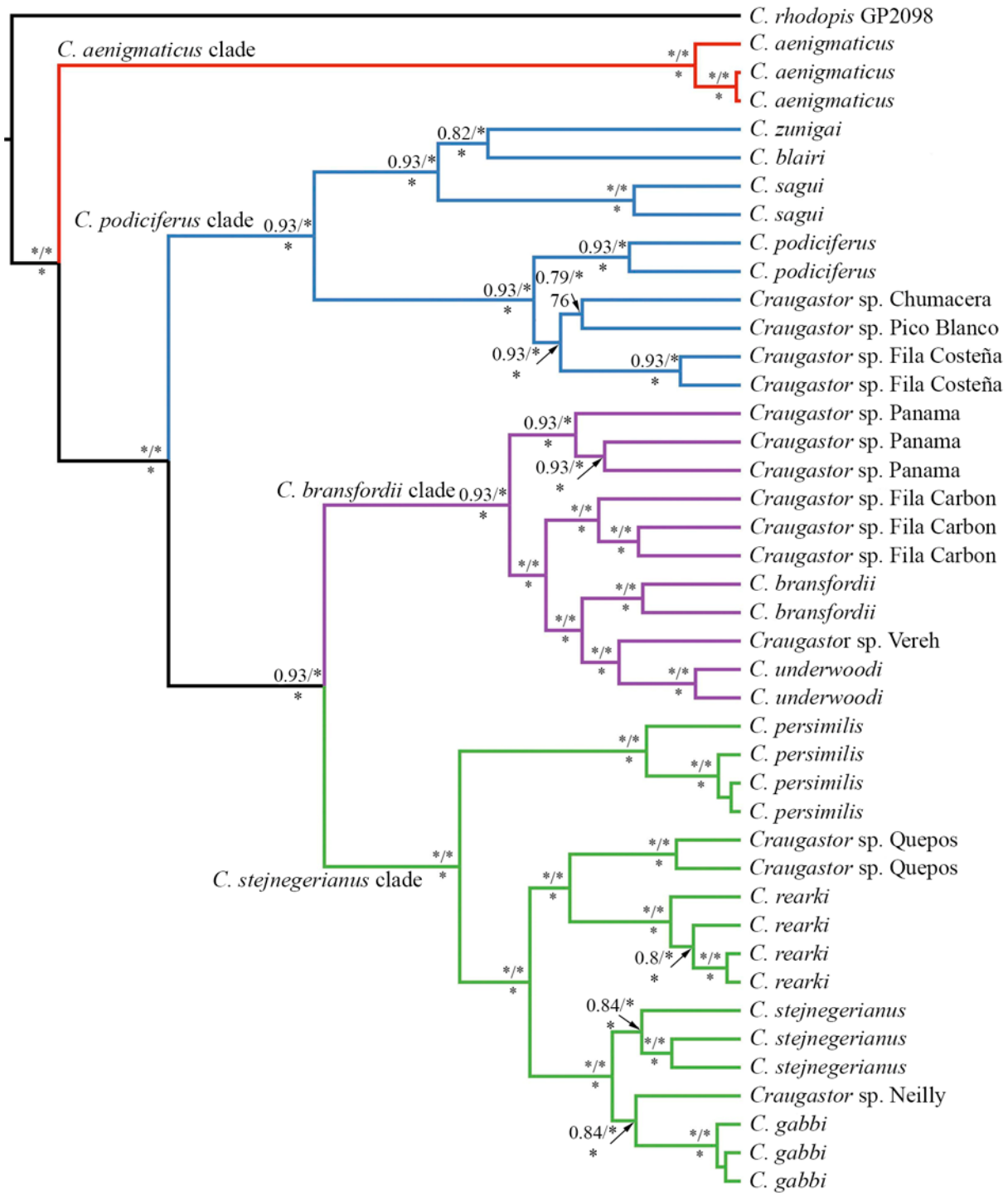

FIGURE 5. Maximum clade credibility tree from the concatenated BEAST analysis of the *Craugastor podiciferus* species group based on ddRAD dataset. Posterior probabilities from BEAST are shown. The scale bar refers to the estimated substitutions per site. The asterisks represent posterior probability values > 0.95.

The *C. bransfordii* clade was composed of *C. bransfordii*, *C. underwoodi*, *Craugastor* sp. Fila Carbon, *Craugastor* sp. Panama, and *Craugastor* sp. Vereh which occur from northern Nicaragua to central Panama. The relationships within the *C. bransfordii* clade differ from the mitochondrial topology. In the nuclear tree, the lineage *Craugastor* sp. Panama is sister to the other lineages within the clade (essentially from Costa Rica). The fourth major clade (*C. stejnegerianus* clade) was highly discordant with the mitochondrial phylogeny. In the nuclear tree, *C. stejnegerianus* is paraphyletic, with some populations of *C. stejnegerianus* (Quepos) sister to *C. rearki*, whereas *C. stejnegerianus* (*sensu stricto*) was sister to *C. gabbi* + other population of *C. stejnegeriaus* (Neilly). The basal position of *C. persimilis* from Caribbean Costa Rica was identical in mitochondrial and nuclear phylogenies.

3.2 Species delimitation

The results from the species delimitation analyses are shown in Figs 6 and 7. Considerably differences were found between the mitochondrial and nuclear data sets and between the different methods. In the mitochondrial analyses, some methods (*i.e.* GMYC and PTP) identified > 60 species, though others (*i.e.* mPTP Bayesian) only identified 13 species. With the mitochondrial data, all methods identified all described and nearly all-putative species except the three lineages within the species *C. stejnegerianus* that were identified in the nuclear analysis (*Craugastor* sp. Neilly, *Craugastor* sp. Quepos, and *C. stejnegerianus*).

The nuclear data, which only had 42 specimens, had several differences among the species delimitation methods, some (*i.e.* mPTP Bayesian with MrBayes and RAxML trees) only recognized a single species for the entire complex whereas others (*i.e.* mPTP maximum likelihood) identified up to 36 species. Incongruence as also found between analyses with the

complete phylogeny and those with the phylogenies by clade. Within the *C. stejnegerianus* clade, the GMYC and PTP methods identified more species using the phylogeny by clade than the complete phylogeny. However, within the *C. podiciferus* clade, the GMYC and PTP methods identified more species using the complete phylogeny than using the phylogeny for the clade. These results highlight the impact of the selection of the initial phylogeny used in the methods GMYC, PTP, and mPTP, which are tree-based methods.

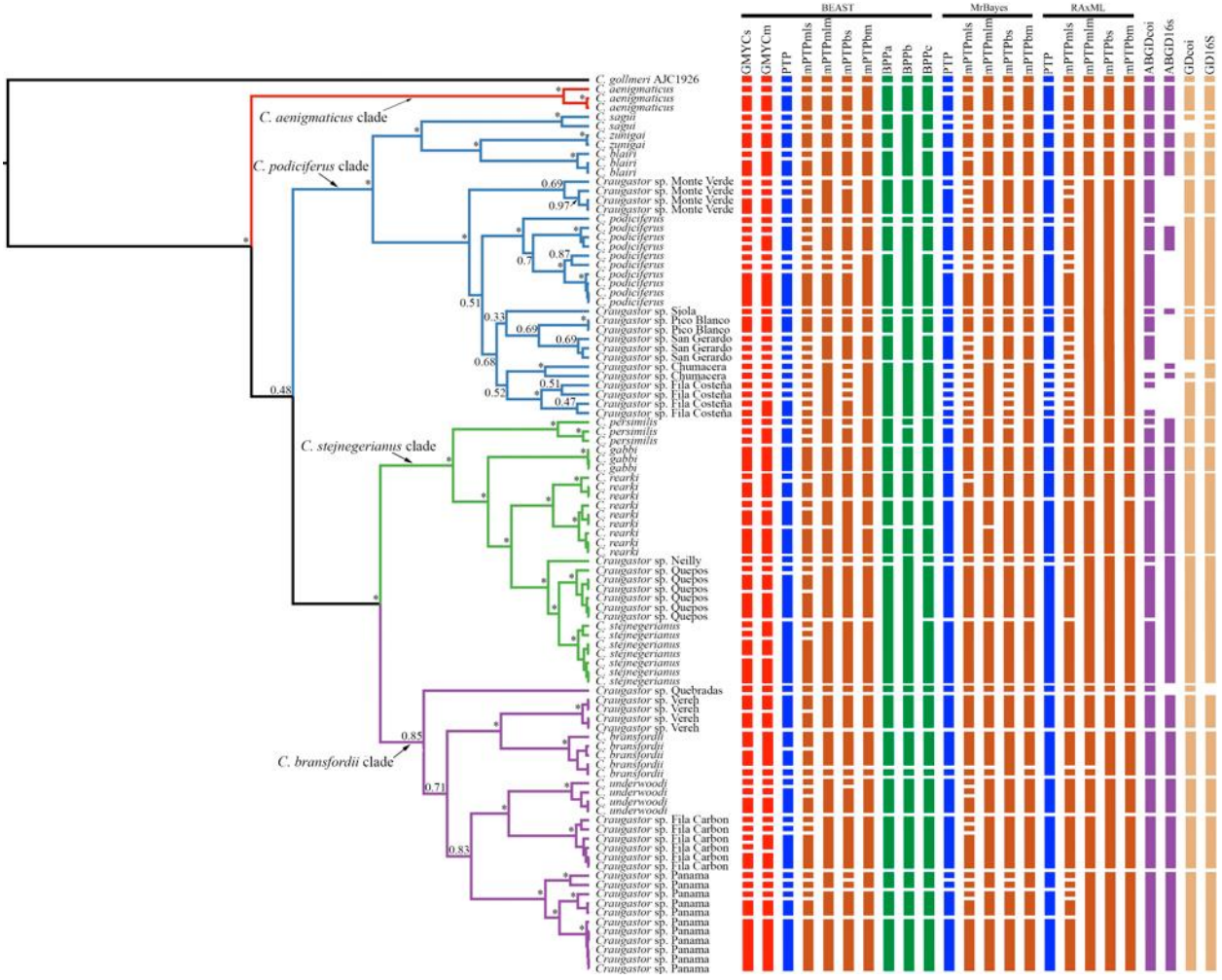

FIGURE 6. Comparison of species delimitation results of the *Craugastor podiciferus* species group based on the 16S and COI mitochondrial DNA gene markers. Each colored bar represents a species delimited by each method tested.

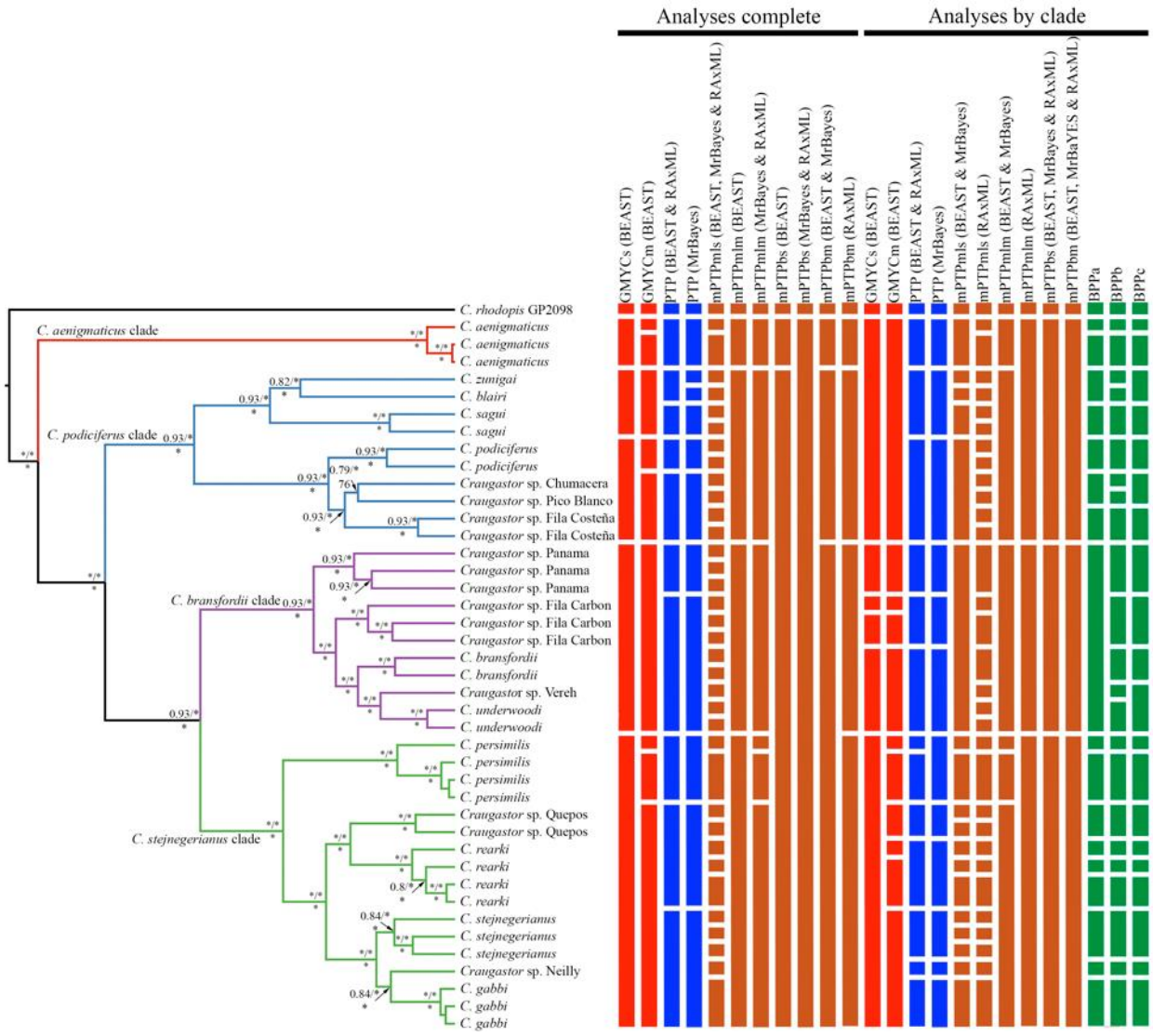

FIGURE 7. Comparison of species delimitation results of the *Craugastor podiciferus* species group based ddRAD dataset. Each colored bar represents a species delimited by each method tested.

The nuclear data, which only had 42 specimens, had several differences among the species delimitation methods, some (*i.e.* mPTP Bayesian with MrBayes and RAxML trees) only recognized a single species for the entire complex whereas others (*i.e.* mPTP maximum likelihood) identified up to 36 species. Incongruence as also found between analyses with the complete phylogeny and those with the phylogenies by clade. Within the *C. stejnegerianus* clade,

the GMYC and PTP methods identified more species using the phylogeny by clade than the complete phylogeny. However, within the *C. podiciferus* clade, the GMYC and PTP methods identified more species using the complete phylogeny than using the phylogeny for the clade. These results highlight the impact of the selection of the initial phylogeny used in the methods GMYC, PTP, and mPTP, which are tree-based methods.

3.3 Biogeography

The result of the ancestral area reconstruction is presented in combination with the BEAST tree for ddRAD data (Fig. 8). A Talamancan origin for the *C. podiciferus* species group was inferred during the Middle Miocene. A Talamancan origin for the *C. podiciferus* clade was also supported, with a dispersal event to Isthmian-Pacific moist forest (lowland) and another dispersal event to Costa Rican seasonal moist forest. The origin of the clade *C. stejnegerianus* + *C. bransfordii* was supported as Isthmian-Atlantic moist forest, with five independent dispersal events from the Isthmian-Atlantic moist forest to Talamancan montane forest during the Pleistocene and three dispersal events in Isthmian-Pacific moist forest, explaining the current patterns of distribution. The elevational distribution of the 23 lineages identified is shown in Fig. 9. The two basal clades, *Craugastor aenigmaticus* clade (in red) and *C. podiciferus* clade (in blue), are restricted to highlands (1000–2700 m a.s.l.), however, all the 23 lineages have populations above 750 m a.s.l., at the Talamanca montane forest (Fig. 1, 9). The lineages distributed at highlands have narrower elevational ranges (Fig. 9); all lineages distributed under 1000 m a.s.l. have altitudinal ranges higher than 750 m but four lineages restricted to highlands have altitudinal ranges smaller than 250 m. In addition, the highlands of the Pacific versant are more structured; in the Pacific versant eight lineages are distributed exclusively over 1000 m a.s.l. whereas in the Atlantic versant only five lineages are restricted to highlands.

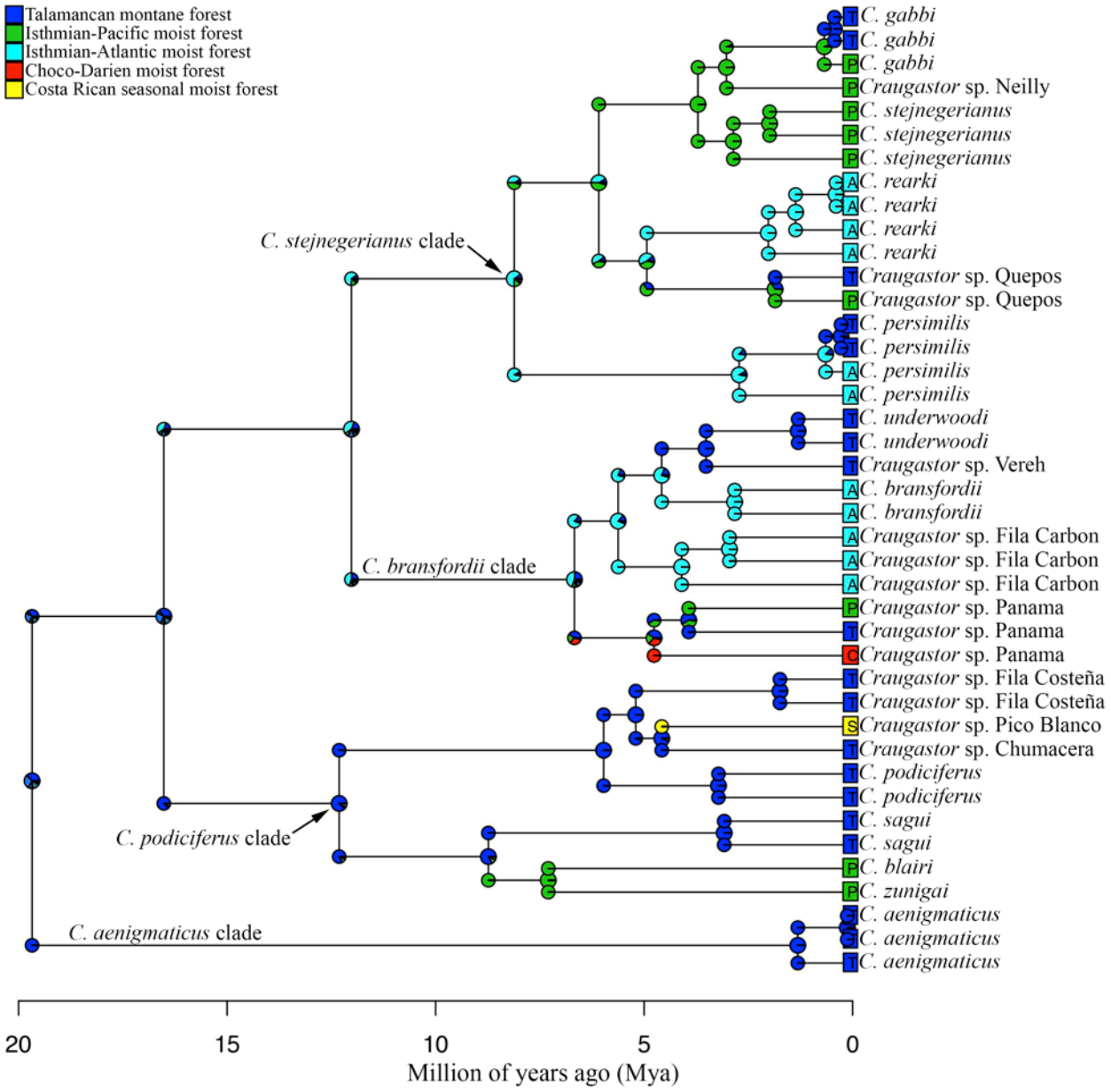

FIGURE 8. Summary of results of LAGRANGE biogeographical analysis.

4. Discussion

4.1 Systematics and biogeography of the C. podiciferus species group

The highlands of ICA played an important role in the diversification of several groups of vertebrates (Savage 2002; García-París *et al*. 2000; Castoe *et al*. 2009; Boza-Oviedo *et al*. 2012;

Duellman *et al.* 2016; Arias *et al.* 2018). However, there is controversy in the chronological dating of the processes responsible for this diversification. Based on the evidence of climatic oscillations and assuming a recent uprising of Isthmian Central America in the Pliocene-Pleistocene (~ 5 Mya), Savage (2002) proposed a model of speciation for highland taxa (Montane speciation). This author suggested that the oscillations between glacial and interglacial periods that began in the Pliocene (~ 5 Mya) and continued during the Pleistocene were responsible for the origin and distribution of many highlands species. Nevertheless, phylogeographic analyses of the frog *Craugastor podiciferus* (Streicher *et al.* 2009) and Neotropical snakes (*Atropoides, Bothriechis,* and *Cerrophidion*; Castoe *et al.* 2009) have disagreed with these events in the Pliocene as a major factor associated with cladogenesis of these highland species. Castoe *et al.* (2009) found that the diversity of Middle American highland pitvipers is explained by diversification during the Miocene and Pliocene. The chronological results shown by Castoe *et al.* (2009) partly overlaps (in the Pliocene) with the time suggested by Savage (2002), however it predates the Pleistocene.

Streicher *et al.* (2009) estimated that the time of origin for the most recent common ancestor (MRCA) of the *C. podiciferus* species group occurred between 16.82 and 27.84 Mya. The origin of the *C. podiciferus* species group is explained by the events of dispersion from Nuclear Central America to ICA when the last emerged as a peninsula at its current position (Montes *et al.* 2015). Our results indicate that the four major clades diverged during the Miocene, supporting Castoe *et al.*'s (2009) results. In addition, it is possible that climatic fluctuations occurred during the Pliocene-Pleistocene favored the origin of several lineages within the *C. podiciferus* species group. Streicher *et al.* (2009) argued that climatic fluctuations during the Pliocene-Pleistocene favored the diversification within the *C. podiciferus* clade. These

climatic fluctuations affected the Pacific slopes mainly (Savage 1966; Crawford *et al.* 2007); that explains their high genetic structure, with species having narrow elevational and latitudinal ranges (Fig. 1, 9). Also, the Pacific slopes have more lineages at highlands and less at lowlands compared to the Caribbean slope. On the Caribbean slopes the lineages having broad elevational and latitudinal ranges, this distribution could indicate that the Caribbean slope was less impacted by climatic fluctuations in the past. However, the fewer species at intermediate lands of Caribbean slopes can also be explained by the lack of surveys in that region and could increase with more surveys conducted in the future.

The *Craugastor podiciferus* species group is distributed from eastern Honduras to central Panama. The 23 identified lineages have populations in Costa Rica and western Panama in an area smaller than 40,000 km^2. The *Craugastor aenigmaticus* clade is restricted to Montane Rainforest, ranging from 2330–2700 m a.s.l, the highest altitudinal distribution for a species in this group (Arias *et al.* 2018). Given that *Craugastor aenigmaticus* is basal within the species group, it is suggested that the ancestor was distributed on the Montane zonation and diversified to lower elevations. The clade *C. podiciferus*, as shown by Streicher *et al.* (2009), possibly diversified due to climatic fluctuations that isolated the suitable habitat on peaks in the mountain ranges. The *C. bransfordii* + *C. stejnegerianus* clade contains species ranging from sea level to 1600 m a.s.l. The *C. bransfordii* clade diversified mainly on the Caribbean slopes of Nicaragua, Costa Rica, and Panama, and only one lineage was found on the Pacific slopes of Costa Rica. No obvious barriers separate the species of the *C. bransfordii* clade, except for paleo-climatic differences. The *C. stejnegerianus* clade contains more species on the Pacific slope of Costa Rica, with two species distributed on the Caribbean slopes of Nicaragua, Costa Rica, and Panama. Possibly, the driest conditions of the Pleistocene on the pacific slope of Costa Rica

(Crawford *et al.* 2007) marked the diversification of this clade on the pacific slope from isolated ancestors of the species on wet patches.

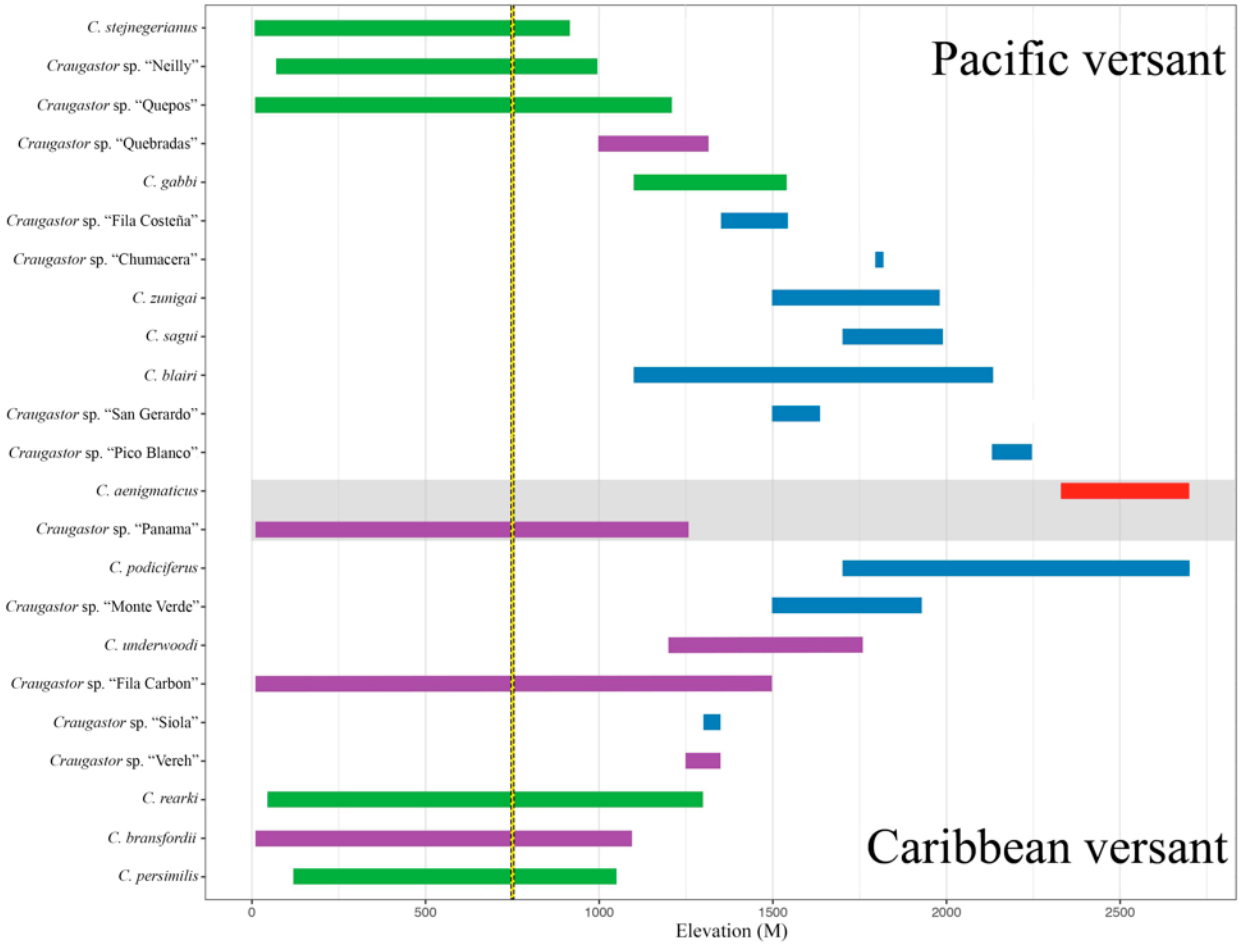

FIGURE 9. Elevational distribution of the lineages within of the *Craugastor podiciferus* species group, color bars correspond with the four major clades. The gray bar represents the continental divisor.

4.2 Species delimitation and taxonomic comments

Seven described and putative species were supported as distinct evolutionary lineages in all analyses. Nevertheless, large differences were found between mitochondrial and nuclear analyses

and between methods. There is not a consensus for taxonomic decision from the delimitation results, and our suggestions are based on both mitochondrial and nuclear analyses. We identified 23 lineages, including described species and putative species. This approach aims to minimize taxonomic instability. Below are details the major clades and its species.

4.2.1 The Craugastor aenigmaticus clade.

This clade was consistently supported as a distinct evolutionary lineage in all analyses. This species was named recently, is microendemic to the Montane Rainforest of the Cordillera de Talamanca, southwestern Costa Rica, ranging from 2390–2700 m a.s.l. (Arias *et al.* 2018). This species corresponds with the higher altitudinal distribution for the species group. This species is separated from other members of the *C. podiciferus* species group by very significant genetic distances, with mean uncorrected genetic distances higher than 13.3 % in 16S and 19.3 % in COI (Table 1).

4.2.2. The Craugastor podiciferus clade

The species *C. blairi*, *C. sagui*, and *C. zunigai* each represent separate species from the topotypic *C. podificiferus* specimens. Several delimitation analyses identified these populations as separate evolutionary lineages. The mitochondrial phylogenies (RAxML and Bayesian) did not recovered these populations clustered with *C. podiciferus*. Instead, they were placed at the base of the complex. The three species are allopatric, distributed latitudinally in southwestern Costa Rica and western Panama. The clade *Craugastor blairi* corresponds to the species *Craugastor* sp. B of Crawford & Smith (2005) and Streicher *et al*. (2009). These three clades are separated from each other by mean uncorrected genetic distances higher than 3.4 % in 16S and 17.3 % in COI (Table 1).

The number of species that form the *C. podiciferus* subclade, that also includes populations from the type locality of *C. podiciferus*, is unclear. Several species were consistently identified with mitochondrial sequence data, but only some were included in the nuclear analysis. Streicher *et al*. (2009) performed a phylogenetic analysis for this clade, suggesting that it was composed of six species. Of the seven putative species in the mitochondrial analysis, only three were included in the nuclear data set, which were supported as separate species in BPP analyses. We identified seven clades within this subclade, which differ morphologically (Arias Unpublished data) and are separated from each other by mean uncorrected genetic distances higher than 2.0 % in 16S and 7.3 % in COI (Table 1). Thus, we suggest that these seven clades may represent separate species. We recognize seven species within the *C. podiciferus* clade, four of Streicher *et al*. (2009) and three additional putative species that were no included in any previous work. Streicher *et al.* (2009) estimated a time to MRCA of the *C. podiciferus* clade of between 4.70 and 8.18 Ma. We hypothesize that the lack of support for the distinctness of these taxa in some analyses may reflect the fact that they are recently derived, and argue that they are nonetheless on evolutionary independent trajectories and therefore should be recognized as separate species.

We included for the first time populations from the type locality of *C. podiciferus.* This type locality was scope of discussion by Savage (1970) and Arias & Chaves (2014), who later corrected the type locality to Caribbean slopes of Cerro Kamuk. We restrict *C. podiciferus* to the populations of Cordillera Volcánica Central from Costa Rica and Cordillera de Talamanca on Costa Rica and western Panama. In the Cordillera de Talamanca *C. podiciferus* is restricted to the Caribbean slopes. This clade corresponds to the clades C and D of Streicher *et al*. (2009). The clade *Craugastor* sp. Monte Verde corresponds to the clade A of Streicher *et al.* (2009), a

species restricted to the Cordillera de Tilarán and Volcánica Central. The clade *Craugastor* sp. San Gerardo corresponds to the clade B of Streicher *et al.* (2009), a species distributed on Cordillera de Tilarán and Volcánica Central. In Monte Verde both species, *Craugastor* sp. Monte Verde and *Craugastor* sp. San Geardo, are in plausible simpatry due to the proximity of both localities. However, the micro-sympatry remains unknown. Also, an occurrence in sympatry of *C. podiciferus* and *Craugastor* sp. Monte Verde seems possible. The clade *Craugastor* sp. Fila Costeña corresponds to the clades E and F of Streicher *et al.* (2009), a species restricted to South Pacific Costa Rica. The clade *Craugastor* sp. Pico Blanco is known only by a population on the Valle Central. The clade *Craugastor* sp. Chumacera is known only by a population on the Pacific slopes of the Cordillera de Talamanca, just as the clade *Craugastor* sp. Siola that is known from a single population on the Caribbean slope of the Cordillera de Talamanca.

The species *C. jota* was referred to the *C. podiciferus* species group (Hedges *et al*. 2008) and assigned to the *C. podiciferus* clade based in morphology. This species was described with specimens from the Changena River in western Panama, from 760 m a.s.l. collected by Linda Trueb in 1966. In the description, Lynch (1980) defined the type locality on the basis of the map shown by Trueb (1968). However, the map was drawn without GPS coordinates and the rivers name could not match with the now rivers names. Here were included specimens from Changena River, western Panama, very near to the type locality of *C. jota* (Linda Trueb pers. comm.). These specimens were grouped within *C. podiciferus sensu stricto*, because we suggest *C. jota* should be referred as a junior synonym of *C. podiciferus.*

4.2.3. The Craugastor bransfordii clade

The nuclear phylogeny supports the monophyly of the *C. bransfordii* clade, but it was not

supported in some mitochondrial analyses. We suggest that the *C. bransfordii* clade is composed of six separate species, only five of which were included in the nuclear analysis. These six lineages are separated from each other by mean uncorrected genetic distances higher than 4.1 % in 16S and 9.5 % in COI (Table 1). *Craugastor bransfordii* included specimens (UCR20559) collected near the type locality, San Juan River on the Costa Rica-Nicaragua border. *Craugastor bransfordii* is distributed from north Nicaragua to central Caribbean Costa Rica.The nominal species *C. polyptychus* was described with specimens from the same type locality as *C. bransfordii* and in the same publication (Cope 1886). It was seen as a synonym of *C. bransfordii* (Savage & Emerson 1970; Miyamoto 1983) until Savage (2002) tentatively resurrected this name and assigned it to specimens from Caribbean Costa Rica, but pointed out that further taxonomic work is needed to clarify the status of the species. Since only one clade within the *C. bransfordii* clade was found in the northern Costa Rica and southern Nicaragua we suggest that *C. polyptychus* should be referred as a junior synonym of *C. bransfordii.* The clade *Craugastor* sp. Fila Carbon corresponds –in part– to *C. polyptychus* of Savage (2002). This clade represents a putative species undescribed distributed in south Caribbean Costa Rica and western Panama.

The clade *C. underwoodi* includes specimens from Cascajal and Cinchona, near the type locality. This species is distributed in the premontane forest of the Cordillera de Guanacaste, Tilarán, Volcánica Central, and the northern edge of the Cordillera de Talamanca. The clade *Craugastor* sp. Quebradas includes specimens from the only locality known on the Pacific slopes for the *C. bransfordii* clade. We consider that the clade *Craugastor* sp. Quebradas represents a separate species due to its allopatric distribution and the large genetic distances to all other samples of 15.3 % or higher in COI (Table 1). The clade *Craugastor* sp. Vereh includes specimens from two localities in the premontane forest in the central Caribbean of Costa Rica.

The phylogenetic position of this clade varies between mitochondrial and nuclear phylogenies. Finally, the clade *Craugastor* sp. Panama is composed of populations from Panama; these populations were attributed to *C. bransfordii* (Leenders 2016), but based on the mitochondrial and nuclear phylogenies this clade from Panama is not closely related to *C. bransfordii*. We suggest that these populations from Panama represent at least one separate species.

4.2.4. The Craugastor stejnegerianus clade

Within the *C. stejnegerianus* clade large differences were found between the mitochondrial and nuclear phylogenies, mainly in the relationships between *C. gabbi* and *C. stejnegerianus.* Arias *et al*. (2016) recently described *C. gabbi* and discussed the differences between *C. gabbi* and *C. stejnegerianus.* We suggest that the *C. stejnegerianus* clade is composed of six separate lineages. The clade *C. persimilis* includes specimens from Suretka, Talamanca, what is the type locality of that species. *Craugastor persimilis* is distributed on the central and south Caribbean slopes of Costa Rica. The specimens from Honduras and Nicaragua that were initially referred to *C. lauraster* are closely related to specimens from central Caribbean of Costa Rica. The populations from central Caribbean of Costa Rica correspond to *C. rearki* (Taylor 1952), synonymized under *C. bransfordii* by Savage & Emerson (1970). We suggest that the name *C. rearki* should be resurrected to include populations from Caribbean of Costa Rica, Nicaragua, and Honduras and *C. lauraster* should be referred as a junior synonym of *C. rearki.*

The populations of the *C. stejnegerianus* clade on the pacific slope are highly conflictive in the mitochondrial and nuclear phylogenies. Arias *et al*. (2016) supported the distinctiveness of this species based on their mitochondrial phylogeny, morphology, and ecological preferences. However, in the nuclear phylogeny *C. gabbi* is sister to *C. stejnegerianus sensu stricto.* If both

species are recognized, it would also be necessary to recognize *Craugastor* sp. Neilly and *Craugastor* sp. Quepos as separate species. The clade *Craugator* sp. Quepos is the sister clade to *C. rearki* because we suggest that this represent a separate species distributed in the central pacific and Central Valley of Costa Rica. We also recognized the clade *Craugastor* sp. Neilly as a separate evolutionary lineage restricted to southeast Pacific of Costa Rica. *Craugastor stejnegerianus* is restricted to south Pacific of Costa Rica. To exclude the three species within the *C. stejnegerianus* (*Craugastor* sp. Neilly, *Craugastor* sp. Quepos, and *C. stejnegerianus*) that were identified only in the nuclear analysis, these are separated from each other by mean uncorrected genetic distances higher than 4.2 % in 16S and 11.6 % in COI (Table 1).

The phylogenetic relationships and the delimitation of species suggest the presence of several undescribed, overlooked species, highlighting the problem of potential cryptic species. The clades herein identified should be morphologically re-evaluated following the phylogenetic relationships. However, a preliminary morphological review of the genetic lineages within the *C. bransfordii* clade show few morphological characteristics that would allow for a differentiation of those lineages. Similar problems occur within the *C. stejnegerianus* clade and it is very difficult to identify these lineages using morphological characters with the exception of *C. persimilis* (Arias *et al.* 2016). It is very important that extensive studies about the use of habitat, acoustic, behavior, alimentation, and other data will be carried out to better understand the dynamic of these species.

5. Conclusion

The *C. podiciferus* species group represents an ideal model for studies on amphibian overlooked diversity, given its high abundance, wide geographic distribution (collectively), high genetic

diversity, high levels of polymorphism, direct development (lack of larval stage), some species are miniaturized (*C. persimilis* reaches to 23 mm in female adults), and only some vocalization species are known (intraspecific recognition unknown). However, to date there are only few studies on the members of this species group due the lack of clear limits between its species. Following our objectives, we report 1) the first phylogeny for the *C. podiciferus* species group sampling extensively its entire geographic distribution, 2) although there is disagreement about the species delineation methods that were performed, 23 lineages were consistently recovered, revealing several undescribed species, and 3) the diversification of this group is strongly associated to the highlands of Isthmian Central America, all lineages ranges above the 750 m. a.s.l.

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Appendix 1. Institutional voucher numbers and locality information for the specimens used in the molecular phylogenetic analyses. Museum collection acronyms follow Frost (2019), with the addition of AJC refers to Andrew J. Crawford field numbers, EAP refers to Erick Arias field numbers, and CRARC refers to Costa Rica Amphibian Research Center private collection.

Species	Institutional vouchers	Collection locality	Elevation (m)	Geographic coordinates	
				Lat	Lon
Craugastor aenigmaticus clade					
Craugastor aenigmaticus	SMF: 104020	Changuinola, Bocas del Toro, PA	2388	8.9139	-82.7088
Craugastor aenigmaticus	UCR: 21951	Telire, Talamanca, CR	2700	9.3488	-83.1750
Craugastor aenigmaticus	UCR: 22737	Buenos Aires, Puntarenas, CR	2660	9.3224	-83.2028
Craugastor podiciferus clade					
Craugastor sagui	SMF: 104014	Nole Duima, Ngöbe Buglé, PA	1762	8.5571	-81.8245
Craugastor sagui	SMF: 104015	Nole Duima, Ngöbe Buglé, PA	1700	8.4997	-81.7724
Craugastor zunigai	UCR: 20389	Buenos Aires, Puntarenas, CR	1500	9.1112	-83.1006
Craugastor zunigai	UCR: 22709	Coto Brus, Puntarenas, CR	1980	8.9751	-82.8243
Craugastor blairi	FMNH: 257562	Gualaca, Chiriquí, PA	1000	8.7500	-82.217
Craugastor blairi	SMF: 104023	Gualaca, Chiriquí, PA	1280	8.6781	-82.2101
Craugastor blairi	SMF: 102024	Gualaca, Chiriquí, PA	1730	8.6775	-82.1980
Craugastor blairi	SMF: 104027	Bugaba, Chiriquí, CR	2134	8.8494	-82.5154
Craugastor podiciferus	CRARC: 0012	Turrialba, Cartago, CR	2250	10.0192	-83.7132
Craugastor podiciferus	EAP: 0536	El Guarco, Cartago, CR	2395	9.7126	-83.9488
Craugastor podiciferus	EAP: 0810	Talamanca, Limón, CR	1860	9.3659	-83.0417
Craugastor podiciferus	SMF: 104005	Changuinola, Bocas del Toro, PA	1766	8.9908	-82.6716
Craugastor podiciferus	UCR: 19853	Telire, Talamanca, CR	1817	9.3580	-83.2294
Craugastor podiciferus	UCR: 19856	Telire, Talamanca, CR	1817	9.3580	-83.2294
Craugastor podiciferus	UCR: 19860	Telire, Talamanca, CR	2108	9.3645	-83.2164
Craugastor podiciferus	UCR: 19862	Telire, Talamanca, CR	2108	9.3645	-83.2164
Craugastor podiciferus	UCR: 20992	Alfaro Ruiz, Alajuela, CR	2143	10.2272	-84.3482
Craugastor podiciferus	UCR: 22146	Vázquez de Coronado, San José, CR	1700	10.0263	-83.9448
Craugastor podiciferus	UCR: 22201	Dota, San José, CR	2395	9.7126	-83.9488
Craugastor sp. Chumacera	UCR: 22120	Buenos Aires, Puntarenas, CR	1821	9.3218	-83.4546
Craugastor sp. Chumacera	UCR: 22690	Pérez Zeledón, San José, CR	1793	9.3267	-83.4706
Craugastor sp. Fila Costeña	EAP: 0509	Golfito, Puntarenas, CR	1546	8.7878	-83.0306
Craugastor sp. Fila Costeña	EAP: 0519	Pérez Zeledón, San José, CR	1350	9.4415	-83.6848
Craugastor sp. Fila Costeña	FMNH: 257651	Coto Brus, Puntarenas, CR	1350	8.7833	-82.9833
Craugastor sp. Fila Costeña	UCR: 16585	Dota, San José, CR	1400	9.5353	-83.8580
Craugastor sp. Fila Costeña	UCR: 22091	Pérez Zeledón, San José, CR	1488	9.4410	-83.6830
Craugastor sp. Monte Verde	FMNH: 257669	Monte Verde, Puntarenas, CR	1500	10.2773	-84.5891
Craugastor sp. Monte Verde	FMNH: 257673	Monte Verde, Puntarenas, CR	1500	10.2773	-84.5891
Craugastor sp. Monte Verde	UCR: 16361	Alfaro Ruiz, Alajuela, CR	1930	10.2176	-84.3671
Craugastor sp. Monte Verde	UCR: 22675	Puntarenas, Puntarenas, CR	1726	10.3202	-84.7987
Craugastor sp. San Gerardo	CRARC: 0247	Tilarán, Guanacaste, CR	1470	10.3600	-84.8000
Craugastor sp. San Gerardo	FMNH257671	Monte Verde, Puntarenas, CR	1500	10.2773	-84.5891
Craugastor sp. San Gerardo	UCR: 16353	Sarapiquí, Heredia, CR	1500	10.2022	-84.1625
Craugastor sp. Pico Blanco	UCR: 22226	Escazú, San José, CR	2242	9.8646	-84.1429
Craugastor sp. Pico Blanco	UCR: 22228	Escazú, San José, CR	2242	9.8646	-84.1429
Craugastor sp. Siola	EAP: 0817	Talamanca, Limón, CR	1300	9.3987	-83.0200
Craugastor stejnegerianus					
Craugastor gabbi	EAP: 0626	Coto Brus, Puntarenas, CR	1541	8.9515	-82.8346
Craugastor gabbi	UCR: 21863	Coto Brus, Puntarenas, CR	1200	8.7889	-82.9583
Craugastor gabbi	UCR: 21864	Coto Brus, Puntarenas, CR	1200	8.7889	-82.9583

Appendix 1. *Continued.*

Species	Institutional vouchers	Collection locality	Elevation (m)	Geographic coordinates	
				Lat	Lon
Craugastor persimilis	EAP: 0586	Talamanca. Limón, CR	121	9.5773	-82.9343
Craugastor persimilis	FMNH: 257567	Turrialba, Cartago, CR	550	9.8917	-83.6500
Craugastor persimilis	FMNH: 257571	Turrialba, Cartago, CR	550	9.8917	-83.6500
Craugastor persimilis	UCR: 22211	Paraíso, Cartago, CR	1050	9.7841	-83.7517
Craugastor persimilis	UCR: 22212	Paraíso, Cartago, CR	1050	9.7841	-83.7517
Craugastor rearki	EAP: 0554	Upala, Guanacaste, CR	764	10.7109	-85.0406
Craugastor rearki	EAP: 0555	Upala, Guanacaste, CR	764	10.7109	-85.0406
Craugastor rearki	EAP: 0572	Siquírres, Limón, CR	537	10.0595	-83.5452
Craugastor rearki	MVZ: 263735	Altagracia, Altagracia, NI	466	11.4687	-85.5069
Craugastor rearki	SMF: 79759	Matagalpa, Matagalpa, NI	1300	12.9993	-85.9092
Craugastor rearki	UCR: 20600	Los Chiles, Alajuela, CR	45	11.0513	-84.7393
Craugastor rearki	UCR: 16343	Bagaces, Guanacaste, CR	640	10.7072	-85.0844
Craugastor rearki	UCR: 22240	Pococí, Limón, CR	228	10.2257	-83.7712
Craugastor rearki	UCR: 21149	Limón, Limón, CR	400	9.9260	-83.1880
Craugastor rearki	UCR: 21152	Limón, Limón, CR	400	9.9260	-83.1880
Craugastor rearki	USNM: 559393	Puerto Lempira, Gracias a Dios, HN	190	14.9275	-84.5339
Craugastor stejnegerianus	EAP: 0508	Golfito, Puntarenas, CR	28	8.6906	-83.4815
Craugastor stejnegerianus	EAP: 0512	Buenos Aires, Puntarenas, CR	812	9.0905	-83.1247
Craugastor stejnegerianus	EAP: 0514	Osa, Puntarenas, CR	45	8.9655	-83.4411
Craugastor stejnegerianus	UCR: 20346	Buenos Aires, Puntarenas, CR	900	9.0860	-83.1110
Craugastor stejnegerianus	UCR: 20352	Buenos Aires, Puntarenas, CR	900	9.0863	-83.1105
Craugastor stejnegerianus	UCR: 21494	Golfito, Puntarenas, CR	100	8.4046	-83.1197
Craugastor stejnegerianus	UCR: 22070	Buenos Aires, Puntarenas, CR	416	9.1520	-83.4256
Craugastor stejnegerianus	UCR: 22101	Pérez Zeledón, San José, CR	740	9.3009	-83.7714
Craugastor stejnegerianus	UCR: 22280	Osa, Puntarenas, CR	30	9.1966	-83.7870
Craugastor sp. Neilly	EAP: 0506	Golfito, Puntarenas, CR	182	8.6985	-82.0489
Craugastor sp. Quepos	CRARC: 0148	San Ramón, Alajuela, CR	1140	10.2021	-84.4843
Craugastor sp. Quepos	CRARC: 0266	Montes de Oro, Puntarenas, CR	1325	10.1800	-84.6700
Craugastor sp. Quepos	EAP: 0524	Aguirre, Puntarenas, CR	12	9.3245	-83.9498
Craugastor sp. Quepos	EAP: 0527	Aguirre, Puntarenas, CR	180	9.4776	-84.0473
Craugastor sp. Quepos	UCR: 20907	Aguirre, Puntarenas, CR	200	9.4619	-84.0631
Craugastor sp. Quepos	UCR: 21007	Aguirre, Puntarenas, CR	200	9.4620	-84.0630
Craugastor sp. Quepos	UCR: 22208	Montes de Oca, San José, CR	1210	9.9374	-84.0495
Craugastor bransfordii clade					
Craugastor bransfordii	CRARC: 0176	Siquírres, Limón, CR	755	10.0600	-83.6300
Craugastor bransfordii	EAP: 0558	Siquírres, Limón, CR	537	10.0595	-83.5452
Craugastor bransfordii	UCR: 20559	Los Chiles, Alajuela, CR	45	11.0513	-84.7393
Craugastor bransfordii	UCR: 20951	San Ramón, Alajuela, CR	1095	10.1862	-84.5075
Craugastor bransfordii	UCR: 22269	Alajuela, Alajuela, CR	466	10.3121	-84.1778
Craugastor underwoodi	EAP: 0534	Paraíso, Cartago, CR	1412	9.7518	-83.7792
Craugastor underwoodi	EAP: 0540	Vázquez de Coronado, San José, CR	1708	10.0254	-83.9456
Craugastor underwoodi	EAP: 0593	Puntarenas, Puntarenas, CR	1566	10.3160	-84.8061
Craugastor underwoodi	CRARC: 0245	Tilarán, Guanacaste, CR	1470	10.3600	-84.8000
Craugastor sp. Fila Carbon	EAP: 0577	Talamanca, Limón, CR	94	9.7116	-82.8344
Craugastor sp. Fila Carbon	EAP: 0583	Talamanca, Limón, CR	198	9.6064	-82.9115
Craugastor sp. Fila Carbon	EAP: 0585	Talamanca, Limón, CR	198	9.6064	-82.9115
Craugastor sp. Fila Carbon	EAP: 0797	Talamanca, Limón, CR	1500	9.3773	-83.0371
Craugastor sp. Fila Carbon	UCR: 20050	Talamanca, Limón, CR	900	9.6178	-83.2681
Craugastor sp. Fila Carbon	UCR: 20052	Talamanca, Limón, CR	900	9.6178	-83.2681
Craugastor sp. Fila Carbon	UCR: 20150	Limón, Limón, CR	500	9.8550	-83.1517
Craugastor sp. Fila Carbon	UCR: 21122	Limón, Limón, CR	400	9.9260	-83.1880

Appendix 1. *Continued.*

Species	Institutional vouchers	Collection locality	Elevation (m)	Geographic coordinates	
				Lat	Lon
Craugastor sp. Fila Carbon	UCR: 22533	Limón, Limón, CR	1123	9.8679	-83.2406
Craugastor sp. Quebradas	UCR: 16326	Pérez Zeledón, San José, CR	1313	9.4375	-83.6869
Craugastor sp. Panama	AJC: 1921	Panamá, Panamá, PA	810	9.3198	-79.2889
Craugastor sp. Panama	AJC: 1960	Panamá, Panamá, PA	624	9.2908	-79.3027
Craugastor sp. Panama	CH: 6808	Panamá, Panamá, PA	871	9.2744	-79.3178
Craugastor sp. Panama	FMNH: 257677	Gualaca, Chiriquí, PA	1000	8.7500	-82.2167
Craugastor sp. Panama	FMNH: 257698	Kuna Yala, Kuna Yala, PA	420	9.3167	-78.9833
Craugastor sp. Panama	MVUP: 1803	La Pintada, Coclé, PA	800	8.6670	-80.5920
Craugastor sp. Panama	MVUP: 1841	La Pintada, Coclé, PA	800	8.6670	-80.5920
Craugastor sp. Panama	USNM: 572220	La Pintada, Coclé, PA	800	8.6670	-80.5920
Craugastor sp. Panama	USNM: 572221	La Pintada, Coclé, PA	800	8.6670	-80.5920
Craugastor sp. Panama	USNM: 572222	La Pintada, Coclé, PA	800	8.6670	-80.5920
Craugastor sp. Panama	USNM: 572223	La Pintada, Coclé, PA	800	8.6670	-80.5920
Craugastor sp. Panama	SMF: 104010	Changuinola, Bocas del Toro, PA	1258	9.0090	-82.6644
Craugastor sp. Vereh	CRARC: 0047	Turrialba, Cartago, CR	1350	9.8681	-83.4621
Craugastor sp. Vereh	CRARC: 0048	Turrialba, Cartago, CR	1350	9.8681	-83.4621
Craugastor sp. Vereh	CRARC: 0228	Turrialba, Cartago, CR	1650	9.7500	-83.5500
Craugastor sp. Vereh	EAP: 0754	Turrialba, Cartago, CR	1650	9.7500	-83.5500

CAPÍTULO II

REVISIÓN TAXONÓMICA DEL GRUPO DE ESPECIES *CRAUGASTOR PODICIFERUS*

CAPÍTULO II.I

TAXONOMIC ASSESSMENT OF *CRAUGASTOR PODICIFERUS* (ANURA: CRAUGASTORIDAE) IN LOWER CENTRAL AMERICA WITH THE DESCRIPTION OF TWO NEW SPECIES

Autores: Erick Arias, Andreas Hertz y Gabriela Parra-Olea
En revisión: Amphibian & Reptile Conservation
Fecha de envío: Enero de 2019

Taxonomic assessment of *Craugastor podiciferus* (Anura: Craugastoridae) in lower Central America with the description of two new species

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Abstract.—The systematics and taxonomy of the polytypic species *Craugastor podiciferus* are poorly understood due to the high level of phenotypic polymorphism between and within species and the lack of molecular data from topotypic specimens. We performed a well-sampled study including all known species of the *C. podiciferus* species group, several localities from highlands in Costa Rica and western Panama, and for the first time, samples from the type locality of *C. podiciferus.* We performed a phylogenetic analysis based on the DNA sequences of the mitochondrial 16S rRNA (16S) and cytochrome oxidase 1 (COI) genes and a morphometric analysis. Based on our results, we restrict *C. podiciferus* to the populations from the Cordillera Volcánica Central and Cordillera de Talamanca in Costa Rica and western Panama. *Craugastor podiciferus sensu stricto* and six additional clades from the highlands of Costa Rica constitute the well-supported *C. podiciferus sensu lato* clade. Our analyses support the existence of three additional species from the Pacific slope of southwestern Costa Rica and western Panama. Herein, we describe two lineages as new species and provide revised descriptions for *C. podiciferus* and *C. blairi.* The name *C. blairi* is resurrected and used for populations from the Cordillera de Talamanca and Cordillera Central in western Panama. Two additional species are named, one from Cordillera Central in western Panama. This species is easily differentiated by the presence of nuptial pads in adult males, a smooth venter, and flat subarticular tubercles. The second species is named for populations from southwestern Costa Rica. This species is recognized by its coarsely areolate venter, projecting subarticular tubercles, and heel without a projecting tubercle. The recognition of these three species from the lower montane rainforest highlights the role of the highlands on the pacific slope of Costa Rica and Panama in the diversification of the *C. podiciferus* species group.

Keywords. Brachycephaloidea, Central America, cryptic species, DNA barcoding, Talamanca, Terrarana.

Introduction

The direct-developing frogs of the *Craugastor podiciferus* species group (Hedges et al. 2008) are found from eastern Honduras to Central Panama (AmphibiaWeb 2019; Savage 2002) with most of the species (nine out of ten) restricted to Isthmian Central America. The distribution of this group ranges from sea level to 2700 m a.s.l. in a wide variety of habitats, from tropical rain forest, cloud forest, to montane forest (Savage 2002). The morphological delimitation between members of the *C. podiciferus* species group is difficult due to the extremely conserved morphological characters and the high level of phenotypic polymorphism within species and populations. The systematics and taxonomy of the *C. podiciferus* species group has been poorly studied; however, previous molecular studies have suggested the existence of several unnamed species that are masked under the current names (Savage 2002; Crawford 2003; Crawford and Smith 2005; Streicher et al. 2009). For example, Crawford and Smith (2005) used 10 samples (seven species) of the *C. podiciferus* species group and found the presence of two unnamed species (*Craugastor* sp. B and *Craugastor* sp. C); they also found *C. stejnegerianus* (Cope, 1893) to be paraphyletic. Recently, Arias et al. (2016) showed that populations formerly considered to be part of the *C. stejnegerianus* from southwestern Costa Rica and western Panama belonged to a different species and described them as *C. gabbi* Arias, Chaves, Crawford, and Parra-Olea, 2016.

Craugastor podiciferus (Cope, 1875) is the most complicated taxon within the *C. podiciferus* species group. Its morphological polymorphism has been recognized since its description by Cope (1875), who described it based on four varieties plus two additional species (*C. muricinus* and *C. habenatus*) in the same paper, with specimens from the same locality. In addition, Cope (1875) stated that “the colors of this species vary remarkably, more than I have observed to be the case in any other frog”. Subsequently, Barbour (1928) described *C. blairi* using specimens from western Panama, which Taylor (1952) later synonymized together with *C.*

muricinus and *C. habenatus* under *C. podiciferus.* An additional species, *C. jota* (Lynch, 1980), was named using specimens from western Panama, and it was suggested to be related to *C. podiciferus.* More recently, the name *C. podiciferus* has been used for specimens from several highlands populations (1089–2650 m a.s.l.) on both slopes of the mountain ranges in Costa Rica and western Panama (Savage 2002). Streicher et al. (2009) used specimens from several populations referred to as *C. podiciferus* to perform a well-sampled phylogenetic study of this complex. They found that *C. podiciferus* is represented by six clades, each likely representing distinct species. In addition, the populations from western Panama were not grouped with the main *C. podiciferus* clade, which they called *Craugastor* sp. B.

Although the molecular and geographical evidence shown by Streicher et al. (2009) supported the presence of several species under the name *C. podiciferus,* taxonomic changes have not yet been implemented, mainly due to the lack of topotipic specimens of *C. podiciferus*. The correct type locality has been the subject of discussion by Savage (1970), and later, Arias and Chaves (2014) concluded that it is actually on the Caribbean slopes of Cerro Kamuk.

Here, we included specimens of *Craugastor podiciferus* from the type locality for the first time together with several additional localities from Costa Rica and western Panama. We used mitochondrial sequences to address the following goals: 1) to identify *C. podiciferus sensu stricto* in a phylogenetic context, 2) to evaluate the phylogenetic relationships of the *C. podiciferus* species complex, and 3) to evaluate the taxonomic status of the populations from the Pacific slopes of southwestern Costa Rica and western Panama. The result is a comprehensive revision of the *C. podiciferus* species complex, in which we describe two new species from southwestern Costa Rica and western Panama. Additionally, we resurrect an old name for a third species in mountainous western Panama.

Materials and Methods

Species criterion: We follow the general metapopulation lineage species concept (Simpson 1951; Wiley 1978; de Queiroz 2007). Since we adhere to this concept, we recognize a species when there is evidence of the separation of metapopulation lineages preferably based on multiple lines of evidence following a consensus protocol for integrative taxonomy (Dayrat et al. 2005; Padial et al. 2010).

Taxon sampling: The frogs were collected in the field, euthanized, fixed in a 10 % formalin solution and processed in 70 % ethanol for long-term storage. A tissue sample was preserved in 96 % ethanol or RNAlater and used for genetic analysis. Museum collection acronyms follow Frost (2019) with the addition of AH referring to Andreas Hertz field numbers. EAP refers to Erick Arias field numbers, and CRARC refers to the Costa Rica Amphibian Research Center private collection.

Amplification and sequencing: We determined partial sequences of the large subunit ribosomal RNA (16S) mitochondrial gene for six specimens of *Craugastor* sp. 1 (Arias et al. 2018), *Craugastor* sp. 2 (Arias et al. 2018), and *Craugastor* sp. B (Crawford and Smith 2005) from the pacific slopes of southwestern Costa Rica and western Panama (Fig. 1). We compared the sequences obtained herein with those available in GenBank for the *C. podiciferus* species group. The protocols for DNA extraction, amplification, sequencing, and editing follow those of Arias et al. (2018). The list of vouchers and GenBank accession numbers used in this study are provided in Appendix I.

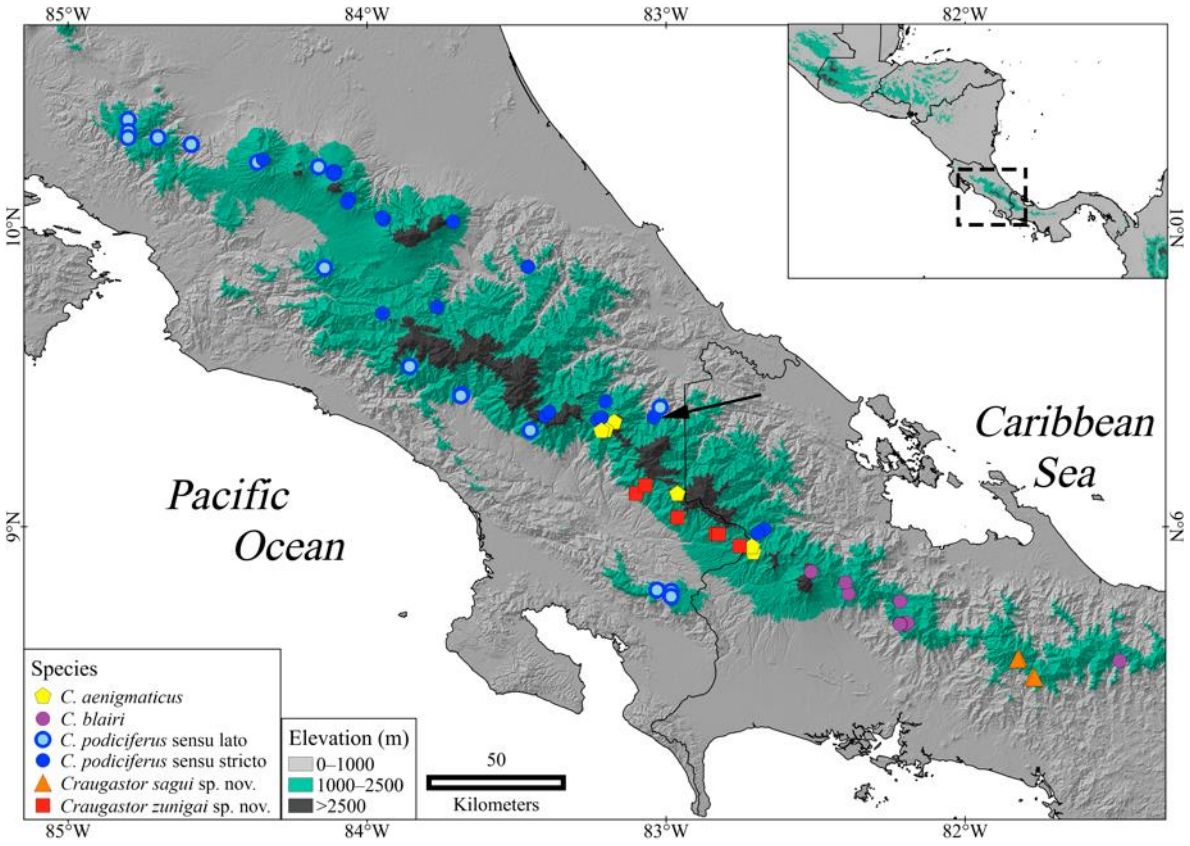


Fig. 1. Map showing the known populations of *Craugastor blairi*, *C. sagui* sp. nov., and *C. zunigai* sp. nov. from the lower montane rainforest in Southwestern Costa Rica and western Panama, and populations of other species of the *C. podiciferus* species group inhabiting the highlands of Costa Rica and western Panama. The arrow indicates the type locality of *C. podiciferus.*

Phylogenetic analyses: Sequence alignments were performed using the MAFFT software (Katoh et al. 2017) under the "auto" strategy, default parameters and trimmed to the point where a majority of the taxa had sequence data. We partitioned the sequence data by gene and further partitioned the COI by codon position. We used PartitionFinder v1.1.1 (Lanfear et al. 2012) and the Bayesian information criterion (BIC) to select the best partition scheme and the best model of sequence evolution for each partition. We used a single set of branch-lengths across all partitions

(*branchlengths=linked*), and the search of the best partition scheme was implemented using a heuristic search (*scheme=greedy*). We defined, *a priori,* four partitions, one for 16S and three for COI (one for each codon).

Phylogenetic analyses were performed using both the maximum likelihood (ML) and Bayesian inference (BI) methods. We performed the maximum likelihood analysis using Garli 2.01 (Zwickl 2006), with 10 search replicates with the following default setting values: *streefname=random, attachmentspertaxon=24, genthreshfortopoterm=100000, significanttopochange=0.00001*. For bootstrapping, we ran 1000 replicates with the previous settings with the following changes: *genthreshfortopoterm=10000, significanttopochange=0.01, treerejectionthreshold=20,* as suggested in the Garli manual, to accelerate the bootstrapping. The bootstrap consensus tree was performed using Sumtrees (Sukumaran and Holder 2010b) from the DendroPy package version 4.4.0 (Sukumaran and Holder 2010a). Bayesian phylogenetic analysis was performed using MrBayes 3.2.6 (Ronquist et al. 2012) with the partition scheme and the model of sequence evolution for each partition as selected previously. Two separate analyses were run, each consisting of 20 million generations, sampled every 1000 generations, and four chains with default heating parameters. We examined a time-series plot of the likelihood scores of the cold chain to check the stationarity using Tracer 1.6 software (Rambaut et al. 2014). We discarded the first 25 % of trees as burn-in and used the remaining trees to estimate the consensus tree along with the posterior probabilities for each node and each parameter. Maximum likelihood and Bayesian analyses were run on the CIPRES portal (Miller et al. 2010). Genetic distances (uncorrected p-distances) were computed using MEGA6 (Tamura et al. 2013).

Morphometric analyses: We performed a morphometric analysis comparing the three populations from the highlands of Southwestern Costa Rica and western Panama. We examined 19 specimens of *Craugastor* sp. 1, 7 specimens of *Craugastor* sp. 2, and 25 specimens of

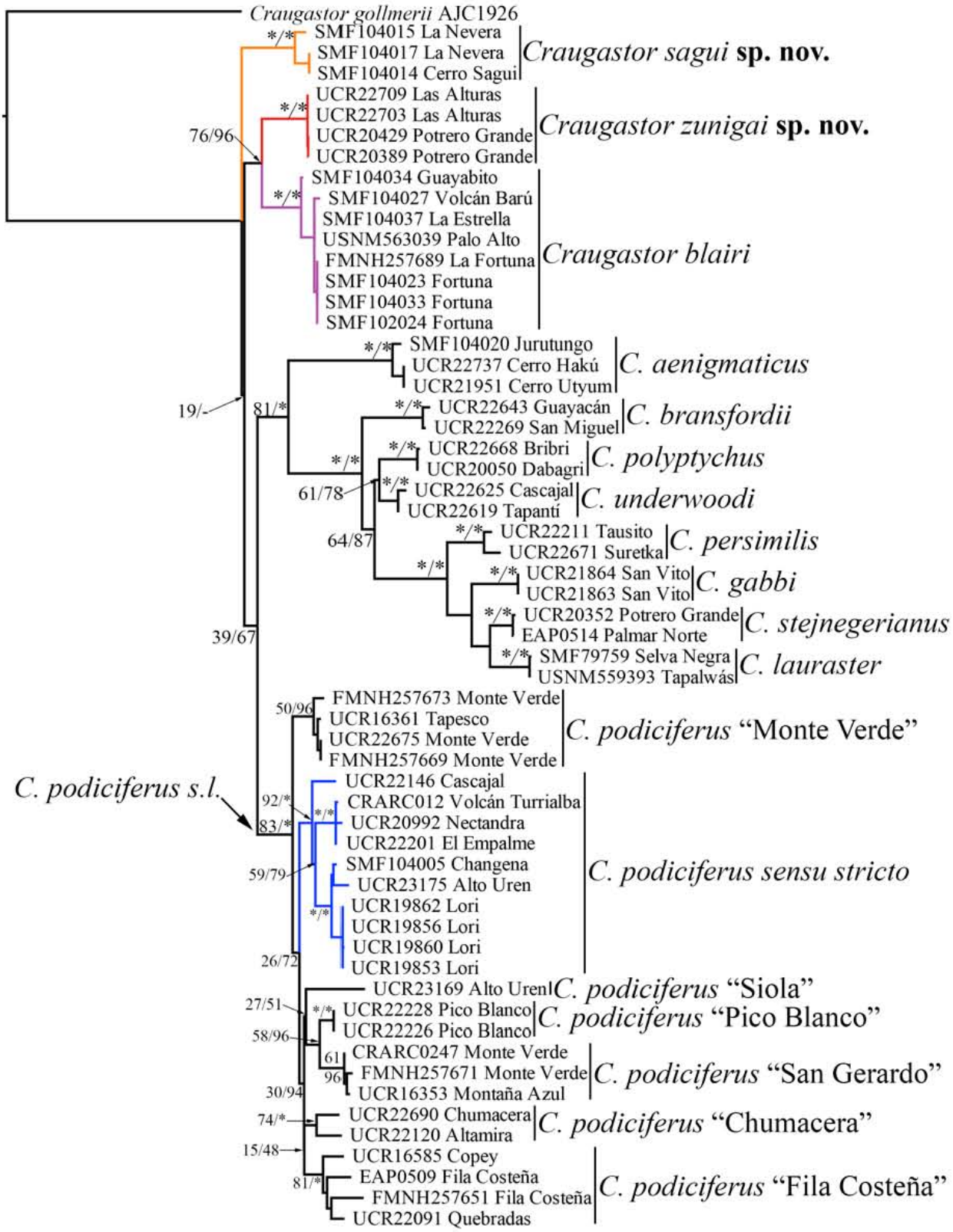
Craugastor sp. B (Appendix II). The specimens were deposited at the Museo de Zoología (UCR), San José, Costa Rica and the Senckenberg Research Institute and Nature Museum, Frankfurt, Germany (SMF). The following morphological measurements were recorded as described by Savage (2002), Duellman and Lehr (2009), and Arias et al. (2016): snout-vent length (SVL), head length (HL), head width (HW), inter orbital distance (IOD), width of the upper eyelid (EW), eye-nostril distance (EN), eye diameter (ED), and tympanum diameter (TY). Measurements were performed using dial calipers and were rounded to the nearest 0.1 mm. To avoid allometric effects relative to differences in size and shape between species and between individuals, we transformed the data using the method of Lleonart et al. (2000). Additional proportions reported herein include the following: EW/IOD, IOD/HW, TY/ED, EN/ED, ED/HL, HL/HW, and EN/HL. The sex of the individuals was determined by the gonadal morphology; the specimens with opaque seminal vesicles were assumed to be adult males, and those with developed oviducts were assumed to be adult females. The general terminology for the morphological characteristics follows Duellman and Lehr (2009). We followed Savage (2002) for the term "supernumerary tubercles", which we use to refer to the tubercles on the phalanges (between subarticular tubercles); this is different from the tubercles referred to herein as accessory palmar or plantar tubercles.

We calculated the mean, standard deviation, and range for each morphometric variable without correction. We used all variables to perform a linear discriminant analysis to determine whether the morphometric variables were effective to predict the species. We validated the proportion of correctly classified individuals using jackknife accuracy (Manly 1994). All analyses were performed using R v3.3.3 (R Core Team 2017).

Results

Molecular: The resulting mitochondrial data matrix included 59 sequences with a total sequence length of 1222 bp including gaps; 565 bp for 16S and 657 bp for COI. The best strategy partition contains four partitions, one for 16S and one for each codon in COI. The following substitution models were selected: GTR+G for 16S, K80+I+G for COI codon position 1, HKY+I+G for COI codon position 2, and GTR+I+G for COI codon position 3. The mitochondrial genetic distances are shown in Table 1. Genetic distances between *Craugastor* sp. 1 and all other members of the *Craugastor podiciferus* species group are 4.9–15.2 % for 16S and 13.5–21.6 % for COI. *Craugastor* sp. 2 is separated by an uncorrected genetic distance to other members of the *Craugastor podiciferus* species group of 2.9–15.7 % for 16S and 14.3–21.3 % for COI. Genetic distances between *Craugastor* sp. B and other members of the *Craugastor podiciferus* species group are 2.9–16.2 % for 16S and 14.3–19.6 % for COI.

The ML and Bayesian trees were similar in topology and show six well-supported clades (Fig. 2). The first is formed by specimens from La Nevera and Cerro Saguí from western Panama. A second and third clade are formed by specimens from southwestern Costa Rica and western Panama, respectively. The fourth clade comprises *C. aenigmaticus* from the montane rainforest of the Cordillera de Talamanca. A fifth major clade includes seven species of the *C. podiciferus* species group that mainly occur from the lowlands to mid-elevations from eastern Honduras to central Panama. Finally, a sixth major clade contains specimens from the type locality of *C. podiciferus* and several other localities from the highlands of Costa Rica and western Panama tentatively referred to this species. The main difference between the ML and the Bayesian topology is the position of the clade that contains the samples from La Nevera and Cerro Saguí. In the ML tree, this group was the sister to all other members of the *C. podiciferus* species group (although with very low support), while in the Bayesian tree (not shown), this was the sister clade to a clade containing the samples from Las Alturas and Potrero Grande and *C. blairi*.


Craugastor gollmerii AJC1926
SMF104015 La Nevera
SMF104017 La Nevera
SMF104014 Cerro Sagui
Craugastor sagui sp. nov.
UCR22709 Las Alturas
UCR22703 Las Alturas
UCR20429 Potrero Grande
UCR20389 Potrero Grande
Craugastor zunigai sp. nov.
SMF104034 Guayabito
SMF104027 Volcán Barú
SMF104037 La Estrella
USNM563039 Palo Alto
FMNH257689 La Fortuna
SMF104023 Fortuna
SMF104033 Fortuna
SMF102024 Fortuna
Craugastor blairi
SMF104020 Jurutungo
UCR22737 Cerro Hakú
UCR21951 Cerro Utyum
C. aenigmaticus
UCR22643 Guayacán
UCR22269 San Miguel
C. bransfordii
UCR22668 Bribri
UCR20050 Dabagri
C. polyptychus
UCR22625 Cascajal
UCR22619 Tapantí
C. underwoodi
UCR22211 Tausito
UCR22671 Suretka
C. persimilis
UCR21864 San Vito
UCR21863 San Vito
C. gabbi
UCR20352 Potrero Grande
EAP0514 Palmar Norte
C. stejnegerianus
SMF79759 Selva Negra
USNM559393 Tapalwás
C. lauraster
FMNH257673 Monte Verde
UCR16361 Tapesco
UCR22675 Monte Verde
FMNH257669 Monte Verde
C. podiciferus "Monte Verde"
C. podiciferus s.l.
UCR22146 Cascajal
CRARC012 Volcán Turrialba
UCR20992 Nectandra
UCR22201 El Empalme
SMF104005 Changena
UCR23175 Alto Uren
UCR19862 Lori
UCR19856 Lori
UCR19860 Lori
UCR19853 Lori
C. podiciferus sensu stricto
UCR23169 Alto Uren
C. podiciferus "Siola"
UCR22228 Pico Blanco
UCR22226 Pico Blanco
C. podiciferus "Pico Blanco"
CRARC0247 Monte Verde
FMNH257671 Monte Verde
UCR16353 Montaña Azul
C. podiciferus "San Gerardo"
UCR22690 Chumacera
UCR22120 Altamira
C. podiciferus "Chumacera"
UCR16585 Copey
EAP0509 Fila Costeña
FMNH257651 Fila Costeña
UCR22091 Quebradas
C. podiciferus "Fila Costeña"
76/96
19/-
81/*
61/78
64/87
39/67
50/96
92/*
83/*
59/79
26/72
27/51
58/96
61
96
30/94
74/*
15/48
81/*

← **Fig. 2.** Maximum likelihood phylogram of the *Craugastor podiciferus* species group based on the 16S and COI mitochondrial DNA gene markers. Bootstrap proportions and posterior probability (multiplied by 100) values were obtained with MrBayes before and after the slash, respectively. The scale bar refers to the estimated substitutions per site. Asterisks represent support > 95. The blue clade corresponds to *C. podiciferus sensu stricto.*

Morphometry: Morphometric variation and comparisons among the species are shown in Table 2. The proportion of specimens that could be correctly assigned to the species was 82 %, showing a clear morphological separation between the specimens of the three populations of southwestern Costa Rica and western Panama (Fig. 3).

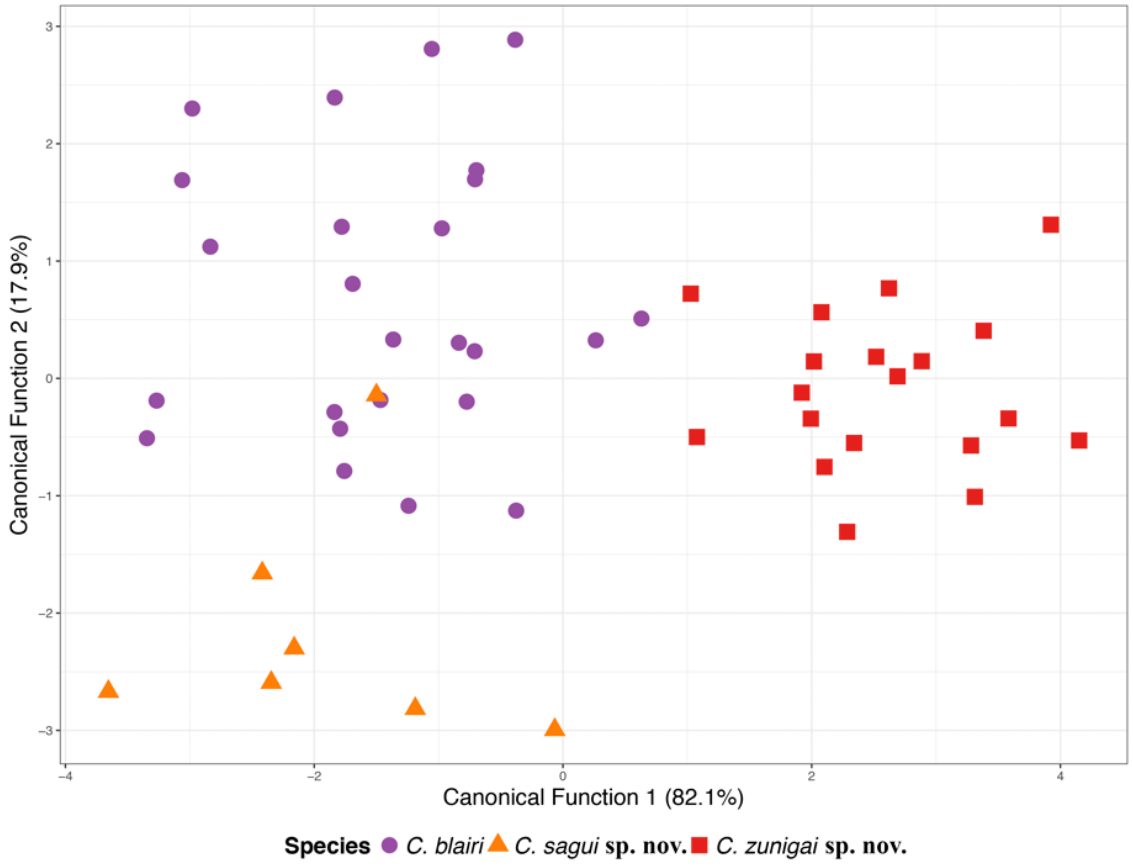


Fig. 3. Linear discriminant analysis showing the morphological separation among the three species from the lower montane rainforest in Southwestern Costa Rica and Western Panama.

Table 1. Mean uncorrected genetic distances among lineages of the *Craugastor podiciferus* species group using the COI (above) and 16S (below) mitochondrial genes.

	16S/COI	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1	*C. sagui* sp. nov.	—	14.25	16.51	18.02	16.28	17.60	17.13	18.85	21.34	21.50	19.63	14.43	15.09	16.67	15.58	15.50	15.89	15.19
2	*C. zunigai* sp. nov.	6.05	—	14.62	19.37	18.30	18.54	18.22	20.79	21.34	19.94	19.24	15.71	15.44	18.61	16.59	17.45	17.21	18.61
3	*C. blairi*	6.54	3.19	—	16.34	18.41	18.69	16.85	19.29	19.42	18.35	19.11	16.10	16.04	16.51	14.49	16.85	15.21	16.74
4	*C. aenigmaticus*	13.73	10.95	12.13	—	19.47	18.38	19.21	19.21	20.25	18.30	18.64	16.41	18.31	19.42	17.34	18.30	16.51	17.58
5	*C. bransfordii*	12.50	12.01	11.27	13.97	—	13.08	11.21	17.25	17.83	18.30	18.46	17.50	17.75	19.78	17.13	18.22	17.68	18.11
6	*C. polyptychus*	8.91	10.05	9.80	13.73	8.33	—	8.72	16.43	15.89	16.67	15.89	18.51	19.50	20.02	19.39	20.95	19.00	18.93
7	*C. underwoodi*	8.99	9.31	9.07	13.56	7.11	3.19	—	15.50	16.59	16.90	17.60	16.33	16.88	18.30	16.43	18.38	17.52	17.60
8	*C. persimilis*	11.52	11.52	11.34	15.69	11.27	7.84	8.09	—	14.02	13.71	14.88	18.93	19.28	19.47	19.94	21.03	20.72	21.18
9	*C. gabbi*	13.73	13.73	14.09	17.32	12.99	11.03	10.29	7.11	—	12.93	13.24	19.06	18.82	20.72	19.47	20.48	20.87	21.18
10	*C. stejnegerianus*	14.54	14.22	14.09	16.34	12.99	11.52	10.78	6.62	4.41	—	10.20	19.65	19.95	21.88	19.78	20.95	21.34	21.34
11	*C. lauraster*	14.87	15.69	15.81	17.16	12.01	11.03	10.78	7.60	7.84	5.39	—	18.48	20.75	21.50	20.33	21.34	20.87	20.72
12	*C. podiciferus* "Monte Verde"	6.59	6.21	7.07	12.25	11.03	9.23	8.01	10.54	12.75	13.40	13.73	—	10.20	10.80	9.76	10.93	10.23	10.51
13	*C. podiciferus sensu stricto*	5.69	5.29	5.54	10.62	10.20	8.92	7.75	9.34	12.01	11.91	12.99	3.09	—	11.40	9.11	11.06	10.22	10.88
14	*C. podiciferus* "Siola"	7.84	7.35	6.99	12.91	12.01	9.31	8.33	11.03	12.25	11.76	14.71	6.37	5.20	—	10.44	11.92	10.28	11.84
15	*C. podiciferus* "Pico Blanco"	6.37	5.88	6.13	11.11	10.05	9.56	8.33	10.05	13.24	13.24	12.25	2.45	1.96	5.88	—	6.93	8.88	10.75
16	*C. podiciferus* "San Gerardo"	8.50	8.66	8.78	12.75	11.19	10.38	9.15	11.36	13.56	13.56	12.58	3.65	4.85	7.84	2.94	—	10.98	12.46
17	*C. podiciferus* "Chumacera"	7.35	7.84	7.78	12.34	12.01	10.05	9.31	11.52	13.73	13.24	15.20	4.58	4.00	7.35	4.17	5.07	—	8.72
18	*C. podiciferus* "Fila Costeña"	7.52	7.03	7.11	12.91	11.36	10.05	8.82	10.54	12.42	13.40	13.07	4.25	3.63	7.19	2.45	4.96	5.64	—

Systematics

Redefinition of *Craugastor podiciferus* (Cope, 1875) and *C. blairi* (Barbour, 1928)

Craugastor podiciferus: The precise type locality of *Craugastor podiciferus* has been a matter of some uncertainty. The taxon was described by Cope (1875) based on material collected by W.M. Gabb from "slope of Cerro Pico Blanco" in 1874. Savage (1974) corrected it to Cerro Utyum, Cantón de Talamanca, Provincia de Limón, 1524–2134 m a.s.l. but collected no additional specimens because he did not reach that site. Recently, Arias and Chaves (2014) corrected the type locality to a place between Cerro Pat and the headwaters of the Río Lari, elev. 1520–2135, Provincia de Limón, Caribbean slope of Cerro Kamuk. The type locality is a remote site within the Parque Internacional La Amistad, in the Cordillera de Talamanca. It is only accessible on foot and requires three days of hiking from the last village (Amubri, Talamanca). We collected specimens in the surroundings of the type locality according to Arias and Chaves (2014) (Fig. 1 and 4a). Based on the phylogenetic relationships, we propose to restrict the taxon *Craugastor podiciferus* to the populations from Cordillera Volcánica Central from Costa Rica and Cordillera de Talamanca (Caribbean slopes) in Costa Rica and western Panama (Fig. 1).

Craugastor jota: This species was described by Lynch (1980) based on specimens from the Changena river in western Panama, at 760 m a.s.l., collected by Linda Trueb in 1966. This species was placed in the *C. podiciferus* species group based on morphology (Hedges *et al*. 2008). In this study, we included specimens from Changena river, western Panama, very near the type locality of *C. jota* (Linda Trueb pers. comm.). In our phylogenetic analyses these specimens fall within the *C. podiciferus sensu stricto* clade. As a result, we suggest that *C. jota* should be referred to as a junior synonym of *C. podiciferus.*

Craugastor blairi: The precise type locality of *Craugastor blairi* is unknown. Barbour (1928) indicated the type locality as "from Gutierrez, Bocas del Toro Province, Panama (near Costa Rican frontier)". The type series was collected by Emmett R. Dunn and Chester Duryea in the summer of 1923. Their expedition followed a trail from Chiriquicito at the Laguna de Chiriquí (today in the Corregimiento of Miramar, Bocas del Toro) to Boquete, Province of Chiriquí. Most of their trail climbs up the Atlantic slopes of the Cordillera Central (Savage 1970) before descending into the high valley of Boquete. On the same route, Dunn and Duryea collected several other new species and identified the type localities as either "Gutierrez" or "La Loma". Species with the type locality "La Loma" are *Dermophis parviceps* (Dunn, 1924), *Hyloscirtus colymba* (Dunn, 1931), *Pristimantis pardalis* (Barbour 1928), and *Pristimantis caryophyllaceus* (Barbour 1928). All these species have a vertical distribution range that enters the lowlands to almost sea level (Köhler 2011). Dunn gives the elevation of La Loma as 2000 feet (610 m) in the description of *D. parviceps* and as 1500 feet (460 m) in the description of *H. colymba*. In comparison to "La Loma", a species with the type locality "Gutierrez" is *Craugastor obesus* (Barbour 1928). Dunn (1940) also mentioned that he collected *C. monnichorum* at "Gutierrez", and both species had a more premontane to montane distribution. Therefore, we believe that "Gutierrez" is further uphill than "La Loma" and thus closer to the continental divide and much closer to Boquete than to the Caribbean lowlands. We collected several specimens at different sites in the vicinity of Boquete on the foothills of Barú Volcano that form a separate clade with specimens collected at Cerro La Estrella, Cerro Guayaba, Guayabito, and La Fortuna (Fig. 1 and 4b). For the above reasons, we resurrect the name *C. blairi* for this clade.

Restricting the taxon *Craugastor podiciferus* to populations inhabiting the Caribbean slopes of Cordillera de Talamanca of Costa Rica and extreme western Panama and *C. blairi* to populations inhabiting the Cordillera Central from Barú Volcano to the east, results in two allopatric lineages

on the Pacific slopes of Cordillera de Talamanca, Costa Rica and those from Cordillera Central, Panama without assignment to an existing taxon. With no available name present in the synonymy of *C. podiciferus* for either of these populations, here we describe these two lineages as new species and provide redescriptions for *C. podiciferus* and *C. blairi*.

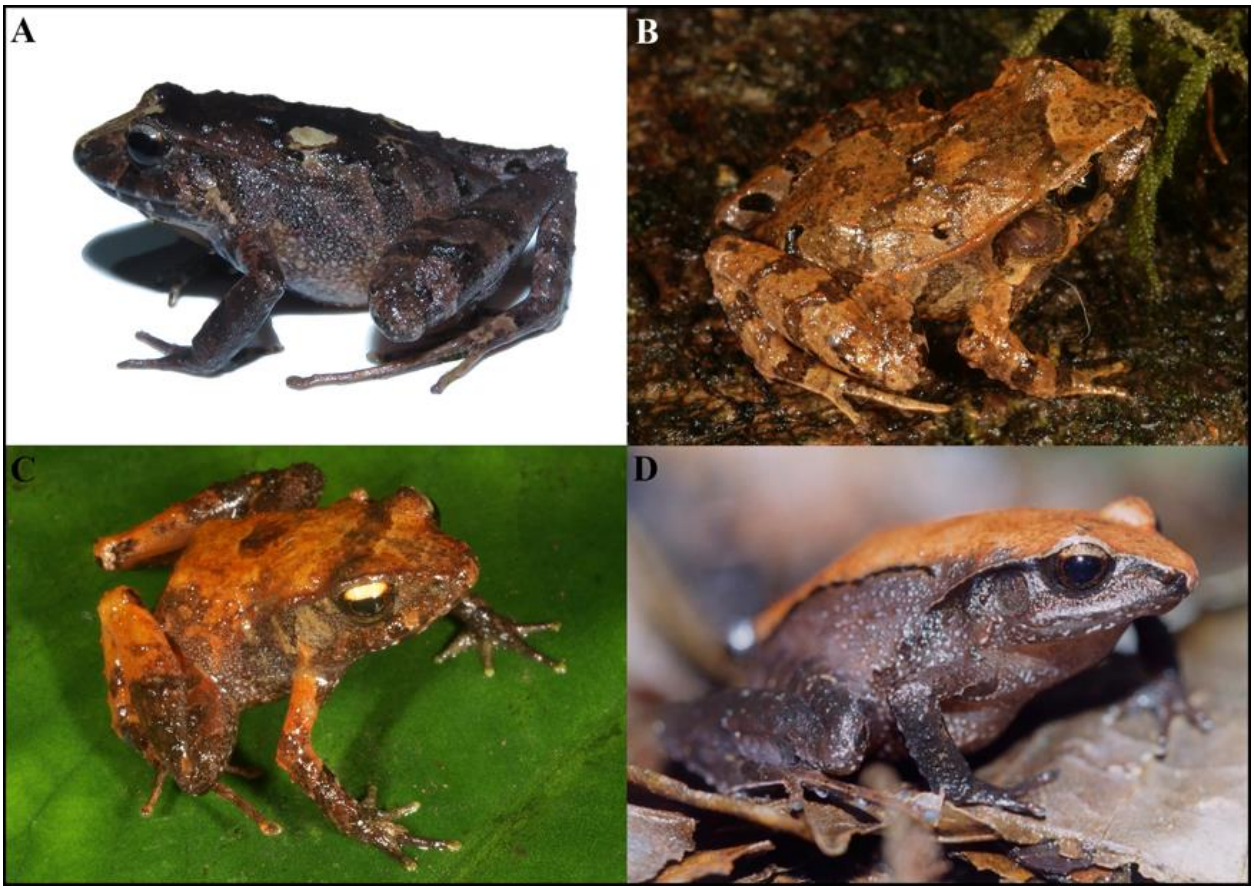


Fig. 4. In life photographs of (**a**) *Craugastor podiciferus* (UCR 23169) from the Caribbean slope of Cerro Kamuk, Costa Rica, (**b**) *C. blairi* (SMF 104032) from Fortuna, Panama, (**c**) *C. sagui* sp. nov. (SMF 104018) from La Nevera, Panama, and (**d**) *C. zunigai* sp. nov. (UCR 20389) from Potrero Grande, Costa Rica. Photo A by E. Arias, B-C by A. Hertz, and D by E. Boza-Oviedo.

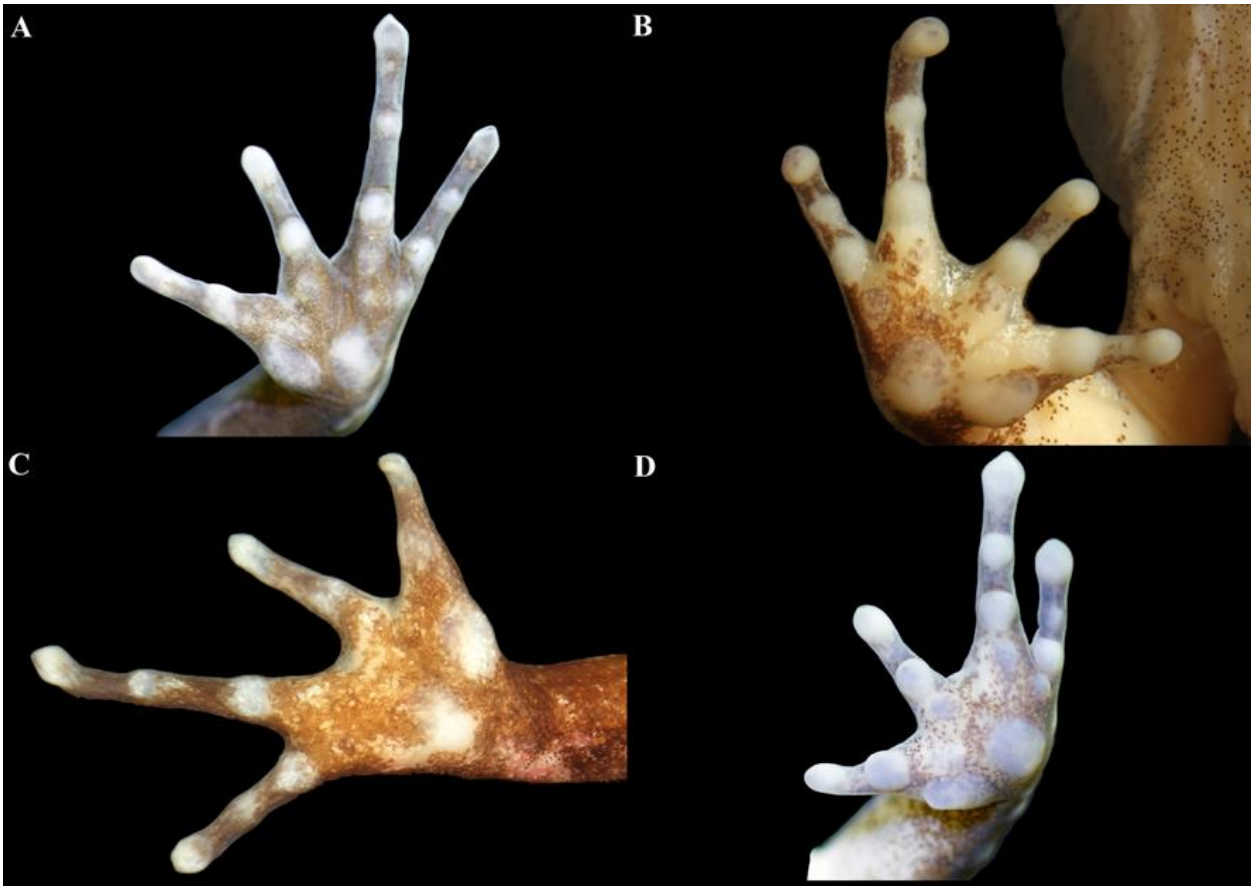


Fig. 5. Variation of the ventral views of the right hands. (**a**) *Craugastor podiciferus* (UCR 23175), (**b**) *C. blairi* (SMF 104032), (**c**) *C. sagui* sp. nov**.** holotype (SMF 104018), and (**d**) *C. zunigai* sp. nov. holotype (UCR 22703). Photos A and D by E. Arias, B-C by G. Köhler.

***Craugastor podiciferus* (Cope, 1875)**

Common name: Polymorphic Dirt Frog

(Figs. 4a and 6)

Syntypes: USNM 30662, USNM 30664–75, and MCZ 11841. All specimens from " 5000 to 7000 feet (elevation), on the Caribbean slopes of Cerro Pico Blanco", collected by William M. Gabb on 1874.

Genetic reference specimen: UCR 23175 (EAP 0810), an adult female from Costa Rica: Provincia de Limón: Cantón de Talamanca: Distrito de Telire: Parque Internacional La Amistad, (9.366º, -83.042º; 1860 m a.s.l.), collected by Erick Arias and Omar Zúñiga on 27 October 2016.

Referred specimens: UCR 23155 (EAP 0803), adult female, same data as the genetic reference specimen. UCR 23145 (EAP 0792), an adult male from Costa Rica: Provincia de Limón: Cantón de Talamanca: Distrito de Telire: Cerro Pat, Parque Internacional La Amistad, (9.393º, -83.025º; 1450 m a.s.l.), collected by Erick Arias and Omar Zúñiga on 26 October 2016.

Assignment to group: Assigned to *Craugastor* based on molecular analysis and on the following characters: cranial crest absents and Toe III larger than Toe V.

Diagnosis: The combination of the following characteristics can be used to distinguish *Craugastor podiciferus* (Fig. 5a–6) from other described species in the genus: 1) skin on the dorsum is smooth to scattered tubercles; 2) skin on the venter is smooth, at least in the midline; 2) vocal slits in adult males; 3) nuptial pads absent; 4) unwebbed toes; 5) heel with a projecting tubercle; 6) accessory palmar and plantar tubercles absent, usually no supernumerary tubercles under the digits; and 7) subarticular tubercles flat in form.

Craugastor (*Craugastor*) *podiciferus* is a small species with the following characteristics: (1) skin on the dorsum smooth to scattered tubercles; head smooth; venter smooth; flanks smooth with scattered tubercles to warty; posterior surface of hind limbs surrounding cloaca strongly areolate; some specimens with a pair of scapular, dorsolateral or lateral folds; discoidal fold complete laterally and posteriorly; (2) tympanic membrane round, heavily pigmented; prominent in males, evident in females; annulus evident through the skin; (TY/ED = 44.9–100 %); usually with a pair of supratympanic folds; (3) snout subovoid in the dorsal view, rounded in profile;

loreal region concave; canthus rostralis usually rounded; (4) eyelid granular, with several low tubercles forming a more or less distinct ridge on the outer edge of the eyelid continuous with the supratympanic fold (EW/IOD = 40.5–71.2 %); cranial crests absent; (5) vomerine teeth in two transverse fasciculi, behind the choanae; choanae smaller than the dentigerous; (6) vocal slits and large single vocal sac in adult males; nuptial pads absent; (7) Fingers I and II subequal; discs absent, some specimens with terminal transverse grooves on fingers, especially in males; tips symmetric, usually rounded but pointed in Fingers III-IV in some specimens; pads ovoid to triangular; (8) fingers lack lateral fringes; webbing absent; thenar and palmar tubercles low, ovoid, similar in size; supernumerary tubercles absent; accessory palmar tubercles usually absent but 1-2 low barely distinct tubercles visible in some specimens; subarticular tubercles round in basal outline, flat in form and globular in profile; (9) ulnar fold absent but tubercles sometimes visible; (10) heel with a projecting tubercle; inner edge tarsal with an indistinct short ridge, outer smooth or with tubercles; (11) toes lacking lateral fringes; inner metatarsal tubercle elongate, outer rounded, much smaller than inner, inner and outer metatarsal tubercles projecting; supernumerary and plantar tubercles absent; subarticular tubercles rounded to ovoid in the basal outline, flat in form and obtuse in profile; (12) Toe III larger than Toe V; discs and terminal transverse grooves present on all fingers; tips symmetrical, disc covers spadate; pads triangular; webbing absent; (13) coloration very variable; dorsum tan to light or dark brown, nearly uniform or suffused with black or reddish pigment; frequently paired suprascapular dark spots, area between dorsal folds usually contrasting with flank color; several specimens with a dark mask from the snout continuing above the tympanum and often bordered above by a narrow light line; venter yellow, grayish, or reddish, uniform or with light or dark spots; usually forelimbs and hind limbs with dark bars, some specimens with paired dark spots on the anterior surface of the hind limbs; the upper lip has dark bars with white pigment in the form of faded bars; and (14) SVL in males 21–28 mm; SVL in females 23–40 mm.

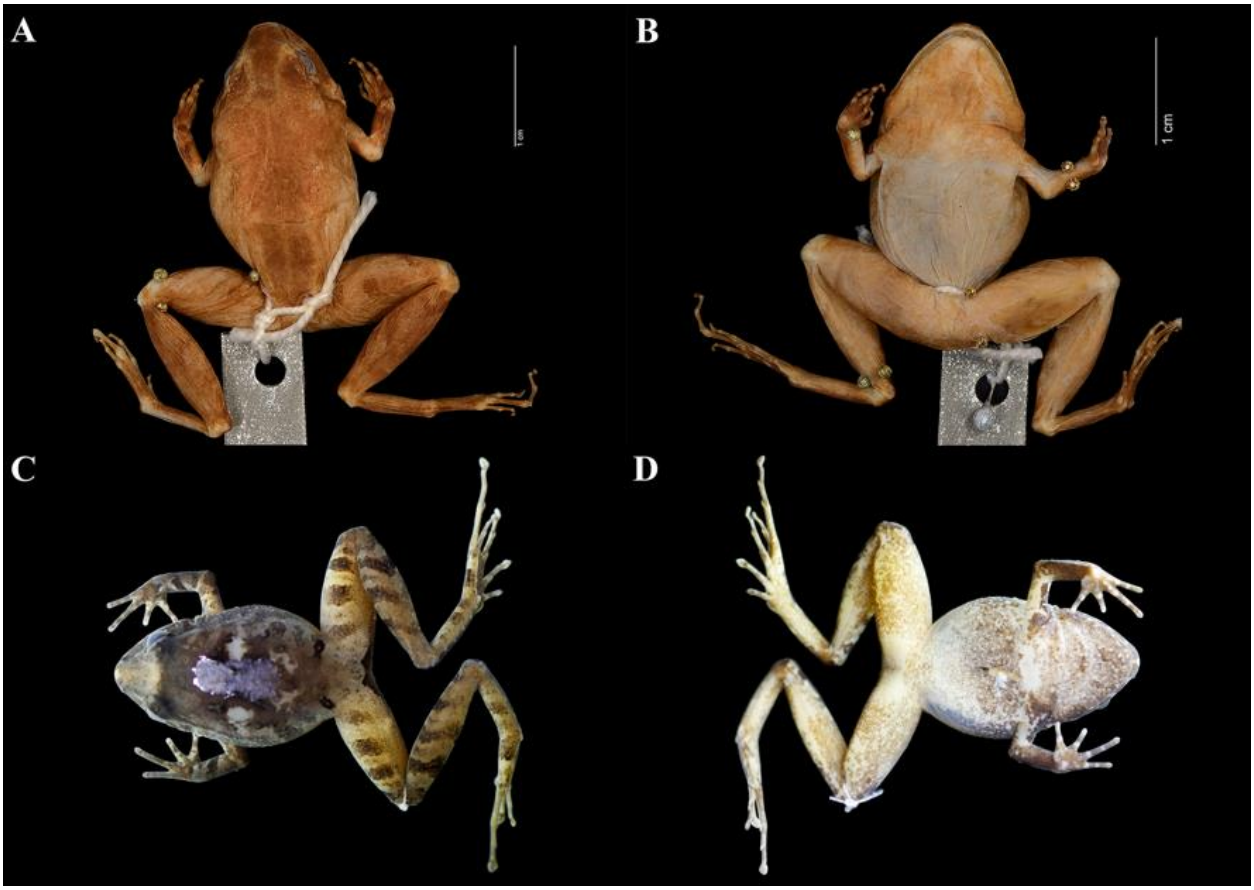


Fig. 6. Dorsal and ventral views in ethanol of *Craugastor podiciferus* (**a,b**) syntype (USNM 30672) and (**c,d**) molecular specimen reference (UCR 23175). Photos A-B by E.M. Langan, C-D by E. Arias.

Comparison: *Craugastor podiciferus* differs from all other craugastorids of Isthmian Central America, excluding those in the *C. podiciferus* species group, by having unwebbed toes and a narrow head (HW 37.0–43.3 % of SVL). *Craugastor podiciferus* differs from other members of the *C. podiciferus* species group by having the following characteristics (condition for *C. podiciferus* in parentheses).

Craugastor bransfordii (Cope, 1886), *C. gabbi*, *C. lauraster* (Savage, McCranie, and Espinal, 1996), *C. persimilis* (Barbour, 1926), *C. polyptychus* (Cope, 1886), *C. stejnegerianus*

(Cope, 1893), and *C. underwoodi* (Boulenger, 1896) differ from *C. podiciferus* by the following features: a) dorsum usually granular or warty (dorsum smooth to scattered tubercles); b) subarticular tubercles projecting (subarticular tubercles flat); and c) venter coarsely areolate, including the midline (venter smooth, at least in midline). *Craugastor aenigmaticus* Arias, Chaves, and Parra-Olea, 2018 differ from *C. podiciferus* by the following features: a) absence of a prominent calcar tubercle on the heel, although some specimens can have one to three small tubercles (a prominent calcar tubercle on the heel); b) venter violet-brown with white blotches (venter yellow, orange, grayish or olive in adults); and c) white prominent folds between subarticular tubercles on the hands of adults (absence of white folds between subarticular tubercles on the hands of adults). *Craugastor blairi* (Barbour, 1928) differs from *C. podiciferus* by the following features: a) skin on the venter coarsely areolate (venter smooth, at least in midline); b) absence of a prominent calcar tubercle on the heel (a prominent calcar tubercle on the heel); c) having accessory palmar tubercles (accessory palmar tubercles absent); and d) having subarticular tubercles projecting (subarticular tubercles flat). *Craugastor sagui* differs from *C. podiciferus* by the following features: a) nuptial pads in adult males (nuptial pads absent); b) absence of a prominent calcar tubercle on the heel (a prominent calcar tubercle on the heel); and c) absence of vocal slits in adult males (vocal slits in adult males). *Craugastor zunigai* differs from *C. podiciferus* by the following features: a) skin on the venter coarsely areolate (venter smooth, at least in midline); b) absence of a prominent calcar tubercle on the heel (a prominent calcar tubercle on the heel); c) accessory palmar tubercles (accessory palmar tubercles absent), and d) subarticular tubercles projecting (subarticular tubercles flat).

Natural history: *Craugastor podiciferus* inhabits the lower montane rainforest (Holdridge 1967; Bolaños et al. 2005), which is characterized by a very short dry season (one to three months), annual precipitation ranging from 3600 to 7500 mm and annual temperature from 12 to 17 ºC.

Very little is known about the natural history of *C. podiciferus*; however, it is important to note that the species was abundant during the months of fieldwork. The specimens were always found on the floor jumping during the active search. Schlaepfer and Figeroa-Sandí (1998) described the call of *C. podiciferus* from Las Cruces, herein referred to as *C. podiciferus* "Fila Costeña". The advertising call of *C. podiciferus sensu stricto* is unknown, although it is known to vocalize.

Distribution: *Craugastor podiciferus sensu stricto* is restricted to the highlands of the Cordillera Volcánica Central in Costa Rica and Caribbean slopes of the Cordillera de Talamanca in Costa Rica and western Panama (Fig. 1). The altitudinal range of *C. podiciferus* is 1700–2700 m a.s.l. All populations of *C. podiciferus* are found in primary forests, and several localities are within protected areas, (*i.e.*, Parque Internacional La Amistad, Parque Nacional Tapantí-Macizo de la Muerte, Parque Nacional Braulio Carrillo, and Parque Nacional Juan Castro Blanco). More fieldwork is necessary to clarify the distribution of *C. podiciferus*, especially on the northern end of the Cordillera de Talamanca and in the adjacent zone between the Cordillera Volcánica Central and the Cordillera de Tilarán.

Remarks: We tentatively assign several populations from the highlands of Costa Rica to *Craugastor podiciferus.* However, these populations are phylogenetically structured and show uncorrected p-distances in the 16S rRNA gene between 2.45 and 6.37 % (Table 1), and some differ morphologically from typical *C. podiciferus* and thus may represent as many as six additional unnamed species. These six populations are as follows: 1) the *C. podiciferus* "Monte Verde" clade, which corresponds to clade A of Streicher et al. (2009), a clade restricted to Cordillera de Tilarán and Volcánica Central, at 1500–1931 m a.s.l. The specimens in this clade are morphologically very similar to *C. podiciferus sensu stricto.* 2) The *C. podiciferus* "San Gerardo" clade, which corresponds to clade B of Streicher et al. (2009), a clade restricted to

Cordillera de Tilarán and Cordillera Volcánica Central, at 1470–1500 m a.s.l. The specimens in this clade differ from *C. podiciferus s.s.* in having projecting subarticular tubercles. 3) The *C. podiciferus* "Pico Blanco" clade, which contains a single population from the northern end of the Cordillera de Talamanca in the Central valley, at 2242 m a.s.l. The specimens in this clade differ from *C. podiciferus s.s.* in having an areolate venter and projecting subarticular tubercles. 4) The *C. podiciferus* "Fila Costeña" clade, which corresponds to clades E and F of Streicher et al. (2009), a clade restricted to South Pacific Costa Rica, at 1350–1550 m a.s.l. The specimens in this clade differ from *C. podiciferus s.s.* by having an areolate venter and accessory palmar tubercles. 5) The *C. podiciferus* "Chumacera" clade, which is known for only one population on the pacific slopes of Cordillera de Talamanca, at 1750–1850 m a.s.l. The specimens in this clade differ from *C. podiciferus s.s.* by having accessory palmar tubercles. 6) The *C. podiciferus "*Siola", which is known for only one population on the Caribbean slope of the Cordillera de Talamanca, at 1300–1350 m a.s.l. The specimens in this clade differ from *C. podiciferus s.s.* by having an areolate venter and by the prominent pungent calcar tubercle on the heel.

Despite the molecular results and morphological differences between some of these clades, there is little to distinguish some populations in particular characters, especially *C. podiciferus sensu stricto, C. podiciferus* "Monte Verde", and *C. podiciferus* "Chumacera". In addition, this group forms a monophyletic group, thus agreeing with the definition of the taxon by Savage (2002), Leenders (2016), and Cossel and Kubicki (2018). For these reasons and until new morphological evidence support the distinctiveness, we refrain from raising these clades to the species level.

Table 2. Mean, standard deviation (S.D.), and range for morphometric variables by species.

Variable	*Craugastor podiciferus*		*Craugastor blairi*		*Craugastor sagui* sp. nov.		*Craugastor zunigai* sp. nov.	
	Mean ± S.D.	**Range**	**Mean ± S.D.**	**Range**	**Mean ± S.D.**	**Range**	**Mean ± S.D.**	**Range**
SVL	25.6±4.3	16.9–33.9	23.2±4.8	13.4–30.6	21.5±6.5	13.0–30.1	20.8±3.9	13.8–26.5
HL	10.4±1.5	7.0–12.9	8.3±1.5	5.6–11.0	8.1±2.5	4.7–10.9	8.4±1.5	5.7–10.3
HW	10.2±1.6	6.5–13.3	8.9±1.8	5.5–12.0	8.0±2.4	4.9–11.5	8.2±1.6	5.6–10.7
ED	3.0±0.5	2.0–3.9	2.9±0.6	1.9–3.8	2.9±0.7	2.0–4.0	2.5±0.3	1.9–2.9
TY	1.8±0.4	1.0–2.7	1.8±0.4	0.8–2.6	1.7±0.6	0.8–2.6	2.0±0.3	1.6–2.9
EW	1.9±0.3	1.2–2.4	1.6±0.3	0.9–2.1	1.5±0.4	0.9–2.0	1.6±0.3	1.2–2.1
IOD	3.3±0.5	2.5–4.2	3.3±0.6	1.8–4.3	2.9±0.8	1.7–3.9	2.7±0.5	1.8–3.7
EN	2.6±0.4	1.9–3.2	2.2±0.4	1.3–3.0	2.1±0.8	1.1–3.2	2.0±0.4	1.3–2.6
EW/IOD	2.8±0.3	2.3–3.6	0.50±0.09	0.34–0.73	0.52±0.06	0.46–0.61	0.59±0.08	0.44–0.76
IOD/HW	0.6±0.1	0.4–0.7	0.37±0.04	0.30–0.45	0.37±0.03	0.34–0.40	0.34±0.02	0.30–0.38
TY/ED	0.6±0.1	0.4–1.0	0.66±0.23	0.40–1.09	0.58±0.18	0.40–0.81	0.81±0.17	0.62–1.34
EN/ED	0.9±0.1	0.7–1.0	0.77±0.08	0.63–0.91	0.71±0.11	0.55–0.88	0.81±0.10	0.63–0.98
ED/HL	0.3±0.1	0.2–0.3	0.35±0.03	0.30–0.43	0.37±0.04	0.31–0.43	0.29±0.02	0.26–0.33
HL/HW	1.0±0.1	0.9–1.1	0.94±0.05	0.84–1.05	1.01±0.06	0.95–1.08	1.03±0.05	0.95–1.14
EN/HL	0.2±0.1	0.2–0.3	0.27±0.02	0.23–0.30	0.26±0.02	0.23–0.29	0.24±0.02	0.20–0.27

***Craugastor blairi comb. new.* (Barbour, 1928)**

Common name: Blair's Dirt Frog

(Figs. 4b and 7)

Holotype: MCZ 13036 from Gutierrez, Bocas del Toro Province, Panama (near Costa Rican border), collected by E. R. Dunn and Chester Duryea on summer 1925.

Genetic reference specimen: SMF 104032 (AH 379), an adult male from Panama: Provincia de Chiriquí: Distrito de Gualaca: La Fortuna, western slope of Cerro Pata de Macho (8.679º, -82.193º; 1793 m a.s.l.), collected by Andreas Hertz and Sebastian Lotzkat on 20 May 2010.

Referred specimens: SMF 104030 (AH 377) and SMF 104031 (AH 378), adult males; same date as the genetic reference specimen. SMF 104025 (AH 196), adult female from Panama: Provincia de Chiriquí: Distrito de Gualaca: La Fortuna, (8.672º, -82.200º; 1400 m a.s.l.), collected by Andreas Hertz and Sebastian Lotzkat on 20 March 2009. SMF 104026 (AH 238)

and SMF 104027 (AH 239), adult females from Panama: Provincia de Chiriquí: Distrito de Bugaba: Volcán Barú, Sendero Los Quetzales (8.849º, -82.515º; 2134 m a.s.l.), collected by Andreas Hertz and Sebastian Lotzkat on 8 April 2009.

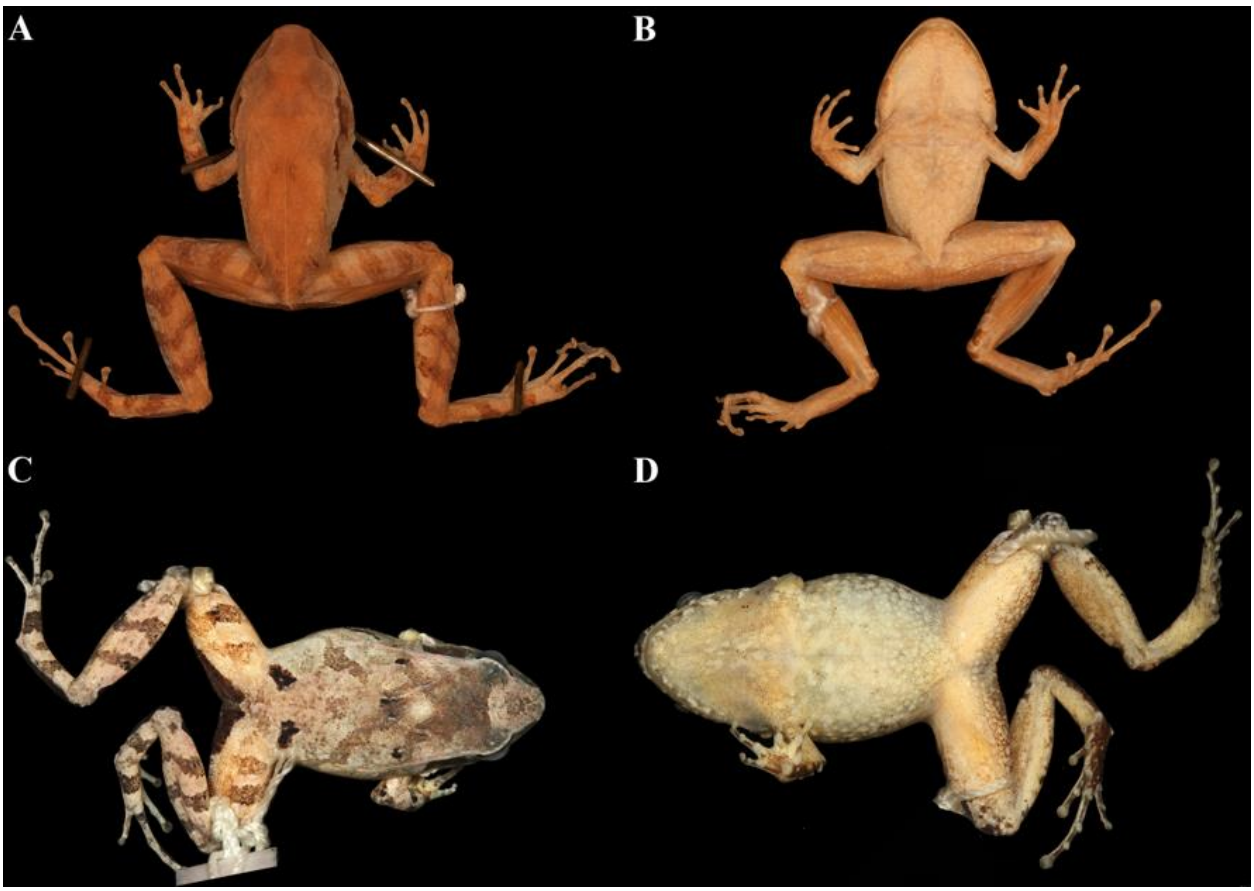


Fig. 7. Dorsal and ventral views in ethanol of *Craugastor blairi* (**a,b**) holotype (MCZ A-13036) and (**c,d**) molecular specimen reference (SMF 104032). Photos A-B by the Museum of Comparative Zoology, Harvard University, C-D by G. Köhler.

Assignment to group: Assigned to the genus *Craugastor* based on molecular data and on the following characters: cranial crest absents and Toe III larger than Toe V. Assigned to the *C. podiciferus* species group based on the following features: narrow head (HW/SVL = 34.6–42.8 %), dorsum smooth to scattered tubercles, unwebbed toes, vocal slits in adult males, and nuptial pads absent.

Diagnosis: The combination of the following characteristics can be used to distinguish *Craugastor blairi* (Fig. 5b and 7) from other described species of the genus: 1) skin on the dorsum is smooth or has scattered tubercles; 2) skin on the venter is coarsely areolate; 3) vocal slits and vocal sac present in adult males; 4) nuptial pads absent; 5) unwebbed toes; 6) heel without an enlarged calcar tubercle, although one to three small tubercles or granules can be present on the heel; 7) accessory palmar and plantar tubercles present, and absence of supernumerary tubercles; and 8) subarticular tubercles projecting.

Craugastor (*Craugastor*) *blairi* is a small species with the following characteristics: (1) skin on dorsum smooth or has scattered tubercles; head smooth; venter coarsely areolate: flanks smooth with scattered tubercles to warty; posterior surface of hind limbs surrounding the cloaca strongly areolate; some specimens with a pair of scapular, dorsolateral or lateral folds; discoidal fold complete laterally and posteriorly; (2) tympanic membrane round, heavily pigmented in females, translucent in adult males; prominent in males, evident in females; annulus evident through the skin; (TY/ED = 40–109 %); usually with a pair of supratympanic folds; (3) snout subovoid in the dorsal view, rounded in profile; loreal region concave; canthus rostralis usually rounded; (4) eyelid granular, with an evident supraocular tubercle and a more or less distinct ridge on outer edge of eyelid continuous with the supratympanic fold (EW/IOD = 37.2–72.7 %); cranial crests absent; (5) vomerine teeth in two transverse fasciculi, behind the choanae; choanae smaller than the dentigerous; (6) vocal slits and a single vocal sac that is large in adult males; nuptial pads absent; (7) Finger I and II subequal; discs absent, some specimens with terminal transverse grooves on fingers, especially in males; tips symmetric, usually rounded; pads ovoid to triangular; (8) fingers lacking lateral fringes; webbing absent; thenar and palmar tubercles low, ovoid, similar in size; supernumerary tubercles absent; 1–2 accessory palmar tubercles; subarticular tubercles round in the basal outline, projecting in form and globular in the profile; (9) ulnar fold absent but tubercles visible; (10) heel without an enlarged calcar tubercle, although

one to three small tubercles or granules can be present on the heel; inner edge tarsal smooth, outer with an incomplete fold and/or tubercles; (11) toes lacking lateral fringes; inner metatarsal tubercle elongate, outer rounded, much smaller than inner, inner and outer metatarsal tubercles projecting; supernumerary tubercles absent; several low plantar tubercles; subarticular tubercles rounded to ovoid in the basal outline, projecting in form and obtuse in profile; (12) Toe III larger than Toe V; discs and terminal transverse grooves present on all fingers; tips symmetrical, disc covers palmate to spadate; pads triangular; webbing absent; (13) coloration very variable; dorsum tan to light or dark brown, nearly uniform or suffused with black or reddish pigment; frequently paired suprascapular dark spots; some specimens with a dark mask from the snout continuing above the tympanum and downward behind the axilla, often bordered above by a narrow light line; venter yellow, grayish, or reddish, uniform or with light or dark spots; usually forelimbs and hind limbs with dark bars, some specimens with paired dark spots on the anterior surface of the hind limbs; upper lip with dark bars with white pigment in the form of faded bars; and (14) SVL in males 15.9–21 mm; SVL in females 13.4–30.6 mm.

Comparisons: *Craugastor blairi* differs from all the other Isthmian Central America craugastorids (except for those in the *C. podiciferus* species group) by having unwebbed toes and a narrow head (HW 34.6–42.8 % of SVL). *Craugastor blairi* differs from other members of the *C. podiciferus* species group by having the following characteristics (condition for *C. blairi* in parentheses). *Craugastor bransfordii, C. gabbi, C. lauraster, C. persimilis, C. polyptychus, C. stejnegerianus,* and *C. underwoodi* differ from *C. blairi* by the following features: a) dorsum usually granular or warty (dorsum smooth to having scattered tubercles); b) subarticular tubercles obtuse to pointed, at least the distal subarticular tubercles under Toe III and IV (subarticular tubercles projecting, globular); and c) altitudinal range, 0–1600 m a.s.l. (altitudinal range is 1280–2134 m a.s.l. for *C. blairi*). *Craugastor podiciferus* differs from *C. blairi* by the

following features: a) a prominent calcar tubercle on the heel (absence of a prominent calcar tubercle on the heel); b) subarticular tubercles flat (subarticular tubercles projecting); and c) venter smooth, at least in the midline (venter coarsely areolate, including the midline). *Craugastor aenigmaticus* differs from *C. blairi* by the following features: a) venter smooth (venter coarsely areolate); b) subarticular tubercles flat (subarticular tubercles projecting, globular); and c) prominent white folds between subarticular tubercles (absence of white folds between subarticular tubercles). *Craugastor sagui* differs from *C. blairi* by the following features: a) venter smooth (venter coarsely areolate); b) nuptial pads in adult males (nuptial pads absent); c) absence of vocal slits (vocal slits in adult males); and d) subarticular tubercles flat (subarticular tubercles projecting). *Craugastor zunigai* differs from *C. blairi* by the absence of an evident supraocular tubercle (eyelid with an evident supraocular tubercle).

Natural history: *Craugastor blairi* inhabits the lower montane rainforest (Holdridge 1967; Bolaños et al. 2005), which is characterized by a very short dry season (one to three months). Annual precipitations range from 3600 to 7500 mm and annual temperature from 12 to 17 ºC. Very little is known about the natural history of *C. blairi*; however, it is important to note that the species was abundant during the months of fieldwork. The specimens were always found on the floor in leaf litter. It is possibly active during the day. Males call during periods of lower light due to dark clouds or in the evening hours. Calling activity is greater during the rain.

Vocalization: Vocalizations of four male specimens (AH 375, 1430 m, dusk, 19.5 °C, 100 % RH, AH 377-379, 1793 m, 18.5 °C, 100 % RH) have been recorded on the slopes of Cerro Pata de Macho. All specimens were calling at dusk, between 18:00 h and 19:00 h after a moderate rain. Calling sites were elevated positions only a few centimeters above the ground such as low vegetation, twigs, or roots. Calling stopped completely after dark. One male (AH375) was recorded at 19.5 °C and 100 % relative humidity. The other three males were recorded at 18.5 °C

and 100 % relative humidity. Two very different call types could be distinguished. The first that was emitted by all four males was a “squeak” of 0.035–0.065 (0.050±0.007) seconds in length, given in frequent intervals after 7.938–29.124 (11.974±4.825) seconds, resulting in a call rate of 4.66–9.63 (6.67±2.41) calls per minute. The call has two harmonics, the fundamental of which also contain the dominant frequency at 6844–8063 (7306±313) Hz. The dominant frequency is reached in the middle or towards the end of the call, 0.009–0.053 (0.030±0.012) seconds after ignition of the call. The frequency range of the fundamental harmonic is between 4705–6674 (5680±634) Hz low frequencies and 7181–9343(7965±467) Hz high frequencies. The higher harmonic has a frequency range of 9566–14057 (12057±1159) Hz low frequencies and 14182–16617 (15175±650) Hz high frequencies, with the highest acoustic intensity at 13313–15656 (14147±626) Hz.

The second call type could only be recorded in two of the four males. It was identified as a trilling, *i.e.*, strongly pulsed “chirp” composed of mostly two, or less often one or three, notes. The notes are 0.266–0.692 (0.450±0.116) seconds in length, and the total call duration is 0.745–1.551 (0.914±0.206), depending on how many notes are contained in the call. Calls are repeated after 5.911–18.265 (10.196±3.522) seconds. A single note contains 12–25 (19±4) pulses that are emitted at a pulse rate of 36–46 (43±2) pulses per second. The dominant frequency of 8813–10313 (9654±236) Hz is reached in the middle or towards the end of the note at pulse 6–21 (13±5). Calls are frequency modulated with the lowest frequencies of 6044–7640 (6617±508) Hz in the first pulses gradually rising towards 10298–12002 (10848±466) Hz high frequencies in the last pulses of each note.

Distribution: *Craugastor blairi* is restricted to western Panama, on the Cordillera Central from Barú Volcano to the east over the La Fortuna depression into the Serranía de Tabasará. The specimens were collected around Barú Volcano, Cerro La Estrella, Cerro Guayaba, Guayabito, and La Fortuna (Fig. 1). The altitudinal range of the species is 1280–2134 m a.s.l. To our current

knowledge, the continuous distribution of *C. blairi* is interrupted between La Fortuna and Guayabito. Both sites are separated by ~80 airline kilometers. Within this gap, we find another distinct clade that seems to be restricted to the surroundings of Cerro Saguí and Cerro Santiago and that we describe below as *C. sagui*. More fieldwork will be necessary to clarify the distribution of *C. blairi* with respect to *C. sagui,* especially in the area between La Fortuna and Guayabito. It would also be interesting to explore the eastern distribution limits of *C. blairi* that could be found as far east as Santa Fé, Veraguas.

***Craugastor sagui* sp. nov.**

Common name: Sagui Dirt Frog

(Figs. 4c and 8)

Holotype: SMF 104018 (AH481), an adult male from Panama: Comarca Ngöbe-Buglé: Distrito de Nole Duima: southeastern slope of Cerro Saguí (8.563º, -81.821º; 1991 m a.s.l.), collected by Andreas Hertz and Sebastian Lotzkat on 9 September 2010.

Paratypes: SMF 104014 (AH483), adult female; same date as the holotype. SMF 104017 (AH342), adult male and SMF 104015 (AH045), adult female from Panama: Comarca Ngöbe-Buglé: Distrito de Nole Duima: La Nevera, southern slopes of Cerro Santiago (8.500º, -81.772º; 1700 m a.s.l.), collected by Andreas Hertz and Sebastian Lotzkat on 13 November 2009.

Assignment to group: Assigned to the genus *Craugastor* based on our molecular data and on the following morphological characters: cranial crests absent, and Toe III longer than Toe V. Assigned to the *C. podiciferus* species group based on the following features: a narrow head (HW/SVL = 35.8–40.9 %), length of Finger I equal to Finger II, dorsum smooth, toes unwebbed, and nuptial pads in adult males.

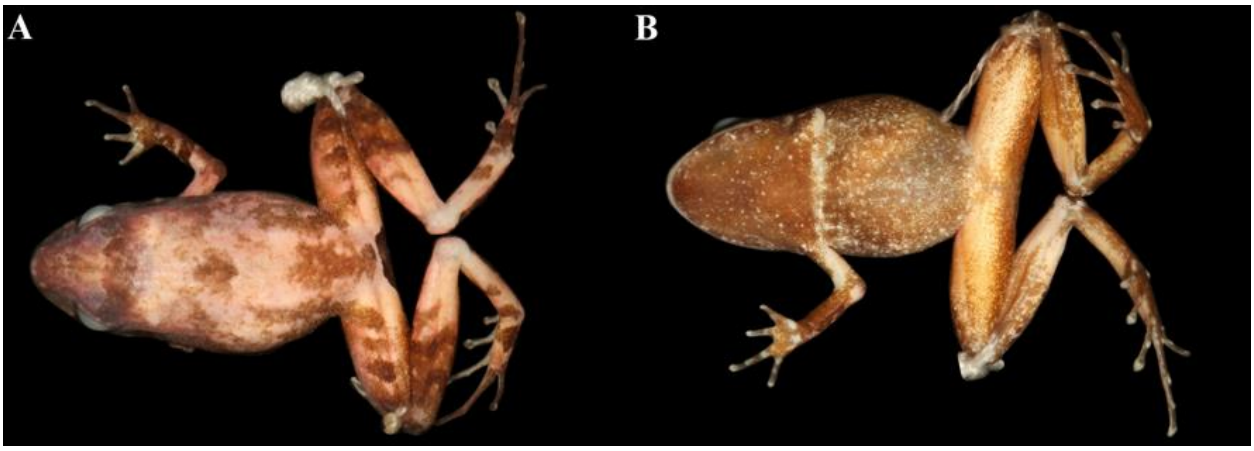


Fig. 8. Dorsal and ventral views in ethanol of the holotype (SMF 104018) of *Craugastor sagui* sp. nov. Photographs taken by G. Köhler.

Diagnosis: The combination of the following characteristics can be used to distinguish *Craugastor* (*Craugastor*) *sagui* (Fig. 4c, 5c and 8) from the other described species in the genus: 1) skin on the dorsum smooth; 2) skin on the venter smooth; 3) vocal slits absent; 4) nuptial pads in adult males; 5) unwebbed toes; 6) heel without an enlarged calcar tubercle, although one to three small tubercles or granules may be present on the heel; 7) accessory palmar, plantar, and supernumerary tubercles absent; and 8) subarticular tubercles flat.

Comparison: *Craugastor sagui* differs from all other Isthmian Central America craugastorids (except for those in the *C. podiciferus* species group) by having unwebbed toes and a narrow head (HW 35.8–40.9 % of SVL). *Craugastor sagui* differs from other members of the *C. podiciferus* species group by having the following characteristics (condition for *C. sagui* in parentheses). *Craugastor bransfordii*, *C. gabbi*, *C. lauraster*, *C. persimilis*, *C. polyptychus*, *C. stejnegerianus*, and *C. underwoodi* differ from *C. sagui* by the following features: a) dorsum usually granular or warty (dorsum smooth to shagreen with scattered tubercles); b) subarticular tubercles projecting (subarticular tubercles flat); and c) venter coarsely areolate, including the midline (venter smooth, at least in midline). *Craugastor podiciferus* differs from *C. sagui* by the

following features: a) a prominent calcar tubercle on the heel (Fig. 6) (calcar tubercle absent, although some specimens can have one to three small tubercles); b) vocal slits present in adult males (vocal slits absent); and c) absence of nuptial pads (nuptial pads present in adult males). *Craugastor aenigmaticus* differs from *C. sagui* by the following features: a) venter coloration of violet-brown with white blotches (venter yellow, orange, grayish or olive in adults); b) white prominent folds between subarticular tubercles on the hands of adults (absence of white folds between subarticular tubercles on the hands of adults); and c) absence of nuptial pads (nuptial pads present in adult males). *Craugastor blairi* differs from *C. sagui* by the following features: a) venter coarsely areolate (venter smooth); b) absence of nuptial pads (nuptial pads present in adult males); c) vocal slits present in adult males (vocal slits absent in adult males); and d) projecting subarticular tubercles (flat subarticular tubercles). *Craugastor zunigai* differs from *C. sagui* by the following features: a) venter coarsely areolate (venter smooth); b) subarticular tubercles projecting (subarticular tubercles flat); c) absence of nuptial pads (nuptial pads present in adult males); and d) vocal slits in adult males (vocal slits absent).

Description of the holotype: Adult male have an SVL of 18.7 mm (Fig. 4–5). Head relatively narrow, HW = 35.8 % of SVL; snout subovoid in the dorsal view, rounded in profile; snout relatively long (HL = 7.0 mm, 37.4 % of SL), with nostrils directed laterally; in the ventral view, tip of the snout protruding slightly beyond the edge of the lower lip. Internarial area convex; canthus rostralis rounded; intercanthal area flat; loreal region slightly concave; vocal slits absent. Eye moderate (EN/ED = 74.07 %), not protruding beyond the dorsal and ventral outline of the head, directed laterally. Tympanic membrane distinct, covered in skin; tympanic annulus prominent, round, relatively large (77.77 % of ED). Skin on all dorsal and lateral surfaces of the head smooth to shagreen. Upper eyelid granular, without superciliar or supraocular tubercles but with a more or less discernible ridge on the outer edge of eyelid continuous with the

supratympanic fold and downward behind the axilla. Postrictal tubercles fused to form a short ridge postero-ventral to the tympanum. Skin on the dorsum and limbs smooth to shagreen. Skin of the chest and throat smooth, venter smooth; ventral surfaces of the thighs smooth; skin of the groin nearly smooth. Flanks shagreen, especially along the antero-ventral flank region. Discoidal fold complete.

Forelimb relatively short and slim; fingers moderately long and slim without lateral fringes. Discs absent; fingers with grooves; tips of fingers unexpanded, rounded in the dorsal view; pads ovoid. Supernumerary tubercles absent; accessory palmar tubercles absent; subarticular tubercles rounded in the basal outline, flat in form and globular in profile; thenar and palmar tubercles elongate and flat; thenar and palmar tubercles similar in size. Ulnar fold absent but several tubercles visible. Fingers not webbed.

Legs relatively long and slim; heel granular, lacking enlarged tubercles. Discs and grooves in all toes, palmate in Toe IV, spadate in others; pads triangular in Toe IV, ovoid in others. Supernumerary and plantar tubercles absent; subarticular tubercles rounded in the basal outline, flat in form and obtuse in profile; inner metatarsal tubercle elongate, globular; outer metatarsal tubercle rounded, globular; outer much smaller than inner; outer edge tarsal with several tubercles, inner smooth. Cloacal opening directed posteriorly at the mid-level of the thighs.

Coloration of the holotype in life: Dorsal background color dark brown-orangish with dark blotches, and a dark interorbital bar. Dorsal surfaces of legs and arms with dark bars, anterior surface of legs with white spots. Upper lip brown-orange with dark bars and scattered white spots. Flanks with a similar dorsal coloration but with a paler orange, with fine white-bluish mottling. Ventral surface of the body and legs dark brown-reddish with white-bluish pigment-forming blotches; ventral surface of the throat similar to the venter but with fewer pale spots. The sole of the feet and hands dark brown with cream-colored tubercles.

Coloration of the holotype in ethanol: After eight years in ethanol (70 %), the overall dark brown-orangish on the dorsum faded to pale cream-pinkish with dark brown blotches. The dark brown-orangish on the ventral surface of the body and legs has faded to dark brown.

Measurements of the holotype (mm): SL 18.7; HL 7.0; HW 6.7; IOD 2.7; EW 1.4; EN 2.0; ED 2.7; TY 2.1. Measurements in related percentages: EW/IOD 51.85 %; IOD/HW 40.3 %; TY/ED 77.78 %; EN/ED 74.07 %; ED/HL 38.57 %; HL/HW 104.48 %; EN/HL 28.57 %.

Etymology: The species was discovered on Cerro Saguí, in western Panama. The scientific name is a noun in the apposition.

Natural history: *Craugastor sagui* inhabits the lower montane rainforest (Holdridge 1967; Bolaños et al. 2005), which is characterized by a very short dry season (one to three months), annual precipitation ranging from 3600 to 7500 mm and annual temperature from 12 to 17 ºC. Very little is known about the natural history of *C. sagui*; however, it is important to note that the species was relatively abundant during the months of fieldwork. The specimens were exclusively found on the floor in the leaf litter, jumping during the active search. We never recorded a vocalization that could be attributed to *C. sagui*; however, it is possible that the species does vocalize.

Distribution: *Craugastor sagui* is restricted to western Panama, on the pacific slopes of Cordillera Central. The specimens were collected on the surrounding slopes of Cerro Saguí and Cerro Santiago (Fig. 1). The known altitudinal range of the new species is 1700–1991 m a.s.l.

***Craugastor zunigai* sp. nov.**

Common name: Zúñiga's Dirt Frog

(Figs. 4d and 9)

Holotype: UCR 22703 (EAP 0618), adult male from Costa Rica: Provincia de Puntarenas: Cantón de Coto Brus: Distrito de Sabalito: Finca Las Alturas, Zona Protectora Las Tablas, (8.976º, -82.834; 1732 m a.s.l.), collected by Erick Arias, Gerardo Chaves, and Omar Zúñiga on 15 September 2015.

Paratypes: UCR 23014 (EAP 0725), UCR 23016 (EAP 0727), UCR 23017 (EAP 0728), and UCR 23018 (EAP 0729), adult females from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Potrero Grande: Tres Colinas, Parque Internacional La Amistad, (9.123º, -83.066º; 1846 m a.s.l.), collected by Erick Arias, Fanny Hernández, and Omar Zúñiga on September 10, 2016. UCR 23176 (EAP 0831), adult male and UCR 23177 (EAP 0832), adult female from Costa Rica: Provincia de Puntarenas: Cantón de Coto Brus: Distrito de Pittier: Santa María de Pittier, Parque Internacional La Amistad, (9.031º, -82.962; 1920 m a.s.l.), collected by Erick Arias and Omar Zúñiga on 27 March 2018.

Assignment to group: Assigned to the genus *Craugastor* based on molecular data and on the following characters: cranial crests absent and Toe III larger than Toe V. Assigned to the *C. podiciferus* species group based on our phylogeny and on the following characters: narrow head (HW/SVL = 35.8–41.9 %), dorsum smooth to shagreen with scattered tubercles, unwebbed toes, vocal slits in adult males, and absence of nuptial pads.

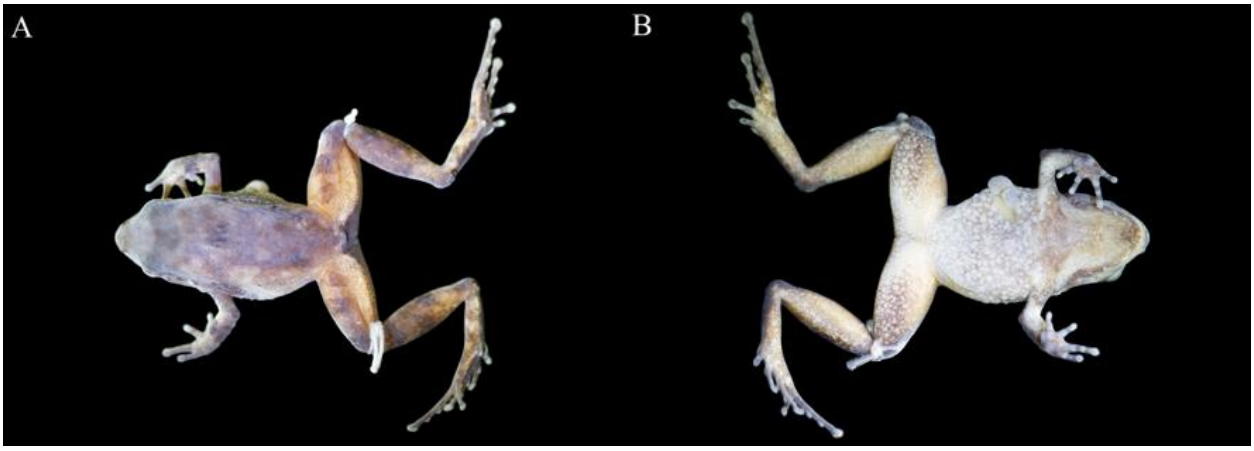


Fig. 9. Dorsal and ventral views in ethanol of the holotype (UCR 22703) of *Craugastor zunigai* sp. nov. Photographs taken by E. Arias.

Diagnosis: The combination of the following characteristics can be used to distinguish *Craugastor* (*Craugastor*) *zunigai* (Fig. 4d, 5d and 9) from other described species in the genus: 1) skin on the dorsum smooth to shagreen with scattered tubercles; 2) skin on the venter coarsely areolate; 3) vocal slits present in adult males; 4) nuptial pads absent; 5) unwebbed toes; 6) heel without a projecting tubercle, although one to three low tubercles or granules can be present; 7) accessory palmar and plantar tubercles present, usually with no supernumerary tubercles under the digits; and 8) subarticular tubercles projecting.

Comparison: *Craugastor zunigai* differs from all other Isthmian Central America craugastorids (except for those in the *C. podiciferus* species group) by having unwebbed toes and a narrow head (HW 35.8–41.9 % of SVL). *Craugastor zunigai* differs from other members of the *C. podiciferus* species group by having the following characteristics (condition for *C. zunigai* in parentheses). *Craugastor bransfordii, C. gabbi, C. lauraster, C. persimilis, C. polyptychus, C. stejnegerianus,* and *C. underwoodi* differ from *C. zunigai* by the following features: a) dorsum usually granular or warty (dorsum is smooth to shagreen with scattered tubercles); b) subarticular tubercles obtuse to pointed, at least distal subarticular tubercles under Toe III and IV

(subarticular tubercles projecting, globular); and c) altitudinal range, 0–1600 m a.s.l. (altitudinal range is 1500–2100 m a.s.l. for *C. zunigai*). *Craugastor podiciferus* differs from *C. zunigai* by the following features: a) a prominent calcar tubercle on the heel (Fig. 6) (calcar tubercle absent, although some specimens can have one to three small tubercles in *C. zunigai*); b) venter smooth (venter coarsely areolate); c) subarticular tubercles flat (subarticular tubercles projecting); and d) absence of accessory palmar tubercles (accessory palmar tubercles present). *Craugastor aenigmaticus* differs from *C. zunigai* by the following features: a) venter smooth (venter coarsely areolate); b) subarticular tubercles flat (projecting subarticular tubercles, globular); and c) prominent white folds between subarticular tubercles (absence of white folds between subarticular tubercles). *Craugastor blairi* differs from *C. zunigai* by the presence of an evident supraocular tubercle (eyelid smooth to granular but without an evident supraocular tubercle). *Craugastor sagui* differs from *C. zunigai* by the following features: a) nuptial pads in adult males (nuptial pads absent); b) absence of vocal slits (vocal slits in adult males); c) venter smooth (venter coarsely areolate); and d) flat subarticular tubercles (projecting subarticular tubercles, globular).

Description of the holotype: Adult male having an SVL of 19.8 mm (Fig. 9). Head relatively narrow, HW = 35.86 % of SVL; snout subovoid in the dorsal view, rounded in profile; snout relatively long (HL = 7.6 mm, 38.38 % of SL), with nostrils directed laterally; in the ventral view, tip of the snout protruding markedly beyond the edge of the lower lip. Internarial area convex (IN 2.2 mm); canthus rostralis rounded; intercanthal area flat (IC = 3.6 mm); loreal region slightly concave; vomerine teeth transverse, in two fascicles behind the choanae. Tongue round in shape, lacking a distinct posterior notch; teeth absent; choanae moderately large, rounded on the posterior half but flat on the anterior half, hemispherical; paired elongate vocal slits under the posterolateral margins of the tongue and a single internal subgular vocal sac. Eye

moderate (EN/ED = 84.1 %), protruding beyond the dorsal outline of head in the ventral view, directed laterally. Tympanic membrane prominent, translucent, and slightly pigmented; tympanic annulus prominent, round, large (134 % of ED). Skin on the dorsal and lateral surfaces of the head smooth. Upper eyelid smooth, without superciliar or supraocular tubercles but with a more or less discernible ridge on the outer edge of the eyelid continuous with the supratympanic fold and downward behind the axilla. Elongate and projecting postrictal tubercle, postero-ventral to the tympanum. Skin on the dorsum and limbs smooth to shagreen with a pair of incomplete dorso-lateral folds, extending from the axillary to the inguinal level. Skin of the chest and throat smooth, venter coarsely areolate with low granules; ventral surfaces of the thighs areolate; skin of the groin smooth. Flanks shagreen with scattered tubercles to areolate, especially along the antero-ventral flank region. Discoidal fold complete.

Forelimb relatively short and robust; fingers moderately long and slim without lateral fringes. Discs absent; fingers III-IV with grooves; tips of fingers unexpanded, rounded in dorsal view; pads ovoid. Supernumerary tubercles absent; four small and rounded accessory palmar tubercles; subarticular tubercles rounded in the basal outline, slightly projecting in form, and globular in profile; thenar tubercle elongate and palmar tubercle rounded, flat, similar in size. Ulnar fold absent but some tubercles visible. Fingers not webbed.

Legs relatively long and slim (TL = 55.3 % SVL); heel smooth with two barely visible low granules, lacking a projecting tubercle. Discs and grooves in all toes, palmate in Toe IV, spadate in others; pads triangular in Toe IV, ovoid in others. Supernumerary tubercles absent; plantar tubercles small and rounded; subarticular tubercles ovoid in the basal outline, projecting in form, and obtuse in profile; inner metatarsal tubercle elongate, projecting; outer metatarsal tubercle rounded, globular; outer metatarsal tubercle much smaller than inner; outer edge tarsal with an indistinct short ridge and low granules, inner smooth. Cloacal opening directed posteriorly at the mid-level of the thighs.

Coloration of the holotype in life: Dorsal background color dark brown suffused laterally with pale brown, head dark brown uniform. Dorsal surfaces of legs and arms with dark bars, posterior surface of legs cream suffused with red. Cloacal opening darker than the posterior surface of the legs. Flanks pale brown, groin suffused with red. Ventral surface of the body and legs cream-yellowish with dark pigment; throat cream with dark pigment. Absence of bars on the lips, mask, and blotches on the dorsal surface.

Coloration of the holotype in ethanol: After three years in ethanol (70 %), the overall dark brown on dorsum has remained very similar to that in life. The cream-yellowish color on the ventral surface of the body and legs has faded to pale brown.

Measurements of the holotype (mm): SL 25.9; HL 10.3; HW 10.3; IOD 3.7; EW 1.7; EN 2.5; ED 2.9; TY 1.9. Measurements in related percentages: EW/IOD 68.82 %; IOD/HW 28.18 %; TY/ED 54.12 %; EN/ED 81.12 %; ED/HL 27.24 %; HL/HW 107 %; EN/HL 24.34 %.

Variation: The morphometric variation is summarized in Table 2. *Craugastor zunigai* shows a relatively high level of intraspecific polymorphism. In some specimens, a pair of lateral folds is present, and some specimens show a supraocular tubercle. In some specimens, two unfused postrictal tubercles are visible. Palmar and thenar tubercles are equal in size in some specimens, thenar slightly smaller than palmar in others. The palmar tubercle is heart-shaped in some specimens and ovoid in others. UCR 20389 with a pair of lateral folds from the axillar level to the cloaca bordered by black pigment, the area between folds and from the snout to the cloaca a lighter, cream-yellowish color in ethanol. UCR 20428 with a pattern of dark brown on the dorsum with a lighter interorbital mark and area anterior to it paler than the dorsum. The mask is absent in some specimens. The throat has a nearly uniform cream color in UCR 20401, heavily

mottled in UCR 20421, and nearly uniform dark brown in UCR 20389. The venter ranges from a nearly uniform cream color without dark pigment in UCR 20401 to heavily mottled in UCR 20389.

Etymology: The name *zunigai* is a patronym honoring the field-guide Omar Zuñiga in recognition of his important aid during the fieldwork on the Cordillera de Talamanca. Omar Zuñiga took part in the fieldwork that yielded the specimens from Tres Colinas, Las Alturas, Las Tablas, and Santa María de Pittier.

Natural history: *Craugastor zunigai* inhabits the lower montane rainforest (Holdridge 1967; Bolaños et al. 2005), which is characterized by a very short dry season (one to three months), annual precipitation ranging from 3600 to 7500 mm and annual temperature from 12 to 17 ºC. Very little is known about the natural history of *C. zunigai*; however, is important to note that the species was relatively abundant during the months of fieldwork. The specimens were always found on the floor jumping during the active search. We never recorded a vocalization that could be attributed to *C. zunigai*; however, it is possible that the species vocalize. At Las Alturas, *C. zunigai* occur very near the *C. gabbi* localities; however, we did not find them in sympatry since they are altitudinally structured. In Tres Colinas, the pattern is similar but with *C. stejnegerianus*.

Distribution: *Craugastor zunigai* is restricted to Southwestern Costa Rica, on the Pacific slopes of Cordillera de Talamanca. The specimens were collected from Santa María de Pittier, Tres Colinas, Las Alturas de Cotón, and road to Las Tablas (Fig. 1). The altitudinal range of the new species is 1500–2100 m a.s.l. All populations were found in primary forests, the populations from Santa María de Pittier and Tres Colinas are in La Amistad International Park and the population from Las Alturas de Cotón and road to Las Tablas is in Zona Protectora Las Tablas. The populations of *C. zunigai* are fragmented over ~43 km. This species was not found on the

pacific slope of Cerro Utyum and Cerro Dúrika (to the west). Additional fieldwork is necessary to clarify the distribution of *C. zunigai*. The locality Road to Las Tablas is very close to the Costa Rica-Panama border, so *C. zunigai* is likely also present in Panama.

Discussion

With the recognition of *Craugastor blairi*, *C. zunigai*, and *C. sagui*, the *C. podiciferus* species group is now formed by 12 species, all of which are collectively distributed in Costa Rica and western Panama (Savage 2002; AmphibiaWeb 2019). The species in this group have been difficult to delineate historically because they are morphologically variable between and within populations. However, using molecular sequence data, we found well-supported clades and large genetic distances between several of these populations (Table 1). Although the sole use of genetic distances for species delimitation is not a recommended practice, we believe that large genetic distances between phylogenetically related and geographically close species are one important measure to identify cryptic species in species groups with a conservative morphology. The genetic distances we present herein between members of the *C. podiciferus* species group are above the thresholds of 3 % in the 16S rRNA and 10 % in the COI mitochondrial genes suggested by Fouquet et al. (2007) and Vences et al. (2005). For amphibians, the 16S rRNA gene fragment has been suggested as a DNA barcode marker for amphibian diversity inventories (Vences et al. 2005) to complement the standard COI-5' marker used in general for animals (Smith et al. 2008). Although the species recognized herein are not cryptic *sensu stricto* (Pérez-Ponce de León and Nadler 2010), they are in taxonomic practice. As has been suggested by Pérez-Ponce de León and Nadler (2010), the use of molecular data within morphologically conserved groups allow us to delineate taxa that otherwise would be considered a single taxon. The use of phylogenies based on molecular data has been essential to solve taxonomic problems in this group.

Table 3. Main diagnostic characteristic and character states for secondary sexual characteristics of the species forming the *Craugastor podiciferus* species group.

Species	Dorsal skin texture	Venter skin texture	Thenar and palmar tubercles in size relation	Subarticular in profile	Super-numeray tubercles	Accessory palmar tubercles	Heel skin texture	Nuptial pads	Vocal slits	Vocal sac
C. aenigmaticus	Smooth to weakly granular	Smooth with granules on laterally	Thenar tubercle much smaller than palmar tubercle	Flat	Absent	Absent	Smooth to three low granules not projecting	Absent	Absent	Absent
C. bransfordii	Granular to warty	Coarsely areolate	Thenar tubercle equal or slightly smaller than palmar tubercle	Projecting	Present	Present	Granular	Present	Absent	Absent
C. blairi	Smooth to shagreen with scattered tubercles	Coarsely areolate	Thenar tubercle equal or slightly smaller than palmar tubercle	Projecting	Absent	Present	Smooth to several low granules not projecting	Absent	Present	Present
C. gabbi	Shagreen with scattered granules to granular	Coarsely areolate	Thenar tubercle much smaller than palmar tubercle	Projecting	Present	Present	Granular	Absent	Absent	Absent
C. lauraster	Granular to warty	Coarsely areolate	Thenar tubercle much smaller than palmar tubercle	Projecting	Present	Present	Granular	Absent	Absent	Absent
C. persimilis	Granular	Coarsely areolate	Thenar tubercle much smaller than palmar tubercle	Projecting	Present	Present	Granular	Absent	Absent	Absent
C. podiciferus	Smooth to scattered tubercles	Smooth	Thenar tubercle equal or slightly smaller than palmar tubercle	Flat	Absent	Absent	A projecting tubercle	Absent	Present	Present
C. polyptychus	Granular to warty	Coarsely areolate	Thenar tubercle equal or slightly smaller than palmar tubercle	Projecting	Present	Present	Granular	Absent	Absent	Absent
C. sagui sp. nov.	Smooth to shagreen with scattered tubercles	Smooth	Thenar tubercle equal or slightly smaller than palmar tubercle	Usually flatted, but projecting in some specimens	Absent	Absent	Smooth to several low granules not projecting	Present	Absent	Absent
C. stejnegerianus	Granular to warty	Coarsely areolate	Thenar tubercle much smaller than palmar tubercle	Projecting	Present	Present	Granular	Absent	Absent	Absent
C. underwoodi	Granular to warty	Coarsely areolate	Thenar tubercle equal or slightly smaller than palmar tubercle	Slightly projecting	Usually absent	Present	Granular	Present	Absent	Absent
C. zunigai sp. nov.	Smooth to shagreen with scattered tubercles	Coarsely granular	Thenar tubercle equal or slightly smaller than palmar tubercle	Slightly projecting	Absent	Present	Granular to two low granules not projecting	Absent	Present	Present

Taylor (1952) synonymized *C. blairi* under *C. podiciferus* without further discussion, nor was it discussed in the comprehensive seminar work of Savage (2002). Our results support the validity of *Craugastor blairi* (previously shown as *Craugastor* sp. B), which it is not a sister to *C. podiciferus* (Crawford and Smith 2005; Streicher et al. 2009). However, *C. blairi* and *C. podiciferus* are morphologically similar and show a high level of phenotypic polymorphism, and thus the taxonomic decision of Taylor (1952) is not surprising given that the technical capabilities at that time were limited. The distinctiveness of *C. blairi* is provided by its phylogenetic position. The same rules apply to *C. sagui*, the phylogenetic position of which provides significant evidence for its different evolutionary trajectory as a species beyond the observation that it resembles *C. podiciferus* and *C. blairi* in morphology. The morphological similarity among nonsister species can be explained either as plesiomorphy or convergence (Castroviejo-Fisher et al. 2017). All species analyzed herein have in common that they inhabit the ground leaf litter in highland forest habitats (lower montane zonation), which has likely played a role in the maintenance of this morphology.

A more complicated situation is observed the recognition of *Craugastor zunigai*, given its morphological resemblance to its sister species *C. blairi*. Although the morphological divergence between *C. blairi* and *C. zunigai* is weak, we believe that both are valid species supported by phylogenetic distinctiveness based on mitochondrial analysis. These species are geographically very close (~10 airline km); thus, it seems unlikely that the large genetic distances (2.9 % in 16S and 14.3 % in COI) are explained by isolation due to distance. Our taxonomic decision to recognize these two as separate species will be strengthened with additional evidence, especially the comparison of mating calls of *C. zunigai* with the call of *C. blairi*, which we have described herein. Future sampling efforts must also be conducted on the highlands of extreme western Panama and adjacent Costa Rica between Barú Volcano and Cerro Pando to explore the species

distribution limits and possible contact zones of *C. aenigmaticus*, *C. blairi*, *C. zunigai*, and *C. podiciferus.*

The presence of several species within *Craugastor podiciferus* is uncertain. Streicher et al. (2009) suggested that the current concept of *C. podiciferus* could be masking six distinct species that are geographically structured but with two instances of sympatry. However, the findings of Streicher et al. (2009) and our own results agree with *C. podiciferus* being a monophyletic entity; thus, there is currently no need to split *C. podiciferus* in several species until such division is supported by detailed morphological or other evidence. We strongly suggest the performance of an extensive, integrative analysis of the *C. podiciferus* species complex to re-evaluate the taxonomic status of its different genetic clades. For now, the recognition of *C. podiciferus sensu latu* as a single species results in a highly variable morphological group, given that even those few useful characters (*i.e.*, skin on venter, subarticular tubercles, vocal slits, and nuptial pads) are highly variable between populations. The clades shown herein should be reevaluated using other types of data that can provide evidence of lineage divergence, such as the geographical distribution, ecological niche, mating calls, or detailed morphometric data.

The members of the *Craugastor podiciferus* species group have qualities such as high abundance, broad distribution (collectively), and high genetic diversity that make them suitable for use in a variety of different studies in ecology and evolution. However, these species have been poorly studied likely because of the difficulty in clearly identifying the species. Therefore, it is necessary to clarify the taxonomy of this species group. Upon revisiting our study objectives, we report the following: 1) *Craugastor podiciferus sensu stricto* is restricted to the populations of Cordillera Volcánica Central, Costa Rica and Cordillera de Talamanca on Costa Rica and western Panama, and the latter is restricted to the Caribbean slopes; 2) six well-supported clades are found within the current concept of *C. podiciferus*, these six clades require

extensive taxonomic revision; and 3) the populations from southwestern Costa Rica and western Panama are grouped in three clades, one potentially referring to an existing name, *C. blairi*, which is resurrected herein, and two representing new species that are herein named *C. sagui* from western Panama and *C. zunigai* from southwestern Costa Rica.

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Appendix I. Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Museum collection acronyms follow Frost (2019) with the addition of EAP to refer to Erick Arias field numbers and CRARC to refer to the Costa Rica Amphibian Research Center private collection.

Species	**Institutional vouchers**	**Collection locality**	**Elevation (m)**	**Geographic coordinates**		**GenBank Number**	
				Lat	Lon	16S	COI
C. aenigmaticus	SMF: 104020	Changuinola, Bocas del Toro, PA	2388	8.9139	-82.7088	MK211615	MK211577
C. aenigmaticus	UCR: 21951	Telire, Talamanca, CR	2700	9.3488	-83.1750	MK211616	MK211578
C. aenigmaticus	UCR: 22737	Buenos Aires, Puntarenas, CR	2660	9.3224	-83.2028	MK211617	MK211579
C. blairi	FMNH: 257689	Gualaca, Chiriquí, PA	1000	8.7500	-82.2170	EF562353	ND
C. blairi	SMF: 102024	Gualaca, Chiriquí, PA	1730	8.6775	-82.1980	MK211627	MK211583
C. blairi	SMF: 104023	Gualaca, Chiriquí, PA	1280	8.6781	-82.2101	MK211628	MK211584
C. blairi	SMF: 104027	Bugaba, Chiriquí, CR	2134	8.8494	-82.5154	MK211629	MK211585
C. blairi	SMF: 104033	Gualaca, Chiriquí, PA	1456	8.6740	-82.2154	MK279367	ND
C. blairi	SMF: 104034	Ñurum, Ngöbe Buglé, PA	1541	8.5512	-81.4833	MK279368	ND
C. blairi	SMF: 104037	Boquete, Chiriquí, PA	1952	8.7757	-82.3901	MK279369	ND
C. blairi	USNM: 563039	Boquete, Chiriquí, PA	1663	8.3136	-82.4000	EF562356	ND
C. bransfordii	UCR: 22269	Alajuela, Alajuela, CR	466	10.3121	-84.1778	KT950295	MK211571
C. bransfordii	UCR: 22643	Siquírres, Limón, CR	537	10.0595	-83.5452	MK211610	MK211572
C. gabbi	UCR: 21863	Coto Brus, Puntarenas, CR	1200	8.7889	-82.9583	KT950271	MK211567
C. gabbi	UCR: 21864	Coto Brus, Puntarenas, CR	1200	8.7889	-82.9583	KT950272	MK211568
C. lauraster	SMF: 79759	Matagalpa, Matagalpa, NI	1300	12.9993	-85.9092	MK211608	MK211565
C. lauraster	USNM: 559393	Puerto Lempira, Gracias a Dios, HN	190	14.9275	-84.5339	KU323364	MK211566
C. persimilis	UCR: 22211	Paraíso, Cartago, CR	1050	9.7841	-83.7517	KT950293	MK211570
C. persimilis	UCR: 22671	Talamanca. Limón, CR	121	9.5773	-82.9343	MK211609	MK211569

Appendix I. *Continued*.

Species	Institutional vouchers	Collection locality	Elevation (m)	Geographic coordinates		GenBank Number	
				Lat	Lon	16S	COI
C. podiciferus sensu stricto	CRARC: 0012	Turrialba, Cartago, CR	2250	10.0192	-83.7132	MK211633	MK211589
C. podiciferus sensu stricto	SMF: 104005	Changuinola, Bocas del Toro, PA	1766	8.9908	-82.6716	MK211641	MK211597
C. podiciferus sensu stricto	UCR: 19853	Telire, Talamanca, CR	1817	9.3580	-83.2294	MK211639	MK211595
C. podiciferus sensu stricto	UCR: 19856	Telire, Talamanca, CR	1817	9.3580	-83.2294	MK211637	MK211593
C. podiciferus sensu stricto	UCR: 19860	Telire, Talamanca, CR	2108	9.3645	-83.2164	MK211636	MK211592
C. podiciferus sensu stricto	UCR: 19862	Telire, Talamanca, CR	2108	9.3645	-83.2164	MK211638	MK211594
C. podiciferus sensu stricto	UCR: 20992	Alfaro Ruiz, Alajuela, CR	2143	10.2272	-84.3482	MK211632	MK211588
C. podiciferus sensu stricto	UCR: 22146	Vázquez de Coronado, San José, CR	1700	10.0263	-83.9448	MK211635	MK211591
C. podiciferus sensu stricto	UCR: 22201	Dota, San José, CR	2395	9.7126	-83.9488	MK211634	MK211590
C. podiciferus sensu stricto	UCR: 23175	Talamanca, Limón, CR	1860	9.3659	-83.0417	MK211640	MK211596
C. podiciferus "Chumacera"	UCR: 22120	Buenos Aires, Puntarenas, CR	1821	9.3218	-83.4546	MK211642	ND
C. podiciferus "Chumacera"	UCR: 22690	Pérez Zeledón, San José, CR	1793	9.3267	-83.4706	MK211631	MK211587
C. podiciferus "Fila Costeña"	EAP: 0509	Golfito, Puntarenas, CR	1546	8.7878	-83.0306	ND	MK211605
C. podiciferus "Fila Costeña"	FMNH: 257651	Coto Brus, Puntarenas, CR	1350	8.7833	-82.9833	EF562367	ND
C. podiciferus "Fila Costeña"	UCR: 16585	Dota, San José, CR	1400	9.5353	-83.8580	MK211647	ND
C. podiciferus "Fila Costeña"	UCR: 22091	Pérez Zeledón, San José, CR	1488	9.4410	-83.6830	MK211646	MK211604
C. podiciferus "Monte Verde"	FMNH: 257669	Monte Verde, Puntarenas, CR	1500	10.2773	-84.5891	EF562372	MK211598
C. podiciferus "Monte Verde"	FMNH: 257673	Monte Verde, Puntarenas, CR	1500	10.2773	-84.5891	EF562343	MK211603
C. podiciferus "Monte Verde"	UCR: 16361	Alfaro Ruiz, Alajuela, CR	1930	10.2176	-84.3671	EF562371	ND
C. podiciferus "Monte Verde"	UCR: 22675	Puntarenas, Puntarenas, CR	1726	10.3202	-84.7987	ND	MK211606
C. podiciferus "Pico Blanco"	UCR: 22226	Escazú, San José, CR	2242	9.8646	-84.1429	MK211644	MK211601
C. podiciferus "Pico Blanco"	UCR: 22228	Escazú, San José, CR	2242	9.8646	-84.1429	MK211643	MK211600

Appendix I. *Continued*.

Species	Institutional vouchers	Collection locality	Elevation (m)	Geographic coordinates		GenBank Number	
				Lat	Lon	16S	COI
C. podiciferus "San Gerardo"	CRARC: 0247	Tilarán, Guanacaste, CR	1470	10.3600	-84.8000	MK211645	ND
C. podiciferus "San Gerardo"	FMNH: 257671	Monte Verde, Puntarenas, CR	1500	10.2773	-84.5891	EF562374	MK211599
C. podiciferus "San Gerardo"	UCR: 16353	Sarapiquí, Heredia, CR	1500	10.2022	-84.1625	EF562349	MK211602
C. podiciferus "Siola"	UCR: 23169	Talamanca, Limón, CR	1300	9.3987	-83.0200	MK211630	MK211586
C. polyptychus	UCR: 20050	Talamanca, Limón, CR	900	9.6178	-83.2681	MK211614	MK211576
C. polyptychus	UCR: 22668	Talamanca, Limón, CR	198	9.6064	-82.9115	MK211613	MK211575
C. sagui sp. nov.	SMF: 104014	Nole Duima, Ngöbe Buglé, PA	1762	8.5571	-81.8245	MK211623	ND
C. sagui sp. nov.	SMF: 104015	Nole Duima, Ngöbe Buglé, PA	1700	8.4997	-81.7724	MK211624	MK211580
C. sagui sp. nov.	SMF: 104017	Nole Duima, Ngöbe Buglé, PA	1815	8.4955	-81.7672	MK279370	ND
C. stejnegerianus	EAP: 0514	Osa, Puntarenas, CR	45	8.9655	-83.4411	MK211607	MK211563
C. stejnegerianus	UCR: 20352	Buenos Aires, Puntarenas, CR	900	9.0863	-83.1105	KT950284	MK211564
C. underwoodi	UCR: 22619	Paraíso, Cartago, CR	1412	9.7518	-83.7792	MK211611	MK211573
C. underwoodi	UCR: 22625	Vázquez de Coronado, San José, CR	1708	10.0254	-83.9456	MK211612	MK211574
C. zunigai sp. nov.	UCR: 20389	Buenos Aires, Puntarenas, CR	1500	9.1112	-83.1006	MK211625	MK211581
C. zunigai sp. nov.	UCR: 20428	Buenos Aires, Puntarenas, CR	1800	9.1381	-83.0700	MK279371	ND
C. zunigai sp. nov.	UCR: 22703	Coto Brus, Puntarenas, CR	1732	8.9759	-82.8344	MK279372	ND
C. zunigai sp. nov.	UCR: 22709	Coto Brus, Puntarenas, CR	1980	8.9751	-82.8243	MK211626	MK211582

Appendix II. Specimens used in the morphometric analysis. Museum collection acronyms follow Frost (2019) with the addition of AH to refer to Andreas Hertz field numbers and HAU to refer to Frank Hauenschild field numbers.

Craugastor blairi: PANAMA: *Chiriquí*: Bajo Mono, Los Naranjos, Boquete (AH: 0289–0290; SMF: 104028); Cerro La Estrella, Jaramillo, Boquete (SMF: 104037); Cerro Guayaba, Caldera, Boquete (HAU: 023; SMF: 104035); Volcán Barú, Los Naranjos, Boquete (AH: 0240–1; SMF: 104026–7); Fortuna, Hornito, Gualaca (AH: 0079–0080, 0372, 00376; HAU: 008, 010; SMF: 102024, 104023, 104025, 104029–33). *Ngöbe Buglé:* Guayabito, Ñurüm (SMF: 104034).

***Craugastor sagui* sp. nov.:** PANAMA: *Ngöbe Buglé:* Cerro Saguí, Jädeberi, Nole Duima (SMF: 104014, 104018–9); La Nevera, Nole Duima (AH: 0168; SMF: 104015–7).

***Craugastor zunigai* sp. nov.:** COSTA RICA: *Puntarenas*: Las Alturas, Pittier, Coto Brus (UCR: 22703–4, 22709–10); Tres Colinas, Potrero Grande, Buenos Aires (UCR: 20257–8, 20389, 20395, 20401, 20411, 20419, 20421, 20423, 20428, 23014, 23016–8); road to Las Tablas, Sabalito, Coto Brus (UCR: 23170).

CAPÍTULO II.II

A NEW SPECIES OF THE *CRAUGASTOR PODICIFERUS* SPECIES GROUP (ANURA: CRAUGASTORIDAE) FROM THE PREMONTANE FOREST OF SOUTHWESTERN COSTA RICA

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A new species of the *Craugastor podiciferus* species group (Anura: Craugastoridae) from the premontane forest of southwestern Costa Rica

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Abstract

In this report, we describe a new species of the *Craugastor podiciferus* species group from the premontane forest of the Pacific versant along the Costa Rican-Panamanian border. Mitochondrial DNA and karyotype analyses previously showed a marked genetic divergence between populations of the premontane forest of the Fila Costeña and the lowlands South Pacific Costa Rica near Panama. Analyses of the mitochondrial DNA sequences and the morphological variation revealed significant differences between the populations of the premontane forest relative to the other populations of *C. stejnegerianus*, including the type locality. We recognize these premontane populations as a new species and show that they differ from the typical *C. stejnegerianus* in the coloration of the venter, the head and the body proportions, and mtDNA divergence. With the addition of this new species, the *C. podiciferus* species group now contains nine species.

Key words: Brachycephaloidea, Costa Rica, *Craugastor gabbi* **sp. nov.**, *Craugastor stejnegerianus*, cryptic species, Panama, taxonomy, Terrarana

Introduction

Terrarana (Brachycephaloidea, sensu Padial *et al.* 2014) is a clade of New World frogs that use a terrestrial breeding mode and direct development (Hedges *et al.* 2008). Currently, this clade is composed of more than 1000 species distributed throughout the New World tropics (Padial *et al.* 2014). Due to its size and complexity, this group has been the target of multiple molecular phylogenetic analyses, resulting in the extensive changes of its composition at all levels; *Eleutherodactylus* (Duméril & Bibron), the largest vertebrate genus at one time, was split into multiple genera and families (Hedges *et al.* 2008; Heinicke *et al.* 2009; Padial *et al.* 2014), and numerous non-monophyletic subgeneric taxa have been identified (Padial *et al.* 2014). *Craugastor,* one of the largest components of the Terraranas, is distributed from the southern USA to northwestern Colombia (Hedges *et al.* 2008) and is composed of 114 species (AmphibiaWeb 2016). This genus is considered monophyletic based on jaw morphology (Lynch 1986) and mitochondrial and nuclear markers (Crawford & Smith 2005; Frost *et al.* 2006; Heinicke *et al.* 2007; Hedges *et al.* 2008; Pyron & Wiens 2011; Padial *et al.* 2014). Several species complexes are recognized within *Craugastor* (Hedges *et al.* 2008; Padial *et al.* 2014); however, a lack of morphological distinction among these taxa have made taxonomic work difficult (Savage 2002), and several cryptic new species that are masked under some of the nominal species remain undescribed (Savage 2002; Crawford 2003; Crawford *et al.* 2007; Hedges *et al.* 2008; Streicher *et al.* 2009; McCranie 2015).

Craugastor stejnegerianus (Cope 1893), a member of the *C. podiciferus* species group, was described by Cope (1893) as *Hylodes stejnegerianus*, based on a specimen from Palmar de Osa, Puntarenas, Costa Rica. Günther

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(1885-1902) placed this species in the synonymy of *H. polyptychus* (Cope 1886); subsequently, however, Taylor (1952) resurrected and re-described this species and placed it in the genus *Microbatrachylus* (Taylor). Based on an analysis of the color pattern polymorphisms, Savage & Emerson (1970) concluded that *C. stejnegerianus*, together with the rest of the *C. podiciferus* species group, were synonyms of *Eleutherodactylus bransfordii* (Cope 1886).

Using biochemical data, Miyamoto (1983) determined that the populations of *E. bransfordii* from the Pacific versant of Costa Rica were markedly divergent from the Caribbean versants; thus the name *E. stejnegerianus* was resurrected and applied to the populations of the Pacific versant of Costa Rica. This finding was subsequently supported by karyotypic differences (Chen 2001, 2005). Savage (2002) found morphological differences between *E. bransfordii* and *E. stejnegerianus* and consolidated the hypothesis of specific differentiation. Phylogenetic and population genetic studies based on mitochondrial and nuclear DNA sequence data (Crawford 2003) revealed deep genetic divergences between the populations of *C. stejnegerianus* and showed that this species was more closely related to *C. persimilis* (Barbour 1926) than to *C. bransfordii*.

In this study, we perform morphological and molecular phylogenetic analyses of all populations referred to as *C. stejnegerianus* of the South Pacific Costa Rica and western Panama to determine the taxonomic status of the lowland and premontane populations. We propose the recognition of the premontane populations as a distinct species closely related to *C. stejnegerianus.*

Materials and methods

Species criterion. Our definition of species follows the general metapopulation lineage species concept (de Queiroz 2007). Because we adhere to this concept, we recognize a species when there is evidence of the separation of metapopulation lineages, preferably based on multiple lines of evidence following the consensus protocol for integrative taxonomy (Padial *et al.* 2010).

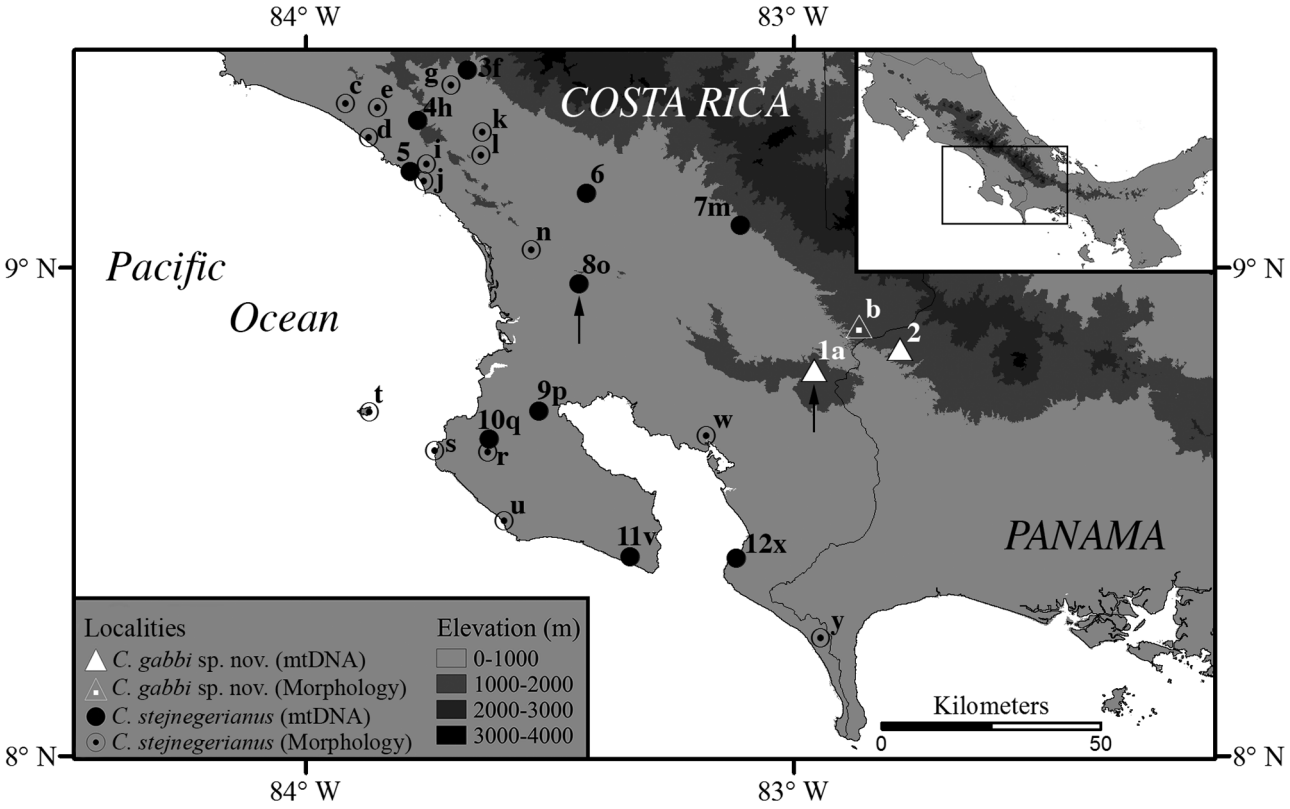

FIGURE 1. Map showing the sampling sites. Samples indicated by the solid color were included in the molecular analyses, and the sites indicated by a number plus letters were included in the morphological analyses. Samples from the sites indicated by open circles and open triangles were included only in the morphological analyses. The numbers correspond to the locality ID in Table 1. Letter codes refer to Table 2.

Taxon sampling. Tissue samples were collected from 26 *Craugastor podiciferus* species group specimens from 16 localities of Costa Rica (Table 1, Fig. 1). The frogs were collected in the field and euthanized, and liver or muscle tissue was preserved in 95% ethanol or RNAlater. Voucher specimens were fixed in 10% formalin, stored in 70% ethanol, and deposited at the Museo de Zoología, Universidad de Costa Rica (UCR) and the Division of Amphibians and Reptiles at the Field Museum of Natural History (FMNH). Additional tissue samples of five specimens of three localities of Panama and Honduras were kindly provided (see Acknowledgments).

Amplification and sequencing. We extracted and sequenced a fragment of the mitochondrial 16S rRNA gene from seven samples of the new species described here, 15 samples referred to as *Craugastor stejnegerianus* from 10 populations, two *C. persimilis,* two *C. bransfordii,* two *C. lauraster* (Savage, McCranie & Espinal 1996), two *C. underwoodi* (Boulenger 1896), and a specimen referred to *C. podiciferus* as an outgroup (Table 1). The total genomic DNA was extracted from the preserved tissues (liver or muscle) using the Animal Genomic DNA Kit (BioBasic Canada Inc.). We used the polymerase chain reaction (PCR) to amplify the mitochondrial 16S rRNA gene (16S) using the primers 16Sbr and 16Sar (Palumbi *et al.* 1991). The PCR amplifications were performed using a total volume of 15 µL, which contained 1 µL DNA template (*c.* 50 ng μL^{-1}), 0.75 U Taq polymerase (Amplificasa®, Biotecnologias Moleculares), 1X PCR buffer with 1.5 mm $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), and 0.3 µM forward and reverse primers. The PCR conditions consisted of an initial cycle of 5 min at 94ºC, followed by 35 cycles of 45 s at 94ºC, 30 or 45 s at 50ºC or 55ºC, 45 or 120 s at 72ºC, plus a final cycle of 5 min at 72ºC. The fragments were sequenced in both directions using the original amplification primers and BigDye termination reaction chemistry (Applied Biosystems). After cycle sequencing, the products were column-purified using a Sephadex G-50 (GE Healthcare) and were run on an ABI PRISM 3100 DNA Analyzer (Applied Biosystems). Consensus sequences for each individual were constructed using SEQUENCHER 5.3 (Genes Codes Corp.). The resulting sequences were deposited in GenBank (Table 1).

Phylogenetic analyses. 16S sequences were aligned using the MUSCLE 3.7 software (Edgar 2004). We used the MrModelTest 2.3 (Nylander 2004) and the Akaike Information Criterion (AIC) to select an appropriate model of the DNA sequence evolution, which was the GTR + I model. Analyses were performed using both the maximum likelihood (ML) and Bayesian analyses (BA). The ML analyses were performed using RAxML 8.1.11 (Stamatakis 2014), including 1000 bootstrap replicates to evaluate nodal support. The Bayesian phylogenetic analyses were performed using MrBayes 3.2.2 (Huelsenbeck & Ronquist 2001) and 10 heated MCMC samples of every 1000 generations for 50 million generations. We examined a time-series plot of the likelihood scores of the cold chain to check stationarity using Tracer 1.6 software (Rambaut *et al*. 2014). We discarded the first 25% of trees as burn-in and used the remaining trees to estimate the consensus tree along with the posterior probabilities for each node and each parameter. Estimates of pairwise evolutionary genetic divergence between and within groups were computed using MEGA6 (Tamura *et al.* 2013), assuming corrected distances based on the Tamura 3-parameter model (Tamura 1992), with rate variation among the sites modeled as a gamma distribution with the shape parameter = 4 as the default of the software.

Morphometric analyses. We examined 35 *Craugastor* specimens from the premontane forest of Fila Costeña and the Cordillera de Talamanca near the Costa Rican-Panamanian border and 155 specimens representing several populations from lowland South Pacific Costa Rica, including the type locality of *C. stejnegerianus* (Table 2, Fig. 1; Appendix). All material was deposited at the Museo de Zoología (UCR), Universidad de Costa Rica, San José, Costa Rica. The following morphological measurements were recorded, as described by Savage (2002) and Duellman & Lehr (2009): snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), inter orbital distance (IOD), tympanum diameter (TY), width of the upper eyelid (EW), tibia length (TL), eye-nostril distance (E-N), lengths of the toes (T1, T2, T3, T4, T5), and lengths of the fingers (F1, F2, F3, F4). Measurements were made using dial calipers and were rounded to the nearest 0.1 mm. To remove the effect of body size, each morphological measurement was regressed against the SVL, and the residuals from a linear fit with the SVL were used in further analyses. The additional proportions reported here include: EW/IOD, TY/ED, E-N/ED, ED/HL, IOD/HW, and T4/TL. The sex of the individuals was determined based on the TY/ED ratio and gonadal morphology. TY/ED > 0.6 corresponds to males. The specimens with opaque seminal vesicles were assumed to be adult males, and those with developed oviducts were assumed to be adult females. The general terminology for the morphological characteristics follows Duellman & Lehr (2009). We followed Savage (2002) in our usage of the term "supernumerary tubercles," which we use to refer to the tubercles on the phalanges (between subarticular tubercles); this is different from the tubercles referred to here as accessory palmares or plantares tubercles.

TABLE 1. Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Numbers in the ID column correspond to the collection sites indicated in Figure 1. UCR voucher number refers to the Museo de Zoología, Universidad de Costa Rica. SMF refers to the Senckenberg Research Institute and Nature Museum, Frankfurt, Germany. FMNH refers to the Division of Amphibians and Reptiles at the Field Museum of Natural History, Chicago, USA.

ID	Species	Institutional vouchers	Collection locality	Geographic coordinates	Elevation (m)	GenBank Number
1	*C. gabbi* **sp. nov.**	UCR: 21863	Fila Costeña, Coto Brus, Puntarenas, CR	+8.789°, -82.958°	1200	KT950271
1	*C. gabbi* **sp. nov.**	UCR: 21864	Fila Costeña, Coto Brus, Puntarenas, CR	+8.789°, -82.958°	1200	KT950272
1	*C. gabbi* **sp. nov.**	UCR: 21865	Fila Costeña, Coto Brus, Puntarenas, CR	+8.789°, -82.958°	1200	KT950273
1	*C. gabbi* **sp. nov.**	UCR: 21876	Fila Costeña, Coto Brus, Puntarenas, CR	+8.789°, -82.958°	1200	KT950274
2	*C. gabbi* **sp. nov.**	SMF: 99863	Santa Clara, Renacimiento, Chiriquí, PA	+8.833º, -82.783º	1200	KT950275
2	*C. gabbi* **sp. nov.**	SMF: 99864	Santa Clara, Renacimiento, Chiriquí, PA	+8.833º, -82.783º	1200	KT950276
2	*C. gabbi* **sp. nov.**	SMF: 99865	Santa Clara, Renacimiento, Chiriquí, PA	+8.833º, -82.783º	1200	KT950277
3	*C. stejnegerianus*	UCR: 22128	Rivas, Pérez Zeledón, San José, CR	+9.404º, -83.669º	857	KT950278
4	*C. stejnegerianus*	UCR: 22103	Tinamaste, Pérez Zeledón, San José, CR	+9.301º, -83.771º	740	KT950279
5	*C. stejnegerianus*	UCR: 22280	Playa Hermosa, Osa, Puntarenas, CR	+9.197º, -83.787º	30	KT950280
6	*C. stejnegerianus*	UCR: 22070	Volcán, Buenos Aires, Puntarenas, CR	+9.152º, -83.426º	415	KT950281
6	*C. stejnegerianus*	UCR: 22071	Volcán, Buenos Aires, Puntarenas, CR	+9.152º, -83.426º	415	KT950282
7	*C. stejnegerianus*	UCR: 20346	Potrero Grande, Buenos Aires, Puntarenas, CR	+9.086º, -83.111º	900	KT950283
7	*C. stejnegerianus*	UCR: 20352	Potrero Grande, Buenos Aires, Puntarenas, CR	+9.086º, -83.111º	900	KT950284
8	*C. stejnegerianus*	UCR: 22134	Palmar Norte, Osa, Puntarenas, CR	+8.966°, -83.441°	45	KT950285
8	*C. stejnegerianus*	UCR: 22136	Palmar Norte, Osa, Puntarenas, CR	+8.966°, -83.441°	45	KT950286
8	*C. stejnegerianus*	UCR: 22137	Palmar Norte, Osa, Puntarenas, CR	+8.966°, -83.441°	45	KT950287
9	*C. stejnegerianus*	FMNH: 257803	Rincón, Osa, Puntarenas, CR	+8.705°, -83.524°	125	KT950288
9	*C. stejnegerianus*	FMNH: 257804	Rincón, Osa, Puntarenas, CR	+8.705°, -83.524°	125	KT950289
10	*C. stejnegerianus*	UCR: 16276	Agujas, Osa, Puntarenas, CR	+8.648º, -83.625º	200	KT950290
11	*C. stejnegerianus*	UCR: 20454	Piro, Golfito, Puntarenas, CR	+8.407º, -83.336º	100	KT950291
12	*C. stejnegerianus*	UCR: 21494	El Higo, Golfito, Puntarenas, CR	+8.405°, -83.119°	10	KT950292
NA	*C. persimilis*	UCR: 22211	Tausito, Paraiso, Cartago, CR	+9.784º, -83.752º	1050	KT950293
NA	*C. persimilis*	UCR: 22212	Tausito, Paraiso, Cartago, CR	+9.784º, -83.752º	1050	KT950294
NA	*C. bransfordii*	UCR: 22269	San Miguel, Alajuela, Alajuela, CR	+10.312º, -84.178º	465	KT950295
NA	*C. bransfordii*	UCR: 22270	San Miguel, Alajuela, Alajuela, CR	+10.312º, -84.178º	465	KT950296
NA	*C. underwoodi*	UCR: 22147	Cascajal, Coronado, San José, CR	+10.026º, -83.945º	1700	KT950297
NA	*C. underwoodi*	UCR: 20203	Fila Matama, Limón, Limón, CR	+9.813º, -83.168º	1200	KT950298
NA	*C. podiciferus*	UCR: 22201	El Empalme, Dota, San José, CR	+9.713º, -83.949º	2400	KT950299
NA	*C. lauraster*	USNM: 559393	Bodega del Río, Tapalwás, Gracias a Dios, HN	+14.928°, -84.534°	150	KU323364
NA	*C. lauraster*	ENS: 10761	Sierra de Agalta, Olancho, HN	+14.935°, -86.143°	1317	KU323365

TABLE 2. Institutional voucher numbers and locality information of the specimens used in the morphological analyses. Letters in the ID column correspond to the collection sites indicated in Figure 1.

ID	Collection locality	Geographic coordinates	Elevation (m)	Individuals	Institutional voucher (UCR)
a	Fila Costeña, Coto Brus, Puntarenas, CR	+8.789°, -82.958°	1200	34	8644, 8645, 12507–12512, 12514, 12524–12526, 12935, 13237, 13240–13242, 14671–14673, 15830, 15832–15834, 18531–5, 21863–21865, 21867, 21876
b	Río Negro, Coto Brus, Puntarenas, CR	+8.878º, -82.867º	1100	1	8684
c	Dos Bocas, Aguirre, Puntarenas, CR	+9.336º, -83.919º	350	2	14536, 14569
d	Dominical, Aguirre, Puntarenas, CR	+9.167º, -83.872º	100	6	14306, 14706, 14734, 15980, 15986, 16163
e	Tres Piedras, Pérez Zeledón, San José, CR	+9.328º, -83.854º	100	6	5010, 5011, 14737, 16330, 16334, 16336
f	Rivas, Pérez Zeledón, San José, CR	+9.404º, -83.669º	857	7	16278–16280, 22127, 22128, 22131, 22132
g	San Isidro, Pérez Zeledón, San José, CR	+9.373º, -83.703º	700	5	5097–5101
h	Tinamaste, Pérez Zeledón, San José, CR	+9.301º, -83.771º	740	6	16331–16333, 22100, 22101, 22103
i	San Josecito, Osa, Puntarenas, CR	+9.212º, -83.754º	400	8	19353, 19527–19529, 19546–19548, 19589
j	Uvita, Osa, Puntarenas, CR	+9.177º, -83.759º	10	10	19272, 19277, 19278, 19282–19284, 19324–19326, 19328
k	Juntas de Pacuar, Pérez Zeledón, San José, CR	+9.278º, -83.639º	560	6	4234–4239
l	Mollejones, Pérez Zeledón, San José, CR	+9.229º, -83.642º	800	3	15991, 15993, 15994
m	Potrero Grande, Buenos Aires, Puntarenas, CR	+9.086º, -83.111º	900	5	20307–20309, 20346, 20352
n	Cerrón, Osa, Puntarenas, CR	+9.036°, -83.539°	700	3	14230, 14235, 14244
o	Palmar Norte, Osa, Puntarenas, CR	+8.966°, -83.441°	45	6	7865, 7866, 9057, 20885, 22136, 22137
p	Rincón, Osa, Puntarenas, CR	+8.705°, -83.524°	125	12	799, 800, 1081–1084, 1158, 4576, 8733, 11350, 11351, 11621
q	Agujas, Osa, Puntarenas, CR	+8.648º, -83.625º	200	3	16275, 16276, 16345
r	Agujas, Osa, Puntarenas, CR	+8.622º, -83.628°	100	7	13683, 13684, 16571–16575
s	San Pedrillo, Osa, Puntarenas, CR	+8.624°, -83.737°	100	1	6400
t	Isla del Caño, Osa, Puntarenas, CR	+8.704°, -83.870°	20	10	3558–3560, 11309, 13673, 13674, 16346–16349
u	Sirena, Golfito, Puntarenas, CR	+8.481°, -83.594°	100	10	11302, 11305, 11306, 11308, 11311–11313, 16277, 16340, 16341
v	Piro, Golfito, Puntarenas, CR	+8.407º, -83.336º	100	12	18339, 18340, 18356–18359, 18361–18364, 19230, 20454
w	Naranjal, Golfito, Puntarenas, CR	+8.656°, -83.181°	35	23	11969, 11970, 12246, 12272–19289, 14958, 14959
x	El Higo, Golfito, Puntarenas, CR	+8.405°, -83.119°	10	1	21494
y	Pavones, Golfito, Puntarenas, CR	+8.241°, -82.946°	100	3	12717–12719

Dorsal color pattern. We followed Savage & Emerson (1970) in defining the dorsal color pattern polymorphisms as follows: M = mottled, U = uniform, L = striped, Z_1 = tympanic stripe, Z_2 = tympanic stripe in combination with eye mask, N = skin dorsum smooth, O = skin dorsum irregularly granular, and Q = a pair of dorsal ridges.

Statistical analyses. We calculated the mean, standard deviation, and range for each morphometric variable using the program JMP 12 (SAS Institute). We compared the populations using ANOVA ($\alpha = 0.05$) when the assumptions of normality (Shapiro-Wilk, $\alpha = 0.05$) and homoscedasticity (Levene, $\alpha = 0.05$) were met. When the assumption of normality failed, we used the Kruskal-Wallis test ($\alpha = 0.01$); when there was heteroscedasticity, we used Welch's ANOVA ($\alpha = 0.01$). The following variables were subject to principal components analyses (PCA) with subsequent ANOVA of the PC1 and PC2 scores: HL/SVL, TL/SVL, E-N/SVL, F2/SVL, and IOD/HW. The same variables were used in a discriminant analysis to calculate the probability of the correct classification of the specimens to their original population.

TABLE 3. Results from the morphological analyses. Significant *P*-values are indicated in bold. Abbreviations: S.D. = standard deviation, P = probability. The statistical test used is indicated in the last column as follows: † = ANOVA, †† = Welch's ANOVA, § = Kruskal-Wallis test.

Variable	*Craugastor gabbi* **sp. nov.**		*Craugastor stejnegerianus*		P*
	Mean±S.D.	Range	Mean±S.D	Range	
SVL	16.72±2.18	13.60–21.55	15.58±2.63	9.50–21.40	**0.018**†
HL/SVL	0.33±0.03	0.29–0.41	0.36±0.03	0.30–0.45	**<0.001**§
HW/SVL	0.34±0.02	0.31–0.38	0.35±0.02	0.32–0.40	0.047§
ED/SVL	0.12±0.02	0.08–0.15	0.12±0.01	0.08–0.16	0.472§
IOD/SVL	0.11±0.01	0.10–0.13	0.11±0.01	0.08–0.17	**<0.001**†
TY/SVL_{males}	0.08±0.02	0.06–0.11	0.09±0.01	0.06–0.11	0.873††
$TY/SVL_{females}$	0.06±0.01	0.05–0.08	0.06±0.01	0.03–0.08	0.113§
EW/SVL	0.08±0.01	0.06–0.11	0.08±0.01	0.05–0.10	0.273†
TL/SVL	0.55±0.02	0.50–0.61	0.52±0.03	0.46–0.58	**<0.001**†
E-N/SVL	0.09±0.02	0.04–0.11	0.10±0.01	0.06–0.12	**<0.001**§
T1/SVL	0.10±0.02	0.06–0.14	0.10±0.01	0.07–0.14	0.590††
T2/SVL	0.19±0.03	0.14–0.24	0.19±0.02	0.12–0.24	0.398†
T3/SVL	0.32±0.03	0.26–0.41	0.31±0.03	0.25–0.38	0.433††
T4/SVL	0.48±0.04	0.37–0.55	0.47±0.04	0.37–0.54	0.228§
T5/SVL	0.30±0.03	0.23–0.35	0.29±0.03	0.22–0.34	0.226§
F1/SVL	0.10±0.02	0.08–0.14	0.11±0.01	0.07–0.15	**0.006**§
F2/SVL	0.13±0.02	0.11–0.17	0.14±0.02	0.09–0.18	**0.001**†
F3/SVL	0.21±0.02	0.16–0.25	0.21±0.02	0.16–0.26	0.148†
F4/SVL	0.14±0.02	0.10–0.19	0.15±0.02	0.10–0.20	0.279†
EW/IOD	0.67±0.12	0.50–1.03	0.74±0.13	0.41–1.31	**0.001**§
TY/ED_{males}	0.76±0.14	0.60–1.11	0.74±0.09	0.60–0.92	0.818§
$TY/ED_{females}$	0.52±0.06	0.39–0.59	0.49±0.05	0.31–0.59	0.090†
E-N/ED	0.74±0.16	0.36–1.05	0.81±0.12	0.50–1.20	**0.002**††
ED/HL	0.35±0.05	0.25–0.49	0.34±0.03	0.24–0.41	0.121§
IOD/HW	0.33±0.02	0.28–0.40	0.31±0.04	0.22–0.48	**<0.001**§
T4/TL	0.86±0.06	0.69–1.01	0.90±0.06	0.74–1.02	**<0.001**§

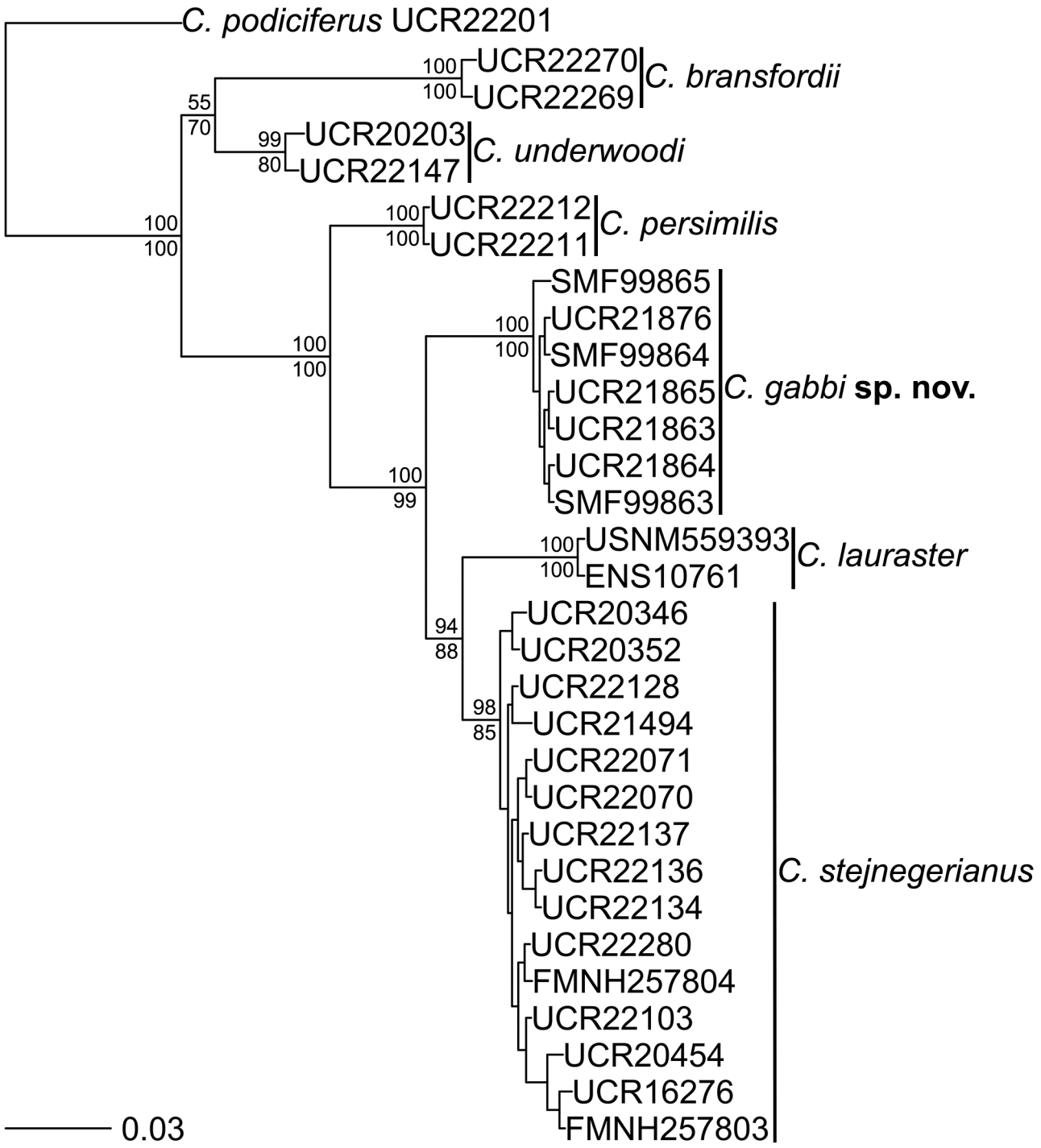

FIGURE 2. Bayesian phylogenetic inference of the relationships of *Craugastor gabbi* **sp. nov.** within the *C. podiciferus* species group based on the 16S mitochondrial DNA gene fragment. Bayesian posterior probabilities (multiplied by 100) are shown above the branch; maximum likelihood bootstrap values from the RAxML analysis are shown below the branches. The scale bar refers to the estimated substitutions per site. The support values of any node within species are not shown.

Results

Molecular analyses. The resulting data matrix had a total length of 539 bp, including gaps. The phylogenies inferred using ML and BA were concordant in supporting the tree shown in Fig. 2. The phylogeny shows two well-supported clades. One is formed of all the samples from the premontane forest of the South Pacific Costa Rica and western Panama (populations "a" and "b", Fig. 1), and the second is formed of all the samples from lowland South

Pacific Costa Rica (populations "c-y", Fig. 1). The genetic divergence between these two clades was 4.5% for the 16S gene. These two clades are nonetheless not sister taxa as *Craugastor stejnegerianus* + *C. lauraster* form a clade separated from *C. gabbi* and show mean-corrected genetic divergence of 4.7%, supporting that *C. stejnegerianus,* is more closely related to *C. lauraster* than to *C. gabbi.* Our results highlight the independent evolutionary status of the populations from the premontane forest and lead us to recognize it as a separate evolutionary unit: *Craugastor gabbi* **sp. nov.**

Morphometric analyses. Morphometric variation and comparisons among the populations are shown in Table 3. The following characteristics were significantly different between the lowland and premontane populations: SVL, HL/SVL, IOD/SVL, TL/SVL, E-N/SVL, F1/SVL, F2/SVL, EW/IOD, E-N/ED, IOD/HW, and T4/TL. The PCA efficiently differentiated the premontane and lowland frogs (Fig. 3). The first principal component (PC1) explained 40.6% of the total variance, with 68.0% of the total variance explained by the first two components. In PC2, TL/SVL and IOD/HW were positively loaded, while HL/SVL, E-N/SVL, and F2/SVL were negatively loaded. PC1 and PC2 differentiated the two populations (PC1: F=12.03, df=189, p=0.001; PC2: F=114.51, df=189, p=<0.001). The discriminant analysis correctly classified 91.55% of the specimens to the populations, demonstrating a clear separation between the lowland and premontane populations studied here (Wilks' lambda 0.512, p=<0.001).

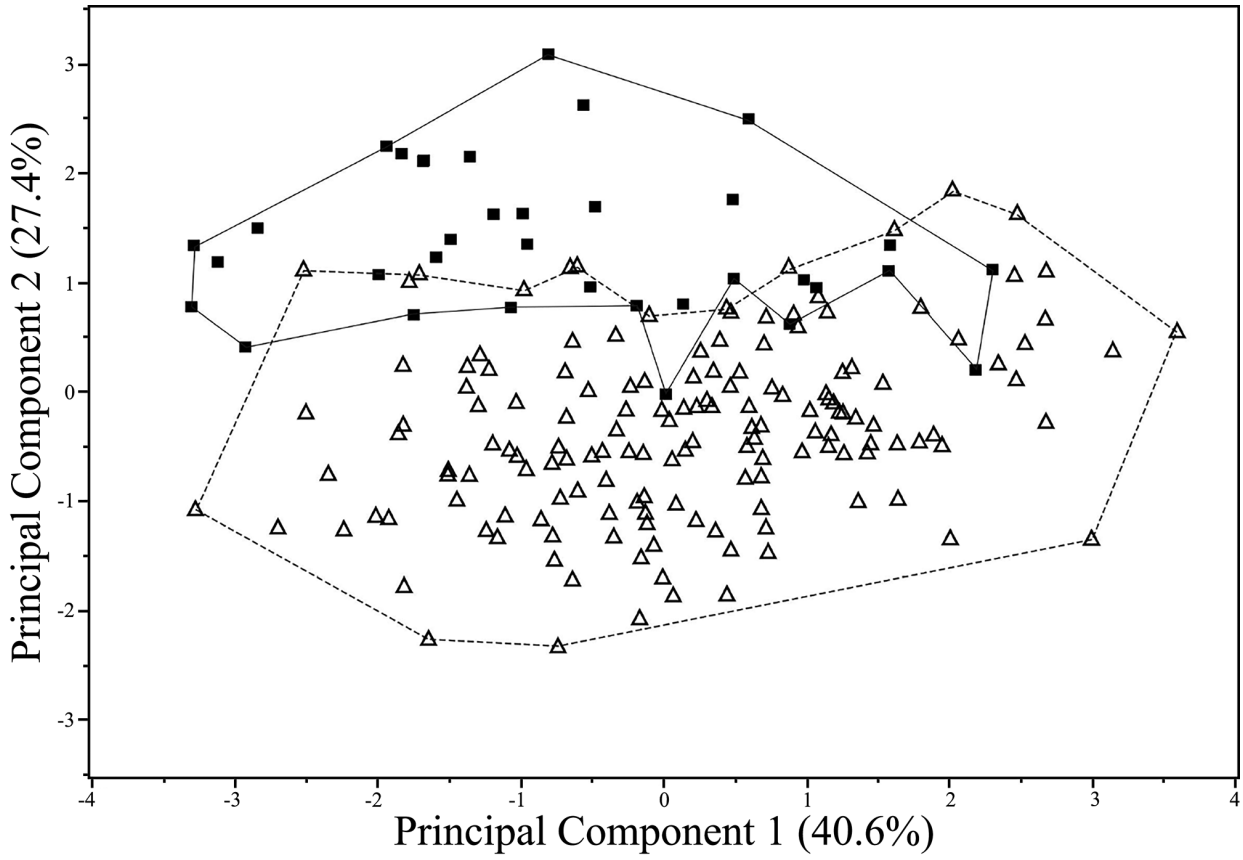

FIGURE 3. Principal component analysis showing the morphological separation among the 35 individuals of *Craugastor gabbi* **sp. nov.** (solid squares) from the premontane forest near the Costa Rican-Panamanian border and 155 individuals of *C. stejnegerianus* (open triangles) from the lowlands of South Pacific Costa Rica.

Description of new species

***Craugastor gabbi* sp. nov.**
Gabb's Dirt Frog
(Figures 4–6)

Craugastor stejnegerianus (part): Scott 1976; Savage 2002; Crawford 2003; Santos-Barrera *et al.* 2008

Holotype. UCR 21864, an adult female from the Organization for Tropical Studies' Las Cruces Biological Station (+8.7889º, -82.9583º; 1200 m elevation), Fila Costeña, San Vito de Coto Brus, Puntarenas Province, Costa Rica; collected by Erick Arias, Gerardo Chaves, Adrián García-Rodríguez, and Federico Bolaños on 16 March 2013.

Paratypes. Adult male UCR 21865 and UCR 21867; adult female UCR 21863 and UCR 21876; same data as the holotype.

Assignment to group. The following inclusion characteristics were used to assign frogs to the *C. podiciferus* species group: narrow head (31–38% SVL), presence of venter areolate, a shorter Finger I than Finger II, absence of inner tarsal fold, and absence of nuptial pads.

Diagnosis. A small species of the *Craugastor* (*Craugastor*) *podiciferus* species group with the following characteristics: (1) skin on the dorsum is shagreen with scattered enlarged granules; venter and flanks are coarsely areolate, usually with dorsolateral and lateral folds; discoidal fold complete laterally and posteriorly; (2) tympanum round, usually with membrane differentiated and annulus prominent, (TY/ED = 39–111%), usually without supratympanic fold; (3) snout subovoid in dorsal view, rounded in profile; loreal region concave; canthus-rostralis usually rounded; (4) upper eyelid granular (EW/IOD = 50–103%); cranial crests absent; (5) vomerine teeth transverse, in two fascicles well behind the choanae; choanae smaller than the dentigerous; (6) vocal slits absent; nuptial pads absent; (7) Finger I slightly shorter than Finger II; disks usually absent, but with terminal transverse grooves; tips usually lanceolate; pads triangular; (8) fingers lacking lateral fringes; thenar and palmar tubercles ovoid, thenar much smaller than palmar; proximal supernumerary tubercles rounded; usually two accessory palmar tubercles; subarticular tubercles round, projecting and obtuse; (9) ulnar fold absent, but tubercles sometimes are visible; (10) heel lacking tubercles; inner tarsal folds usually absent; (11) inner metatarsal tubercle elongate, outer rounded, much smaller than inner; proximal supernumerary tubercles rounded; numerous (usually 10–15) rounded plantar tubercles; subarticular tubercles ovoid, projecting and obtuse; (12) Toe III larger than Toe V; disks expanded, asymmetric; disk cover usually lanceolate; disk pad triangular; toe webbing basal, usually does not reach the proximal subarticular tubercle on Toes I-II-III-IV; however, in some specimens webbing reaches the proximal subarticular tubercle or beyond; (13) dorsum gray brown to dark brown, uniform, mottled, some specimens have a middorsal light stripe, or, rarely, paired dorsolateral light stripes; venter cream with dark pigment reaching the midline; throat usually yellowish with dark mottling; upper surfaces of thighs usually with dark bars; posterior surface of thigh uniform reddish brown; usually labial bars and supratympanic mark (Fig. 4); (14) SVL in males 14.35–21.35 mm; SVL in females 13.60–21.55 mm.

Comparisons with other species. *Craugastor gabbi* differs from all the other craugastorids of Lower Central America, except for those in the *C. podiciferus* species group, which have basal webbing between the toes and a narrow head, i.e., head width 31–38% SVL. *Craugastor gabbi* differs from other members of the *C. podiciferus* species group by having the following characteristics (condition for *C. gabbi* in parentheses, see Table 4). *Craugastor gabbi* differs from *C. bransfordii, C. polyptychus* and *C. underwoodi* by having a thenar tubercle equal to or slightly smaller than the palmar tubercle (thenar tubercle definitely much smaller than palmar tubercle, Fig. 5); *C. gabbi* differs from *C. podiciferus* by having a prominent calcar tubercle on the heel (calcar tubercle absent) and by the absence of supernumerary tubercles (supernumerary tubercles well defined in fingers and toes); from *C. jota* (Lynch 1980) by having a prominent calcar tubercle on the heel (calcar tubercle absent); from *C. lauraster* by having an immaculate white venter (having a cream-colored venter with dark pigment reaching the midline, Fig. 4) and by having a much shorter Finger I than Finger II (Finger I slightly shorter than Finger II); from *C. persimilis* by having toes unwebbed (basal webbing between toes), by having dorsum and forelimbs areolate (dorsum and forelimbs shagreen), and by having a much shorter Finger I than Finger II (Finger I slightly shorter than Finger II); from *C. stejnegerianus* by having an immaculate white venter, [only the 8% of the specimens reviewed here had a venter with dark pigment reaching the midline] (venter cream-colored with dark pigment reaching the midline, Fig. 6) and by having a significantly larger TL/SVL and IOD/HW and a significantly smaller HL/SVL and E-N/SVL. Is important to note that the specimens of the northernmost populations of the Central Pacific of Costa Rica had the venter with dark pigment reaching the midline; however, these populations are under taxonomic revision because they could represent a separate species.

Description of holotype. Adult female; head width 36.5% of SVL; head length 38.6% of SVL; snout subovoid in dorsal view, rounded in profile; canthus-rostralis indistinct; loreal region slightly concave; nostrils small, directed laterally; vomerine teeth transverse, in two fascicles well behind the choanae; eye larger, diameter equal to 126.67% of E–N; tympanum small, 57.69% of ED, round, with membrane undifferentiated and annulus

prominent; skin on dorsum shagreen, venter coarsely areolate, throat and head smooth, flanks shagreen like the dorsum; a pair of dorsolateral folds extend from the orbit to the sacrum, and a pair of lateral folds extend from the axillar level to the sacrum; discoidal fold complete; upper eyelid granular; postrictal tubercles fused forming a short ridge posterior-ventral to the tympanum.

Forelimb slim; ulnar tubercles and fold absent; thenar and palmar tubercles ovoid, with the thenar much smaller than palmar; proximal supernumerary tubercles rounded, medial supernumerary tubercles only on Finger III; three accessory palmar tubercles much smaller than the supernumerary tubercles; subarticular tubercles larger, round, projecting and obtuse; fingers slim; disks absent; fingers with grooves; tips of fingers lanceolate in dorsal view; pads triangular; fingers not webbed.

Hindlimb slightly slim; heel smooth, inner tarsal fold absent; inner metatarsal tubercle elongate, outer rounded, much smaller than inner; proximal supernumerary tubercles rounded, medial supernumerary tubercles only on Toes III and IV; numerous rounded plantar tubercles; subarticular tubercles rounded, projecting and pungent; disks expanded, asymmetric; disk cover lanceolate; disk pad triangular; webbing basal between Toes I-II-III-IV, reaching the proximal subarticular tubercle on Toe I.

FIGURE 4. a) Dorsal and **b)** ventral photos of *Craugastor gabbi* **sp. nov.** holotype (UCR 21864). The scale bar represents 0.5 cm. Photos by Mauricio Calderón-Rivera.

Coloration of the holotype in ethanol (Fig. 4). Dorsum of head and back dark brown to grayish with a pair of dark spots on each flank, a pair of dark spots on the back, an oblique dark stripe crossing the sacrum, and a middorsal light stripe; upper lip with diffuse dark bars; a dark supratympanic stripe extending from the orbit to the suprascapular shoulder; venter cream-colored and dotted with dark pigment; throat cream with dark mottling, not contrasting with the venter; groin cream, with a few small dark dots, not contrasting with the flanks; flanks light brown but transition dorsally to dark brown and ventrally to cream with dark dots, which penetrate the venter; dorsal surface of hind limbs similar to dorsal background with dark bars, which extend over the tibia and feet; posterior and anterior surfaces of hind limbs uniform dark brown.

Measurements of holotype (mm). SVL 19.7; HL 7.6; HW 7.2; ED 2.6; IOD 2.3; TY 1.5; EW 1.8; TL 10.4; E–N 2.1; T1 1.7; T2 3.7; T3 5.1; T4 9.50; T5 6.1; F1 2.4; F2 3.0; F3 4.2; F4 3.20.

Morphometric (mm) and morphological variation of paratypes. Morphometric variation of all specimens analyzed is summarized in Table 3. Here we provide the mean and standard deviation and, in parentheses, the range of each measurement of all the paratypes. SVL 16.9±2.0 (14.8–19.3); HL 6.7±0.81 (6.0–7.8); HW 6.2±0.8 (5.4–7.1); ED 2.3±0.3 (1.9–2.5); IOD 2.1±0.3 (1.8–2.5); TY in males 1.6, in females 1.4±0.2 (1.2–1.4); EW 1.3±0.3 (1.1–1.7); TL 9.3±0.9 (8.4–10.4); E-N 1.7±0.3 (1.5–2.1); T1 1.4±0.3 (1.1–1.7); T2 2.3±0.4 (2.7–3.6); T3

5.1±0.6 (4.5–5.9); T4 7.9±0.6 (7.2–8.4); T5 4.9±0.4 (4.6–5.4); F1 2.0±0.2 (1.8–2.3); F2 2.2±0.4 (1.8–2.6); F3 3.6±0.4 (3.0–4.1); F4 2.4±0.5 (1.9–3.0).

The scant variation among the paratypes is described as follows. Tympanic membrane differentiated in UCR 21863, UCR 21865, and UCR 21876; a pair of dorsolateral folds extending from the axillar level to the sacrum in UCR 21865 and UCR 21867; a pair of lateral folds extending from the orbit to the sacrum in UCR 21865; discoidal fold indistinguishable in UCR 21863, UCR 21865, UCR 21867 and UCR 21876; two tubercles postrictal in UCR 21867, semifused in UCR 21863 and UCR 21865; inner tarsal fold incomplete in UCR 21863. Fingers without medial supernumerary tubercles in UCR 21863 and UCR 21865; only two palmar accessory tubercles in UCR 21863, UCR 21865, and UCR 21876; toes without medial supernumerary tubercles in UCR 21863 and UCR 21865; dorsum with a pair of broad lateral light stripes extending from the snout to the groin, these stripes are divided by a pair of dark stripes extending from the axillar level to the groin, forming a stripe pattern in UCR 21876, with a pair of lateral light stripes extending from the orbit to the groin in UCR 21865; labial marks absent in UCR 21863; supratympanic mark absent in UCR 21876.

Variation in other specimens not observed in paratypes. Some specimens with the snout subelliptical in dorsal view; canthus-rostralis rounded in some; others with vomerine teeth obtuse; some specimens with a pair of dorsolateral folds extending from the orbit to the anus; lateral folds variable to absent, from the orbit to groin, from the orbit to anus, from the axillary level to sacrum and from the axillary level to groin; supratympanic fold distinctly curved downwards present in several specimens; some with only one postrictal tubercle; a specimen with inner tarsal fold complete; the inguinal gland was elevated and prominent in some individuals, in others this was not evident, although we did not observe a relation between this characteristic and sex. Some specimens with four or five ulnar tubercles in a row; some without grooves in fingers; at least one specimen with disk expanded in Fingers III and IV; webbing between Toes I-II can reach proximal subarticular tubercle, the web between Toes II-III-IV can reach least half of the proximal phalanx.

Patterns variation. In comparison with the other species of the *C. podiciferus* species group, *C. gabbi* had low levels of dorsal color pattern variation. The holotype had the formula MZ_1OQ (mottled dorsal pattern with stripe supratympanic, skin texture irregularly granular and a pair of dorsal ridges); this formula was present in 25% of the specimens. The most common formula was UZ_1OQ at 34.4%, described by Savage & Emerson (1970) as Morpho V. The following formulae were also observed: LZ_1OQ present in 9.4%; UZ_1NQ, MOQ, and MO present in 6.3%; UZ_2OQ, LZ1NQ, LOQ, and MZ_1NQ present in 3.1%.

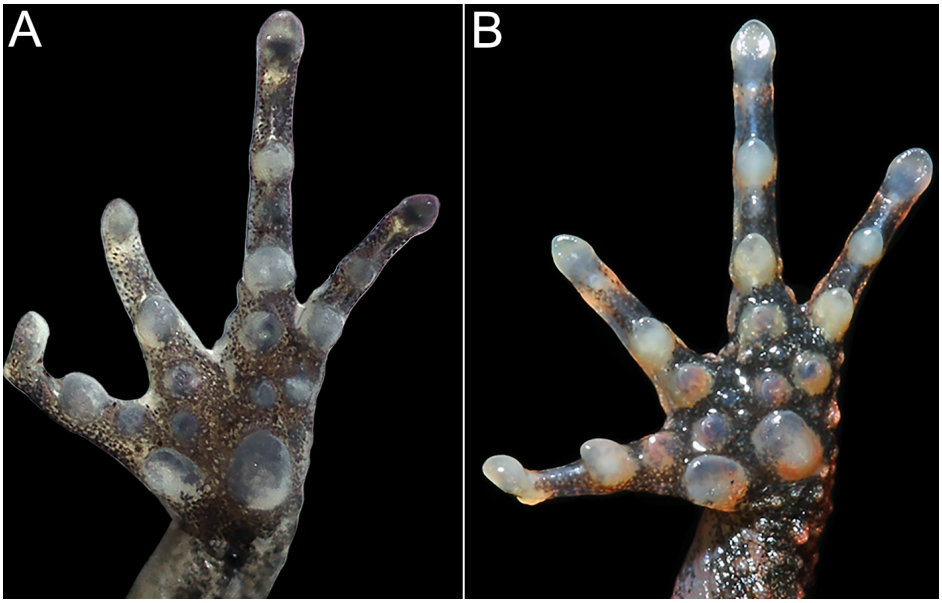

FIGURE 5. Comparison of the thenar and palmar tubercles. **a)** *Craugastor gabbi* **sp. nov.** holotype (UCR 21864) showing smaller size of thenar tubercle compared to the palmar tubercle and **b)** *C. bransfordii* (CRARC0177) of Turrialba, Costa Rica showing thenar tubercle to be equal to or slightly smaller than the palmar tubercle. This condition is also seen in *C. underwoodi* and *C. polyptychus*. Photo A by E.A and B by Brian Kubicki.

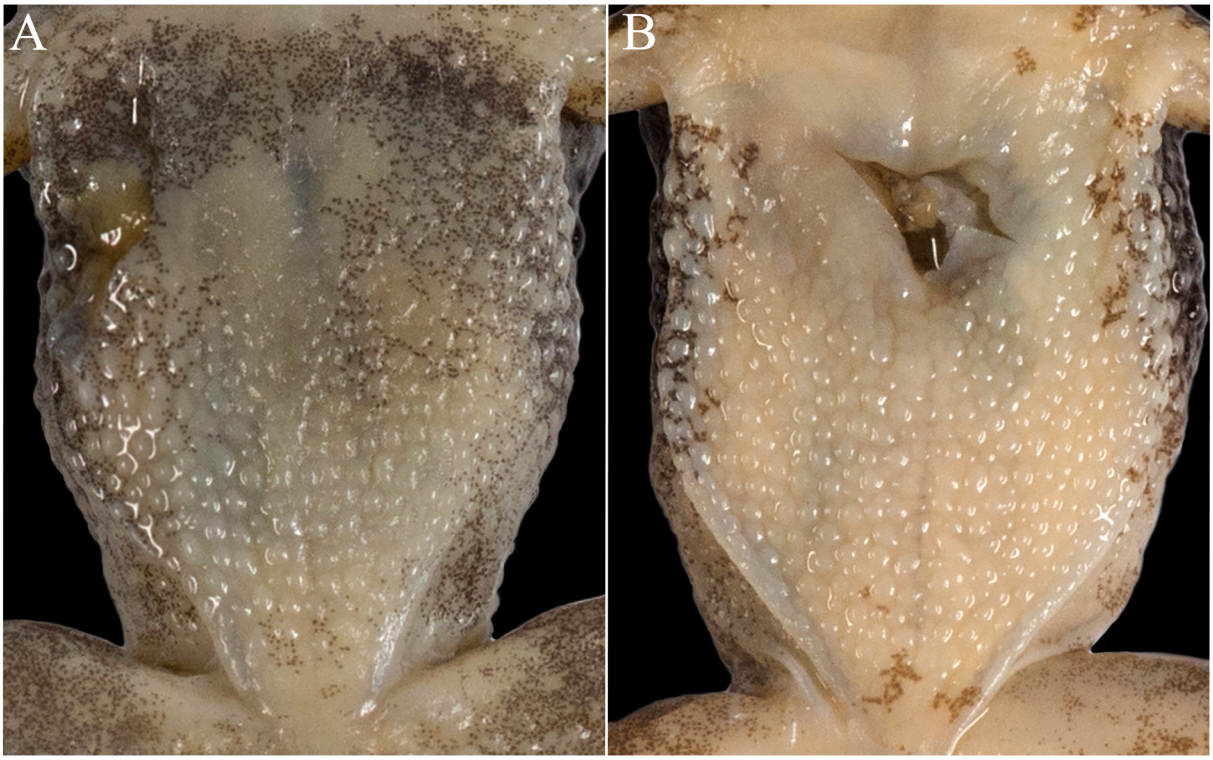

FIGURE 6. Ventral coloration. **a)** *Craugastor gabbi* **sp. nov.** paratype (UCR 21876), with the dark pigment reaching the midline and **b)** *C. stejnegerianus* (UCR 20885) of Palmar Norte, Costa Rica showing immaculate venter. Photos by Mauricio Calderón-Rivera.

The dorsal background color ranged from light gray to blackish brown; at least one individual presented longitudinal lines from the orbit to groin, light color on a dark background, and another two specimens presented dark lines on a light background. In eight individuals, the pattern of contrasting mottles was strongly prominent. The throat color varied from cream to dark brown, strongly contrasting with the lighter-colored venter. No association between the throat color and sex was observed. At least one individual had a well-defined mask, and the head of another was completely black. The supratympanic dark mark was present in most, but not all, individuals; labial bars were also present in most, but not all, specimens.

Natural history notes. *Craugastor gabbi* is abundant in the Fila Costeña, Coto Brus region. Santos-Barrera *et al.* (2008) reported this species at 21 of the 27 study sites, stating that *C. gabbi* was the most abundant amphibian in the forest and the coffee plantations and was also present in pastures. Usually, this species is associated with leaf litter. This species may reach densities of up 4586 individuals/ha (Scott 1976). *Craugastor gabbi* is diurnal (Savage 2002) and reproductively active throughout the year (Santos-Barrera *et al*. 2008). The type series was found together in a patch of primary forest, active at approximately 12:00 h on the leaf litter and away from any body of water. No reproductive activity was observed, and no calls were recorded. The call is unknown, but it is likely an inconspicuous single slow squeak, as reported for *C. stejnegerianus* (Savage 2002). We did not find gravid females, and pairs in amplexus were not observed.

Geographic distribution. *Craugastor gabbi* is restricted to the premontane forest near the type locality of Fila Costeña, Costa Rica and the premontane forest of Cordillera de Talamanca in the extreme southwestern region of Costa Rica and western Panama near the present international border (Fig. 1). The altitudinal range of the new species is 1100–1280 m elevation. *Craugastor gabbi* overlaps with the range of *C. underwoodi* and *C. podiciferus* at the Las Cruces Biological Station, Fila Costeña, Coto Brus.

Etymology. This species is named in honor of paleontologist William M. Gabb in recognition of his important contribution to the herpetology of Costa Rica as an explorer and collector, mainly in the Talamanca region.

TABLE 4. Main diagnostic characteristics and character states for the secondary sexually characteristics of the members of the *Craugastor podiciferus* species group.

Species	Thenar and palmar tubercles size relation	Finger I and Finger II size relation	Subarticular in profile	Supernumerary tubercle	Heel tubercle	Venter skin texture	Nuptial pads	Vocal sac	Vocal slits
C. gabbi **sp. nov.**	Thenar tubercle much smaller than palmar tubercle	Finger I slightly shorter than Finger II	Projecting	Present	Absent	Areolate	Absent	Absent	Absent
C. stejnegerianus	Thenar tubercle much smaller than palmar tubercle	Finger I equal to or slightly shorter than Finger II	Projecting	Present	Absent	Areolate	Absent	Absent	Absent
C. persimilis	Thenar tubercle much smaller than palmar tubercle	Finger II definitively much shorter that Finger I	Projecting	Present	Absent	Areolate	Absent	Absent	Absent
C. lauraster	Thenar tubercle much smaller than palmar tubercle	Finger II definitively much shorter that Finger I	Projecting	Present	Absent	Areolate	Absent	Absent	Absent
C. bransfordii	Thenar tubercle equal to or slightly smaller than palmar tubercle	Finger I equal to or slightly shorter than Finger II	Pointed	Present	Absent	Areolate	Present	Absent	Absent
C. underwoodi	Thenar tubercle equal to or slightly smaller than palmar tubercle	Finger I equal to or slightly shorter than Finger II	Projecting and low	Absent	Absent	Areolate	Present	Absent	Absent
C. polyptychus	Thenar tubercle equal to or slightly smaller than palmar tubercle	Finger I equal to or slightly shorter than Finger II	Projecting, often pointed	Present	Absent	Areolate	Absent	Absent	Absent
C. podiciferus	Thenar tubercle much smaller than palmar tubercle	Finger I equal to or slightly shorter than Finger II	Flattened, low	Absent	Present	Smooth	Absent	Present	Present

Discussion

In previous studies, molecular (Crawford 2003) and cytogenetic analyses (Chen 2001) of *Craugastor stejnegerianus* from the premontane forest of the Fila Costeña and the lowlands South Pacific of Costa Rica near Panama showed large genetic distances. Our taxonomic re-assessment using morphological and molecular data led us to elevate to the species level populations of the premontane forest as distinct from the lowlands populations. We found that *C. gabbi* differ from typical *C. stejnegerianus* by a combination of morphological traits as determined using discriminant function analyses, differences in ventral coloration and genetic differentiation at the 16S gen. Following the criteria of Pérez-Ponce de León & Nadler (2010), *C. gabbi* is not a cryptic species *sensu stricto*, although in the past this species was masked under *C. stejnegerianus* due their high degree of morphological similarity (functional definition of cryptic species). Our molecular analyses rejected the null hypothesis that *C. stejnegerianus* represent a single species, and our detailed morphological analyses showed morphological differences between both species, allowing a proper morphological diagnosis and species description.

The 16S gene fragment used here has been suggested as a DNA barcode marker for amphibian diversity inventories (Vences *et al.* 2005) to complement the standard COI-5' marker used in general for animals (Smith *et al.* 2008). The uncorrected genetic distance of 4.5% at the 16S gene observed between the premontane populations now assigned to *C. gabbi* and the lowlands South Pacific populations of *C. stejnegerianus*, is greater than the suggested threshold of 3% for flagging the potential cryptic species of Neotropical frogs (Fouquet *et al.* 2007). It is also substantially higher than the 1.8% genetic distance at 16S found by Hertz *et al.* (2012) in separating a recently described new species of arboreal Terrarana from Panama. Thus, our single mtDNA marker offers additional support for the specific status of the new species. Furthermore, based on genetic divergence at the mitochondrial ND2 gene, Crawford (2003) estimated that the Las Cruces Biological Station (Fila Costeña) population (now *C. gabbi*) diverged from the lowland populations (Palmar Norte and Rincón de Osa) of *C. stejnegerianus* between 6.25 and 10.3 million years ago (Ma), demonstrating the long-term evolutionary independence of these two lineages, which were formerly regarded as conspecific. Although the Fila Costeña and Palmar Norte localities are only 25 km apart (Fig. 1), these populations are separated by at least 1100 m in elevation. Based on the genetic data, their estimated divergence time corresponds perfectly with the rise of the Fila Costeña at 12.80–11.67 Ma (MacMillan *et al.* 2004). Our findings, therefore, agree with Bickford *et al.* (2007) in rejecting the common assumption that cryptic species are recently diverged and remind us that morphological homoplasy presents a challenge to amphibian taxonomy (Wake 2009).

We believe that the separation of *C. gabbi* from *C. stejnegerianus* is the beginning of the resolution of this group of highly similar frogs, treated as "cryptic species" in the taxonomic practice and in agreement with the functional definition of cryptic species by Pérez-Ponce de León & Nadler (2010). *Craugastor stejnegerianus* is distributed along the Pacific coast of Costa Rica from the Panamanian border northward to the Cordillera de Tilarán (Crawford 2003). The Cordillera de Tilarán populations could potentially represent yet one more undescribed species distinct from *C. stejnegerianus* (Crawford 2003; J. Savage, pers. comm.), which could eventually leave *C. stejnegerianus* restricted to the Pacific lowlands, roughly south of the City of Puntarenas. Additional taxonomic work is needed to complete the understanding of these diverse and locally abundant leaf-litter frogs.

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APPENDIX. **Specimens examined.**

All voucher numbers below are 'UCR' numbers, and the specimens were housed at the Museo de Zoología at the Universidad de Costa Rica.

Craugastor stejnegerianus

COSTA RICA: *Puntarenas*: Bahía Ballena, Osa (19272, 19277, 19278, 19282–19284, 19324–19326, 19328, 19353, 19527–19529, 19546–19548, 19589); Potrero Grande, Buenos Aires (20307–20309, 20346, 20352); Golfito, Golfito (11969, 11970, 12246, 12272–12289, 14958, 14959); Palmar, Osa (7865, 7866, 9057, 20885, 22136, 22137); Pavón, Golfito (12717–12719, 21494); Puerto Cortés, Osa (14230, 14235, 14244); Puerto Jiménez, Golfito (11302, 11305, 11306, 11308, 11311–11313, 16277, 16340, 16341, 18339, 18340, 18356–18359, 18361–18364, 19230, 20454); Savegre, Aguirre (14306, 14536, 14569, 14706, 14734, 15980, 15986, 16163); Sierpe, Osa (799, 800, 1081–1084, 1158, 3558–3560, 4576, 6400, 8733, 11309, 11350, 11351, 11621, 13673, 13674, 13683, 13684, 16275, 16276, 16345–16349, 16571–16575). *San José*: Barú, Pérez Zeledón (5010, 5011, 14737, 16330–16334, 16336, 22100, 22101, 22103); Daniel Flores, Pérez Zeledón (4234–4239); Platanares, Pérez Zeledón (15991, 15993, 15994); Rivas, Pérez Zeledón (16278–16280, 22127, 22128, 22131, 22132); San Isidro del General (5097–5101)

Craugastor gabbi **sp. nov.**

COSTA RICA: *Puntarenas*: Aguabuena, Coto Brus (13242, 15830, 15832, 15833); Sabalito, Coto Brus (8684, 15834); San Vito, Coto Brus (8644, 8645, 12507–12512, 12514, 12524–12526, 12935, 13237, 13240, 13241, 14671–14673, 18531–18535, 21863–21865, 21867, 21876).

CAPÍTULO II.III

A NEW SPECIES OF *CRAUGASTOR* (ANURA: CRAUGASTORIDAE) FROM THE MONTANE RAINFOREST OF THE CORDILLERA DE TALAMANCA, COSTA RICA

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A new species of *Craugastor* (Anura: Craugastoridae) from the montane rainforest of the Cordillera de Talamanca, Costa Rica

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Abstract

A new species of *Craugastor* (Anura: Craugastoridae) from the montane rainforest of the Cordillera de Talamanca, Costa Rica. A new dirt frog of the *Craugastor podiciferus* Species Group is described from Costa Rica; it is restricted to elevations between 2330 and 2700 m a.s.l. in the montane rainforest of the Cordillera de Talamanca. Analysis of DNA sequences of the mitochondrial 16S rRNA (16S) and cytochrome oxidase I (COI) genes reveals a distinct lineage within the *C. podiciferus* Species Group. Additional morphological and morphometric analyses support the distinctiveness of this lineage that is described as a new species herein. The species is distinguished from other members of the *C. podiciferus* Species Group by its unique coloration: a violet-brown to blackish brown venter with white pigment forming blotches, and dark brown palmar surfaces with prominent white folds between subarticular tubercles in the adults. The genetic divergence of the species from other members of the *C. podiciferus* Species Group is significant (higher than 9.2% in 16S and 13.3% in COI). Although not closely related, it resembles *C. podiciferus* morphologically, a species that also inhabits montane rainforest. The discovery of this new species highlights the importance of montane rainforest as a center of species richness and endemism.

Keywords: Brachycephaloidea, Central America, *Craugastor podiciferus* Species Group, Panama, Terrarana.

Resumen

Una especie nueva de *Craugastor* (Anura: Craugastoridae) del bosque montano lluvioso en la Cordillera de Talamanca, Costa Rica. Se describe una especie nueva para Costa Rica de rana de hojarasca perteneciente al grupo de especies *Craugastor podiciferus,* restringida a elevaciones entre 2330–2700 m s.n.m. en el bosque montano lluvioso de la Cordillera de Talamanca. Análisis de las secuencias del ADN de los genes mitocondriales 16S ARNr (16S) y citocromo oxidasa 1 (COI) reveló un linaje distinto dentro del grupo de especies *C. podiciferus.* Los análisis complementarios

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de morfología y morfometría apoyaron la diferenciación de este linaje, el cual describimos aquí como una especie nueva. Esta especie se distingue de los miembros del grupo de especies *C. podiciferus* por su coloración única: vientre violeta-marrón a marrón negruzco con pigmento blanco formando manchas, la superficie palmar en adultos es marrón oscuro con pliegues blancos prominentes entre los tubérculos subarticulares. Geneticamente esta nueva especie es significativamente divergente de los demás miembros del grupo de especies *C. podiciferus* (mayores a 9.3% en el 16S y 13.3% en COI). Aunque no están estrechamente relacionadas, la nueva especie es morfológicamente similar a *C. podiciferus,* especie que también habita en el bosque montano lluvioso. El descubrimiento de esta nueva especie resalta la importancia del bosque montano lluvioso como un centro de riqueza de especies y endemismos.

Palabras clave: América Central, Brachycephaloidea, grupo de especies *Craugastor podiciferus,* Panamá, Terrarana.

Resumo

Uma nova espécie de *Craugastor* (Anura: Craugastoridae) do bosque montano chuvoso da Cordilheira de Talamanca, Costa Rica. Descrevemos aqui uma nova espécie do grupo de *Craugastor podiciferus* para a Costa Rica, restrita a altitudes entre 2330–2700 m acima do nível do mar no bosque montano chuvoso da Cordilheira de Talamanca. Análises das sequências de DNA dos genes mitocondriais16S ARNr (16S) e da citocromo oxidase 1 (COI) revelaram uma linhagem distinta dentro do grupo de espécies de *C. podiciferus.* Análises morfológicas e morfométricas complementares apoiaram a diferenciação desta linhagem, que descrevemos aqui como uma espécie nova. Essa espécie distingue-se dos membros do grupo de espécies de *C. podiciferus* por sua coloração única: ventre marrom-violeta a marrom enegrecido com pigmento branco formando manchas e superfície palmar nos adultos marrom escura com pregas brancas proeminentes entre os tubérculos sub-articulares. Geneticamente esta nova espécie é significativamente divergente dos demais membros do grupo de espécies de *C. podiciferus* (maiores em 9.3% no 16S e 13.3% no COI). Ainda que não estejam estreitamente relacionadas, a nova espécie é morfologicamente similar a *C. podiciferus,* que também habita o bosque montano chuvoso. A descoberta dessa nova espécie ressalta a importância do bosque montano chuvoso como um centro de riqueza de espécies e endemismos.

Palavras-chave: América Central, Brachycephaloidea, grupo de espécies de *Craugastor podiciferus,* Panamá, Terrarana.

Introduction

The highlands of isthmian Central America are characterized by a high level of species richness and endemism. The isthmian highlands (1000–3820 m a.s.l.) are an isolated topographic unit in Central America (Campbell 1999, Gutiérrez-García and Vázquez-Domínguez 2013) formed by the Guanacaste, Tilarán, Central, and Talamanca mountain ranges. The Cordillera de Talamanca extends from the central valley in Costa Rica to western Panama and contains both the highest mountain peaks of the isthmus (Campbell 1999, Savage 2002) and the most endemic amphibians of Costa Rica (Campbell 1999, Olson *et al.* 2001, Savage 2002, Boza-Oviedo *et al.* 2012). The summits of the Cordillera de Talamanca (ranging from 2500–3500 m a.s.l.) are dominated by Montane Rainforest Life Zone (Holdridge 1967, Bolaños *et al.* 2005), which is extremely fragmented and isolated. The Talamanca montane rainforest (TMR) is relatively poorly studied and is thought to have a lower species diversity than the premontane forest of Talamanca Range (Kubicki 2008, Santos-Barrera *et al.* 2008, Arias and Bolaños 2014). Nevertheless, the TMR is home to several micro endemic amphibians—*viz. Atelopus chirripoensis* Savage and Bolaños, 2009, *Bolitoglossa kamuk* Boza-Oviedo, Rovito,

Chaves, García-Rodríguez, Artavia, Bolaños, and Wake, 2012, *B. pesrubra* Taylor, 1952, *B. pygmaea* Bolaños and Wake, 2009, *B. robinsoni* Bolaños and Wake, 2009, *B. splendida* Boza-Oviedo, Rovito, Chaves, García-Rodríguez, Artavia, Bolaños, and Wake, 2012, and *Diasporus ventrimaculatus* Chaves, García-Rodríguez, Mora, and Leal, 2009.

During recent fieldwork in the TMR we found frogs of the *Craugastor podiciferus* Species Group Hedges *et al.* (2008). The anurans were collected at the summits of Cerro Arbolado, Cerro Hakú, Cerro Utyum, and Caribbean slopes of Cerro Pando. The population of Cerro Utyum is near (~ 10 km airline distance) to the type locality of *C. podiciferus* (Cope, 1875) (Cope 1875, Arias and Chaves 2014) and both species of anurans are morphologically similar. *Craugastor podiciferus* is abundant, with a broad distribution across the highlands of all the mountain ranges of Costa Rica and western Panama (1000–2650 m a.s.l.); it has been thought to represent a species complex with several unnamed species (Savage 2002, Streicher *et al.* 2009). Molecular analyses of two mitochondrial genes showed that despite the morphological resemblance to *C. podiciferus,* the recently discovered populations from the TMR are not *C. podiciferus,* but instead represent a new member of the species group, which is described here based on molecular and morphological data.

Materials and Methods

Taxon Sampling

Frogs of the new species were collected at five localities along the Cordillera de Talamanca, at the summits of Cerro Arbolado, Cerro Hakú, Cerro Utyum, and the Caribbean slopes of Cerro Pando in southwestern Costa Rica (Figure 1). In addition, we collected several specimens from highlands of Costa Rica and western Panama, including several populations referred to *C. podiciferus, Craugastor* sp.B of Crawford & Smith (2005), and two populations that we record herein as unnamed species (*Craugastor* sp.1 and *Craugastor* sp.2). The frogs were euthanized in the field and extracted liver or muscle tissue was preserved in 95% ethanol or RNAlater. Voucher specimens were fixed in 10% formalin, stored in 70% ethanol, and deposited at the Museo de Zoología, Universidad de Costa Rica (UCR) and Senckenberg Research Institute and Nature Museum, Frankfurt, Germany (SMF). Museum codes follow those of Frost (2018), with the addition of CRARC in reference to the Costa Rica Amphibian Research Center private collection; EAP denotes field numbers of Erick Arias.

Amplification and Sequencing

We extracted total genomic DNA from the preserved tissue samples using the Animal Genomic DNA Kit (BioBasic Canada Inc.), DNeasy Blood & Tissue Kit (Qiagen), or the phenol-chloroform standard protocol (Sambrook & Russell 2006). We amplified the large subunit ribosomal RNA (16S) and cytochrome oxidase subunit I (COI) mitochondrial genes. The primers 16Sar and 16Sbr (Palumbi *et al.* 1991) were used for 16S and dgLCO and dgHCO (Meyer 2003) for COI. The PCR amplifications were performed using a total volume of 15 µL, which contained 1 µL DNA template (*c.* 50 ng/µL), 0.75 U Taq polymerase (Amplificasa®, Biotecnologias Moleculares), 1X PCR buffer with 1.5 mm $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), and 0.3–0.5 µM forward and reverse primers. The PCR conditions are as follow: 16S, an initial cycle of 5 min at 94°C, followed by 35 cycles of 45 s at 94°C, 30 s at 50 or 55°C, 45 or 120 s at 72°C, plus a final cycle of 3 min at 72°C; COI, an initial cycle of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 50°C, 45 s at 72°C, plus a final cycle of 3 min at 72°C. PCR products were cleaned with ExoSap-IT (USB Corporation) and sequenced in both directions using the original amplification primers and BigDye termination reaction chemistry (Applied Biosystems). The cycle-

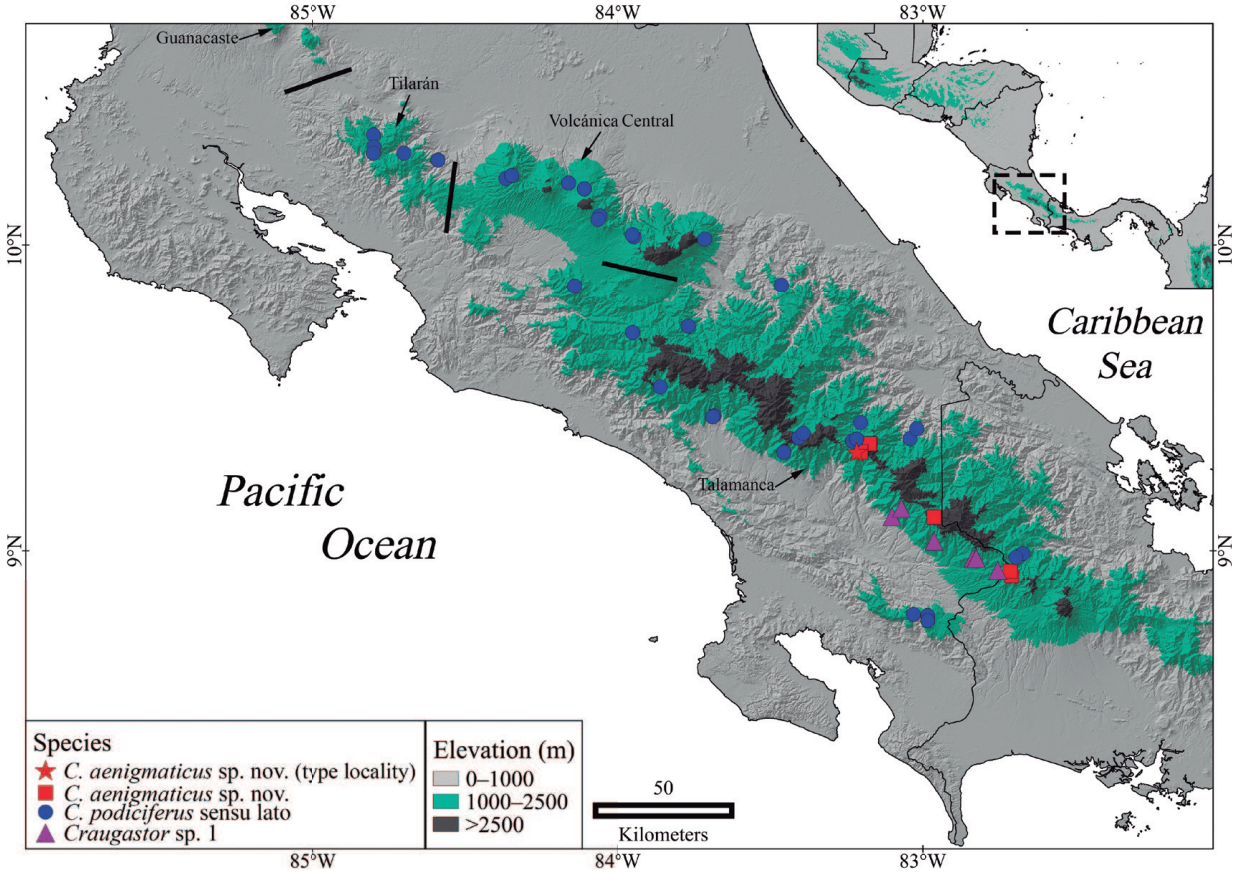

Figure 1. Map showing the known populations of *Craugastor aenigmaticus* sp. nov. (red star = type locality) in the Cordillera de Talamanca, and the populations of *C. podiciferus* used in our molecular analysis. Solid (black) lines depict the geographical limits between each mountain range.

sequencing products were column-purified with Sephadex G-50 (GE Healthcare) and run on an ABI 3500xL Genetic Analyzer (Applied Biosystems). Consensus sequences for each individual were constructed using SEQUENCHER 5.3 (Genes Codes Corp.).

Phylogenetic Analyses

The sequences obtained were compared to those available in GenBank for the *Craugastor podiciferus* Species Group and sequences of *C. gollmeri* (Peters, 1863) were used as the outgroup. See Appendix I for the list of DNA voucher and GenBank Accession numbers used in this study. Sequence alignments were performed using the MUSCLE 3.7 software (Edgar 2004) with default parameters and trimmed to the point at which a majority of taxa had sequence data. We partitioned the sequence data by gene, and further partitioned COI by codon position. We used PartitionFinder v1.1.1 (Lanfear *et al.* 2012) and the Bayesian Information Criterion (BIC) to select the best partition scheme and the best model of sequence evolution for each partition. We used a single set of branch-lengths across all partitions (branchlengths = linked); the search of the best partition scheme was using a heuristic search (scheme = greedy). We defined, *a priori,* four partitions: one for 16S and three for COI (one for each codon).

We performed analyses using both the maximum likelihood (ML) and Bayesian analyses (BA). For ML we used Garli 2.01 (Zwickl 2006), with 10 search replicates with the following default setting values changed: streefname = random, attachmentspertaxon = 24, genthreshfortopoterm = 100000, significanttopochange = 0.00001. For bootstrapping, we ran 1000 replicates with the previous settings with the following changes: genthreshfortopoterm = 10000, significanttopochange = 0.01, treerejectionthreshold = 20, as suggested in the Garli manual to speed up bootstrapping. The bootstrap consensus tree was performed using Sumtrees (Sukumaran and Holder 2010a) from DendroPy packages Version 4.4.0 (Sukumaran and Holder 2010b). Bayesian phylogenetic analysis was performed using MrBayes 3.2.6 (Ronquist *et al.* 2012) with the partition scheme and the model of sequence evolution for each partition as selected previously. Two separate analyses were run; each consisted of 20 million generations, sampled every 1000 generations, and four chains with default heating parameters. We examined a time-series plot of the likelihood scores of the cold chain to check stationarity using Tracer 1.6 software (Rambaut *et al.* 2014). We discarded the first 25% of trees as burn-in and used the remaining trees to estimate the consensus tree along with the posterior probabilities for each node and each parameter. The ML and Bayesian analyses were run on the CIPRES portal (Miller *et al.* 2010). Estimates of pairwise evolutionary genetic divergence between species were computed using MEGA7 (Kumar *et al.* 2016), assuming uncorrected distances based on the Tamura 3-parameter model (Tamura 1992), with rate variation among the sites modeled as a gamma distribution with the shape parameter = 4 as the default of the software.

Morphometric Analyses

We performed a morphometric analysis to compare the new species with *Craugastor podiciferus* because the taxa closely resemble one another and both inhabit the TMR (allopatrically). In addition we compared the new species with a third undescribed species (*Craugastor* sp.1) because populations of this frog are located nearby (~ 5 km airline distance) (Figure 1). We examined 20 specimens of the new species from four localities, 86 *C. podiciferus* from several localities in Costa Rica, and 19 *Craugastor* sp.1 (Appendix II). Specimens are deposited at UCR. The following morphological measurements were recorded, as described by Savage (2002), Duellman and Lehr (2009), and Arias *et al.* (2016): snout–vent length (SVL), head length (HL), head width (HW), interorbital distance (IOD), width of upper eyelid (EW), intercanthal distance (IC), internarial distance (IN), upper lip–nostril distance (TN), eye–nostril distance (E–N), eye diameter (ED), tympanum diameter (TY), ulna length (UL), hand length (HaL), lengths of the Fingers I (F1) and III (F3), femur length (FL), tibia length (TL), tarsus length (TaL), foot length (FoL), and lengths of the Toes III (T3) and V (T5). Measurements were taken with dial calipers and were rounded to the nearest 0.1 mm.

We transformed the morphometric data using the method of Lleonart *et al.* (2000) to avoid allometric effects relative to the differences in the size and shape between species and between individuals. In this method, a logarithmic transformation of the continuous variables is performed to reduce the extreme values. All transformed variables are used in the allometric transformation by means of equation $Y_i^* = Y_i (X_0/X_i)^b$, where Y_i^* is the value of each of the dependent variable corrected for size and shape; Y_i is the value of each of the dependent morphometric variable; X_0 is the average of the SVL variable for all populations; X_i is the SVL value for each individual; and *b* is the regression line intercept with the Y-axis resulting from the regression of each dependent variable with X_0. The intercept is used as an allometric transformation factor and is unique for each

variable. The additional proportions reported here include: EW/IOD, IOD/HW, TY/ED, EN/ED, ED/HL, IC/HL, IN/EN, IN/TN, FL/TL, TL/TaL, TaL/FoL, T3/FoL, T5/FoL, UL/HaL, F1/HaL, and F3/HaL. The sex of individuals was determined by gonadal morphology. Specimens with opaque seminal vesicles were assumed to be adult males, and those with developed oviducts were assumed to be adult females. The general terminology for the morphological characteristics follows that of Duellman and Lehr (2009). We adopted Savage's (2002) usage of the term "supernumerary tubercles" to refer to the tubercles on the phalanges (between subarticular tubercles); this differs from the tubercles denoted as accessory palmar or plantar tubercles.

We calculated the mean, standard deviation, and range for each morphometric variable without correction. We performed a discriminant analysis to determine whether the morphometric variables were effective to predict the species, using the following variables: EW/IOD, IOD/HW, TY/ED, EN/ED, ED/HL, IC/HL, IN/EN, IN/TN, FL/TL, TL/TaL, TaL/FoL, T3/FoL, UL/HaL, F1/HaL, and F3/HaL. We also conducted a Principal Component Analysis (PCA) to explore the degree of structure within the sample and which variables have more loads in the segregation of groups. All analyses were performed using R v3.3.3 (R Core Team 2017).

Results

Molecular Data

The data matrix includes 56 sequences, with a total sequence length of 1222 bp, including gaps: 565 bp for 16S and 657 bp for COI. Three partition schemes were identified with the following substitution models: GTR + I + G for 16S and COI codon position 3, K80 + I + G for COI codon position 1, and HKY+I for COI codon position 2. Genetic distances between the new species and other members of the *Craugastor podiciferus* Species Group are of 9.2–18.5% for 16S and 18.9–24.8% for COI. Specifically, the new species is separated by a mean-uncorrected genetic distance to *C. podiciferus* of 11.57–16.15% for 16S and 19.16–24.35% for COI and to *Craugastor* sp.1 of 12.25–12.9% in 16S and 23.57–24.72% in COI.

The phylogenies inferred by Garli and MrBayes were mostly congruent (Figure 2), with six well-supported clades. The three most basal clades represent three unnamed species (*Craugastor* sp.B, *Craugastor* sp.1, and *Craugastor* sp.2) that occur in the highlands of the southwestern end of Cordillera de Talamanca. The fourth clade contains the samples from Cerro Hakú, Cerro Utyum, and Cerro Pando at Cordillera de Talamanca (Figure 1). A fifth major clade includes seven species of the *C. podiciferus* Species Group that mainly occur from the lowlands to intermediate elevations from eastern Honduras to central Panama. The sixth major clade is formed by all the samples of *C. podiciferus* sensu lato that are broadly distributed in the highlands of Costa Rica and western Panama (Figure 1).

The main differences between the ML and Bayesian topologies are in the relationships of *Craugastor* sp.B, *Craugastor* sp.1, and *Craugastor* sp.2 within the phylogeny. Their placement is unresolved in the ML tree, whereas in the Bayesian tree (not shown), *Craugastor* sp.1 is the most basal taxon for the entire group. *Craugastor* sp.1 + *Craugastor* sp.B form a clade that is sister to the rest of the species group.

Morphometric Analysis

Morphometric variation among the species is shown in Table 1. The PCA did not differentiate specimens of the new species from those of *Craugastor podiciferus* and *Craugastor* sp.1. The discriminant analysis correctly classified 87.9% of the specimens to the species, showing a clear separation between the specimens of the new taxon and the specimens referred to *C. podiciferus* and *Craugastor* sp.1 (Figure 3).

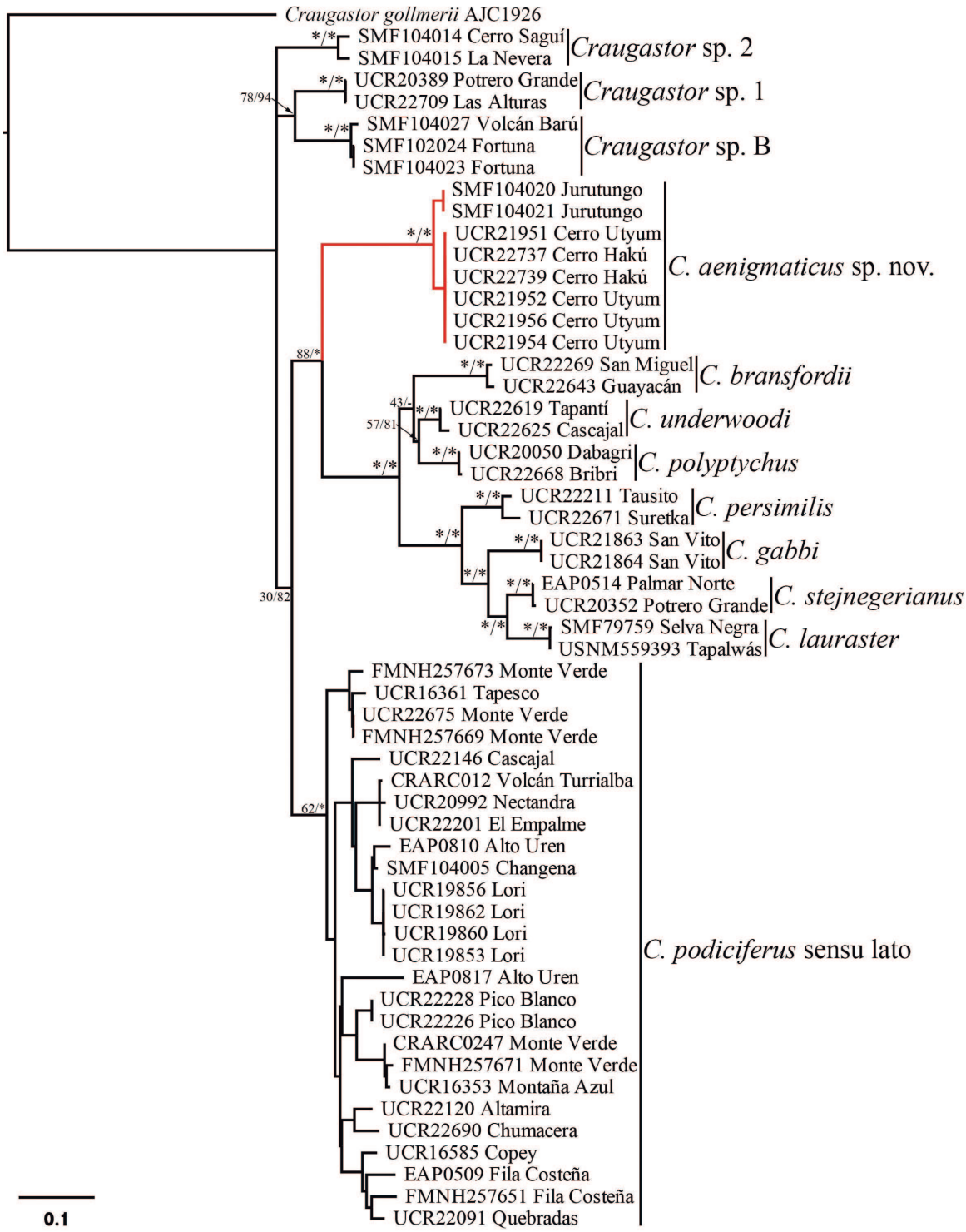

Figure 2. Maximum likelihood phylogeny of *Craugastor podiciferus* Species Group based on 16S and COI mitochondrial DNA genes. Bootstraps proportions are before the slash, and posterior probabilities (multiplied by 100) from MrBayes analysis are following the slash. The scale bar refers to the estimated substitutions per site. The support values of any node within species are not shown. The asterisks represent support of > 95.

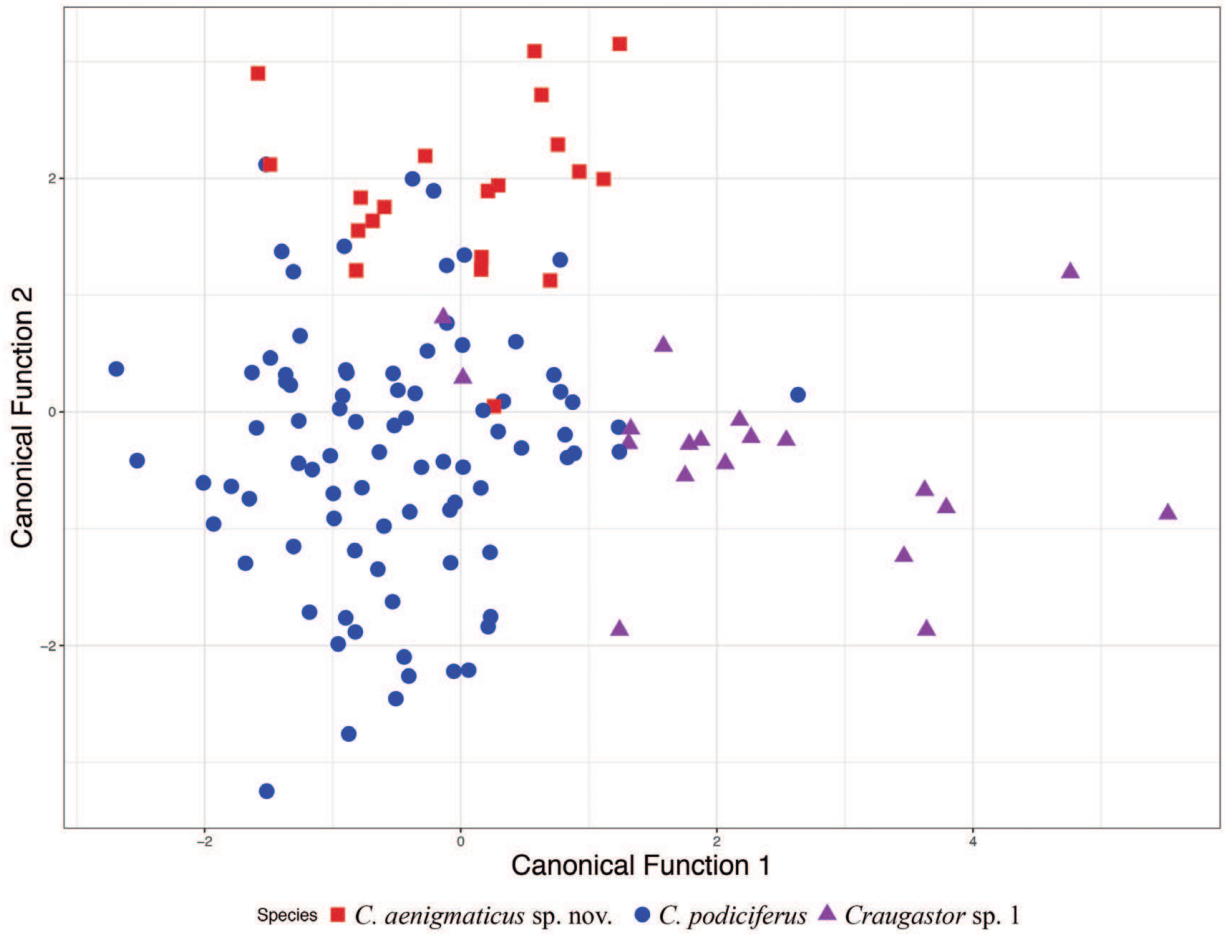

Figure 3. Linear discriminant analysis showing the morphological separation among the 20 individuals of *Craugastor aenigmaticus* sp. nov. and the individuals of *C. podiciferus* and *Craugastor* sp.1.

***Craugastor aenigmaticus* sp. nov.**
Montane Dirt Frog
(Figures 4–6)

Holotype.—UCR 22961 (EAP 0762), an adult female from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: summit of Cerro Arbolado, Parque Internacional La Amistad, (09°19'12.0" N, 83°12'57.6" W; 2600 m a.s.l.), collected by Erick Arias and Omar Zúñiga on 19 October 2016.

Paratopotypes.—UCR 22957 (EAP 0758), subadult male; UCR 22958 (EAP 0759) and UCR 22960 (EAP 0761), adult females; UCR 22962 (EAP 0763), subadult female; UCR 22959 (EAP 0760), juvenile; same date as the holotype.

Paratypes.—UCR 21951 (EAP 0303), adult female from Costa Rica: Provincia de Limón: Cantón de Talamanca: Distrito de Telire: Caribbean slopes of Cerro Utyum, Parque Internacional La Amistad, (09°20'56.4" N, 83°10'30.0" W; 2700 m a.s.l.), collected by Erick Arias, Gerardo Chaves, Olmer Cordero, and Omar Zúñiga on 12 July 2013. UCR 22414 (EAP 0490) and UCR 22415 (EAP 0491), adult females, same data as UCR 21951 but collected on 29 March 2013. UCR 22737 (EAP 0674) and

Table 1. Mean ± SD and range (in mm) for morphometric variables by species. Abbreviations: SVL, snout–vent length; HL, head length; HW, head width; IOD, interorbital distance; EW, width of the upper eyelid; IC, intercanthal distance; IN, internarial distance; TN, upper lip–nostril distance; E–N, eye–nostril distance; ED, eye diameter; TY, tympanum diameter; UL, ulna length; HaL, hand length; F1, length of Finger I; F3, length of Finger III; FL, femur length; TL, tibia length; TaL, tarsus length; FoL, foot length; T3, length of Toe III; T5, length of Toe V.

Variable	***Craugastor aenigmaticus* sp. nov.**		***Craugastor podiciferus***		***Craugastor* sp.1**	
	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max
SVL	26.38 ± 9.33	16.10–41.10	24.27 ± 4.76	15.10–35.10	20.79 ± 3.94	13.80–26.50
HL	10.65 ± 3.52	6.20–15.95	9.91 ± 1.76	6.45–13.60	8.44 ± 1.47	5.70–10.30
HW	10.61 ± 3.68	6.25–16.50	9.66 ± 1.88	6.20–14.15	8.22 ± 1.64	5.60–10.70
IOD	3.265 ± 1.10	1.80–5.55	3.23 ± 0.53	2.10–4.55	2.74 ± 0.51	1.80–3.70
EW	2.08 ± 0.70	1.20–3.20	1.80 ± 0.38	1.15–2.90	1.62 ± 0.34	1.15–2.10
IC	5.20 ± 1.64	3.25–8.00	5.02 ± 0.77	3.55–6.95	4.22 ± 0.73	3.15–5.30
IN	3.29 ± 0.99	2.15–4.85	3.04 ± 0.51	2.20–4.30	2.57 ± 0.39	1.95–3.25
TN	1.54 ± 0.46	1.00–2.30	1.25 ± 0.24	0.90–2.10	1.04 ± 0.18	0.70–1.25
EN	2.59 ± 0.77	1.55–3.70	2.43 ± 0.46	1.50–3.60	1.99 ± 0.42	1.30–2.60
ED	3.05 ± 0.95	2.05–4.90	2.88 ± 0.49	1.90–3.90	2.46 ± 0.32	1.90–2.90
TY	2.01 ± 0.73	0.95–3.15	1.75 ± 0.41	1.00–2.70	1.96 ± 0.32	1.55–2.95
UL	6.08 ± 2.34	3.10–9.65	5.56 ± 1.09	3.50–8.20	4.80 ± 0.99	3.35–6.30
HaL	6.93 ± 2.57	3.70–11.00	5.83 ± 1.24	3.55–9.50	5.03 ± 0.99	3.10–6.55
F1	2.62 ± 1.31	1.10–4.85	2.10 ± 0.55	1.10–3.95	1.93 ± 0.56	0.90–2.95
F3	4.16 ± 1.62	2.20–6.50	3.46 ± 0.79	1.75–6.10	2.97 ± 0.62	1.80–3.90
FL	14.73 ± 5.85	7.90–23.00	12.40 ± 2.80	7.60–20.30	10.67 ± 1.96	7.05–14.00
TL	16.75 ± 6.54	8.50–25.85	14.07 ± 3.04	8.25–22.50	12.25 ± 2.26	8.25–15.25
TaL	9.44 ± 3.38	5.45–14.25	8.28 ± 1.66	5.10–12.85	7.36 ± 1.35	5.10–9.30
FoL	14.87 ± 5.76	7.95–23.50	12.79 ± 2.75	7.65–20.15	10.94 ± 2.24	7.05–14.15
T3	5.40 ± 2.32	2.05–9.70	4.46 ± 0.99	2.60–7.25	3.79 ± 0.77	2.45–5.05
T5	4.27 ± 1.82	1.70–7.00	3.67 ± 0.95	1.90–6.70	2.99 ± 0.58	1.90–3.80
EW/IOD	0.64 ± 0.07	0.46–0.75	0.56 ± 0.09	0.38–0.89	0.59 ± 0.08	0.44–0.76
EN/ED	0.86 ± 0.11	0.67–1.02	0.84 ± 0.07	0.64–1.04	0.81 ± 0.10	0.63–0.98
ED/HL	0.29 ± 0.03	0.25–0.35	0.29 ± 0.02	0.25–0.33	0.29 ± 0.02	0.26–0.33
IOD/HW	0.31 ± 0.03	0.27–0.38	0.34 ± 0.03	0.27–0.43	0.34 ± 0.02	0.30–0.38
TY/ED	0.66 ± 0.15	0.45–1.03	0.61 ± 0.10	0.41–1.00	0.81 ± 0.17	0.62–1.34
IC/HL	0.49 ± 0.02	0.43–0.53	0.51 ± 0.03	0.45–0.61	0.50 ± 0.05	0.36–0.58
IN/EN	1.27 ± 0.12	1.03–1.55	1.26 ± 0.11	0.95–1.53	1.31 ± 0.16	1.10–1.62
IN/TN	2.14 ± 0.13	1.94–2.44	2.45 ± 0.20	1.90–2.94	2.50 ± 0.22	2.21–3.00
FL/TL	0.88 ± 0.03	0.80–0.94	0.88 ± 0.05	0.54–0.96	0.87 ± 0.03	0.79–0.92
TL/TaL	1.76 ± 0.10	1.51–1.94	1.70 ± 0.07	1.48–1.83	1.67 ± 0.07	1.58–1.91
TaL/FoL	0.64 ± 0.04	0.58–0.77	0.65 ± 0.03	0.58–0.73	0.68 ± 0.03	0.59–0.72
T3/FoL	0.36 ± 0.04	0.24–0.46	0.35 ± 0.02	0.30–0.39	0.35 ± 0.01	0.32–0.36
T5/FoL	0.28 ± 0.04	0.18–0.34	0.28 ± 0.02	0.22–0.38	0.27 ± 0.02	0.22–0.31
UL/HaL	0.88 ± 0.05	0.77–0.95	0.96 ± 0.06	0.81–1.16	0.96 ± 0.08	0.82–1.11
F1/HaL	0.36 ± 0.06	0.27–0.47	0.36 ± 0.04	0.24–0.43	0.38 ± 0.05	0.29–0.46
F3/HaL	0.60 ± 0.02	0.56–0.64	0.59 ± 0.04	0.49–0.67	0.59 ± 0.04	0.51–0.68

UCR 22747 (EAP 0684), adult males from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: summit of Cerro Hakú, Parque Internacional La Amistad, (09°19'19.2" N, 83°12'10.8" W; 2660 m a.s.l.), collected by Erick Arias and Omar Zúñiga on 28 December 2015.

Group assignment.—Assigned to the genus *Craugastor* based on possessing the following characters: differentiated tympanum; absence of cranial crest; and Toe III longer than Toe V. Assigned to the *C. podiciferus* Species Group based on having a narrow head (HW/SVL = 36.1–43.64%) and a rugose dorsum, but lacking inner tarsal folds, webbing between the toes, nuptial pads, and vocal slits.

Diagnosis.—The combination of the following characteristics distinguish *Craugastor aenigmaticus* from its congeners (Figure 4 and 5): (1) skin on venter smooth, but with large granules laterally; (2) vocal slits absent; (3) nuptial pads absent; (4) toes lacking webbing; (5) heels lacking enlarged calcar tubercle, but can have one to three small tubercles or granules on heels; (6) supernumerary, accessory palmar, and plantar tubercles absent; and (7) unique coloration consisting of a dark brown, olive, olive-brown or dark violet-brown dorsal ground color, violet-brown to blackish brown venter with white to white-bluish pigment forming blotches, dark brown palmar surface in adults with prominent white folds between subarticular tubercles, and in some specimens, white bones apparent through the skin (Figure 6C).

Comparisons with other species.—*Craugastor aenigmaticus* differs from all the other craugastorids of isthmian Central America except for those in the *C. podiciferus* Species Group by having a narrow head (HW 36.1–43.64% SVL) and toes that lack webbing. *Craugastor aenigmaticus* differs from other members of the *C. podiciferus* Species Group by having the following characteristics (condition for *C. aenigmaticus* in parentheses). In *C. bransfordii* (Cope, 1886), *C. gabbi* Arias, Chaves, Crawford, and Parra-Olea, 2016, *C. lauraster* (Savage, McCranie, and Espinal, 1996), *C. persimilis* (Barbour, 1926), *C. polyptychus* (Cope, 1886), *C. stejnegerianus* (Cope, 1893), and *C. underwoodi* (Boulenger, 1896): (1) the venter is cream (olive-brown in life, and dark brown in ethanol); (2) the venter, as well as the midline, is completely areolate to tuberculate (smooth, at least in the midline in *C. aenigmaticus*); and (3) they range in altitude from 0–1600 m a.s.l. (range 2330–2700 m a.s.l.). *Craugastor jota* (Lynch, 1980) differs from *C. aenigmaticus* by having a prominent calcar tubercle on the heel (evident calcar tubercle absent, although some individuals have one to three small tubercles). *Craugastor podiciferus* differs from *C. aenigmaticus* by: (1) having a prominent calcar tubercle on the heel (Figure 7) (evident calcar tubercle absent although some individuals could have one to three small tubercles); (2) venter yellow, orange, grayish or olive in adults (adults with venter violet-brown with white blotches); and (3) by lack of white prominent folds between subarticular tubercles on hands of adults (prominent white folds between the subarticular tubercles on the hands of adults) (Figure 6D).

Figure 4. *Craugastor aenigmaticus* sp. nov. Photograph taken by EA.

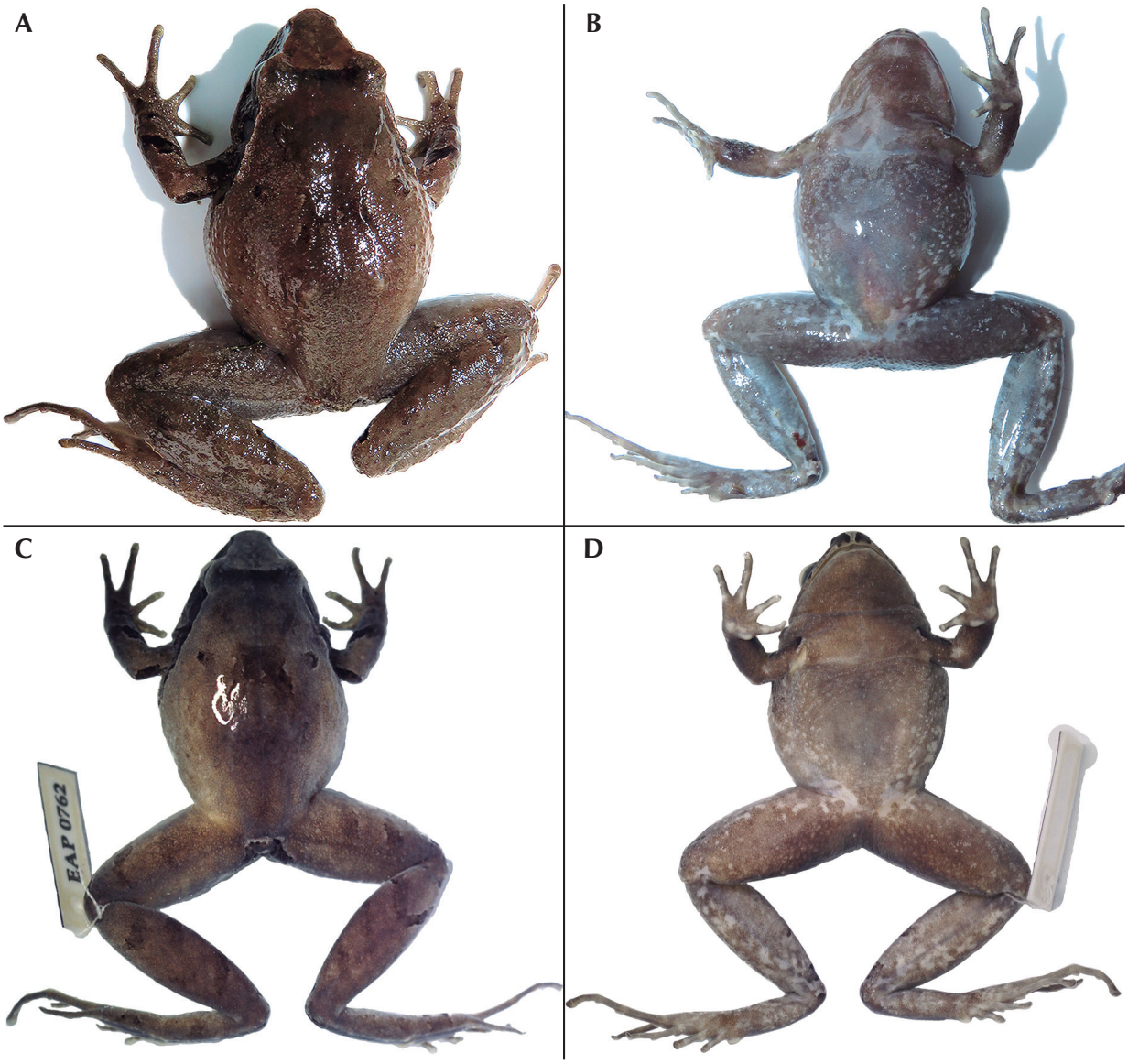

Figure 5. Dorsal (right) and ventral (left) color in life (above) and in ethanol (below) of the holotype (UCR 22961) of *Craugastor aenigmaticus* sp. nov. Photographs taken by EA.

Description of the holotype.—Adult female, SVL = 40.1 mm (Figures 4, 5). Head relatively narrow, width = 41.15% SVL; snout subovoid in dorsal view, rounded in profile; snout relatively long (HL = 15.6 mm, 38.9% SL), with nostrils directed laterally; in ventral view, tip of snout protruding markedly beyond edge of lower lip. Internarial area convex (IN 4.85 mm); canthus rostralis rounded; intercanthal area flat (IC = 8.0 mm); loreal region slightly concave; vomerine teeth transverse, in two fascicles well behind the choanae. Tongue round, lacking a distinct posterior notch; teeth absent; choanae moderately large, rounded on posterior half, but flat on anterior half, hemispherical; vocal slits absent. Eye moderate (EW = 92.75% E-N), not

protruding beyond dorsal and ventral outline of head, directed laterally. Tympanum distinct, round, and covered by skin; tympanic annulus prominent, round, small (54.12% of ED). Skin on all dorsal and lateral surfaces of head moderately granular. Upper eyelid granular, without superciliar or supraocular tubercles. Postrictal tubercles fused forming short ridge posteroventral to tympanum. Skin on dorsum and limbs weakly granular. Skin of chest smooth,

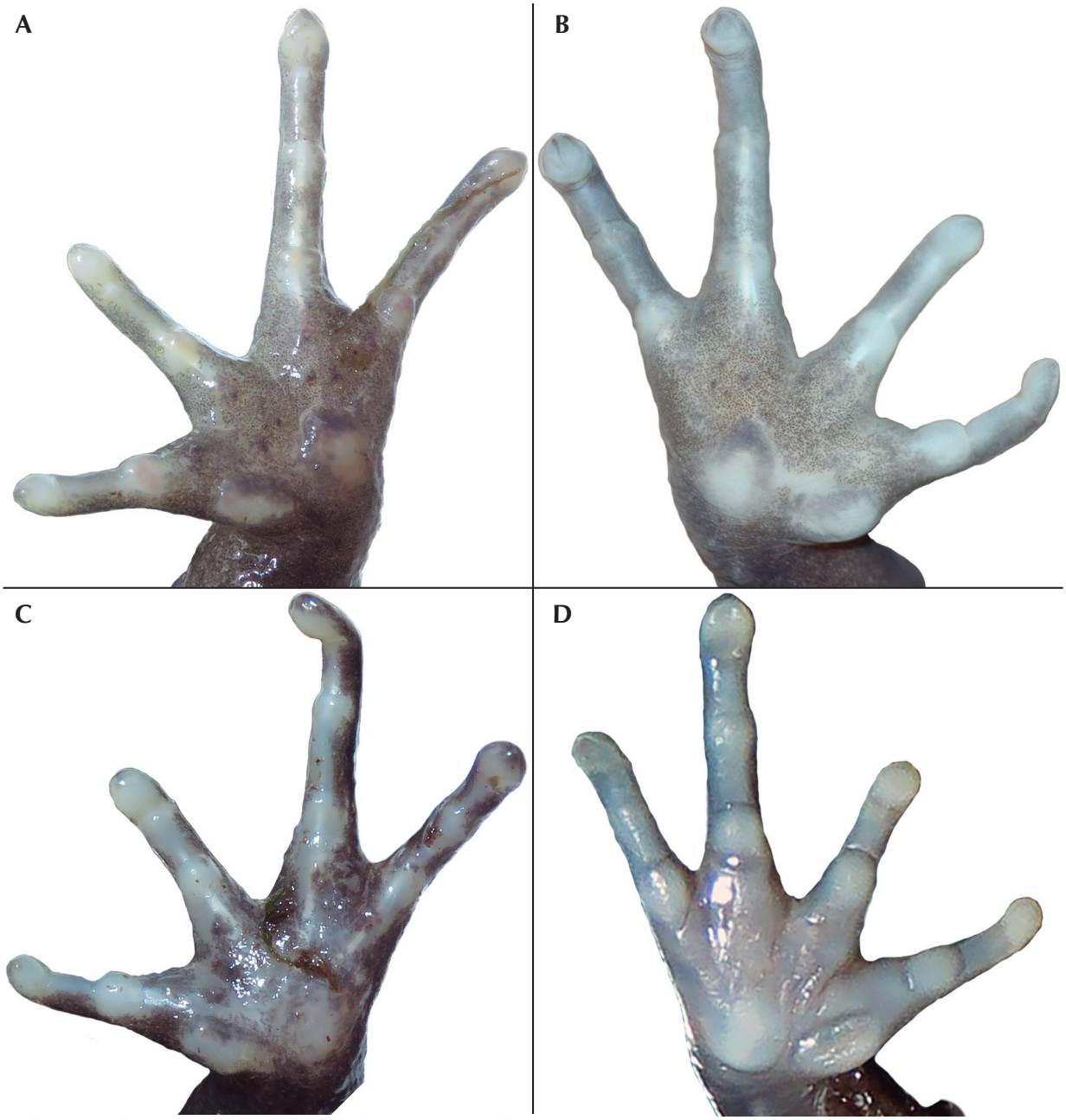

Figure 6. Variation of the ventral views of the hands for comparison. (**A**) *Craugastor aenigmaticus* sp. nov. holotype in life (UCR 22961); (**B**) holotype (UCR 22961) in ethanol; (**C**) paratopotype (UCR 22958); (**D**) *C. podiciferus*. Photographs taken by EA.

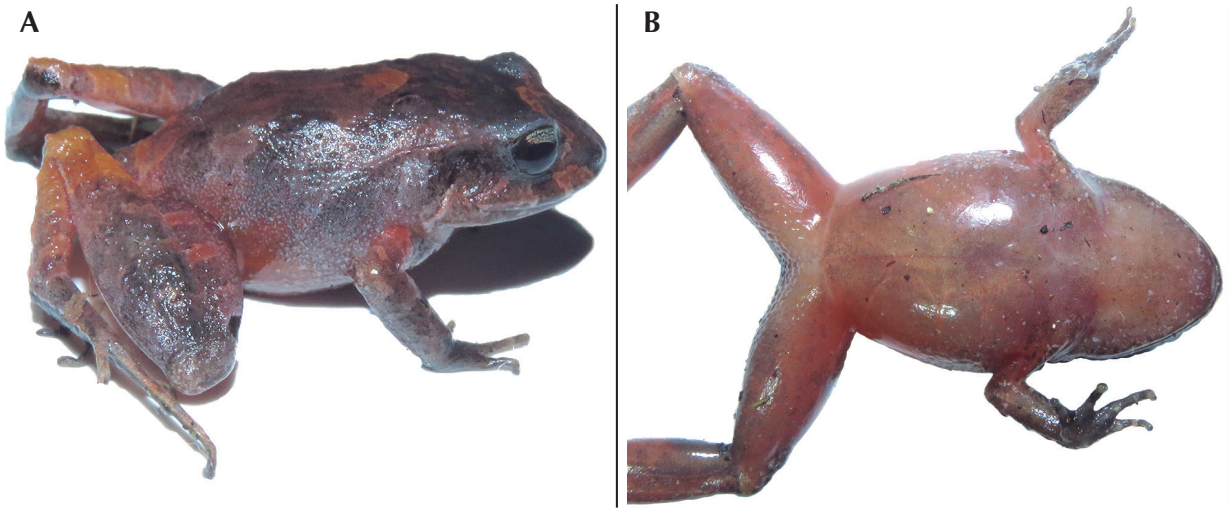

Figure 7. Dorsal (**A**) and ventral (**B**) color in life views of *Craugastor podiciferus* (EAP 0803), for comparison. Note the enlarged calcar tubercle and coloration on dorsum and venter. Photographs taken by EA.

venter smooth and encroached on laterally by low granules; ventral surfaces of thighs smooth to weakly granular; skin of groin and ventral surfaces of arms and lower legs nearly smooth. Flanks areolate, especially along the anteroventral flank region; skin on chin smooth. A pair of complete hourglass-shaped dorsal ridges present between posterior margin of eye and sacrum. Pair of supratympanic folds extending from posterior margin of eye, above tympanum, bifurcating at the axillary level, one fold continues laterally and other continues to the venter. Discoidal fold complete.

Forelimb relatively short and robust; fingers moderately long and slim without lateral fringes. Discs absent; fingers with grooves; tips of fingers unexpanded, rounded in dorsal view; pads ovoid. Proximal subarticular tubercles indistinct. Supernumerary tubercles absent; accessory palmar tubercles absent; distal subarticular tubercles rounded in basal outline, flattened, and globular in profile; thenar tubercle ovoid, flattened, palmar tubercle rounded, flattened; thenar tubercle much smaller than palmar. Ulnar tubercles and fold absent. Fingers are not webbed.

Legs relatively long and robust; heel granular but lacking enlarged tubercles. Discs absent; toes with grooves; tips of toes unexpanded, lanceolate in dorsal view; pads triangular. Supernumerary tubercles absent; plantar tubercles absent; subarticular tubercles ovoid in basal outline, flattened, and globular in profile; inner metatarsal tubercle elongate, globular; outer metatarsal tubercle rounded, barely discernible, flattened; outer metatarsal tubercle much smaller than inner; inner tarsal fold absent; toes are not webbed. Cloacal opening directed posteriorly at midlevel of thighs.

Coloration of the holotype in life.—Dorsal ground color is uniform dark brown, but with two dark spots on the scapular region (Figure 4). A dark interorbital mark is present, lying just posterior to an adjacent paler area. Dorsal surfaces of the legs and arms with dark bars. The surfaces below the canthus rostralis and the supratympanic fold, from the snout to the axilla, are darker (forming a type of mask); the dark pigment of the mask continues posteriorly bordering the supratympanic fold. The upper lip has dark bars with white pigment in form of

faded bars. The flanks are the same color as dorsum, but with white pigment in form of small spots. Ventral surfaces of the body and legs are violet-brown with bluish-white pigment in the form of blotches; ventral surface of tibia-tarsus grayish; ventral surface of the throat violet-brown is relatively uniform, slightly paler than the venter (Figure 5). The palmar surface of the hands with violet coloration, especially on thenar, palmar, and proximal subarticular tubercles; the fingers are whitish, with a white prominent fold between subarticular tubercles (Figure 6A).

Coloration of the holotype in ethanol.—After 2 years in ethanol (70%), the overall dark brown dorsum has changed little from its color in life. The violet-brown on ventral surfaces of the body and legs has faded to dark brown and the bluish-white blotches have changed to light brown. The overall patterns of the blotches and other markings on the holotype have remained identical to those that were observed while alive.

Measurements of holotype (mm).—SL 40.1; HL 15.6; HW 16.5; IOD 4.65; EW 3.2; IC 8.0; IN 4.85; TN 2.3; EN 3.45; ED 4.25; TY 2.3; UL 9.65; HaL 10.25; F1 4.85; F3 6.5; FL 22.65; TL 25.85; TaL 14.25; FoL 23.5; T3 8.1; T5 7.0. Measurements in related percentages: EW/IOD 68.82%; IOD/HW 28.18%; TY/ED 54.12%; EN/ED 81.12%; ED/HL 27.24%; IC/HL 51.28%; IN/EN 140.58%; IN/TN 210.87%; FL/TL 87.62%; TL/TaL 181.40%; TaL/FoL 60.64%; T3/FoL 34.47%; T5/FoL 29.79%; UL/HaL 94.15%; F1/HaL 47.32%; F3/HaL 63.42%.

Variation.—Morphometric variation is summarized in Table 1. *Craugastor aenigmaticus* has a relatively high level of intraspecific polymorphisms (Figure 8). The skin on venter ranges from almost completely smooth to heavily areolate, but always with a smooth midline. Some frogs have supraocular tubercles; additionally, some individuals have two postrictal tubercles that are not fused. The palmar tubercle is heart shaped in some specimens, but ovoid in others; the proximal subarticular tubercles are not visible in some specimens. In the adult male, UCR 22747, an accessory palmar tubercle is visible. In some specimens, the heel is areolate; in others one to three small tubercles are visible. Some specimens have flattened subarticular tubercles, whereas others have projecting subarticular tubercles. Some individuals have globular subarticular tubercles in profile, whereas others have obtuse subarticular tubercles. UCR 21951 and UCR 22734 have a pattern of dark brown lateral stripes on a pale brown background (Figure 8E); UCR 21956 has an olive-green dorsum (Figure 8F). The mask is absent in some specimens. The throat is a uniform cream coloration in some frogs, whereas it is uniform violet-brown, heavily mottled, or uniformly dark brown in others. The venter usually is dark-brown or grayish in coloration, but in some individuals, especially in juveniles, it is cream (Figure 8D). The juveniles have light brown arms in contrast to the dark brown dorsum (Figure 8C). The palmar surfaces of the hands in adults usually are dark brown with prominent white folds between subarticular tubercles, but in some frogs, the white bones are visible beneath the skin; in juveniles, the palmar surfaces are dark brown or cream.

Habitat and natural history notes.—*Craugastor aenigmaticus* inhabits the montane rainforest of the Cordillera de Talamanca (Holdridge 1967, Bolaños *et al.* 2005), which has a short dry season (1 or 2 mo), annual precipitation ranging between 2200 and 4500 mm, and annual temperatures ranging from 6–12°C. The type locality (Cerro Arbolado) and the other known localities consist of primary forest dominated by oak trees (genus *Quercus* L.) that are abundantly covered with bryophytes and epiphytes (Figure 9). The forest floor is covered by a thick layer of leaf litter and other types of decomposing organic material. Little is known about the natural history of *C. aenigmaticus,* but it is important to note that the species was abundant

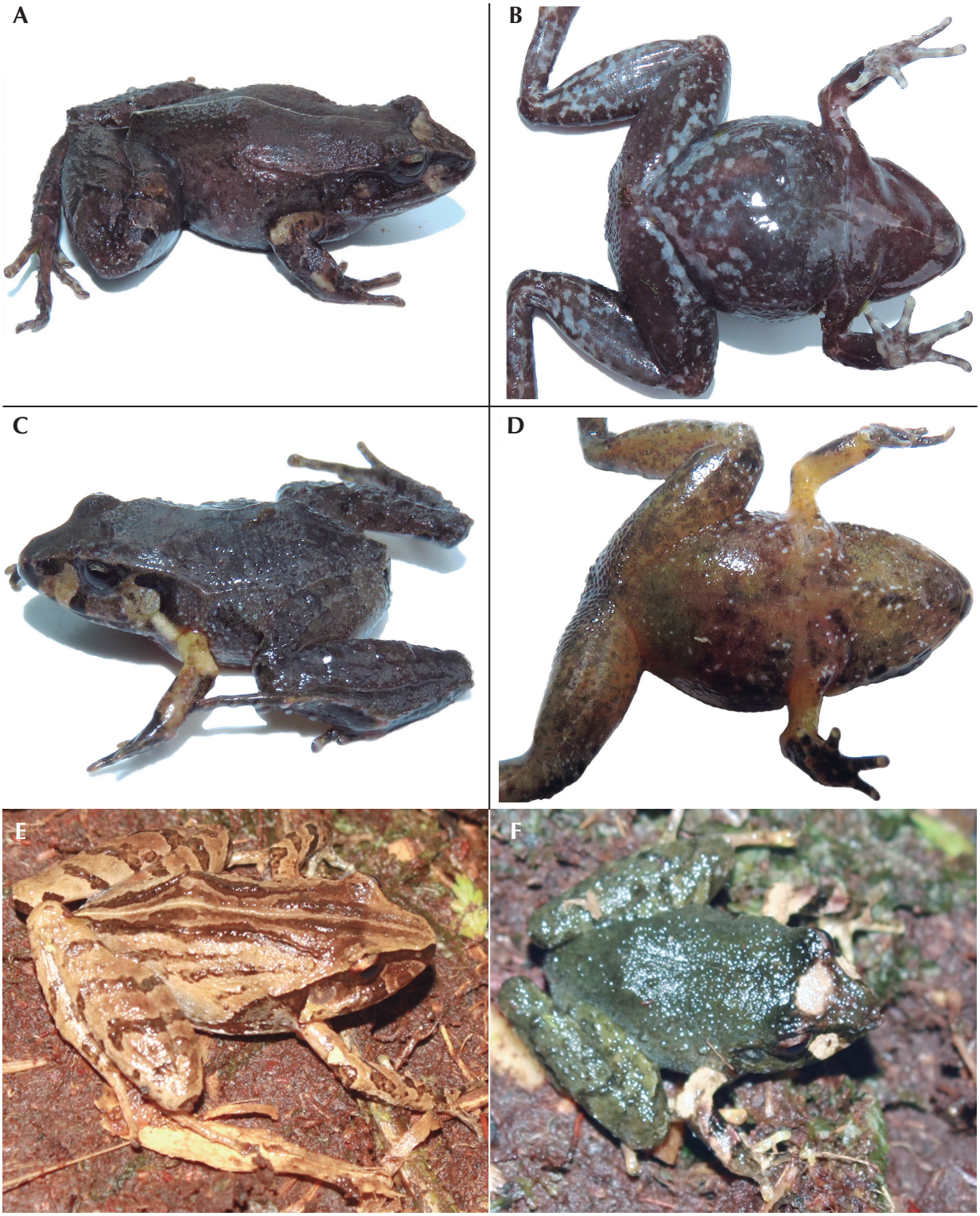

Figure 8. Variation on dorsum and venter of *Craugastor aenigmaticus* sp. nov. (**A, B**) Paratopotype UCR 22958, adult female; (**C, D**) paratopotype UCR 22959, juvenile; (**E**) paratype UCR 21951, adult female; (**F**) UCR 21956, juvenile. Photographs taken by EA.

Figure 9. Cloud forest at type locality of *Craugastor aenigmaticus* sp. nov., summit of Cerro Arbolado at 2600 m a.s.l. Photograph taken by Omar Becerra Soria.

during our fieldwork in the months of April and July 2013, December 2015, and October 2016. During December 2015 and October 2016, gravid females were observed. Juveniles were observed during all the periods of fieldwork; the smallest size recorded was with a SVL of 16.1 mm. All specimens of *C. aenigmaticus* collected were discovered as they were jumping on the forest floor. We did not record any vocalization that we could attribute to *C. aenigmaticus,* though we think that the frog may vocalize. *Craugastor aenigmaticus* is sympatric with *Diasporus ventrimaculatus* in Cerro Arbolado, Cerro Hakú, on the Caribbean slopes of Cerro Utyum, and Valle del Silencio, which is the type locality of *D. ventrimaculatus.*

Distribution.—Craugastor aenigmaticus is restricted to the summit of Cerro Arbolado, Cerro Hakú, Caribbean slopes of Cerro Utyum, Valle del Silencio, and Caribbean slopes of Cerro Pando (Figure 1). The altitudinal range of this taxon is 2330–2700 m a.s.l. The species occurs in primary forest; and the populations on Cerro Arbolado, Cerro Hakú, Cerro Utyum, and Valle del Silencio are within the La Amistad International Park and that on Cerro Pando is within the Zona Protectora Las Tablas. The distribution of *C. aenigmaticus* is fragmented along a line of ~ 80 km. We did not find this species during fieldwork carried out on Cerro Kamuk and Cerro Echandi. More fieldwork is required to assess the range of this species more

accurately. Given that this species is found at Cerro Pando, which is on the Costa Rica–Panama border, *C. aenigmaticus* is recognized as having a distribution within southeastern Costa Rica and at least for now, marginally into Panama.

Etymology.—The specific name is derived from the Latin word for enigmatic. We propose this name in light of the taxonomic confusion surrounding this species. *Craugastor aenigmaticus* was first collected in 2009 but was erroneously identified as *C. podiciferus* because of its great morphological resemblance to the latter, and its close proximity to the type locality of *C. podiciferus.* However, based on molecular data, we confirmed that this taxon is highly divergent genetically from other species in the *C. podiciferus* Species Group and represents a new species.

Discussion

With the recognition of *Craugastor aenigmaticus,* the *C. podiciferus* Species Group now comprises 10 species, all of which are endemic to Costa Rica and western Panama (Savage 2002, AmphibiaWeb 2018). However, the diversity within the *C. podiciferus* Species Group is underestimated and several species remain unnamed (Streicher *et al.* 2009). The high genetic divergence between *C. aenigmaticus* and all other members of the *C. podiciferus* Species Group strongly supports the distinctiveness of *C. aenigmaticus.* The above-mentioned genetic distances are greater than those suggested by Fouquet *et al.* (2007) for recognizing new taxa of Neotropical frogs, which is 3% 16S divergence as threshold to define candidate species. However, it is important to point out that although not closely related, *C. aenigmaticus* and *C. podiciferus* are morphologically similar. Furthermore, unlike other members of the group, both of these species inhabit the montane rainforest, although *C. podiciferus* is not restricted to it.

The montane rainforest of the Cordillera de Talamanca is naturally fragmented in two relatively large patches (Bolaños *et al.* 2005). One extends from Cerro Vueltas to Cerro Dúrika and the other from Cerro Arbolado to Cerro Echandi, and both are separated by a depression (2300 m a.s.l.). *Craugastor aenigmaticus* inhabits the patch that extends from Cerro Arbolado to Cerro Echandi. *Bolitoglossa kamuk, B. pygmaea, B. robinsoni,* and *B. splendida* are endemic to the same patch of montane rainforest. *Craugastor aenigmaticus* occurs in sympatry with *D. ventrimaculatus* in four localities, with the former only being known from one additional locality (Cerro Pando).

The restricted distribution of *Craugastor aenigmaticus* to montane rainforest highlights the importance of this habitat as center of diversification and endemism, and suggests that the highlands of the Cordillera de Talamanca have had an important role in the process of speciation, possibly in association with climatic fluctuations (Savage 2002, Streicher *et al.* 2009). At least eight amphibians are restricted to montane rainforest in Cordillera de Talamanca (Savage 2002, AmphibiaWeb 2018), a habitat that was naturally reduced along the last 2 million years and that is fragmented (Foster 2001).

Further studies are needed to help us resolve the phylogenetic relationships of *Craugastor aenigmaticus* within the *C. podiciferus* Species Group and to evaluate the biological significance of the high genetic distances that exist within the group. Additionally, it is important to continue inventory work in the montane rainforest to justify the need to protect the flora and fauna of this life zone, much of which may remain unknown.

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Appendix I. *Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Museum codes follow those of Frost (2018), with the addition of CRARC in reference to the Costa Rica Amphibian Research Center private collection and EAP denotes field numbers of Erick Arias.*

Species	Institutional vouchers	Collection locality	Geographic coordinates	Elevation (m a.s.l.)	GenBank Number	
					16S	COI
C. aenigmaticus sp. nov.	SMF: 104020	Jurutungo, Changuinola, Bocas del Toro, PA	8°54' N, 82°42' W	2388	MK211615	MK211577
C. aenigmaticus sp. nov.	SMF: 104021	Jurutungo, Changuinola, Bocas del Toro, PA	8°55' N, 82°42' W	2330	MK211621	-
C. aenigmaticus sp. nov.	UCR: 21951	Cerro Utyum, Talamanca, Limón, CR	9°20' N, 83°10' W	2700	MK211616	MK211578
C. aenigmaticus sp. nov.	UCR: 21952	Cerro Utyum, Talamanca, Limón, CR	9°20' N, 83°10' W	2690	MK211618	-
C. aenigmaticus sp. nov.	UCR: 21954	Cerro Utyum, Talamanca, Limón, CR	9°20' N, 83°10' W	2627	MK211620	-
C. aenigmaticus sp. nov.	UCR: 21956	Cerro Utyum, Talamanca, Limón, CR	9°20' N, 83°10' W	2690	MK211619	-
C. aenigmaticus sp. nov.	UCR: 22737	Cerro Hakú, Buenos Aires, Puntarenas, CR	9°19' N, 83°12' W	2660	MK211617	MK211579
C. aenigmaticus sp. nov.	UCR: 22739	Cerro Hakú, Buenos Aires, Puntarenas, CR	9°19' N, 83°12' W	2660	MK211622	-
C. bransfordii	UCR: 22269	San Miguel, Alajuela, Alajuela, CR	10º18' N, 84º10' W	466	KT950295	MK211571
C. bransfordii	UCR: 22643	Guayacán, Siquirres, Limón, CR	10º03' N, 83º32' W	537	MK211610	MK211572
C. gabbi	UCR: 21863	Fila Costeña, Coto Brus, Puntarenas, CR	8º47' N, 82º57' W	1200	KT950271	MK211567
C. gabbi	UCR: 21864	Fila Costeña, Coto Brus, Puntarenas, CR	8º47' N, 82º57' W	1200	KT950272	MK211568
C. lauraster	SMF: 79759	Selva Negra, Matagalpa, Matagalpa, NI	12º59' N, 85º54' W	1300	MK211608	MK211565
C. lauraster	USNM: 559393	Bodega del Río, Tapalwás, Gracias a Dios, HN	14º55' N, 84º32' W	150	KU323364	MK211566
C. persimilis	UCR: 22211	Tausito, Paraiso, Cartago, CR	9º47' N, 83º45' W	1050	KT950293	MK211570
C. persimilis	UCR: 22671	Suretka, Talamanca, Limón, CR	9º34' N, 82º56' W	121	MK211609	MK211569
C. podiciferus s.l.	CRARC: 012	Volcán Turrialba, Turrialba, Cartago, CR	10º01' N, 83º42' W	2250	MK211633	MK211589
C. podiciferus s.l.	CRARC: 247	Monte Verde, Tilarán, Guanacaste, CR	10º21' N, 84º48' W	1470	MK211645	-
C. podiciferus s.l.	EAP: 509	Fila Costeña, Golfito, Puntarenas, CR	8º47' N, 83º01' W	1546	-	MK211605
C. podiciferus s.l.	EAP: 810	Alto Uren, Talamanca, Limón, CR	9º21' N, 83º02' W	1860	MK211640	MK211596
C. podiciferus s.l.	EAP: 817	Alto Uren, Talamanca, Limón, CR	9º23' N, 83º01' W	1500	MK211630	MK211586

Appendix I. *Continued.*

Species	Institutional vouchers	Collection locality	Geographic coordinates	Elevation (m a.s.l.)	GenBank Number 16S	COI
C. podiciferus s.l.	FMNH: 257651	Fila Costeña, Coto Brus, Puntarenas, CR	8º47' N, 82º59' W	1350	EF562367	-
C. podiciferus s.l.	FMNH: 257669	Monte Verde, San Ramón, Alajuela, CR	10º18' N, 84º47' W	1500	EF562372	MK211598
C. podiciferus s.l.	FMNH: 257671	Monte Verde, San Ramón, Alajuela, CR	10º18' N, 84º47' W	1500	EF562374	MK211599
C. podiciferus s.l.	FMNH: 257673	Monte Verde, San Ramón, Alajuela, CR	10º18' N, 84º47' W	1500	EF562343	MK211603
C. podiciferus s.l.	SMF: 104005	Changena, Changuinola, Bocas del Toro, PA	8º59' N, 82º40' W	1766	MK211641	MK211597
C. podiciferus s.l.	UCR: 16353	Montaña Azul, Sarapiquí, Heredia, CR	10º12' N, 84º09' W	1500	EF562349	MK211602
C. podiciferus s.l.	UCR: 16361	Tapesco, Alfaro Ruiz, Alajuela, CR	10º13' N, 84º22' W	1930	EF562371	-
C. podiciferus s.l.	UCR: 16585	Copey, Dota, San José, CR	9º32' N, 83º51' W	1400	MK211647	-
C. podiciferus s.l.	UCR: 19853	Lori, Talamanca, Limón, CR	9º21' N, 83º13' W	1817	MK211639	MK211595
C. podiciferus s.l.	UCR: 19856	Lori, Talamanca, Limón, CR	9º21' N, 83º13' W	1817	MK211637	MK211593
C. podiciferus s.l.	UCR: 19860	Lori, Talamanca, Limón, CR	9º21' N, 83º12' W	2108	MK211636	MK211592
C. podiciferus s.l.	UCR: 19862	Lori, Talamanca, Limón, CR	9º21' N, 83º12' W	2108	MK211638	MK211594
C. podiciferus s.l.	UCR: 20992	Nectandra, Alfaro Ruiz, Alajuela, CR	10º13' N, 84º20' W	2143	MK211632	MK211588
C. podiciferus s.l.	UCR: 22091	Quebradas, Pérez Zeledón, San José, CR	9º26' N, 83º40' W	1488	MK211646	MK211604
C. podiciferus s.l.	UCR: 22120	Altamira, Buenos Aires, Puntarenas, CR	9º19' N, 83º27' W	1821	MK211642	-
C. podiciferus s.l.	UCR: 22146	Cascajal, Coronado, San José, CR	10º01' N, 83º56' W	1700	MK211635	MK211591
C. podiciferus s.l.	UCR: 22201	El Empalme, El Guarco, Cartago, CR	9º42' N, 83º56' W	2395	MK211634	MK211590
C. podiciferus s.l.	UCR: 22226	Pico Blanco, Escazú, San José, CR	9º51' N, 84º08' W	2242	MK211644	MK211601
C. podiciferus s.l.	UCR: 22228	Pico Blanco, Escazú, San José, CR	9º51' N, 84º08' W	2242	MK211643	MK211600
C. podiciferus s.l.	UCR: 22675	Monte Verde, Puntarenas, Puntarenas, CR	10º19' N, 84º47' W	1726	-	MK211606
C. podiciferus s.l.	UCR: 22690	Chumacera, Pérez Zeledón, San José, CR	9º19' N, 83º28' W	1793	MK211631	MK211587
C. polyptychus	UCR: 20050	Dabagri, Talamanca, Limón, CR	9º37' N, 83º16' W	900	MK211614	MK211576
C. polyptychus	UCR: 22668	Bribri, Talamanca, Limón, CR	9º36' N, 82º54' W	198	MK211613	MK211575

Appendix I. *Continued.*

Species	Institutional vouchers	Collection locality	Geographic coordinates	Elevation (m a.s.l.)	GenBank Number 16S	COI
C. stejnegerianus	EAP: 0514	Palmar Norte, Osa, Puntarenas, CR	8º57' N, 83º26' W	45	MK211607	MK211563
C. stejnegerianus	UCR: 20352	Potrero Grande, Buenos Aires, Puntarenas, CR	9º05' N, 83º06' W	900	KT950284	MK211564
C. underwoodi	UCR: 22619	Tapantí, Paraíso, Cartago, CR	9º45' N, 83º46' W	1412	MK211611	MK211573
C. underwoodi	UCR: 22625	Cascajal, Coronado, San José, CR	10º01' N, 83º56' W	1708	MK211612	MK211574
Craugastor sp.1	UCR: 20389	Potrero Grande, Buenos Aires, Puntarenas, CR	9º06' N, 83º06' W	1500	MK211625	MK211581
Craugastor sp.1	UCR: 22709	Las Alturas, Coto Brus, Puntarenas, CR	8º58' N, 82º49' W	1980	MK211626	MK211582
Craugastor sp.2	SMF: 104014	Cerro Saguí, Nole Duima, Ngöbe Buglé, PA	8º33' N, 81º49' W	1762	MK211623	-
Craugastor sp.2	SMF: 104015	La Nevera, Nole Duima, Ngöbe Buglé, PA	8º30' N, 81º46' W	1700	MK211624	MK211580
Craugastor sp.B	SMF: 102024	Fortuna, Gualaca, Chiriquí, PA	8º40' N, 82º11' W	1730	MK211627	MK211583
Craugastor sp.B	SMF: 104023	Fortuna, Gualaca, Chiriquí, PA	8º40' N, 82º12' W	1280	MK211628	MK211584
Craugastor sp.B	SMF: 104027	Volcán Barú, Bugaba, Chiriquí, PA	8º50' N, 82º30' W	2134	MK211629	MK211585

Appendix II. *Specimens used in the morphometric analysis. Museum collection acronyms follow Frost (2018), with the addition of EAP refers to Erick Arias field numbers.*

Craugastor aenigmaticus sp. nov. COSTA RICA: Limón: Cerro Utyum, Telire, Talamanca (UCR: 21951–21952, 22413–22415); Valle del Silencio, Telire, Talamanca (UCR: 21194, 21931–21932). Puntarenas: Cerro Arbolado and Cerro Hakú, Buenos Aires, Buenos Aires (EAP: 758–763; UCR: 22731–22734, 22737, 22747).

Craugastor podiciferus. COSTA RICA: Alajuela: Poasito, Sabanilla, Alajuela (UCR: 21317–21319); Zarcero, Palmira, Alfaro Ruiz (UCR: 20992–20994); Los Alpes, Piedades Sur, San Ramón (UCR: 13961–13962). Santa María, Aguas Claras, Upala (UCR: 10528). Cartago: Coris, Quebradilla, Cartago (UCR: 3490–3491); Empalme, San Isidro, Guarco (UCR: 22200, 22202, 22204–22205); Tapantí, Orosi, Paraíso (UCR: 11542, 12010, 21708); Vereh, Chirripó, Turrialba (EAP: 748–750). Heredia: San José de la Montaña, Barva (UCR: 21299, 21352); Cerro Chompipe, Varablanca, Heredia (UCR: 18403–18404); La Legua, Varablanca, Heredia (UCR: 17464, 17478); Montaña Azul, Cureña, Sarapiquí (UCR: 16354). Limón: Fila Matama, Matama, Limón (UCR: 20177, 20213); Cerro Pat, Telire, Talamanca (EAP: 0792, 801–803, 807, 810, 814, 817–818); Sabanas Dúrika, Telire, Talamanca (UCR: 21662, 21682, 21825, 21836); Transtalamanca, Telire, Talamanca (UCR: 19849, 19853, 19860, 19870, 19874, 19895, 19933). Puntarenas: Cerro Quemado, Biolley, Buenos Aires (UCR: 21191, 21700); Las Cruces, San Vito, Coto Brus (UCR: 12934, 12936, 13239); Las Tablas, Sabalito, Coto Brus (UCR: 8379, 8381–8382, 10738, 10747–10748, 12560); Monte Verde, Puntarenas (UCR: 13646–13647, 17243, 21718, 22674–22676). San José: Pico Blanco, San Antonio, Escazú (UCR: 22228); Tinamaste, Barú, Pérez Zeledón (UCR: 14510, 14513, 14518); Quebradas, San Isidro del General, Pérez Zeledón (EAP: 738–740; UCR: 16359–16360, 22606); Chumacera, San Pedro, Pérez Zeledón (EAP: 721–723; UCR: 22119); Cascajal, Vazquez de Coronado (UCR: 16090, 16092, 21918).

Craugastor sp.1. COSTA RICA: Puntarenas: Las Alturas, Pittier, Coto Brus (UCR: 22703–22704, 22709–22710); Tres Colinas, Potrero Grande, Buenos Aires (EAP: 725, 727–729; UCR: 20257–20258, 20389, 20395, 20401, 20411, 20419, 20421, 20423, 20428); road to Las Tablas, Sabalito, Coto Brus (EAP: 0823).

DISCUSIÓN Y CONCLUSIONES GENERALES

La importancia de las tierras altas de América Central Ístmica

Este trabajo contiene la mejor representatividad geográfica para cualquier grupo de anfibios en ACI, incluyendo todas las localidades tipo. Esto permitió obtener resultados sólidos sobre la distribución de las especies, pero en especial de la importancia de las tierras altas de Costa Rica y Panamá en la diversificación del grupo de especies *Craugastor podiciferus.* Todos los linajes encontrados contienen poblaciones por encima de los 750 m de elevación en las tierras altas de Costa Rica y el oeste de Panamá. Lo anterior apoya resultados previos que sugieren que las tierras altas de ACI tuvieron un papel importante en la diversificación de la biota regional (García-París *et al.* 2000; Savage 2002; Crawford *et al.* 2007; Boza-Oviedo *et al.* 2012).

Los resultados obtenidos son similares a los hallazgos previos sobre la importancia de la diversificación en tierras altas de ACI . No obstante, existe controversia en la datación cronológica de los procesos responsables de esta diversificación. Savage (2002), con base en la evidencia de oscilaciones climáticas y asumiendo un rápido levantamiento de las tierras altas de América Central en el Plioceno-Pleistoceno (~5 Ma), propuso un modelo de especiación de tierras altas (especiación montana). Este autor sugirió que las oscilaciones entre periodos glaciares e interglaciares que comenzaron en el Plioceno (~5 Ma) y continuaron durante el Pleistoceno, son las responsables del origen y distribución actual de muchas especies de tierras altas. Durante periodos glaciares los bosques montanos estuvieron conectados, y periodos subsecuentes de calentamiento llevaron al aislamiento de estos bosques en las cumbres de las cordilleras en América Central, lo cual originó muchas de las especies actuales (Savage 2002). Sin embargo, los resultados encotrados en este estudio junto con los encontrados por Castoe *et*

al. (2009) y Streicher *et al.* (2009) rechazan que estos eventos del Plioceno-Pleistoceno estén asociados con cladogénesis dentro de especies de altura. Castoe *et al.* (2009) encontraron que la diversidad de serpientes de foseta neotropicales en las tierras altas de América Central, es mejor explicada por eventos tectónicos durante el Mioceno-Plioceno. Aunque esto coincide parcialmente (Plioceno) con la propuesta de Savage (2002), lo cierto es que definitivamente la diversificación es anterior al Pleistoceno.

La datación cronológica del origen del grupo de especies *C. podiciferus* realizada en este trabajo fue similar a aquella obtenida por Streicher *et al.* (2009), quienes calcularon el origen del mismo en aproximadamente 20 Ma. Asumiendo un origen temprano durante el mioceno para ACI (Montes *et al.* 2012a,b, 2015). Nuestros datos sugieren que la llegada a ACI del ancestro común del grupo de especies *C. podiciferus* corresponde con las primeras colonizaciones de ACI, previo al cierre del Istmo de Panamá. Este momento histórico en América Central no debe ser entendido como un evento aislado, sino, como pulsos discontinuos de dispersión de la fauna ya presente en América Central Nuclear hacia el sur colonizando las tierras de Costa Rica y Panamá a medida que estas se levantaban. Es probable que estos pulsos de dispersión hayan sido separados por altos niveles del mar, lo cual aisló temporalmente estas dos unidades de América Central propiciando la evolución de diferentes grupos (Castoe *et al.* 2009; Gutiérrez-García & Vázquez-Domínguez 2013).

Posiblemente el inicio de estos pulsos de dispersión se remonta al Mioceno Temprano (~23 Ma), cuando ACI había emergido y se prolongaba como una península en su posición actual (Montes *et al.* 2012a). Estos primeros eventos de dispersión corresponden con la aparición del grupo de especies *C. podiciferus* hace ~20 Ma (Streicher *et al.* 2009). Otros grupos apoyan esta primera dispersión desde América Central Nuclear hacia ACI. Duellman *et al.* (2016)

encontraron que el origen y diversificación del género de ranas arborícolas *Isthmohyla* en ACI se remonta a ~19.5 Ma. Říčan *et al.* (2013) encontraron que los peces dulceacuícolas de ACI llegaron desde el norte entre 19–13 Ma. Rovito *et al.* (2015) sugirieron que las salamandras neotropicales de la tribu Bolitoglossine se dispersaron hacia el sur, y que hace 16.4–8 Ma arribaron a Sudamérica, por lo cual debieron llegar a ACI previamente.

Aunque el presente trabajo contiene una amplia representación geográfica para el grupo de especies *C. podiciferus*, es necesario continuar con el trabajo de campo y la recolección de muestras, especialmente en las tierras altas de Costa Rica y el oeste de Panamá. Es posible que otras especies permanecen sin describir en sitios remotos de las cordilleras, lo anterior dado que es posible que las especies de alturas tengan áreas de distribución muy restringidas como el caso de *Craugastor* sp. Chumacera y *Craugastor* sp. Siola. En particular, es necesario realizar esfuerzos intensivos en las tierras altas de la vertiente pacífica de la cordillera de Talamanca, dado que *C. podiciferus* parece no distribuirse sobre el pacífico de Talamanca y que existe alta estructuración con varias especies distintas.

Comentarios taxonómicos al grupo de especies *Craugastor podiciferus*

Este es el primer estudio filogenético con datos moleculares para el grupo de especies *C. podiciferus* en América Central, ya que estudios anteriores analizaron algunos clados (Crawford 2003; Streicher *et al.* 2009). Como se había sugerido previamente, se encontró gran diversidad de especies no descritas y enmascaradas por los nombres actuales dentro del grupo. Al inicio del estudio, el grupo contenía ocho especies (Padial *et al.* 2014) y al final de este se detectaron hasta 23 linajes independientes dentro del grupo. Con algunas excepciones (*C. aenigmaticus*) la gran mayoría de estos grupos han pasado desapercibidos en colecciones científicas por muchos años,

resaltando la complejidad de delimitar morfológicamente las especies y reconocer aquellas distintas.

Este trabajo incluyó marcadores mitocondriales y una gran cantidad de SNPs nucleares, siendo el más extensivo para el grupo de especies *C. podiciferus,* y para el género *Craugastor,* hasta la fecha. El empleo de ambos marcadores en combinación con métodos de delimitación molecular permitió dilucidar la diversidad presente en el grupo, mucha de la cual ha sido enmascrada por los nombres actuales. En el presente estudio se describieron cuatro especies nuevas (*C. aenigmaticus, C. gabbi, C. sagui* y *C. zunigai*), se sinonimizaron tres especies (*C. jota, C. lauraster* y *C. polyptychus*) y dos más fueron resucitadas (*C. blairi* y *C. rearki*). Otras especies necesitan ser nombradas y descritas; sin embargo, estas deben estar acompañadas de exhaustivos análisis morfológicos que respalden su distinción y permitan una correcta diagnosis.

El principal aporte de este trabajo es la comprensión de las relaciones filogenéticas dentro del grupo de especies *C. podiciferus.* Se encontró que el grupo está formado por cuatro clados, tres de los cuales coinciden con los tres grupos morfológicos que se mencionaron previamente (Savage 2002). Un grupo adicional, el más basal según la filogenia nuclear, fue identificado y nombrado (*C. aenigmaticus*). Esta especie es la más divergente del grupo y no había sido previamente colectada, es decir, no estuvo enmascarada por alguna de las especies nombradas. *C. aenigmaticus* es una especie microendémica a la Cordillera de Talamanca en el extremo sureste de Costa Rica, conocida únicamente entre los 2390–2650 m de elevación.

Un segundo gran clado contiene dos subclados, el primero está formado por tres especies distribuidas en el extremo sureste de Costa Rica y oeste de Panamá en la Cordillera de Talamanca y Cordillera Central de Panamá. Estas tres especies están distribuidas alopátridamente sobre el eje de las Cordilleras. Dos de estas especies fueron descritas en el

presente estudio, *C. sagui* y *C. zunigai*, una especie adicional corresponde con *C. blari* (Barbour, 1928) nombre que estaba bajo sinonimia de *C. podiciferus* y que fue resucitado en este trabajo. Un segundo subclado contiene a *C. podiciferus* y una serie de grupos estrechamente relacionados a esta. Con la obtención de material topotípico de *C. podiciferus* se logró restringir a *C. podiciferus sensu stricto* a las poblaciones de la Cordillera Volcánica Central de Costa Rica y la Cordillera de Talamanca en Costa Rica y el oeste de Panamá, en esta última restringida a la vertiente caribeña. Esta especie corresponde con los clados C y D de Streicher *et al.* (2009).

El subclado conteniendo a *C. podiciferus* incluye una serie de grupos que necesitan ser evaluados morfológicamente, ya que posiblemente representen al menos seis especies no nombradas. El grupo *Craugastor* sp. Monte Verde corresponde con el clado A de Streicher *et al.* (2009), un grupo restringido a la Cordillera de Tilarán y Volcánica Central. *Craugastor* sp. San Gerardo corresponde con el grupo B de Streicher *et al.* (2009), distribuido en la Cordillera de Tilarán, Volcánica Central y Valle Central. En la zona de Monte Verde los grupos *Craugastor* sp. Monte Verde y *Craugastor* sp. San Gerardo están en simpatría potencial por la cercanía de sus localidades, sin embargo, no se conocen las dos especies en el mismo punto. Lo mismo sucede en Zarcero entre los grupos *C. podiciferus* y *Craugastor* sp. Monte Verde. *Craugastor* sp. Fila Costeña corresponde con los clados E y F de Streicher *et al.* (2009), un grupo restringido al pacífico sur de Costa Rica en la Fila Costeña y las faldas de Talamanca. Adicionalmente, se encontraron tres grupos que no fueron incluidos por Streicher *et al.* (2009). *Craugastor* sp. Chumacera conocido de una única población en el pacífico de la Cordillera de Talamanca. *Craugastor* sp. Pico Blanco, restringido al Cerro Pico Blanco en el Valle Central de Costa Rica. Y *Craugastor* sp. Siola también restringido a una única población, pero en el caribe de la Cordillera de Talamanca.

La especie *C. jota*, fue atribuida al grupo de especies *C. podiciferus* por Hedges *et al.* (2008) y basado en la morfología esta debe pertenecer al clado *C. podiciferus.* Esta especie fue descrita con especímenes provenientes del río Changena en el oeste de Panamá sobre los 760 m, colectados por Linda Trueb en 1966. La localidad tipo dada por Lynch (1980) se basa en el mapa mostrado por Trueb (1968), sin embargo, esta localidad es discutida, ya que se basa en un mapa de poca calidad y con nombres de ríos que en la actualidad no coinciden. Aquí se incluyen especímenes provenientes de "Río Changena", muy cerca de la localidad tipo de *C. jota* (Linda Trueb comunicación personal), no obstante, estos son conespecíficos con *C. podiciferus sensu stricto*. Por lo tanto, se decidió sinonimizar a *C. jota* bajo *C. podiciferus*.

Un tercer gran clado contiene las especies relacionadas a *C. bransfordii*, conformado por seis especies, aunque solo dos están nombradas. La especie *C. bransfordii* fue descrita con especímenes del Río San Juan en la región limítrofe entre Costa Rica y Nicaragua, aquí se incluye un espécimen cuya localidad es muy cercana a la posible localidad tipo. De esta manera se pudo restringir *C. bransfordii* al sur de Nicaragua y el Caribe norte y central de Costa Rica. La especie nominal *C. polyptychus* fue descrita con especímenes de Río San Juan, Nicaragua, la misma localidad tipo de *C. bransfordii.* Savage (2002) resucitó este nombre de la sinonimia de *C. bransfordii* para asignarlos a las poblaciones del Caribe norte y sur de Costa Rica, sin embargo, mencionó que es probable que estas poblaciones deban recibir otro nombre. Dado que en la región del Río San Juan (límite entre Costa Rica y Nicaragua) no se encontraron dos clados distintos dentro del gran clado *C. bransfordii* se decidió sinonimizar el nombre *C. polyptychus* bajo *C. bransfordii.* La especie *Craugastor* sp. Fila Carbón corresponde –en parte– al *C. polyptychus* de Savage (2002), esta especie está conformada por especímenes provenientes del

Caribe Sur de Costa Rica y extremo oeste de Panamá, con una distribución de tierras bajas a intermedias.

Craugastor underwoodi fue descrita con especímenes de Bajo La Hondura, Costa Rica, aquí se incluyen especímenes de Cascajal y Cinchona dos localidades geográfica y biológicamente cercanas a la localidad tipo. *Craugastor underwoodi* se distribuye en la región premontana de la Cordillera Volcánica Central, Cordillera de Tilarán y extremo norte de la Cordillera de Talamanca. La especie *Craugastor* sp. Quebradas incluye la única localidad en el Pacífico costarricense para el clado *C. bransfordii.* La especie *Craugastor* sp. Vereh es representada por dos poblaciones en el Caribe Central de Costa Rica, su gran distancia genética con respecto a los demás grupos y su cambio en la posición filogenética entre las filogenias mitocondrial y nuclear respalda su validez como nueva especie. *Craugastor* sp. Panamá está conformada por poblaciones de Panamá, poblaciones atribuida por Leenders (2016) a *C. bransfordii,* su validez como nueva especie es también apoyada por su cambio en la posición filogenética entre las filogenias mitocondrial y nuclear.

El cuarto gran clado contiene a las especies relacionadas a *C. stejnegerianus,* conformado por seis especies, cuatro de estas nombradas. *Craugastor persimilis* agrupa especímenes de varias localidades en el Caribe Central y Sur de Costa Rica. Este grupo incluye la localidad tipo de *C. persimilis,* Suretka, Talamanca. Un caso muy particular es *C. rearki* (Taylor, 1952), esta fue descrita del Caribe de Costa Rica, Savage & Emerson (1970) la asignaron bajo sinonimia de *C. bransfordii.* En este trabajo el nombre *C. rearki* se asigna a las poblaciones del caribe central y norte de Costa Rica (incluyendo una localidad de donde se asignaron paratipos para *C. rearki*), pero además, están incluidos especímenes de Nicaragua y Honduras cercanas a la localidad tipo

de *C. lauraster*. Bajo el principio de prioridad debe prevalecer *C. rearki* y sinonimizar a *C. lauraster* bajo el primero.

Las poblaciones del clado *C. stejnegerianus* del pacífico costarricense son altamente conflictivas, dado los cambios significativos en las posiciones filogenéticas entre las filogenias mitocondrial y nuclear. Basado en la filogenia mitocondrial y la evidencia morfológica se respalda a *C. gabbi,* la cual fue recientemente descrita (Arias *et al.* 2016) y distribuida en el hábitat premontano del Pacífico Sur de Costa Rica y el oeste de Panamá. Sin embargo, en la filogenia nuclear *C. gabbi* es el clado hermano a *C. stejnegerianus sensu stricto,* por lo cual, si se reconoce a *C. gabbi* como especie válida entonces se deben reconocer a *Craugastor* sp. Quepos y *Craugastor* sp. Neilly. La primera restringida a las poblaciones del Pacífico Central y el Valle Central de Costa Rica, constituyendo el clado hermano a *C. rearki*. *Craugastor* sp. Neilly está restringida a el extremo sureste del pacífico costarricense y el clado hermano a *C. gabbi* + *C. stejnegerianus sensu stricto,* el último restringido al pacífico sur de Costa Rica.

El presente trabajo constituye una primera aproximación al entendimiento de las relaciones filogenéticas y los patrones biogeográficos para el grupo de especies *C. podiciferus*; sin embargo, persisten grandes retos por resolver en el futuro. Los resultados de este trabajo demostraron que el grupo *C. podiciferus* está compuesto por cuatro grandes clados, no obstante, solo el grupo basal y un subclado del segundo fueron taxonómicamente resueltos. Es necesario realizar una revisión morfológica exhaustiva de *C. podiciferus* con el fin de delimitar morfológicamente las distintas especies que permanecen enmascaradas por este nombre y que fueron identificadas en este estudio. Dentro del clado *C. bransfordii* cuatro especies carecen de nombre y formal descripción. La experiencia previa sugiere que las especies dentro del clado *C. bransfordii* son morfológicamente muy conservadas, por lo cual, es necesario realizar un

exhaustivo análisis morfológico que permita una correcta diagnosis y separación de las especies nombradas. Finalmente, dentro del clado *C. stejnegerianus,* es necesario determinar el estatus de los grupos asociados a *C. stejnegerianus* del pacífico costarricense, ya que estos fueron altamente diferentes entre la filogenia mitocondrial y la nuclear.

El uso de RAD-seq permitó obtener una gran cantidad de SNPs nucleares para el grupo de especies *C. podiciferus.* Esto permitió obtener filogenias robustas para el grupo, pero también, hipótesis mejor apoyadas para la delimitación de las especies dentro del grupo de estudio. Es importante resaltar que la técnica utilizada ddRAD-seq (Peterson *et al.* 2012; Leaché *et al.* 2015), fue altamente eficiente en la obtención de gran cantidad de loci homólogos para este grupo de organismos no-modelo. Estos SNPs permitieron una adecuada delimitación de las especies, dado que estos marcadores son considerados una fuente de evidencia independiente a aquella obtenida con los marcadores mitocondriales. Además, la técnica de ddRAD-seq tiene como ventaja el bajo costo relativo a la cantidad de secuencias obtenidas y el poco tiempo necesario para la obtención de las mismas. Sin embargo, los datos obtenidos con RAD-seq también presentan una serie de limitaciones, como por ejemplo dado que los SNPs son de longitud corta (~100 pb) son inadecuados para ciertos métodos de delimitación molecular como BPP, donde no se pueden tratar como loci independientes. Asimismo, la longitud de los SNPs es un impedimento para la otención de árboles de máxima credibilidad a través de BEAST.

Delimitación y especies crípticas

Hasta la fecha ninguna de las especies del grupo *C. podiciferus* puede ser considerada críptica bajo el concepto *sensu stricto*, es decir, morfológicamente idénticas (Pérez-Ponce de León & Nadler 2010). Sin embargo, en la práctica estas han sido tratadas como crípticas y su

delimitación se ha basado en análisis moleculares que permitieron delimitar grupos, que posteriormente fueron revisados morfológicamente y permitió identificar características diagnósticas para todas las especies analizadas según lo sugerido por Pérez-Ponce de León & Nadler (2010). Es necesario continuar realizando análisis morfológicos para todos los clados identificados, con el fin de determinar si existen caracteres diagnósticos para todas las especies dentro del grupo. En especial para aquellas dentro del grupo *C. bransfordii,* las cuales son morfológicamente muy similares (sin realizar análisis exhaustivos hasta la fecha). Pero también, está latente la posibilidad de que existan especies crípticas *sensu stricto* dentro del grupo *C. podiciferus,* lo cual sería un hallazgo novedoso de un fenómeno poco documentado.

El empleo de marcadores moleculares es fundamental cuando se trata con especies crípticas, al menos en el sentido funcional, es decir, aquellas que el taxónomo no puede reconocer en su tratamiento actual (Pérez-Ponce de León & Nadler 2010), dado que una de las propiedades intrínsecas de las especies crípticas es el conservadurismo morfológico. Sin embargo, desde una perspectiva de taxonomía integradora (Dayrat 2005; Padial *et al.* 2010) son necesarias al menos dos fuentes de evidencia independientes para el reconocimiento de separación de linajes. Lo anterior no debe ser un problema cuando se trata de especies que no son hermanas, por ejemplo, cuando se debe describir una especie para evitar la parafilia de especies. Ya que en el caso anterior la posición filogenética de la especie es evidencia suficiente para reconocer su distintividad como linaje evolucionando independientemente (de Queiroz 2007; Padial *et al.* 2010). Un ejemplo fue la descripción de *C. gabbi,* cuyas poblaciones fueron históricamente referidas como *C. stejnegerianus,* sin embargo, análisis mitocondriales demostraron que *C. gabbi* es el grupo hermano al clado formado por *C. stejnegerianus* + *C.*

lauraster. En el ejemplo anterior, se logró identificar un carácter diagnóstico que permite la diferenciación de la recién nombrada especie de las demás dentro del grupo.

Es importante tomar en cuenta la diferencia entre caracteres diagnósticos e historia evolutiva única (Frost & Kluge 1994). Por ejemplo, en *C. gabbi* la principal evidencia apoyando la historia evolutiva única de esta es su posición filogenética. Una alternativa para evitar la parafilia era reconocer como una sola especie a todo el clado que contiene a *C. gabbi, C. lauraster* y *C. stejnegerianus*. Una opción que debe ser formalmente considerada y evaluada antes de nombrar alguna nueva especie, no obstante, es necesario evitar especies altamente variables en múltiples caracteres fenotípicos.

Resulta complicado tomar la decisión de nombrar una nueva especie la cual es muy similar a su especie hermana, ya que en este caso la similitud morfológica es mejor explicada como el resultado de la escasa divergencia evolutiva desde el proceso de especiación. En este caso, la posición filogenética no es suficiente para justificar la presencia de dos especies hermanas morfológicamente muy similares (Castroviejo-Fisher *et al.* 2017). Dado que la estructura jerárquica de variación de caracteres que típicamente se observa durante la especiación, puede ser simulado por procesos como variación geográfica dado a aislamiento por distancia o por selección/deriva génica en poblaciones pequeñas (Padial *et al.* 2010; Castroviejo-Fisher *et al.* 2017). En estos casos es necesario otros tipos de evidencia que apoyen la separación de linajes, como encontrar ambas especies en simpatría, divergencia de cantos reproductivos o la combinación de marcadores mitocondriales y nucleares (Padial *et al.* 2010; Castroviejo-Fisher *et al.* 2017). Un ejemplo de este caso fue el reconocimiento de *C. blairi* y *C. zunigai* como especies válidas a pesar de que son especies hermanas y que era también válido reconocer solamente una especie sin problemas de parafilia. En este caso se logró identificar caracteres morfológicos que

permiten la diferenciación de ambas especies, lo cual apoya la hipótesis de que ambas especies poseen una trayectoria evolutiva única.

En el caso hipotético donde ninguna evidencia adicional, más que la filogenética, apoye la divergencia de linajes entre dos clados hermanos, lo mejor es reconocer una sola especie ya que se podría caer en inflación taxonómica (Isaac *et al.* 2004). Lo anterior es fundamental ante el escenario de sobreestimación de especies por los métodos de delimitación molecular (Sukumaran & Knowles 2017). Los resultados encontrados en este estudio sugieren que varios de los métodos de delimitación molecular sobreestiman la diversidad real dentro de un grupo, por lo cual, es necesario la utilización de al menos dos fuentes de evidencia para dividir una especie en dos o más. En este sentido, es válida la recomendación de ser cauteloso con los resultados generados por métodos de delimitación molecular, estos deben ser entendidos como prospección molecular, mas no como evidencia irrefutable de separación de linajes. Pueden ser utilizados como evidencia preliminar para realizar otra serie de análisis (morfológico, acústico, genético, etc) que apoyen la divergencia de especies.

Por último, es necesario realizar estudios con las especies del grupo *C. podiciferus* que permitan un mejor entendimiento de la historia natural de estas especies. A la fecha se conoce poco sobre los miembros del grupo, a pesar de que estos son relativamente abundantes y fáciles de encontrar. Por ejemplo, aunque posiblemente todas las especies del grupo realizan cantos de aviso (Savage 2002), este solo ha sido detalladamente descrito para dos especies (Schlaepfer & Figeroa-Sandi 1994; Twining & Cossel 2017) y recientemente descrito para otras dos (Cossel *et al.* En prensa). Adicionalmente, se desconoce la alimentación, los depredadores, uso de hábitat, picos de actividad, épocas reproductivas, cortejo, etc. Es posible que en el pasado el grupo haya

sido poco estudiado dada la gran complejidad para discernir entre especies y el alto grado de polimorfismos fenotípicos intrapoblacional que sugería que más de una especie estaba presente. La presente contribución aporta información sobre las relaciones filogenéticas y la delimitación geográfica de las especies del grupo, que puede servir para estudios futuros donde se ahonde en la biología particular de cada especie.

Conclusiones

- El presente trabajo contiene la mayor representación geográfica y la mayor cantidad de datos moleculares para el grupo *Craugastor podiciferus* a la fecha, y representa uno de los trabajos sistemáticos más exhaustivos dentro de la familia Craugastoridae.
- El grupo de especies *C. podiciferus* contiene diversidad considerable de especies bajo los nombres actuales, los cuales están conformados por al menos 23 especies distintas.
- Aunque existe alto conservadurismo morfológico dentro del grupo de especies *C. podiciferus*, se logró reconocer cuatro especies nuevas y se redefinieron otras ya descritas, incluyendo una adecuada diagnosis y comparación entre especies.
- El origen biogeográfico del grupo de especies *C. podiciferus* corresponde con las tierras altas de Costa Rica y el oeste de Panamá, área donde se distribuyen todos los linajes actualmente conocidos.
- Las especies del grupo *C. podiciferus* ameritan claras estrategias de conservación, ya que a pesar de que son relativamente abundantes sus áreas de distribución son restringidas.

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APÉNDICES

APÉNDICE I

140 YEARS AFTER WILLIAM M. GABB`S CLIMB TO CERRO PICO BLANCO

Autores: Erick Arias y Gerardo Chaves

Fuente: Mesoamerican Herpetology, 1 (1): 176–180. 2014

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140 years after William M. Gabb's climb to Cerro Pico Blanco

On 4 February 1873, the American geologist William M. Gabb arrived in Puntarenas, Costa Rica, commissioned by the Keith brothers and the Costa Rican president, Tomás Guardia. He remained in Costa Rica until August of 1874, and returned to the United States where he died on 30 May 1878 at the age of 39. We will not provide a more extensive biography on W. M. Gabb, since other authors have written extensively about his life (Dall, 1909) and contributions to herpetology (Savage, 1970), geology and cartography (Denyer and Soto, 1999; Denyer and Lücke, 2007), and ethnic and socio-political studies in Costa Rica (Gabb, 1978). Instead, the purpose of this note is to analyze the route William M. Gabb traveled on his expedition to Cerro Pico Blanco, because of its importance in the study of the Costa Rican herpetofauna.

During his 19 months in Costa Rica, Gabb primarily was engaged in studying the Talamanca region (Gabb, 1894). As part of his research he was required to climb Cerro Pico Blanco, currently known as Cerro Kamuk. Whether he actually went to Kamuk or another peak has been debated (see below). During this trip, Gabb collected specimens of 20 species of amphibians and reptiles (Cope, 1875), of which 15 were described as new species (Cope, 1875; Savage, 1970). To this day, 12 specimens from Gabb's collection are maintained as holotypes (Frost, 2014; Table 1). Due the great scientific value of Gabb's collections, it is important to clarify the actual collecting localities from where the specimens came. Identifying the type locality of a species is an essential consideration when attempting to visit the site to collect new specimens to replace lost material, or to obtain tissues for molecular studies. Thus, the goals of this contribution are as follows: (1) to clarify which peak Gabb actually climbed during his trip to Cerro Pico Blanco in Costa Rica, (2) to suggest the possible ascent route he followed, and (3) to suggest the possible type localities for the majority of the specimens he collected.

Table 1. Species described with specimens Gabb collected. The elevations are according to the sites suggested on Fig. 3.

Species	Locality
Amphibia	
Craugastor gulosus	Near an unnamed hill close to the Río Lari canyon, elev. 1,830 m
Craugastor megacephalus	Near an unnamed hill close to the Río Lari canyon, elev. 1,830 m
Craugastor melanostictus	Near another unnamed hill at the headwaters of the Río Lari, elev. 2,135 m
Craugastor podiciferus	Between Cerro Pat and the headwaters of the Río Lari, elev. 1,520–2,135 m
Diasporus hylaeformis	Near another unnamed hill at the headwaters of the Río Lari, elev. 2,135 m
Incilius fastidiosus	Around the small village of Ourut, elev. 760 m
Incilius epioticus	Near Cerro Pat, elev. 1,520 m
Isthmohyla pictipes	Between Cerro Pat and the headwaters of the Río Lari, elev. 1,520–2,135 m
Pristimantis cerasinus	Slope of Cerro Kamuk, elev. possibly 760–1,520 m
Reptilia	
Anolis pachypus	Slope of Cerro Kamuk, elev. possibly 760–1,520 m
Ninia psephota	Between Cerro Pat and the headwaters of the Río Lari, elev. 1,520–2,135 m
Mesaspis monticola	Summit of Cerro Kamuk, elev. ca. 3,500 m

Among the documents Gabb generated during his stay in Costa Rica was a report on the geology of the country (Gabb, 1874). This report, however, remained unpublished until it was transcribed in 2007 (Gabb, 2007). In this report, Gabb wrote that he reached the summit of Cerro Pico Blanco or Kamuk on 13 June 1874, after 12 days of the hardest work in his life. He reported an elevation of 11,877.8 feet (3,620 m), close to the 3,549 m in elevation calculated for Kamuk by the Instituto Geográfico Nacional of Costa Rica (IGN). Nonetheless, in a map Gabb produced from his studies on Costa Rica (Petermann 1877, Fig. 1) an elevation of 9,652 feet (2,942 m) is assigned to Cerro Pico Blanco. This confusion grew when Enrique Pittier translated Gabb's reports (Gabb, 1894), since Pittier attributed an elevation of 9,652 feet to Cerro Pico Blanco. This discrepancy in the reported elevations led some researchers to conclude that William Gabb ascended Cerro Utyum and not Cerro Kamuk (Carballo, 1960; Gutiérrez, 1960; Savage, 1970), a conclusion largely motivated by the similarity of elevations in the 1894 translation of Gabb's report and that of the Cerro Utyum (3,060 m). We believe, however, that this interpretation from the 1960s and 70s is the result of the difficulty in accessing Gabb's unpublished 1874 manuscript. The inaccessibility of the original copy at the U.S. Geological Survey obligated researchers to consult only Pittier's translation (Gabb, 1894) in referring to Gabb's work. Denyer and Lücke (2007) suggested that Gabb actually climbed Cerro Kamuk, based on the similarity of the hydrography and position of the mountains between Gabb's map (Petermann, 1877, Fig. 1) and recent ones. These authors, however, did not discuss the issues raised by other researchers (Carballo, 1969; Gutiérrez, 1960; Savage, 1970). Hence, Gabb's zoological contributions from Cerro Pico Blanco still are attributed to Cerro Utyum (Frost, 2014).

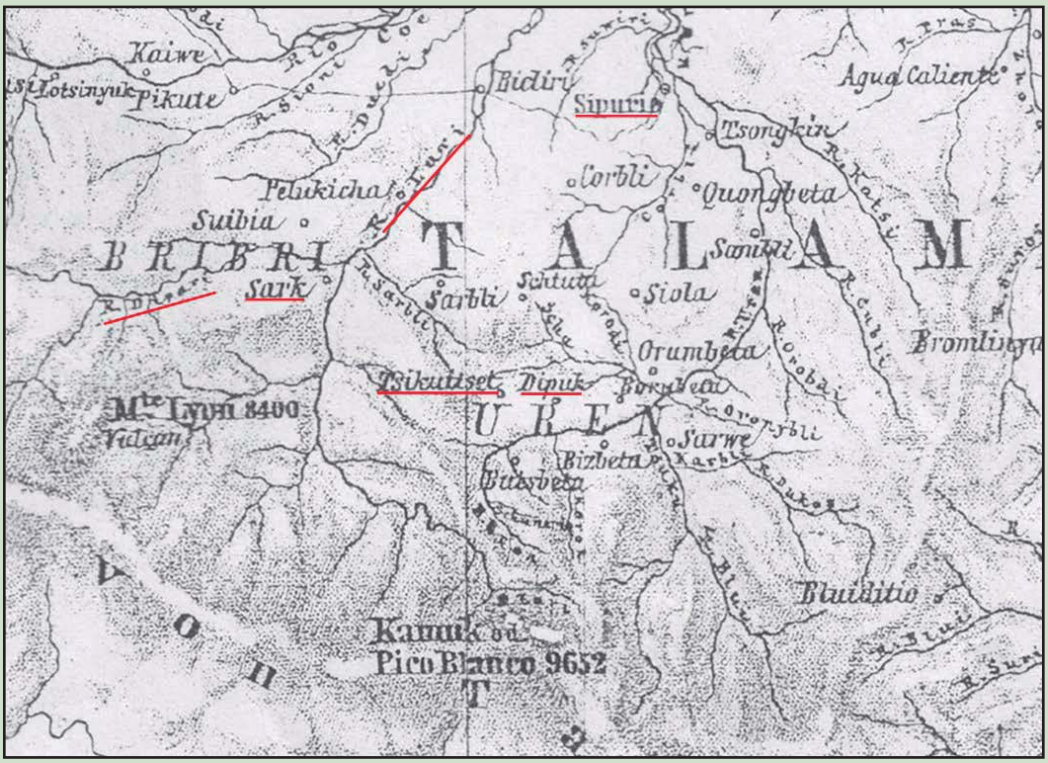

Fig. 1. A portion of Gabb's map showing the region between Sipurio and Cerro Kamuk. Compare sites underlined in red with those in Fig. 3. Reproduced from Petermann (1877).

We performed a comprehensive analysis of Gabb's original manuscript (1874), his map (Petermann, 1877), Pittier's translation (Gabb, 1894), Savage's interpretation (1974), Denyer and Lücke's analysis (2007), and our personal surveys at the summits of Kamuk and Utyum. Based on our re-evaluation of these sources, we conclude that Gabb indeed climbed Cerro Kamuk, as he reported. This conclusion is based on several lines of reasoning. First, when Gabb sent the specimens he collected to the Smithsonian Institution, he attributed an elevation of at least 11,800 feet to Cerro Pico Blanco (Cope 1875), which is very close to the actual elevation of Cerro Kamuk. Also,

in some of Gabb's other documents (Gabb, 1894; Petermann, 1877), he consistently miscalculated the elevation of several peaks, other than Cerro Kamuk. For example, the well-known Volcán Barva was reported at an elevation of 2,827 m in Gabb (1894), but it was reduced to 2,652 m in his map (Petermann, 1877); indeed, it would be difficult to believe that Gabb misidentified Volcán Barva for another peak. Thirdly, Gabb (1874) reported that the Lari and Uren rivers originate on Cerro Pico Blanco; both are known to originate at Cerro Kamuk, and it is difficult to imagine that Gabb could have reported this accurately if he actually had reached the summit of another peak. For example, if he in fact had climbed Cerro Utyum his descriptions of the Lari and Coen rivers would have been the most detailed, but the Río Coen is poorly described in his map (Petermann, 1877). Finally, a detailed description of the summit of Cerro Pico Blanco in the original 1874 report is not included in the translation of Pittier (Gabb, 1894). Gabb described the difficult access to the summit, with large cliffs and vegetation similar to that in American deserts. This description differs greatly from the summit of Cerro Utyum, where we currently conduct studies. An extensive peat bog is present on Cerro Utyum, and the summit is accessed easily from the treeline (Fig. 2a). The description, however, closely matches that of Cerro Kamuk (G. Chaves, pers. observ.), in which the slopes are very steep and it is difficult to reach the summit, the vegetation is limited, and cliffs dominate the landscape (Fig. 2b).

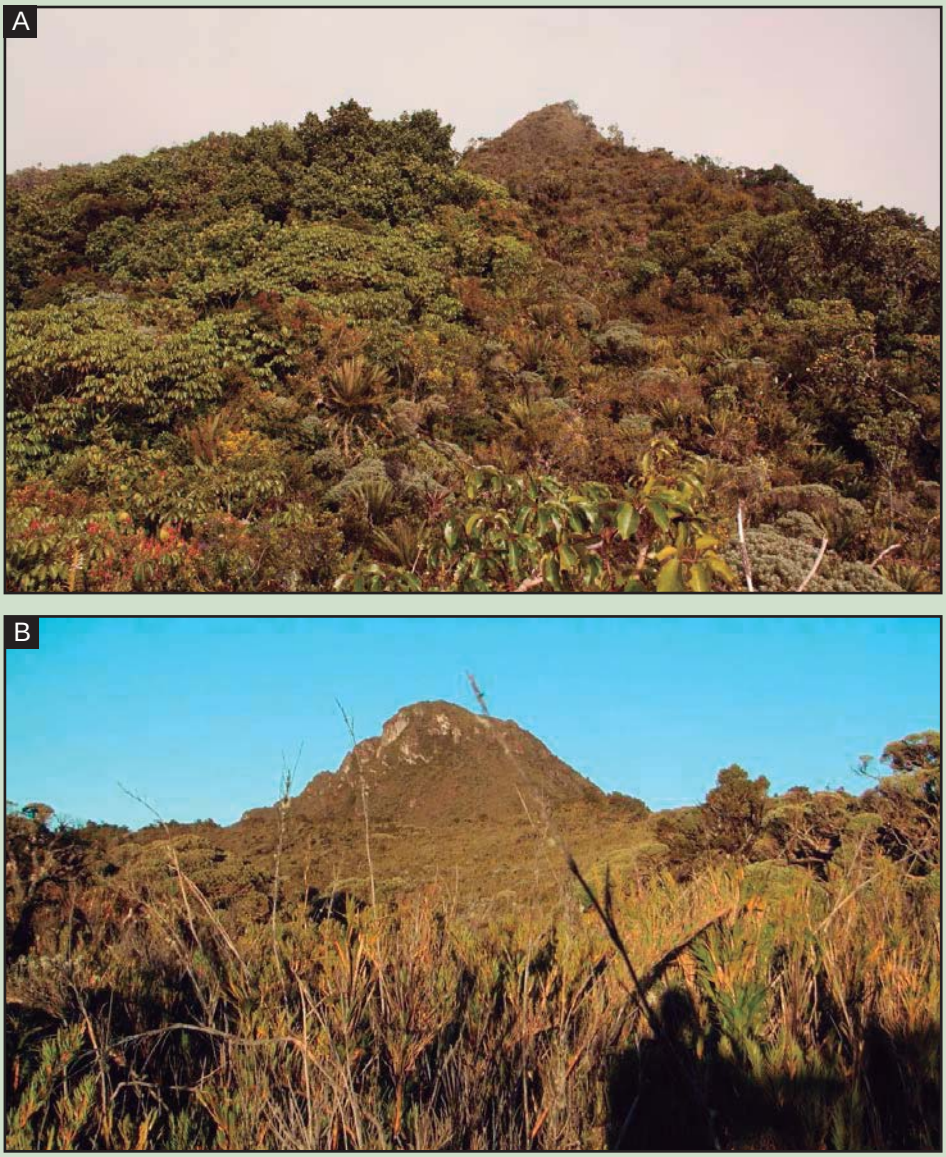

Fig. 2 Summits of (A) Cerro Utyum and (B) Cerro Kamuk in southwestern Costa Rica. © (A) E. Arias, (B) Luis G. Artavia

We analyzed Gabb's map (Petermann, 1877, Fig. 1) to determine the possible route he followed while ascending Cerro Kamuk. We inferred that the more accurate Gabb's description of a landmark, the better he was able to inspect it, and therefore, the closer his route took him to it. Gabb (1874) indicated that he ascended between the Lari and Uren rivers. According to Denyer and Lücke (2007), Gabb's map (Petermann, 1877) adheres closely to current knowledge of the hydrography of the Talamanca region; they especially noted the excellent fit of the Río Lari with the IGN's current maps (Fig. 1). A noteworthy consideration, however, is that the section of the Río Uren between Sipurio (9.5360°N, -82.9513°W, WGS84, elev. 60 m) and Dipuk (see below) is different than in the current maps (Figs. 1 and 3). Given this detail, we suggest that Gabb did not use the river line of the Uren to reach Dipuk, but rather walked along the Lari. We propose (see Fig. 3) that he started in the town of Sipurio, walked to the small village of Sarke or Alto Lari (9.4345°N, -83.0466°W, elev. 400 m) following the Río Lari, then walked to Cerro Pat (9.3957°N, -83.0231°W, elev. 1,500 m). At an elevation of about 2,000 m, he climbed to Cerro Kamuk following a ridge that divides the Uren and Lari rivers. When Gabb's map (Petermann, 1877) is superimposed on IGN's current map, the locations Gabb called Isikoilset and Dipuk correspond to Cerro Pat and the small village of Guachalaba (9.3730°N, -82.9987°W, elev. 900 m), respectively (Figs. 1 and 3). Gabb likely visited Dipuk on a separate trip taken before climbing Cerro Kamuk, since he indicated that he crossed the Río Uren and continued southeastward until reaching Panama. We infer that this visit to Dipuk was part of an earlier trip, because the journey to Panama would have been very difficult to complete following the strenuous trip to Kamuk. Notably, the trail between Sipurio and Guachalaba

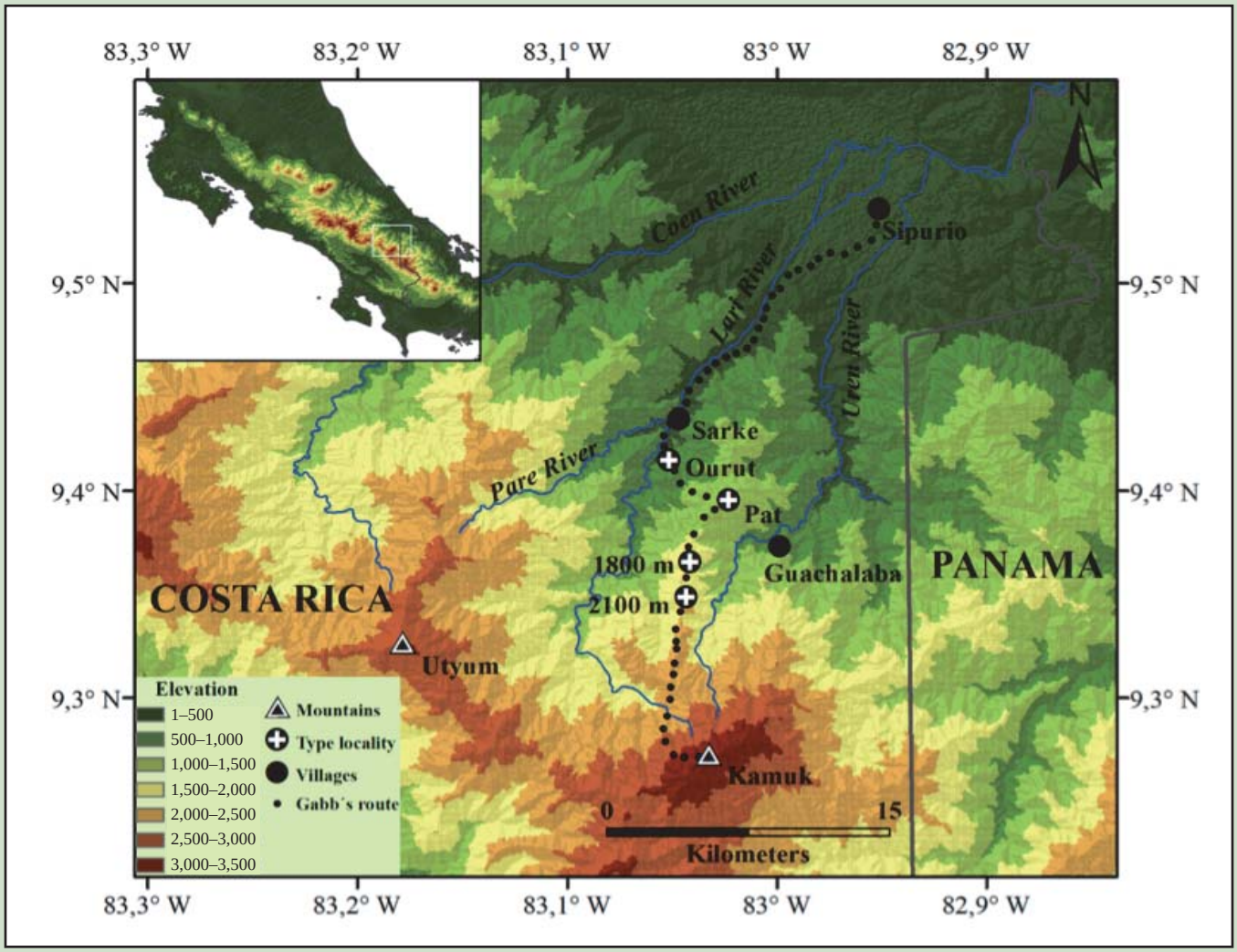

Fig. 3 Map of the Costa Rica–Panama border region showing the route we suggest Gabb followed.

is an old indigenous route recognized on IGN maps from 1968. Finally, Gabb (1874) noted that he climbed to Pico Blanco by the narrow ridge dividing the Lari and Uren, a crest that runs from Cerro Pat to Cerro Kamuk.

Based on the proposed route, we suggest some localities as potential collection sites (Table 1, Fig. 3). The specimens collected by Gabb on his trip to Cerro Kamuk are restricted to four sites, at 760, 1,520, 1,830 and 2,135 m in elevation (Cope, 1875). We suggest the following four areas are an approximation of Gabb's original sites (Table 1, Fig. 3): the site at 760 m is around the small village of Ourut (9.4149°N, -83.0514°W, elev. 700 m); the 1,520 m site is near the Cerro Pat; the 1,830 m site is near an unnamed hill close to the Río Lari canyon (9.3657°N, -83.0415°W, elev. 1,800 m); and the 2,135 m site is near another unnamed hill at the headwaters of the Río Lari (9.3488°N, -83.0434°W, elev. 2,100 m).

Clarifying the route followed by Gabb on his trip to Cerro Kamuk allows us to understand the fieldwork of a pioneer collector of Costa Rica's herpetofauna. On 13 June 2014 we celebrated the 140th anniversary of William Gabb's climb to Cerro Pico Blanco. This feat fills us with admiration, but also imposes the task of replicating his expedition, particularly since the comforts and technologies of the present facilitate fieldwork.

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APÉNDICE II

BIOGEOGRAFÍA HISTÓRICA DE LOS ANFIBIOS DE AMÉRICA CENTRAL: UNA REVISIÓN DE HIPÓTESIS ACTUALES Y PERSPECTIVAS FUTURAS

Autores: Erick Arias y Gabriela Parra-Olea
Ensayo evaluado durante el examen de candidatura.

Biogeografía histórica de los anfibios de América Central: una revisión de hipótesis actuales y perspectivas futuras.

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CONTENIDO

INTRODUCCIÓN

La biogeografía histórica estudia la distribución de los seres vivos, trata de establecer las causas y factores responsables de esta distribución en una escala de tiempo geológico (Crisci *et al.* 2003). Su origen multidisciplinario hace de la biogeografía histórica una disciplina sensible a los avances en otros campos de conocimiento como la geología, geografía y biología. Según Wiley (1988) el desarrollo de la teoría de la tectónica de placas y los avances conceptuales-metodológicos en la sistemática filogenética explican el surgimiento de la biogeografía cladística a principios de 1980, misma que dio origen a la gran mayoría de los enfoques teóricos y prácticos de la actualidad (Morrone 2005). Además, el entendimiento de la historia biogeográfica de una región se beneficia con nuevas y más robustas reconstrucciones filogenéticas y geológicas para dicha región.

Tres características hacen de América Central una región de gran interés para la biogeografía. La primera de estas es el cierre del Istmo de Panamá en el extremo sureste de América Central que permitió el Gran Intercambio de la Biota Americana, el intercambio entre dos grandes masas continentales (Norte y Sudamérica) hasta entonces aisladas. El interés por este gran evento biogeográfico ha generado mucha investigación en la región, desde sus orígenes como modelo en mamíferos hasta recientemente con otros organismos como anfibios (Simpson 1940; Marshall *et al.* 1982, 1988; Webb 1991, 2006; Pinto-Sánchez *et al.* 2012; Bacon *et al.* 2016). La segunda de estas características es su compleja historia geológica con fuertes implicaciones para la biogeografía regional. Las discusiones generadas desde la geología sobre la formación de América Central y la posibilidad de una conexión temporal entre Norteamérica y Sudamérica previo al cierre del Istmo de Panamá (Pindell & Barrett 1990; Iturralde-Vinent & MacPhee 1999) han sido de gran interés para la biogeografía (Savage 1966, 1982; Hedges 2006; Alí 2012). La tercera característica de América Central es su extremadamente rica biodiversidad actual. Por ejemplo, alberga el 7% de los anfibios del mundo, muchos de los cuales son endémicos a la región (Duellman 2001; Savage 2002). Esta alta diversidad se ha atribuido a su compleja historia geológica y su amplia heterogeneidad geográfica y climática (Savage 1966, 1982; Campbell 1999). La presencia de una biodiversidad rica y única ha propiciado diversos estudios biogeográficos con diferentes grupos de organismos (Savage 1996, 1982; Bussing 1976, 1985; Bagley & Johnson 2014).

Los anfibios son un grupo con alta diversidad en América Central (Duellman 2001), con un alto nivel de endemismo, lo que motivó a Savage (1966) a sugerir que esta fauna debe ser considerada una unidad independiente al mismo nivel de la fauna neotropical o neártica. Uno de los grupos diversos de anfibios en la región son las ranas de desarrollo directo (Brachycephaloidea), que en su totalidad representan el 23% de las especies de anfibios de América Central (Frost 2019). Siguiendo a Padial *et al.* (2014) las ranas de desarrollo directo constituyen un clado de 25 géneros en tres familias neotropicales (Craugastoridae, Eleutherodactylidae y Brachycephalidae). Cinco de estos géneros (*Craugastor, Diasporus, Eleutherodactylus, Pristimantis* y *Strabomantis*) habitan América Central, pero, *Craugastor* es el más diverso con 96 especies en la región. Estos géneros tienen orígenes distintos y su presencia en América Central responde a diferentes momentos espacio-temporales (Heinicke *et al.* 2007; Hedges *et al.* 2008). Los distintos orígenes e historias biogeográficas hacen de las ranas de desarrollo directo un grupo ideal para una revisión de la biogeografía histórica de los anfibios en América Central.

El presente trabajo revisa la biogeografía histórica de los anfibios en América Central, mas no se pretende repetir lo que de forma extensa y detallada se ha documentado en las revisiones sobre biogeografía histórica de anfibios para la región (Savage 1966, 1982, 2002; Campbell 1999; Flores-Villela & Goyenechea 2001; Duellman 2001). Este trabajo se enfoca en la revisión de las hipótesis actuales y como estas responden a avances en reconstrucciones filogenéticas y geológicas para la región. Para alcanzar este objetivo el presente trabajo se divide en cinco secciones. La primera sección da un marco histórico para América Central, su definición, unidades ecogeográficas, su historia geológica, el cambio en el clima y el nivel del mar en el pasado. En la segunda sección se revisa brevemente la diversidad de anfibios en América Central, sus patrones de diversidad en la región y las principales hipótesis biogeográficas que surgieron previo al advenimiento de las herramientas moleculares. En la tercera sección se revisan las hipótesis actuales de la biogeografía histórica de América Central a la luz de las nuevas evidencias moleculares y geológicas, haciendo especial énfasis a estudios realizados con ranas de desarrollo directo. En la cuarta sección se analiza la biogeografía de la región desde la perspectiva actual con nuevas herramientas metodológicas y conceptuales utilizando ejemplos puntuales con ranas de desarrollo directo. En la quinta y última sección se

revisan los principales retos que debe atender el estudio de la biogeografía histórica de los anfibios en América Central.

AMÉRICA CENTRAL

Definición. Siguiendo la definición de Savage (1982) y Gutiérrez-García & Vázquez-Domínguez (2013) América Central es el área comprendida entre el Istmo de Tehuantepec, México y la Cordillera de los Andes en Colombia (Fig. 1). Es importante remarcar que en la literatura biogeográfica existen nombres ambiguos para la delimitación geográfica de la región. El término en inglés "*Middle America*" que se puede traducir como Medio de América o Centro de América, se ha utilizado para referirse al área comprendida entre los Estados Unidos de América y Colombia (Villa *et al.* 1988; Duellman 2001), a veces incluyendo las Islas Caribeñas (Winker 2011). El término Mesoamérica ("*Mesoamerica*" en inglés) ha sido utilizado como un sustituto para "*Middle America"* (Johnson *et al.* 2001; Wilson *et al.* 2010), sin embargo, Winker (2011) recomienda el uso de "*Middle America*" sobre "*Mesoamerica*", principalmente porque este último término se acuñó en antropología para referirse a una entidad social. A pesar de la confusión que puede causar el uso de América Central, especialmente con respecto a "*Middle America*", la delimitación geográfica aquí propuesta corresponde con la región nombrada "*Central America*" por Savage (1982) y Gutiérrez-García & Vázquez-Domínguez (2013); Savage (19829 además distinguió entre "*Central America*" y "*Middle America*". El término centroamericano, se utilizará como relativo a América Central. ***América Central Nuclear*** hace referencia a las tierras entre el Istmo de Tehuantepec y el sur de Nicaragua y ***América Central Ístmica*** se refiere a las tierras de Costa Rica y Panamá en su conjunto.

Unidades ecogeográficas. América Central, a pesar de ser una región relativamente pequeña, poco más de 770 077 km^2, alberga una compleja y heterogénea fisiografía, que a su vez, genera una amplia gama de climas y hábitats. La región se ubica entre los 7º y 21º N, corriendo de Noroeste a Sureste, separando el Océano Pacífico al Noroeste y el Golfo de México y el Mar Caribe al Noreste. Su rango de elevación va desde el nivel del mar hasta los 4 220 m.s.n.m. (Volcán Tajumulco, Guatemala). En el extremo sureste, la franja terrestre se reduce a 65 km formando el Istmo de Panamá.

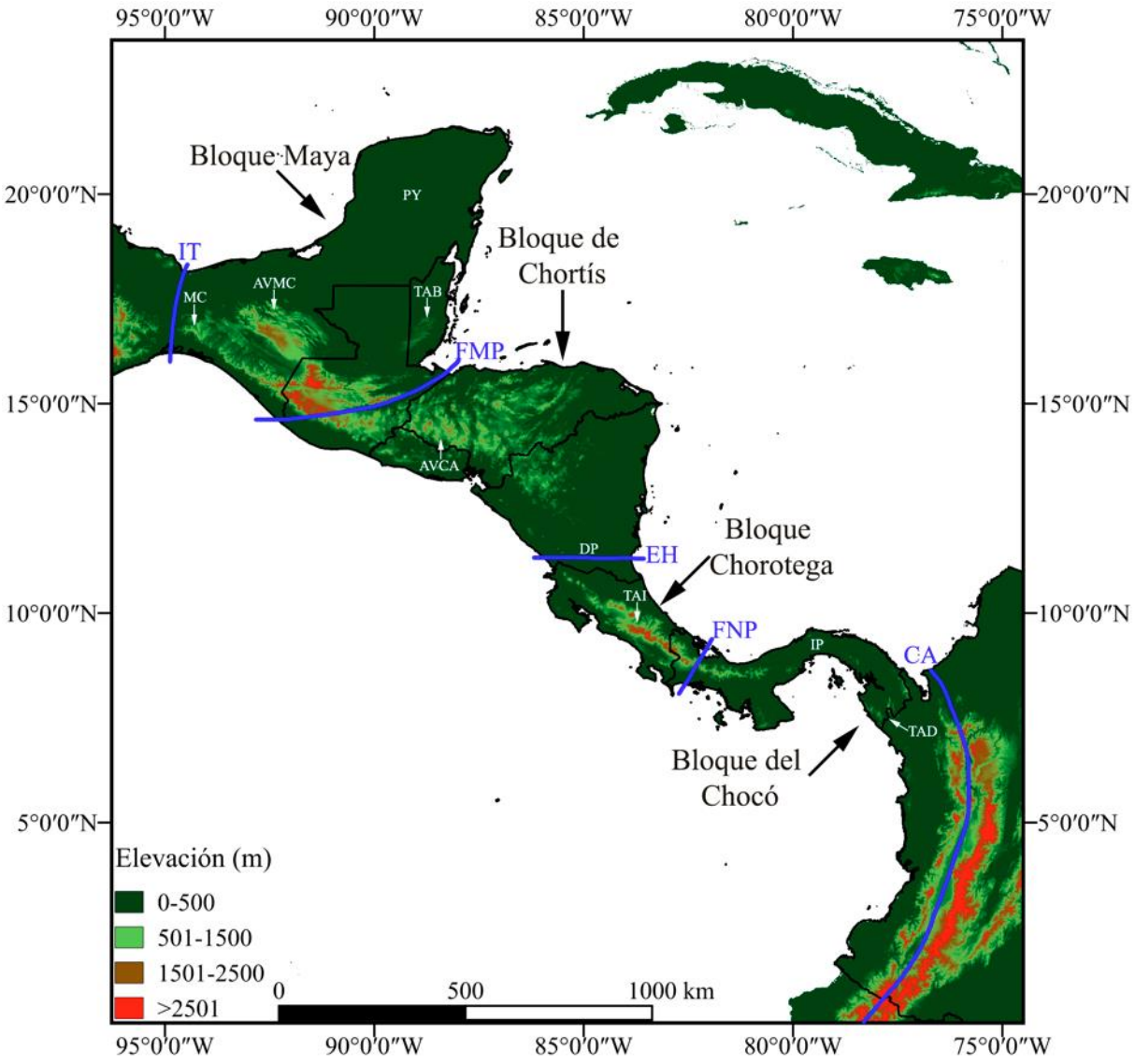

Figura 1. Mapa de América Central, se muestran los cuatro bloques tectónicos (delimitados en azul) y las principales unidades ecogeográficas de la región (en blanco). Las abreviaciones corresponden a: IT = Istmo de Tehuantepec, FMP = Falla Motagua-Polochic, EH = escarpada de Hess, FNP = fractura norte de Panamá, CA = Cordillera de los Andes, MC = macizo de Chiapas, AVMC = arco volcánico moderno de Chiapas, TAB = tierras altas de Belice, PY = península de Yucatán, AVCA = arco volcánico de Centro América, DP= depresión de Nicaragua, TAI = tierras altas del Istmo, IP = Istmo de Panamá y TAD = tierras altas del Darién.

Las unidades ecogeográficas de la región han recibido diversos nombres. Siguiendo a Campbell (1999) y Duellman (2001) con algunas modificaciones a partir de Gutiérrez-García & Vázquez-Domínguez (2013) (Fig. 1), las tierras altas de la región se pueden agrupar en seis unidades geomorfológicas: 1) el Macizo de Chiapas, 2) las Tierras Altas de Belice, 3) el Arco Volcánico Moderno de Chiapas [Campbell (1999) y Duellman (2001) trataron estas últimas dos unidades como una sola "Tierras Altas del Este de América Central Nuclear"], 4) el Arco Volcánico de Centro América [las "Tierras Altas del Oeste de América Central Nuclear" en Campbell (1999) y Duellman (2001)], 5) las Tierras Altas del Istmo y 6) las Tierras Altas del Darién. Asimismo, las tierras bajas de la región se agrupan en ocho unidades: 1) la Península de Yucatán, 2) las Tierras Húmedas del Caribe Norte, 3) las Tierras Húmedas del Caribe Sur, 4) las Tierras Húmedas del Pacífico, 5) las Tierras Secas de América Central, 6) las Tierras Húmedas del Golfo Dulce, 7) las Tierras Secas de Panamá y 8) las Tierras Húmedas del Chocó.

Nivel del mar y clima en el pasado. El nivel del mar desde el Paleoceno hasta el Mioceno ha sido controversial. Miller *et al.* (2005) sugirieron que el nivel del mar desde el Paleoceno (~60 Ma) hasta el presente se mantuvo casi de forma constante bajo el nivel actual con un descenso acelerado durante los últimos 2 Ma y con un aumento hasta su nivel actual tan solo en los últimos 18 ka –miles de años antes–. Posteriormente, Kominz *et al.* (2008) revisaron y corrigieron la curva de Miller *et al.* (2005) y sugirieron que el nivel del mar desde el Cretácico Superior (~100 Ma) hasta el Oligoceno (~30 Ma) se mantuvo siempre por encima del nivel actual. A partir del Oligoceno y hasta el presente, el nivel del mar se mantuvo oscilando cerca de los niveles actuales, con al menos dos momentos donde el nivel del mar fue significativamente inferior al actual, uno al inicio del Mioceno (~23 Ma) y otro en el Mioceno Medio (~10 Ma). Los resultados de Kominz *et al.* (2008) son concordantes con trabajos previos (Kominz 1984; Haq *et al.* 1987) y apoya la hipótesis de que la Depresión de Nicaragua permaneció inundada desde el Cenozoico Tardío hasta el comienzo del Pleistoceno (Duellman 2001; Gutiérrez-García & Vázquez-Domínguez 2013).

Savage (1966) sugiere que durante el Cenozoico el clima en la región fue mésico y argumenta a favor de una barrera semi-árida a árida entre América Central y Norteamérica en el Oligoceno Medio (~28 Ma). El cambio climático mejor documentando en América Central es la transición de la región hacia condiciones más xéricas, especialmente en las tierras bajas de la costa pacífica, a partir del Plioceno (5 Ma) (Savage 1966; Graham & Dilcher 1995; Duellman

2001). Durante el Pleistoceno Tardío (39.4-28.1 ka) se asume que las temperaturas fueron elevadas y el nivel del mar superior al actual, posteriormente (28-14.5 ka) y coincidente con la última máxima glaciación las temperaturas fueron inferiores y el nivel del mar disminuyó (González *et al.* 2006), en los últimos 18 ka se dio un aumento acelerado del nivel del mar hasta su nivel actual (Miller *et al.* 2005; González *et al.* 2006). Durante la última máxima glaciación las tierras altas de América Central Nuclear e Ístmica estuvieron cubiertas por glaciares que terminaron su desglaciación hace ~10 ka (Lachniet & Vazquez-Selem 2005; Kapelle & Horn 2005).

Historia geológica. La historia geológica de América Central es compleja e incompleta. Existen diversos trabajos extensos revisando este tema (Coates & Obando 1996; Coates *et al.* 2004, 2005; Bundschuh & Alvarado 2007; Rogers *et al.* 2007; Silva-Romo 2009; Montes *et al.* 2012a,b, 2015) y otros que han realizado recopilaciones de la historia geológica de la región como marco para análisis biogeográficos (Savage 1982; Campbell 1999; Duellman 2001; Gutiérrez-García & Vázquez-Domínguez 2013; Bagley & Johnson 2014). Sin embargo, es necesario dar una breve reseña de la geología regional. América Central se puede subdividir en 4 bloques tectónicos (Fig. 1): Maya, Chortís, Chorotega y Chocó. En conjunto los bloques Maya y de Chortís conforman lo que se denomina América Central Nuclear, representando las tierras más antiguas de la región. Estas tierras por su mayor tiempo emergidas, han jugado un papel importante en la biogeografía de la región, recibiendo biodiversidad de las grandes masas norte y sudamericanas. Por su parte, los bloques Chorotega y Chocó conforman América Central Ístmica, tierras relativamente más jóvenes y que han tenido un papel más reciente en la conformación actual de la biodiversidad regional, en especial permitiendo el Gran Intercambio de la Biota Americana.

El bloque Maya se extiende desde el Istmo de Tehuantepec hasta la Falla Motagua-Polochic (Fig. 1), son las tierras más viejas de América Central. Se ha sugerido que el Macizo de Chiapas y el domo de los Cuchumatanes existieron durante el Pérmico-Triásico (~250 Ma) (Weber *et al.* 2007; Martens *et al.* 2010). El bloque de Chortís se extiende desde la Falla Motagua-Polochic hasta el Escarpa de Hess, su evolución es más compleja y discutida. Rogers *et al.* (2007) revisaron las diferentes hipótesis del origen del bloque de Chortís, ellos sugirieron que el bloque colisionó con el extremo sur de la placa Norteamérica (sur de México) en el Cretácico Tardío (~72 Ma) y se mantuvo en contacto con el continente hasta alcanzar su posición actual.

Keppie & Morán-Zenteno (2005) sugirieron una hipótesis alternativa, en la que el bloque de Chortis llegó a su posición actual por deriva desde el Océano Pacífico Este entre el Cretácico Tardío y el Eoceno Temprano (65-55 Ma). Otra hipótesis sugiere que el bloque de Chortís estuvo en contacto con el bloque Maya, en su posición actual, al menos desde el Cretácico Tardío (~66 Ma) (James 2005). Más allá de la discusión sobre el origen del Bloque de Chortís, es probable que este haya estado en su posición actual o al menos adherido al sur del actual México desde el Eoceno (~56 Ma).

Los bloques Chorotega y Chocó se extienden desde el Escarpa de Hess hasta la Fractura Norte de Panamá y desde esta última hasta la zona de subducción entre las placas Nazca y Sudamericana en Colombia (Cordillera de los Andes), respectivamente (Fig. 1). Aunque ambos bloques tienen características únicas, se revisan en conjunto dada su estrecha relación histórica. El conocimiento sobre la historia geológica de la región está cambiando aceleradamente. La hipótesis tradicional, denominada "modelo de isla" propone un origen para América Central Ístmica reciente, del Mioceno Medio (~15 Ma) a ~3 Ma (Coates & Obando 1996, Coates *et al.* 2004), bajo este modelo en el Mioceno Medio (~15 Ma) América Central Ístmica estaba conformada por un archipiélago de islas. Este modelo plantea que el levantamiento regional generó un continuo de tierras emergidas pero desconectadas de Sudamérica hace ~6.5 Ma y data el cierre del Istmo de Panamá hace ~3 Ma. Una hipótesis más reciente, denominada "modelo de península" propone un levantamiento y un cierre mucho más antiguo (Montes *et al.* 2012a,b, 2015). Este modelo expone que América Central Ístmica existe como tierra emergida pero desconectada de Sudamérica desde el Oligoceno Tardío al Mioceno Temprano (~25 Ma) y data el cierre del Istmo de Panamá entre 15-13 Ma.

Aún persisten grandes vacíos de conocimiento sobre la formación geológica de América Central. Es de especial importancia para las hipótesis biogeográficas de la región, un modelo que explique si hubo una conexión terrestre entre América Central Nuclear y Sudamérica previo al cierre del Istmo de Panamá, y si la hubo cómo fue. La evidencia es concordante en apoyar que diversos grupos biológicos llegaron a América Central Nuclear desde Sudamérica previo al cierre del Istmo de Panamá (Duellman 2001; Savage 2002). Dos hipótesis se han sugerido como modelos para explicar esta primera conexión. La primera, la hipótesis de las "proto-Antillas" asume que existió una conexión entre América Central Nuclear y Sudamérica al menos hace 80-70 Ma, este puente terrestre estuvo ubicado en la posición actual de América Central Ístmica

(Pindell & Barrett 1990; Hedges 2006). Una segunda hipótesis denominada "*Greater Antilles + Aves Ridge GAARlandia*" menciona que las Antillas mayores estuvieron conectadas a Sudamérica al menos hace 35-33 Ma (Iturralde-Vinent & MacPhee 1999). Esta última hipótesis no implica una conexión directa entre América Central Nuclear y Sudamérica, pero sí una ruta intermedia que acortó la distancia y aumentó el éxito de dispersión desde las Antillas Mayores hacia América Central Nuclear. La conexión temporal entre América Central Nuclear y Sudamérica sí existió, sigue siendo controversial y la hipótesis de las proto-antillas es altamente improbable, asumiendo un origen temprano de América Central Ístmica bajo el modelo de península de Montes *et al.* (2012a,b).

DIVERSIDAD DE ANFIBIOS EN AMÉRICA CENTRAL

Diversidad de anfibios y sus patrones de distribución en América Central

Los anfibios (Amphibia Linnaeus) conforman un grupo monofilético, distribuido de forma cosmopolita excepto en la Antártida, Groenlandia, el desierto del Sahara y la Meseta Tibetana (Frost *et al.* 2006; Pyron & Wiens 2011). Los anfibios actuales se remontan al carbonífero (~320 Ma) (San Mauro 2010) y el registro fósil presenta una gran diversidad de grupos anfibios (Pyron 2011). La diversidad de anfibios vivientes es de 7 959 especies nombradas (Frost 2019) distribuidas en tres órdenes: 1) Anura (Fischer von Waldheim) con 7021 especies, 2) Caudata (Fischer von Waldheim) con 726 especies y 3) Gymnophiona (Müller) con tan solo 212 especies. La diversidad de anfibios en América Central es de 529 especies, representando el 7% de los anfibios en el mundo en tan solo el ~0.7% de la superficie mundial habitada por anfibios. De esta diversidad el 65.5% (346 especies) corresponde a Anura, el 31.5% (167 especies) corresponde a Caudata y tan solo el 3% (16 especies) corresponde a Gymnophiona.

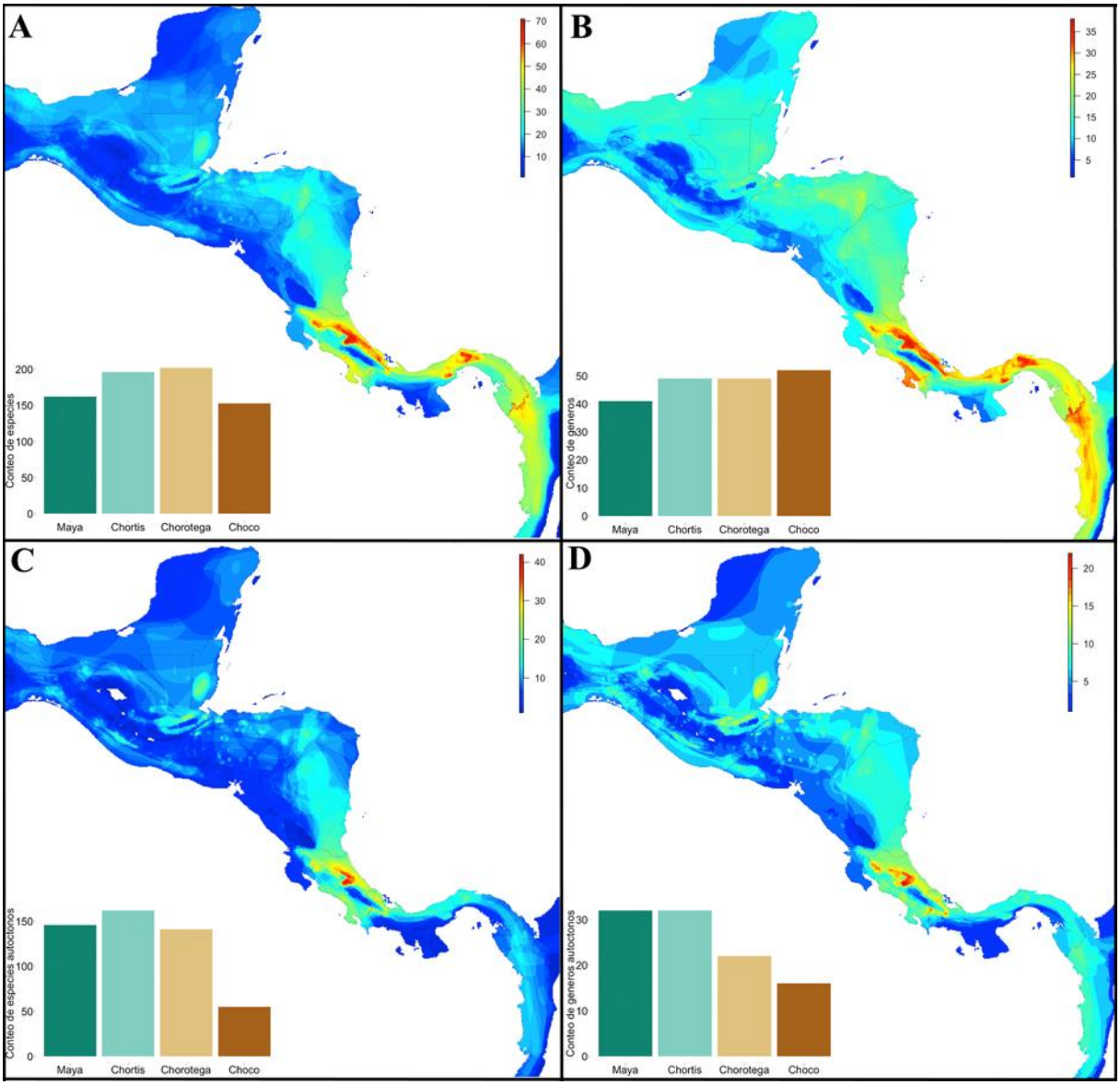

Figura 2. Patrones de distribución de riqueza de anfibios en América Central. **A**) riqueza total de especies, **B**) riqueza total de géneros y subgéneros, **C**) riqueza autóctona de especies y **D**) riqueza autóctona de géneros y subgéneros. Las gráficas de barras corresponden a los conteos por bloque tectónico. Rangos de distribución obtenidos de la IUCN (http://www.iucnredlist.org/).

Los anfibios de América Central se pueden agrupar con base en tres orígenes distintos (ver abajo): 1) el grupo centroamericano (CA) correspondiendo a aquellos que se originaron en la región, 2) el grupo Norteamericano (NA) que representan especies que ampliaron su rango de distribución hacia el sur y 3) el grupo Sudamericano (SA) que representan especies que ampliaron su rango de distribución hacia el norte. En la Tabla 1, se resume la conformación de los tres grupos de anfibios de América Central siguiendo a Duellman (2001), Savage (2002) y Rovito *et al.* (2015a). De las 529 especies de anfibios habitando en América Central, 401 (75.8%) se atribuyen a géneros que se originaron en la región. Las restantes 128 especies corresponden a especies pertenecientes a géneros de origen sudamericano (114) o norteamericano (14). Aunque muchas de estas especies atribuidas a Sudamérica son endémicas a la región, no se consideran parte del grupo originario de América Central. Esta alta diversidad de anfibios en América Central retrata una historia evolutiva regional compleja que incluye sitios de alto endemismo, principalmente en las tierras altas (Savage 1982, 2002; García-París *et al.* 2000; Rovito *et al.* 2015a).

La diversidad de anfibios en América Central no está distribuida de forma proporcional, la riqueza de especies de Costa Rica y Panamá (América Central Ístmica) es por mucho superior al resto de la región (Fig. 2). La mayor cantidad de especies en Costa Rica y Panamá se ha explicado históricamente como el producto de múltiples factores, el primero, es la colonización por especies que se originaron en América Central Nuclear, segundo, una diversificación *in situ* y tercero, la colonización por especies precedentes de Sudamérica que alcanzan sus límites más norteños en Costa Rica o Panamá (Savage 1982, 2002; Campbell 1999). Sin embargo, el factor que posiblemente más ha contribuido a la conformación actual de la fauna anfibia en estos dos países, es la diversificación *in situ* promovida por una alta heterogeneidad ambiental en las tierras altas del Istmo (Costa Rica y oeste de Panamá) (García-París *et al.* 2000; Savage 2002; Boza-Oviedo *et al.* 2012). Con el fin de ilustrar el patrón desproporcional de la riqueza de anfibios en América Central, se presenta en la Tabla 2 el número de especies de anfibios por país en la región.

Se realizó un análisis de riqueza de especies para 459 anfibios distribuidos en América Central. Las áreas de distribución son aquellas disponibles en IUCN y el análisis fue realizado con lestR (Vilela & Villalobos 2015). Se obtuvieron acumulados de especies para cuadrículas de 1ºx1º, y el análisis fue replicado excluyendo las especies de Sudamérica. Al analizar los patrones

de distribución de los anfibios en América Central (Fig. 2) se puede observar lo siguiente. Cuando se analiza toda la diversidad actual es evidente una mayor diversidad de especies y linajes mayores (géneros y subgéneros) en Costa Rica y Panamá. Al excluir las especies de origen sudamericano se observa como estas tienen una gran influencia en la diversidad de Panamá pero contribuye poco al resto de América Central (Fig. 2A vs Fig. 2C) y en especial, se sigue observando como Costa Rica sigue siendo la región con más riqueza de especies. Otro resultado llamativo es que al analizar solamente la fauna autóctona, se puede observar que existe mayor cantidad de linajes mayores habitando los bloques geológicos que conforman América Central Nuclear (Fig. 2B vs Fig. 2D). Otro patrón llamativo es la mayor predominancia de especies en zonas de elevación intermedia (Fig. 3), patrón que se pronuncia cuando se incluyen únicamente las especies autóctonas.

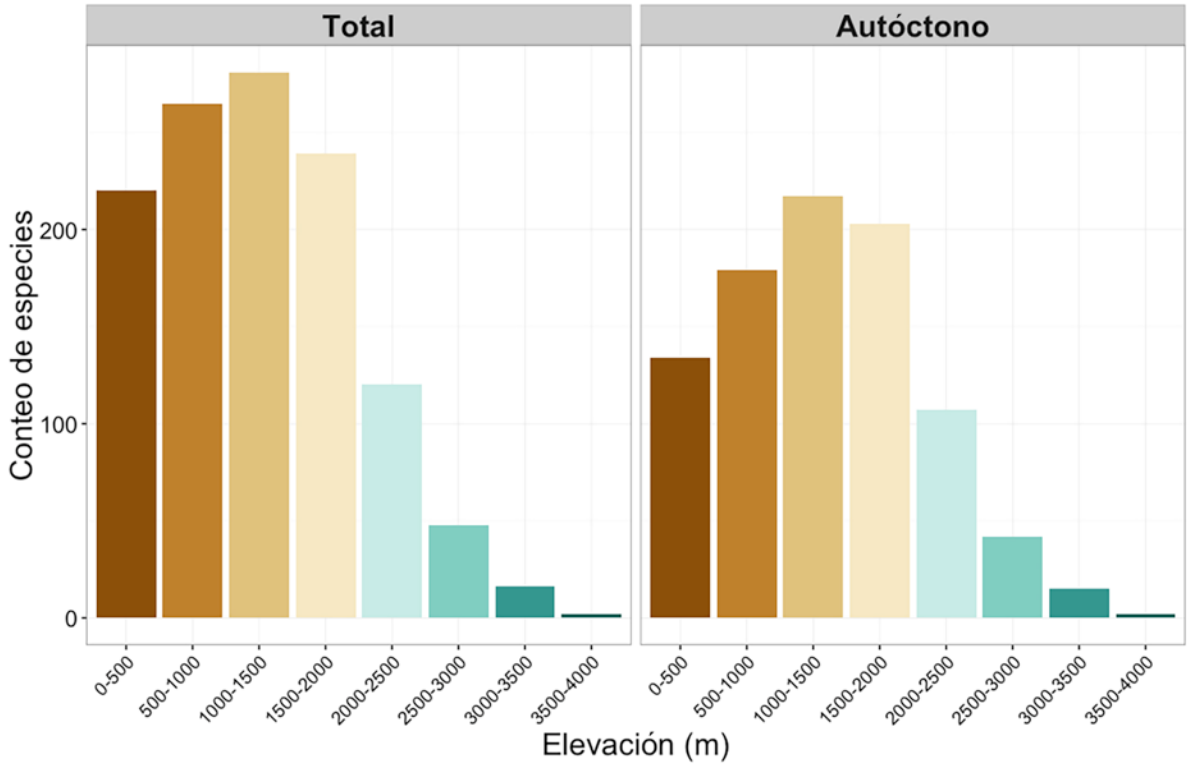

Figura 3. Cantidad de especies según rango altitudinal (m.s.n.m) en América Central, en la izquierda se observa el conteo para el total de especies en la región y en la derecha se observa solamente las especies autóctonas.

Tabla 1. Componente de géneros en América Central según sus tres distintos orígenes. El número entre paréntesis corresponden con el número de especies.

Norte América	**América Central**	**Sur América**
Anura		
Lithobates (11)	*Agalychnis* (6)	*Allobates* (1)
Rana (1)	*Anotheca* (1)	*Ameerega* (1)
Rhinophrynus (1)	*Bromeliohyla* (1)	*Andinobates* (4)
	Charadrahyla (1)	*Anomaglossus* (2)
	Craugastor (96)	*Atelopus* (8)
	Cruziohyla (1)	*Cochranella* (2)
	Ctenophryne (1)	*Colostethus* (3)
	Diasporus (8)	*Dendrobates* (1)
	Dryophytes (2)	*Dendrosophus* (5)
	Duellmanohyla (7)	*Elachistocleis* (3)
	Ecnomiohyla (7)	*Engystomops* (1)
	Eleutherodactylus (3)	*Espadarana* (1)
	Exerodonta (5)	*Gastroteca* (2)
	Gastrophryne (2)	*Hemiphractus* (1)
	Hypopachus (4)	*Hyalinobatrachium* (8)
	Incilius (29)	*Hyloscirtus* (2)
	Isthmohyla (15)	*Hyloxalus* (1)
	Plectrohyla (18)	*Hypsiboas* (5)
	Ptychohyla (9)	*Leptodactylus* (7)
	Smilisca (6)	*Oophaga* (5)
	Tlalocohyla (2)	*Osteopilus* (1)
	Triprion (1)	*Phyllobates* (2)
		Phyllomedusa (1)
		Pipa (1)
		Pleuroderma (1)
		Pristimantis (14)
		Rhaebo (1)
		Rhinella (6)
		Sachatamia (2)
		Scinax (6)
		Silverstoneia (2)
		Strabomantis (2)
		Teratohyla (2)
		Trachycephalus (1)

Tabla 1. *Continua*.

Caudata		
	Bolitoglossa (93)	
	Bradytriton (1)	
	Cryptotriton (6)	
	Dendrotriton (8)	
	Ixalotriton (2)	
	Nototriton (17)	
	Nyctanolis (1)	
	Oedipina (35)	
	Pseudoeurycea (4)	
Gymnophiona		
	Dermophis (6)	*Caecilia* (4)
	Gymnopis (2)	*Oscaecilia* (4)

Tabla 2 Riqueza de especies anfibias en América Central por país.

País	Área (Km^2)	Familias	Géneros	Especies	Especies / 1000 Km^2
México[1]	246 226	12	37	119	0.48
Guatemala	108 889	12	36	167	1.53
Belice	22 966	12	24	41	1.78
Honduras	112 492	12	41	144	1.28
El Salvador	21 041	11	21	40	1.90
Nicaragua	129 494	14	37	76	0.59
Costa Rica	51 100	16	47	203	3.97
Panamá	78 569	16	55	217	2.76

[1] Incluye los Estados al este del Istmo de Tehuantepec (Campeche, Chiapas, Quintana Roo, Tabasco y Yucatán).

Áreas de endemismo para los anfibios de América Central

Un área de endemismo es definida como el área de congruencia distribucional no azarosa entre al menos dos taxones (Morrone 1994), esta congruencia implica simpatría. Las áreas de endemismo son unidades básicas para los estudios evolutivos y biogeográficos, para identificar estas áreas de congruencia es sugerido el uso del Análisis de Parsimonía de Endemismos (PAE, Morrone 1994). Se realizó un PAE para identificar las áreas de endemismo de los anfibios de América Central. América Central fue dividido en 110 celdas de 1ºx1º y utilizamos las mismas 459 áreas

de distribución de los anfibios de América Central. La matriz de presencia-ausencia fue analizada con NONA y WINCLADA, los taxones no-informativos fueron descartados y el análisis de parsimonía fue usado con 50 réplicas y la búsqueda heurística TBR+TBR. Los clados con al menos dos sinapomorfías (especies) fueron considerados áreas de endemismos, la simpatría de estas áreas de endemismo fueron evaluadas en el espacio geográfico. Nosotros identificamos siete áreas de endemismo (Fig. 4), cuatro de estas en América Central Nuclear y tres en América Central Ístmica. Estas áreas de endemismo no fueron correlacionadas con la riqueza de especies, excepto en Costa Rica donde hay alta riqueza de especies y dos áreas de endemismo. Todas las áreas de endemismo corresponden con áreas de elevación intermedia resaltando la importancia de la complejidad topográfica en la diversificación de los anfibios de América Central. La simpatría de las especies en estas áreas de endemismo fue alta, resaltando el rol de estas áreas en la diversificación de diferentes taxones, por ejemplo, la simpatría de cinco especies apoya el área de endemismo del caribe de Costa Rica (Fig. 5)

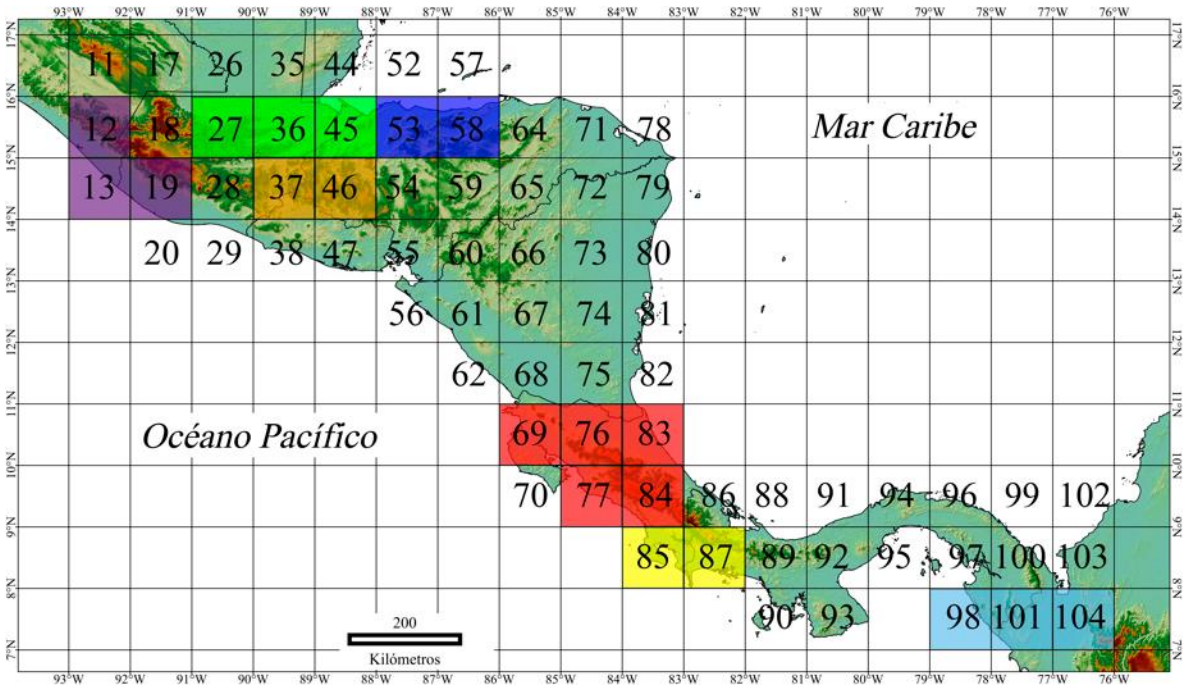

Figura 4. Áreas de endemismo de los anfibios de América Central.

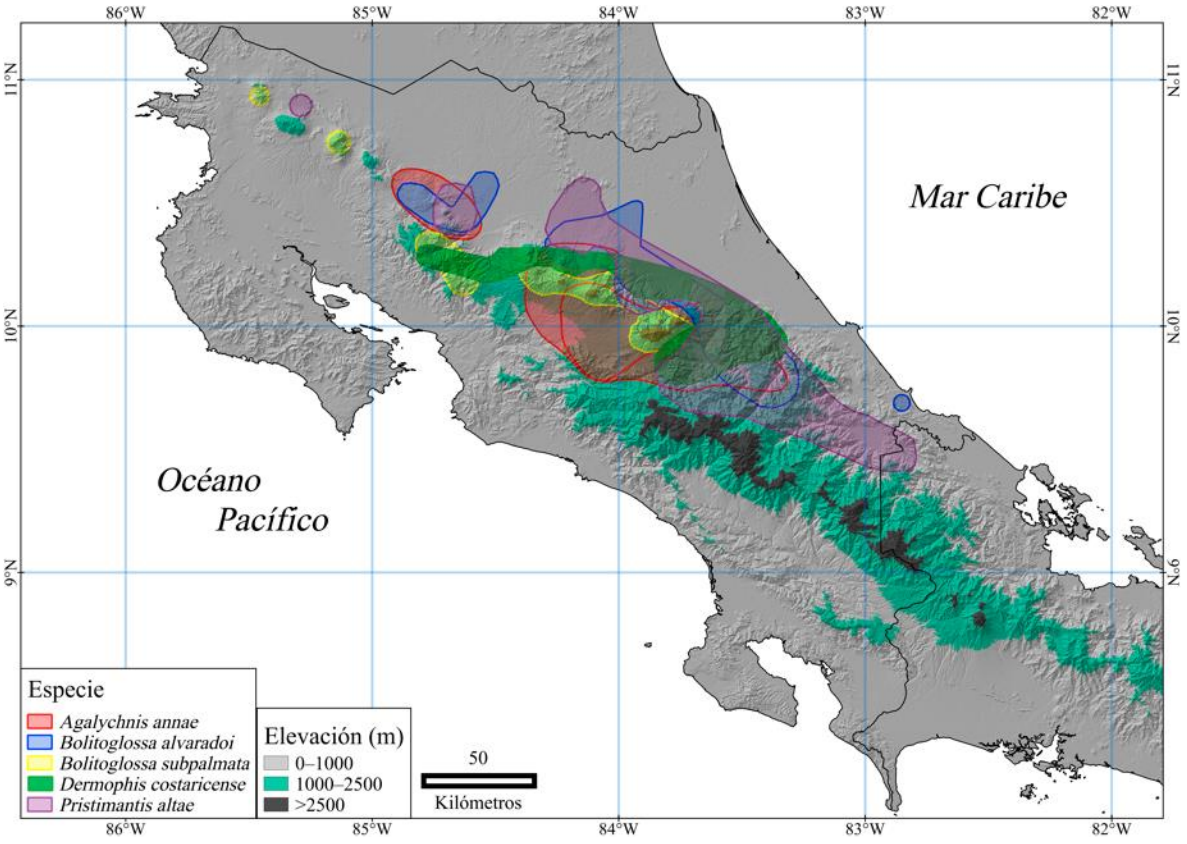

Figura 5. Mapa de Costa Rica mostrando un área de endemismo apoyada por cinco especies de anfibios.

BIOGEOGRAFÍA HISTÓRICA DE LOS ANFIBIOS DE AMÉRICA CENTRAL

Principales estudios durante el siglo XX

El primer gran trabajo abordando la historia biogeográfica de los anfibios de América Central fue presentado por Savage (1966). El autor concluyó que la fauna anfibia de la región es producto de la confluencia de tres grandes grupos: 1) un grupo centroamericano con su origen en la región, 2) un grupo sudamericano el cual el autor considera que contribuye poco a la diversidad y se restringe principalmente en el extremo este de Panamá y 3) un grupo norteamericano que tiene sus más cercanos ancestros fuera de América Tropical (ej. salamandras). Savage (1966) concluye que los anfibios de América Central derivan en su gran mayoría de un ancestro en común con clados presentes en Sudamérica, él propone que esto es producto de una conexión terrestre entre América Central Nuclear y Sudamérica en el Paleoceno (66-56 Ma). Savage (1966) sugiere que posterior a la conexión entre América Central Nuclear y

Sudamérica estas estuvieron separadas durante el Eoceno al Plioceno (56-5 Ma), permitiendo la evolución de forma aislada. Por último, el autor sugiere que durante el Plioceno (~5 Ma) se dio el inicio de la formación del Istmo, permitiendo la colonización de América Central por especies sudamericanas. Savage (1966) también basado en su análisis de distribución y endemismo de los géneros de anfibios y reptiles en América Central, plantea que la fauna anfibia de esta región debe catalogarse como una unidad independiente al mismo nivel que los ensamblajes neárticos o neotropicales.

El trabajo de Savage (1966) sirvió como base para la revisión de la historia biogeográfica de los anfibios y reptiles de América Central por el mismo autor (Savage 1982). En esta extensa revisión el autor discute las diferentes posiciones metodológicas y teóricas de la biogeografía en aquel momento (vicarianza *vs.* dispersión) utilizando como modelo las especies de anfibios y reptiles de la región. Savage (1982) llega a las mismas conclusiones que él alcanzó en Savage (1966), él refinó que los eventos que en conjunto originaron la fauna anfibia de América Central son los siguientes: 1) Un evento de dispersión desde Sudamérica, aunque evita sugerir la forma en que esta dispersión ocurrió, 2) un evento de vicarianza entre América Central y Sudamérica, 3) un segundo evento de dispersión desde Norteamérica, 4) un segundo evento de vicarianza entre América Central y Norteamérica y 5) un tercer evento de dispersión desde Sudamérica al completarse el cierre del Istmo de Panamá. Savage (1982) también reanaliza los datos de angiospermas de Raven & Axelrod (1974) y peces de agua dulce de Bussing (1976) y demuestra que estos dos grupos son concordantes con los patrones que él encontró.

El modelo de conformación propuesto por Savage (1966, 1982) se ha mantenido como el modelo más aceptado. Otros trabajos biogeográficos para la región Bussing (1985), Duellman (1979, 2001) y Savage (2002) han respaldado en lo general esta propuesta. Sin embargo, la principal discrepancia se ha centrado en la forma en que ocurrió la primera dispersión desde Sudamérica hacia América Central. Duellman (1979) argumentó a favor de una primera dispersión sobre el mar o a través de islas. Savage (1982) evita argumentar a favor de alguna hipótesis y enumera las posibles explicaciones incluyendo una conexión por las proto-Antillas (~80 Ma) o el modelo propuesto por Duellman (1979). Savage (1982) sugiere que una hipótesis alternativa sería una primera dispersión post-Mioceno (~23 Ma) a través de la actual América Central (asumida como un complejo de islas) que conllevó a una rápida diversificación en la

región. Más recientemente Duellman (2001) y Savage (2002) han argumentado a favor de una primera dispersión a través de las proto-Antillas durante el Cretácico Tardío (~84 Ma).

Hipótesis actuales a la luz de la evidencia molecular

El siglo XXI trajo un uso cada vez más generalizado del ADN en la construcción de filogenias. Filogenias moleculares existen hoy para los principales grupos anfibios de América Central como las ranas arborícolas (Faivovich *et al.* 2005, 2010; Duellman *et al.* 2016), salamandras neotropicales (Parra-Olea *et al.* 2004; Wiens *et al.* 2007; Rovito *et al.* 2015a) y los sapos mesoamericanos (Mendelson *et al.* 2011). Posiblemente, el entendimiento de las relaciones filogenéticas en las ranas de desarrollo directo ha sido el más beneficiado con el uso de las herramientas moleculares. El alto grado de homoplasia y polimorfismos fenotípicos entre dichas ranas impidió resolver sus relaciones filogenéticas hasta el uso de ADN en el siglo presente. Casi 1000 especies conformaban hasta entonces el gran género *Eleutherodactylus* (Frost *et al.* 2004). Las relaciones filogenéticas entre las ranas de desarrollo directo continúa cambiando (Hedges *et al.* 2008; Pyron & Wiens 2011; Padial *et al.* 2014), pero existe amplia concordancia a favor de muchos de sus géneros, familias y subfamilias. Actualmente, dichas ranas se agrupan en tres familias y 25 géneros (Padial *et al.* 2014).

Las hipótesis biogeográficas para América Central también se ha beneficiado del uso de las herramientas moleculares. En la biogeografía cladística un requisito fundamental es conocer la monofilia de los grupos en cuestión. Con relaciones filogenéticas pobremente entendidas entre las ranas de desarrollo directo estas carecían de utilidad el estudio biogeográfico de la región, pero, a medida que sus relaciones filogenéticas son mejor entendidas su importancia para evaluar hipótesis biogeográficas se hace notable. Además, las ranas de desarrollo directo como grupos de estudio tienen las siguientes ventajas: su alta diversidad en la región, su alta abundancia relativa y su ubicuidad. En esta sección se revisa cómo el estudio de las ranas de desarrollo directo en la era de la sistemática molecular han tenido un papel importante en apoyar las hipótesis actuales de la biogeografía de América Central. Además, otros grupos biológicos son utilizados para respaldar dichos hallazgos.

La primera dispersión y diversificación en América Central. Como propuso Savage (1966), la explicación de la diversidad actual en América Central requirió una primera dispersión hacia la

región desde Sudamérica y Norteamérica (salamandras). El único grupo de origen norteamericano con diversificación regional son las salamandras de la tribu Bolitoglossini (Savage 1966, 1982, 2002; Rovito *et al.* 2015a). El arribo de las salamandras a América Central desde Norteamérica se entiende como un evento de dispersión sobre las tierras continentales actuales, esto debido a la conexión terrestre entre dichas unidades es anterior al arribo de las salamandras a América Central. Rovito *et al.* (2015a) reconstruyen la historia filogenética y biogeográfica para las salamandras neotropicales (Bolitoglossini), encontrando que todas las salamandras de América Central constituyen un grupo monofilético y rechazando la parafilia encontrada por Wiens *et al.* (2007). Además, encontraron que el origen de las salamandras neotropicales se remonta al Eoceno Medio (47-37 Ma) y resaltaron que América Central Nuclear tuvo un papel importante en la diversificación temprana de las salamandras neotropicales, en la diversificación de géneros y subgéneros. Basado en los hallazgos de Rovito *et al.* (2015a) y en los argumentos utilizados por Savage (1966, 1982) para clasificar los orígenes de la fauna anfibia, las salamandras neotropicales deben ser consideradas como un clado centroamericano y no un clado norteamericano como lo consideró Savage (1966, 1982, 2002).

La primera dispersión desde Sudamérica hacia América Central es más conflictiva; Savage (1966) concluyó que una primera dispersión tuvo que ocurrir previo al cierre del Istmo de Panamá, seguido de un aislamiento que llevó al origen de la mayor parte de la fauna anfibia autóctona de la región. El origen sudamericano de todo el clado de las ranas de desarrollo directo (Hedges *et al.* 2008) y su presencia actual en América Central lo convierte en un grupo ideal para evaluar esta primera dispersión. Crawford & Smith (2005) evaluaron la evolución y biogeografía de ranas de desarrollo directo en América Central. Estos autores asumen *a priori* que la primera dispersión hacia América Central desde Sudamérica ocurrió a través de las proto-Antillas y siguiendo a Savage (1966, 1982) restringen el origen de *Craugastor* al intervalo de 80-60 Ma. Con esta calibración ellos encontraron que el origen de *Craugastor* se remonta al Cretácico-Paleoceno (75-66 Ma) y argumentan a favor de una primera dispersión sobre las proto-Antillas. Crawford & Smith (2005) argumentan que el movimiento de las proto-Antillas hacia el este, aisló los ancestros de *Craugastor* en América Central Nuclear lo que llevó a su origen. Esta conclusión la basan en el hecho de que los clados más basales de *Craugastor* (*C. milesi* y *C. augusti* + *C. alfredi*) tienen como extremo sur de su distribución a Honduras. Crawford & Smith (2005) también proponen que los ancestros de *Eleutherodactylus* arribaron a las Antillas

mediante GAARlandia hace unos 46-25 Ma y posteriormente los *Eleutherodactylus* continentales llegaron a América Central Nuclear desde las Antillas en el Oligoceno (~35-26 Ma).

El estudio de Crawford & Smith (2005) tenía dos limitantes, primero, la calibración restringida por un modelo biogeográfico y segundo, la falta de fósiles o grupos filogenéticamente cercanos a *Craugastor* en las Antillas como lo predice un modelo de colonización mediante las proto-Antillas. Heinicke *et al.* (2007) aumentaron el muestreo de especies y caracteres moleculares y revisaron el origen de las ranas de desarrollo directo en América Central y el Caribe, pero esta vez calibrando con el registro fósil. Los autores encontraron que los orígenes de *Craugastor* en América Central y de *Eleutherodactylus* en el Caribe son producto de dispersiones oceánicas. Heinicke *et al.* (2007) sugieren que el origen de *Craugastor* y *Eleutherodactylus* se ubica en el Eoceno, ~42-31 Ma y ~47-29 Ma respectivamente, mientras que el origen del clado continental de *Eleutherodactylus* se data en ~19 Ma producto de otra dispersión oceánica desde Cuba a Yucatán. Los autores rechazan las hipótesis de dispersión sobre las conexiones terrestres de proto-Antillas y GAARlandia. Sin embargo, es importante resaltar que los tiempos propuestos para la dispersión de Sudamérica hacia América Central y las Antillas (~47-29 Ma) son coincidentes con la propuesta de GAARlandia que se ubica en ~35-33 Ma.

Recientemente Duellman *et al.* (2016) revisaron las relaciones filogenéticas de las ranas arborícolas (Hylidae), encontrando que el origen de Hylinae (subfamilia con origen en América Central) se remonta al Oligoceno Temprano (35.6-30.2 Ma). Aunque esta dispersión es congruente también con la hipótesis de GAARlandia los autores no la sugieren como posible medio, quizás, porque tampoco existen fósiles ni grupos filogenéticamente cercanos a Hylinae en las Antillas. Ř íčan *et al.* (2013) encontraron que la dispersión de peces dulceacuícolas hacia América Central y las Antillas desde Sudamérica se ajusta a la hipótesis de GAARlandia. Ř íčan *et al.* (2013) proponen que una primera dispersión desde Sudamérica hacia las Antillas corresponde con la presencia de GAARlandia (~35-29 Ma), posteriormente, América Central Nuclear fue colonizado por al menos dos eventos de dispersión desde las Antillas. El trabajo de Ř íčan *et al.* (2013) rechaza los hallazgos de Chakrabarty (2006) quien había sugerido que el origen de estos peces se remontaba al Cretácico-Paleoceno (~66 Ma) y quien propuso que la vía de colonización era coincidente con las proto-Antillas. Alonso *et al.* (2012) al revisar la filogenia

y biogeografía del género de sapos *Peltophryne* en Cuba, encontraron que la dispersión de estos anfibios a las Antillas desde Sudamérica es concordante con la conexión de GAARlandia.

La primera dispersión desde Sudamérica hacia América Central sigue bajo discusión, los tres ejemplos anteriores (Heinicke *et al.* 2007; Řícan *et al.* 2013; Duellman *et al.* 2016) rechazan la hipótesis de las proto-Antillas. Más discutida sigue siendo la hipótesis de GAARlandia (Iturralde-Vinent & MacPhee 1999), ejemplificado anteriormente (Řícan *et al.* 2013) y como lo notó Alí (2012) dicha hipótesis ha sido bien recibida como explicación espacio-temporal de dispersión desde Sudamérica hacia las Antillas y América Central. Alí (2012) argumenta en contra de GAARlandia como una conexión continua entre Sudamérica y las Antillas y como medio generalizado de dispersión entre estas dos masas terrestres. Alí (2012) sugiere que si bien pudo existir la conexión de GAARlandia, es difícil que haya sido ampliamente utilizada como medio de dispersión y expone que muchos otros eventos de dispersión debieron ocurrir sobre el agua. La evidencia plantea que el origen de *Craugastor* y la subfamilia Hylinae, se explica mejor con eventos aislados de dispersión oceánica. También, es concordante la evidencia de que los *Eleutherodactylus* continentales tienen su origen en las Antillas, pero el origen de estos en las Antillas es incierto, Heinicke *et al.* (2007) argumentan a favor de dispersión oceánica, pero también es cierto que pudieron arribar por GAARlandia. Más investigación será necesaria para esclarecer el origen de este clado.

Una hipótesis alternativa que Savage (1982) sugirió pero sin detallar, es que los ancestros de *Craugastor* e Hylinae hayan dispersado por una América Central Ístmica primigenia durante el Oligoceno Temprano (~34-28 Ma). Está hipótesis alternativa tiene la ventaja de que ubica una conexión directa entre Sudamérica y América Central Nuclear y evita recurrir a la dispersión de largas distancias sobre el agua, eventos que son altamente improbables para fisiologías de organismos como anfibios o peces de agua dulce. La desventaja de esta hipótesis es que los centros de diversificación basal para *Craugastor* e Hylinae se encuentran en América Central Nuclear, lo cual obliga a recurrir a una extinción de los ancestros en América Central Ístmica posterior a la colonización de América Central Nuclear. La evidencia revela que durante el Eoceno (~56-33 Ma), América Central Ístmica existía como un estrecho cinturón de tierras emergidas de baja elevación que variaba de forma peninsular en el Norte (Costa Rica) a insular en el sureste (Panamá) y separado de Sudamérica por el Canal Marítimo Centroamericano (Montes *et al.* 2012a,b, 2015). El levantamiento continuó y para el Mioceno Temprano (~23 Ma)

América Central Ístmica ya existía como una península que se extendía desde el extremo sur de América Central Nuclear hasta el Darién (extremo este de Panamá) separado de Sudamérica por ~200 Km de océano (Kirby & MacFadden 2005; Montes *et al.* 2012b). Se ha propuesto que durante el Oligoceno Temprano (~30 Ma) el nivel del mar fue inferior al actual (Haq *et al.* 1987), descenso que fue argumentado a favor de la hipótesis de GAARlandia (Alí 2012). A pesar de que los estudios sobre la reconstrucciones del nivel del mar han sido contrastantes (ver *Clima y nivel del mar en el pasado*), es posible que este descenso –si existió– pudo haber permitido la primera dispersión desde Sudamérica hacia América Central Nuclear. El posterior aumento del nivel del mar pudo haber inundado las tierras apenas superficiales de América Central Ístmica y extinguido su fauna incipiente.

La colonización de América Central Ístmica previo al cierre del Istmo de Panamá. Este momento histórico en América Central no debe ser entendido como un evento aislado, sino, como pulsos discontinuos de dispersión de la fauna ya presente en América Central Nuclear hacia el sur colonizando las tierras de Costa Rica y Panamá a medida que estas se levantaban. Es probable que estos pulsos de dispersión hayan sido separados por altos niveles del mar lo cual aislaba temporalmente estas dos unidades de América Central, propiciando la evolución de diferentes grupos (Castoe *et al.* 2009; Gutiérrez-García & Vázquez-Domínguez 2013). Posiblemente el inicio de estos pulsos de dispersión se remonta al Mioceno Temprano (~23 Ma), cuando América Central Ístmica había emergido y se prolongaba como una península en su posición actual (Montes *et al.* 2012a). Estos primeros eventos de dispersión corresponden con la aparición del grupo de especies *Craugastor podiciferus* hace ~20 Ma (Streicher *et al.* 2009). El grupo de especies *C. podiciferus* contiene diez especies de ranas de desarrollo directo que tiene su diversificación en Costa Rica y el oeste de Panamá, el grupo hermano del clado *C. podiferus* es el clado *C. rhodopis* que habita desde el sur de México hasta el norte de Honduras (Hedges *et al.* 2008; Padial *et al.* 2014). Otros grupos apoyan esta primera dispersión desde América Central Nuclear hacia América Central Ístmica, Duellman *et al.* (2016) encontraron que el origen y diversificación del género *Isthmohyla* en América Central Ístmica data de ~19.5 Ma. Říčan *et al.* (2013) encontraron que los peces dulceacuícolas de América Central Ístmica llegaron desde el norte entre 19-13 Ma. Rovito *et al.* (2015a) sugirieron que las salamandras se dispersaron hacia

el sur y que entre 16.4-8 Ma arribaron a Sudamérica, por lo cual debieron llegar a América Central Ístmica previamente.

El cierre del Istmo de Panamá y el Gran Intercambio de la Biota Americana. El cierre del Istmo de Panamá se asumió históricamente en ~3 Ma (Coates & Obando 1996; Coates *et al.* 2004), sin embargo, evidencia reciente ubica el cierre entre 15-13 Ma (Montes *et al.* 2015). Previo a los descubrimientos del cierre del Istmo de Panamá en el Mioceno (Montes *et al.* 2012a,b, 2015) algunos estudios indicaban que varias dispersiones desde Sudamérica hacia América Central habían ocurrido previo a ~3 Ma (excluyendo las primeras dispersiones de *Craugastor* e Hylinae). Wang *et al.* (2008) estudiaron la filogeografía de la rana de desarrollo directo *Pristimantis ridens*, género de origen sudamericano, y encontraron que el origen del clado conteniendo las poblaciones de Costa Rica y Honduras ocurrió ~12 Ma, por lo que esta especie dispersó desde Sudamérica mucho antes del cierre del Istmo de Panamá. Pinto-Sánchez *et al.* (2012) realizaron un estudio intensivo con ranas *Pristimantis* tanto al norte como al sur del Istmo de Panamá, en busca de dispersiones desde Sudamérica hacia América Central previas al cierre del Istmo de Panamá. Ellos descubrieron que el género *Pristimantis* efectivamente tiene su origen en Sudamérica, donde habita la mayoría de su diversidad (489 especies), y encontraron que los *Pristimantis* de América Central son producto de múltiples invasiones desde Sudamérica entre 12-6 Ma. Estos autores refirieron ocho eventos individuales de dispersión de *Pristimantis* que se distribuyen tanto en América Central como en Sudamérica, pero también, encontraron tres eventos de dispersión que han generado linajes endémicos de América Central, entre ellos el grupo de especies *P. pardalis* que diversificó en al menos tres especies.

Los resultados anteriores son concordantes con otros encontrados en peces dulceacuícolas (Bermigham & Martin 1998) y pseudoescorpiones (Zeh *et al.* 2003). Estas primeras dispersiones desde Sudamérica hacia América Central originaron algunos géneros de anfibios y reptiles autóctonos para la región como *Agalychnis, Bothriechis, Atropoides* y *Cerrophidion.* Duellman *et al.* (2016) en un estudio con las ranas arborícolas, encontraron que posterior a la primera dispersión que dio origen a Hylinae, al menos otros seis eventos de dispersión ocurrieron desde Sudamérica hacia América Central, todos durante el Mioceno (23-5 Ma). Una de estas dispersiones dio origen al género *Agalychnis* que divergió de sus ancestros sudamericanos entre 12.3-7.9 Ma. Castoe *et al.* (2009) encontraron dos eventos de dispersión en serpientes de foseta

neotropicales entre 15-10 Ma, que dieron origen a los géneros *Bothriechis, Atropoides* y *Cerrophidion*. La única dispersión bien documentada de un anfibio desde América Central hacia Sudamérica es la del género de salamandras *Bolitoglossa*, que ha sido datada entre 16.4-8 Ma (Rovito *et al.* 2015a), concordante con los resultados anteriores en haber ocurrido previo a ~3 Ma.

El Gran Intercambio de la Biota Americana se refiere al gran intercambio biótico que siguió al cierre del Istmo de Panamá (Webb 1991, 2006). Bacon *et al.* (2015) basados en la evidencia reciente que ubica el cierre del Istmo de Panamá entre 15-13 Ma (Montes *et al.* 2015), evaluaron si este cierre temprano es concordante con un Gran Intercambio de la Biota Americana previo a ~3 Ma. Bacon *et al.* (2015) encontraron que el Gran Intercambio de la Biota Americana ocurrió de forma generalizada antes de los 3 Ma, en forma de pulsos entre 20-6 Ma, resultados concordantes para anfibios, reptiles, aves, artrópodos y plantas. Sin embargo, múltiples estudios realizados con mamíferos apoyan el Gran Intercambio de la Biota Americana entre 3.5-2.5 Ma (Webb 1991, 2006). Recientemente, Bacon *et al.* (2016) analizaron por qué el intercambio de mamíferos entre Norte- y Sudamérica no concuerda con el cierre del Istmo de Panamá hace 15-13 Ma, habiendo encontrado que el cruce tardío de los mamíferos se debió a factores climáticos y medioambientales, más que la configuración geológica de la región.

Todos los resultados anteriores son contundentes en apoyar una serie de pulsos discontinuos de dispersión desde Sudamérica hacia América Central y viceversa, posterior al cierre del Istmo de Panamá hace 15-13 Ma. Esto demuestra que para los anfibios, el Gran Intercambio de la Biota Americana es concordante con el cierre del Istmo de Panamá en el Mioceno. Sin embargo, existe un grupo grande de especies de origen sudamericano que carecen de estudios que daten su presencia en América Central. Estas especies pertenecen a géneros (ej. *Atelopus, Elachistocleis* y *Gastroteca*) o inclusive familias (Dendrobatidae, Leptodactylidae y Centrolenidae) con sus centros de origen y mayor diversidad en Sudamérica. Muchas de estas especies poseen distribución compartida entre América Central y Sudamérica, lo cual se ha interpretado como el producto de dispersiones recientes (<~3 Ma) desde Sudamérica hacia América Central. Es necesario datar la presencia de dichas especies en América Central, porque podría ser que efectivamente un grupo grande de estas especies hayan ingresado tan recientemente como ~3 Ma, quizás respondiendo a los mismos factores climáticos que impulsaron la migración de mamíferos (Bacon *et al.* 2016). Pero quizás, a pesar de que

efectivamente estas especies pertenecen a géneros de origen sudamericano, su presencia en América Central corresponda con el cierre del Istmo de Panamá hace 15-13 Ma, mucho antes de ~3 Ma, tal como lo encontró Pinto-Sánchez *et al.* (2012) en *Pristimantis.*

PERSPECTIVAS ACTUALES: UNA MIRADA DESDE LAS RANAS DE DESARROLLO DIRECTO

Especies crípticas

Con el desarrollo de nuevas tecnologías que facilita el obtener secuencias moleculares en poco tiempo y a bajo costo, surgió un cambio de paradigma taxonómico: transformar la taxonomía tradicional en una taxonomía integradora (Dayrat, 2005; Will *et al.* 2005). El objetivo es claro, robustecer las conclusiones taxonómicas con el apoyo de evidencia filogenética, morfológica, filogeográfica y demás en cuanto sea posible (Dayrat, 2005; Will *et al.* 2005). A pesar de que la implementación de la taxonomía integradora traería grandes beneficios para la taxonomía y el entendimiento de la biodiversidad (Padial *et al.* 2010) aún su implementación no se generaliza en la práctica taxonómica (Pante *et al.* 2015). La implementación de herramientas taxonómicas integrativas en grupos con alta diversidad críptica puede ser especialmente beneficiosa (Padial & De la Riva 2009; Padial *et al.* 2010). Según Bickford *et al.* (2007) las especies crípticas son aquellas que han sido tratadas como un único taxón dado que son al menos superficialmente indistinguibles. Esta característica intrínseca de la diversidad críptica, justifica el atender este fenómeno desde un enfoque integrativo con adecuados protocolos de delimitación, que incluya el uso de herramientas moleculares, morfológicas, ecológicas u otras fuentes de evidencia particulares a cada grupo biológico. El fenómeno de especies crípticas tiene profundas implicaciones en la sistemática, la conservación, la biología evolutiva y en la biogeografía (Nadler & Pérez-Ponce de León 2010). La presencia de especies crípticas no delimitadas correctamente implica el enmascaramiento de biodiversidad, puede conllevar a conclusiones biogeográficas erróneas y a la pérdida de evidencia para apoyar o refutar patrones biogeográficos.

Recientemente se han propuestos enfoques integradores para enfrentar el fenómeno de las especies crípticas. Pérez-Ponce de León & Nadler (2010) proponen atender el problema desde un enfoque molecular de prueba de hipótesis, sugiriendo que si la hipótesis nula de un solo taxón se rechaza, entonces una re-evaluación morfológica de los clados moleculares puede revelar

diferencias hasta entonces ocultas. También, se han propuesto protocolos detallados para delimitar diversidad críptica en anfibios (Padial *et al.* 2010; Ortega-Andrade *et al.* 2015). Y de forma general, grandes avances se han dado en el campo de la delimitación de especies, cuyas propuestas pueden contribuir a delimitar la diversidad críptica (Sites & Marshall 2003; Wiens 2007; Flot 2015). El consenso sugiere que la delimitación de la diversidad críptica en anfibios requiere en un primer paso, generar una filogenia molecular que permita agrupar la diversidad en grupos monofiléticos. Posteriormente, con base en estos clados se selecciona una muestra de la cual se recupera información morfológica, morfométrica, acústica o ecológica y finalmente la delimitación es basada en la concordancia de la evidencia.

Se ha sugerido que la distribución de especies crípticas no es proporcional entre distintos grupos biológicos (Pérez-Ponce de León & Poulin 2016) y que los anfibios, en especial los Anuros, pueden albergar mayor diversidad críptica entre los vertebrados (Bickford *et al.* 2007; Bagley & Johnson 2014). Las ranas de desarrollo directo son uno de los grupos sugeridos a albergar gran diversidad de especies crípticas (Padial & De la Riva 2009). En América Central el género *Craugastor* es el más diverso de las ranas de desarrollo directo y se presume que contiene muchas especies no descritas, ocultas por las especies actualmente reconocidas (Savage 2002; Crawford *et al.* 2007; Streicher *et al.* 2009). En este apartado del ensayo se revisa como la taxonomía integrativa, la delimitación de especies y la filogeografía, contribuyen a resolver problemas de especies crípticas y con esto a mejorar el entendimiento de la sistemática y biogeografía en Costa Rica y Panamá. Como se comentó anteriormente (ver *Diversidad de anfibios en América Central y sus patrones de distribución*) Costa Rica y Panamá representan la región más diversa en anfibios de América Central, en especial Costa Rica y el oeste de Panamá son los sitios de mayor diversificación y endemismo regional.

Un estudio actualmente en desarrollo, donde se revisa la sistemática y taxonomía de las ranas del grupo de especies *Craugastor podiciferus* en América Central, sugiere la presencia de varias especies no descritas dentro del grupo (Datos sin publicar). Una de estas especies, *C. gabbi* Arias, Chaves, Crawford & Parra-Olea, 2016 estuvo históricamente bajo el nombre *C. stejnegerianus* (Cope, 1893) producto de una morfología extremadamente conservada. Sin embargo, análisis moleculares apoyan su monofilia y ubican al clado *C. stejnegerianus* + *C. rearki* (Taylor, 1952) como su grupo hermano (Fig. 6). Con base en la inferencia molecular se realizaron análisis morfométricos y de morfología externa y se encontró una característica

diagnóstica que permitió su correcta delimitación y descripción. La delimitación de esta especie tiene implicaciones biogeográficas, pues, esta es endémica de la región premontana (1200-1500 m.s.n.m) del pacífico sur de Costa Rica, respaldando descubrimientos previos de anfibios endémicos para la región (Brame & Duellman 1970; García-París & Wake 2000; Wake *et al.* 2007). Crawford (2003) calculó que el origen de la nueva especie data entre 10.3-6.3 Ma, respaldando la importancia biogeográfica del levantamiento de la Fila Costeña hace 12.8-11.7 Ma (MacMillan *et al.* 2004). El uso de herramientas moleculares en el grupo de especies *C. podiciferus*, también ha permitido esclarecer especies mal clasificadas dado su alto grado de similitud morfológica. Gracias a las inferencia moleculares se pudo determinar que la especie *C. rearki*, hasta entonces sinónimo de *C. bransfordii*, es una especie válida (Fig. 6). Además, se demostró que *C. rearki* se distribuye hasta el este de Honduras incluyendo las poblaciones anteriormente asignadas a *C.* lauraster, la cual se colocó bajo sinonimia de la primera.

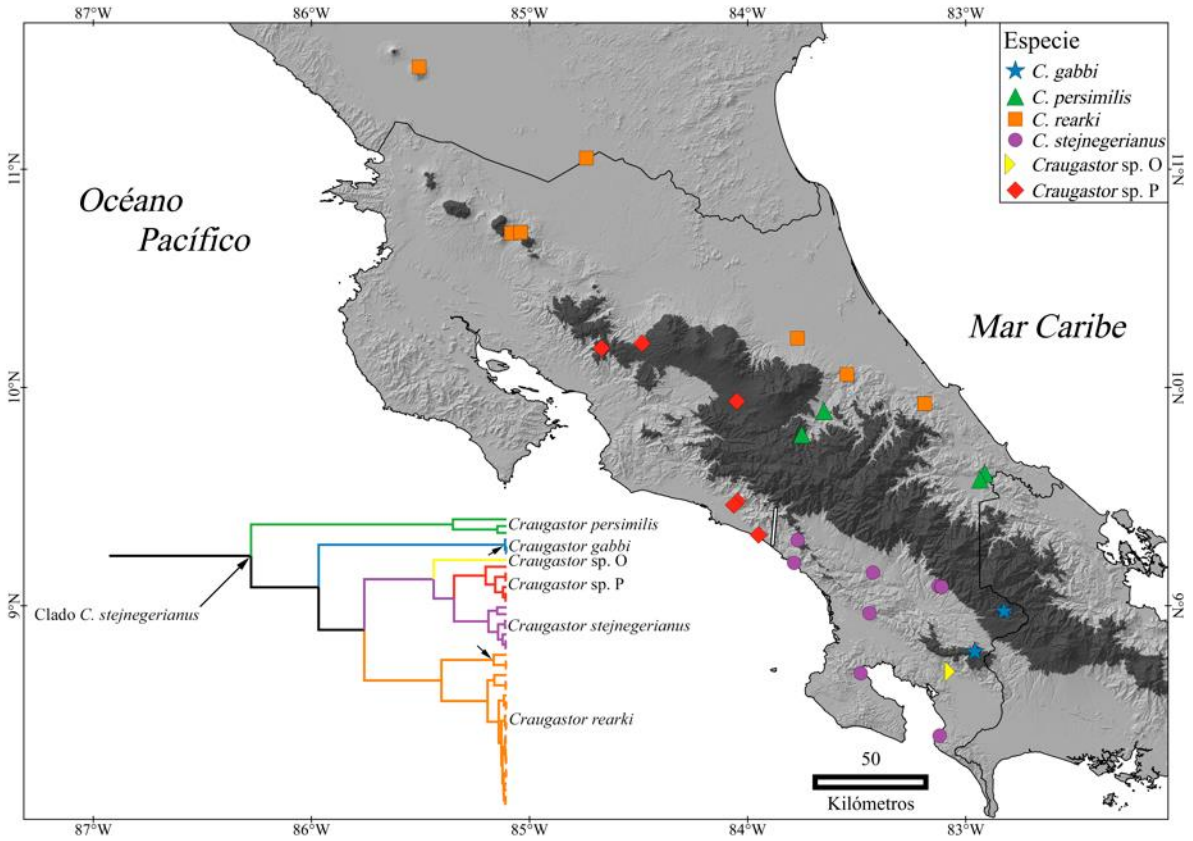

Figura 6. Mapa de América Central mostrando la distribución de un clado dentro del grupo de especies *Craugastor podiciferus.* Las flechas indican poblaciones que fueron en el pasado clasificadas como *C. stejnegerianus*, incluyendo a la especie *C. gabbi.* La línea blanca señala el río Savegre.

Filogeografía como herramienta para descubrir especies crípticas

La filogeografía estudia la distribución espacial de linajes genéticos dentro de una especie o especies estrechamente relacionadas (Avise 2009), esto hace de la filogeografía una herramienta especialmente útil para el descubrimiento de especies crípticas. Bagley & Johnson (2014) revisaron los estudios filogeográficos realizados en América Central Ístmica y encontraron que en promedio los estudios registraron 2.1 linajes genéticos independientes por cada taxón. Los grupos con mayor diversidad críptica registrada son los peces dulceacuícolas y los anuros. Las ranas de desarrollo directo se han convertido en un excelente modelo para estudios filogeográficos en la región (Wang *et al.* 2008; Crawford *et al.* 2007; Streicher *et al.* 2009; Crawford *et al.* 2013; Paz *et al.* 2015). En un estudio filogeográfico con la rana *Craugastor podiciferus* en Costa Rica y el oeste de Panamá, se encontró la presencia de una especie críptica y se dató la divergencia entre 11.8–6.6 Ma (Streicher *et al.* 2009). En otro estudio filogeográfico con *Craugastor* en Costa Rica y Panamá, se encontró que al menos tres especies crípticas permanecen sin describir (Crawford *et al.* 2007). Estas tres especies no descritas de *Craugastor* se distribuyen por encima de los 1000 m de elevación, resaltando la importancia de las tierras intermedias en la diversificación regional (ver Fig. 3).

Los ejemplos anteriores junto con la distribución altitudinal de las especies de América central (Fig. 3) son concordantes en apoyar la importancia de la diversificación en tierras intermedias. Sin embargo, existe controversia en la datación cronológica de los procesos responsables de esta diversificación. Savage (2002), basado en la evidencia de oscilaciones climáticas y el rápido levantamiento de las tierra altas de América Central en el Plioceno-Pleistoceno (~5 Ma), propuso un modelo de especiación de tierras altas (especiación montana). Savage (2002) sugiere que las oscilaciones entre periodos glaciares e interglaciares que comenzaron en el Plioceno (~5 Ma) y se continuaron durante el Pleistoceno, son las responsables del origen y distribución de muchas especies de tierras altas. Durante periodos glaciares los bosques montanos estuvieron conectados, periodos subsecuentes de calentamiento llevaron al aislamiento de estos bosques en las cumbres de las cordilleras centroamericanas lo cual conllevó al origen de muchas de las especies actuales (Savage 2002). Sin embargo, análisis filogeográficos con la rana *Craugastor podiciferus* (Streicher *et al.* 2009) y con serpientes de foseta neotropicales (Castoe *et al.* 2009) han rechazado que estos eventos del Plioceno-

Pleistoceno estén asociados con cladogénesis dentro de estas especies de altura. Más estudios son necesarios para entender la diversificación en las tierras altas de la región.

En un estudio actualmente en desarrollo, la evidencia molecular sugiere que los eventos durante el Pleistoceno provocaron la formación de al menos 11 especies de salamandras de musgo (*Nototriton*) en la tierras altas de Costa Rica (Datos sin publicar). Estos resultados respaldan la propuesta de Savage (2002) de que los eventos climáticos del Plioceno-Pleistoceno generaron diversificación en los anfibios de América Central. La discrepancia entre los resultados encontrados en *Nototriton* y aquellos encontrados por Streicher *et al.* (2009) y Castoe *et al.* (2009) podrían reflejar las diferentes capacidades de dispersión entre los grupos estudiados. Las salamandras del género *Nototriton* son altamente miniaturizadas (longitud estándar = ~30mm), semifosoriales y de baja movilidad (Savage 2002). Estos resultados con las salamandras *Nototriton* de Costa Rica, también respaldan la evidencia que sugiere que existe alta diversidad críptica dentro de las salamandras miniaturizadas del neotrópico en los géneros *Chiropterotriton, Nototriton* y *Thorius* (Darda 1994; Savage 2002; Townsend *et al.* 2011; Rovito *et al.* 2013). Nuestros resultados sugieren la presencia de cuatro especies no descritas, todas con alta similitud morfológica entre sí y con las demás especies descritas para el país (Datos sin publicar). Pero la evidencia molecular y la historia de vida de estos organismo como: la restricción altitudinal (1000-2500 m.s.n.m), la baja movilidad, y la ausencia de conectividad biogeográfica (Savage 2002) respalda el reconocimiento de las especies y la descripción de las mismas.

La filogeografía comparada busca patrones filogeográficos compartidos dentro de especies codistribuidas (Gutiérrez-García & Vázquez-Domínguez 2011). El objetivo principal, es la búsqueda de patrones generales que permitan construir explicaciones para una gama amplia de organismos (Bagley & Johnson 2014). El filtro de Bocas del Toro en el oeste de Panamá se ha propuesto como una barrera geográfica, asociada a eventos de altos niveles del mar que afectó las poblaciones de tierras bajas (Coates *et al.* 2005). Esta barrera se ha correlacionado con fragmentaciones filogeográficas en peces (Perdices *et al.* 2002) y anfibios (Crawford *et al.* 2007), pero en los anfibios el nivel de fragmentación varió entre tres especies de *Craugastor* con diferentes requerimientos ecológicos (Crawford *et al.* 2007). Sin embargo, según Bagley & Johnson (2014) en América Central Ístmica hay una carencia generalizada de historia

biogeográfica compartida y sugieren que la respuesta a barreras varía entre especies y en temporalidad.

A pesar de la carencia de patrones generales, es importante continuar en la búsqueda de historias compartidas que apoyen ciertas fragmentaciones, al menos de forma parcial. El descubrimiento de nuevas zonas de fragmentación donde no existen claras barreras actuales, obliga a profundizar en el estudio geológico y paleoclimático de la región. Profundas fragmentaciones entre poblaciones de la lagartija *Anolis aquaticus* en la costa pacífica de Costa Rica, llevó a la delimitación de una nueva especie cuya única barrera visible es la cuenca del río Savegre (Chaves *et al.* En revisión). Este mismo río es la única barrera visible para explicar la fragmentación encontrada en la rana *Craugastor stejnegerianus* en el pacífico costarricense (Fig. 6), cuya distancia genética entre poblaciones de ambos márgenes del río asciende al 1.8% en el gen mitocondrial 16S y 5.5% en el gen COI (Datos sin publicar). No existe explicación para soportar estas fragmentaciones concordantes, más allá del río, mismo que no varía de los otros ríos que se encuentran dentro del rango geográfico de estas especies. Quizás alguna explicación climática del Plioceno-Pleistoceno pueda ser hallada en el futuro.

RETOS FUTUROS

Se han realizado grandes avances en el entendimiento biogeográfico de América Central y en especial para anfibios. Sin embargo, persisten grandes vacíos de conocimiento por llenar. Los novedosos y controversiales hallazgos sobre el cierre temprano en el Mioceno Medio (15-13 Ma) del Istmo de Panamá (Montes *et al.* 2015) tiene implicaciones cruciales para el entendimiento biogeográfico de la región. Es necesario más evidencia que soporte o refute estos hallazgos y que consolide las reconstrucciones paleogeográficas del Istmo de Panamá. Asimismo, para entender las primeras dispersiones desde Sudamérica hacia América Central Nuclear es necesario generar un cuerpo de evidencia que apoye o refute la posibilidad de una conexión terrestre entre Sudamérica y las Antillas (GAARlandia), esto cuanto la evidencia actual es controversial y dudosa (Iturralde-Vinent & MacPhee 1999; Alí 2012). Como se detalló anteriormente, muchas de las hipótesis biogeográficas para América Central tienen supuestos sobre el nivel del mar en distintos momentos históricos, no obstante, las reconstrucciones sobre el nivel del mar carecen de concordancia (Miller *et al.* 2005; Kominz *et al.* 2008). La

biogeografía histórica y las hipótesis sobre América Central se verán beneficiadas de futuros hallazgos.

Desde el campo de la sistemática filogenética son necesarios grandes avances. Más y mejores reconstrucciones filogenéticas son necesarias. Aún persisten vacíos de conocimiento en las relaciones filogenéticas de diversos grupos de anfibios en la región. Diversos grupos de ranas de desarrollo directo (*Craugastor*, *Pristimantis* y *Diasporus*) y salamandras (*Bolitoglossa*) carecen de filogenias sólidas (Padial *et al.* 2014; Boza-Oviedo *et al.* 2012). Si bien, la carencia de fósiles anfibios centroamericanos es generalizada (Rovito *et al.* 2015a; Duellman *et al.* 2016) las dataciones moleculares pueden mejorar significativamente. Recientemente Sheng *et al.* (2016) encontraron que el origen de la familia de salamandras neotropicales Plethodontidae es al menos 24 Ma más recientes que los cálculos anteriores. Dataciones moleculares a partir de grandes matrices de ADN nuclear, generó dataciones más recientes que aquellas alcanzadas con la utilización de ADN mitocondrial, los autores sugieren que las dataciones a partir de ADN mitocondrial deben ser analizadas con precaución y construir nuevas dataciones con el uso de ADN nuclear (Shen *et al.* 2016).

Avances recientes han permitido el desarrollo de modelos y software que reconstruyen la evolución de rangos geográficos a partir de árboles filogenéticos (Ree *et al.* 2005; Ree & Smith 2008; Ree & Sanmartín 2009). Estos avances aún no se han implementado extensivamente en estudios con anfibios de América Central, a excepción de algunos trabajos con salamandras neotropicales (Rovito *et al.* 2012, 2015a, 2015b). Estos avances en biogeografía histórica tienen la ventaja de que permiten probar hipótesis sobre centros de dispersión y los posibles rangos ancestrales de un clado particular (Ree & Sanmartín 2009). Además, la implementación de modelos de dispersión-cladogénesis-extinción en el software Lagrange (Ree & Smith 2008) tiene la ventaja de que se basa en máxima verosimilitud para reconstruir el rango ancestral para una especie utilizando un árbol filogenético. En el futuro las reconstrucciones biogeográficas pueden tomar ventaja de estos avances en biogeografía paramétrica.

CONCLUSIONES

El análisis de los patrones de distribución de anfibios en América Central apoyó una distribución desproporcional, con una mayor concentración de especies en Costa Rica y Panamá. Costa Rica y el oeste de Panamá constituyen el sitio con la mayor riqueza de anfibios en la

región, tanto en el análisis con el total de las especies, como también al analizar únicamente las especies autóctonas. Esto resalta la importancia biogeográfica de Costa Rica y el oeste de Panamá, visto como un reservorio de especies que alcanzan en esta región su límite de la distribución y también como sitio de alta diversificación. De igual forma, en el ensayo se comprobó como el uso de herramientas moleculares han ayudado a un mejor entendimiento biogeográfico para América Central. Se pudo observar como los estudios moleculares recientes han rechazado la posibilidad de las proto-Antillas como medio de dispersión desde Sudamérica hacia América Central y como la evidencia sobre GAARlandia es todavía controversial. Se demostró como el uso de secuencias de ADN junto con los descubrimientos geológicos están cambiando aceleradamente el entendimiento sobre el Gran Intercambio de la Biota Americana, ahora entendida como pulsos de dispersión posterior al cierre del Istmo de Panamá hace 15-13 Ma. Las herramientas moleculares también han permitido descubrir gran diversidad de especies crípticas dentro de las ranas de desarrollo directo en América Central, resultando en mejores hipótesis biogeográficas para la región.

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