

IMPLICACIONES ADAPTATIVAS DEL SISTEMA DE APAREAMIENTO Y "FEMALE- GUARDING" EN RANAS ARLEQUINES (BUFONIDAE: ATELOPUS)



Luis Alberto Rueda Solano

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MSc. Luis Alberto Rueda Solano
Doctorando en Ciencias-Biología
Universidad de los Andes

Director:

Dr. Andrew J. Crawford
Universidad de los Andes

Asesores:

Dr. Fernando Vargas - Salinas
Universidad del Quindío

Dr. Karen Warkentin
Boston University

Dr. Carlos Arturo Navas
Universidade de São Paulo

Evaluador Interno:
Dr. Susana Caballero
Universidad de los Andes

Evaluador Externo:
Dr. Jennifer L. Stynoski.
Universidad de Costa Rica

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La rana negra era una mujer muy hermosa y orgullosa, que no quiso irse a vivir con el dios sol. El dios sol la castigó, transformandola en sapo. Ella no se podía vengar del dios sol, por eso castiga a los hombres, sapo macho, a que mueran de amor por ella en la espalda. El dios sol está en el cielo con su esposa la luna. Ella sigue aquí abajo, arrastrándose como castigo y castigando a los hombres.

Mitología Kogui



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PRESENTACIÓN

Las ranas o sapos arlequines del género *Atelopus* son un célebre grupo de anuros neotropicales perteneciente a la familia de sapos verdaderos (Bufonidae). Sus especies, se constituyen como maravillosos modelos de estudio debido a sus hábitos diurnos, abundancia, colores llamativos, lento caminar y son fácilmente reconocibles. Habitán casi estrictamente en las quebradas de las selvas, bosques húmedos montanos y páramos andinos, desde el nivel del mar hasta la línea de nieves perpetuas. Lamentablemente, en las últimas décadas hemos sido testigos de la desaparición vertiginosa de estos extraordinarios anuros, incluso en zonas remotas donde la intervención antrópica es nula. La quitridiomicosis producida por el hongo patógeno *Batrachochytrium dendrobatidis* es la mayor causa de la catastrófica desaparición de sus poblaciones en sus hábitats, llevándolos a la extinción casi completa de su linaje. Afortunadamente, muchas personas y organizaciones han aportado tiempo y esfuerzo para entender esta problemática y salvar las especies sobrevivientes. Esta tesis doctoral se suma a los esfuerzos de conservación de los *Atelopus* en Latinoamérica, especialmente las que habitan en la Sierra Nevada de Santa Marta - Colombia, integrando estudios comportamentales, ecológicos y eco-morfológicos, para entender las implicaciones evolutivas de algunos rasgos fenéticos asociados a la reproducción en *Atelopus*. El conocimiento de la historia natural de las especies de *Atelopus* constituye las bases de su conservación, generando una esperanza para este clado altamente amenazado.

INTRODUCCIÓN GENERAL

SISTEMAS DE APAREAMIENTO ANIMAL Y SELECCIÓN SEXUAL

Los sistemas de apareamiento animal, clasifican las especies según las particularidades del comportamiento de apareo, la adquisición de pareja, entre otros aspectos, incluyendo cuidado parental, que influyen en el éxito reproductivo individual (Emlen & Oring 1977; Shuster & Wade 2003).

Esta clasificación obedece a la disponibilidad de información reproductiva de la especie o grupo de especies a estudiar, lo que genera gran controversia alrededor de la categorización de estos sistemas (Klug 2011). No obstante, en su versión básica, los sistemas de apareamiento son determinados según el número de parejas y la manera de obtenerlas en cada temporada reproductiva (Emlen & Oring 1977). La monogamia, solo una pareja en cada temporada reproductiva, y la poligamia, varias parejas en cada temporada, serían los posibles resultados de esta clasificación. La poliginia, un macho con varias hembras; y la poliandria, una hembra con varios machos por temporada reproductiva, conformarían sub-categorías de la poligamia (Emlen & Oring 1977; Reynolds 1996). Una consideración importante con respecto al sistema de apareamiento polígamo, es que aumenta la varianza en el éxito reproductivo del sexo que tiene más de un compañero por temporada reproductiva (Sullivan et al. 1995; Dugatkin 2013).

En vertebrados, los sistemas de apareamiento se definen según la proporción sexual operativa (número de machos maduros/números de hembras receptivas) y los niveles de poligamia (Emlen & Oring 1977). Estos últimos, se presentan teniendo en cuenta el grado de monopolización del sexo limitante (mayormente hembras), o de los recursos involucrados en la reproducción, por parte del sexo no limitante (mayormente machos) (Emlen & Oring 1977). La monopolización dependerá del potencial

ambiental para la poligamia, lo cual hace referencia a que tan concentrados estén las hembras o los recursos en el espacio y tiempo (Emlen & Oring 1977; Sullivan et al. 1995; Shuster 2009). Cuando las hembras y/o recursos se concentran en lugares específicos, y además las hembras receptivas se presentan asincrónicamente, es más probable para una especie ser polígama (Emlen & Oring 1977). Así mismo, la calidad de los recursos monopolizados, influye en gran medida sobre los niveles de poligamia (Verner, 1964; Verner & Willson 1966; Orians, 1969; Emlen & Oring 1977). Como es el caso del umbral de poliginia (Verner & Willson 1966), donde las hembras eligen convertirse en hembras secundarias de un macho polígamo con un territorio de alta calidad, en lugar de la pareja de un macho monógamo con territorio de baja calidad (Verner & Willson 1966; Orians 1969).

Los sistemas de apareamiento son el marco referencial donde selección sexual opera (Darwin 1874; Emerson 1994; Shuster & Wade 2003; Shuster 2009; Klug 2011). A pesar de esto, los sistemas de apareamiento por sí solos no pueden predecir en su totalidad los patrones generados por selección sexual (ej. dimorfismo sexual) (Clutton-Brock 1983; Wells 2007; Fairbairn, et al. 2007), se hace necesaria información sobre las características fenotípicas sujetas a selección sexual, influyentes en el éxito reproductivo de ambos sexos (Clutton-Brock 1983). Selección es una de las fuerzas evolutivas más poderosas (Shuster 2009; Futuyma & Kirkpatrick 2017). Específicamente, selección sexual promueve la evolución acelerada de caracteres fenotípicos que hacen que un individuo sea más exitoso que otro a la hora de conseguir pareja (Shuster 2009; Hoquet 2015; Futuyma & Kirkpatrick 2017). Este éxito reproductivo puede ser mediado por selección intra-sexual (competencia macho-macho) o selección inter-sexual (escogencia de las hembras) (Andersson & Iwasa 1996; Hoquet 2015), a través de las cuales se promueve la selección direccionada de características fenotípicas adaptativas al contexto reproductivo de cada especie (Shuster 2009). El resultado de este tipo selección, está fuertemente influenciado por la proporción sexual operativa y la competencia macho-macho (Emlen &

Oring 1977). En especies monógamas, dado que existen pocas oportunidades para que selección favorezca la adaptación sexual, se espera un conflicto sexual nulo y que los sexos muestren poco o ningún dimorfismo (Sullivan et al. 1995; Shuster & Wade 2003). Por el contrario, en especies polígamias, debido a que los machos y las hembras se aparean más de una vez, se espera un incremento considerable en la varianza del éxito reproductivo entre individuos y mayor conflicto sexual dentro de este sistema de apareamiento, promoviendo a su vez, una mayor variedad de comportamientos reproductivos y dimorfismo sexual (Sullivan et al. 1995; Shuster & Wade 2003). Regularmente, ocurre mayor varianza en los sucesos reproductivos de los machos, lo que ofrece una mayor oportunidad para que selección sexual opere sobre ellos (Bateson 1983; Futuyma & Kirkpatrick 2017), generando patrones notorios de dimorfismo sexual en este sexo (Rensch 1950; Fairbairn et al. 2007; Berns 2013). En especies polígamias, una competencia macho-macho más intensa, generará un patrón de dimorfismo sexual más marcado (Fairbairn et al. 2007; Dugatkin 2013; Futuyma & Kirkpatrick 2017).

DIMORFISMO SEXUAL

Dimorfismo sexual, es resultado de procesos evolutivos que han llevado a machos y hembras de la misma especie, a diferir en algunas características morfológicas, ecológicas o comportamentales (Slatkin 1984; Hedrick & Temeles 1989; Fairbairn et al. 2007; Berns 2013). Selección sexual es una de las principales causas de los patrones de dimorfismo sexual (Darwin 1874; Hedrick & Temeles 1989; Andersson 1994; Andersson & Simmons 2006; Berns 2013; Hoquet 2015). Fundamentalmente, selección intra e inter-sexual son los procesos por el cual estos patrones se manifiestan (Hedrick & Temeles 1989; Berns 2013; Hoquet 2015). Selección intra-sexual compromete a individuos del mismo sexo, mayormente por medio de la competencia macho-macho, donde el ganador monopoliza el sexo

opuesto o recursos biológicos necesarios para este (Andersson & Iwasa, 1996; Vitt & Caldwell 2013; Hoquet 2015). En consecuencia, selección sexual favorecerá individuos con características fenotípicas que superen a sus rivales en combates físicos o de resistencia (Hedrick & Temeles 1989; Andersson & Iwasa, 1996). En contraste, selección inter-sexual compromete los dos性os, siendo uno el que escoge características fenotípicas o genotípicas del sexo opuesto (Hedrick & Temeles 1989; Andersson & Iwasa, 1996; Andersson & Simmons 2006; Hoquet 2015). Las hembras debido a su mayor inversión en gametos son consideradas el sexo que escoge (Trivers 1972; Andersson & Simmons 2006), mediante sus preferencias, selecciona características de los machos o de los recursos que ellos proveen, que pueden ser beneficiosos para su descendencia (Bateson 1983; Andersson 1994; Andersson & Iwasa, 1996; Andersson & Simmons 2006). En este caso, selección sexual favorecerá machos con características fenotípicas conspicuas, preferidas por el sexo opuesto, o que ayuden a la monopolización de recursos preferidos por las hembras (Bateson 1983; Andersson 1994; Hoquet 2015). Como consecuencia de estos procesos, divergencias morfológicas notables entre machos y hembras dan origen al dimorfismo mediante selección sexual (Hedrick & Temeles 1989; Andersson 1994; Andersson & Simmons 2006; Berns 2013). No obstante, procesos ecológicos también podrían influir en los patrones de dimorfismo sexual (Slatkin 1984; Hedrick & Temeles 1989; Arnold & Duvall 1994; Berns 2013).

Dimorfismo sexual puede presentarse como diferencias estadísticamente significativas en talla de los sexos (Lovich & Gibbons 1992; Fairbairn et al. 2007). La magnitud del dimorfismo sexual en talla, es más pronunciada cuando los machos de una especie son más grandes, y tiende hacer menor cuando el patrón es inverso (Rensch 1950; Fairbairn et al. 2007). Este esquema en la magnitud de dimorfismo sexual en talla, está presente en muchos grupos de vertebrados e invertebrados, conocido como la regla Rensch (Fairbairn et al. 2007; Berns 2013). La mayor talla corporal de los machos está

relacionada positivamente con los sucesos reproductivos (número de pajeras reproductivas en una temporada), como resultado de selección sexual (Andersson & Iwasa 1996; Fairbairn et al. 2007; Hoquet 2015). Mientras que en especies donde las hembras son más grandes que los machos, generalmente es relacionado con mayor fecundidad y mayor supervivencia de la descendencia (hipótesis de fertilidad), atribuido al resultado de selección natural (Fairbairn et al. 2007; Hoquet 2015), o debido apareamientos asociativos basados en talla por selección sexual (Lu et al. 2010; Hase & Shimada 2014; Chajma & Vojar, 2016) Por otra parte, dimorfismo sexual puede presentarse en características fenotípicas (forma, color, apéndices, etc.) que están involucradas en el éxito reproductivo y son sujetas a selección sexual, llamados caracteres sexuales secundarios (Darwin 1874; Shine 1979; Hoquet 2015), los cuales reflejan diferencias reconocibles entre machos y hembras dentro de una especie. A menudo, las diferencias entre tallas y morfología de ambos sexos son causadas por los efectos sinérgicos de selección sexual y natural (Hedrick & Temeles 1989; Fairbairn et al. 2007; Berns 2013; Vitt & Caldwell 2013).

SISTEMAS DE APAREAMIENTO EN ANUROS

Los anfibios son polígamlos (Wells 1977; Heatwole & Sullivan 1995; Wells 2007), con escasas excepciones de especies monógamas (Brown et al. 2010). Sus sistemas de apareamiento son definidos según niveles de poligamia y competencia macho-macho (Emlen & Oring 1977; Wells 1977; Heatwole & Sullivan 1995; Wells 2007). Para anfibios anuros, Wells (1977, 2007) propone la clasificación de tres categorías de estrategias de apareamiento; 1) tipo “scramble” (competencia por el amplexo); 2) tipo leks (competencia en coros); y 3) tipo defensa de recursos (competencia territorial). Cabe aclarar que entre estas categorías puede existir plasticidad, dependiendo de la duración de la temporada reproductiva (explosiva o prolongada) (Wells 1977; 2007). Cada categoría de sistema de

apareamiento en anuros, presentará una competencia macho-macho dominante, y selección sexual operará favoreciendo características fenotípicas de los machos, acorde al contexto comportamental del apareamiento, generando así, patrones de dimorfismo sexual dentro de cada sistema (Arak 1983; Emerson 1994; Duellman & Trueb 1994; Wells 2007).

El sistema de apareamiento tipo “scramble” (Wells 1977; 2007), se presenta mayormente en especies de anuros con temporadas reproductivas cortas o explosivas (Duellman & Trueb 1994; Wells 2007). Los machos acuden a los lugares reproductivos en altas densidades, buscando activamente a las hembras, lo que produce una competencia macho-macho intensa por interferencia (Duellman & Trueb 1994), con el objetivo de monopolizar el amplexo. Este tipo de sistema genera patrones de dimorfismo sexual marcados, donde machos con mayor talla o extremidades hipertrofiadas con callosidades nupciales que ayuden a sujetar a la hembra durante el amplexo, podrían tener mayor éxito reproductivo (Davies and Halliday 1977; Lee & Corrales 2002).

El sistema de apareamiento tipo leks, es el mayoritario entre anuros (Wells 1977; 2007), se presenta en especies con temporada reproductiva corta o prolongada, donde los machos se congregan en coros y despliegan a través de una competencia macho-macho intensa, sus cantos de advertencia, y solo defienden su sitio de despliegue temporalmente (Duellman & Trueb 1994; Wells 2007). En este sistema, las hembras tienen mayor oportunidad de escoger características fenotípicas de los machos (Emlen & Oring 1977; Gerhardt 1982; Arak 1983; Heatwole & Sullivan 1995; Wells 2007), que generalmente están relacionadas con rasgos espectrales y/o temporales de su llamado de advertencia, que pueden estar correlacionados a mayores tallas corporales (Gerhardt 1982; Sullivan & Hinshaw 1992; Wells 2007).

El sistema de apareamiento tipo defensa de recursos (Wells 1977; 2007), se presenta en especies de reproducción prolongada, donde los machos defienden agresivamente una porción del rango de hogar, monopolizando recursos valiosos para la reproducción, ya sean sitios de ovoposición, recursos alimenticios, refugio ante predadores, entre otros (Duellman & Trueb 1994; Wells 2007). La edad y la talla de los machos son factores fundamentales a la hora de ocupar y mantener exitosamente un territorio (Duellman & Trueb 1994), igualmente se pueden desarrollar caracteres secundarios ventajosos en la confrontación entre machos (ej. colmillos, punzones en las extremidades) (Wells 2007).

DIMORFISMO SEXUAL EN ANUROS

Dimorfismo sexual está presente en todos los linajes de anfibios (Duellman & Trueb 1994; Kupfer 2007). En anfibios anuros, el 90% de las especies presenta un patrón de dimorfismo donde las hembras son más grandes que los machos, el patrón inverso está presente en pocos linajes (Shine 1979; Kupfer 2007). Aunque la explicación generalizada para este patrón es la hipótesis de fertilidad (Shine 1979; Woolbright 1983; Vitt & Caldwell 2013), otros factores podrían estar involucrados, afectando la talla corporal en machos, como por ejemplo, la alta demanda energética asociada a la reproducción (Woolbright 1983), o altas tasas de depredación en machos impactando negativamente en su longevidad y crecimiento (Shine 1979), o diferencias entre nacimientos de machos y hembras, afectando la estructura de edades entre sexos dentro de una población (Monnet & Cherry 2002). Por otra parte, dimorfismo sexual en anuros se manifiesta frecuentemente en caracteres sexuales secundarios (Shine 1979; Duellman & Trueb 1994; Wells 2007), tales como diferencias en morfología de las extremidades, principalmente en patas anteriores, y/o estructuras tegumentarias como las excrecencias nupciales (ej. callosidades nupciales), espinas pectorales, entre otras (Shine 1979;

Duellman & Trueb 1994; Emerson 1994; Hoffman & Bloin 2000; Lee 2001; Fabrezi & Emerson 2003; Wells 2007; Greene & Funk 2009). En muchos casos, la funcionalidad de estas estructuras y del mismo dimorfismo sexual en anuros no es bien entendida (Wells 2007).

Selección intra-sexual a través de la competencia macho-macho provee un marco referencial para estudiar la funcionalidad del dimorfismo sexual en talla y caracteres secundarios en anuros (Davis & Halliday 1979; Arak 1983; Andersson 1994). La competencia macho–macho afecta los sucesos reproductivos, y esta depende de factores intrínsecos dentro de cada sistema de apareamiento de los anuros, donde selección sexual influye sobre las características fenotípicas de los machos (Arak 1983; Emerson 1994; Duellman & Trueb 1994; Wells 2007). Debido a que, diferentes características son probablemente favorecidas dependiendo si los machos compiten directamente por la hembra (competencia “scramble”), defienden recursos atractivos para estas (territorialidad) o llaman en coros para atraerlas (Leks) (Wells 2007).

Estos factores que impactan relativamente en el éxito reproductivo de los machos incluyen, el tiempo invertido y/o sus niveles de actividad en sitios reproductivos (Davis & Halliday 1979; Greer & Wells 1980; Godwin & Roble 1983; Sullivan & Hinshaw 1992; Basto-Riasco et al. 2017); la habilidad de obtener sitios adecuados para emitir llamados (Greer & Wells 1980; Wells & Schwartz 1982; Narins & Hurley 1982); la persistencia, complejidad, duración, o frecuencia dominante del canto (Gerhardt 1982; Rand & Ryan 1981; Ryan 1983; Gerhardt 1991; Tárano & Herrera 2003; Amézquita et al. 2005); la calidad del territorio (Howard 1978; Wells 1977; Roithmair 1992; Candolin & Voigt 2001; da Rocha et al. 2018), entre otros (Wells 2007). Por ejemplo, para muchas especies de anuros con reproducción explosiva, la competencia macho–macho es directamente por la monopolización de la hembra, a través de competición “scramble” (Emlen & Oring 1977; Wells 1977; Davis & Halliday 1979), donde la

habilidad de desplazar a otros machos o de evitar ser desplazado del amplexo aumenta las probabilidades de éxito reproductivo (Davies & Halliday 1977; Davis & Halliday 1979; Wells 1977; Howard & Kluge 1985; Duellman & Trueb 1994; Lee 2001; Lee & Corrales 2002; Wells 2007).

EL GÉNERO *ATELOPUS* EN COLOMBIA Y LA SIERRA NEVADA DE SANTA MARTA

Atelopus (Bufonidae) es un clado de anuros altamente especioso del neotrópico (Lötters 1996; Rueda-Almonacid et al. 2005; Frost 2018). Tradicionalmente, las especies de este género han sido asociadas taxonómicamente a grupos morfológicos no-filogenéticos (*longirostris* y *ignescens*) (Peters, 1973; Lynch, 1993). El grupo fenético *longirostris*, se caracteriza por poseer cuerpos estilizados y extremidades largas y delgadas (Peters, 1973). Mientras que especies del grupo fenético *ignescens*, poseen cuerpos y extremidades robustas (Peters, 1973). Peters (1973) hace amplia claridad sobre su propuesta de grupos fenéticos más que grupos filogenéticos. En contraste, Lynch (1993) reconoce como monofilético el grupo *flavescens* (extraído como un subgrupo de *longirostris*), debido a que algunas especies presentan una sinapomorfía en su fórmula manual, ligada a la reducción de las falanges en el primer dedo (pulgar). No obstante, esta sinapomorfía ha evolucionado independientemente en varios linajes (Lötters et al., 2011).

El género *Atelopus*, fue recuperado como monofilético a partir de evidencia molecular por Lötters et al., (2011), en uno de los estudios más completos de sistemática del género hasta la fecha (Coloma, L. A. 1997; Lötters, 2003; Noonan & Gaucher 2005; Guayasamin et al., 2010; Lötters et al., 2011). En concordancia con Peters (1973), los análisis moleculares, en los cuales incluyeron especies guyanenses, amazónicas, ecuatorianas, panameñas y una colombiana, revelaron la no monofilia de los grupos taxonómicos tradicionales en *Atelopus* (Lötters et al., 2011). Es decir, que los grupos fenéticos

reflejan convergencias más que una historia evolutiva cercanamente compartida. Adicionalmente, Lötters et al., (2011) revelaron que las especies amazónicas y guyanesas (clado *flavescens-spumarius*) de cuerpos estilizados y pulgar reducido, en la actualidad está compuesto por uno de los clados más diversificados al interior de *Atelopus*. Sin embargo, aún desconocemos la posición filogenética de la mayoría de las especies del género.

A pesar de la alta diversidad intra-genérica (>100 especies) (Frost 2020), *Atelopus* es uno de los grupos de anfibios anuros más amenazados del mundo (La Marca et al. 2005; Lötters 2007). Enfermedades emergentes como la quitridiomicosis, producida por el *Batrachochytrium dendrobatidis* (Bd) (Lips et al. 2008), el cambio climático (Pounds et al., 2006) y la destrucción parcial o total de su hábitat (Tarvin et al., 2014) han llevado al 90% de su diversidad a estar en peligro de extinción (IUCN 2020). *Atelopus* alcanza su mayor número de especies dentro de los límites geográficos colombianos (Lötters 1996; Rueda Almonacid et al., 2005; Frost 2018), 45 de 99 especies descritas están presentes en Colombia (Frost 2020). Se distribuyen desde el nivel del mar hasta más de 4000 m de elevación; habitando las quebradas de las selvas húmedas del choco biogeográfico y el amazonas, hasta los páramos y súper-paramos Andinos (Rueda Almonacid et al. 2005). La mayoría de especies se encuentra en las cordilleras de los Andes colombianos, en bosques húmedos montanos entre los 1000 y 2000 m de elevación (Rueda Almonacid et al. 2005; Frost 2017), siendo la Cordillera Oriental de los Andes colombianos, la zona biogeográfica que ostenta el mayor número de especies descritas (Lynch et al., 1997; Rueda Almonacid et al., 2005; Frost 2017).

Así mismo, la Sierra Nevada de Santa Marta (SNSM), un macizo montañoso costero, de más de 5.700 m de elevación, se encuentra aislado de las cordilleras de los Andes, lo que ha permitido la evolución de una biota única al norte de Colombia (Rangel-Ch, 2012; Le Saout et al., 2013). Este macizo,

representa una isla biogeográfica para los anfibios que la habitan (Lynch et al., 1997), y es uno de los centros de endemismo más importantes del país, con alta diversidad de este tipo de fauna, entre los que se encuentran varias especies de *Atelopus* (Ruiz-Carranza et al. 1994; Lynch et al., 1997; Le Saout et al., 2013). *Atelopus carrikeri* (Ruthven 1916; Coloma, 2002), *A. arsyecue* (Rueda-Almonacid 1994), *A. walkeri* (Rivero 1963), *A. laetissimus* y *A. nahumae* (Ruiz-Carranza et al. 1994) son endémicas de la SNSM y muestran una distribución con reemplazo altitudinal, desde los 1000 hasta los 4800 metros de elevación (Rueda-Almonacid 1994; Rueda-Solano et al. 2016a). Todas las especies de *Atelopus* de la SNSM poseen abundancias relativamente altas (Rueda Solano et al. 2016a; Rueda Solano et al. 2016b). Sin embargo, se encuentran amenazadas de extinción (IUCN 2020), y la presencia del hongo Bd (Flechas et al., 2017) aumenta la vulnerabilidad e incertidumbre sobre el estado de conservación futuro de sus poblaciones.

ESTRATEGIAS DE APAREAMIENTO Y DIMORFISMO SEXUAL EN ATELOPUS

Tradicionalmente, se conoce la competencia macho-macho en *Atelopus*, por especies como *A. cruciger*, *A. varius*, *A. zeteki*, *A. chiriquiensis*, y *A. senex*, donde los machos pueden ser agresivos con sus congéneres. Esta agresividad se manifiesta desplegando cantos, señales visuales e incluso combates físicos (Sexton 1958; Jaslow 1979; Crump 1988; Lindquist & Hetherington 1998; Barrantes-Cartín 1986; Criswell 2008), lo que se ha interpretado como territorialidad (Crump 1988; Lötters 1996; Lindquist & Hetherington 1998; Wells 2007; Criswell 2008). Recientemente ha sido documentada la competencia tipo “lek cercano al scramble” en *Atelopus* (Rocha-Usuga et al. 2017). Los machos de *A. laetissimus* no forman territorios ni presentan agresión a sus congéneres, su reproducción no es necesariamente explosiva, sin embargo, buscan activamente hembras en quebradas de reproducción y la competencia macho-macho se da en forma cantos y/o de bolas reproductivas alrededor de una

hembra (Rocha-Usuga et al. 2017). Las demostraciones de competencia macho-macho en *Atelopus*, ocurren en contextos comportamentales diferentes, es decir en sistemas de apareamiento distintos y por ende deberían impactar de manera distinta en el dimorfismo sexual de las especies. En *Atelopus*, las hembras son más grandes que los machos (Lötters 1996), y la proporción en las extremidades diferencia machos y hembras (Peters 1973); además se ha reportado que en algunas especies la coloración ventral y lateral es diferente entre sexos; igualmente, dimorfismo sexual en *Atelopus* se presenta en caracteres sexuales secundarios, como callosidades nupciales, entre otras estructuras tegumentarias (Lötters 1996). La funcionalidad de las diferentes expresiones de dimorfismo sexual en *Atelopus* es desconocida.

COMPORTAMIENTO DE GUARDIA DE COMPAÑERO EN ATELOPUS

Guardia de compañero es un comportamiento en el que un individuo es monopolizado por otro del sexo opuesto durante la reproducción (Emlen y Oring 1977). Este comportamiento evita que el individuo monopolizado busque parejas adicionales y evita la aproximación de posibles parejas reproductivas. Por lo tanto, asegura el éxito reproductivo del individuo monopolizador (Breed & Moore 2012). El comportamiento de guardia de compañero es similar al comportamiento territorial. Sin embargo, los machos protegen a las hembras directamente, en lugar de los recursos de interés para ellas (Härdling et al. 2004). En el reino animal, se ha reportado guardia de compañero en invertebrados (Conrad y Pritchard 1990; Alcock 1994; Dick y Elwood. 1996; Jormalainen 1998), peces (Yamamoto et al., 1999; Alonso & Warner 2000; Yokoi et al., 2015), reptiles (Olsson & Shine 1998; Cuadrado 2001), aves (Hasselquist & Bensch 1991; Van Rhijn 1991) y mamíferos (Brotherton & Komers 2003). En anfibios, el comportamiento de guardia de compañero es bien conocido en salamandras (Duellman y True 1994; Wells 2007; Deitloff et al. 2014). Sin embargo, hay poco conocimiento sobre este

comportamiento en los anuros, el cual se reporta mediante de un extenso amplexo que puede durar semanas o meses. Como la rana japonesa, *Rana sakuraii*, las parejas en amplexus viajan varias semanas en una migración reproductiva (Miwa 2007).

El género *Atelopus* exhibe un comportamiento de guardia de compañero (“female guarding”; como fue descrito por primera vez por Wells, 1977). Los machos monopolizan a las hembras a través de amplexos prolongados, que pueden durar meses (Wells 1977; 2007; Lötters 1996). Se ha reportado un caso extremo de amplexus prolongado de hasta 125 días para *A. carbonerensis* (*A. oxyrhynchus*; Dole y Durant 1974). La protección de la pareja podría afectar la supervivencia de los individuos de *Atelopus*. Dado que, durante los amplexos prolongados, los machos rara vez o nunca se alimentan (Durant & Dole 1974), y las hembras pueden colapsar al tener que cargar a los machos hasta el desove (Durant & Dole 1974; Lötters 1996; Rocha-Usuga et al.2017). La supresión de la alimentación durante el tiempo de amplexo afecta negativamente la condición corporal del macho, hasta el punto de la desnutrición. Se ha documentado entre un 25 y un 30% de pérdida de masa corporal durante los períodos reproductivos de *A. laetissimus* (Rocha-Usuga et al.2017).

Una alta proporción sexual operativa sesgada hacia los machos y una baja probabilidad de desplazamiento del amplexo parecen estar involucrados en la evolución del comportamiento de guardia de compañero en *Atelopus* (Durant y Dole 1974; Wells 1977; Duellman y Trueb 1994; Lötters 1996; Wells 2007). Wells (2007) planteó la hipótesis de que los amplexos prolongados evolucionaron como una extensión de la competencia macho-macho tipo “scramble”, porque es difícil reemplazar a los machos durante los amplexos. Bajo esta hipótesis, ser el primero en encontrar y monopolizar a una hembra, además de una baja posibilidad de reemplazo de amplexo, habría promovido el comportamiento de guardia de compañero en *Atelopus* (Wells 2007).

CONTEXTUALIZACIÓN DE CAPÍTULOS DOCTORALES

El sistema de apareamiento es el marco donde selección sexual opera. Selección favorecerá la evolución de características fenotípicas que ayuden a los individuos a ser más exitosos al momento de la reproducción. En anfibios-anuros, la disponibilidad espacial y temporal de las hembras hará que selección intra-sexual o inter-sexual predominen dentro de sus estrategias comportamentales de obtención de hembras. La territorialidad, la competición tipo *lek* y *scramble* son un continuo de estrategias comportamentales empleadas por los machos de anuros para adquirir hembras, en las cuales selección sexual actúa sobre las características fenotípicas de manera distinta. Estas tácticas reproductivas han sido documentadas en *Atelopus*, un grupo de sapos bufonidos neo-tropicales altamente amenazado, que además presentan comportamiento de guardia de compañero, manifestándose en amplexos prolongados durante sus períodos reproductivos.

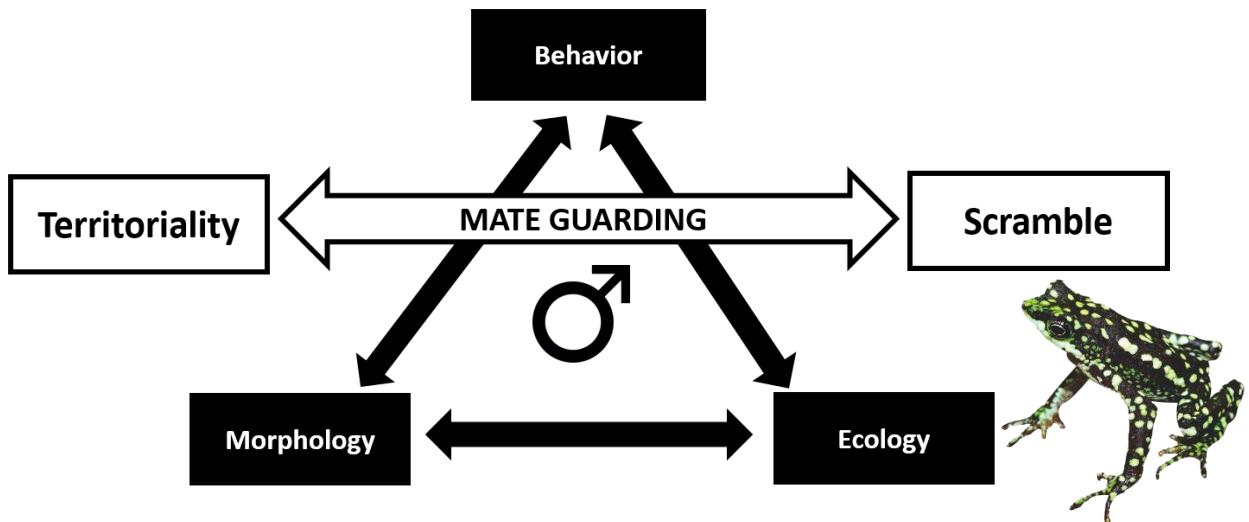
El género *Atelopus* brinda una excelente oportunidad para conocer las implicaciones de estos sistemas de apareamiento y guardia de compañero sobre la evolución fenotípica dentro de un mismo clado de anfibios anuros. Por esta razón, este estudio doctoral pretendió entender las implicaciones evolutivas de rasgos fenotípicos asociados a la reproducción en sapos arlequín del género *Atelopus*, empleando mayormente *Atelopus laetissimus* como especie focal, la cual es endémica y abundante de la Sierra Nevada de Santa Marta. Para esto nos propusimos estudiar primero, la funcionalidad del dimorfismo sexual y los amplexos prolongados en la selección intra-sexual de *Atelopus laetissimus*, una especie con estrategia de apareamiento *lek* cercano al *scramble*. Segundo, documentar la diversidad en señales acústicas de las especies de *Atelopus* y explorar la potencial relación de algunos rasgos morfológicos con parámetros acústicos. Por último, conocer el rol de las estrategias de apareamiento en la evolución del dimorfismo sexual en rasgos morfológicos y como están potencialmente relacionados con variables ambientales y los grupos fenéticos intra-genéricos de *Atelopus*.

El conocimiento de la historia natural de las especies de Atelopus constituye las bases de su conservación, generando una esperanza para este linaje de anuros altamente amenazado.



DOCTORAL GOAL QUESTION.....

WHAT ARE THE IMPLICATIONS OF MATE-GUARDING BEHAVIOR AND
MATING STRATEGIES ON THE EVOLUTION OF MORPHOLOGICAL,
BEHAVIORAL AND ECOLOGICAL TRAITS IN *ATELOPUS*?



**CHAPTER 1: SEXUAL DIMORPHISM AND MALE-MALE COMPETITION
ASSOCIATED WITH THE LEK LIKE SCRAMBLE MATING STRATEGY WITH
FEMALE-GUARDING IN *ATELOPUS* (ANURA: BUFONIDAE).**

MAIN QUESTION CHAPTER 1:

**What are the adaptive morphological traits within the lek-scramble
mating strategy of *Atelopus* with mate-guarding behavior?**



**MATE-GUARDING BEHAVIOR IN ANURANS: INTRA-SEXUAL SELECTION AND THE
EVOLUTION OF PROLONGED AMPLEXUS IN THE HARLEQUIN TOAD *ATELOPUS*
*LAETISSIMUS.***

Luis Alberto Rueda-Solano^{1,2,3}, Fernando Vargas-Salinas⁴, José Luis Pérez-González^{1,3}, Arantxa Sánchez-Ferreira¹, Alejandro Ramírez-Guerra⁵, Carlos A. Navas⁶ & Andrew J. Crawford².

¹*Grupo de Investigación en Biodiversidad y Ecología Aplicada (GIBEA)*, Facultad de Ciencias Básicas, Universidad del Magdalena, Santa Marta, Magdalena, Colombia.

²Department of Biological Sciences, Universidad de los Andes, Bogotá, 111711, Colombia

³*Fundación Atelopus*, Santa Marta, Magdalena, Colombia.

⁴*Grupo de Investigación en Evolución, Ecología y Conservación (EECO)*, Universidad del Quindío, Armenia, Quindío, Colombia.

⁵*Grupo Biociencias Parque Explora*, Corporación Parque Explora, Medellín, Antioquia, Colombia.

⁶Departamento de Fisiología, Instituto de Biociências, Universidade de São Paulo.

Corresponding Author:

Luis Alberto Rueda Solano

Universidad del Magdalena, Santa Marta, Magdalena, 470003, Colombia

Email address: biologoluisrueda@gmail.com

ABSTRACT

The term 'mate guarding' refers to the monopolization of the reproductive potential of a conspecific. Mate guarding has been recorded in invertebrates and vertebrates, but is poorly known in anuran amphibians (frogs and toads), the terrestrial vertebrates with the highest diversity of reproductive modes. Mate guarding in anurans may consist of a prolonged amplexus or copulatory clasp by the male starting well prior to egg laying. In this study we combined field observations and experimental studies of the Santa Marta Harlequin Toad, *Atelopus laetissimus*, to elucidate whether the prolonged amplexus (more than one-month) in *A. laetissimus* evolved as a mate-guarding strategy promoted by intense intra-sexual selection in males engaging in amplexus displacement attempts. First, we asked how the ability of a male to successfully defend its amplexus status against interlopers is related to body condition and 13 other morphological traits. Second, we tested whether the following characteristics were associated with mating success and mate guarding in *A. laetissimus*: clasp force, amplexus duration, and males' reproductive effort. Under natural conditions, amplexant males had significantly higher body condition, were larger, and had wider forearms relative to unmated males. In experimental trials, successful mate guarding in *A. laetissimus* was associated positively with body condition and clasp force, yet the overall probability of successful displacement of amplexant males was low (< 0.20). Our results support the hypothesis that intra-sexual selection in *A. laetissimus* have favored the mating success of males clasping females each time earlier, even before females are ready for breeding. This study contributes to our knowledge of the diverse strategies of mate guarding in vertebrates and the processes underlying them.

Keywords: amplexus displacement, clasp force, female guarding, reproductive effort.

INTRODUCTION

Sexual selection is the evolutionary process arising from competition among individuals for access to mates and their gametes (Darwin, 1874; Andersson, 1994). Given that females invest more in offspring than males in most species, males show higher variance in reproductive success and have a higher potential rate of reproduction than females (Trivers, 1972). In such species, females are not ready to re-mate as quickly as males. As a consequence, the sex ratio is often biased towards males during breeding events, there is more intense competition among males than among females for mates (Andersson, 1994; Jennions & Kokko, 2010, but see Clutton-Brock, 2007). Such competition for mates can happen through two processes termed by Darwin (1874) as intra-sexual and inter-sexual selection. The former process consists of competition between individuals of the same sex, often males, to be conspicuous or monopolize resources that are attractive or necessary to individuals of the opposite sex (Andersson & Iwasa, 1996). The latter process consists of mate selection by individuals of one sex, often females, for individuals of the opposite sex with particular phenotypic traits or monopolized resources (Bateson, 1983; Rosenthal, 2017). Sexual selection includes several non-exclusive mechanisms of competition over mates, which in turn, are associated with traits selected by the competing sex (Andersson & Iwasa, 1996). One such mechanism is mate-guarding (Grafen & Ridley, 1983).

Mate guarding is the behavior in which an individual monopolizes a conspecific of the opposite sex during reproduction in order to prevent that individual from accessing other potential mates, and may occur before or after the guarded individual has mated (Emlen & Oring, 1977). Mate guarding differs from territorial behavior because one sex monopolizes the other directly, instead of monopolizing space or other resources required to breed (Härdling, Kokko & Elwood, 2004). Mate guarding can increase

the reproductive success of the monopolizing individual if the cost of guarding is lower than the benefits (Breed & Moore, 2012). Mate guarding is apparently more commonly practiced by males and has been recorded among invertebrates (Conrad & Pritchard, 1990; Alcock, 1994; Dick & Elwood, 1996; Jormalainen, 1998) and vertebrates (fishes: Yamamoto et al., 1999; Alonso & Warner, 2000; Yokoi et al., 2015; Yokoi et al., 2016; reptiles: Olsson & Shine, 1998; Cuadrado, 2001; birds: Birkhead, 1987; Hasselquist & Bensch, 1991; Van Rhijn, 1991; mammals: Brotherton & Komers, 2003). Among amphibians, mate-guarding behavior is well known in salamanders, but much less known in anurans (Duellman & Trueb, 1994; Deitloff et al., 2014). Studies of mate guarding are necessary in anurans, the terrestrial vertebrates with the highest diversity of reproductive modes, because variation in behavioral ecology among lineages can inform theories for the evolution of mating systems.

Anurans can be classified as either prolonged or explosive breeders (Wells, 1977). Prolonged breeders include species that breed during several weeks or months of the year, thanks to the extended availability of environmental conditions and resources for reproduction. Explosive breeders, on the other hand, are those species whose breeding lasts only one to a few nights because of the brief availability of environmental conditions and resources necessary for reproduction. According to the variability of duration and timing for breeding, there are important interspecific differences in male-male competition and mate-acquisition tactics, ranging from calling while perched in specific sites in the microenvironment to scramble competition (Halliday & Tejedo, 1995; Duellman & Trueb, 1994; Pough et al., 2015). In anurans with explosive breeding, scramble competition happens when the density of males reaches such a high level that they stop calling and begin searching actively for mates (Arak, 1983; Han & Brooks, 2013). In such cases, unmated males may try to remove amplexant males from a female's dorsum, or several males may engage in physical combat for amplexing a single female (i.e., the formation of 'mating balls'; Halliday & Tejedo, 1995; Zamudio & Chan, 2008). In contrast,

prolonged breeders and some explosive breeders tend to have males that call from specific sites that can be actively defended from other conspecific males (Duellman & Trueb, 1994; Pough et al., 2015). In these species, acoustic displays may inform the females about the genetic quality of the signaler (Welch, Semlitsch & Gerhardt, 1998; Jennions & Petrie, 2000; Forsman & Hagman, 2006; Ryan et al., 2019), his body size (McClelland, Wilczynski & Ryan, 1996; Tonini et al., 2020) or even parental care ability (Pettitt, Bourne & Bee, 2020). In both explosive and prolonged breeders, the operative sexual ratio (OSR) in anuran breeding aggregations is frequently biased toward males, which makes male-male competition intense (Halliday & Tejedo, 1995; Shuster & Wade, 2003; Bee, 2016).

In anurans, mate guarding has been associated with males performing prolonged amplexus (i.e., extended copulatory clasp) (Miwa, 2007; Wells, 2007). In most anuran species, amplexus lasts only a few hours or one night, but in a few species amplexus can last for weeks or months (Duellman & Trueb, 1994; Lötters, 1996). Mate guarding in the form of prolonged amplexus may have evolved in response to male-male scramble competition, in species where the breeding season is short and the male has a fair probability of resisting removal by females and by rival amplexant males (Wells, 2007). In such circumstances, being the first male to amplex with a female would be tremendously advantageous, and hence, intra-sexual selection might lead to males initiating amplexus ever earlier, even to the point where the female is not yet ready for reproduction. The few studies on males' probability of successfully displacing a rival male in amplexus come from anurans with scramble competition and suggest that the chances of displacement are relatively high. For instance, in the European Common Toad, *Bufo bufo*, more than one-third of displacement attempts were successful, especially when the unmated male was larger than the amplexant male (Davies & Halliday, 1979; Reading, 2001). In species with scramble competition, intra-sexual selection would favor the reproductive success of larger males with higher body condition (Davies & Halliday, 1977; Davies & Halliday, 1979; Howard & Kluge, 1985) or with

specific traits, such as stronger limbs, that might increase chances for resisting displacement from a female's dorsum by a rival male (Lee & Corrales, 2002; Greene & Funk, 2009).

The Neotropical genus *Atelopus* (Anura: Bufonidae), commonly known as harlequin toads, contains more than 100 species and is considered among the most threatened genera in the world (La Marca et al., 2005; Lötters, 2007; Frost, 2020). Courtship and mating occur in vegetation alongside or away from streams, but eggs are laid in long strings in small streams or pools (Lötters, 1996). These species may provide an excellent model system for testing hypotheses related to the evolution of mate guarding in anurans because amplexus in *Atelopus* can last for weeks or months (Lötters, 1996), e.g., up to 125 days in *A. carbonerensis* (Dole & Durant, 1974). In addition, species of *Atelopus* show sexual size dimorphism (Lötters, 1996) with females being larger than males, but with males exhibiting wider forelimbs than females (Ruiz-Carranza & Osorno-Muñoz, 1994; Peters, 1973), suggesting that male-male competition may be strong in these species (Halliday & Tejedo, 1995; Lee & Corrales, 2002; Greene & Funk, 2009). The Santa Marta Harlequin Toad, *Atelopus laetissimus* (Ruiz-Carranza, Ardila-Robayo & Hernández-Camacho, 1994), is endemic to the Sierra Nevada de Santa Marta, a mountain range in northern Colombia. Amplexus in this species can last more than one month, and adults exhibit sexual dimorphism in body size and other morphological traits (Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017). *Atelopus laetissimus* seems to exhibit a lek-like breeding system (Halliday & Tejedo, 1995; Hoglund & Atalalo, 1995) where males call from ground or understory vegetation located in forested habitat next to or up to 500 m away from streams (Rueda-Solano et al., 2020). Males of *A. laetissimus* seem not to establish territories or otherwise monopolizing resources essential for female reproduction (Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017). When a female approaches the edge of a stream looking for an oviposition site, she is typically already in amplexus since weeks before and the pair is often detected by unmated males (LARS, personal observations), leading to the

formation of mating balls and possible amplexus displacement attempts (Vargas-Salinas, 2006; Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017). Based on our long-term studies of this population, the operative sex ratio (OSR) is biased towards males by approximately 3:1 and the acoustic signal repertory in this species is well known (Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017; Rueda-Solano et al., 2020).

The aim of this study was to test whether the prolonged amplexus in *A. laetissimus* evolved as a mate-guarding strategy promoted, at least partially, by intense intra-sexual selection in males engaging in amplexus-displacement attempts, based on observation of individuals during natural breeding events and on manipulative experiments. We, first, corroborated the degree of sexual dimorphism in body size and forelimbs development in our long-term study population of *A. laetissimus*. Second, we tested whether amplexant versus unmated males differs in body size and maximum forearm width, which are morphological traits relevant to mating success in males of diverse anurans. We hypothesize that larger males with wider forearms exhibit higher mating success than smaller males with thinner forearms. Third, we quantified the maximum clasp force that a male could perform in defense of his amplexant position against unmated intruders, and using manipulative experiments, we quantified the capacity of males to defend their amplexant position against interference attempts by unmated males. We hypothesize that males with wider forearms, which may reflect greater muscle development, show increased strength for amplexus defense. We also hypothesize that the probability of successful amplexus displacement in *A. laetissimus* is null or very low if the amplexant male exhibit a higher or similar body condition than the interfering unmated male. We predict a negative correlation between forelimb size (and higher body condition) and the probability of a male being displaced from their amplexant position. Finally, under ex-situ conditions, we estimated the amplexus duration and the rate of change in body weight of the males during and after the prolonged amplexus. To our knowledge,

this is the first study quantitatively and experimentally testing behaviors associated with the evolution of mate-guarding in anurans.

METHODOLOGY

Study Area

Our study population of the Santa Marta Harlequin Toad, *A. laetissimus*, breeds in Pascual Stream, located in the Serranía de Cebolletas, San Pedro de la Sierra, on western slope of the Sierra Nevada de Santa Marta, Colombia (10.9058 N, -73.9649 W; 2500 m a.s.l., Fig. 1A). This stream crosses conserved and paddock areas with vegetation mostly corresponding to pastures (Poaceae) and small shrubs. Individuals in amplexus are observed mostly in conserved areas of riparian forest (Fig. 1B). Fieldwork was performed during July 2018 and July 2019, the reported time of peak reproductive activity in *A. laetissimus* (Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017). During fieldwork, the average air temperature across both field seasons was 12.93+4.27°C, and the relative humidity was 94.40+47.0% (Data-Logger HOBO U23-002). We search for individuals between 18:00 and 00:00 h, given the nocturnal behavior of *A. laetissimus* (Rueda-Solano et al., 2016). Individuals were uniquely identified based on their pattern of ventral spots, which is a common technique for unambiguously discriminating among individuals in several species of anurans (Donnelly et al., 1994; Ferner, 2010).

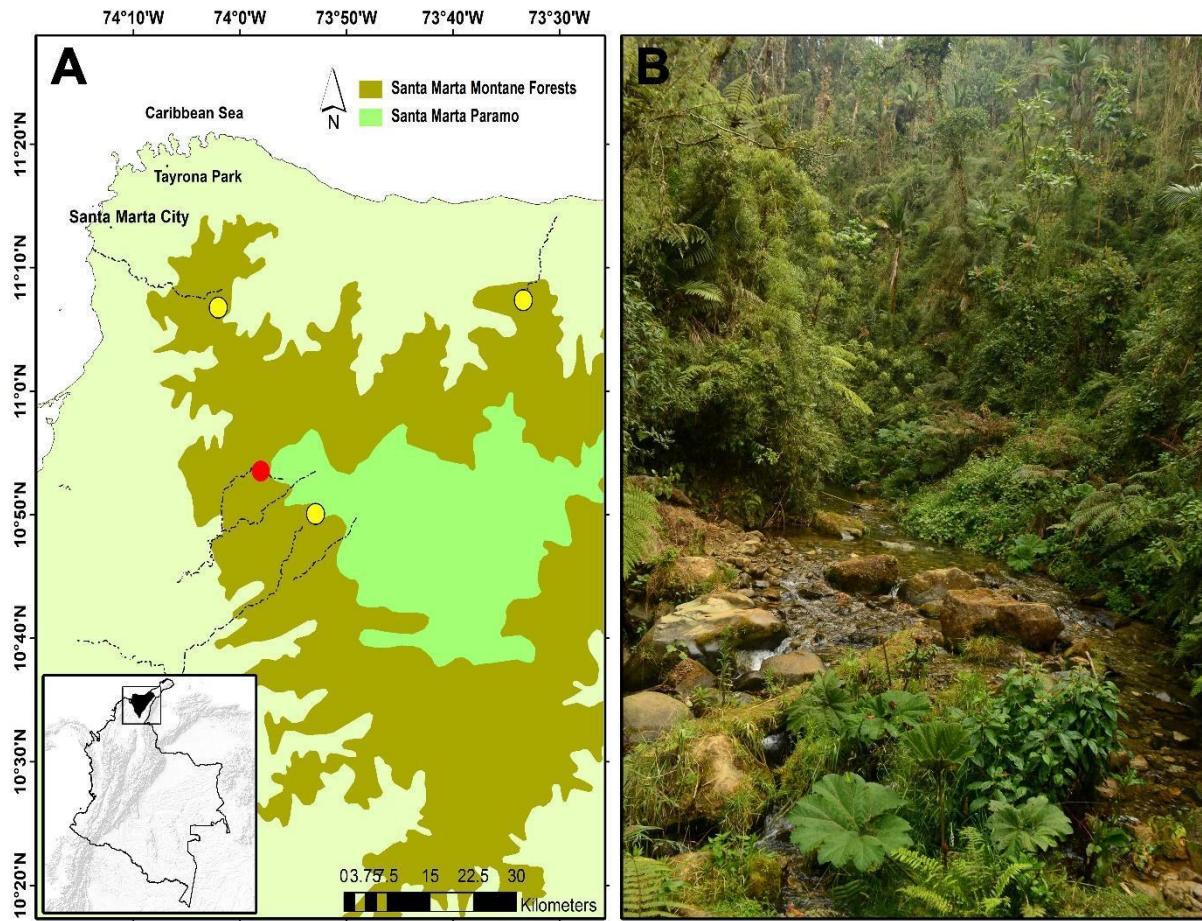


Figure 1. Sierra Nevada de Santa Marta, northern Colombia. The location of the study population of *Atelopus laetissimus* at San Pedro de la Sierra is indicated by a red dot, and additional populations of *A. laetissimus* are indicated by yellow dots (A). The stream Pascual, located in the Serranía de Cebolletas (B), western slope of the Sierra Nevada de Santa Marta.

Sexual Dimorphism and Morphological Traits by Mating Pattern

Morphological characterization of *A. laetissimus* was undertaken to quantify the degree of sexual dimorphism in body size and forelimbs development (Sánchez-Ferreira & Rueda-Solano, 2019). The weight of each frog was recorded with a digital pocket balance (Peso-PPS200, d = 0.02 g; Pesola AG, Switzerland). We photographed each frog using a Sony Cyber-shot DSC-HX400V digital camera, with

an automatic program (P) and flash level to maximum (+2.0); each frog was placed in a standard dorsal position with upper arms directed laterally and at an angle of 90° from the body axis and forearms directed anteriorly. We used a millimeter grid as a reference in each image for distance calibration and subsequent processing. From the above digital images we characterized the morphological variation of *A. laetissimus* based on 13 linear morphometric traits (Greene & Funk, 2009; Fig. 2) using Image J software version 1.50 (Abrámoff *et al.*, 2004). We measured the nutritional condition of each individual using the body condition index (BCI), which consists of the ratio between body weight and body length from snout to vent (SVL) (Stevenson & Woods, 2006). We compared the morphological traits between amplexant and unmated males in order to test their relevance in the mating pattern of *A. laetissimus*. Body size and the maximum forearm width were chosen a priori as potential predictors of mating success, due to their relevance in other anurans (Duellman & Trueb, 1994; Lee, 2001; Lee & Corrales, 2002).

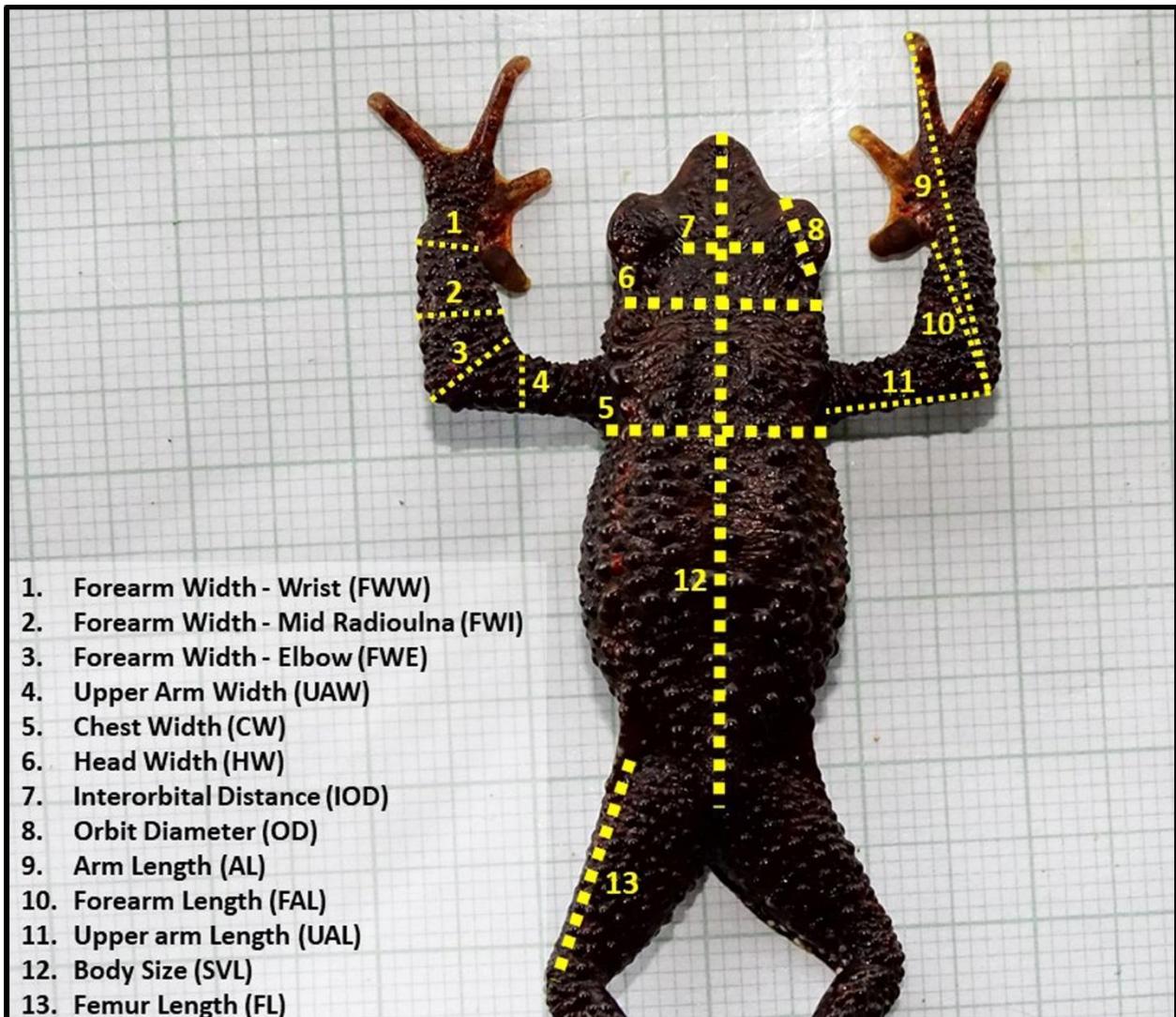


Figure 2. Standard dorsal position for morphological characterization to quantify the degree of sexual dimorphism based on 13 linear morphometric traits of male and female *Atelopus laetissimus*. To account for possible asymmetries, all measures of the appendicular skeleton were taken on left and right sides and averaged.

The following statistical analyses were performed in the statistical computing environment, *R* version 23 for Windows (R Development Core Team, 2012). We performed an analysis of covariance to measure the degree of sexual dimorphism in *A. laetissimus* using each morphological trait (see Fig. 2)

separately as a response variable, sex as a categorical predictor, and body size as a covariate. To account for any bilateral asymmetry, we recorded the mean of left and right values for each morphological trait. Morphological measurements were transformed using log10 to better fit the data to a normal distribution in the response variable, as our linear modeling assumed errors were normal distributed. We compared body size, body weight, and body condition between males and females using a general linear model (lm function; *Bruce & Bruce*, 2017).

Because most of the morphological traits were correlated among each other and with body size, a principal component analysis (PCA) was performed to reduce redundancy. This analysis reduces the dimensionality among variables and generates meta-variables called principal components (PCs). PC1 expressed variation in body size; PC2 was related to body shape (*Zelditch, Swiderski & Sheets*, 2012). For understanding the morphological dimorphism that we hypothesized is related to the strength of the nuptial embrace in *Atelopus laetissimus*, we performed two additional PCs analysis, associated exclusively with the pectoral girdle (forelimbs and chest variables) between sexes and only using the pectoral girdle measurements for males. These PCs were then used as metavariables and regressed to body length (SVL) and body weight. Additionally, they were used in means contrast analyses using a general linear model (lm function; *Bruce & Bruce*, 2017) where the differences between sexes and between amplexant and unmated males were both modeled independently as a function of SVL, body weight, and forelimb thickness.

Finally, we obtained an amplexus probability for males, performing fitness function analyses through binary logistic regressions (glm function; *Bruce & Bruce*, 2017). In these analyses, we performed one logistic regression for each morphological traits, included SVL, body weight, forearm width, and body

condition as explanatory variables that could predict a binary response variable (i.e. whether a male was amplexed or unmated).

Clasp Force During Amplexus

To estimate the maximum clasp force that a male could perform in defense of his amplexus, we used amplexant pairs collected *in situ* as well as amplexant pairs created experimentally by us, putting together in a plastic box one male with one female. Pairs that engaged in amplexus were then used in experiments measuring clasp force. Clasp force experiments consisted of putting a small harness or vest made of Teflon tape (Ptfe Basic RT0030) around the axillary region of the male already in amplexus. This was carefully put on without break the male's nuptial embrace. The tape vest was connected to a spring scale (300G, D=2G, ©Pesola AG, Switzerland), which functioned as a manual pulley system (Fig. 3A), allowing us to pull perpendicular to the frontal plane, *i.e.*, in an opposite direction to the male nuptial embrace (Fig. 3B). The clasp force experiments ended when the amplexant male released the clasped female and the force was recorded (as maximum g reading before static equilibrium was broken). In this way, we quantified how much force was necessary to pull the male off of the female's dorsum. Each male was tested one time to avoid fatiguing. After the experiments were completed, those males collected *in situ* were returned to their original position on the dorsum of their original partner and liberated in the stream site where initially captured.

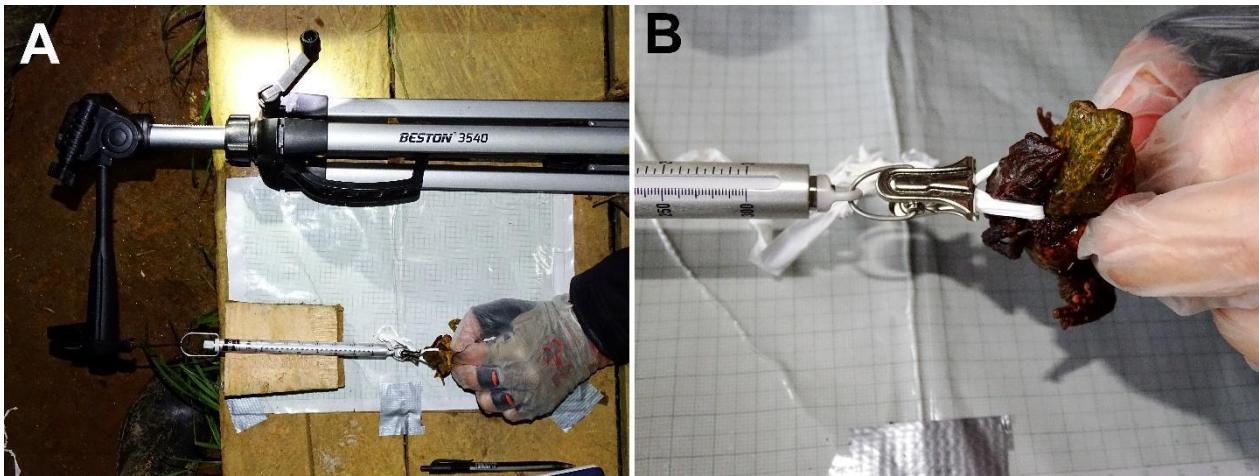


Figure 3. Clasp force experiments in amplexus defense. Manual pulley system (A) allows to pull in an opposite direction and perpendicular to the frontal plane to the male nuptial embrace (B), quantifying the force necessary to pull the male off of the female's dorsum.

We analyzed the clasping force generated during the amplexus defense by males, and compared it between males already amplexed in the wild and those amplexed in experimental containers. The scale measurements in grams (g) obtained in the clasp force experiments were converted to Newtons of force (N). To estimate the extent to which morphology predicts performance, morphology (forearm width at the elbow, SVL, body weight, and body condition) was regressed on clasp force in N exerted during amplexus using multivariate linear regression (`lm` function; *Bruce & Bruce, 2017*). We also performed a covariance analysis (`lm` function; *Bruce & Bruce, 2017*), adding origin of amplexus (in the wild versus in experimentally arranged) as categorical independent variables.

Amplexus Displacement Experiments

We performed 38 amplexus displacement experiments (*Davies & Halliday, 1977; Lamb, 1984*) involving 76 unmated males and 38 unmated females obtained from the study population stream. Sexual maturity was corroborated for males based on the presence of nuptial pads on finger I (aka, 'thumb')

and for females based on the presence of eggs in the abdomen (Peters, 1973; Poole, 2006). Prior to the experiments, individuals were weighed with a pocket digital balance (Peso-PPS200, d = 0.02 g; © Pesola AG, Switzerland), and SVL was measured with a digital caliper (RM 814, d = 0.01 mm; © Black Bull Tools, Guatemala). With these measurements, we calculated the body condition index, BCI (g/cm) of all frogs. With the 38 females we could run 38 trials, and 26 of these were selected randomly for displacement experiments. The males were classified into large versus small body-size groups defined as being either above or below the mean BCI value of 0.93 g/cm ($N= 52$). For each trial, one male from each size class (large and small) was randomly chosen and assigned to an experimental chamber along with one female. Each experimental unit thus consisted of three individuals (two males and one female) in a closed plastic box (14 cm high x 32 cm long x 21 cm wide) covered internally on its sides and bottom with absorbent paper towel, reinforced with industrial tape (duct tape Tesa® 4663). To each box, we added side windows covered with a mesh to allow ventilation, along with a transparent hole in the lid to allow the experimenters to look inside the box without disturbing individuals (Fig. 4). The experiments were carried out 500 meters away from the study stream under local environmental conditions.



Figure 4. Chamber for amplexus displacement experiments. Each experimental unit consisted of three individuals, two males and one female (A). The experiments were carried under local environmental conditions (B).

In the amplexus displacement experiments, two males were randomly selected, and the magnitude of the difference in their BCI was recorded. The pair of rival males was not matched by BCI because we wanted to simulate the natural disparity in body conditions and study its effect on amplexus displacement. Later of acclimation period of around 15 min, a female was put into the box with the pair of males, and we recorded the time elapsed until one male amplexed the female. Once the female was amplexed, we initiated a 24-hour countdown, during which time the unmated male tried to remove the amplexant male from the female's dorsum, and the harassment behavior of the unmated male was monitored. After 24 hours, the trial was ended if the unmated male could not displace the amplexant male and no further signs of pursuing or touching the pair were observed. If the displacement process appeared to be still ongoing, however, we allowed the trial to continue until the unpaired male was successful or appeared to have given up, i.e., the female walked around the box with one amplexant male. The length of the trial was then recorded in hours.

The other 12 of the 38 experiments were assigned to systematic displacement trials. In this type of experiment, we introduced a female along with a male of low body condition (i.e., $BCI < \text{mean BCI value}$) into a box. After these individuals engaged in amplexus, we introduced into the box a second male that had a higher body condition ($BCI > \text{mean BCI value}$). The trial was ended following the same guidelines noted previously (for the 26 randomized displacement experiments), the success or failure of displacement was noted, and the temporal length of the trial recorded.

To estimate the extent to which males with low or high BCI were more likely to engage in amplexus, we used data obtained from the 26 randomized displacement experiments. We compared the percentage of males in each body condition category that amplexed first. We also calculated the probability of successful displacement in the random and systematic experiments by calculating the

ratio of the number of successful displacements over the total number of experiments of each type. In addition, we estimated the probability of displacement for cases where males with the higher BCI were the first to amplex with the female, by selecting a subset ($N = 13$) of trials from the randomized experiments. The 95% confidence intervals of these probabilities were estimated by bootstrap resampling with 1000 replicates. To evaluate whether morphological differences between rival males in arm width, SVL, or body weight were correlated with the probability of amplexus displacement, we used the whole experiments (randomized and systematic) to perform a binary logistic regression model for each morphological trait (glm function in R; *Bruce & Bruce, 2017*).

Amplexus Duration and Male Reproductive Effort

Between July and September 2019, we performed systematic observations of ten *A. laetissimus* housed as five separated pairs that naturally formed amplexus in captivity at *Parque Explora* in Medellín, Colombia. Based on these observations, we estimated the amplexus duration and the rate of change in body weight of males during and after prolonged amplexus.

Each of the five amplexant pairs was placed in its own terrarium (50 cm high, 47.5 cm wide, 84 cm long) that included a dry and wet area, and controlled air (17 °C) and water temperatures (16 °C to 18 °C). We ended our observations if 1) the female oviposited; 2) the amplexus ended naturally prior to oviposition; or 3) because the veterinarians responsible determined that an animal's health was at risk. Amplexant pairs were monitored at least three times per day, to minimize disturbance. To calculate male weight loss during amplexus, each male was weighed twice, once before and once after prolonged amplexus. When amplexus ended, males were fed *ad libitum* with domestic crickets (*Acheta domesticus*) and weighed daily until they reached their original body weight. During this monitoring, males were weighed with a digital balance (Ohaus PA3102 Explorer Precision Balance. 3100g, +

0.01g), and SVL was measured with a digital caliper (Mitutoyo, precision 0.1mm). During these observations females were monitored and fed *ad libitum*.

We obtained the rate of weight loss of males during amplexus by subtracting the body weight in g before amplexus from the weight at the end of the amplexus, and dividing the difference by the amplexus duration in days. We preferred to assume a constant weight loss rate rather than interrupting the amplexus. The weight recovery rate (g/day) was obtained by dividing this same difference in pre-versus post-amplexus body weight of each male by the number of days the male needed to recover its pre-amplexus weight. 95% confidence intervals around these estimates were obtained by bootstrap resampling with replacement over 1000 replicates. Using the mean of our estimated rate of body weight loss under *ex-situ* conditions, we estimated the amplexus duration in *A. laetissimus* under natural conditions based on the body weight loss of males and their reproductive effort (between 25 and 30% of losing body weight during reproduction reported in *Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017*). In this way amplexus duration was estimated from the minimum, maximum, and average body weight loss of males recorded in wild.

Ethical Note

Our study was conducted during the reproductive season, sampling adult frogs that were ready to reproduce. All *in-situ* and *ex-situ* studies and experimental conditions were authorized through permits from the *Corporación Autónoma Regional del Magdalena - Colombia, resolución N° 0425 of 2015*. All research was conducted in compliance with the animal care and use protocols approved by the Ethics Committee of the Universidad de los Andes, Bogotá, Colombia under CICUAL N° C.FUA. 17_006 of 2017. All frogs were returned in healthy condition to the study site, except for some individuals

mentioned in the previous sections, which are being used for a pilot study on captive breeding for an ex-situ conservation program at *Parque Explora*.

RESULTS

Sexual Dimorphism

We found significant inter-sexual differences in all morphological traits based on a sample of 70 males and 23 females (Table 1). Females were on average 1.95 cm larger ($t_{91} = -32.5, P < 0.001$), 10.24 g heavier ($t_{91} = -45.78, P < 0.001$), and with a higher body condition than males ($t_{91} = -37.25, P < 0.001$) (Figs. 5A, 5B). The only body trait that exhibited a higher mean value in males than in females was the width of the mid- radioulna (aka, forearm halfway between hand and elbow) (Table 1). Moreover, this morphological trait was not related with body size in females ($R^2 = 0.016, F_{91} = 0.35, P = 0.56$), but was positively related with body size in males ($R^2 = 0.16, F_{91} = 13.03, P < 0.001$) (Suppl. 1).

Table 1. Morphological trait measurements in cm, mean +SD (Range), for females and males of *Atelopus laetissimus*. Principal components analysis (PCA), using morphological trait measurements between sexes, showing the load of each morphological variable in the meta-variable allometric (PC1) and the meta-variable shape (PC2); and additional PCA, using exclusively the pectoral girdle variables between sexes, showing the load of each morphological variable in the meta-variable arms – chest (PC1) and the meta-variable forelimbs thickness (PC2). SVL= Snout-Vent Length; BCI= Body Condition Index. *Negative value indicates that males had the higher value.

Morphological trait (cm)	Females N = 23	Males N = 70	Diff. between sexes	PC1 (Meta Variable Allometric)	PC2 (Meta Variable Shape)	PC1 (Meta Variable Arms - Chest)	PC2 (Meta Variable Forelimbs Thickness)
Forearm width -wrist	0.423 +0.024 (0.38 – 0.48)	0.344 +0.027 (0.30 – 0.41)	0.078	0.840	0.311	0.844	0.268
Forearm width - mid- radioulna	0.452 +0.035 (0.41 – 0.54)	0.473 +0.041 (0.36 – 0.56)	-0.021*	-0.017	0.934	0.062	0.922
Forearm width - elbow	0.648 +0.060 (0.52 – 0.73)	0.586 +0.041 (0.49 – 0.66)	0.061	0.636	0.628	0.676	0.584
Forearm length	1.174 +0.08 (0.98 – 1.31)	0.955 +0.058 (0.84 – 1.09)	0.218	0.875	-0.110	0.895	-0.191
Arm length	2.941 +0.160 (2.64 – 3.18)	2.185 +0.141 (1.71 – 2.45)	0.755	0.933	-0.149	0.928	-0.222
Upper arm length	1.260 +0.104 (1.06 – 1.43)	1.044 +0.087 (0.84 – 1.22)	0.215	0.775	-0.248	0.805	-0.337
Upper arm width	0.496 +0.031 (0.44 – 0.55)	0.416 +0.027 (0.36 – 0.47)	0.079	0.883	0.133	0.923	0.048
Chest width	1.873 +0.109 (1.75 – 2.21)	1.257 +0.073 (1.07 – 1.41)	0.615	0.943	-0.032	0.911	-0.081
Femur length	2.073 +0.129 (1.80 – 2.27)	1.427 +0.141 (1.13 – 1.73)	0.645	0.880	-0.136	-	-
Head width	1.844 +0.093 (1.61 – 1.93)	1.304 +0.064 (1.17 – 1.44)	0.540	0.961	-0.057	-	-
Orbit diameter	0.680 +0.045 (0.60 – 0.75)	0.516 +0.041 (0.43 – 0.64)	0.163	0.907	0.064	-	-
Interorbital distance	0.64 +0.054 (0.55 – 0.74)	0.451 +0.052 (0.32 – 0.56)	0.188	0.834	-0.105	-	-
SVL (cm)	5.88 +0.295 (5.29 – 6.53)	3.93 +0.206 (3.52 – 4.38)	1.95	0.942	-0.107	-	-
Weight (g)	13.92 +1.328 (10.91 – 15.92)	3.67 +0.431 (2.78 – 4.81)	10.24	-	-	-	-
BCI (g/cm)	2.387 +0.201 (1.91 – 2.71)	0.945 +0.093 (0.70 – 1.15)	1.44	-	-	-	-

There were inter-sexual differences in the allometry meta-variable (variation in body size), such that females were allometrically larger than males ($PC_1 = 72.6\%$; $t_{91} = -27.99$, $P < 0.001$) and body shape tended to be different between females and males ($PC_2 = 10.69\%$; $t_{91} = 1.884$, $P = 0.062$). When we used only the pectoral trait measurements related with nuptial clasp, the meta-variable arms and chest ($PC_1 = 70.95\%$), differed widely between sexes, females these last had larger pectoral measurements ($t_{91} = -19.42$, $P < 0.001$), and chest width had the greatest load (Fig. 4C); however, once the effect of SVL was controlled for, this difference disappeared ($t_{91} = -0.991$, $P = 0.324$). Likewise, the meta-variable forelimb thickness ($PC_2 = 12.85\%$) differed between sexes with forearm width halfway along the radioulna showing the greatest load (Fig. 5C) and males had larger forelimbs thickness ($t_{91} = 2.298$, $P = 0.023$, Fig. 5D). Larger and heavier males had greater forelimb thickness ($R^2 = 0.16$, $F_{57} = 13.55$, $P < 0.001$ and $R^2 = 0.19$, $F_{57} = 14.04$, $P < 0.001$, respectively), but body weight has more predictive power than body size (Fig. 5E). For females there was no significant relationship between body size or body weight and the forelimbs thickness ($R^2 = 0.003$, $F_{21} = 0.074$, $P = 0.788$, and $R^2 = 0.001$, $F_{21} = 0.022$, $P = 0.884$, respectively; Fig. 5E).

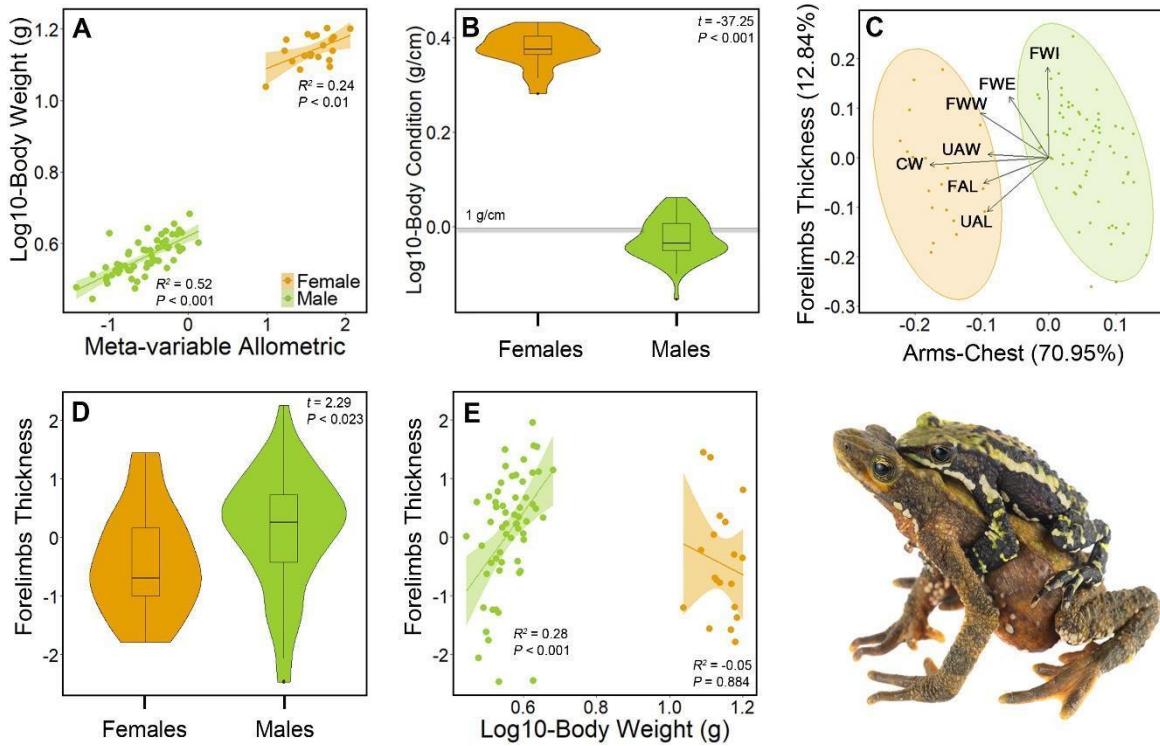


Figure 5. Sexually dimorphic traits in *Atelopus laetissimus*. (A) Relationship between the meta-variable PC1 of combined morphological variables versus weight; (B) difference between body condition in males and females. (C) Meta-variables associated exclusively with the pectoral girdle between sexes: Forearm width at the carpal (FWW), the elbow (FWE), midway along the radioulna (FWI), and forearm length (FAL), Upper arm length (UAL) and width (UAW), and chest width (CW). (D) Difference in forelimbs thickness between sexes. (E) Relationship between the forelimbs thickness (as a meta-variable derived from PC analysis) and the weight of the individuals of *A. laetissimus*. Photo: Amplexus of *Atelopus laetissimus* by Jaime Culebras.

Morphological Traits by Mating Pattern

The mating pattern in *A. laetissimus* was non-random with respect to size of male forearm: under natural conditions, amplexant males exhibit higher forearm width at the carpals (wrist) ($t_{76} = -2.10, P = 0.039, N = 78$) and at the elbow ($t_{76} = -2.53, P = 0.013, N = 78$, Fig. 6A) than unmated males. Standardized width mid-radioulna tends to be larger in amplexant males ($t_{76} = -1.85, P = 0.067, N = 78$). Males in amplexus were larger ($3.95 +0.199$ cm, $N = 29$) than unmated males ($3.80 +0.169$ cm, $N = 49$) ($t_{76} = 3.44, P < 0.001$; Fig 6B). In contrast, the forearm length was longer in unmated males than in amplexant males ($t_{76} = 2.08, P = 0.040$). Amplexant and unmated males were not significantly different in upper arm width ($t_{76} = 0.25, P = 0.803$), upper arm length ($t_{76} = -0.10, P = 0.919$), or in chest width ($t_{76} = 0.17, P = 0.864$). When we only used male pectoral trait measurements, which are associated with nuptial clasp, we found that larger and heavier males exhibit more robust arms and chest than smaller and lighter males ($R^2 = 0.11, F_{76} = 9.62, P = 0.002$; and $R^2 = 0.44, F_{76} = 60.07, P < 0.001, N = 78$; respectively).

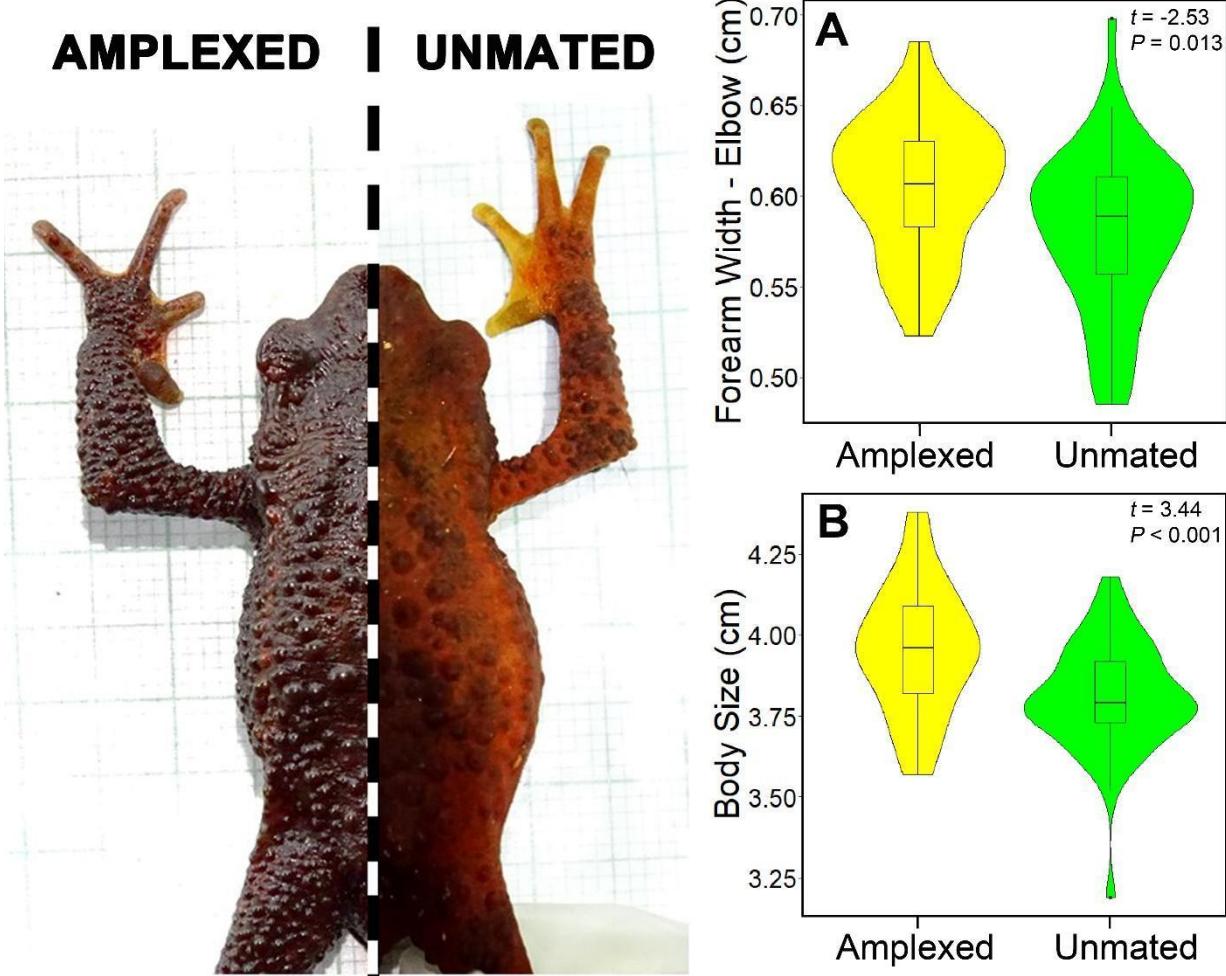


Figure 6. Morphology associated with the mating pattern in *Atelopus laetissimus*. Differences between forearms width at the elbow (A) and body size (B) of amplexed and unmated males of *Atelopus laetissimus*.

Males with larger body size and a wider forearm at the elbow showed a higher probability of engaging in amplexus ($Z_{77} = 3.03, P = 0.002$, and $Z_{77} = 2.36, P = 0.018, N = 78$; respectively; Fig. 7A, 7B). According to the odds-ratio analysis, males in amplexus were up to 4 times more likely to be the male with larger body size (> 4.5 cm) and wider forearm at the elbow (> 0.70 cm). The probability of being

in amplexus was unrelated with body weight or body condition ($Z_{77} = 0.17$, $P = 0.86$, and $Z_{77} = -1.33$, $P = 0.18$, $N = 78$, respectively).

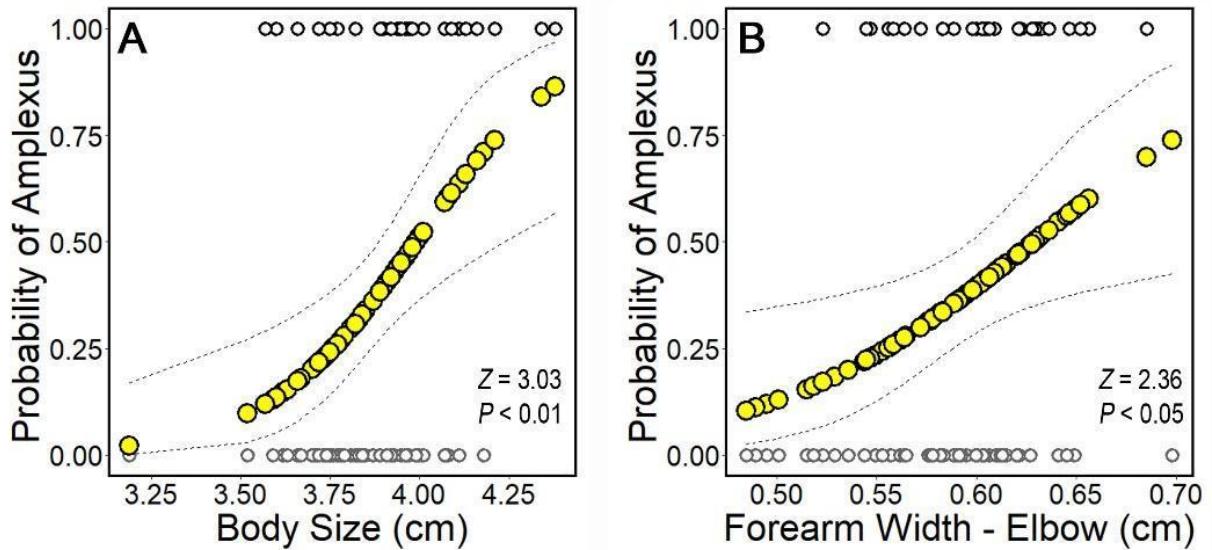


Figure 7. Fitness function of the probability of amplexus and its relationship with the body size (A) and the forearms width at the elbow (B) of the males of *Atelopus laetissimus*. Estimated probability of amplexus (yellow dots) with 95% confident intervals (dashed lines); observed unmated males (gray dots), and observed amplexed males (black dots).

Clasp Force During Amplexus

The maximum recorded clasp force was 29.43 N (equivalent to 300 g of body weight), and the minimum recorded clasp force was 6.87 N (equivalent to 70 g of body weight). The average clasp force during the amplexus defense by males was $18.11 + 5.34$ N ($N = 28$), which is equivalent to 190 g of body weight. In other words, a male generated, on average, around 52 times its body weight in clasp force. Males that began amplexus during our experiments, generated more clasp force ($21.83 + 5.0$ N, $N = 10$) than males already found in amplexus in the stream ($16.05 + 4.41$ N, $N = 18$; $t_{26} = 3.16$, $P = 0.003$; Fig. 8A), which suggests that clasp force weakens as the amplexus duration increases.

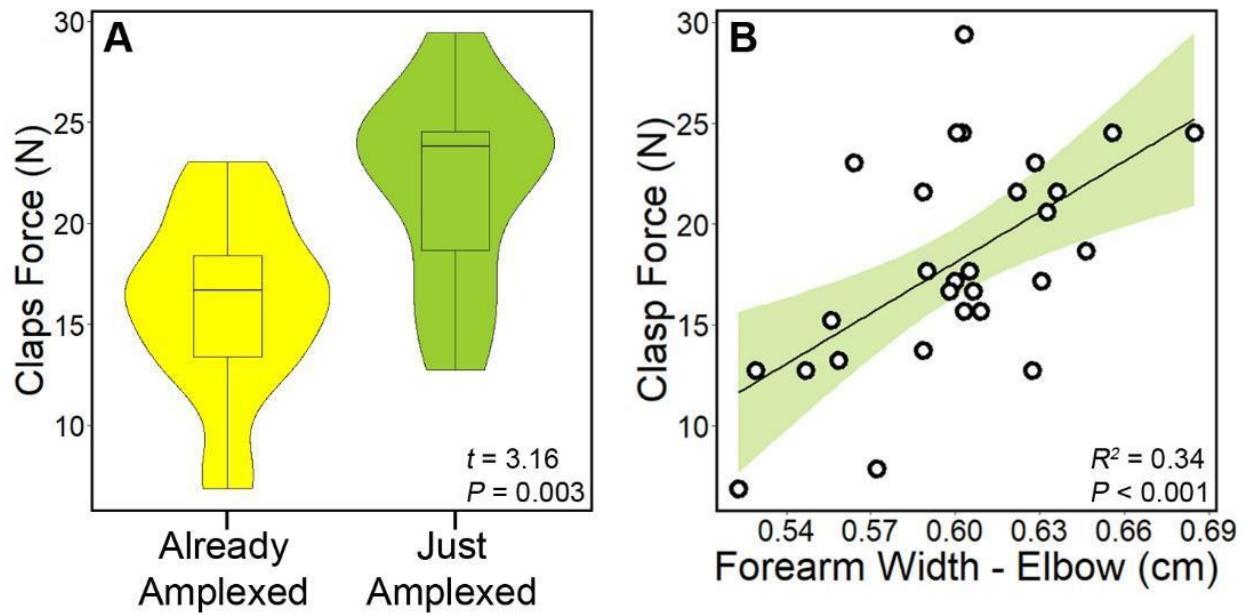


Figure 8. Clasp force differences in *Atelopus laetissimus* between males already amplexed in wild and those just amplexed experimentally (A). Relationship between clasp force and forearm width at the elbow (B) of the males of *A. laetissimus* with 95% confident intervals (green shade).

The wider the forearm (at the elbow), the stronger the clasp force generated by males during the amplexus clasp ($R^2 = 0.34$, $F_{26} = 13.69$, $P < 0.001$, $N = 28$; Fig. 8B); there was a similar tendency between body weight and clasp force ($R^2 = 0.12$, $F_{26} = 3.83$, $P = 0.06$, $N = 28$). However, clasp force was unrelated with body size ($R^2 = 0.05$, $F_{26} = 1.48$, $P = 0.23$, $N = 28$) or body condition of males ($R^2 = 0.09$, $F_{26} = 2.85$, $P = 0.10$, $N = 28$). When we compared males amplexed by us with those found already amplexed, we found that clasp force was unrelated with SVL, body weight, or body condition (Fig. 9A-C). However, the two categories of males differed in the relationship between forearm width (at the elbow) and clasp force generated during amplexus (Fig. 9D); males found *in situ* already amplexed

showed a positive relationship ($R^2 = 0.56$, $F_{16} = 23.53$, $P < 0.001$), while males just amplexed by us showed no significant relationship ($R^2 = 0.19$, $F_8 = 3.14$, $P = 0.11$).

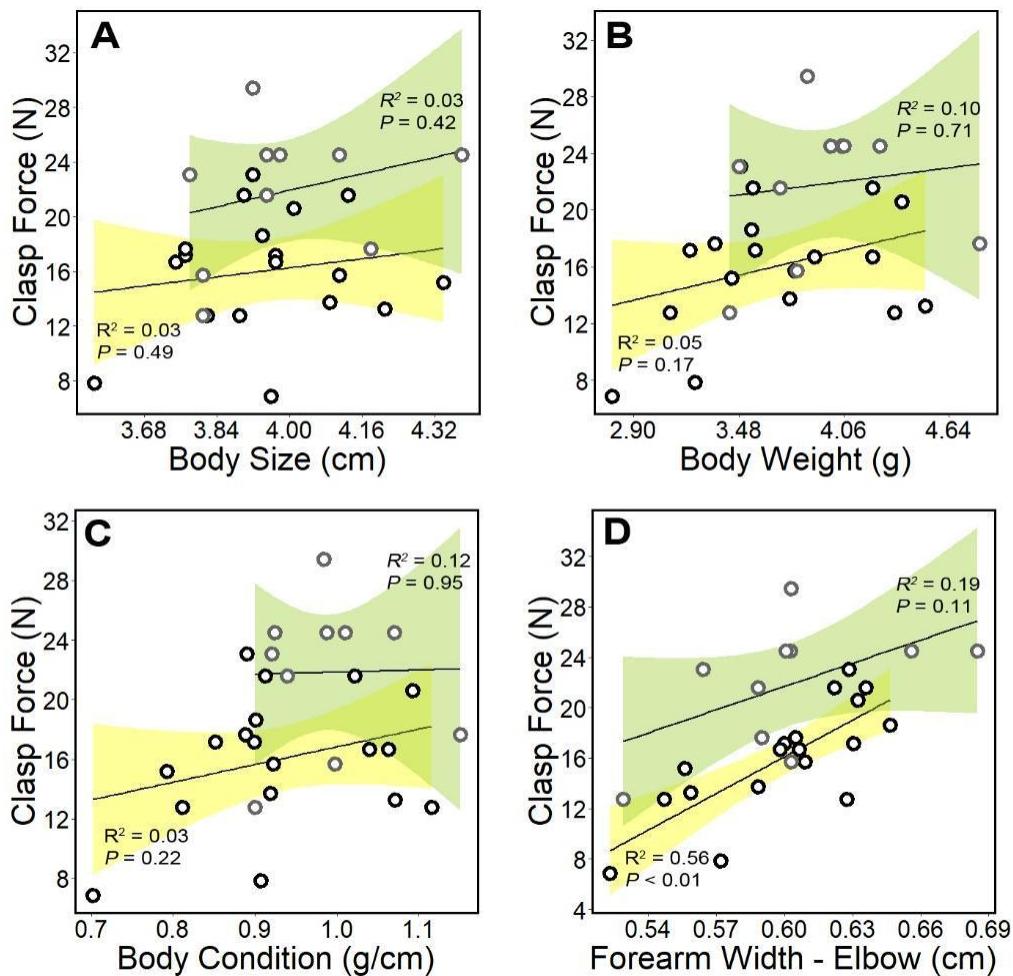


Figure 9. Relationship between clasp force and four morphological traits: body size as snout-vent length (A), body weight (B), body condition index (C), and forearm width at the elbow (D) between already and just amplexed males of *Atelopus laetissimus*. Already amplexed males (black dots) with 95% confident intervals (yellow shade); and just amplexed males (gray dots) with 95% confident intervals (green shade).

Amplexus Displacement Probabilities

In the randomized mating experiment, males with low and high body condition exhibited comparable probability (= 0.54; 95% CI = 0.26 – 0.65) of winning an amplexus. Once the amplexus started, the probability of a successful amplexus displacement was 0.15 (4 of 26 experiments; 95% CI = 0.038 – 0.30). For the sub-group of randomized experiments in which males with the higher body condition (0.901 – 1.150 g/cm) were the first to engage amplexus, the probability of a successful amplexus displacement was 0.076 (1 of 13 experiments; 95% CI = 0.0 – 0.23). Essentially, the probability of a successful amplexus displacement was almost nil when the first male in amplexus has a higher body condition (Fig. 9A). Finally, in the systematic experiments in which males with lower body condition were the first to engage in amplexus, the probability of successful amplexus displacement was 0.33 (4 of 12 experiments; 95% CI = 0.08 – 0.58). Regarding differences between rival males in SVL, body weight, and forearm width at the elbow, we did not find a significant relationship with the probability of successful amplexus displacement (body size: $Z_{37} = -0.403$, $P = 0.687$; body weight: $Z_{37} = 0.675$, $P = 0.499$; forearm width at the elbow: $Z_{37} = 1.177$, $P = 0.239$; $N = 38$).

Successful amplexus displacement processes lasted 5 h – 96 h. (33.62 +28.81 h, $N = 8$). The slowest successful displacement (i.e., 96 h) happened between two males with only a slight difference in body condition ($\Delta\text{BCI} = 0.094$ g/cm, Fig. 10A), while the fastest displacement (i.e., 5 h) was between males with a large difference in body condition ($\Delta\text{BCI} = 0.28$ g/cm; Fig. 10B). During the displacement experiments, we observed that unmated males which tried to remove the amplexant male from the female's dorsum emitted advertisement calls when they stood in front of the amplexant pair; they also made some visual signaling with forelimbs and hindlimbs. Both the male and female in amplexus jointly exhibited defensive behavior against unmated intruder males. Clasped females kicked unmated males

when they approached. The harassment behavior by unmated males was continuous during the whole experiment (Fig. 11, see video in Suppl. 2).

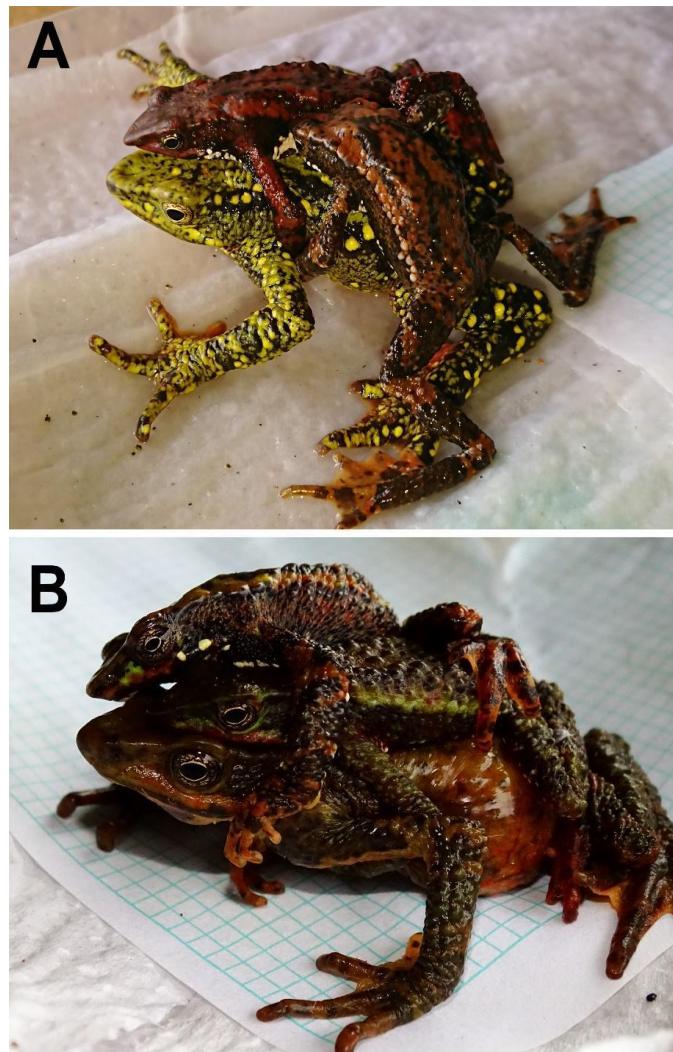


Figure 10. Amplexus displacement processes in *Atelopus laetissimus*. Unsuccessful amplexus displacement when a lower body condition unmated male tried to remove the amplexant higher body condition male from the female's dorsum (A). Successful and faster amplexus displacement processes when higher body condition unmated male had a large difference than an amplexant lower body condition male (B).

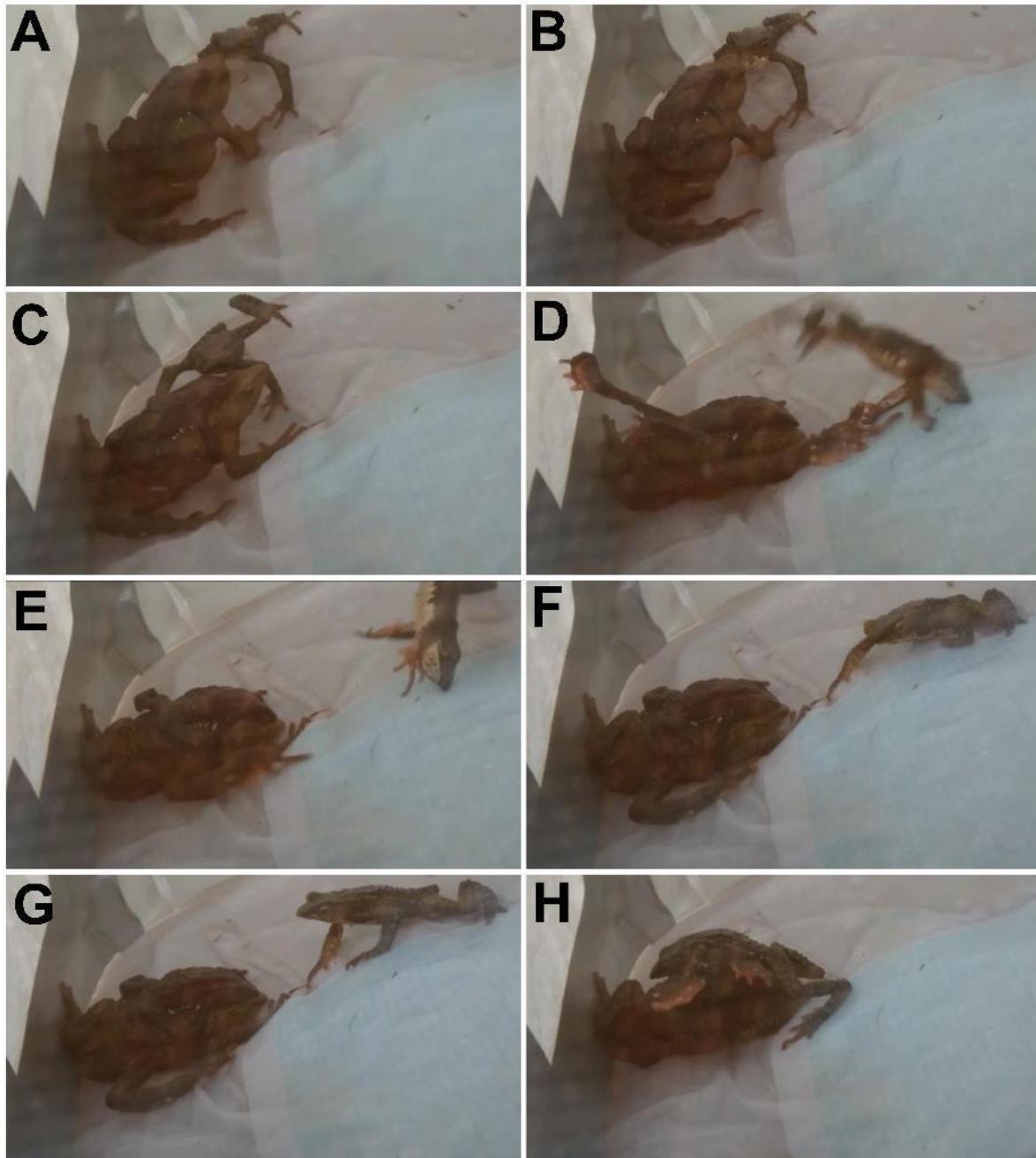


Figure 11. Amplexus displacement experiments of *Atelopus laetissimus*, unmated males tried to remove the amplexant male from the female's dorsum emitted advertisement calls (A-B). Amplexant pair jointly exhibited defensive behavior against unmated intruder males (C-E). The harassment behavior by unmated males was continuous during the whole experiment (F-H).

Amplexus Duration and Male Reproductive Effort

Amplexus in *A. laetissimus* lasted an average of 43 days (for all amplexant pairs, $N= 5$) in *ex-situ* conditions (Fig. 12). All amplexes were suspended by veterinary suggestions. The mean body weight loss in the five males was $0.51+0.38$ g (range= $0.140 – 0.516$ g) and the mean weight loss rate was $0.012+0.009$ g/day ($0.003 – 0.020$ g/day; 95% CI = $0.004 – 0.019$ g/day). Consequently, the reproductive effort by males in terms of loss in body weight varied between 3.11% – 17.83%. Once amplexus ended, these five males recovered their original body weight in only $7.2 +2.29$ days, at a rate of $0.062 +0.02$ g/day ($0.02 – 0.08$ g/day; 95% CI = $0.042 – 0.076$ g/day). Assuming the estimated weight loss rate of 0.012 g/day and applying it as a constant rate, with a reproductive effort of 25% and 30% of male body weight lost during amplexus and the average body weight of 3.6 g ($N = 78$ males) by males in natural conditions, our estimates of amplexus duration in *A. laetissimus* varies between 75 – 90 days, (Table 2). Likewise, assuming a 30% reproductive effort (weight loss), the estimated duration of amplexus would be 120 days for the male with the highest initial body weight (4.8 g) recorded in field, or 138 days for the male with the maximum body weight (5.5 g) recorded in *ex-situ* conditions (Table 2).

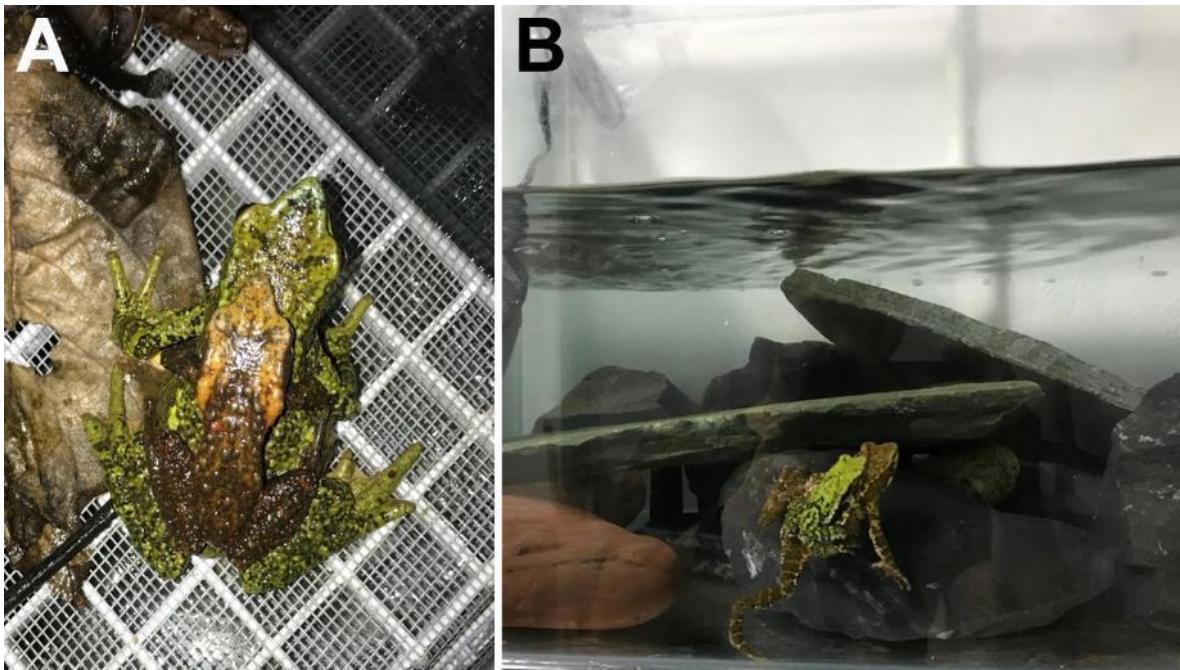


Figure 12. Amplexus of *Atelopus laetissimus* in ex-situ facilities at Parque Explora, Medellín - Colombia. Amplexant pairs during 43 days in dry (A) and wet zone (B).

Table 2. Estimates of amplexus duration of *Atelopus laetissimus*. Assuming between 25% and 30% of the reproductive effort (% reduction in weight during amplexus) for males and a constant rate of weight loss of 0.012 g/day based on the mean body weight loss by five amplexant males during 43 days in captivity. These estimates are shown with the minimum, maximum, and mean of body weight recorded in field. * Maximum body weight recorded in ex-situ experiments.

Male weight at start of amplexus (g)	25% reproductive effort (g)	30% reproductive effort (g)	Constant Weight Loss Rate (g/day)	Estimated days of amplexus with 25% reproductive effort	Estimated months of amplexus with 25% reproductive effort	Estimated days of amplexus with 30% reproductive effort	Estimated months of amplexus with 30% reproductive effort
Min 2.8	0.70	0.84	0.012	58.3	1.9	70	2.3
Mean 3.6	0.90	1.08	0.012	75.0	2.5	90	3.0
Max 4.8	1.20	1.44	0.012	100.0	3.3	120	4.0
Max 5.5*	1.38	1.65	0.012	114.6	3.8	137.5	4.6

DISCUSSION

Prolonged amplexus is expected to evolve in anurans with male-male scramble competition, limited availability of females, and low probability of successful amplexus displacement (Grafen & Ridley, 1983; Wells, 2007). In such species, intra-sexual selection can favor a strong axillary amplexus, and hence, the evolution of traits that promote or reinforce mate-guarding behavior by males (Lee & Corrales, 2002; Carvajal-Castro et al., 2020). Our results with the Santa Marta Harlequin Toad, *A. laetissimus*, agree with these predictions regarding the evolution of traits related to a very low probability of successful amplexus displacement, but expand such predictions to species that exhibit strategies of competition over mates other than scramble (e.g. lek-like systems), and where females are dispersed across the habitat and may exhibit mate choice.

As predicted by Wells (2007) for anurans with prolonged amplexus, the probability of a successful amplexus displacement in *A. laetissimus* was almost none (0.076) when the earlier amplexant male showed better body condition. When the amplexant male had a lower body condition than the unmated male, the probability increased to 0.33. However, this probability remains lower than those reported for other bufonids without prolonged amplexus, e.g., 0.39 in *B. terrestris* under experimental conditions (Lamb, 1984) or 0.40 in *B. bufo* in its natural environment (Davies & Halliday, 1979). Contrary to these latter bufonids, in *A. laetissimus* not all the amplexant males with low body condition were removed from the female's dorsum. In other words, even males with low body condition have a high probability (> 0.6) of defending their amplexant position successfully. This low probability of successful amplexus displacement could explain why amplexus is prolonged in *A. laetissimus* but not in bufonids species of the genus *Bufo* or *Rhinella*. Moreover, unmated males of *A. laetissimus*, when successful, required on average 33 h, even when the amplexant pair could not escape. This estimate is considerably higher

than that reported for *B. bufo* (approx. 7 h; Davies & Halliday, 1977) and *Rhinella marina* (approx. 56 min; Vargas-Salinas, 2005).

Under natural conditions the successful amplexus displacements in *A. laetissimus* is probably even lower than reported here by us, because individuals are dispersed on vegetation, up to 500 m away from the stream margin (Rueda-Solano et al., 2020). Therefore, females can move freely among males and encounters between the amplexant pair and any unmated male would obviously be less frequent than in our experimental boxes. Furthermore, if an encounter happens under natural conditions, females have more opportunities to get away from intruder males and hide among vegetation than under our experimental conditions. This is not necessarily the case for *B. terrestris* and *B. bufo*, and other many bufonids. Males and females of these species congregate around ponds for breeding, and hence, the high density of males promotes scramble behavior where unmated male-amplectant female encounters are frequent (Arak, 1983; Vargas-Salinas, 2007; Bowcock, Brown & Shine, 2013) and there are many more opportunities for successful amplexus displacements than in *A. laetissimus*.

Under natural conditions, larger males of *A. laetissimus* with larger forearms have up to four times more chances of being in amplexus than smaller males. Similar results have been recorded for species of Bufonidae (Reading & Clarke, 1983; Lee, 2001; Lee & Corrales, 2002; Yu & Lu, 2010), Ranidae (Howard & Kluge, 1985; Greene & Funk, 2009), and Hylidae (Lee & Crump, 1981). However, based on our results, we believe that this non-random mating pattern in *A. laetissimus* is not caused mainly by successful amplexus displacements, as happens in *B. bufo* (David & Halliday, 1977). Like other *Atelopus*, some adult males of *A. laetissimus* exhibit high fidelity to specific foraging sites along streams (Granda-Rodríguez et al., 2020). This behavior might be associated with males awaiting the arrival of females during the whole year (Crump, 1986; Himmel, 2013). Based on the positive relationship

between body size and age in anurans (Halliday & Verrell, 1988), larger males in *A. laetissimus* are likely to be older than smaller males, and hence, could have participated in more reproductive seasons and have more expertise than smaller males (in this species males can live more than 14 years, LARS unpublished data). Therefore, larger and more experienced males might decide to arrive earlier to breeding habitats or know better breeding sites than smaller and less experienced males. Such behaviors have been recorded in other bufonids (Gittins, 1983; Crump, 1986; Reading, 2001; Himmel, 2013).

The morphological trait that was larger in males than females (after controlling by differences in body size) was the thickness of the forearm (radioulna), specifically in the central and elbow areas. A similar sexual dimorphism is present in other *Atelopus* species (Peters, 1973). Forearm morphology is associated with a strong and complete nuptial embrace around the pectoral area of females, which is especially important in species with intense male-male competition and frequent amplexus displacement attempts (Davies & Halliday, 1977; Davis & Halliday, 1979; Wells, 1977; Howard & Kluge, 1985; Lee, 1986; Duellman & Trueb, 1994; Lee, 2001; Lee & Corrales, 2002; Wells, 2007; Greene & Funk, 2009). In *A. laetissimus*, the clasping force generated by a male in amplexus could exceed by 50-fold the average of their body weight, up to a maximum of 80-fold. This physical strength during amplexus defense was positively related to the thickness of the male's forearm, and is associated with the ontogenetic development of the *flexor carpi radialis* and other muscles of the forearms (Soliz, Tulli & Abdala, 2020). It would be extremely challenging for an unmated male to overcome this strong amplexus defense. In addition, the forearm thickness in males of *A. laetissimus* was highly related to body weight. Thus, body weight and body condition seem to be very important for mating success in males of this species, as these traits are related to an increased chance of amplexus defense, and possibly endurance, during a prolonged amplexus.

Prolonged amplexus could reduce the survival of males because while clasped they rarely if ever feed (Durant & Dole, 1974; Lötters, 1996). The feeding suppression affects the male's body condition negatively, even until starvation (Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017). However, according to our data, males quickly recover their body weight after a prolonged amplexus, and the rate of body weight recovery exceeded the rate of weight loss, which in turn, is associated with the foraging abilities of each male (Roff, 2002; Rueda-Solano & Warkentin, 2016). Although the quick body weight recovery of males was recorded under experimental condition and *ad libitum* food supply, we expect that also under natural conditions the males have an abundance of prey items. Unlike males, the females of *A. laetissimus* do not lose weight during amplexus because they continue to feed while clasped. Thus, females would not bear a fitness cost associated with starvation if they are clasped too early by a male, and therefore, it would not be an aspect restricting the evolution of prolonged amplexus in this species. However, it is necessary to test why other potential cost associated with being in amplexus for weeks or months (e.g. predation risk, reduction in growth rate) have not restricted the evolution of prolonged amplexus in *Atelopus* and other anurans with similar behavior (Robinson & Doyle, 1985; Ward, 1986; Magnhagen, 1991). Prolonged amplexus has been reported for *B. bufo* (more than one week) only under experimental conditions, that is, without cost associated to predation risk or other factors (Davies & Halliday, 1977). In contrast, prolonged amplexus (more than one month) has been documented under natural conditions in *A. laetissimus* and other *Atelopus* species (Dole & Durant, 1974; Barrantes-Cartín, 1986; Crump, 1988; Lötters, 1996; Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017).

Our estimates of *in-situ* amplexus duration of 70 – 135 days in *A. laetissimus* are close to those reported for some *Atelopus* (125 days in *A. oxyrhynchus*, Dole & Durant, 1974), but longer than reported for *A. cruciger* (19 days, Sexton, 1958) or *A. flavescens* (35 days, Gawor et al., 2012). Understanding in detail

reasons explaining such inter-specific differences in amplexus duration among species of *Atelopus* is beyond the scope of this manuscript. However, differences in ecological interactions and habitat characteristics may play an important role. The temporal asynchrony between amplexus formation and spawning would be particularly appreciable in *Atelopus*, due to its need for pools or stretches with very weak currents within montane streams (Lötters, 1996), as such microhabitats may be difficult for females to find (Karraker, Richards & Ross, 2006). The spawning in *Atelopus* would be particularly delayed in fast-moving streams, like those in mountains and steep topographies, which in turn implies more searching time by females, longer amplexus, and higher reproductive effort by males (Rocha-Usuga, Vargas-Salinas & Rueda-Solano, 2017). Therefore, the difficulty that females face for finding a suitable spawning site may be a factor promoting variability in amplexus duration at the intra-specific level, and possibly also explain variation in such aspect at the inter-specific level.

We are aware of limitations in the present study. Measurement of amplexus displacement probability emphasizes the male phenotype. However, females also can play an important and active role in affecting such probability through diverse behaviors, for example, get turgid for reducing clasping performance by males (Bruning et al., 2010), approaching near unmated males for favoring amplexus take-over attempts (Davies & Halliday, 1979), moving away from water, hide among vegetation and delay oviposition until dawn when unmated males abundance is lower (Vargas-Salinas, 2007), or for mechanical constraint on size-assortative paring success (Lu et al., 2010; Chajma & Vojar, 2016). In *A. laetissimus*, we observed that some amplexant females repel unmated males, which could make a successful amplexus displacement even harder for an unmated male, especially under natural conditions where in addition kicks, the female can retreat. In fact, during experiments we observed that the unmated males that successfully displaced an amplexant male had to overcome first the female defensive behavior, and then the male defensive behavior. Likewise, in other *Atelopus* females offer

resistance to amplexus (Crump, 1988), which also may help them reduce the cost associated with being amplexed early or simultaneously by multiple males (Verrell & McCabe, 1986; Chapman et al., 2003; Menin, Rodrigues & Lima, 2006; Parker, 2006; Bowcock, Brown & Shine, 2009; Izzo et al., 2012).

In addition to intra-sexual selection based on male-male competition, inter-sexual selection through mate choice could also be important in the evolution of extravagant phenotypes in males (Bateson, 1983; Prum, 2017; Rosenthal, 2017) and, of course, could be a driver in the evolution of prolonged amplexus in *A. laetissimus*. Males of *A. laetissimus* have a broad repertory of auditory signals (Rueda-Solano et al., 2020), which suggests they call to attract or court females. Further studies are necessary to test mate choice in this species and how this might relate to the evolution of mate guarding. For instance, females of *A. laetissimus* may choose particular males based on call features, and hence, mate with males that can endure the prolonged amplexus and reduce risk associated with long mating ball formation.

CONCLUSIONS

This study focused on factors that could favor the evolution of prolonged amplexus as a strategy of mate guarding in anurans, using as a model system the Santa Marta Harlequin Toad, *A. laetissimus*. Our field observations and experimental evidence support the role of intra-sexual selection favoring the mating success of males amplexing females earlier and with the capacity to defend their position on the female dorsum. Successful amplexus displacement is very low in *A. laetissimus*, even if the initial amplexant male was small or had a low body condition; hence, it may be adaptive to find and clasp a female early (Härdling, Kokko & Elwood, 2004; Candolin & Voigt, 2003). Possibly a similar evolutionary process happens in other *Atelopus*, especially those distributed in the highlands, which exhibit a high

sexual dimorphism in limb morphology (Peters, 1973). We did not discard the role of inter-sexual selection in the evolution of prolonged amplexus in *A. laetissimus* (Bruning, Phillips & Shine, 2010; Lu et al., 2010; Chajma & Vojar, 2016). When clasped, females physically interfere when an unmated male approaches and hence, lower the chances of a successful amplexus attempt. Moreover, under natural conditions females of *A. laetissimus* can move freely while unmated or while amplexed (LARS personal observations), and hence, they also could be a driver in the evolution of prolonged amplexus in *A. laetissimus* and possibly, in other congeneric species conforming this highly threatened clade of charismatic toads. This study contributes to improving our knowledge of the diverse strategies of mate guarding in vertebrates, and the roll life history characteristics may play in influencing evolutionary processes.

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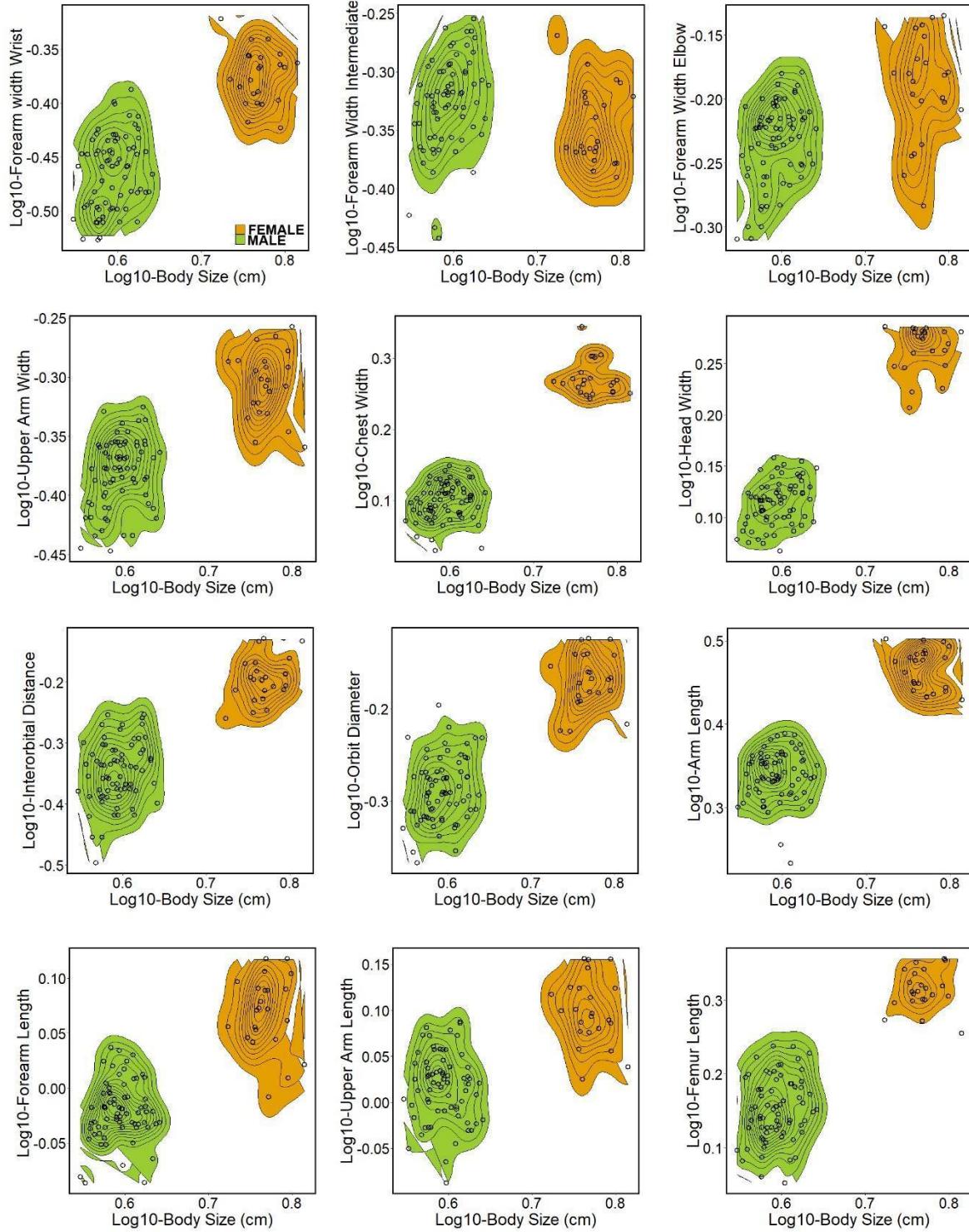
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Supplementary 1. Morphological measurements of male and female *Atelopus laetissimus*. Body size (snout-vent length, SVL); Arm length (AL) was measured from the postaxial elbow to the distal

tip of finger III; Upper arm length (UAL), measured from the point of the armpit to the elbow point; Upper arm width (UAW) was measured by the line of the posterior border of the forearm; Forearm length (FAL) was measured from the elbow to the proximal edge of the nuptial pad; Forearm width (FW) which was measured at three points, at the carpal (aka, 'wrist', FWW), the elbow (FWE), and at the halfway point along the radioulna where the width was greatest (FWI); Femur length (FL) was taken from the groin to the knee; Chest width (CW) was measured from one armpit to the other; Head width (HW) was taken from one extreme to another; Orbit diameter (OD) was taken between the extremes of the orbit; Interorbital distance (IOD) was taken as the minimal distance between the eyes as measured dorsally.

Supplementary 2. Video: Amplexus defensive behavior by male and female of *Atelopus laetissimus*, against harassment unmated male.

CHAPTER 2: ACOUSTIC DIVERSITY AND ITS RELATIONSHIP WITH ADAPTIVE MORPHOLOGICAL TRAITS IN THE LEK LIKE SCRAMBLE MATING STRATEGY OF *ATELOPUS* (ANURA: BUFONIDAE).

MAIN QUESTION CHAPTER 2:

What is the acoustic signal diversity of the genus *Atelopus* and could the advisement call of *Atelopus laetissimus* show a relationship with some adaptive morphological traits of lek-scramble mating strategy?



**SOUNDS RESISTING TO EXTINCTION: ACOUSTIC SIGNAL DIVERSITY IN THE
HARLEQUIN TOAD *ATELOPUS LAETISSIMUS* (ANURA: BUFONIDAE)**

Luis Alberto Rueda-Solano^{1,2,3*}, José Luis Pérez-González^{1,2}, Mauricio Rivera-Correa^{3,4} and
Fernando Vargas-Salinas⁵.

¹Fundación Atelopus, Santa Marta - Colombia

²Grupo de Investigación en Biodiversidad y Ecología Aplicada, Facultad de Ciencias – Básicas,
Universidad del Magdalena, Santa Marta - Colombia

³Biomics, Facultad de Ciencias, Universidad de los Andes, Bogotá - Colombia.

⁴ Semillero de Investigación en Biodiversidad de Anfibios (BIO), Seccional Oriente, Universidad de
Antioquia.

⁵Grupo Herpetológico de Antioquia (GHA), Instituto de Biología, Universidad de Antioquia

⁶Evolución, Ecología y Conservación EECO, Programa de Biología, Facultad de Ciencias Básicas y
Tecnologías, Universidad del Quindío, Armenia, Colombia.

(*) corresponding author: biologoluisrueda@gmail.com

ABSTRACT

The acoustic signals in anurans of the genus *Atelopus* (Bufonidae) have been scarcely reported and described, a neotropical genus currently composed for 97 species. In this study, we describe for the first time the advertisement call, the agonist call, and the release call emitted by males of *Atelopus laetissimus*, an endangered toad species that is endemic to the Sierra Nevada de Santa Marta, a mountain range located in northern Colombia. In addition, we also describe for first time the call produced by a female of the genus. The calling behavior of males was associated to reproductive contexts (advertisement calls), male-male interactions (agonistic and release calls) and possibly in anti-predatory or distress contexts (release calls). The call emitted by the females of *A. laetissimus* was recorded while one male was trying to claps her, hence, it is possible that also being emitted when them are clasped simultaneously by several males. With the acoustic signals described in this study, *Atelopus laetissimus* becomes the species with the most diverse vocal repertoire known so far for the whole genus.

Key words: Acoustic communication, Colombia, Female's call, Anuran Call, Natural History

INTRODUCTION

Anurans use several sensory modalities to transmit information to conspecifics and heterospecifics (Ryan 2001; Starnberger et al. 2013; Rojas 2016). However, the production and perception of acoustic signals can conform the most common sensory modality used by mature individuals looking for a mate and defending a resource (Gerhardt and Huber 2002; Wells 2007). There are eight types of acoustic signals in anurans, being the advertisement call the more conspicuous and studied by biologists. This type of call is emitted only by males and is used for attracting mates and for advertises to other males about the signaler's position; other types of calls are less conspicuous and are differentiated according to the behavioral context in which are emitted, for example, release call and aggressive/agonistic calls (Ryan et al. 1996; Wells 2007; Toledo et al. 2015).

Toads of the genus *Atelopus* Duméril and Bibron 1841, commonly known as Harlequin toads for its striking colorations, is a clade with 97 recognized species, the richest genus of the family (i.e. Bufonidae). The geographical distribution of *Atelopus* goes from sea level to more than 4800 m a.s.l. in Neotropical countries, specifically from Costa Rica to Bolivia (Frost 2019). Reproduction in these toads is associated to streams, where females lay eggs under rocks in small- or medium-sized creeks (Lötters 1996) and with gastromyzophorous tadpoles (Rueda-Solano et al. 2015). Communication in species of *Atelopus* include at least two sensory modalities that commonly are performed simultaneously by males (i.e. bimodal communication); the emission of advertisement calls is commonly accompanied by visual cues consisting in stereotyped movements of the anterior and posterior extremities (Lindquist and Hetherington 1996; Hödl and Amézquita 2001; Criswell 2008).

Acoustic signals in the genus *Atelopus* have been scarcely reported and described; to date, the advertisement call is known only for 17 species (Starrett 1967; Jaslow 1979; Lescure 1981; Cocroft et

al. 1990; Ibañez et al. 1995; Lötters et al. 1999; Coloma et al. 2000; Lötters et al. 2002). Furthermore, to much less is known about other types of acoustic signals for the genus (Lötters et al. 1999; Carvajalino-Fernandez et al. 2017). Increase our knowledge about acoustic signaling in *Atelopus* is important because several non-excluding reasons: call features can be used as diagnostic characters for inferring phylogenetic relationships and delimiting species (Schneider and Sinch 2007; Padial et al. 2008; Köhler et al. 2017; Rivera-Correa et al. 2017); advertisement call features can allow us to identify taxa in automatized acoustic recordings (see Bedoya et al. 2017; Farina 2019), which is especially important for species such as those of the genus *Atelopus*, whose populations have dramatically declined (La Marca et al. 2005; Lötters 2007). And our little knowledge about acoustic communication in *Atelopus* limits our inferences about the natural history and the evolutionary behavioral ecology of those Neotropical anurans (Lindquist and Hetherington 1996; Criswell 2008). Such as, if acoustic displays of these species can inform to the females about the genetic quality of the signaler (Welch, Semlitsch & Gerhardt, 1998; Jennions & Petrie, 2000; Forsman & Hagman, 2006; Ryan et al., 2019), or direct benefits associated with its body size or another morphological trait (McClelland, Wilczynski & Ryan, 1996; Tonini et al., 2020).

Atelopus laetissimus is an endemic species of the Sierra Nevada de Santa Marta (hereafter SNSM), a mountainous enclave located in northern Colombia (Ruiz-Carranza et al. 1994). This species, categorized as endangered (IUCN 2019), and individuals are frequently observed on vegetation alongside small streams in Andean and sub-Andean forests between 1900 to 2800 m a.s.l. (Granda-Rodríguez et al. 2007; Rocha-Usuga et al. 2017; Rueda-Solano pers. obs.). Males of *A. laetissimus* have been observed emitting advertisement calls (Granda-Rodríguez et al. 2007; Pérez-González pers. obs.), and low intensity calls have been reported in males and females during the formation of mating balls in reproductive seasons (Rocha-Usuga et al. 2017). However, the natural history of the

species has been poorly documented and no formal description of those acoustic signals has been previously studied. Here, we describe the acoustic repertoire for males of *Atelopus laetissimus* (i.e. advertisement, agonistic and released call), and we describe the call performed by a female for the first time in the genus *Atelopus*. In addition, we make a comparison of the call features in this species with those reported for other species of the genus. Finally, we examine the potential relationships of advertisement call features, such as, call dominant frequency, call duration and call emission rate with morphological traits of males.

MATERIALS AND METHODS

The population of *Atelopus laetissimus* included in this study is located in the stream Pascual, Serranía de Cebolletas, corregimiento de San Pedro de la Sierra, western slope of the Sierra Nevada de Santa Marta, Colombia (10.905804 N, -73.964919 W; 2500 m a.s.l., Fig. 1A). The stream Pascual crosses conserved and paddocks areas with vegetation mostly corresponding to pastures (Poaceae) and small shrubs (Fig. 1B). Individuals can be found at daytime and nighttime hours in disturbed and conserved areas of riparian vegetation (Fig. 1C).

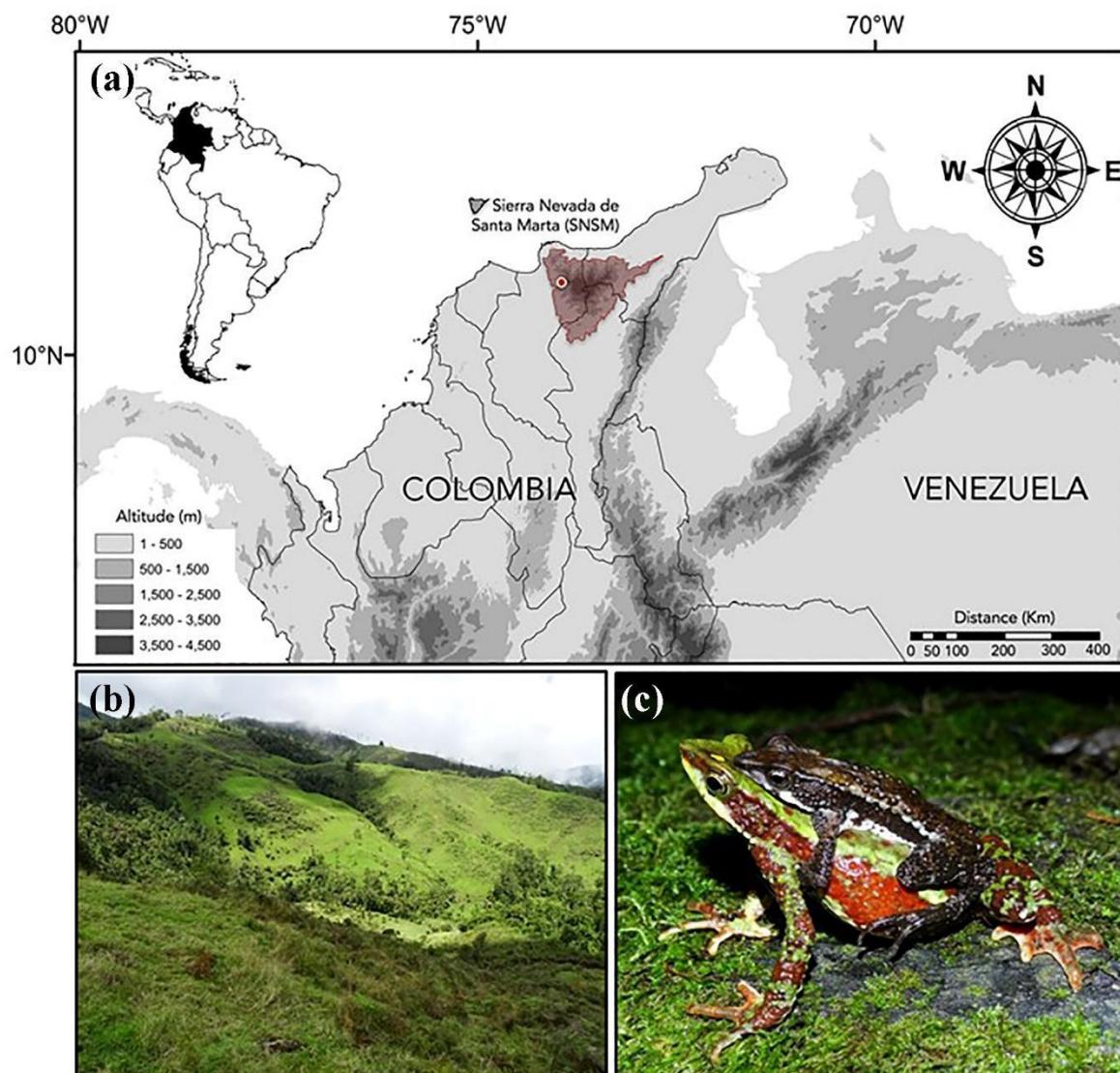


Figure 1. Geographic location of the Sierra Nevada de Santa Marta (SNSM), at north of Colombia, South America, showing the specific location (red dot) of the study area (a). Image of the vegetal coverage in the study area; note the high level of deforestation and the presence of narrow strips of riparian forest (b). Male and female of *Atelopus laetissimus* in amplexus; observe the sexual dimorphism in body size and the polymorphism in coloration between individuals (c).

Data collection

We recorded acoustic signals in one field trip realized between 25 and 28 June 2018, during the performing of reproductive behavior experiments (Rueda-Solano et al. see Chapter 1). Recordings were made with a digital recorder (Tascam DR-22WL) connected to a directional microphone (Sennheiser K6/ME66) at distances between 0.10 – 0.5 m from the signaler. After each recording, we measured the signaler body size (snout–vent length, SVL) with a digital caliper (Mitutoyo, precision 0.1 mm) and its body temperature with a digital infrared thermometer (Oakton Series 35629-00); the air temperature and relative humidity were recorded with an thermo-hygrometer (RH 101 Extech IR).

Bioacoustics analysis

All recordings were analyzed with Raven Pro 1.5 software for Windows (Bioacoustics Research Program 2013) in Hann's sampling window, FFT window size of 256 points, sampling rate of 44.1 kHz, and 16-bit precision. Call features definitions follow McLister et al. (1995); Crocroft and Ryan (1995), and Gerhardt and Huber (2002). Following these authors, we consider the dominant call frequency as the frequency with the greatest amount of acoustic energy, note as the sound unit produced by a single expiratory event of the frog, and pulse as a single unbroken wave train isolated in time by significant amplitude reduction (Köhler et al. 2017). We report numerical call features as mean \pm SD and the respective range in parenthesis. The temporal call features (i.e. call duration, number of notes and pulses, and note and pulse duration) were measured in oscillograms; we used power spectra diagrams to calculate the dominant call frequency and frequency bandwidth (the difference between the 5 and 95% frequencies, hereafter referred to as low frequency and high frequency). Graphs of oscillograms, spectrograms, and power spectra were elaborate with Seewave 1.6 software, R 2.15.1 package using Seewave settings (i.e. window name (Fourier transform window) = Hann; window length = 256

samples; and overlap = 90%). All calls were deposited at Instituto de Investigaciones Alexander von Humboldt under access number IAvH-CSA-34148 to IAvH-CSA-34164. Villa de Leyva - Colombia.

Morphological and Bioacoustics Measurements

Morphological characterization of *A. laetissimus* was undertaken to quantify the body size and forelimbs development (see chapter 1). The weight of each frog was recorded with a digital pocket balance (Peso-PPS200, d = 0.02 g; Pesola AG, Switzerland). We photographed each frog using a Sony Cyber-shot DSC-HX400V digital camera, with an automatic program (P) and flash level to maximum (+2.0); each frog was placed in a standard dorsal position with upper arms directed laterally and at an angle of 90° from the body axis and forearms directed anteriorly. We used a millimeter grid as a reference in each image for distance calibration and subsequent processing. We measured the nutritional condition of each individual using the body condition index (BCI), which consists of the ratio between body weight and body size (length from snout to vent) (Stevenson & Woods, 2006).

We used multiple regression (lm function in R) to test for a linear relationship between advisement call features (dominant frequency, call duration, and calling rate) and morphological traits (forearm width at the elbow, body size, body weight, and body condition). We used the four morphological traits as predictor variables in each of three separate analyses where each of the call features in turn served as the dependent variable. In all multiple regressions, we included body temperature as a covariate.

RESULTS

Acoustic signal repertory

We recorded 141 calls representing three types of calls in males of *Atelopus laetissimus*: 70 advertisement calls (Fig. 2), 18 agonist calls (Fig. 3), and 53 release calls (Fig. 4). Also, we recorded a female emitting a release call (15 calls; Fig. 5). A summary of the temporal and spectral call features, body size and temperature at time of calling is shown for each male recorded in the Table 1.

The advertisement call. The 70 advertisement calls recorded from 10 males consisted of a pulsed trill with a duration of 0.29 ± 0.050 s (range: 0.16–0.46 s). The call tends to be characterized by a rapid and steady amplitude shift from the onset of the pulses until reaching peak amplitude in the last pulses; then, it decreases slightly at the end of the call. The number of pulses was 21.37 ± 3.64 pulses (11–28), and their duration was 0.013 ± 0.002 ms (0.011–0.028 ms). In most of the males, the last pulse of the call was the longest, between 0.011–0.054 ms (0.024 ± 0.014 ms). Pulses were emitted at a rate of 74.71 ± 7.53 pulses/s (52.1–90 pulses/s), no change in the pulse rate was detected through the call. The call dominant frequency was 1.83 ± 0.083 kHz (1.56–1.90 kHz); its low frequency was 1324 ± 0.109 kHz (1.06–1.79 kHz) and its high frequency was 2.25 ± 0.104 Hz (2.08–2.53 kHz) (Fig. 2). Little shifts on frequencies were detected through the call. The advertisement call was emitted by males in solitary conditions, in the presence of other males, or in front of amplexant pairs. These calls were regularly accompanied by visual signaling made with fore and hind limbs. The emission of this type of call was detected during breeding seasons (May-August) and at diurnal hours starting early in the morning (~06:30 h). Males emitted advertisement calls near streams, but we also detected individuals vocalizing up to 500 meters away from the nearest stream.

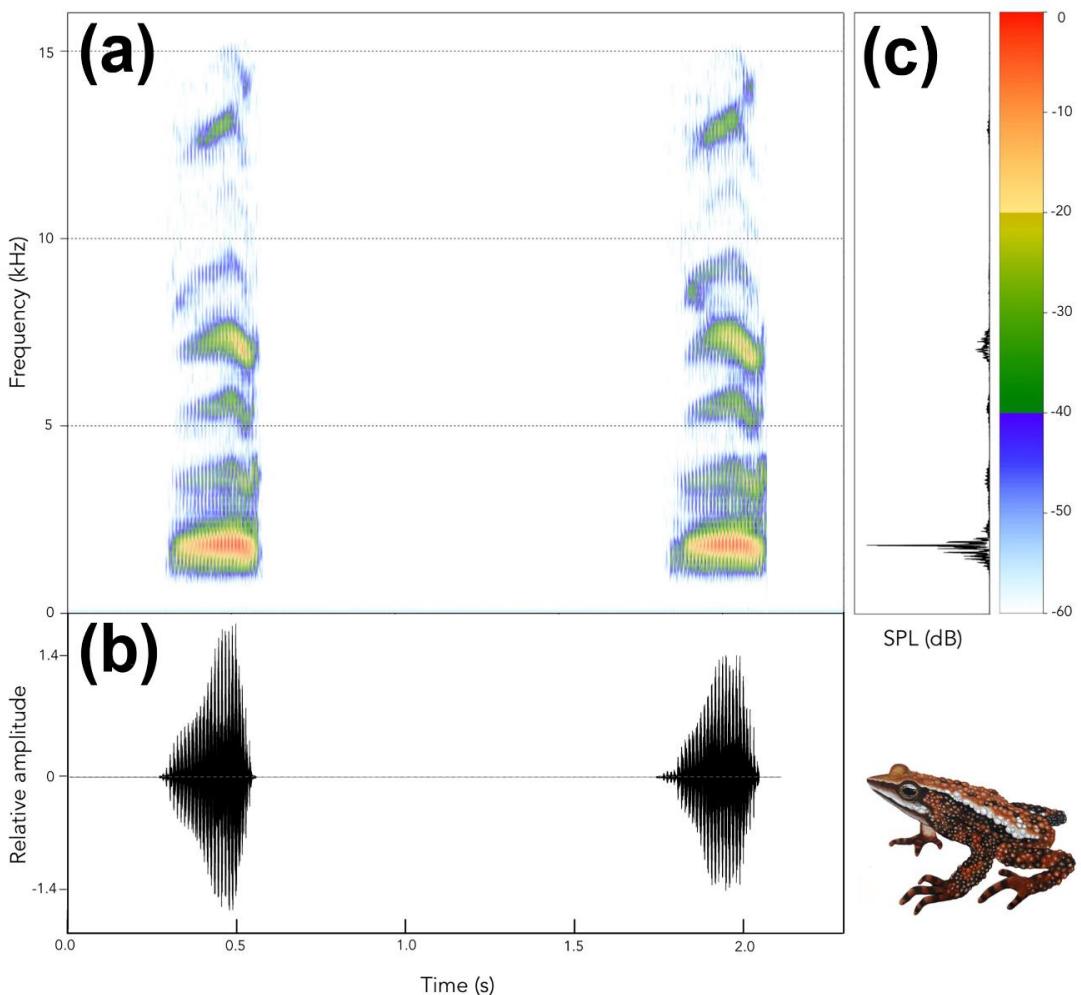


Figure 2. Spectrogram (a), oscillogram (b), and power diagram (c) showing general aspects of the structure of the advertisement call in males of *Atelopus laetissimus*.

The release call. The 38 release calls recorded from four males consisted of a pure tone call with a duration of 0.07 ± 0.0140 s (range: 0.01–0.09 s), and an inter-note period of 3.03 ± 0.71 s (1.13–4.45 s). The dominant frequency of the release call was 1.59 ± 0.09 kHz (1.47–1.73 kHz), its low frequency was 1.23 ± 0.07 kHz (1.12–1.38 kHz), and its high frequency was 1.98 ± 0.12 kHz (1.81–2.25 kHz) (Fig. 3). This type of call was emitted in four behavioral contexts. First, when a single male tries to

displace to an amplexant male, the latter male emits a released call; amplexant males emit this type of call accompanied by a visual display with their hind limbs, especially making undulations with metatarsals and toes. Second, during mating balls; third, when incidentally a male was clasped by another male; and fourth, when the individual was manipulated by humans or was confined in plastic bags or containers.

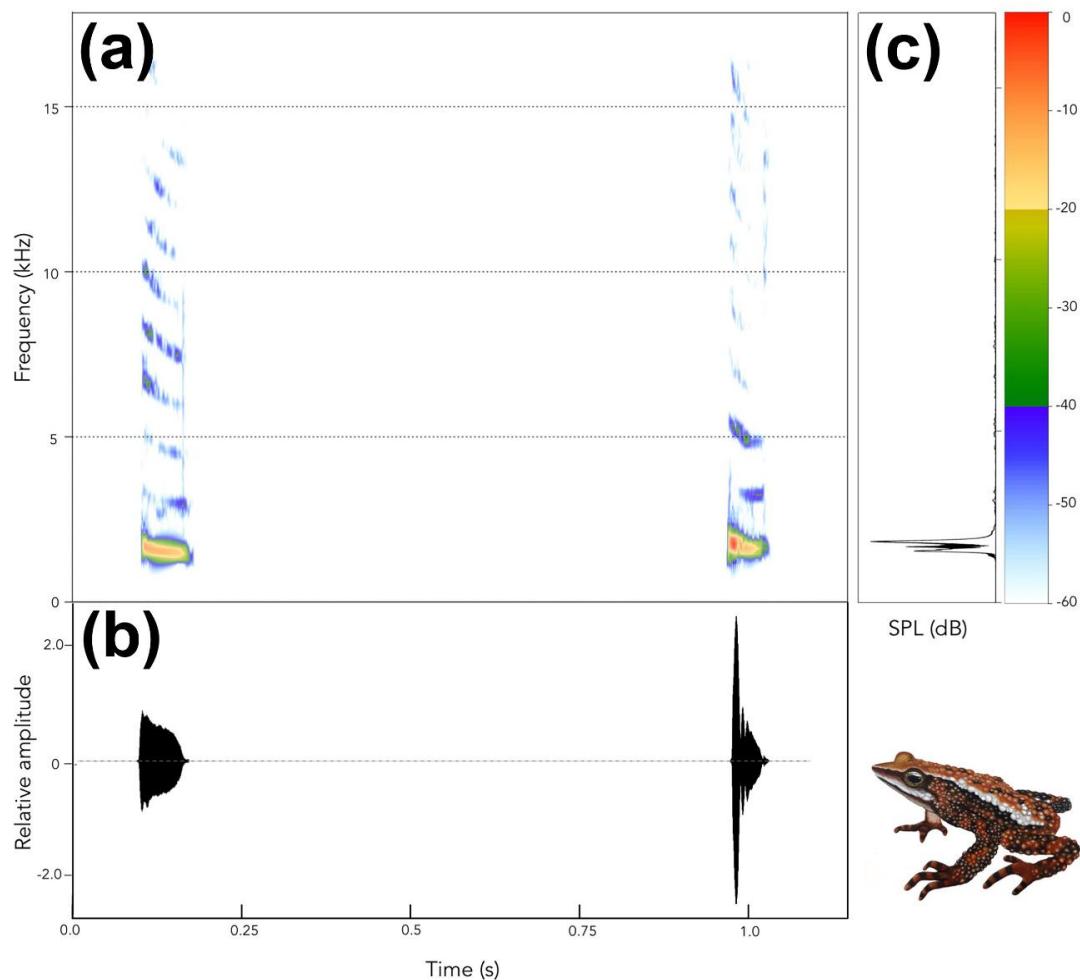


Figure 3. Spectrogram (a), oscillogram (b), and power diagram (c) showing general aspects of the structure of the release call in males of *Atelopus laetissimus*.

The agonistic/aggressive call. The 18 agonistic calls recorded from two males consisted of 8–10 notes. Every single note has a duration of 0.27 ± 0.049 s (0.198–0.34 s), the inter-note period lasted 0.36 ± 0.03 s (0.31–0.42 s), and the whole call duration was 5.58 ± 1.30 s (4.66–6.5 s). The dominant frequency of the agonistic call was 1743 ± 0.186 Hz (1521–2056 Hz), its low frequency was 1351 ± 0.074 Hz (1213–1452 Hz) and its high frequency was 2093 ± 0.212 Hz (1833–2348 Hz). A harmonic was detected in our recordings; it has a dominant frequency of 3499 ± 0.411 Hz (3052–3977 Hz) (Fig. 4). The agonistic call in *A. laetissimus* is characterized by a rapid frequency shift, increasing from the beginning of the call to the end of the -call. This type of call was emitted during male-male interactions, mainly when a male was erroneously clasping another male; we also observed the emission of these calls, when a male was displaced from the amplexant position by another male. Agonistic calls were accompanied by a visual display made with hind limbs.

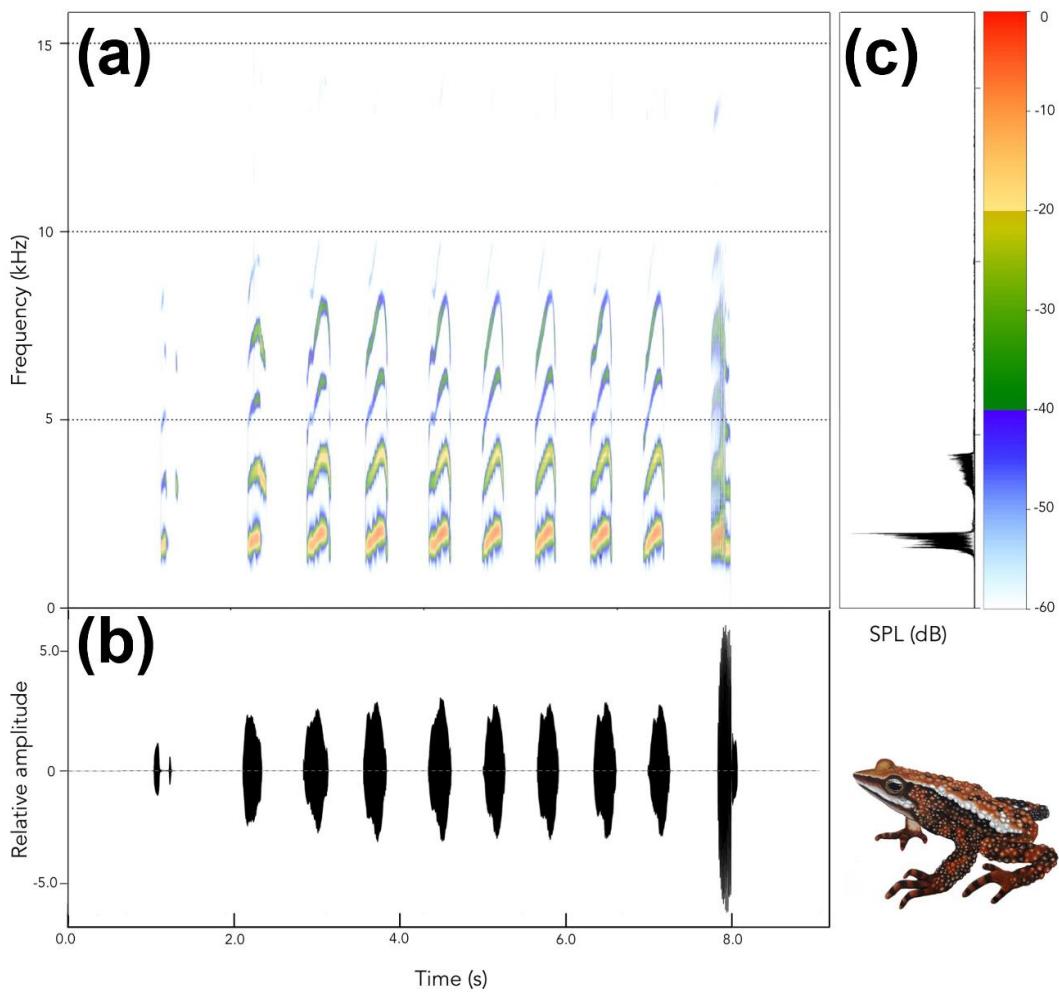


Figure 4. Spectrogram (a), oscillogram (b), and power diagram showing general aspects of the structure of the agonistic call in males of *Atelopus laetissimus*.

The female release call. The 15 release calls recorded from one female, has one or two notes with different duration and was emitted at a very low intensity (Figure 5). The shorter note was emitted more frequently (15 times) than the longer note (3 times), and had a duration of 0.02 ± 0.008 s ($0.008 - 0.03$ s). The dominant frequency of the shorter note was 1.326 ± 0.08 kHz ($1.20 - 1.39$ kHz), its low frequency

was 0.98 ± 0.08 kHz (0.823–1.102 kHz), and its high frequency was 1.68 ± 0.084 Hz (1.52–1.79 kHz). The duration of the longer note was 0.29 ± 0.14 s (0.14 – 0.43 s), its dominant frequency was 1.32 ± 0.08 Hz (1.20–1.39 Hz), its low frequency was 0.92 ± 0.08 kHz (0.87–1.03 Hz) and its high frequency was 1.59 ± 0.07 kHz (1.52–1.67 kHz). When a call consisted in two notes, the inter-note duration was 1.89 ± 0.77 s (0.8–3.8 s) (Fig. 5). The female's call was recorded while one male was trying to claps her.

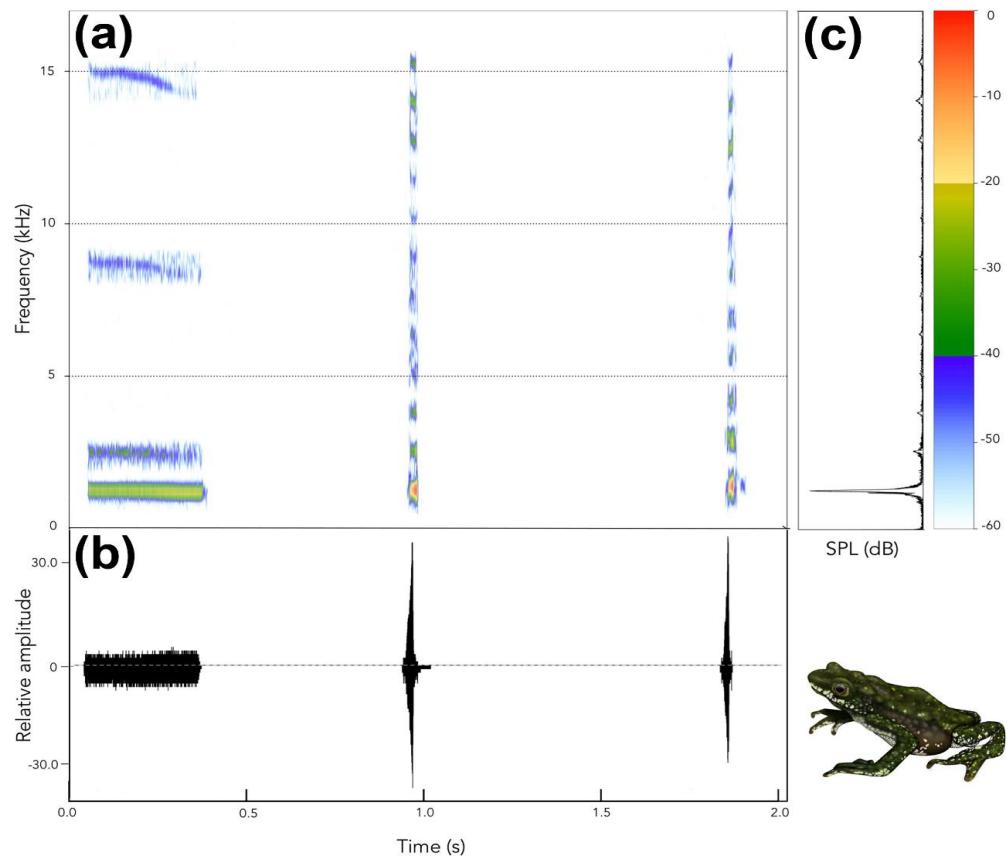


Figure 5. Spectrogram (a), oscillogram (b), and power diagram showing general aspects of the structure of the call emitted by a female of *Atelopus laetissimus*. Body size= 58.0 mm, Temperature at time of recording= 13 °C, in Table 1.

Advisement Call features and morphological traits

The body size of males was positively related with their calling rate ($R^2 = 0.19, F = 5.04, DF = 1, P = 0.039, N = 18$; Fig. 6A), but was unrelated to the dominant frequency of the call ($R^2 = 0.001, F = 0.019, DF = 1, P = 0.891, N = 18$) or call duration ($R^2 = 0.07, F = 1.32, DF = 1, P = 0.267, N = 18$). The forearm width (at the elbow) of males had a minimal relationship with call duration ($R^2 = 0.19, F = 3.37, DF = 1, P = 0.084, N = 18$; Fig. 6B), but was unrelated to call rate ($R^2 = 0.04, F = 0.74, DF = 1, P = 0.400, N = 18$), and call dominant frequency ($R^2 = 0.02, F = 0.34, DF = 1, P = 0.565, N = 18$). Male body weight was unrelated to all of the call features analyzed in this study (Call rate: $R^2 = 0.00, F = 0.005, DF = 1, P = 0.942$; call duration: $R^2 = 0.09, F = 1.58, DF = 1, P = 0.226$; dominant frequency: $R^2 = 0.01, F = 0.24, DF = 1, P = 0.63, N = 18$). The body condition of males was unrelated with their call frequency ($R^2 = 0.01, F = 0.30, DF = 1, P = 0.58, N = 18$), tended to be negatively related to call rate ($R^2 = 0.18, F = 3.69, DF = 1, P = 0.072, N = 18$; Fig. 6C), and was positively related to call duration ($R^2 = 0.27, F = 6.14, DF = 1, P = 0.024, N = 18$; Fig. 6D).

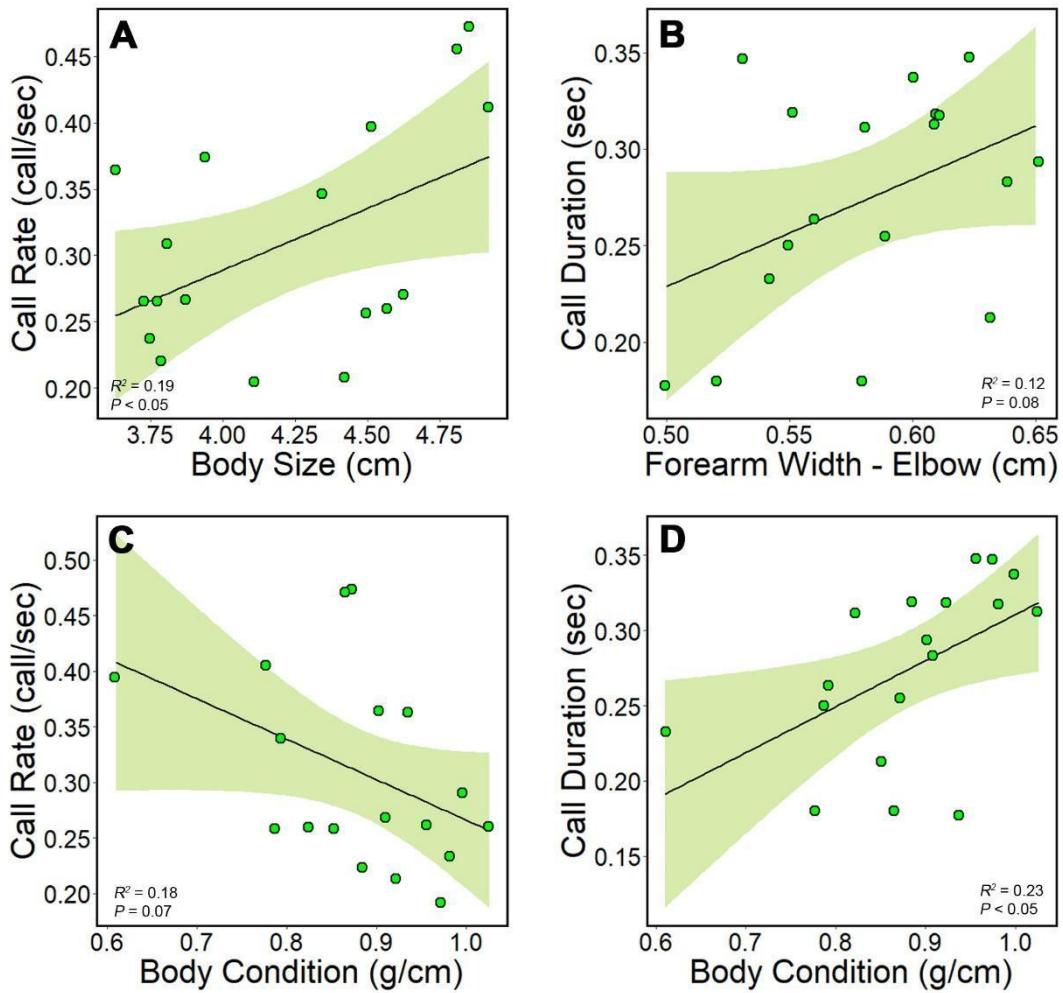


Figure 6. Advisement call features and their relationship with morphological traits in males of *Atelopus laetissimus*. Call rate and body size (A); call duration and forearm width at the elbow (B); call rate (C) and call duration (E) with the body condition of males of *Atelopus laetissimus*.

DISCUSSION

With the acoustic signals described in this study, *Atelopus laetissimus* becomes the species with the most diverse vocal repertoire reported so far for the genus. The males have three types of calls, which are used in reproductive contexts (advertisement calls), male-male interactions (agonistic and release calls) and possibly in anti-predatory or distress contexts (release calls). Males of *A. laetissimus* do not seem to use its advertisement calls on a territorial context, as reported for several species of the genus (see Lötters 1996), for example, *A. varius* (Crump 1988), *A. zeteki* (Lindquist and Hetherington 1996; Criswell 2008), and *A. chiriquiensis* (Jaslow 1979).

Males of *A. laetissimus* emit an advertisement call that is pulsed, which is the structure usually reported in *Atelopus* species (see Table 2). The pulsed structure of the advertisement call in *A. laetissimus* is similar to that in *A. nicefori*, *A. exiguus*, *A. reticulatus*, *A. pulcher*, *A. tricolor*, *A. minutulus*, *A. cruciger*, *A. varius* and *A. zeteki* (Cocroft et al. 1990; Lötters et al. 1999; Lötters et al. 2002; Coloma et al. 2000).

With respect to the number of pulses emitted at a constant rate through the advertisement call of *A. laetissimus*, that resembles those emitted by *A. exiguus* (Coloma et al. 2000), *A. reticulatus* (Lötters et al. 2002), *A. cruciger* and *A. varius* (Cocroft et al. 1990), but contrasts with other species in which the pulse rate increases from the beginning to the end of the call (*A. hoogmoedi*; Costa-Campos and De 2018). A last pulse longer than others in the call also has been reported in *A. nicefori*, *A. reticulatus*, *A. varius* and *A. zeteki*. Advertisement call longer than those emitted by *A. laetissimus* (>1.0 s, according to Lötters et al. 2002) have been registered in *A. spumarius* sensu lato, *A. spumarius* sensu stricto, and *A. hoogmoedi* (Cocroft et al. 1990; Lötters et al. 2002; Costa-Campos and De 2018).

The amplitude structure in the advertisement call in *A. laetissimus* and others *Atelopus* species is similar; the amplitude decay at the end of the call, unlike to *A. nicefori*, where amplitude increases during the first third of its call and remains constant until the end of the signal (Cocroft et al. 1990). On the other hand, the advertisement call in *A. laetissimus* exhibits one of the lowest dominant frequency reported for the genus (see Table 2). The dominant call frequency of *A. laetissimus* is similar to that in *A. zeteki* (Cocroft et al. 1990), but lower than the reported for *A. barbotini*, *A. chiriquiensis*, *A. cruciger* *A. exiguous* *A. flavesiensis*, *A. franciscus*, *A. limosus*, *A. minutulus*, *A. nicefori*, *A. reticulatus*, *A. senex*, *A. spumarius hoogmoedi*, *A. spumarius spumarius*, *A. tricolor*, and *A. varius*. Our study species, *A. laetissimus*, is a mountain species whose individuals have a relatively large body size in comparison to other *Atelopus* species (Ruiz-Carranza et al. 1994; Lötters 1996), which could explain lower frequencies due to pleiotropic effects (Martin 1972; Gerhardt and Huber 2002). Finally, the dominant call frequency shifts parallel with shifts in the call amplitude in species such as *A. nicefori*, *A. exiguous*, *A. reticulatus*, *A. pulcher*, *A. tricolor*, *A. minutulus*, *A. cruciger*, *A. varius* and *A. zeteki* (Cocroft et al. 1990; Lötters et al. 1999; Lötters et al. 2002; Coloma et al. 2000), which contrasts with the little variability in dominant call frequency during the call of *A. laetissimus*.

The advertisement call features in *Atelopus* show a high conservatism (Cocroft et al. 1990, Lötters et al. 1999), which allows inferring some important aspects regardless to the evolutionary history of acoustics signals for those toads. For instance, a pulsed call structure that is independent of the altitudinal distribution of the species suggest a slight influence of the habitat characteristics in the evolution of this temporal call feature (Coloma et al. 2000). The high conservatism in the structure of those advertisement calls also could be explained by the absence of sympatric co-generic species in most of cases (Cocroft et al. 1990; Lötters et al. 1999; Coloma et al. 2000; Lötters et al. 2002). Call feature overlaps found between closely related species is associated with allopatric distribution

patterns (Duellman and Pyles 1983; Crocroft et al. 1990; Kwet et al. 2005; Pereyra et al. 2012; Wen et al. 2012), which, render pre-mating isolation mechanisms to prevent hybridization redundant (Rivera-Corra et al. 2017).

Release calls are emitted by individuals of *A. laetissimus* in distress contexts, mainly when they are grouped, enclosed or manipulated, as has been reported for *A. chiriquiensis*, *A. peruvensis*, *A. tricolor* and *A. nahumae* (Jaslow 1979; Crocroft et al. 1990; Lindquist and Hetherington 1996; Lötters et al. 1999; Carvajalino-Fernández et al. 2017). Although this type of call has been mainly reported as aggression between males on not natural context (Jaslow 1979; Lötters et al. 1999), we do not dismiss that could be used in an interaction with potential predators. Likewise, release calls are used as a signal that helps to minimize the energy expenditure in reproductive interactions such as male recognition during wrong amplexus and mating balls (Carvajalino-Fernández et al. 2017; Rocha-Usuga et al. 2017).

Except for call duration, the temporal and spectral features of the release call in *Atelopus* exhibit slight interspecific variation (Crocoft et al. 1990; Lötters et al. 1999; Table 2). In this type of call, the frequency range in *A. laetissimus* is narrow and similar to that in *A. chiriquiensis*, *A. peruvensis*, *A. tricolor* and *A. limosus* (Crocoft et al. 1990; Ibañez et al. 1995; Lötters et al. 1999). Respect to the dominant frequency, in *A. laetissimus* is similar to that recorded for *A. peruvensis*, *A. zeteki*, and *A. nahumae*, but lower than that reported for *A. chiriquiensis*, *A. tricolor*, *A. cruciger*, *A. minutulus*, *A. spumarius* *spumarius*, and *A. limosus* (Jaslow 1979; Crocoft et al. 1990; Ibañez et al. 1995; Lötters et al. 1999; Carvajalino-Fernández et al. 2017).

Aggressive calls have been recorded in diverse lineages of anurans, and typically do not differ in spectral features from the advertisement call (Wells 2007). This trend is clear in *A. laetissimus*, the

dominant frequency (and the low and high frequency) of the advertisement call overlaps with that in the aggressive calls. Aggressive calls have been overlooked for species of *Atelopus*, the exception is the description of the aggressive calls for *A. chiriquiensis* by Jaslow (1979). However, because physical constraints on call production, it is expected that spectral features of aggressive calls in other *Atelopus* species covaries with body size, as reported for advertisement calls. With respect to temporal features, the aggressive call of *A. laetissimus* seems to be a modification of the advertisement call; while the former type of call consists in 8–10 notes, the latter type of call consists in a trill of 11 to 28 pulses. Similar modifications are present in species of diverse lineages where both types of call are known (see Gerhard and Huber 2002, and Wells 2007 for a review with specific examples).

Acoustic signals by females are unusual in anurans (Duellman and Trueb 1986; Wells 2007); some examples of this behavior have been reported in various lineages such as *Limnonectes blythii* (Emerson 1992), *Alytes cisternasii* (Márquez and Verrell, 1991) *Didocös calcaratus* (Linzana et al. 1994), and *Xenopus laevis* (Tobias et al. 1998). Vocalization by females in species of Bufonidae is known only in *Rhinella* (Marco and Lizana, 2002, Bowcock et al., 2008, Liao and Lu, 2009), but ours is the first call description for a female of the genus *Atelopus*. Unlike the release call emitted by males of *A. laetissimus*, where only one note is emitted, the release call by the female was composed of two notes. The dominant frequency of this type of call was higher in males (1.47–1.73 kHz) than in the female (1.20–1.39 kHz), which is expected because females are larger in body size. The calling behavior in females of anurans have been associated with territorial or aggressive context in Dendrobatidae (Dole and Durant 1974; Wells 1980; Summers 1989), distress context in Eleutherodactylidae and Ranidae (*Eleutherodactylus coqui*, Stewart and Rand 1991; *Lithobates catesbeianus*, Capranica 1968), and reproductive context in Alytidae, Pelobatidae and Pipidae (McClelland and Wilczynski 1989; Márquez and Verrell 1991; Tobias et al. 1998; Emerson and Boyd

1999; Linzana et al. 1994; Tobias et al. 2014). The functionality of release call in females of *A. laetissimus* it is unknown although was recorded while one male was trying to clap her, hence, it is possible that also being emitted when them are clasped simultaneously by several males (i.e. mating ball).

Males of *A. laetissimus* have a broad repertory of acoustic signals, which suggests males call for attracting or courting females. Larger males of *A. laetissimus* have higher call rates than smaller males, but males with a better body condition seem to reduce their call rates. Calling is energetically costly for anurans (Ryan, 1988; Gerhardt & Huber, 2002; Narins, Feng & Fay, 2006); hence, latter males may reduce call rate to save energy for the prolonged amplexus (See chapter 1). In some anurans males save more energy for reproduction than females (Ryan, Bartholomew & Rand, 1983; Lardner & Loman, 2003), and males of explosive breeding species could save more energy than do males of species with continuous reproduction (Soulisbury, 2019). Therefore, females may choose particular males based on particular call features (using as honest indicator of male quality), and hence, mate with males that can endure the prolonged amplexus, this interesting question should be the next step of our studies through playback experiments.

Atelopus species have an intimate relationship with their home range next to streams (Lötters 1996), which may implies masking of acoustic signals by abiotic noise (Hödl and Amézquita 2001; Vargas-Salinas and Amézquita 2014). In species such as *A. zeteki* or *A. varius*, males use acoustic and visual signals (semaphoring) to be more detectable to females in those noisy habitats (Lindquist and Hetherington 1996; Criswell 2008). In particular, males of *A. laetissimus* seems to counteract the potential masking effect of abiotic noise by calling away from the streams or by restricting their vocal activity at seasons with lower noise intensity, for example, at drought season (see Rocha-Usuga et al.

2017). In addition, males of *A. laetissimus* also display visual signals. The conspicuous colorations in *Atelopus* could help to males be more detectable for females, however, striking colorations seem to be more related to avoiding predation (aposematism) than to attracting mates (Rößler et al. 2019). These aspects related of multimodal communication in *A. laetissimus* should be addressed in future studies.

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Table 1. Summary of call features for harlequin frog *Atelopus laetissimus* in the Sierra Nevada de Santa Marta, Colombia. Data are mean values \pm standard deviation and range where pertinent. Male body size corresponds to snout-vent length.

SVL (mm)	Call type	Number of calls analized	Body Temperature (°C)	Air Temperature (°C)	Relative Humidity (%)	Call duration (s)	Note/Pulse duration	Inter-call interval (s)	Number of pulses per call	Pulses per second	Frequency range (kHz)	Dominant frequency (kHz)	CV Dominant frequency	Frequency Peak near end of call (kHz)	CV Peak near end of call	Frequency modulation (kHz)
38.7 \pm 0.209 36.3-43.4 n=10	Pulsed call/ Advertisemen t call	70	14.76 \pm 7.13 10.8-15.3	16.56 \pm 8.01 12.4-17.4	72.6 \pm 4.43 67.5-86.0	0.29 \pm 0.050 0.16 - 0.46	0.013 \pm 0.002 0.011-0.028	2.89 \pm 0.931 0.979 - 9.4	21.37 \pm 3.64 11 - 28	74.71 \pm 7.53 52.1 - 90	1.06-1.90	1.83 \pm 0.083 1.56 - 1.90	4.53	1.65 \pm 0.150 1.53 - 1.95	9.09	-0.18 -0.35-+0.11
39.6 \pm 0.26 37.7-43.4 n=4	Pure tone/ Male Release call	38	12.95 \pm 2.48 10.8 - 15.1	14.8 \pm 3.17 12.4-19.1	81.7 \pm 6.48 72.3 -86	0.07 \pm 0.014 0.01-0.09	0.07 \pm 0.014 0.01-0.09	3.03 \pm 0.71 1.13-4.45	unpulsed call	unpulsed call	1.12-2.25	1.59 \pm 0.09 1.47-1.73	5.66	-	-	-
40.0 \pm 0.09 39.4-40.7 n=2	Tonal call/ Agonistic call	18	14.45 \pm 1.20 13.6-15.3	17.8 \pm 0.63 17.4-18.3	74.05 \pm 1.20 73.2-74.9	5.58 \pm 1.30 4.66-6.5	0.274 \pm 0.06 0.22-0.32	0.36 \pm 0.01 0.35-0.37	unpulsed call	unpulsed call	1.29-1.92	1.76 \pm 0.22 1.59-1.92	12.50	2.70 \pm 1.128 1.56 - 4.20	41.78	+0.94 -0.030-+2.18
58.0 n=1	Short /Female Release call	12				0.02 \pm 0.008 0.008-0.038	0.02 \pm 0.008 0.008-0.038	1.89 \pm 0.77 1.44 - 3.85	unpulsed call	unpulsed call	0.82-1.79	1.32 \pm 0.08 1.20-1.39	6.06	-	-	-
			13	15	90.3											
	Large / Female Release call	3				0.29 \pm 0.14 0.14 - 0.43	0.29 \pm 0.14 0.14 - 0.43	-	unpulsed call	unpulsed call	0.87-1.673	1.21 \pm 0.09 1.20 - 1.38	7.44	-	-	-

Table 2. Comparison of call features among species of *Atelopus* (modified from Lötters et al., 2019). * Body size (SVL) data from the original description of call. ** Body size (SVL) data from Lötters (1996). Values listed as mean ± SD (min–max).

Species	Male SVL (mm)	Call type	Number of calls analyzed	Body Temperature (°C)	Call duration (s)	Inter-call interval (s)	Number of pulses per call	Pulses per second	pulse duration (s)	Frequency range (kHz)	Dominant frequency (kHz)	Peak near end of call (kHz)	Frequency modulation (kHz)	Reference
<i>A. barbotini</i>	(25.3–26.5)*	Pulsed	5	25.5	1.49 ± 0.14 (1.30–1.68)	–	48.6 ± 4.7 (41–53)	–	–	–	(2.00–3.00)	–	–	Lescure 1981
		Pulsed	6	17.5	0.575 ± 0.038 (0.478–0.623)	3.601 ± 0.994 (1.473–5.389)	–	0.48	0.014 ± 0.004 (0.004–0.032)	(2.38–4.1)	3.41 ± 0.021 (3.38–3.45)	–	–	
	(31–33)	Short pulsed	8	17.5	0.136 ± 0.025 (0.074–0.171)	5.181 ± 4.172 (1.041–1.3870)	–	0.11	0.014 ± 0.004 (0.007–0.048)	(0.58–2.26)	1.43 ± 0.044 (1.38–1.5)	–	–	Lötters et al. 2019
		Pure tone	11	–	0.273 ± 0.059 (0.202–0.347)	3.732 ± 2.86 (0.872–6.591)	–	–	–	(0.85–2.16)	1.39 ± 0.037 (1.36–1.44)	–	–	
		Short pure tone	13	–	0.021 ± 0.01 (0.008–0.047)	3.059 ± 2.884 (0.14–9.379)	–	–	–	(0.6–2.26)	1.33 ± 0.151 (1.16–1.81)	–	–	
<i>A. chiriquiensis</i>	(26–32)*	Pulsed	25	(17–18)	0.38 ± 0.049 (0.315–0.478)	–	73.8 ± 6.2 (59.5–82.3)	–	–	–	2.37 ± 0.202 (2.0–2.7)	–	–	
		Short pulsed	3	–	–	–	–	–	–	–	2.05 ± 0.5 (2.0–2.7)	–	–	Jaslow 1979
		–	7	–	–	–	–	–	–	–	1.98 ± 1.68 (1.8–2.2)	–	–	
	28.3*	Pure tone	13	18	0.024 ± 0.012 (0.012–0.052)	–	–	–	–	(1.70–2.35)	(1.90–2.15)	–	–	Lötters et al. 1999
		Pulsed	10	–	0.69 (0.620–0.770)	–	91.2 (84–99)	131.9 (128–140)	–	–	(2.65–3.17)	(2.99–3.17)	+0.281 (+0.09 – +0.48)	
<i>A. cruciger</i>	30* (22.0–31.0)**	Short pulsed	12	12	0.048 (0.030–0.080)	–	–	–	–	–	(2.39–2.97)	2.38 (2.31–2.63)	-0.192 (-0.07 – -0.48)	Cocroft et al. 1990
		Pure tone	5	–	0.232 (0.18–0.29)	–	–	–	–	–	(2.40–2.55)	2.86 (2.81–2.87)	+0.370 (+0.32 – +0.47)	
		–	–	–	–	–	–	–	–	–	–	–	–	
<i>A. exiguous</i>	(21.2 – 27.1)*	Pulsed	–	–	(0.48–0.52)	11.6	(19–21)	–	0.012	–	(2.15–2.70)	–	–	Coloma et al. 2000
<i>A. flavescens</i>	(23.3–25.4)*	Pulsed	7	–	1.53 ± 0.22 (1.34–1.82)	–	49.7 ± 4.9 (45–58)	–	–	–	(2.50–3.00)	–	–	Lescure 1981
<i>A. franciscus</i>	– (18.0–21.5)**	Pulsed	130	–	1.33 ± 0.25	–	32 ± 4.5	25.03 ± 6.10	0.003 ± 0.093	(2.9 – 3.3)	3.134 ± 0.187	–	–	Boistel et al. 2011
		–	3	26	1.49 ± 0.15 (1.34–1.64)	–	34.6 ± 4.0 (31–39)	–	–	–	(2.30–3.00)	–	–	Lescure 1981
<i>A. hoogmoedi</i>	(23.3–25.4)*	Pulsed	3	–	1.19 ± 0.01 (1.19–1.20)	–	41.0 ± 1.0 (40–42)	34.35 ± 0.7 (33.61–35.0)	–	–	(2.30–3.00)	–	–	Lescure 1981; Cocroft et al. 1990

			10	-	1.12 (1.06–1.24)	-	61.6 (57–71)	(43.1–74.0) (39.3–47.7) (70.0–81.7)	-	-	(2.67–2.75)	(2.87–3.00)	+0.24 (+0.12 – +0.30)	(as <i>A. spumarius</i> <i>hoogmoedi</i>)	
<i>A. limosus</i>	(29–30.7)*	Pulsed	13	21	0.26 ± 0.03 (0–25–0.295)	-	40 ± 5 (31–45)	-	-	(2.16–3.40)	(2.68–2.80)	-	-	Ibañez et al. 1995	
		Short pulsed	18	-	0.01 ± 0.003 (0.005–0.16)	-	-	-	-	(1.85–2.61)	(2.00–2.16)	-	-		
<i>A. minutulus</i>	19.8* (17.1–19.1)**	Pulsed	9	-	0.28 (0.227–0.381)	-	18 (14–21)	64.8 (59.5–67.9)	-	-	(2.95–3.38)	(3.16–3.69)	+0.205 (+0.08 – +0.31)		
		Short pulsed	-	(17–18)	0.028 (0.022–0.037)	-	-	-	-	-	(3.12–3.43)	2.97 (2.81–3.12)	+0.213 (+0.15 – +0.31)	Cocroft et al. 1990	
		Pure tone	10	-	0.24 (0.18–0.29)	-	-	-	-	-	(2.77–3.27)	3.56 (3.27–3.78)	+0.497 (+0.37 – +0.63)		
<i>A. mucubajensis</i>	34.5	Pure tone	8	13.5	0.243 ± 0.048 (0.159–0.337)	3.704 ± 0.059 (3.179–4.5605)	-	-	-	(1.55–5.35)	3.17 ± 0.836 (2.71–5.35)	-	-	Lötters et al. 2019	
	34.5	Short Pure tone	17	-	0.041 ± 0.01 (0.022–0.057)	2.122 ± 2.059 (0.05–8.395)	-	-	-	(0.86–2.46)	2.24 ± 0.183 (1.96–2.46)	-	-		
<i>A. nahumae</i>	(32.4–37.2)**	Short pure tone	19	-	0.1355 ± 0.02	-	-	-	-	-	1.54	-		Carvajalino-Fernandez et al. 2017	
<i>A. nicefori</i>	21.3* (17.6–22.5)**	Pulsed	5	15.0	0.35 (0.330–0.370)	-	22.8 (21–24)	65.0 (63.9–65.7)	-	-	(2.63–2.67)	-	+0.226 (+0.20 – +0.24)	Cocroft et al. 1990	
<i>A. pulcher</i>	27.27 ± 1.07	Pulsed	7	23	0.0245 ± 0.0056 (0.0185–0.0347)	-	-	-	-	-	-	-	-	Lötters et al. 2002b	
		Short pulsed	5	23	1.2 ± 0.1 (1.1–1.3)	-	35.4 ± 9.2	28.8 ± 5.5 (22–35)	-	-	(2.03–2.82)	-	-		
<i>A. peruensis</i>	37.0* (32.8–38.5)**	Short pulsed	12	23	0.073 ± 0.022 (0.044–0.114)	-	-	-	-	(1.50–2.15)	(1.50–1.75) (1.80–2.10)	-	-	Lötters et al. 1999	
<i>A. reticulatus</i>	24.7*	Pure tone	25	23	0.34 ± 0.04 (0.29–0.42)	-	(27–32)	(75–76)	0.34 ± 0.04 (0.29–0.42)	(3.22–3.49)	3.36	-	-	Lötters et al. 2002a	
									(27–32)	0.43		3.28			
									13	0.33		3.49			
									-	0.08		3.29			
<i>A. senex</i>	27.4	Pulsed	6	-	-	-	32.5 (30–34)	-	-	-	-	-	-	-	Cocroft et al. 1990

<i>A. tamaense</i>	37.0	Short pure tone	15	-	0.022 ± 0.057 (0.013–0.030)	1.209 ± 0.897 (0.103–3.802)	-	-	-	(2.21–2.97)	2.59 ± 0.036 (2.54–2.64)	-	-	Lötters et al. 2019
<i>A. spumarius spumarius</i>	-	Pulsed	7	29	0.83 ± 0.02 (0.81–0.86)	-	34.6 ± 1.5 (32–37)	41.62 ± 2.24 (38.55–45.9)	-	-	(3.60–4.40)	-	-	Lescure 1981
<i>A. tricolor</i>	21.9* (20.4–21.2)**	Pulsed	15	-	0.10 ± 0.0045	-	17.80 ± 1.08 (16–19)	-	-	(2.25–7.0)	(2.97–3.45)	-	-	Lötters et al. 1999
		Short pulsed	10	26.2	0.23 ± 0.21 (0.007–0.077)	-	-	-	-	(2.10–2.15)	(2.10–2.85) (2.15–2.70) (2.45–2.85)	-	-	
<i>A. varius</i>	37.1* (27.0–39.0)**	Pulsed	10	-	0.40 (0.37–0.46)	-	47.7 (43–56)	120.1 (119–123)	-	-	(2.21–2.48)	(2.41–2.52)	+0.120 (+0.01 – +0.23)	Starret 1967; Cocroft et al. 1990
		Short pulse	11	-	0.061 (0.034–0.070)	-	-	-	-	(1.92–2.01)	1.85 (1.75 – 1.92)	+0.160 (-0.10 – 0.26) +0.051 (+0 – +120)		
<i>Atelopus sp. 'Panamá'</i>	(24–29)	Pulsed	5	-	0.42 (0.41–0.43)	-	0.049 (0.048–0.050)	118.2 (115–119)	-	-	(1–62–1.95)	(1.91–2.05)	+0.231 (+0.04 – +0.43)	Starret 1967; Cocroft et al. 1990 (in part as <i>A. zeteki</i>)
		Pulsed	5	-	0.34 (0.29–0.36)	-	0.049 (0.042–0.052)	145.2 (143–146)	-	-	(1.68–1.915)	(1.95–2.22)	+0.165 (+0.04 – +0.39)	
		Pure tone	5	-	0.030 (0.026–0.033)	-	-	-	-	-	1.51	1.38	+0.13	
<i>Atelopus sp. 'Itaya'</i>	19.3	Pulsed	1	26	0.68 (0.67–0.71)	0.022 (0.054–0.008)	-	-	-	-	2.585 (2.51–2.64)	2.460 (2.350– 2.580)	-0.120 (0 – 290)	Asquith and Altig 1987 (as. <i>A. spumarius</i>)
<i>A. cf. loettensi</i>	(23–29)	Shor pulsed	10	17.5	0.06 (0.03–0.1)	-	-	-	-	-	1.965 (1.92–2.01)	3.37 (3.32–3.43)	-0.01 (-0.08 – +0.04)	Cocroft et al. 1990 (cf. <i>A. spumarius spumarius</i>)

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CHAPTER 3: THE ROLE OF MATING STRATEGIES IN THE EVOLUTION OF SEXUAL DIMORPHISM AND INTRA-GENERIC PHENETIC GROUPS IN ATELOPUS (ANURA: BUFONIDAE).

MAIN QUESTION CHAPTER 3:

Does mating strategy drive sexual dimorphism and phenotypic diversity in *Atelopus*?



**SHAPE OF LOVE: MATING STRATEGIES PROMOTE THE INTRA-GENERIC
PHENOTYPIC EVOLUTION OF HARLEQUIN FROGS (BUFONIDAE: ATELOPUS).**

**LA FORMA DEL AMOR: ESTRATEGIAS DE APAREAMIENTO PROMUEVEN LA
EVOLUCIÓN FENOTÍPICA INTRA-GÉNERICA EN RANAS ARLEQUINES (BUFONIDAE:
ATELOPUS).**

Luis Alberto Rueda-Solano^{1,2,3*}, Stefan Lötters⁴, Romario Salas-Nieto¹, Angie Tovar-Ortiz², José Daniel Barros-Castañeda^{1,3}, Lily Santodomingo-Ravelo¹, José Luis Pérez-González^{1,3}, Fernando Vargas-Salinas⁵ & Andrew J. Crawford².

¹*Grupo de Investigación en Biodiversidad y Ecología Aplicada (GIBEA), Facultad de Ciencias Básicas, Universidad del Magdalena, Santa Marta, Magdalena, Colombia.*

²*Department of Biological Sciences, Universidad de los Andes, Bogotá, 111711, Colombia*

³*Fundación Atelopus, Santa Marta, Magdalena, Colombia.*

⁴*Department of Biogeography, Trier University, 54286, Trier, Germany*

⁵*Grupo de Investigación en Evolución, Ecología y Conservación (EECO), Universidad del Quindío, Armenia, Colombia.*

(*) Corresponding Author: biologoluisrueda@gmail.com

RESUMEN

Los sistemas de apareamiento animal dependen del número de parejas que obtienen los individuos a lo largo de su vida. Los anuros son polígamos empleando estrategias de apareamiento en un continuo entre scramble, lek y territorial. *Atelopus* (Bufonidae) presenta estrategias lek cercano al scramble y cercano a la territorialidad. Asimismo, este género exhibe dos tipos de formas corporales, conocidos como grupos fenéticos *longirostris* e *ignescens*. Debido a que los dos grupos fenéticos en *Atelopus* involucran aspectos morfológicos de las extremidades, las cuales juegan un rol importante en el éxito de apareo en *Atelopus*, testeamos la influencia de selección sexual en la evolución morfológica de estos grupos intra-genericos en *Atelopus*. Empleando fotografías estandarizadas obtenidas de ejemplares de colecciones herpetológicas e in-situ, cuantificamos el dimorfismo sexual de rasgos morfológicos en especies del género *Atelopus*. Los patrones de dimorfismos resultantes de la integración de los grupos fenéticos, variables ambientales y filogenia son congruentes con la intensidad de selección sexual sobre los rasgos de tamaño corporal y robustez de los brazos en *Atelopus*. En el grupo *longirostris*, las especies presentan tendencia monomórfica hasta moderadamente dimórfica en talla corporal y robustez de brazos. En el grupo *ignescens*, las especies presentan tendencia desde moderada hasta extremadamente dimórfica en talla corporal y robustez de brazos. Este estudio muestra evidencia que sugiere un continuo en los niveles de competencia macho-macho en el clado *Atelopus*, correspondiente a la transición de las estrategias de apareamiento a lo largo del gradiente altitudinal y estacionalidad, generando paralelismos fenéticos entre especies de como resultado de la presión de selección en ambientes similares. Los resultados apoyan la hipótesis sobre el reflejo causado por la contribución de selección sexual y las estrategias de apareamiento en la evolución fenética dentro del género *Atelopus*.

Palabras clave: Selección sexual, sistemas de apareamiento, dimorfismo sexual, Anura

INTRODUCCIÓN

La clasificación de los sistemas de apareamiento en animales se basa en el número de parejas que obtienen tanto machos como hembras en una temporada reproductiva (Shuster & Wade 2003). En animales los sistemas de apareamiento se definen según los niveles de poligamia (Emlen & Oring 1977) y son el marco referencial en el cual selección sexual opera (Darwin 1874; Emerson 1994; Shuster 2009; Klug 2011). Específicamente, selección sexual promueve la evolución de caracteres fenotípicos que incrementan la probabilidad de apareamiento de los individuos (Shuster 2009; Hoquet 2015; Futuyma & Kirkpatrick 2017). Sin embargo, los sistemas de apareamiento por sí solos no permiten predecir todos los patrones fenotípicos generados por selección sexual (ej. dimorfismo sexual) (Clutton-Brock 1983; Fairbairn et al. 2007); para ello se hace necesario determinar las características fenotípicas que influyen en el éxito reproductivo de los individuos (Clutton-Brock 1983, 2007; Shuker & Kvarnemo 2021). En especies polígamias, selección sexual puede ser particularmente intensa en machos lo que podría generar rasgos corporales distintivos en ellos (Fairbairn et al. 2007; Dugatkin 2013).

La gran mayoría de anfibios anuros son polígamos (Halliday & Tejedo 1995; pero ver Brown et al. 2010). En ellos se han propuesto tres categorías de estrategias de apareamiento no excluyentes entre sí: 1) tipo “scramble” o competencia por el amplexo, 2) tipo leks o competencia en coros y 3) defensa de recursos o competencia territorial (Wells 2007). Una misma especie puede exhibir estrategias de apareo intermedias o alternar entre ellas, dependiendo de cambios en características ambientales que determinan la duración de la temporada reproductiva y la densidad de individuos en un evento reproductivo dado (Wells 1977; Arak 1983). En cada estrategia de apareamiento predomina un tipo de competencia entre machos, y selección sexual operará favoreciendo determinadas características

fenotípicas, generando así, patrones de dimorfismo sexual distintivos entre especies (Arak 1983; Emerson 1994; Duellman & Trueb 1994; Sullivan et al. 1995). El resultado entre sistemas de apareamiento y estrategias de competencia entre machos sobre los patrones de dimorfismo sexual, ha sido escasamente investigada en anuros (Nali et al. 2014; Pough et al. 2015).

Atelopus (Bufonidae) es un género compuesto por más de 100 especies de anuros con distribución neotropical (Lötters 1996; Frost 2021). En *Atelopus*, existe una considerable inversión en comunicación inter e intra-sexual, ya sea a través de señales acústicas (Jaslow 1979; Crocroft et al. 1990; Lötters 1996; ver capítulo 2) o complejas señales visuales (Jaslow 1979; Crump 1988; Lindquist & Hetherington 1998; Lötters 1996; Criswell 2008). Además, en este género de anuros se han reportado al menos dos estrategias de competencia entre machos por lograr acceso a hembras, las cuales pueden ser extremos de un continuo moldeado por características ambientales: lek-scramble (Rocha-Usuga et al. 2017, ver capítulo 1) y territorialidad (Crump 1988, Lötters 1996). En la estrategia de apareamiento tipo “leks-scramble”, el canto de advertencia de los machos es combinado con la búsqueda activa de las hembras y la interacción física entre machos se limita a intentos de desplazamiento de amplexo (Arak 1993; Rocha-Usuga et al., 2017; ver capítulos 1 y 2). En contraste, en la estrategia territorial, hay altos niveles de agresión entre conespecíficos por la posesión de un territorio (Jaslow 1979; Crump 1988; Criswell 2008). La competencia macho-macho incluye combates físicos (Jaslow 1979; Crump 1988; Criswell 2008), intimidación a través de despliegues ritualizados (Lindquist & Hetherington 1998; Criswell 2008) e interferencia física (Sexton 1958; Crump 1988). Las implicaciones evolutivas de las dos estrategias de apareamiento predominantes en *Atelopus* son desconocidas. Sin embargo, selección sexual podría actuar de manera divergente entre especies, exhibiendo predominancia de uno u otra estrategia, y así, promover la evolución de caracteres morfológicos distintivos entre ellas.

En *Atelopus*, las hembras exhiben tamaños corporales mayores a los registrados en machos (Lötters 1996). Además, las extremidades son proporcionalmente diferentes entre sexos, con extremidades anteriores más robustas y presencia de callosidades nupciales en el primer dedo manual de los machos (Peters 1973; ver capítulo 1); también hay diferencias en otros caracteres anatómicos que conforman las manifestaciones de dimorfismo sexual dentro del género (Peters 1973; Duellman & Trueb 1994; Lötters 1996; Rueda-Almonacid et al. 2005). Algunas de estas diferencias morfológicas entre machos y hembras en especies de *Atelopus* se podrían acentuar acorde a como selección sexual actúe en ellas, lo cual, estaría determinado por la estrategia de competencia macho-macho predominante en la especie (Fairbairn et al. 2007; Berns 2013). Por ejemplo, una estrategia de competencia macho-macho por interferencia de amplexo podría generar un dimorfismo sexual acentuado en las extremidades, exhibiendo machos con extremidades muy robustas y callosidades nupciales conspicuas sin necesariamente maximizar diferencias intersexuales en tamaño corporal (Shine 1979; Duellman & Trueb 1994; Lee & Corrales 2002; Kupfer 2007; ver capítulo 1). Por el contrario, una estrategia basada en territorialidad podría minimizar diferencias en dimorfismo sexual en tamaño corporal, al favorecer tamaños corporales grandes en machos, sin necesariamente maximizar diferencias en la morfología de extremidades (Duellman & Trueb 1994; Halliday & Tejedo 1995; Fairbairn et al. 2007; Berns 2013). La funcionalidad de las diferentes expresiones de dimorfismo sexual en *Atelopus* es desconocida (pero ver capítulo 1 de la presente tesis doctoral).

Adicional al dimorfismo sexual en características morfológicas, las estrategias de competencia macho-macho en *Atelopus* podrían tener otras consecuencias sobre la evolución morfológica. En general, las especies de *Atelopus* exhiben dos formas corporales, las cuales han sido categorizadas tradicionalmente como grupos fenéticos *longirostris* e *ignescens* (Peters 1973, Lötters 1996); los cuales no son ensamblajes monofiléticos (Lötters et al. 2011). Las diferencias morfológicas entre

especies pertenecientes a estos dos formas corporales se basan principalmente en robustez corporal; las especies del grupo *longirostris* exhiben cuerpos estilizados y extremidades delgadas (Fig. 1A), mientras que especies del grupo *ignescens* exhiben cuerpos y extremidades robustas (Fig. 1B) (Peters 1973; Lötters 1996). Ambos grupos fenéticos representan los extremos de un continuo morfológico que, al parecer, ha evolucionado múltiples veces dentro del género *Atelopus* (Lötters et al. 2011, Fig. 1C, D). Hasta la fecha no se le ha conferido ninguna funcionalidad a esta variabilidad morfológica; sin embargo, las especies del grupo *longirostris* predominan en hábitats a baja altitud y las del grupo *ignescens* en hábitats a mayor altitud (Peters 1973; Lötters 1996, Fig. 1). Debido a que estos dos grupos fenéticos involucran rasgos morfológicos de las extremidades, que en Bufonidae juegan un rol importante en el éxito de apareo de los machos (Reading & Clarke 1983; Lee 2001; Lee & Corrales 2002; Yu & Lu 2010; ver capítulo 1), es posible que dichos grupos fenéticos en *Atelopus* sean el resultado de selección sexual actuando de forma divergente entre especies, o pueden haber evolucionado como adaptaciones al clima frío, pero aun así, tendrían implicaciones sobre la evolución de sistemas de apareamiento a través de la evolución de la longitud y diámetro de los brazos.

El presente estudio tiene como principal objetivo examinar si los dos grupos fenéticos en *Atelopus* son, en parte, el reflejo morfológico de sus estrategias de apareamiento y paralelismo causados por selección sexual entre especies de tierras altas y bajas. De cumplirse esta hipótesis, esperaríamos que las especies del grupo fenético *longirostris* (tierras bajas) no presenten un dimorfismo sexual marcado en talla y robustez de las extremidades, causado por una estrategia de apareamiento cercana a lek o territorialidad. En contraparte, las especies del grupo fenético *ignescens* (tierras altas), presentarían un dimorfismo marcado en talla y robustez de extremidades, causado por una estrategia de apareamiento cercana a “scramble”.

Asimismo, debido a la amplia distribución altitudinal de las especies de *Atelopus* (desde 0 hasta 4800 msnm), podría existir un continuo en las características anatómicas sujetas a selección sexual, reflejando una transición de estrategias de apareamiento e intensidad de selección sexual operando diferencialmente entre rasgos corporales a lo largo del gradiente altitudinal; donde la mayor pendiente de las quebradas de tierras altas, limitaría temporalmente las zonas de remanso, claves como lugares de ovoposición. De cumplirse esta segunda hipótesis, esperaríamos que los niveles de competencia macho-macho por interferencia física (estrategia cercana a “scramble”) se incrementen con la altitud, lo que promovería mayor dimorfismo sexual en extremidades anteriores en especies de alta montaña en comparación con especies de elevaciones intermedias y bajas.

Por último, si el ambiente causa estos paralelismos en las formas corporales en *Atelopus* (Lötters et al. 2011), proponeros que la estacionalidad ambiental podría influir en la duración de los períodos reproductivos, donde la mayor estacionalidad en zonas de alta montaña de páramo, promovería períodos reproductivos restringidos, en el cual una competencia cercana a tipo “scramble” sería una estrategia reproductiva más adecuada para *Atelopus* de estos hábitats. Por el contrario, la menor estacionalidad y mayor estabilidad ambiental en zonas de tierras bajas y medias de bosque húmedo, constituiría condiciones para una reproducción más prolongada y la monopolización de recursos a largo plazo. De cumplirse esta tercera hipótesis, esperaríamos que los niveles de competencia macho-macho por monopolización de recursos (estrategia cercana a la territorialidad) se incrementen con la estabilidad ambiental, lo que promovería menor dimorfismo en tamaños corporales en especies de elevaciones intermedias y bajas en comparación con especies de alta montaña.

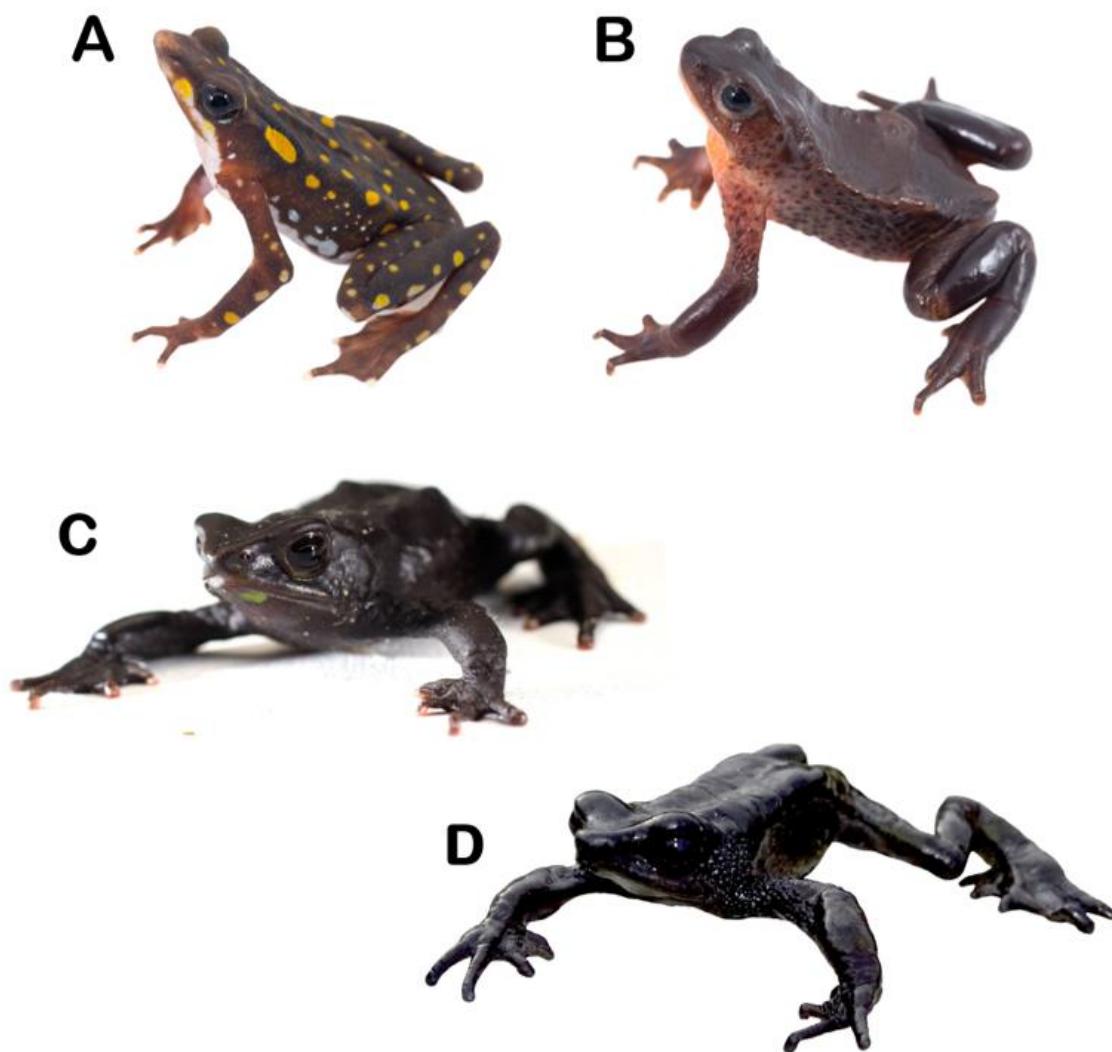


Figura 1. Grupos fenéticos intra-genéricos de *Atelopus* (Peters, 1973). Individuo macho de *Atelopus longirostris* de las tierras bajas de Ecuador representante de la anatomía del grupo fenético *longirostris* (A); Individuo macho de *Atelopus ignescens* de las tierras altas de Ecuador representante de la anatomía del grupo fenético *ignescens* (B). Anatomía similar de especies de páramos pertenecientes al grupo fenético *ignescens*. Machos de *Atelopus nanay* en Ecuador (C) y *Atelopus carrikeri* en Colombia (D). Fotografías: Jaime Culebras (A-B); Carlos Martínez (C); Luis Alberto Rueda Solano (D).

METODOLOGÍA

Rasgos Morfológicos y Dimorfismo Sexual en Atelopus

Para el registro de rasgos morfológicos en especies de *Atelopus*, utilizamos ejemplares depositados en las colecciones herpetológicas del Instituto de Ciencias Naturales (ICN), Universidad Nacional de Colombia, Colombia; Centro Jambatu (CJ), Ecuador; *Smithsonian Tropical Research Institute*, Círculo Herpetológico (STRI-CH), Panamá; el Museo de Historia Natural de Universidad Nacional Mayor de San Marcos (MHNSM), Perú; y *Zoologisches Forschungs Museum Alexander Koenig* (ZFMK-HERP), Alemania. Entre los especímenes revisados, se escogieron ejemplares fijados con las extremidades delanteras abiertas, formando un ángulo de 90° desde el eje del cuerpo. Estos especímenes fueron fotografiados en posición dorsal estandarizada (Fig. 2A). Todas las fotografías se tomaron con una cámara digital Sony Cyber-shot DSC-HX400V, configuración automática, a 10 cm de distancia del espécimen y sobre un fondo blanco con cuadrícula milimetrada que sirviese de referencia. Adicional a las fotografías de especímenes de colecciones herpetológicas, utilizamos fotografías de ejemplares vivos *in-situ* (Fig. 2B). Estas fotografías fueron donadas por el programa de monitoreo de especies de *Atelopus* en la Sierra Nevada de Santa Marta, llevado a cabo por la Fundación Atelopus y la Universidad del Magdalena (Colombia). Dentro de este programa de monitoreo, el registro de los individuos se lleva a cabo con base en fotografías bajo las condiciones estandarizadas ya mencionadas. Por último, completamos la información de caracteres morfológicos en *Atelopus*, mediante información bibliográfica disponible en la descripción original de cada especie (ver compendio en Lötters 1996; Rueda-Almonacid et al. 2005; Frost 2021).

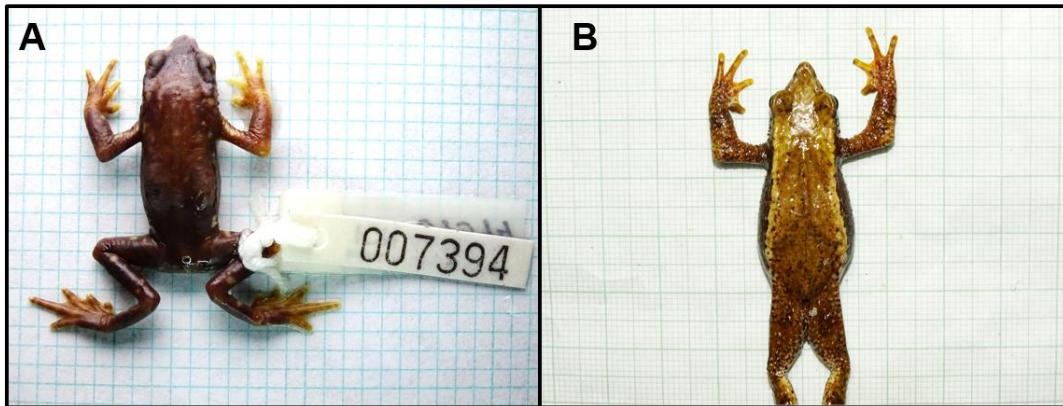


Figura 2. Fotografías estandarizadas para cuantificar los rasgos morfológicos de individuos y el nivel de dimorfismo sexual en especies de *Atelopus*. En especímenes de colecciones herpetológicas e *in-situ*. Macho de *Atelopus sp.* (A), Instituto de Ciencias Naturales (ICN), Universidad Nacional de Colombia. Macho *in-situ* de *Atelopus nahumae* (B), Sierra Nevada de Santa Marta, Colombia.

Para cuantificar los rasgos morfológicos utilizamos el software ImageJ V1.8 (Abrámoff et al., 2004; Scheneider et al., 2012). Se midieron un total de 12 variables morfológicas asociadas al cuerpo y extremidades (Ver capítulo 1): El ancho del antebrazo fue medido a nivel de la muñeca, el codo y en la zona intermedia con mayor diámetro; el ancho del brazo superior fue medido en la parte distal del húmero. Estas medidas representan la robustez de las extremidades anteriores. Además, registramos la longitud del brazo superior medida desde el punto de la axila hasta el punto del codo; la longitud del antebrazo medida desde el punto del codo hasta el punto donde la almohadilla nupcial se encuentra con el antebrazo; el largo del brazo tomada desde el punto del codo hasta donde termina el dedo más largo de la mano; el ancho del pecho fue medido desde una axila hasta la otra axila; el ancho de la cabeza fue tomada a nivel occipital desde un extremo a otro de esta; la distancia inter-orbital fue tomada en la zona intermedia entre los ojos; el diámetro de ojo fue medido de manera dorsal de un

extremo a otro de la órbita; por último, registramos el tamaño corporal o la longitud rostro cloaca (SVL), la cual fue medida desde el extremo del hocico hasta el orificio de la cloaca.

Para cuantificar la magnitud del dimorfismo sexual en tamaño corporal y demás rasgos morfológicos, realizamos un análisis de frecuencias de los tamaños de machos y de hembras dentro del género *Atelopus* (R software versión 4.1; R Core Team, 2020). Luego, calculamos el índice de dimorfismo sexual (SDI) sugerido por Lovich & Gibbons (1992); la tamaño corporal del sexo más grande es usada como numerador, y la del sexo más pequeño como denominador; con esto se obtiene la magnitud del dimorfismo sexual. Finalmente, al resultado de esta división se le resta 1, con lo cual se obtiene la direccionalidad del dimorfismo (ver más detalles en Lovich & Gibbons 1992). En nuestro estudio, los resultados positivos (+) hacen referencia a que las hembras superan a los machos en el carácter morfológico evaluado, mientras que en resultados negativos (-) los machos sobrepasan a las hembras. El SDI fue estimado para tamaño corporal y otros rasgos morfológicos, enfocándonos en las mediciones de robustez de extremidades anteriores (Tabla1, Suppl. 1), las cuales son rasgos adaptativos en el sistema de apareamiento *lek-scramble* de *Atelopus* (ver capítulo 1). Previo al cálculo del SDI, realizamos un control por alometría a cada uno de los rasgos morfológicos, dividiéndolos sobre la medida de tamaño corporal promedio de cada especie.

Altitud y Estacionalidad del Hábitat

Para cada especie de *Atelopus* adquirimos la altitud mínima y máxima de su distribución con base en información bibliográfica y estimamos la altitud mediana de cada especie (ver detalles Suppl. Table S2). Posteriormente, para cada altitud mediana por especie realizamos una búsqueda de las coordenadas geográficas reportadas en el GBIF (<https://www.gbif.org/>). Con estas coordenadas y

utilizando los paquetes raster (Hijmans, 2021) y rgdal (Bivand et al., 2021) del software R versión 4.1, obtuvimos a partir de datos históricos (1970 – 2000) de cada localidad la estacionalidad promedio en precipitación, BIO15 - *Precipitation Seasonality (Coefficient of Variation)* (Booth et al. 2014). Empleamos las capas climáticas con resolución de 2.5 min de WorldClim versión 2.1 (<https://worldclim.org/>; Hijmans et al. 2005) (Suppl. Table S3).

Para estimar la relación entre altitud y estacionalidad (variables ambientales) y el SDI en *Atelopus*, utilizamos regresiones lineales múltiples (función lm en R, Bruce & Bruce 2017) y regresiones filogenéticas [función pgls, paquetes Caper (Orme et al. 2018), APE (Paradis & Schliep 2019), y Phytools (Revell 2012)]. Estos modelos de regresión se realizaron para cada uno de los rasgos morfológicos (ver Tabla 1, Suppl. Tabla S1). La variables ambientales y la hipótesis filogenética para el género *Atelopus* (Lötters et al. unpubl.) se incluyeron en los modelos como predictoras, mientras que la variable dependiente fueron los valores de SDI.

Clasificación Estadística de Grupos Fenéticos de Atelopus

Para analizar la relación entre los grupos fenéticos y el dimorfismo sexual en *Atelopus*, agrupamos las especies con base en su morfología, empleando el análisis multivariado de “vecino más cercano” (Mack & Rosenblatt 1979) en el software estadístico IBM - SPSS 25 (Mallery 2018). Esto se hizo con los 11 rasgos morfológicos (SVL incluido como control alométrico) cuantificados para los machos de cada especie. En este análisis de ordinación multivariado, obtuvimos el número de posibles agrupaciones fenéticas (K) y la probabilidad de que una especie pertenezca a cada uno de ellos, acorde a sus rasgos morfológicos ponderantes. Finalmente, las tres variables con mayor peso dentro de la clasificación en este análisis, se graficaron en un espacio multivariado.

Una vez realizada la clasificación estadística fenotípica, utilizamos una ANOVA filogenética (función `pgls`) para determinar las diferencias en la magnitud del dimorfismo sexual entre grupos fenéticos estimados de *Atelopus*. Además, para testear el efecto de la agrupación fenotípica, variables ambientales y filogenia sobre la magnitud del dimorfismo sexual en rasgos morfológicos de *Atelopus*, procedimos a realizar dos ANCOVAs filogenéticas (función `pgls`), empleando independientemente la altitud y luego la estacionalidad como co-variables. Finalmente, para reducir la colinealidad entre las variables ambientales (altitud y estacionalidad), ejecutamos un Análisis de Componentes Principales (PCA), obteniendo una meta-variable ambiental, la cual fue integrada junto a la agrupación fenotípica y filogenia como predictoras del SDI en una ANCOVA filogenética (función `pgls`).

RESULTADOS Y DISCUSIÓN

Dimorfismo Sexual en Tamaño Corporal vs Altitud y Estacionalidad del Hábitat.

Con información disponible del promedio en tamaño corporal para hembras y machos de 87 de las 99 especies descritas del género (Frost 2021), pudimos establecer un panorama general del dimorfismo sexual en tamaño corporal en *Atelopus* (Fig. 3A). La mayor frecuencia de tamaños corporales para las hembras se presenta entre 3.5 - 4 cm, aunque en algunas especies pueden alcanzar tamaños corporales de hasta 7 cm. En machos, los tamaños corporales (SVL) varían principalmente entre 2.5 - 3 cm y en ninguna especie sobrepasaron 5.5 cm (Fig. 3A). Como consecuencia de lo anterior, el índice de dimorfismo sexual en tamaño corporal (SDI en SVL) resultó en valores positivos (+). No obstante, la magnitud del SDI en SVL varió considerablemente entre las especies de *Atelopus*; desde especies casi monomórficas hasta especies extremadamente dimórficas en tamaño corporal (Fig. 3B, Suppl. Table S2).

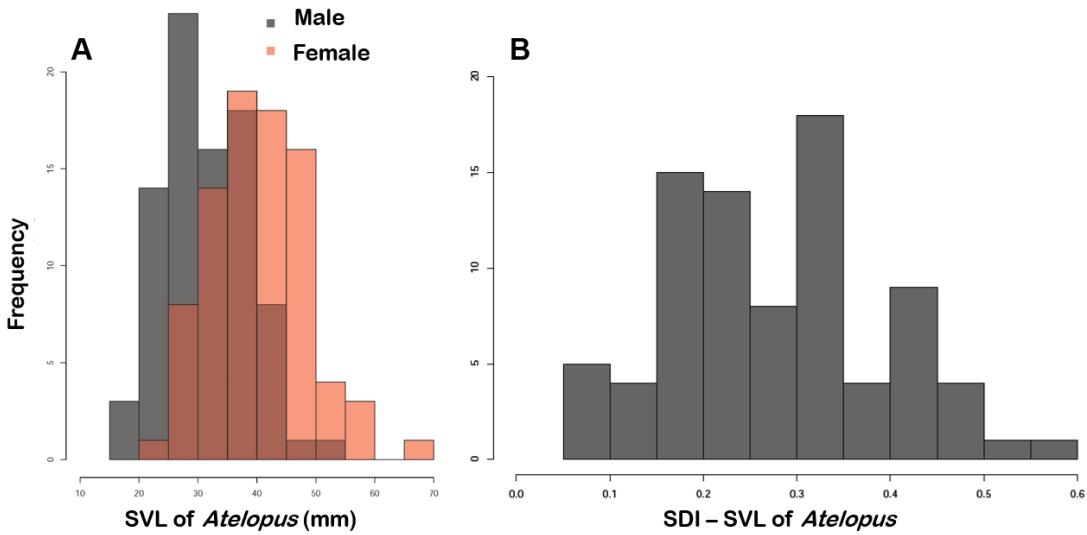


Figura 3. Frecuencias de los promedios en tamaños corporales de machos y hembras de 87 especies de *Atelopus* (A) y el índice de dimorfismo sexual (SDI) en tamaño corporal dentro de este género. SDI positivo (+) hace referencia a que las hembras superan a los machos.

Obtuvimos información de la altitud y estacionalidad para cada una de las localidades de 84 y 73 especies de *Atelopus*, respectivamente (Suppl. Tablas S2 y S3). Estas especies se distribuyen desde el nivel del mar hasta los 4800 m de elevación (Fig. 4A, Suppl. Tabla S2), lo cual incluye la presencia de especies de *Atelopus* en selvas húmedas tropical, bosque montano, puna y páramo (Lötters 1996; Rueda-Almonacid et al., 2005). Asociado a esta diversidad de hábitats, se evidenció una alta variación en el nivel de estacionalidad de condiciones ambientales en las cuales viven las especies de *Atelopus* (Fig. 4B, Suppl. Tabla S3). Con excepción de dos especies, las cuales parecen habitar ambientes altamente estacionales ($CV > 90\%$), la gran mayoría habita ambientes con CV entre 10 y 70% (CV) de estacionalidad en precipitación (Fig. 4B, Suppl. S3). La influencia de la altitud sobre el SDI en SVL

parece ser mínima ($R^2 = 0.06$, $F = 5.59$, $gl = 1$, $P = 0.02$; Fig. 4A), mientras que la influencia de la estacionalidad sobre SDI-SVL es inexistente ($R^2 = 0.00$, $F = 0.09$, $gl = 1$, $P = 0.75$; Fig. 4B).

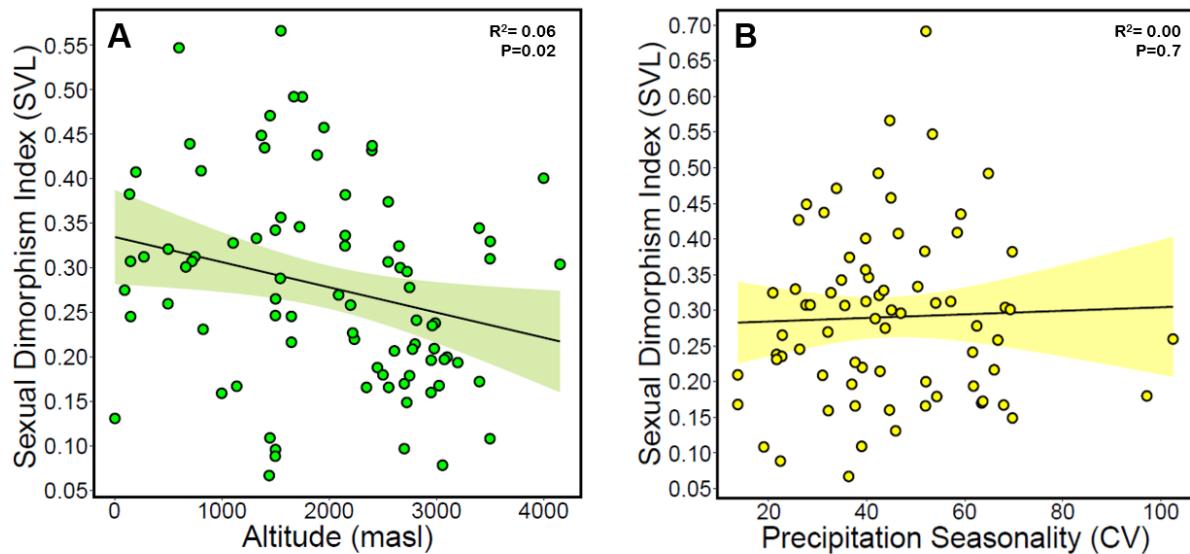


Figura 4. Relación de las variables ambientales, altitud (A) y estacionalidad (B), sobre el índice dimorfismo sexual (SDI) en SVL de machos y hembras de 84 y 73 especies de *Atelopus*, respectivamente. SDI positivo (+) hace referencia a que hembras superan a los machos. Coeficientes sin incluir efecto filogenético.

Dimorfismo Sexual en Robustez Extremidades y Otros Rasgos Corporales vs Altitud y Estacionalidad.

Para un sub-set de 12 especies incluidas en la hipótesis filogenética de *Atelopus* (Lötters et al. unpubl.), con las cuales obtuvimos datos de rasgos morfológicos corporales de 261 machos y 116 hembras, además, la altitud y estacionalidad de cada una de sus localidades (Tabla 1); evidenciamos que todos los rasgos morfológicos asociados a la robustez de las extremidades anteriores, y casi todos los demás rasgos corporales, obtuvieron valores negativos (-) en el SDI. En otras palabras, después de controlar por alometría, los valores de los rasgos morfológicos en machos superan los valores en hembras (tabla 1).

Tabla 1. Índice de dimorfismo sexual (SDI) en tamaño y otros rasgos corporales de 261 machos y 116 hembras, para un total de 377 individuos de 12 especies de *Atelopus* (ver detalles Suppl. Tabla S1). Valores SDI presentados con control alométrico. SDI positivo (+) hace referencia a que hembras superan a los machos, mientras que SDI negativo (-) los machos sobrepasan a las hembras. Grupo fenético estimado estadísticamente = "ignescens" o "longirostris".

Species	Estimated group	Median Altitude (m)	Seasonality precipitation (CV)	N Males	N Females	SDI - Body Size	SDI - Forearm width (wrist)	SDI - Forearm width (elbow)	SDI - Forearm width (middle)	SDI - Upper arm width	SDI - Upper arm length	SDI - Forearm Length	SDI - Arm length	SDI - Chest width	SDI - Head width	SDI - Distance between orbits	SDI - Eye diameter
<i>Atelopus arsyecue</i>	"ignescens"	2300	62.46	27	11	0.331	-0.097	-0.133	-0.258	-0.175	-0.072	-0.099	-0.070	-0.107	-0.113	0.091	-0.146
<i>Atelopus carrikeri</i>	"ignescens"	3500	68.25	22	3	0.149	-0.218	-0.288	-0.337	-0.270	-0.077	-0.033	-0.064	-0.089	-0.066	0.007	0.033
<i>Atelopus flavescens</i>	"longirostris"	40	52.08	3	4	0.076	-0.147	-0.040	-0.155	-0.095	-0.088	0.056	-0.012	0.085	-0.003	0.025	-0.071
<i>Atelopus glyphus</i>	"longirostris"	1600	50.41	6	2	0.392	-0.053	-0.276	-0.251	-0.181	0.054	-0.021	-0.024	0.071	-0.064	-0.152	-0.223
<i>Atelopus gracilis</i>	"longirostris"	150	28.47	11	10	0.357	-0.080	-0.164	-0.194	-0.144	-0.001	-0.182	-0.102	0.013	-0.028	0.118	-0.143
<i>Atelopus laetissimus</i>	"ignescens"	2200	66.76	70	23	0.498	-0.180	-0.262	-0.362	-0.205	-0.195	-0.180	-0.102	-0.005	-0.056	-0.054	-0.121
<i>Atelopus limosus</i>	"longirostris"	500	51.84	6	1	0.386	-0.167	-0.251	-0.280	-0.220	-0.004	0.041	-0.021	-0.058	-0.076	0.036	-0.166
<i>Atelopus nahumae</i>	"ignescens"	1500	69.70	23	10	0.401	-0.208	-0.258	-0.412	-0.136	0.033	-0.026	-0.032	-0.103	-0.124	-0.114	-0.151
<i>Atelopus peruvensis</i>	"ignescens"	3500	63.69	3	3	0.405	-0.229	-0.340	-0.338	-0.314	-0.191	-0.136	-0.109	-0.104	-0.081	-0.202	0.090
<i>Atelopus pulcher</i>	"longirostris"	350	21.63	7	3	0.131	0.006	-0.087	-0.091	-0.139	0.054	0.016	0.018	0.033	-0.063	0.069	-0.081
<i>Atelopus spurrelli</i>	"longirostris"	280	39.84	10	10	0.297	-0.140	-0.209	-0.219	-0.185	-0.006	-0.063	-0.019	-0.087	-0.140	-0.153	-0.257
<i>Atelopus subornatus</i>	"ignescens"	2500	36.48	9	10	0.395	-0.203	-0.339	-0.394	-0.246	-0.032	-0.058	-0.068	-0.094	-0.166	-0.180	-0.159

Los rasgos morfológicos asociados a la robustez de los brazos, tal como el ancho de antebrazo, están relacionados directamente a la defensa de la hembra amplexada y son adaptativos al sistema de apareamiento *lek-scramble* en *Atelopus* (ver capítulo 1). El dimorfismo sexual en rasgos de robustez de los brazos parece estar influenciado por la altitud y la estacionalidad (Figs. 5). La altitud está relacionada con todos los rasgos morfológicos asociados a la robustez de los brazos, tales como el ancho de antebrazo en el intermedio ($R^2 = 0.49, F = 9.8, gl = 1, P = 0.01$), codo ($R^2 = 0.47, F = 9.18, gl = 1, P = 0.01$), muñeca ($R^2 = 0.33, F = 4.93, gl = 1, P = 0.05$), y ancho del brazo superior ($R^2 = 0.62, F = 16.96, gl = 1, P = 0.002$). La estacionalidad solo se relacionó significativamente con ancho máximo de antebrazo (intermedio) ($R^2 = 0.43, F = 7.71, gl = 1, P = 0.01$) y en la muñeca ($R^2 = 0.44, F = 7.87, gl = 1, P = 0.01$), pero no con el ancho de antebrazo en el codo ($R^2 = 0.14, F = 1.63, gl = 1, P = 0.23$) ni con el ancho del brazo superior ($R^2 = 0.11, F = 1.25, gl = 1, P = 0.28$).

Al tener en cuenta la relación filogenética sobre la variación entre especies de los rasgos morfológicos y variables ambientales, los resultados siguen indicando influencia de las variables ambientales en el dimorfismo sexual de rasgos corporales en *Atelopus* (Fig. 5). Las hembras tienden a incrementar su tamaño corporal en relación al macho, a medida que se incrementa la altitud de las localidades donde habitan ($R^2 = 0.40, F = 6.75, gl = 1, P = 0.02$; Fig. 5A), mientras que, en los machos se acentúa la robustez de los brazos ($R^2 = 0.40, F = 6.75, gl = 1, P = 0.02$; Fig. 5B). La estacionalidad no se relacionó con el dimorfismo sexual en tamaño corporal ($R^2 = 0.12, F = 1.48, gl = 1, P = 0.25$; Fig. 5C) pero si se relacionó positivamente con el dimorfismo sexual en el ancho de antebrazo ($R^2 = 0.40, F = 7.60, gl = 1, P = 0.02$; Fig. 5D). La meta-variable ambiental (que contiene la co-variación entre altitud y estacionalidad) y la historia evolutiva (filogenia) de *Atelopus* muestran un mayor poder predictivo sobre el dimorfismo sexual en la robustez del brazo, medida en el ancho de antebrazo ($R^2 = 0.68, F = 21.87,$

$gl = 1, P = 0.0008$), que para el dimorfismo sexual en tamaño corporal ($R^2 = 0.29, F = 4.17, gl = 1, P = 0.06$).

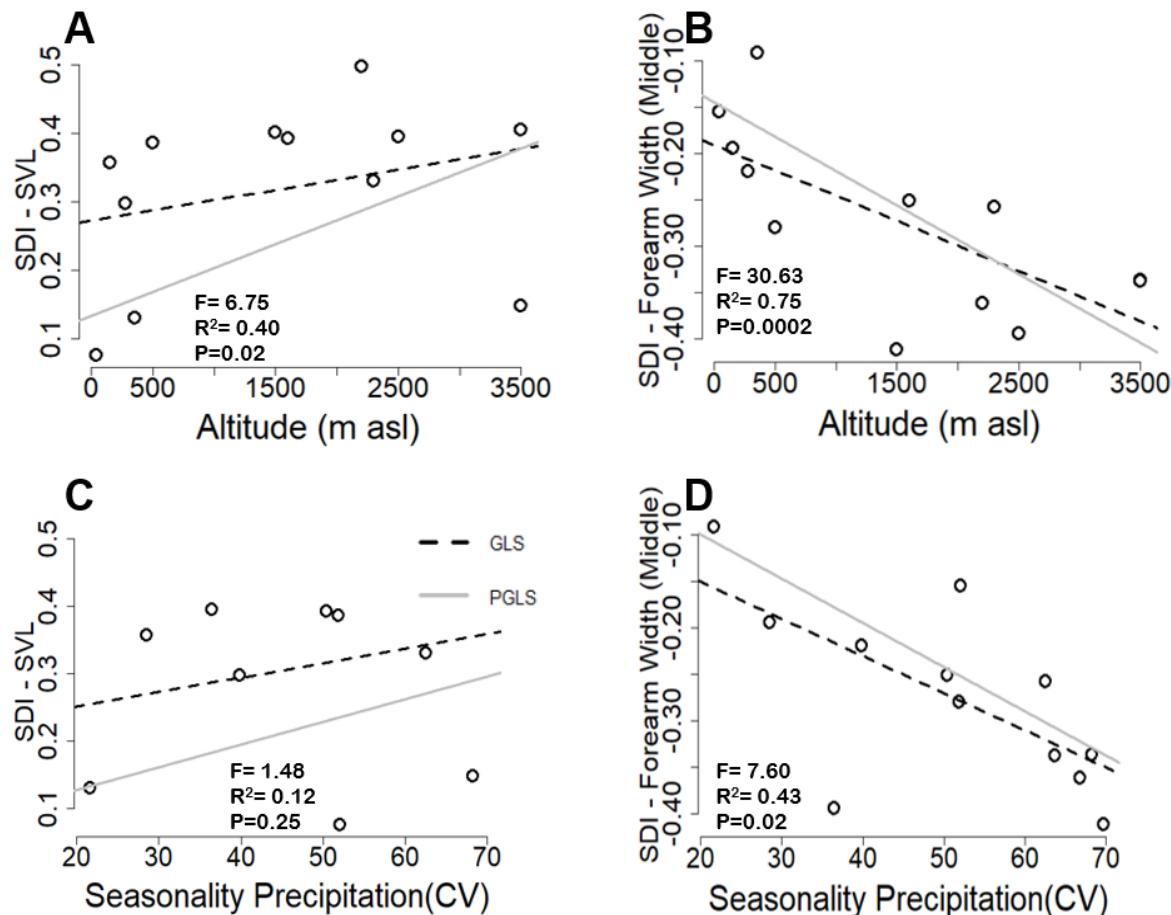


Figura 5. Regresiones filogenéticas de las variables ambientales sobre el índice dimorfismo sexual (SDI) en tamaño corporal y robustez de los brazos de las 12 especies de *Atelopus* listadas en la tabla 1. Relación de la altitud y el dimorfismo sexual en tamaño corporal (A) y ancho de antebrazo intermedio (B); Relación de la estacionalidad y el dimorfismo en tamaño (C) y ancho de antebrazo intermedio (D). SDI positivo (+) hace referencia a que hembras superan a los machos, mientras que SDI negativo (-) los machos sobrepasan a las hembras. Coeficientes incluyen efecto filogenético (PGLS, línea sólida); para comparar regresión sin efecto filogenético (GLS, línea punteada).

Los resultados son acorde a nuestras hipótesis y predicciones sobre selección sexual actuando de forma diferente en especies de tierras bajas y especies de tierras altas, contribuyendo a la evolución fenotípica en *Atelopus*. Especies de tierras bajas tienden al monomorfismo en talla corporal y robustez de los brazos, y a medida que aumenta la altitud y la estacionalidad en precipitación, las especies de *Atelopus* tienden a ser más dimórficas en estos mismos caracteres morfológicos (Fig. 5). En especies de *Atelopus* que habitan tierras altas y mayormente estacionales (e.i. menor estabilidad climática), las condiciones apropiadas para reproducirse pueden estar limitadas en el tiempo, tales como sitios de remanso en las quebradas en épocas de bajas precipitaciones (Karraker et al. 2006; Rocha-Usuga et al. 2017); en este escenario, la alta densidad de machos puede promover que ellos adopten una estrategia tipo *lek-scramble*, en la cual la defensa de la hembra amplexada es particularmente relevante para el fitness de los machos en *Atelopus* (ver capítulo 1). Por lo tanto, selección sexual promovería que machos evolucionaran antebrazos robustos y fuertes. Por el contrario, en especies que habitan tierras bajas con menor estacionalidad (e.i., mayor estabilidad climática) las condiciones favorables para reproducirse pueden estar disponibles en un mayor rango de tiempo, durante semanas y meses. Bajo estas condiciones, los individuos pueden exhibir un comportamiento tipo *lek-territorial*, y selección favorecer la evolución de mayores tamaños corporales en machos que les permita entre otras cosas, monopolizar recursos contenidos en pequeños territorios (Crump 1988; Lötters 1996; Lindquist & Hetherington 1998; Criswell 2008). Como consecuencia de esto, se daría una anatomía más cercana al monomorfismo entre machos y hembras. Futuros estudios de territorialidad en *Atelopus* de tierras bajas son necesarios para corroborar esta hipótesis.

Nuestro estudio demuestra la contribución de selección sexual sobre la evolución fenética en *Atelopus*, aunque no descartamos los efectos, a través la temperatura ambiental de las localidades, que la regla de Bergmann (Ashton 2002) y la de Allen (Rivas et al. 2018), tendrían sobre la morfología de este

linaje. Sin embargo, creemos que selección sexual jugaría un rol preponderante, pues las reglas Bergmann y Allen no predicen el dimorfismo sexual que documentamos para *Atelopus*.

Las estrategias de apareamiento adoptadas por las especies de *Atelopus* a través del gradiente altitudinal y estacional también impactarían en el desarrollo amplexos prolongados (“female guarding”, Wells 1977), los cuales están presentes en ambas estrategias de apareamiento, sin embargo, parecen ser mucho más extensos en especies de alta montaña (Dole & Durant 1974; Wells 1977; Rocha-Usuga et al. 2017, ver discusión del capítulo 1). Dado que, sí a lo largo gradiente ambiental aumenta la relevancia de una estrategia como la defensa de la hembra, mediante la robustez corporal, es esperado que este tipo de competencia macho-macho aumente el tiempo de guardia de compañero, al ser progresivamente más ventajoso y adaptativo amplexar cada vez más temprano a una hembra. Se requieren estudios detallados de la variación intra-generica de la duración del amplexo y como este aspecto estaría relacionado a condiciones ambientales y sociales, las cuales podrían variar con altitud y otras condiciones ambientales.

Agrupaciones Fenéticas, Dimorfismo Sexual, Altitud y Estacionalidad.

La estimación multivariada resultó en dos ($K=2$) grupos fenotípicos (Fig. 6, Tabla 1). Los rasgos de la robustez de los brazos fueron los más ponderantes en la clasificación de estos (Fig. 6). La probabilidad de pertenecer a uno de los dos grupos fue de 0.75 para cada especie, teniendo en cuenta la robustez de sus brazos. Etiquetamos a estos grupos fenéticos como “grupo ignescens” y “grupo longirostris”, debido a la concordancia en la clasificación de robustez de los brazos entre las dos agrupaciones fenéticas estimadas estadísticamente y la tradicional categorización fenética *ignescens* y *longirostris* en *Atelopus* (Peters, 1973) (Fig. 6, Tabla 1).

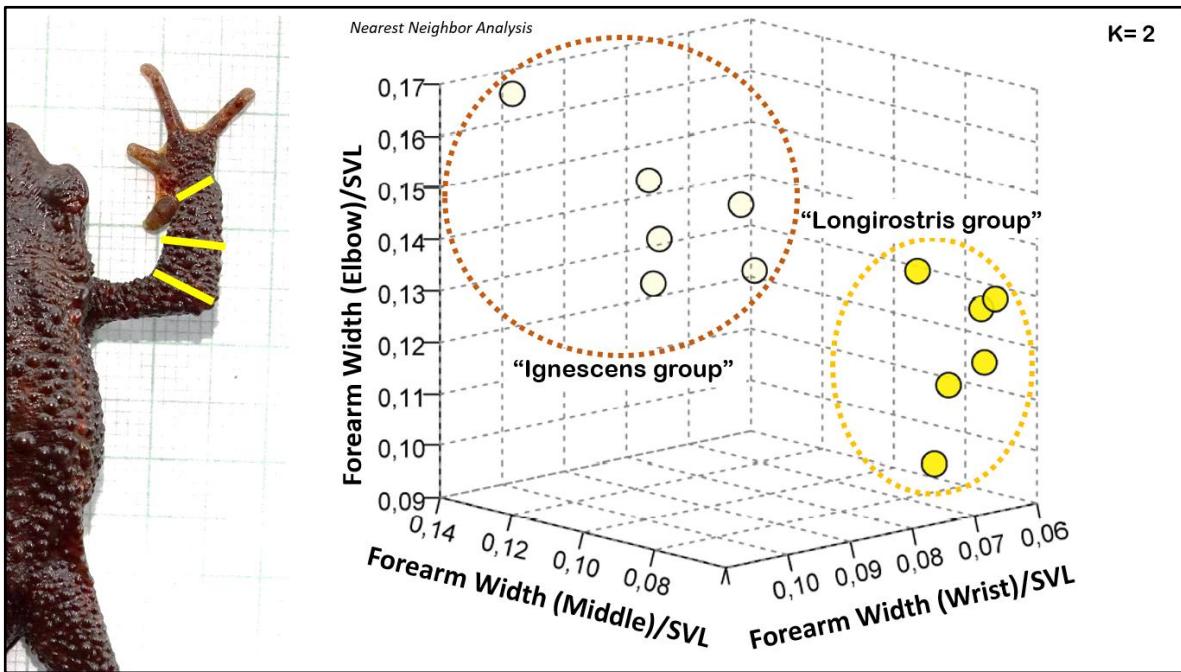


Figura 6. Grupos fenéticos estimados estadísticamente mediante análisis multivariado de “vecino más cercano”. La clasificación de los grupos estimados “ignescens” y “longirostris” fue mayormente según los rasgos morfológicos que representan la robustez del brazo.

Estos grupos fenéticos estimados presentan diferencias en los patrones de dimorfismo sexual dentro de *Atelopus* (Fig. 7). Las especies que se encuentran en el grupo fenético estimado “longirostris” presentan patrones de dimorfismo sexual en tamaño corporal menores a los del grupo fenético estimado “ignescens” (ANOVA filogenética; $R^2 = 0.37$, $t = -2.45$, $gl = 1$, $P = 0.03$). Es decir, las especies del “grupo longirostris” tienden a exhibir un dimorfismo sexual desde moderado hasta cercano al monomorfismo en tamaño corporal, mientras que especies clasificadas en el “grupo ignescens” tienden a ostentar dimorfismo sexual en tamaño corporal de moderado a marcado (Fig. 7A). De igual forma, el patrón de dimorfismo sexual en robustez del brazo, muestra a las especies pertenecientes

del grupo estimado “ignescens”, con valores más acentuados en comparación con especies pertenecientes al grupo estimado “longirostris” ($R^2 = 0.78$, $t = -5.99$, $gl = 1$, $P = 0.0001$) (Fig. 7B). Las especies de “longirostris” tienden al monomorfismo en robustez en brazos, hasta moderadamente dimórficas, mientras que en “ignescens” la tendencia es a contener especies moderadamente dimórficas hasta extremadamente dimórficas. La agrupación fenética en *Atelopus* explicó mejor la varianza en la magnitud de dimorfismo sexual en la robustez de los brazos (78%) que para el dimorfismo sexual en tamaño corporal (37%) (Fig. 7A, B).

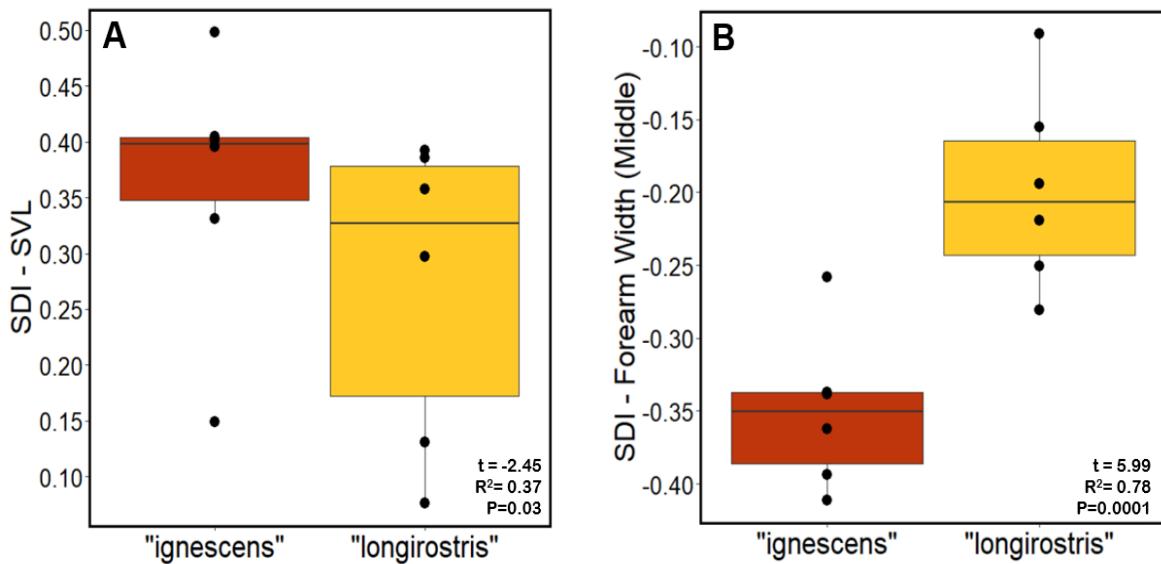


Figura 7. Comparación de la magnitud del índice de dimorfismo sexual en tamaño (A) y robustez de los brazos, medida en el ancho de antebrazo intermedio (B) entre los grupos fenéticos estimados “ignescens” y “longirostris”. Coeficientes incluyen efecto filogenético (PGLS).

Los grupos fenéticos al coviar con la altitud y la estacionalidad tienen un gran poder predictivo sobre el dimorfismo sexual en *Atelopus* (Fig. 8). Cuando estos grupos fenéticos se analizan con respecto a la altitud, la magnitud del dimorfismo sexual en tamaño corporal es explicado en un 40% (ANCOVA filogenética; $R^2 = 0.40$, $F = 6.75$, $gl = 2$, $P = 0.02$; Fig. 8A); mientras que, el dimorfismo en la robustez del brazo, medido en el ancho del antebrazo intermedio, es explicado en un 81% ($R^2 = 0.81$, $F = 20.35$, $gl = 2$, $P = 0.0004$; Fig. 8B). Por otra parte, cuando los grupos fenéticos se analizan con respecto a estacionalidad, no encontramos relación sobre el dimorfismo sexual en tamaño corporal ($R^2 = 0.12$, $F = 1.48$, $gl = 2$, $P = 0.25$; Fig. 8C), pero si una fuerte relación y poder predictivo para la robustez del brazo ($R^2 = 0.79$, $F = 16.98$, $gl = 2$, $P = 0.0008$; Fig. 8D). Finalmente, al integrar la agrupación fenotípica, el efecto filogenético, y las variables ambientales (previa reducción de la colinealidad entre la altitud y la estacionalidad, ejecutando un PCA y obteniendo una meta-variable ambiental); observamos que este modelo explica mejor la variación en el dimorfismo en ancho de antebrazo ($R^2 = 0.80$; $F = 18.88$, $gl = 1$, $P = 0.000$; Fig. 9A), que el dimorfismo en tamaño corporal ($R^2 = 0.37$; $F = 2.74$, $gl = 1$, $P = 0.11$; Fig. 9B).

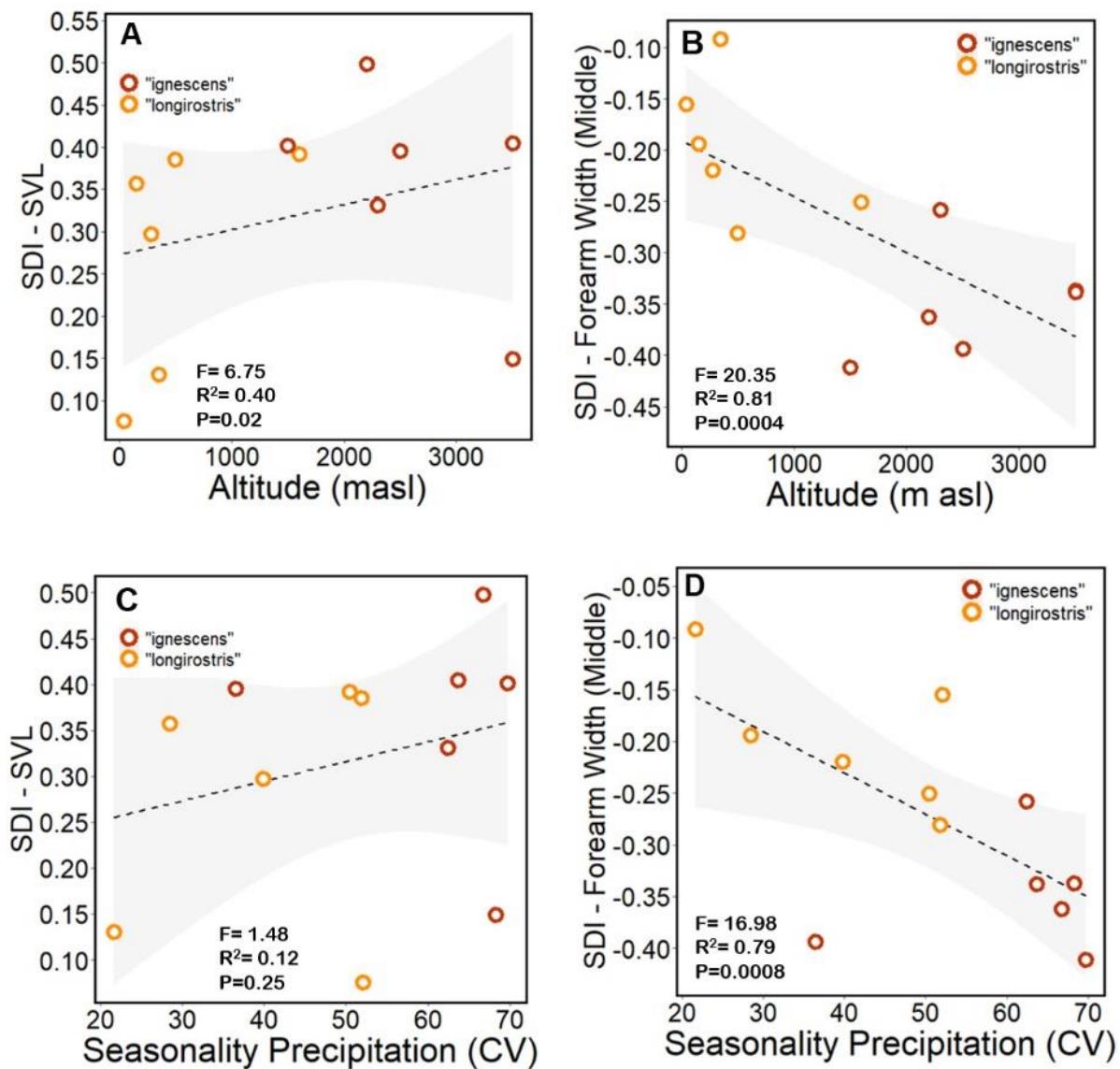


Figura 8. Relación entre variables ambientales (altitud y estacionalidad en precipitación) y grupos fenéticos estimados ("ignescens" y "longirostris") sobre el índice de dimorfismo sexual (SDI) en tamaño corporal y robustez de los brazos en *Atelopus*. Covariación entre los grupos fenéticos y la altitud sobre el dimorfismo sexual en talla (A) y ancho de antebrazo intermedio (B); Covariación entre los grupos fenéticos y estacionalidad sobre el dimorfismo en talla (C) y ancho de antebrazo intermedio (D). Coeficientes incluyen efecto filogenético (PGLS).

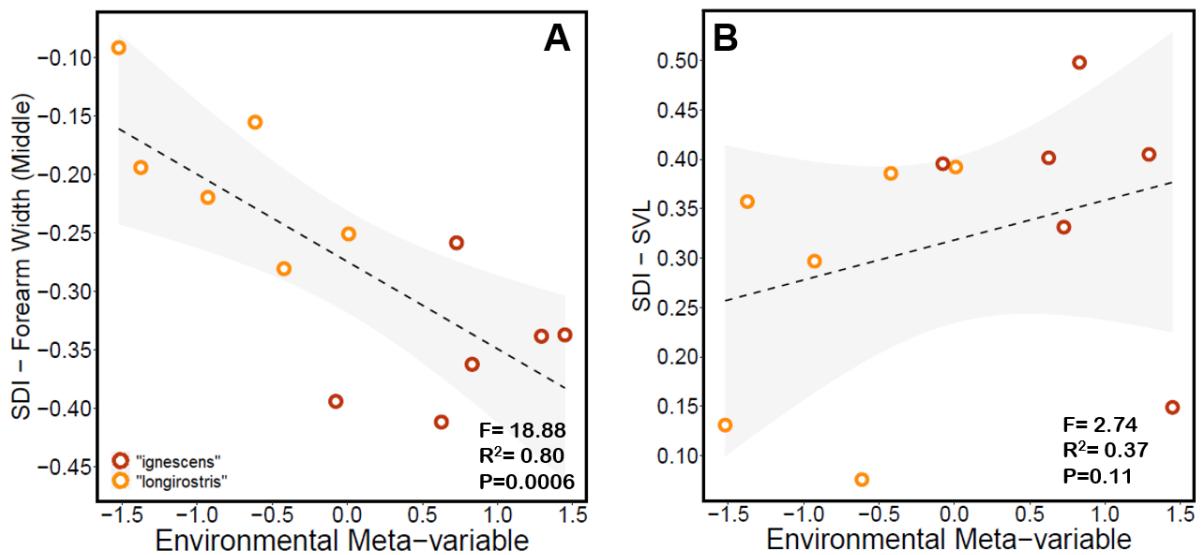


Figura 9. Relación entre la meta-variable ambiental, grupos fenéticos (“ignescens” y “longirostris”) y el dimorfismo sexual en talla corporal y robustez de los brazos del clado *Atelopus*. Covariación entre los grupos fenéticos y la meta-variable ambiental sobre el dimorfismo sexual en ancho de antebrazo intermedio (A) y tamaño corporal (B). Coeficientes incluyen efecto filogenético (PGLS).

CONCLUSIONES

Los patrones de dimorfismo resultantes de la integración de los grupos fenéticos estimados y las variables ambientales, son congruentes con nuestras predicciones sobre la influencia de selección sexual sobre los rasgos corporales dentro de *Atelopus*. Lo anterior apoya la hipótesis sobre el reflejo causado por la intensidad de la selección sexual operando mediante las estrategias de apareamiento en la evolución morfológica en *Atelopus*.

Las formas corporales conocidas como grupos fenéticos *ignescens* y *longirostris* podrían considerarse, en parte, como el reflejo de estrategias divergentes de competencia macho-macho en *Atelopus*. Las especies del grupo fenético *longirostris* (tierras bajas) exhiben patrones de dimorfismo sexual no marcado en tamaño corporal ni en robustez de las extremidades anteriores (tendencia monomórfica en estos rasgos), las cuales podrían presentar una estrategia de apareamiento cercana a la territorialidad (o lek cercana a la territorialidad), en el cual rasgos como tamaño corporal u otros parámetros comportamentales confieran mayor éxito de apareo a los machos. Por el contrario, las especies del grupo fenético *ignescens* (tierras altas) exhiben patrones de dimorfismo marcado en tamaño corporal y robustez de las extremidades anteriores (tendencia dimórfica en estos rasgos), las cuales presentan una estrategia de apareamiento lek cercana a scramble; dentro de esta estrategia un mayor tamaño corporal y brazos más robustos son rasgos adaptativos y confieren mayor éxito reproductivo a los machos (ver capítulo 1).

Este estudio muestra evidencia de un continuo en los niveles de competencia macho-macho en *Atelopus*, promoviendo mayor dimorfismo en tamaño corporal y robustez de los brazos. Esto corresponde a la transición de las estrategias de apareamiento a lo largo del gradiente altitudinal y estacionalidad, desde cercanas a la defensa de recursos en especies de tierras bajas, hasta la competencia por el amplexo en especies de montaña, generando paralelismos fenéticos entre especies de *Atelopus* como resultado de la presión de selección en ambientes similares.

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Supplementary Table S1. Promedio, \pm SD y (Mínimo – Máximo) de 3444 medidas corporales (en cm) de ejemplares de colecciones e in-situ para 261 machos y 116 hembras, para un total de 377 individuos de 12 especies de *Atelopus* incluidas en el análisis de índice de dimorfismo sexual de rasgos corporales.

Species	Males SVL	Females SVL	Males Forearm Width (Wrist)	Females Forearm Width (Wrist)	Males Forearm Width (Elbow)	Females Forearm Width (Elbow)	Males Forearm Width (Middle)	Females Forearm Width (Middle)	Males Upper Arm Width	Females Upper Arm Width	Males Upper Arm Length	Female Upper Arm Length	Males Forearm Length	Females Forearm Length	Males Arm Length	Females Arm Length	Males Chest Width	Females Chest Width	Males Head Width	Females Head Width	Males Distance between eyes	Females Distance between eyes	Males Eye diameter	Females Eye diameter
<i>Atelopus arsyecue</i>	5.11 \pm 0.19 (4.67 – 5.64)	6.80 \pm 0.29 (6.29 – 7.23)	0.46 \pm 0.02 (0.41 – 0.52)	0.556 \pm 0.04 (0.45 – 0.61)	0.66 \pm 0.04 (0.58 – 0.76)	0.77 \pm 0.06 (0.67 – 0.89)	0.58 \pm 0.04 (0.51 – 0.65)	0.57 \pm 0.03 (0.51 – 0.64)	0.50 \pm 0.04 (0.43 – 0.58)	0.55 \pm 0.03 (0.50 – 0.61)	1.12 \pm 0.07 (0.97 – 1.33)	1.38 \pm 0.10 (1.19 – 1.3)	1.15 \pm 0.05 (1.02 – 1.28)	1.38 \pm 0.10 (1.19 – 1.62)	2.55 \pm 0.11 (2.32 – 2.80)	3.16 \pm 0.14 (2.95 – 3.37)	1.60 \pm 0.07 (1.40 – 1.78)	1.91 \pm 0.12 (1.76 – 2.20)	1.61 \pm 0.06 (1.49 – 1.73)	1.90 \pm 0.07 (1.80 – 2.02)	0.57 \pm 0.12 (0.40 – 0.88)	0.83 \pm 0.07 (0.69 – 0.91)	0.59 \pm 0.03 (0.52 – 0.66)	0.67 \pm 0.04 (0.63 – 0.75)
<i>Atelopus carrikeri</i>	4.70 \pm 0.29 (3.93 – 5.11)	5.41 \pm 0.37 (4.9 – 5.66)	0.40 \pm 0.03 (0.35 – 0.46)	0.36 \pm 0.01 (0.36 – 0.38)	0.64 \pm 0.09 (0.52 – 0.81)	0.53 \pm 0.07 (0.45 – 0.59)	0.55 \pm 0.06 (0.49 – 0.68)	0.42 \pm 0.08 (0.37 – 0.52)	0.46 \pm 0.05 (0.38 – 0.57)	0.39 \pm 0.00 (0.38 – 0.39)	0.94 \pm 0.14 (0.73 – 1.2)	0.99 \pm 0.00 (0.99 – 1.00)	0.94 \pm 0.11 (0.77 – 1.12)	1.04 \pm 0.14 (0.95 – 1.21)	2.04 \pm 0.27 (1.75 – 2.78)	2.20 \pm 0.13 (2.11 – 2.35)	1.32 \pm 0.19 (1.03 – 1.73)	1.38 \pm 0.30 (1.15 – 1.73)	1.40 \pm 0.17 (1.15 – 1.66)	1.51 \pm 0.22 (1.37 – 1.77)	0.44 \pm 0.06 (0.30 – 0.56)	0.51 \pm 0.12 (0.50 – 0.53)	0.59 \pm 0.07 (0.46 – 0.72)	0.70 \pm 0.04 (0.66 – 0.75)
<i>Atelopus flavescens</i>	3.39 \pm 0.34 (3.0 – 3.68)	3.64 \pm 0.24 (3.33 – 3.89)	0.24 \pm 0.02 (0.20 – 0.26)	0.22 \pm 0.01 (0.19 – 0.23)	0.33 \pm 0.02 (0.32 – 0.35)	0.34 \pm 0.04 (0.27 – 0.39)	0.23 \pm 0.02 (0.20 – 0.25)	0.21 \pm 0.02 (0.18 – 0.23)	0.21 \pm 0.01 (0.19 – 0.22)	0.20 \pm 0.02 (0.18 – 0.23)	0.78 \pm 0.14 (0.61 – 0.87)	0.76 \pm 0.05 (0.70 – 0.82)	0.80 \pm 0.16 (0.62 – 0.95)	0.91 \pm 0.07 (0.81 – 0.99)	1.52 \pm 0.26 (1.25 – 1.77)	1.62 \pm 0.13 (1.46 – 1.75)	1.12 \pm 0.05 (1.31 – 1.18)	1.31 \pm 0.09 (1.21 – 1.40)	0.98 \pm 0.09 (0.89 – 1.08)	1.06 \pm 0.03 (1.03 – 1.10)	0.48 \pm 0.02 (0.45 – 0.50)	0.53 \pm 0.05 (0.48 – 0.58)	0.38 \pm 0.02 (0.36 – 0.41)	0.38 \pm 0.04 (0.33 – 0.43)
<i>Atelopus glyphus</i>	3.87 \pm 0.19 (3.61 – 4.16)	5.39 \pm 0.13 (5.29 – 5.48)	0.24 \pm 0.02 (0.21 – 0.27)	0.32 \pm 0.01 (0.31 – 0.33)	0.50 \pm 0.03 (0.48 – 0.57)	0.51 \pm 0.06 (0.46 – 0.55)	0.33 \pm 0.04 (0.29 – 0.39)	0.35 \pm 0.03 (0.32 – 0.37)	0.30 \pm 0.01 (0.28 – 0.32)	0.34 \pm 0.03 (0.31 – 0.36)	0.97 \pm 0.10 (0.76 – 1.09)	1.42 \pm 0.07 (1.37 – 1.47)	0.88 \pm 0.06 (0.78 – 0.97)	1.20 \pm 0.01 (1.19 – 1.21)	1.79 \pm 0.13 (1.64 – 2.01)	2.43 \pm 0.03 (2.40 – 2.45)	1.06 \pm 0.14 (0.94 – 1.28)	1.58 \pm 0.01 (1.57 – 1.59)	1.05 \pm 0.03 (1.01 – 1.11)	1.37 \pm 0.11 (1.29 – 1.45)	0.42 \pm 0.04 (0.34 – 0.47)	0.49 \pm 0.07 (0.44 – 0.54)	0.42 \pm 0.04 (0.37 – 0.47)	0.46 \pm 0.01 (0.45 – 0.47)
<i>Atelopus gracilis</i>	2.58 \pm 0.10 (2.44 – 2.84)	3.51 \pm 0.14 (3.33 – 3.80)	0.16 \pm 0.01 (0.14 – 0.18)	0.20 \pm 0.01 (0.16 – 0.22)	0.32 \pm 0.04 (0.26 – 0.39)	0.37 \pm 0.06 (0.31 – 0.53)	0.18 \pm 0.01 (0.14 – 0.20)	0.20 \pm 0.01 (0.17 – 0.22)	0.15 \pm 0.02 (0.11 – 0.18)	0.17 \pm 0.01 (0.15 – 0.19)	0.68 \pm 0.06 (0.58 – 0.81)	0.92 \pm 0.09 (0.74 – 1.06)	0.65 \pm 0.07 (0.52 – 0.78)	0.72 \pm 0.06 (0.62 – 0.83)	1.32 \pm 0.12 (1.14 – 1.59)	1.61 \pm 0.35 (0.99 – 1.99)	0.68 \pm 0.06 (0.60 – 0.81)	0.94 \pm 0.09 (0.87 – 1.20)	0.70 \pm 0.03 (0.65 – 0.79)	0.92 \pm 0.05 (0.84 – 0.98)	0.23 \pm 0.02 (0.19 – 0.28)	0.35 \pm 0.05 (0.27 – 0.47)	0.28 \pm 0.02 (0.23 – 0.32)	0.33 \pm 0.03 (0.28 – 0.38)
<i>Atelopus laetissimus</i>	3.93 \pm 0.20 (3.52 – 4.38)	5.88 \pm 0.29 (5.29 – 6.53)	0.34 \pm 0.02 (0.3 – 0.41)	0.42 \pm 0.02 (0.38 – 0.48)	0.58 \pm 0.04 (0.49 – 0.66)	0.64 \pm 0.06 (0.52 – 0.73)	0.47 \pm 0.04 (0.36 – 0.56)	0.45 \pm 0.03 (0.41 – 0.54)	0.41 \pm 0.02 (0.36 – 0.47)	0.49 \pm 0.03 (0.44 – 0.55)	1.04 \pm 0.08 (0.82 – 1.22)	1.26 \pm 0.10 (1.06 – 1.43)	0.95 \pm 0.05 (0.82 – 1.09)	1.17 \pm 0.08 (0.98 – 1.31)	2.18 \pm 0.14 (1.71 – 2.45)	2.94 \pm 0.16 (2.69 – 3.18)	1.25 \pm 0.07 (1.07 – 1.41)	1.87 \pm 0.10 (1.75 – 2.21)	1.30 \pm 0.06 (1.17 – 1.44)	1.84 \pm 0.09 (1.61 – 1.93)	0.45 \pm 0.05 (0.32 – 0.56)	0.64 \pm 0.05 (0.55 – 0.74)	0.51 \pm 0.04 (0.43 – 0.64)	0.68 \pm 0.04 (0.6 – 0.75)
<i>Atelopus limosus</i>	3.14 \pm 0.07 (3.04 – 3.21)	4.35	0.191 \pm 0.01 (0.16 – 0.20)	0.22	0.36 \pm 0.02 (0.33 – 0.39)	0.37	0.23 \pm 0.02 (0.20 – 0.26)	0.23	0.21 \pm 0.02 (0.18 – 0.23)	0.22	0.76 \pm 0.06 (0.69 – 0.85)	1.05	0.69 \pm 0.04 (0.63 – 0.73)	0.99	1.42 \pm 0.05 (1.36 – 1.51)	1.92	0.89 \pm 0.06 (0.77 – 0.97)	1.16	0.88 \pm 0.04 (0.79 – 0.94)	1.12	0.32 \pm 0.04 (0.26 – 0.4)	0.46	0.35 \pm 0.00 (0.35 – 0.37)	0.41
<i>Atelopus nahumae</i>	3.87 \pm 0.27 (3.22 – 4.34)	5.48 \pm 0.32 (4.9 – 5.89)	0.30 \pm 0.03 (0.23 – 0.34)	0.33 \pm 0.02 (0.30 – 0.37)	0.50 \pm 0.06 (0.36 – 0.59)	0.52 \pm 0.03 (0.47 – 0.59)	0.42 \pm 0.07 (0.26 – 0.54)	0.34 \pm 0.03 (0.29 – 0.42)	0.35 \pm 0.05 (0.27 – 0.46)	0.43 \pm 0.06 (0.30 – 0.51)	0.86 \pm 0.09 (0.69 – 1.05)	1.24 \pm 0.08 (1.08 – 1.35)	0.84 \pm 0.06 (0.71 – 0.96)	1.14 \pm 0.03 (1.09 – 1.20)	1.80 \pm 0.14 (1.52 – 2.05)	2.44 \pm 0.16 (1.98 – 2.57)	1.22 \pm 0.10 (1.01 – 1.37)	1.54 \pm 0.09 (1.39 – 1.71)	1.19 \pm 0.09 (1.00 – 1.34)	1.47 \pm 0.08 (1.31 – 1.56)	0.43 \pm 0.04 (0.33 – 0.50)	0.54 \pm 0.04 (0.49 – 0.66)	0.47 \pm 0.04 (0.36 – 0.53)	0.56 \pm 0.05 (0.46 – 0.62)
<i>Atelopus peruvensis</i>	3.34 \pm 0.18 (3.15 – 3.51)	4.69 \pm 0.25 (4.46 – 4.96)	0.34 \pm 0.04 (0.32 – 0.39)	0.37 \pm 0.02 (0.34 – 0.39)	0.56 \pm 0.01 (0.54 – 0.57)	0.52 \pm 0.04 (0.47 – 0.56)	0.43 \pm 0.03 (0.40 – 0.47)	0.40 \pm 0.03 (0.36 – 0.43)	0.37 \pm 0.01 (0.36 – 0.39)	0.36 \pm 0.05 (0.29 – 0.41)	0.59 \pm 0.04 (0.56 – 0.64)	0.68 \pm 0.11 (0.57 – 0.8)	0.79 \pm 0.04 (0.74 – 0.82)	0.96 \pm 0.03 (0.93 – 0.99)	1.59 \pm 0.01 (1.58 – 1.60)</td									

Supplementary Tabla 2. Índice de dimorfismo sexual en talla corporal y altitudes en *Atelopus* (Lötters 1996; Rueda-Almonacid et al. 2005; Frost 2021). *(Tabatinga - Amazonas, Brasil)

Species	Mean SVL Females_mm	Mean SVL Males_mm	SDI Mean SVL	Altitude Min masl	Altitude Max masl	Altitude Median masl
<i>Atelopus andinus</i>	34.03	27.30	0.25	1000	2000	1500
<i>Atelopus angelito</i>	41.00	34.27	0.20	2900	3000	2950
<i>Atelopus ardila</i>	46.60	38.37	0.21	2800	2800	2800
<i>Atelopus arsyecue</i>	56.67	44.33	0.28	2000	3500	2750
<i>Atelopus balios</i>	35.90	28.50	0.26	350	650	500
<i>Atelopus barbotini</i>	36.60	26.00	0.41	200	200	200
<i>Atelopus bomolochos</i>	51.00	38.50	0.32	2500	2800	2650
<i>Atelopus boulengeri</i>	65.17	44.30	0.47	900	2000	1450
<i>Atelopus carauta</i>	44.45	34.50	0.29	1300	1790	1545
<i>Atelopus carbonerensis</i>	50.10	42.97	0.17	2100	2600	2350
<i>Atelopus carrikeri</i>	57.25	43.90	0.30	3500	4800	4150
<i>Atelopus certus</i>	42.00	32.00	0.31	500	1000	750
<i>Atelopus chiriquiensis</i>	47.00	31.50	0.49	1400	2100	1750
<i>Atelopus chrysocorallus</i>	48.60	40.90	0.19	2200	2700	2450
<i>Atelopus coynei</i>	32.10	27.50	0.17	900	1380	1140
<i>Atelopus cruciger</i>	41.00	26.50	0.55	100	1100	600
<i>Atelopus dimorphus</i>	31.30	23.25	0.35	1650	1800	1725
<i>Atelopus ebenoides</i>	48.00	40.00	0.20	2500	3700	3100
<i>Atelopus elegans</i>	33.15	25.35	0.31	300	1140	720
<i>Atelopus sp. cf. erythrops</i>	28.00	20.95	0.34	1800	2500	2150
<i>Atelopus eusebianus</i>	41.85	33.80	0.24	2990	2990	2990
<i>Atelopus exiguus</i>	32.05	24.10	0.33	3150	3850	3500
<i>Atelopus famelicus</i>	40.55	38.00	0.07	1300	1580	1440
<i>Atelopus farci</i>	36.25	28.55	0.27	2090	-	2090
<i>Atelopus flavescens</i>	36.40	33.90	0.07	10	70	40
<i>Atelopus franciscus</i>	25.45	22.50	0.13	5	5	5
<i>Atelopus galactogaster</i>	41.00	37.40	0.10	1500	1500	1500
<i>Atelopus gigas</i>	50.25	45.80	0.10	2700	2700	2700
<i>Atelopus gracilis</i>	33.15	25.35	0.31	0	300	150
<i>Atelopus glyphus</i>	48.00	36.00	0.33	1200	1445	1322.5
<i>Atelopus guanujo</i>	40.50	33.50	0.21	2600	2950	2775
<i>Atelopus guitarraensis</i>	39.00	29.00	0.34	3400	3400	3400
<i>Atelopus halihelos</i>	36.10	25.30	0.43	1875	1900	1887.5
<i>Atelopus hoogmoedi</i>	36.40	27.55	0.32	500	500	500
<i>Atelopus ignescens</i>	41.90	37.80	0.11	2500	4500	3500
<i>Atelopus laetissimus</i>	46.75	37.15	0.26	1500	2900	2200
<i>Atelopus limosus</i>	39.55	28.60	0.38	10	270	140
<i>Atelopus longirostris</i>	43.90	32.70	0.34	500	2500	1500
<i>Atelopus lozanoi</i>	38.00	29.00	0.31	3100	3900	3500
<i>Atelopus lynchii</i>	50.00	37.65	0.33	800	1410	1105
<i>Atelopus manauensis</i>	28.35	22.23	0.28	61	125	93

Species	Mean SVL Females_mm	Mean SVL Males_mm	SDI Mean SVL	Altitude Min masl	Altitude Max masl	Altitude Median masl
<i>Atelopus mandingues</i>	36.00	29.00	0.24	2580	3050	2815
<i>Atelopus marinkellei</i>	48.00	44.50	0.08	2660	3450	3055
<i>Atelopus mindoensis</i>	27.86	19.42	0.43	700	2100	1400
<i>Atelopus minutulus</i>	25.10	18.50	0.36	1500	1600	1550
<i>Atelopus mittermeieri</i>	43.14	33.18	0.30	2525	2800	2662.5
<i>Atelopus monohernandezii</i>	37.90	26.00	0.46	1700	2200	1950
<i>Atelopus mucubajensis</i>	41.95	35.85	0.17	2300	3100	2700
<i>Atelopus muisca</i>	38.80	32.50	0.19	2900	3500	3200
<i>Atelopus nahumae</i>	48.10	34.80	0.38	1500	2800	2150
<i>Atelopus nanay</i>	35.65	25.45	0.40	4000	4000	4000
<i>Atelopus nepiozomus</i>	32.40	25.00	0.30	2000	3450	2725
<i>Atelopus nicefori</i>	24.40	20.00	0.22	1800	2670	2235
<i>Atelopus nocturnus</i>	33.65	22.55	0.49	1670	1670	1670
<i>Atelopus onorei</i>	44.90	38.05	0.18	2500	2500	2500
<i>Atelopus orcesi</i>	42.10	29.40	0.43	2400	2400	2400
<i>Atelopus oxyrhynchus</i>	47.40	41.25	0.15	2100	3350	2725
<i>Atelopus pachydermus</i>	58.75	50.30	0.17	2750	3300	3025
<i>Atelopus palmatus</i>	29.70	20.50	0.45	1000	1740	1370
<i>Atelopus pastuso</i>	40.15	32.50	0.24	2200	3720	2960
<i>Atelopus patazensis</i>	45.75	38.80	0.18	2500	3000	2750
<i>Atelopus peruvensis</i>	41.80	35.65	0.17	2800	4000	3400
<i>Atelopus petersi</i>	46.75	38.65	0.21	2660	3300	2980
<i>Atelopus pictiventris</i>	36.10	27.25	0.32	1700	2600	2150
<i>Atelopus pinangoi</i>	38.20	31.65	0.21	2300	2920	2610
<i>Atelopus planispina</i>	34.35	31.55	0.09	1000	2000	1500
<i>Atelopus podocarpus</i>	46.20	37.65	0.23	1014	3423	2218.5
<i>Atelopus pulcher</i>	33.55	27.25	0.23	450	1200	825
<i>Atelopus quimbaya</i>	34.50	26.40	0.31	2200	2900	2550
<i>Atelopus reticulatus</i>	27.40	24.70	0.11	1300	1600	1450
<i>Atelopus seminiferus</i>	40.00	34.50	0.16	1000	1000	1000
<i>Atelopus senex</i>	36.50	30.00	0.22	1100	2200	1650
<i>Atelopus sernai</i>	27.15	23.40	0.16	2800	3100	2950
<i>Atelopus simulatus</i>	34.35	23.90	0.44	2000	2800	2400
<i>Atelopus sonsonensis</i>	31.00	24.50	0.27	1500	1500	1500
<i>Atelopus sorianoi</i>	46.35	39.75	0.17	2400	2710	2555
<i>Atelopus spumarius*</i>	31.20	25.05	0.25	0	300	150
<i>Atelopus surrelli</i>	42.00	32.00	0.31	50	500	275
<i>Atelopus subornatus</i>	38.00	27.65	0.37	2300	2800	2550
<i>Atelopus tamaense</i>	44.90	37.50	0.20	2950	3200	3075
<i>Atelopus tricolor</i>	32.50	20.75	0.57	600	2500	1550
<i>Atelopus varius</i>	46.50	33.00	0.41	16	1600	808
<i>Atelopus vogli</i>	36.20	25.15	0.44	700	700	700
<i>Atelopus walkeri</i>	47.40	38.05	0.25	1500	1800	1650
<i>Atelopus zeteki</i>	54.00	41.50	0.30	430	900	665

Supplementary Tabla S3. Variables climáticas de WorldClim 2.1 (datos históricos - 1970-2000; resolución espacial 2.5 min) extraídas de localidades reportadas en GBIF para especies de *Atelopus*.

Species	BIO1 Average Annual Temperature (° C)	BIO12 Annual Precipitation (mm)	BIO15 Seasonality precipitation (CV)	BIO16 Precipitation of wettest quarter (mm)	BIO17 Precipitation of driest quarter (mm)
<i>Atelopus angelito</i>	9.14	1799	36.96	577	271
<i>Atelopus ardila</i>	13.29	1422	42.72	515	162
<i>Atelopus arsyecue</i>	10.47	1595	62.46	623	87
<i>Atelopus balios</i>	23.78	1066	102.50	657	44
<i>Atelopus barbotini</i>	24.53	2692	46.43	1051	284
<i>Atelopus bomolochos</i>	10.08	1062	20.85	343	212
<i>Atelopus boulengeri</i>	14.11	1340	33.82	490	202
<i>Atelopus carauta</i>	18.99	2619	41.74	885	271
<i>Atelopus carbonerensis</i>	22.38	1396	37.64	460	169
<i>Atelopus carrikeri</i>	10.16	1579	68.25	653	68
<i>Atelopus certus</i>	26.02	3018	57.15	1207	258
<i>Atelopus chiriquiensis</i>	26.94	2177	64.82	934	63
<i>Atelopus coynei</i>	17.33	2312	67.94	1096	193
<i>Atelopus cruciger</i>	21.49	1028	53.41	402	62
<i>Atelopus dimorphus</i>	26.52	4293	40.39	1502	494
<i>Atelopus ebenoides</i>	11.69	1028	52.07	401	82
<i>Atelopus elegans</i>	24.68	2556	27.61	848	433
<i>Atelopus eusebianus</i>	11.87	1898	21.61	585	348
<i>Atelopus exiguus</i>	9.88	1032	25.44	347	183
<i>Atelopus famelicus</i>	19.53	1625	36.32	555	260
<i>Atelopus farci</i>	17.40	1806	32.06	573	299
<i>Atelopus flavescens</i>	25.55	3094	52.08	1238	260
<i>Atelopus franciscus</i>	25.68	2871	45.90	1131	322
<i>Atelopus glyphus</i>	25.70	2868	50.41	1034	255
<i>Atelopus gracilis</i>	25.39	4903	28.47	1514	737
<i>Atelopus guanjuo</i>	7.55	821	30.99	298	133
<i>Atelopus halihelos</i>	20.73	1570	26.13	530	273
<i>Atelopus hoogmoedi</i>	24.77	2545	42.55	967	271
<i>Atelopus ignescens</i>	9.90	1180	19.00	372	243
<i>Atelopus laetissimus</i>	17.98	1649	66.76	707	83
<i>Atelopus limosus</i>	24.26	3064	51.84	1125	171
<i>Atelopus longirostris</i>	19.03	1964	34.85	614	235
<i>Atelopus lozanoi</i>	11.86	1925	54.08	829	176
<i>Atelopus lynchii</i>	17.54	1306	43.46	453	129
<i>Atelopus manauensis</i>	26.44	2339	43.78	878	276

Species	BIO1 Average Annual Temperature (° C)	BIO12 Annual Precipitation (mm)	BIO15 Seasonality precipitation (CV)	BIO16 Precipitation of wettest quarter (mm)	BIO17 Precipitation of driest quarter (mm)
<i>Atelopus mandingues</i>	14.82	1938	61.60	894	157
<i>Atelopus mindoensis</i>	18.26	2410	59.20	1080	244
<i>Atelopus minutulus</i>	19.49	2429	39.76	869	269
<i>Atelopus mittermeieri</i>	13.18	1167	45.05	439	134
<i>Atelopus monohernandezii</i>	15.14	1182	44.94	403	126
<i>Atelopus mucubajensis</i>	7.20	976	63.46	396	41
<i>Atelopus mucisca</i>	10.81	1872	61.79	871	150
<i>Atelopus nahumae</i>	17.53	1660	69.70	709	55
<i>Atelopus nanay</i>	8.34	998	39.77	386	128
<i>Atelopus nepiozomus</i>	18.09	1238	46.96	518	160
<i>Atelopus nicefori</i>	13.80	2356	39.08	795	290
<i>Atelopus nocturnus</i>	22.00	3147	42.35	1133	348
<i>Atelopus onorei</i>	18.28	856	97.17	514	25
<i>Atelopus oxyrhynchus</i>	5.24	927	69.74	439	33
<i>Atelopus pachydermus</i>	7.17	1154	13.69	322	251
<i>Atelopus palmatus</i>	17.97	2339	27.71	799	415
<i>Atelopus pastuso</i>	7.31	1043	22.69	306	185
<i>Atelopus patazensis</i>	10.73	1210	54.29	472	95
<i>Atelopus peruvensis</i>	8.03	796	63.69	352	40
<i>Atelopus petersi</i>	7.17	1154	13.69	322	251
<i>Atelopus pictiventris</i>	15.17	1743	32.70	555	287
<i>Atelopus planispina</i>	13.60	1591	22.39	503	292
<i>Atelopus podocarpus</i>	13.25	543	37.63	190	70
<i>Atelopus pulcher</i>	23.60	1658	21.63	503	315
<i>Atelopus quimbaya</i>	16.03	2021	35.53	664	305
<i>Atelopus reticulatus</i>	23.13	2209	38.96	786	290
<i>Atelopus seminiferus</i>	19.88	740	32.16	247	103
<i>Atelopus senex</i>	14.29	2298	66.01	1018	117
<i>Atelopus sernai</i>	12.23	2201	44.64	783	288
<i>Atelopus simulatus</i>	9.39	1613	31.28	493	273
<i>Atelopus sonsonensis</i>	19.34	2824	22.78	871	542
<i>Atelopus sorianoi</i>	14.87	1252	51.99	500	104
<i>Atelopus spumarius</i>	26.54	2930	26.32	935	476
<i>Atelopus spurrelli</i>	25.22	4153	39.84	1560	538
<i>Atelopus subornatus</i>	11.33	1689	36.48	596	247
<i>Atelopus tricolor</i>	15.95	936	44.71	377	123
<i>Atelopus varius</i>	21.63	2803	58.48	1151	164
<i>Atelopus zeteki</i>	25.02	2666	69.28	1159	74



CONCLUSIONES GENERALES

Este estudio doctoral se enfocó en comprender las implicaciones de la selección sexual y estrategias de apareamiento sobre rasgos fenotípicos adaptativos asociados a la reproducción de los anuros tropicales del género *Atelopus*. Orientado mayormente en la competencia macho-macho para entender algunas implicaciones asociadas a la reproducción y evolución fenotípica dentro de este linaje.

Estudiamos primero la funcionalidad del dimorfismo sexual y los amplexos prolongados en la selección intra-sexual de una especie de *Atelopus* (*A. laetissimus*) con sistema de apareamiento *lek* cercano al *scramble*. Nuestras observaciones de campo y evidencia experimental apoyan el papel de la selección intra-sexual favoreciendo el éxito de apareamiento de los machos amplexando antes a las hembras y con la capacidad de defender, mediante mayor tamaño y robustez corporal, su posición en el dorso de la hembra. El desplazamiento exitoso del amplexus es muy bajo, por lo tanto, puede ser adaptativo encontrar y abrazar a una hembra antes que otros machos.

Así mismo, documentamos la diversidad en señales acústicas de las especies del clado *Atelopus*, revelando un amplio repertorio acústico en *Atelopus*; además, la relación de algunos rasgos morfológicos adaptativos en la reproducción *lek-scramble* (tamaño corporal y ancho de antebrazo) con parámetros acústicos sugiere la importancia (probablemente indicador honesto) de este tipo de señales en sus estrategias de apareamiento.

Por último, estudiamos la influencia de selección sexual y el rol de las estrategias de apareamiento en la evolución del dimorfismo sexual en tamaño y robustez corporal a través del género; además, como estos están relacionados con variables ambientales y los tradicionales grupos taxonómicos fenéticos intra-genéricos de *Atelopus*. Nuestros resultados apoyan la hipótesis sobre el reflejo causado por la

intensidad de la selección sexual operando mediante las estrategias de apareamiento en la evolución fenotípica de *Atelopus*. Las formas corporales conocidas como grupos fenéticos *ignescens* y *longirostris* podrían considerarse como el resultado parcial de las estrategias de apareamiento presentes dentro de este género.

Esta tesis doctoral obtuvo importantes resultados para el avance del conocimiento en tópicos relacionados a la ecología reproductiva y biología evolutiva de las especies de ranas amenazadas del género *Atelopus*, haciendo énfasis en la competencia macho-macho dentro de la estrategia de apareamiento *lek* cercano al *scramble*. No obstante, son necesarios estudios comportamentales en especies de *Atelopus* con estrategias de apareamiento cercanos a la territorialidad, o diferentes estrategias de apareamiento aun no documentadas dentro del género. Estos estudios podrían corroborar nuestras predicciones sobre la influencia de selección sexual en evolución fenotípica dentro del género.

Así mismo, se hizo explícita la importancia de las hembras dentro de las estrategias de apareamiento, evolución de la guardia de compañero y evolución fenética de *Atelopus*. Por consiguiente, las futuras perspectivas de investigación, se dirigen a experimentos comportamentales para comprender los costos y beneficios en el que podrían incurrir las hembras dentro de las estrategias de apareamiento; tales como, el esfuerzo reproductivo invertido en cantidad y calidad de los huevos, costos asociados a los amplexos prolongados en locomoción, forrajeo y otros aspectos ecológicos. Además, estudios relacionados a un mayor entendimiento de la selección inter-sexual en *Atelopus*; particularmente la selección de la hembra sobre parámetros acústicos, los cuales podrían ser indicadores honestos de la calidad de los machos. Entre otros tópicos que conlleven a una visión holística de la biología reproductiva de este linaje de anuros altamente amenazados.

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Not every drought is bad: quantifying reproductive effort in the harlequin frog *Atelopus laetissimus* (Anura: Bufonidae)

Andres A. Rocha Usuga^a, Fernando Vargas-Salinas^b and Luis Alberto Rueda Solano^{a,c}

^aGrupo de Investigación en Biodiversidad y Ecología Aplicada (GIBEA), Universidad del Magdalena, Santa Marta D.T.C.H, Colombia; ^bGrupo de Investigación en Evolución, Ecología y Conservación EECO, Universidad del Quindío, Armenia, Colombia; ^cGrupo Biomics, Universidad de los Andes, Bogotá, Colombia

ABSTRACT

Atelopus laetissimus is an endemic and threatened harlequin frog from the high mountain forests of Sierra Nevada de Santa Marta, Colombia. Knowledge of its reproductive biology is essential for understanding the intraspecific interactions that can help the conservation of *Atelopus* species. We quantified the energy, measured in body weight, invested by males and females of *A. laetissimus* for reproduction, and how this energetic investment is related to the survival of individuals and rainfall conditions in habitats during two years (2014 and 2015). Our results show plasticity in terms of reproductive phenology linked to rainfall with short- and long-duration breeding strategies. The first year of this study, 2014, had a precipitation level in accordance with the annual averages at the area. During this time frogs exhibit a short breeding period. Contrary to 2014, 2015 was a year with little precipitation, below the annual averages, which probably facilitated the females' quick spawning in the creeks and a consequent reduction in the duration of amplexus and low breeding efforts by males. This, in turn, was related to a long breeding period that favors the survival and reproduction of males during the entire year. In 2014 we found a decrease of 25% to 30% body weight of potentially reproductive males, which may be attributed to a prolonged duration of amplexant events.

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Introduction

Reproductive effort is defined as the total specific and biologically significant energy invested by an individual in breeding during a defined time interval (Stearns 1976). Reproductive effort depends on the food resources in the habitat, and the activities that the individual carries out before and during the breeding process (Lemckert & Shine 1993). The current reproductive effort invested by an individual directly impacts its survival and breeding probabilities in the future (Stearns 1992; Nilsson & Svensson 1996; Roff 2002). Predictions about reproductive effort have been supported by empirical evidence in plants (Reekie & Bazzaz 1987), invertebrates (e.g. Ward et al. 2009) and all vertebrate groups (e.g. fishes: Jones & Reynolds 1999; birds:

CONTACT Luis Alberto Rueda Solano biologoluisrueda@gmail.com

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Acoustic Signal Diversity in the Harlequin Toad *Atelopus laetissimus* (Anura: Bufonidae)

Luis Alberto Rueda-Solano^{1,2,3}, José Luis Pérez-González^{1,2}, Mauricio Rivera-Correa⁴, and Fernando Vargas-Salinas⁵

The acoustic signals in *Atelopus* (Anura: Bufonidae), a Neotropical genus currently composed of 97 species, are poorly known. In this study, we describe for the first time, the advertisement, release, and agonistic calls of *Atelopus laetissimus*, an endangered species endemic to the Sierra Nevada de Santa Marta, in northern Colombia. The calling behavior of males was associated with reproduction (advertisement calls) and male-male aggression (agonistic and release calls). We also describe, for the first time, calls from female *A. laetissimus*, constituting the first case of a female's vocalization in the genus, recorded while one male was trying to clasp her. The diverse acoustic signals described here for *Atelopus laetissimus* suggest the vocal repertoire in the genus *Atelopus* may be more complex than is currently realized.

ACOUSTIC signals are the most common sensory modality used by mature anurans when choosing mates and defending resources (Rand, 1988; Gerhardt and Huber, 2002; Wells, 2007). Eight types of acoustic signals have been described in anurans, with advertisement calls being the most commonly studied (Toledo et al., 2015; Köhler et al., 2017; Guerra et al., 2018). Advertisement calls are produced only by males and are used for attracting mates and communicating to other males the signaler's position and status (Ryan, 1991, 2001; Wells, 2007). Other types of calls are more specialized, being produced within narrower behavioral contexts; these include release calls and aggressive/agonistic calls (Wells, 2007; Toledo et al., 2015).

The genus *Atelopus*, consisting of 97 recognized species, is the most species-rich genus in the family Bufonidae, and its distribution ranges from Costa Rica to Bolivia including habitats from sea level to >4,800 m a.s.l. (Frost, 2020). Female *Atelopus* lay eggs under rocks in small- or medium-sized creeks, and their tadpoles are classified in the gastro-myophorous ecomorphological guild (Lötters, 1996; Rueda-Solano et al., 2015); that is, stream-dwelling tadpoles with an enormous disc posterior to the mouth and extending over much of the ventral surface of the body (Altig and Johnston, 1989; Wells, 2007). During pre-mating rituals, advertisement calls are usually accompanied by stereotyped movements of the anterior and posterior extremities (Lindquist and Hetherington, 1996; Hödl and Amézquita, 2001; Criswell, 2008).

Acoustic signals in the genus *Atelopus* are poorly known; to date, the advertisement calls of only 18 species have been described (Starrett, 1967; Jaslow, 1979; Lescure, 1981; Crocott et al., 1990; Ibáñez et al., 1995; Lötters et al., 1999, 2002a, 2019; Coloma et al., 2000). Similarly, descriptions of other types of acoustic signals are lacking (Lötters et al., 1999,

2019; Carvajalino-Fernández et al., 2017). Increasing our knowledge of acoustic call features in *Atelopus* is essential for delimiting species and inferring phylogenetic relationships (Schneider and Sinch, 2007; Padial et al., 2008; Köhler et al., 2017; Rivera-Correa et al., 2017). Moreover, advertisement call features can allow us to monitor species in automatized acoustic recordings (Bedoya et al., 2017; Farina, 2019), which is especially important for species such as those of the genus *Atelopus*, whose populations have dramatically declined (La Marca et al., 2005; Lötters, 2007). Last, our limited knowledge about acoustic communication in *Atelopus* limits our inferences about the evolutionary behavioral ecology of those Neotropical anurans (Lindquist and Hetherington, 1996; Criswell, 2008).

Atelopus laetissimus is an endemic species of the Sierra Nevada de Santa Marta, a mountainous enclave located in northern Colombia (Ruiz-Carranza et al., 1994). This species is categorized as endangered by the International Union for Conservation of Nature (IUCN, 2020) because of its small range; its known extent of occurrence (EOO) is estimated to be 797 km². It is known only from three localities; in each, the amount and quality of habitat are in decline (Ruiz-Carranza et al., 1994). Individuals are observed on vegetation alongside small streams in Andean and sub-Andean forests between 1900 to 2800 m a.s.l. (Granda-Rodríguez et al., 2007; Rocha Usuga et al., 2017; Rueda-Solano, pers. obs.). Males emit advertisement calls from these sites (Granda-Rodríguez et al., 2007; Pérez-González, pers. obs.). In addition, low-intensity calls are produced by males and females when aggregated into mating balls (Rocha Usuga et al., 2017). However, no formal description of these acoustic signals has been published (but see Granda-Rodríguez et al., 2020 for a brief description based on one male). Here, we describe the

¹ Fundación Atelopus, 47001 Santa Marta, Colombia; Email: (LRS) biologoluisrueda@gmail.com; and (JPG) joseperezgonzalez2407@gmail.com
Send reprint requests to LRS.

² Grupo de Investigación en Biodiversidad y Ecología Aplicada, Facultad de Ciencias Básicas, Universidad del Magdalena, 470004 Santa Marta, Colombia.

³ Biomics, Facultad de Ciencias, Universidad de los Andes, 111711 Bogotá, Colombia.

⁴ Grupo Herpetológico de Antioquia (GHA), Instituto de Biología, Universidad de Antioquia, 054040 Medellín, Colombia; Email: mauriciorivera79@gmail.com.

⁵ Evolución, Ecología y Conservación EECO, Programa de Biología, Facultad de Ciencias Básicas y Tecnologías, Universidad del Quindío, 630004 Armenia, Colombia; Email: fvargas@uniquindio.edu.co.

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Histology and structure of the testicles in three species of *Atelopus* frogs (Anura: Bufonidae) endemic to the Sierra Nevada de Santa Marta, Colombia

Arantxa Sánchez-Ferreira^{1*}; <https://orcid.org/0000-0001-7571-7952>
Edgar Javier Rincón-Barón²; <https://orcid.org/0000-0003-1347-171X>
Luis Alberto Rueda-Solano^{1,3}; <https://orcid.org/0000-0001-6968-0719>

1. Universidad del Magdalena, Facultad de Ciencias Básicas, Grupo de Investigación en Biodiversidad y Ecología Aplicada, calle 32 No 22-08, Santa Marta, Colombia; biologaarantxasanchez@gmail.com (Correspondence*)
2. Universidad de Santander, Facultad de Ciencias de la Salud, Grupo de Investigación Agroambiente y Salud-MICROBIOTA, calle 70 No 55-210, Campus Universitario Lagos del Cacique, Bucaramanga, Colombia; ed.rincon@mail.udes.edu.co
3. Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, Colombia; luisruedasolano@gmail.com

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ABSTRACT

Introduction: Testicular histology constitutes one of the least explored aspects in frogs of the genus *Atelopus*. This taxonomic group shows an alarming population decline; therefore, its reproductive biology is one of the greatest topics of interest for its conservation.

Objective: To describe the testicular morphology and the spermatogenetic lineage cells in adult males of *Atelopus laetissimus*, *Atelopus nahumae*, and *Atelopus carrikeri* in the Sierra Nevada de Santa Marta, Colombia.

Methods: During June – July 2017 and 2018, sampling was conducted in the localities of San Lorenzo and Páramo Cebolletas, Sierra Nevada de Santa Marta (SNSM), to collect 15 adult males, 5 per species. Testes samples were fixed in Bouin to be processed by the standard paraffin-embedding technique. Histological sections (3 µm) were stained with Hematoxylin-eosin and Mallory-Heidenhain-Azan-Gomori's. For the description and photographic register of the germ cells, the photonic microscopy technique was used with the differential interference contrast system.

Results: The testes are oval organs, compact, light yellow color, and with little vascularization. Externally, they are surrounded by a thin albuginea tunic constituted by regular dense connective tissue. Inside this layer, they are composed of numerous seminiferous tubules of hexagonal contour, in which germ cell cysts are distinguished at different stages of spermatogenesis (spermatogonia I and II, spermatocyte I and II, and early and late spermatids) and spermogenesis (spermatozoa in fascicles and free spermatozoa). Separating the seminiferous structures is the interstitial tissue in which Leydig cells and blood vessels stand out. Additionally, in the cranial part of the testis, the Bidder's organ was found, formed by two distinguishable regions, the cortex and the medulla. In the cortex, there are previtellogénic oocytes of different sizes surrounded by a monolayer of flat follicular cells. For its part, the medullary region is the connective tissue that nourishes the oocytes and is constituted by blood capillaries.

Conclusions: The gonads of the three species analyzed present a cystic cellular organization similar to other anurans, where all stages of spermatogenesis and spermogenesis were identified, possibly indicating a continuous reproductive activity. Likewise, the Bidder's organ is reported for the first time in the three *Atelopus* species, which allows suggesting a possible sexual reversion in case of a population decrease of females as a reproductive strategy.

Key words: spermatogenic cells; histology; bidder's organ; sexual reversal; testes.