



<http://dx.doi.org/10.11646/zootaxa.3920.1.4>

<http://zoobank.org/urn:lsid:zoobank.org:pub:D03CFD04-F9BA-47E9-8DBD-920C539FF4F0>

A new species of glassfrog, genus *Hyalinobatrachium* (Anura: Centrolenidae), from the Caribbean foothills of Costa Rica

BRIAN KUBICKI¹, STANLEY SALAZAR² & ROBERT PUSCHENDORF³

¹Costa Rican Amphibian Research Center, Guayacán, Provincia de Limón, Costa Rica. E-mail: crarc@yahoo.com

²Veragua Rainforest, Research & Adventure Park, Provincia de Limón, Costa Rica. E-mail: stanleysalazarn@yahoo.com

³School of Biological Sciences, Plymouth University, Drake Circus, Plymouth PL4 8 AA, UK.

E-mail: Robert.Puschendorf@plymouth.ac.uk

Abstract

We describe a new glassfrog belonging to the genus *Hyalinobatrachium* from the tropical wet forests and premontane rainforests of the Caribbean foothills of Costa Rica, at elevations between 400–800 m. The type locality is approximately 4 km west of Santa Clara, ca. 400 m a.s.l. (N 10.219, W 83.949). This new taxon is distinguished by its morphological characteristics, distinct advertisement call, and genetic distance (COI mRNA). The new species can be distinguished from other species of the genus *Hyalinobatrachium* by the combination of the following characters: (1) snout truncate in dorsal and lateral views; (2) granular dorsal skin; (3) parietal and cardiac peritonea transparent; (4) hand webbing formula III 2⁻–2⁺ IV; (5) in life having a uniform lime green dorsal surface that lacks any evident light or dark spots; (6) iris coloration silvery-white with fine dark spots or reticulation; (7) advertisement call consisting of a single tonal long metallic whistle-like note with a duration of 0.40–0.55 s (average 0.501 s) and a dominant frequency of 3.35–3.44 kHz (average 3.39 kHz); (8) highly divergent DNA sequences for the mitochondrial gene COI from other analysed members of the genus *Hyalinobatrachium*, with 12.4% divergence from its closest known species, *Hyalinobatrachium chirripoi*.

Key words: Amphibia, Central America, COI, *Hyalinobatrachium chirripoi*, *Hyalinobatrachium colymbiphyllum*, *Hyalinobatrachium diana* sp. nov., integrative taxonomy

Resumen

Describimos una nueva rana de vidrio que pertenece al género *Hyalinobatrachium* que se encuentra en los bosques tropicales húmedos y bosques premontanos lluviosos de la vertiente caribeña de Costa Rica, entre los 400–800 m sobre el nivel del mar. La localidad tipo está ubicada aproximadamente 4 km hacia el oeste desde Santa Clara, ca. 400 m.s.n.m. (N 10.219, W 83.949). Este nuevo taxón se distingue por sus características morfológicas, canto y distancia genética (COI mRNA). La nueva especie se distingue de las otras especies de *Hyalinobatrachium* por la combinación de las siguientes características: (1) hocico truncado en vista dorsal y lateral; (2) piel granulada; (3) peritoneos parietal y cardíaco transparentes; (4) fórmula de la membrana de la mano III 2⁻–2⁺ IV; (5) en vida dorso uniforme verde y sin puntos claros o oscuros evidentes; (6) iris blanco con puntos o reticulación fina y oscura; (7) canto consistente en un largo silbido metálico con una duración de 0.40–0.55 s (promedio 0.501 s) y una frecuencia dominante de 3.35–3.44 kHz (promedio 3.39 kHz); (8) secuencias de ADN para el gen mitocondrial COI altamente divergentes respecto a los otros miembros analizados del género *Hyalinobatrachium*, con una divergencia del 12.4% con *Hyalinobatrachium chirripoi*, la especie conocida más cercana.

Palabras clave: América Central, Amphibia, *Hyalinobatrachium chirripoi*, *Hyalinobatrachium colymbiphyllum*, *Hyalinobatrachium diana* sp. nov., secuencia COI, taxonomía integradora

Introduction

The Neotropics harbor a vast diversity of amphibian species, but undoubtedly one of the most charismatic taxa is

that of the Centrolenidae family of glassfrogs. This family, with its 149 species (Frost 2014) is found from southern Mexico, through Central America, into the northern half of South America, and disjunctly along the Atlantic Forest of southeastern Brazil and the extreme northeastern margin of Argentina (Castroviejo-Fisher *et al.* 2014). The region with the highest diversity of species is along the Andean slopes of Colombia, Ecuador, and Peru (Guayasamin *et al.* 2009). Glassfrogs are nocturnal and associated with riparian forests, where they reproduce by laying their eggs attached to the vegetation, rocks, branches, and other surfaces overhanging the water of rivers, streams, and seepages (Kubicki 2007). Many glassfrogs can be difficult to observe due to inhabiting the vegetation high above streams, and often at sites that present difficult access due to rough topography or remoteness. The difficulty involved in detecting some species in their natural habitat has in part resulted in the steady increase of newly described species as more sites become explored.

The tiny Mesoamerican country of Costa Rica, with its mere 51,032 km² of national territory, has played an important part in the history of the study and discovery of glassfrogs. Some of the earliest work and species descriptions of glassfrogs resulted from specimens collected within Costa Rica, starting back in 1892 when Carl Fleischmann collected the syntypes for what Oskar Boettger would describe as *Hyla prosoblepon* Boettger, 1892 and *Hylella fleischmanni* Boettger, 1893. In 1929, while Emmett R. Dunn and Manuel Valerio were exploring the forests in the region of La Palma, they collected two specimens of a glassfrog that would be described as *Centrolene valerioi* Dunn, 1931. In 1947 Edward H. Taylor began his work in Costa Rica, and during his explorations from June–September of that year he managed to collect type material for a variety of new amphibian species, including four glassfrogs that he would describe as *Centrolenella albomaculata*, *Centrolenella colymbiphylum*, *Centrolenella granulosa*, and *Centrolenella spinosa* Taylor, 1949. Taylor (1951) also recognized that the known species of glassfrogs, which were considered to be tree frogs, formed a monophyletic group and proposed the family Centrolenidae to accommodate them. During subsequent trips to Costa Rica, Taylor went on to collect and describe two more new species of glassfrogs; *Cochranella talamancae* Taylor, 1952 and *Cochranella chirripoi* Taylor, 1958. In 1960 Jay M. Savage began working in Costa Rica and he would add to the knowledge of this expanding family. Savage's explorations throughout Costa Rica in the years that followed would result in the discovery and description of three additional species; *Centrolenella ilex* Savage, 1967, *Centrolenella euknemos* Savage & Starrett, 1967, and *Centrolenella vireovittata* Starrett & Savage, 1973. Of the 13 species of glassfrogs previously known from Costa Rica, only one has its type locality lying outside Costa Rica's borders, that of *Teratohyla pulverata*, which was collected by H. Ribbe in "Chiriqui", Panama and described as *Hyla pulverata* Peters, 1873.

Following the extensive herpetological explorations throughout Costa Rica for more than a century by not only the above-mentioned herpetologists, but countless others, we were pleasantly surprised to have discovered yet another species of glassfrog that had evaded detection. In this work we will be presenting an integrative approach through the use of morphology, bioacoustics, and DNA sequences to support the proposal of the new species.

Material and methods

We follow the generic assignment of *Hyalinobatrachium* proposed by Ruiz-Carranza & Lynch (1991), and further modified by Cisneros-Heredia & McDiarmid (2007) and Guayasamin *et al.* (2009). The GPS coordinates are WGS84 datum. Photographs included in the figures were taken by BK.

Morphometrics. Measurements were taken with a ROHS NORM 2002/95/EC digital caliper and rounded to the nearest 0.1 mm. Morphological measurements taken and the abbreviation used herein are as follows: Snout–vent length (SVL), internarial tip of the snout to the posterior edge of cloacal opening; head length (HL), posterior edge of the jaw articulation to the internarial tip of the snout; head width (HW), greatest width of head, at the posterior edge of the jaw articulation; length of crus (TI), outer edge of the flexed knee articulation to the opposing outer edge of the flexed tibiotarsal articulation; foot length (FL), proximal edge of the inner metatarsal tubercle to the tip of Toe IV; hand length (HaL), proximal edge of the thenar tubercle to the tip of Finger III; interorbital distance (IOD), distance between the inner margins of the orbits; eye–naris distance (END), posterior edge of naris to the anterior edge of the orbit; width of disc on Finger III (DW); forearm length (FoL), proximal edge of the thenar tubercle to the exterior margin of the flexed elbow articulation; tarsus length (TaL), proximal edge of the inner metatarsal tubercle to the exterior margin of the flexed tibiotarsal articulation; eye length (EL),

the greatest horizontal length of the orbit between the anterior and posterior margins. The following are also expressed as percentage of snout-vent length (SVL): head length (HL); head width (HW); length of crus (TI); length of foot (FL); hand length (HaL); interorbital distance (IOD); eye–naris distance (END); forearm length (FoL); tarsus length (TaL). The following is expressed as a percentage of eye length (EL): width of disc on Finger III (DW). Note that the tympanic membrane and tympanic annulus are not evident in the specimens of the type series, thus no tympanic diameter measurements were taken.

The capitalized colors and their corresponding color codes (code in parentheses) used in the color in life description of the holotype follow Köhler (2012). Webbing formulae follow Savage & Heyer (1967) as modified by Myers & Duellman (1982) and Savage & Heyer (1997). Specimens were fixed in a 10% formalin solution and processed over to 70% ethanol for long term storage. Tissue samples that were used for genetic analyses were preserved in 96% ethanol. All phenotypic characteristics reported herein are from adult specimens. The type series for *Hyalinobatrachium diana* is deposited in the Museo de Zoología, Escuela de Biología, Universidad de Costa Rica (UCR). Additional specimens examined during this study are listed in Appendix I.

Call data. Nine advertisement calls from the male holotype of the new species were recorded in the field with a Tascam DR-05 digital recorder. The microphones used to record the advertisement calls were the two built-in omni-directional stereo microphones on the Tascam DR-05. The call recordings were taken at a distance of approximately 50 cms from the male holotype. The ambient air temperature at the time of call recording was taken with a Taylor 1441 digital thermometer. Advertisement call data was generated by the examination and measurement of variables in audiospectrograms created in Raven Lite ver. 1.0. Advertisement call variables that were noted are the dominant frequency of each note (frequency provided in Raven Lite 1.0 at the vertically oriented center average of the note image), note duration in seconds (time in seconds measured from the visibly determinable beginning and end of each note or group of distinct pulses that collectively form a note). The additional congener advertisement calls referred to in this work that originated from Costa Rican were recorded in the field and measured in Raven Lite by BK especially for this study; the results are from the examination of a single advertisement call for each specifically mentioned species and locality. Values for the advertisement calls of *H. anachoretus* and *H. pellucidum* were obtained from Twomey *et al.* (2014) and Wen *et al.* (2012) respectively.

Genetic data. One paratype (UCR 22033; Genbank accession number KJ703103) of the new species was sequenced for a fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) using published primers and protocols (Crawford *et al.* 2010). We compared our sample of UCR 22033 with all COI sequences available for the genus *Hyalinobatrachium* on GenBank, which included *H. chirripoi*, *H. fleischmanni*, *H. talamancae*, *H. vireovittatum*, and several sequences of *H. colymbiophyllum*. We also sequenced a sample of *Hyalinobatrachium valerioi* (CRARC 1045) collected by BK in Guayacán de Siquirres, Limón Province, Costa Rica. Sequences were edited in GENEIOUS version 8.0.5 (Biomatters Ltd, Auckland, New Zealand). The corresponding forward and reverse sequences were aligned using the GENEIOUS alignment tool in standard settings (global alignment with free end gaps), followed by the trimming of primer ends. We create a bayesian phylogenetic tree using HK85 substitution model, and MCMC settings as follows: chain length 10 million, subsampling every 1000 generations, burn in length 100,000 and using *Cochranella euknemos* (FJ766601.1) as an outgroup.

***Hyalinobatrachium diana* sp. nov.**

Diane's bare-hearted glassfrog / rana de vidrio de Diane
(Fig. 1)

Holotype. UCR 22038, an adult male from Costa Rica: Provincia de Heredia: Cantón de Sarapiquí: Distrito de Horquetas: approximately 4km west of Santa Clara, ca. 400 m a.s.l. (N 10.219, W 83.949), obtained by Brian Kubicki and Stanley Salazar on 13 October 2013.

Paratopotypes. UCR 22037, adult female, same data as the holotype; UCR 22034, adult male, same data as the holotype but obtained by Stanley Salazar on 18 November 2013.

Paratypes. UCR 22033, an adult male from Costa Rica: Provincia de Limón: Cantón de Turrialba: Distrito de Chirripo: headwaters of Quebrada Surubre, ca. 900 m a.s.l. (N 9.973, W 83.392), obtained by Norberto Solano on 1 August 2012. UCR 22035–36, two adult males from Costa Rica: Provincia Limón: Cantón de Limón: Distrito Río Blanco: head waters of Río Victoria, ca. 400 m a.s.l. (N 9.915, W 83.188), obtained by Stanley Salazar on 22 November 2013.



FIGURE 1. *Hyalinobatrachium diana* new species. A) Adult male holotype UCR 22038, B) Adult female paratopotype UCR 22037.

Generic Placement. We assign this new species to the genus *Hyalinobatrachium* due to the combination of the following characteristics that are outlined as diagnostics for members of this genus (Guayasamin *et al.* 2009): (1) humeral spine absent in adult males; (2) digestive tract and bulbous liver covered in white iridophores; (3) parietal peritoneum transparent, allowing viscera to be fully visible when viewed ventrally; (4) white bones in life; (5) dorsal coloration in preservative cream; (6) lacking vomerine teeth and dentigerous process of the vomer; (7) adult males typically seen calling from the underside of the foliage; (8) egg masses deposited in a single layer on the inferior surface of leaves.

Diagnosis. Herein we follow the standardized character states for diagnosing centrolenids presented by Cisneros-Heredia & McDiarmid (2007). The combination of the following characteristics can be used to distinguish *Hyalinobatrachium diana* from other members of the family Centrolenidae: (1) lack of vomerine teeth and dentigerous process of the vomer; (2) snout truncate in dorsal and lateral views; (3) tympanic membrane and tympanic annulus indistinct, covered in skin and indiscernible in color and texture from adjacent surfaces; (4) skin

on dorsal surfaces of limbs, body and head granular, slightly increased granular texture on body (Fig. 2A); (5) ventral skin of thighs and body slightly granular, with most pronounced granulation on abdomen; chin and ventral surfaces of the arms and lower legs are smooth; subcloacal skin containing a concentration of weakly enameled smooth tubercles, lacking large subcloacal tubercles or folds; (6) parietal and cardinal peritonea transparent; urinary bladder transparent; hepatic and gastrointestinal peritonea containing iridophores; gallbladder transparent yellowish green in living specimens (Fig. 2B); (7) liver bulbous; (8) adult males lack humeral projection; (9) lacking webbing between fingers I and II, basal webbing present between fingers II and III, moderate webbing between fingers III and IV, $\text{III } 2^- - 2^+ \text{ IV}$ (Fig. 3 A); (10) toe webbing $\text{I } 1 \frac{3}{4} - 2 \text{ II } 1 \frac{1}{4} - 2^+ \text{ III } 1 \frac{1}{2} - 2 \frac{3}{4} \text{ IV } 2 \frac{1}{2} - 1 \frac{1}{2} \text{ V}$ (Fig. 3B); (11) a very weak white fleshy fringe extends from the elbow along the ventrolateral margin of forearm and Finger IV to the base of the disc; a very weak white fleshy fringe is present on the lower leg, originating at the heel and extending along the ventrolateral margin of tarsus, metatarsus, and to the base of Toe V; (12) nuptial excrescence (type V) present as a small cluster of minute glandular granules on the basal to medial dorsolateral edge of Finger I; prepollex not enlarged and prepollical spine not protruding; (13) Finger I longer than Finger II when measured from the corresponding distal margin of the palmar tubercle to the digit tip; (14) diameter of eye roughly twice the width of disc on Finger III; (15) living individuals have uniform lime green dorsal surfaces, lacking any evident light or dark spots; a dark oblong spherical structure under the skin of the lower back; bones white in life; (16) preserved specimens have a uniform cream-yellow dorsal coloration, with numerous minute dark star-like melanophores present throughout; bones white in preserved specimens; (17) iris coloration in life: silvery-white with fine dark spots or reticulation that become more concentrated directly surrounding the horizontally elliptical pupil; iris coloration in preservative: similar to that in life with a silvery white background and contrasting dark purplish spots or reticulation; (18) digits of hands and feet uniformly yellow, with the exception of the dorsal surfaces of toes IV and V which have a mixture of pigmentation typical of the adjacent dorsal surface of the metatarsus; melanophores absent on the digits of hands and toes I, II, and III; (19) males have been observed calling from the undersides of leaves; advertisement call is a single tonal note, a long metallic whistle-like note with a very rapid but weakly pulsed intensity; note duration 0.40–0.55 s (average 0.501 s), dominant frequency 3.35–3.44 kHz (average 3.39 kHz) (N=9); (20) egg masses have been observed on the underside of the leaves, in a single layer; clutch size 31–68 eggs (N = 9); early-staged embryos are greenish-yellow; males have been observed attending eggs during the night; (21) SVL in adult males 28.5–29.4 mm (N = 5), adult female 28.0 mm (N = 1). Combat behavior and tadpoles are unknown for the new species.

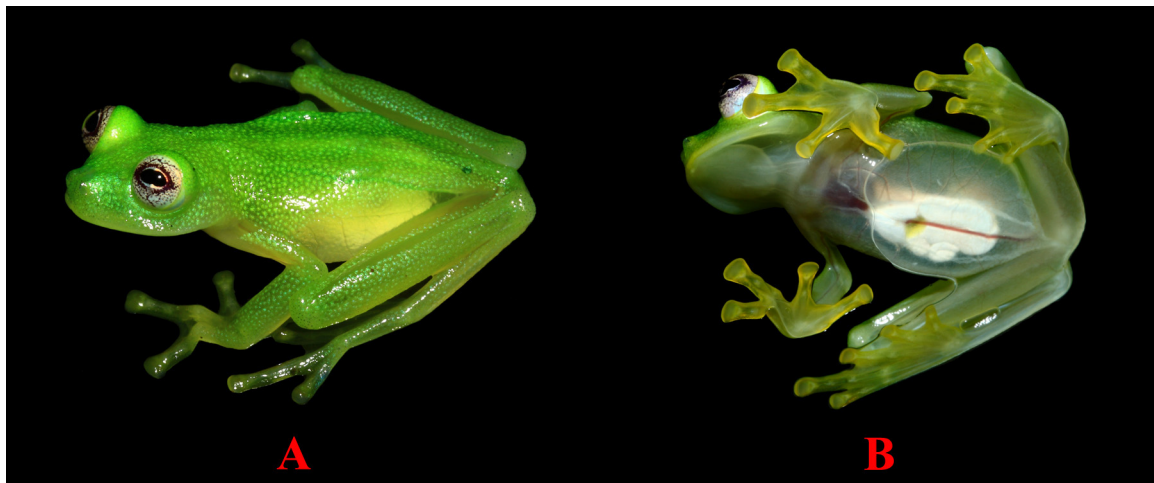


FIGURE 2. Dorsal (A) and ventral (B) views of *Hyalinobatrachium diana*, adult male paratype UCR 22033.

Comparisons. Due to their small size, potential inter/intrapopulational variation, and general lack of evident morphological characters, especially when examining preserved specimens, some members of the genus *Hyalinobatrachium* present difficulty when it comes to making interspecific comparisons based solely on their morphology. This difficulty in discerning some *Hyalinobatrachium* species based solely on morphological characters has been noted by previous authors as well, and resulted in their use of an integrative taxonomy approach that employs a combination of morphological characters, advertisement calls, and phylogenetic

relationships to define the different species addressed in their taxonomic studies (Castroviejo-Fisher *et al.* 2009; 2011; Twomey *et al.* 2014). Herein we will also be utilizing an integrative comparative approach where we look at aspects involving morphological and bioacoustic characteristics, in addition to COI DNA sequences. Being that the new species is only known to occur in Costa Rica, our comparisons are narrowed down to congeners native to Central America, the Magdalena and Cauca river valleys of Colombia, and the Chocó biogeographical region of western Colombia and Ecuador. Additionally, being that our studies have shown *H. diana* to have the closest relation to *H. chirripoi* and *H. colymbiphyllum* we have included in our comparisons two species that are not native to the above-mentioned regions. The two additional species that we included morphological and bioacoustic comparisons are *H. anachoretus* and *H. pellucidum*, both of which occur east of the Andes and were shown to have a close relation to both *H. chirripoi* and *H. colymbiphyllum* according to the phylogeny presented by Twomey *et al.* (2014). We did not include any genetic comparison to *H. anachoretus* and *H. pellucidum* being that COI sequences are not available for these two species.



FIGURE 3. Hand (A) and foot (B) details of *Hyalinobatrachium diana* (holotype UCR 22038).

Contrasting characteristics for *H. diana* are presented in parentheses. *Hyalinobatrachium anachoretus* Twomey *et al.*, 2014 has yellow spots on the dorsum (lacking yellow spots); advertisement call lasting 0.32–0.37 s, containing 5–6 distinct pulses, and having a dominant frequency of 4.67–4.8 kHz (total duration of 0.40–0.55 s [average 0.501 s]), a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity, lacking distinct pulses, and a dominant frequency of 3.35–3.44 kHz [average 3.39 kHz]. *Hyalinobatrachium aureoguttatum* (Barrera-Rodríguez & Ruiz-Carranza, 1989) has distinct large yellow spots on the dorsum that become white in preservative (lacking yellow spots); the advertisement call of *H. aureoguttatum* is unavailable for comparison. *Hyalinobatrachium chirripoi* (Taylor, 1958) has substantial webbing between fingers II–III and III–IV (Kubicki 2004; 2007) (substantial webbing being found only between fingers III–IV), light yellow spots in the dorsal skin (lacking any light yellow spots in the dorsal skin, Fig. 4), the advertisement call of *H. chirripoi* is a rapid high-pitched trill, comprised of numerous individual pulses, a single examined advertisement call of a male *H. chirripoi* from a tributary of Río Banano, Limón Province, Costa Rica, 80 m a.s.l. had a total duration of 0.285 seconds, a dominant frequency of 4.3 kHz, and 18 individual pulses (total duration of 0.40–0.55 s [average 0.501 s]), a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity, lacking distinct pulses, and a dominant frequency of 3.35–3.44 kHz [average 3.39 kHz]. *Hyalinobatrachium colymbiphyllum* (Taylor, 1949) has light yellow spots in the dorsal skin (lacking any light yellow spots in the dorsal skin, Fig. 4), the advertisement call of *H. colymbiphyllum* is a high-pitched trill comprised of numerous distinct pulses, single advertisement calls from two males of *H. colymbiphyllum* from two different locations within Costa Rica were

examined for this study. The first call is from a male near the Santa Elena Reserve in Tilarán, Guanacaste Province, 1550 masl. This call had a total duration of 0.628 seconds, a dominant frequency of 4.0 kHz, and 13 distinct pulses. The second *H. colymbiphyllum* advertisement call is from a male near Rincón de Osa, Puntarenas Province, 40 masl. This call had a total duration of 0.32 seconds, a dominant frequency of 4.6 kHz, and 9 distinct pulses (total duration of 0.40–0.55 s [average 0.501 s], a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity, lacking distinct pulses, and a dominant frequency of 3.35–3.44 kHz [average 3.39 kHz]). *Hyalinobatrachium fleischmanni* (Boettger, 1893) differs by having light yellow spots in the dorsal skin, and a pericardial sac containing iridophores, (lacking light yellow spots in the dorsal skin, and having a transparent pericardial sac); the advertisement call of *H. fleischmanni* differs from that of *H. diana* by being a rapid high-pitched rising whistle. A single examined advertisement call of *H. fleischmanni* from a small stream near Santa Elena, Heredia Province, Costa Rica, 1400 masl, which is located approximately 10 km straight line distance from the type locality for *H. fleischmanni* of San José, Costa Rica, had a total duration of 0.17 seconds, a dominant frequency of 4.1 kHz, and lacking a pulsed intensity (total duration of 0.40–0.55 s [average 0.501 s], a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity, lacking distinct pulses, and a dominant frequency of 3.35–3.44 kHz [average 3.39 kHz]). *Hyalinobatrachium pellucidum* (Lynch & Duellman, 1973), according to Wen *et al.* (2012) has a single tonal note advertisement call, with each note lasting 0.12–0.15 s in duration, and having a dominant frequency of 4.86–5.41 kHz (total duration of 0.40–0.55 s [average 0.501 s], a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity, lacking distinct pulses, and a dominant frequency of 3.35–3.44 kHz [average 3.39 kHz]). *Hyalinobatrachium talamancae* (Taylor, 1952) has light yellow spots in the dorsal skin, and a distinct green mid-dorsal stripe (Kubicki 2006; 2007) (lacking both light yellow spots in the dorsal skin and a distinct green mid-dorsal line); *Hyalinobatrachium talamancae* produces a long whistle-like advertisement call, consisting of a single non-pulsed note. A single examined advertisement call of *H. talamancae* from a small stream near Guayacán de Siquirres, Limón Province, Costa Rica, 500 masl, had a total duration of 0.3 s, and a dominant frequency of 4.9 kHz (total duration of 0.40–0.55 s [average 0.501 s], a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity, lacking distinct pulses, and a dominant frequency of 3.35–3.44 kHz [average 3.39 kHz]). *Hyalinobatrachium valerioi* (Dunn, 1931) has a green reticulation of the dorsal skin, and a fully to partially white pigmented pericardial sac (lacking a green reticulation of the dorsal skin, and pericardial sac completely lacking white iridophores); *Hyalinobatrachium valerioi* produces a short “peep” advertisement call. A single examined advertisement call of *H. valerioi* from a small stream near Guayacán de Siquirres, Limón Province, Costa Rica, 300 m a.s.l., had a total duration of 0.05 s, and a dominant frequency of 7.5 kHz (total duration of 0.40–0.55 s [average 0.501 s], a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity, lacking distinct pulses, and a dominant frequency of 3.35–3.44 kHz [average 3.39 kHz]). *Hyalinobatrachium vireovittatum* (Starrett & Savage, 1973) has light yellow spots in the dorsal skin, and a distinct green mid-dorsal line bordered on both sides by yellow paravertebral stripes (lacking both light yellow spots in the dorsal skin and a distinct green mid-dorsal line bordered on both sides by yellow paravertebral stripes); *Hyalinobatrachium vireovittatum* produces a long whistle-like advertisement call, consisting of a single non-pulsed note. A single examined advertisement call of *H. vireovittatum* from a small stream northwest of Palmar Norte, Puntarenas Province, Costa Rica, 550 masl, had a total duration of 0.264 s, and a dominant frequency of 4.6 kHz (total duration of 0.40–0.55 s [average 0.501 s], a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity, lacking distinct pulses, and a dominant frequency of 3.35–3.44 kHz [average 3.39 kHz]).

Description of holotype. Adult male having a SVL of 28.5 mm. Head slightly wider than body, with greatest width just posterior to the articulation of the jaws; upper lip round in dorsal outline, not flared; snout raised anterodorsally, truncate in dorsal outline and profile; snout short with nearly terminal protuberant nostrils directed laterally; internarial area concave. Eyes large and directed forward. Top of head flat; canthus rostralis distinct; intercanthal area slightly concave, loreal region slightly concave. Skin on all dorsal and lateral surfaces of head moderately granular; tympanic membrane and tympanic annulus indistinct, covered in skin. A weak but discernible supratympanic fold is present. Upper surfaces of body and limbs granular. Flanks smooth to weakly granular, especially along the anterior flank region; skin on the chin smooth. Skin of the chest, ventral surfaces of the body, and thighs weakly granular; skin of the groin and ventral surfaces of the arms and lower legs nearly smooth.

Arms short, with forearm slightly enlarged; humeral spine absent; no distinct transverse dermal fold on wrist; a very weak white fleshy fringe extends from the elbow along the ventrolateral margin of forearm, metacarpus, and

Finger IV to the base of the disc. Hands are large (HaL 8.9 mm, 31.2% of SVL); fingers moderately long and robust with weak lateral fringes; discs on fingers truncate. Discs on fingers I, III, and IV nearly equal in width; disc on Finger I (1.6 mm) minutely wider than discs on fingers III and IV; disc on Finger II narrowest (1.3 mm). Nuptial excrescence (type V), which is present as a small cluster of minute glandular granules on the basal to medial dorsolateral edge of Finger I; prepollex not enlarged and prepollical spine not protruding. Subarticular tubercles on fingers III and IV indistinct. Subarticular tubercles on fingers I and II distinct, round and slightly raised; supernumerary tubercles on the proximal side of subarticular tubercles on fingers I and II distinct, round and slightly raised. Other supernumerary tubercles indistinct; accessory palmar tubercles very weak to indistinct; thenar and palmar tubercles poorly developed, thenar ovoid and palmar round. Webbing absent between fingers I–II and basal between fingers II–III; moderate webbing found between fingers III–IV, reaching the proximal margin of the distal subarticular tubercle on Finger III and the distal margin of the subarticular tubercle on Finger IV. Webbing formula for between fingers III and IV: **III** 2–2⁺ **IV**. Finger lengths radiating from corresponding margin of palmar tubercle to the digit tip: Finger I (4.9 mm), Finger II (4.1 mm), Finger III (6.9 mm), and Finger IV (5.9 mm); relative lengths of fingers II < I < IV < III.



FIGURE 4. Comparison of dorsal coloration between A) *Hyalinobatrachium chirripoi*, CRARC 1005, B) *H. colymbiphyllum* CRARC 1026, and C) a male paratype UCR 22036 of *H. diana*.

Legs relatively long and thin; tibiotarsal articulation extending well beyond tip of snout when hind limb adpressed; a very weak white fleshy fringe is present, originating on the heel and extending along ventrolateral margin of tarsus, metatarsus, and to base of Toe V; discs on toes slightly rounded to somewhat truncate; narrower than those on fingers, widest on toes IV and V (Toe I 0.7 mm, Toe II 0.9 mm, Toe III 1.0 mm, Toe IV 1.3 mm, Toe V 1.2 mm); subarticular tubercles under the toes small, round and slightly elevated; inner metatarsal tubercle elongate, slightly raised, outer metatarsal tubercle not well distinguished from the numerous small accessory tubercles; slight inner tarsal fold present; toes moderately webbed, webbing extending to penultimate phalanges on one margin on four toes; web margin slightly concave; toes webbing formula: **I** 1 3/4–2 **II** 1 1/4–2⁺ **III** 1 1/2–2 3/4 **IV** 2 1/2–1 1/2 **V**. Cloacal opening directed posteriorly at mid-level of thighs with a concentration of small weakly enameled (white) tubercles below cloaca.

Tongue round in shape, lacking a distinct posterior notch; prevomerine dentigerous processes and teeth absent; choanae moderately large, rounded on posterior half, but flat on anterior half, hemispherical; paired elongate vocal slits present, extending from posterolateral base of tongue towards the angle of the jaw; vocal sac single, median, subgular.

A dark oblong spherical structure is found under the skin of the lower back, to left of the center axis of the body in the pelvic region (Fig. 5). This structure has been observed in all of the specimens of *Hyalinobatrachium diana*e examined during this study, whether it serves a function is unknown.

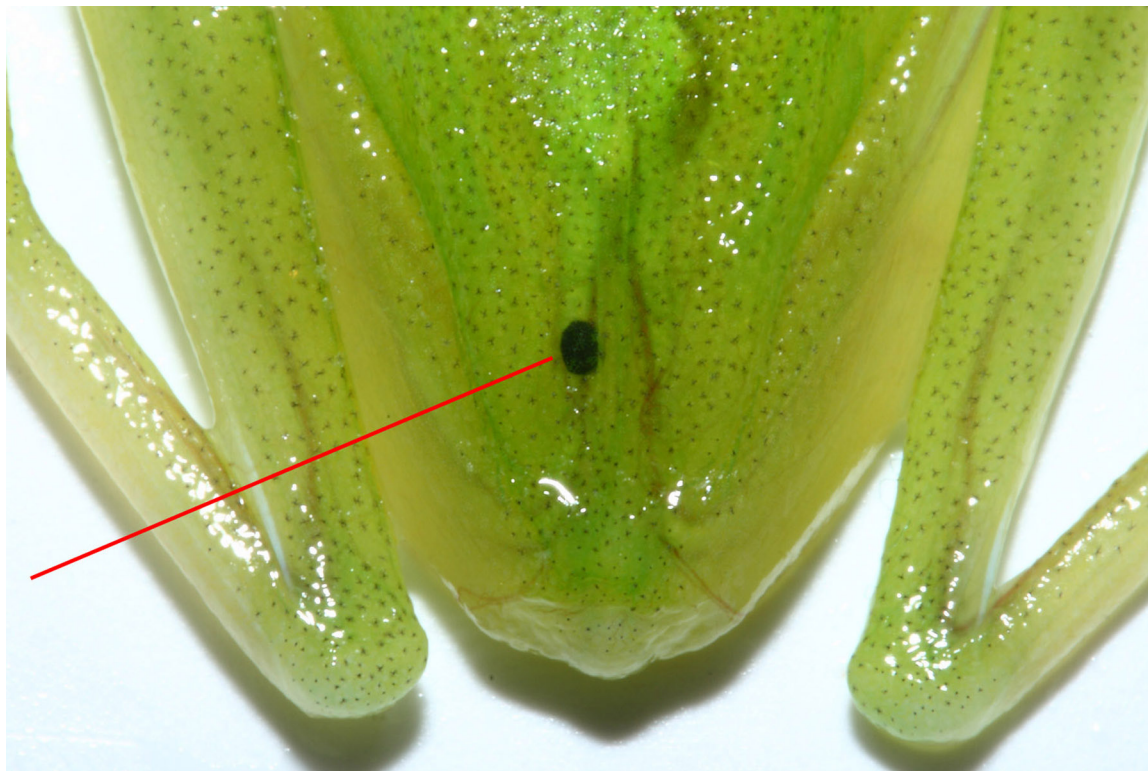


FIGURE 5. Dark oblong spherical structure of dorsal pelvic region (male holotype UCR 22038).

Coloration in life. Dorsal background color uniform Lime Green (105), lacking light yellow spots. Granules of dorsal skin often with a slightly lighter shade, varying from Pale Greenish White (97) to Light Yellow Green (87), subject to metachrosis. The hands and feet have a Pale Greenish Yellow (86) coloration, especially concentrated on the digits and webbing. The iris has a white background color with darker pigments in the form of fine to moderate-sized spots, or an even a fine reticulation in some individuals. The dark spotting or reticulation of the iris is Fuscous (283), and it typically becomes more concentrated directly surrounding the horizontally elliptical pupil. Directly below the cloaca there is a concentration of weakly white-pigmented tubercles. The subdermal oblong spherical structure of the dorsal pelvic region is black.

The ventral skin of the body is transparent; parietal peritoneum transparent; parietal pericardium transparent; bones white. The bulbous liver and digestive organs are covered in white peritonea. The heart and ventral vein are blood red. Lungs transparent, but with a network of red blood vessels. The gall bladder is transparent Sulphur Yellow (91).

Coloration in preservative. The green dorsal coloration has faded to a pale cream-yellow. The only noticeable contrasting structures of the dorsum are the dark star-shaped melanophores, and the dark colored oblong spherical structure under the dorsal skin of the pelvic region.

Measurements. Holotype: SVL 27.4 mm; HW 10.7 mm, 37.5 % of SVL; HL 8.6 mm, 30.2% of SVL; TI 17.0 mm, 59.6% of SVL; FL 12.2 mm, 42.8% of SVL; HaL 8.9 mm, 31.2% of SVL; IOD 3.1 mm, 10.9% of SVL; END 2.6 mm, 9.1% of SVL; FoL 6.0 mm, 21.1% of SVL; TaL 9.1 mm, 31.9% of SVL; EL 3.0 mm; DW 1.6 mm, 53.3% of EL.

TABLE 1. Morphometric data for the type series of *Hyalinobatrachium diana*.

Trait (mm)	UCR 22038 (Holotype)	UCR 22033	UCR 22034	UCR 22035	UCR 22036	UCR 22037	Average (range)
SVL	28.5	29.4	28.6	27.8	29.3	28.0	28.62(27.8–29.4)
HW	10.7	10.4	10.6	10.3	10.7	10.4	10.48(10.3–10.7)
HL	8.6	8.9	8.6	8.5	8.7	8.7	8.68(8.5–8.9)
TI	17.0	16.6	16.1	16.2	17.6	17.0	16.7(16.1–17.6)
FL	12.2	12.3	12.4	11.8	13.3	12.6	12.48(11.8–13.3)
HaL	8.9	9.0	9.0	8.5	9.4	9.0	8.98(8.5–9.4)
IOD	3.1	3.4	2.6	2.9	3.2	3.4	3.1(2.6–3.4)
END	2.6	2.7	2.6	2.2	2.3	2.2	2.42(2–2.7)
DW	1.6	1.5	1.6	1.5	1.5	1.6	1.54(1.5–1.6)

Variation. The dark oblong spherical structure under the skin of the dorsal pelvic region showed slight variation in its orientation to the center axis of the body, being positioned slight off center to the left or right in the different type specimens.

The female paratopotype had more of an acute shape of the snout in lateral view compared to the truncate snout of the five males of the type series. Being that only one female is known for *Hyalinobatrachium diana*, we are unclear if this was simply a case of individual malformation, or if it is possible that this species shows sexual dimorphism in snout profile.

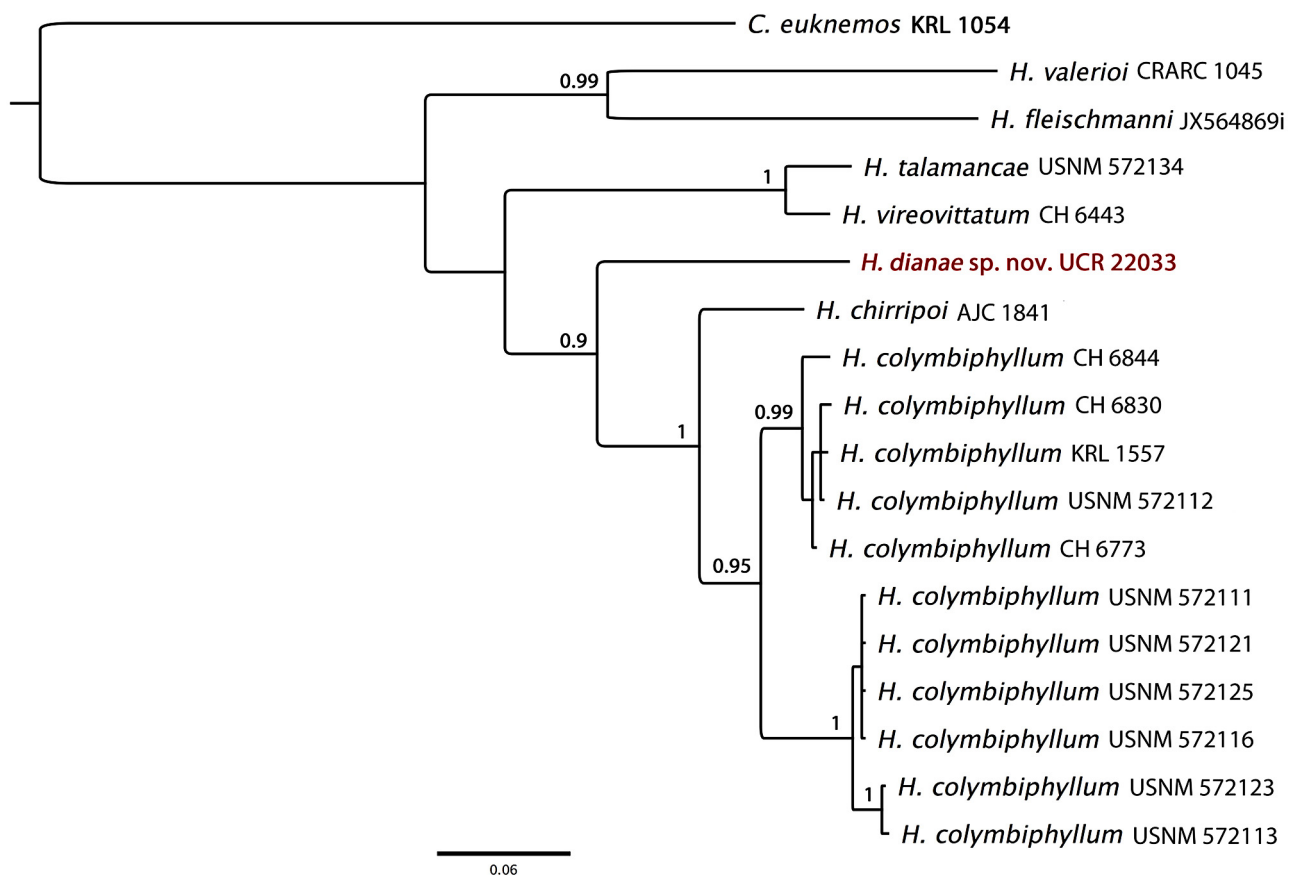


FIGURE 6. Bayesian phylogenetic tree showing phylogenetic relationships of *Hyalinobatrachium diana* and other Central American species of *Hyalinobatrachium* for which sequences were available. Bayesian posterior probability values (>0.9) are shown above the branches.

Molecular genetics. Our studies have shown that *Hyalinobatrachium diana*e is highly divergent from other members of the genus *Hyalinobatrachium* that have COI sequences available on GenBank (Fig. 6). The congener that presents the closest relationship to *H. diana*e is *H. chirri*poi (12.4%). Despite the relatively low number of COI sequences for *Hyalinobatrachium* on GenBank, a good representation of the species native to Central America are available, lacking only *H. aureoguttatum* and *H. valer*ioi. We have included a COI sequence of *H. valer*ioi for additional comparison. Being that our analysis only looked at a single gene (COI) we use our data to infer alpha-level differences among those species which have COI sequences available on GenBank, and do not attempt to imply any broader phylogenetic relationships. Lacking a larger sampling of South American *Hyalinobatrachium* species sequenced for COI makes a phylogenetic assessment of this gene for the genus difficult at this time. One COI mtDNA sequence for both *H. diana*e (accession number: KJ703103), and *H. valer*ioi (accession number: KM925140) have been deposited in GenBank.

Advertisement call. Males of this species call at night from sites among the forest understory. Males have only been observed calling from the underside of the vegetation, but being that some other congeners can at times be observed calling from the upper leaf surface (Kubicki 2007), it is possible that *Hyalinobatrachium diana*e males occasionally call from the upper leaf surfaces as well. Calling males have been observed overhanging the ground instead of moving water like most centrolenids. The advertisement call of *H. diana*e is quite unique, it consists of a single tonal long metallic whistle-like note with a very rapid but weakly pulsed intensity. Despite having a weakly pulsed intensity, the advertisement calls of *H. diana*e lacks distinctly discernible pulses like those in *H. chirri*poi and *H. colymbi*phyllum.

On 13 October 2013, nine advertisement calls were recorded from the male holotype prior to collection. The male was observed calling alongside a single egg mass, and positioned over a trail with a trickle of water flowing down its path due to recent rain showers; normally this trail is dry without flowing water. The calls were recorded at 20:15–20:30 hours: ambient temperature 22.7 C; relative humidity 95–100%. The advertisement call of *Hyalinobatrachium diana*e has total duration of 0.40–0.55 s (average 0.501 s), and a dominant frequency of 3.35–3.44 kHz (average 3.39 kHz). The accompanying audiospectrograms of *H. diana*e (Fig. 7) represent two calls from the nine that were recorded and measured for this study. Call 1 consisted of a single note, with a total duration of 0.53 s and having a dominant frequency of 3.43 kHz. Call 2 consisted of a single note, with a total duration of 0.52 seconds and having a dominant frequency of 3.4 kHz. The “pulses” of the advertisement call are so close together and weak that it is difficult to determine an exact number from visual examination of the audiospectrograms in Raven Lite 1.0. Our estimates from visual examination of the audiospectrograms included in Figure 7 are 33 weak pulses in call 1, and 30 weak pulses in call 2. Extrapolation from this estimated number of pulses in the two advertisement calls examined would result in approximately 60 pulses per second of note.

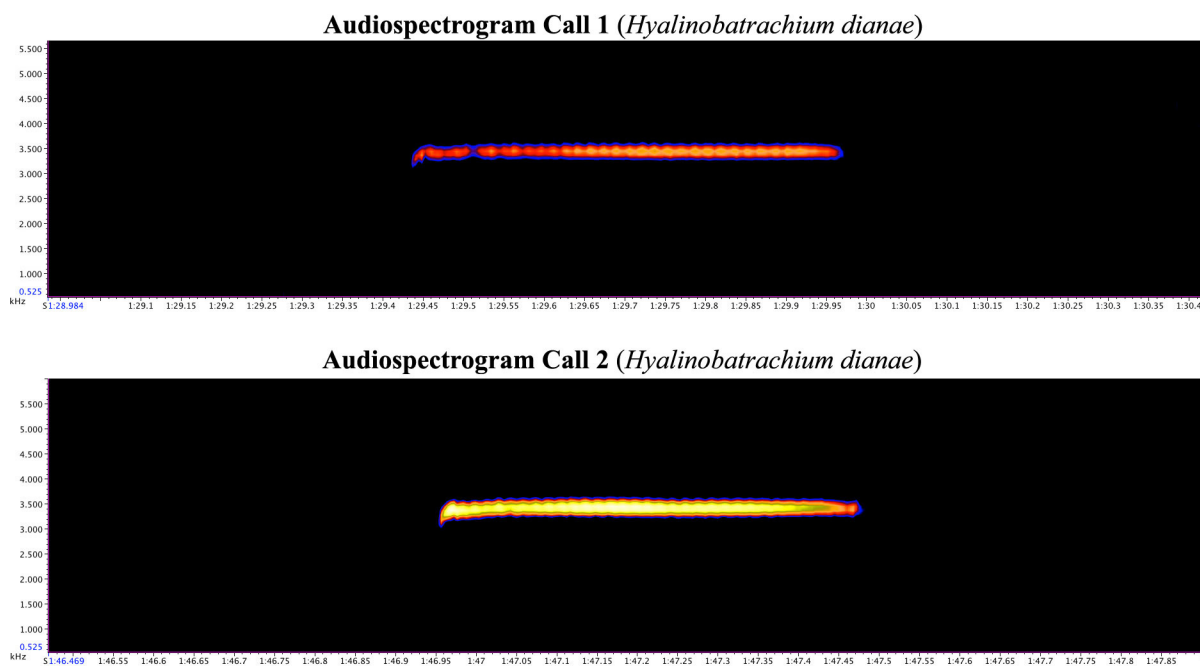


FIGURE 7. Audiospectrograms of two advertisement calls from the male holotype of *Hyalinobatrachium diana*e.

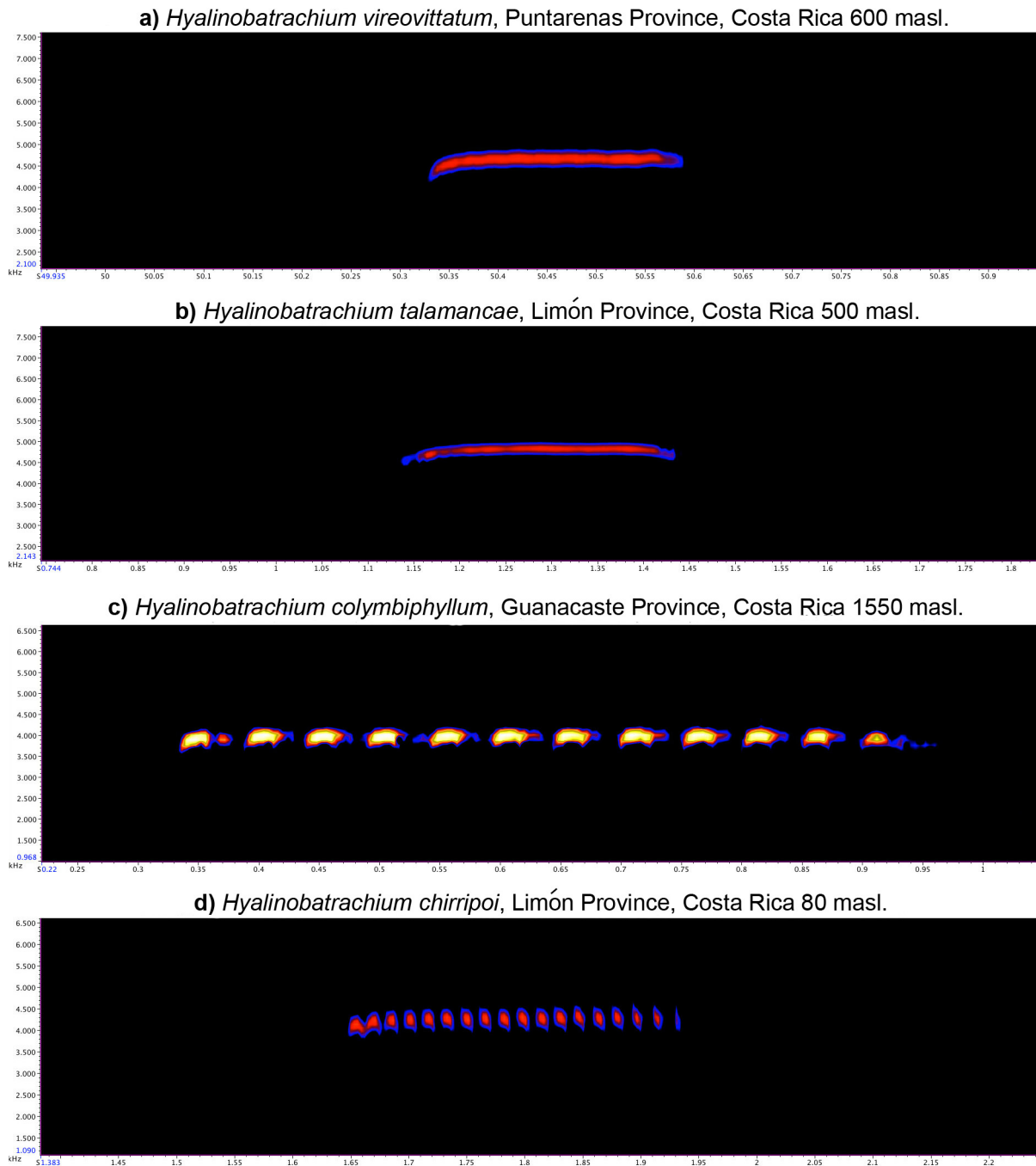


FIGURE 8. Audiospectrograms of the closest related congeners from Costa Rica.

Audiospectrograms of related Costa Rican congeners to *Hyalinobatrachium diana*e are provided for comparison (Fig. 8).

Etymology. We propose the name “*diana*e” in dedication to the senior author’s mother, Janet Diane Kubicki, who always encouraged Brian’s life-long interest with natural history and especially fishes and amphibians. Additionally alluding to the Roman goddess of the hunt, wild animals and woodlands, Diana, who was believed to have a preference of dwelling in sacred forests on high mountains. This being in relation to our own “hunt” among Costa Rica’s mountainous forests to better understand the amphibians dwelling within.

Habitat and ecology. *Hyalinobatrachium diana*e is a nocturnal frog that has been observed to inhabit mature secondary and primary humid forests with varying topography. This species has not been encountered in high densities, during evenings when we have encountered actively calling males typically only one to three individuals

have been heard or observed at a particular site. Egg masses are laid on the underside of the leaf in a single layer (Fig. 9). In nine masses that were counted, the number of eggs ranged from 31–68 eggs. Males have been observed at night calling next to the egg masses on the underside of the vegetation between 0.5–5 meters above the ground. Males have also been noted to have the peculiar behavior of calling and/or attending egg masses over apparently dry forest substrate or dry washes instead of overhanging water, as is typical for members of the family Centrolenidae. The sites from which *H. diana*e has been observed calling and reproducing are located on sloping low areas in relative proximity to streams. It is possible these sites are chosen that in the event of heavy rains the surface runoff water will channel into these sloping low areas and provide flowing water connectivity to a nearby stream for any larvae that hatch. Our field observations (BK & SS) thus far have shown that this species has unpredictable calling activity, with calling males being heard on one night, then on a different night (even with apparently similar climatic and lunar conditions) not a single male can be heard calling.



FIGURE 9. A male paratype (UCR 22033) of *Hyalinobatrachium diana*e attending two egg masses.

Distribution. This species is only known from three sites along the Caribbean foothills of Costa Rica, between the vicinity of Santa Clara, Heredia Province and the headwaters of Río Victoria, Limón Province. *Hyalinobatrachium diana*e has been observed at elevations between 400 to 900 m.a.s.l. (Fig. 10). The known sites for this species are within the life zones of Tropical Wet Forest and Tropical Premontane Rain Forest (Holdridge 1967). It is likely that this species ranges further to the northwest and to the southeast along the same Caribbean foothills of the Cordillera Volcanica Central and the Cordillera de Talamanca within Costa Rica. It is also possible that the distribution of this species extends on to the Caribbean slopes of northwestern Panama.

Remarks. Most of the known geographic range of *Hyalinobatrachium diana*e is covered by large intact tracts of premontane rainforests along the Caribbean slopes of the Central Volcanic and Talamancan mountain ranges of Costa Rica where several protected areas exist under the National System of Areas of Conservation (Sistema Nacional de Areas de Conservacion, SINAC). Very few roads grant access to the overall region that *H. diana*e is known to inhabit, so in the near future we foresee only very limited human threats to this species. How potential infectious disease and climatic change might affect this species in the future is unclear at this moment.

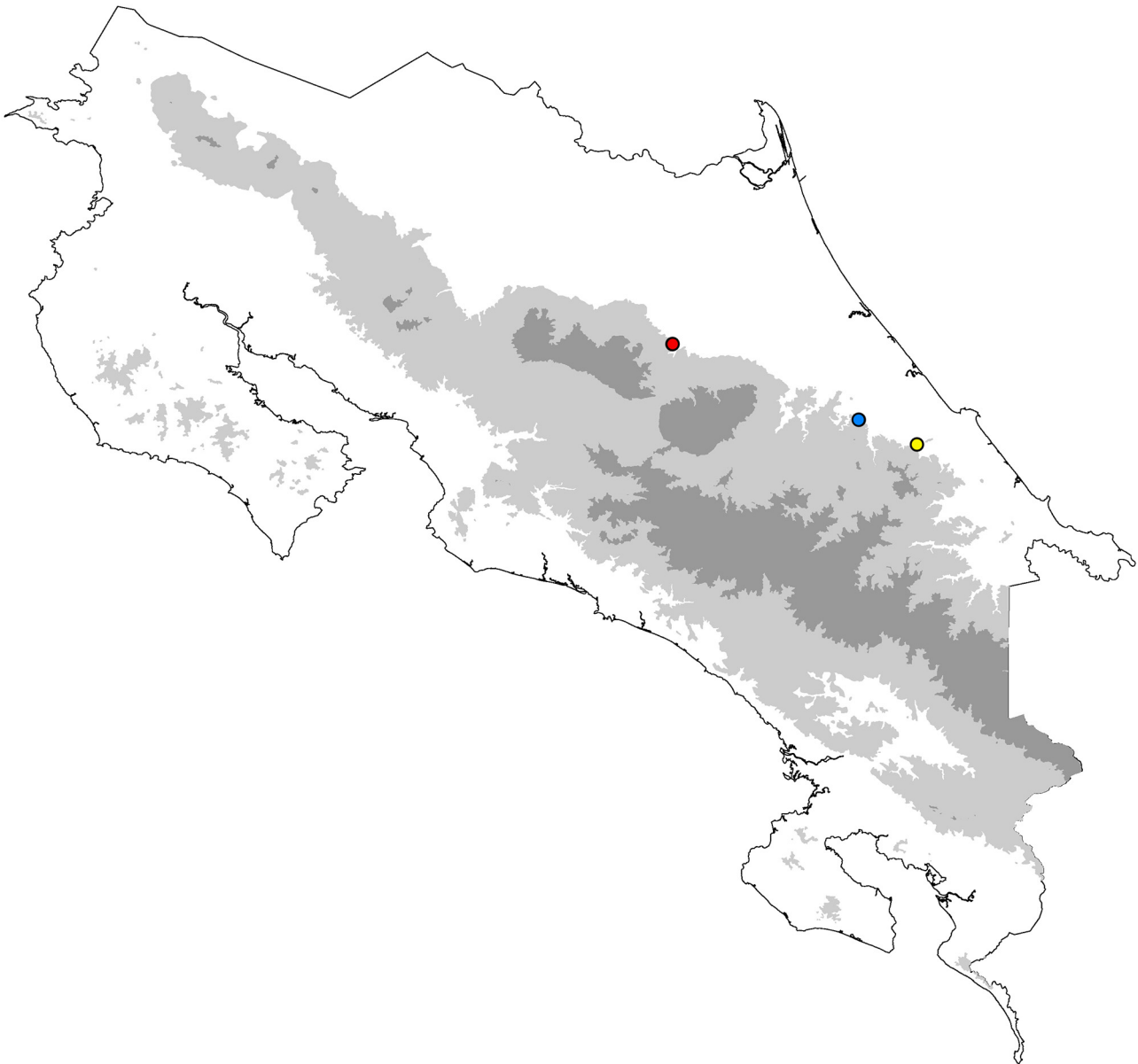


FIGURE 10. Known distribution of *Hyalinobatrachium diana* within the republic of Costa Rica. Red circle represents the type locality near Santa Clara, the blue circle represents the paratype locality at the headwaters of Quebrada Surubre, and the yellow circle represents the paratype locality at the headwaters of Rio Victoria. Light gray shaded area on the map represents 500 to 1500 masl. The dark gray shaded region represents area with an altitude above 1500 masl.

We relate the recent discovery of this species in part to the fact that its known distribution lies in an area that has remained relatively unexplored due to the limited access. Additional factors that may have led to the recent discovery of *Hyalinobatrachium diana* include its unusual call, being more similar to an insect than other centrolenids known to inhabit Costa Rica, and our observations that have shown this species exhibits unpredictable calling activity, in which it can be more common to visit a site and not hear calling males than it is to arrive at a site and find calling males.

Acknowledgments

We would like to thank Javier Guevara and the Costa Rican Ministry of Energy, the Environment, and Telecommunications (MINAET) for providing the senior author the corresponding scientific collecting permits

(081-2011-SINAC, 089-2012-SINAC, and 218-2012-SINAC). Conrad Hoskin provided help during the sequencing of specimens. Andrew Crawford and Santiago Castroviejo for providing advice with the phylogenetic tree. Joseph R. Mendelson and Gunther Köhler who kindly read early drafts of the manuscript and provided useful comments. Miguel and Norberto Solano who provided one of the male paratype specimens (UCR 22033). We would also like to thank Víctor Varela for allowing us to explore his private property, Finca la Guacamaya.

References

- Barrera-Rodriguez, M. & Ruiz-Carranza, P.M. (1989) Una nueva especie del genero *Centrolenella* Noble, 1920 (Amphibia: Anura: Centrolenidae) de la Cordillera Occidental de Colombia. *Trianea*, 3, 77–84. [Bogotá]
- Boettger, O. (1892) *Katalog der Batrachier-Sammlung im Museum der Senckenbergischen Naturforschenden Gesellschaft in Frankfurt am Main*. Gebrüder Knauer, Frankfurt am Main, 73 pp.
- Boettger, O. (1893) Ein neuer Laubfrosch aus Costa Rica. *Bericht der Senckenbergischen Naturforschenden Gesellschaft in Frankfurt am Main*, 1893, 251–252.
- Castroviejo-Fisher, S., Padiá, J.M., Chaparro, J.C., Aguayo, R. & De la Riva, I. (2009) A new species of *Hyalinobatrachium* (Anura: Centrolenidae) from the Amazonian slopes of the central Andes with comments on the diversity of the genus in the area. *Zootaxa*, 2143, 24–44.
- Castroviejo-Fisher, S., Moravec, J., Aparicio, J., Guerrero-Reinhard, M. & Calderon, G. (2011) DNA taxonomy reveals two new species records of *Hyalinobatrachium* (Anura: Centrolenidae) for Bolivia. *Zootaxa*, 2798, 64–68.
- Castroviejo-Fisher, S., Guayasamin, J.M., Gonzalez-Voyer, A. & Vilà, C. (2014) Neotropical diversification seen through glassfrogs. *Journal of Biogeography*, 41, 66–80.
<http://dx.doi.org/10.1111/jbi.12208>
- Cisneros-Heredia, D.F. & McDiarmid, R.W. (2007) Revision of the characters of Centrolenidae (Amphibia: Anura: Athesphatanura), with comments on its taxonomy and the description of new taxa of glassfrogs. *Zootaxa*, 1572, 1–82.
- Crawford, A.J., Lips, K.R. & Bermingham, E. (2010) Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. *Proceedings of the National Academy of Sciences*, 107, 13777–13782. <http://dx.doi.org/10.1073/pnas.0914115107>
- Dunn, E.R. (1931) New frogs from Panama and Costa Rica. *Occasional Papers of the Boston Society of Natural History*, 5, 385–401.
- Frost, D.R. (2014) Amphibian Species of the World: an Online Reference. Version 6.0. (October 8th 2014). Electronic Database accessible. American Museum of Natural History, New York, USA. Available from: <http://research.amnh.org/herpetology/amphibia/index.html> (accessed 3 February 2015)
- Guayasamin, J.M., Castroviejo-Fisher, S., Trueb, L., Ayarzagüena, J., Rada, M. & Vilà, C. (2009) Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa*, 2100, 1–97.
- Holdridge, L.R. (1967) *Life Zone Ecology. Revised Edition*. Tropical Science Center, San José, Costa Rica, 206 pp.
- Lynch, J.D. & Duellman, W.E. (1973) A review of the centrolenid frogs of Ecuador, with descriptions of new species. *The University of Kansas Museum of Natural History Occasional Papers*, 16, 1–66.
- Köhler, G. (2012) *Color Catalogue for Field Biologists*. Herpeton, Offenbach, Germany, 49 pp.
- Kubicki, B. (2004) Rediscovery of *Hyalinobatrachium chirripoi* (Anura: Centrolenidae) in southeastern Costa Rica. *Revista de Biología Tropical*, 52, 214–218.
- Kubicki, B. (2006) Rediscovery of the green-striped glass frog *Hyalinobatrachium talamancae*, (Anura Centrolenidae) in Costa Rica. *Brenesia*, 66, 25–30.
- Kubicki, B. (2007) *Ranas de Vidrio de Costa Rica/Costa Rica Glass Frogs*. Editorial INBio, Santo Domingo de Heredia, Costa Rica, 304 pp.
- Myers, C.W. & Duellman, W.E. (1982) A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. *American Museum Novitates*, 2752, 1–32
- Peters, W.C.H. (1873) Über eine neue Schilddrüsenart, *Cinosternon Effeldtii* und einige andere neue oder weniger bekannte Amphibien. *Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin*, 1873, 603–618.
- Ruiz-Carranza, P.M. & Lynch, J.D. (1991) Ranas Centrolenidae de Colombia. 1. Propuesta de una nueva clasificación genérica. *Lozania (Acta Zoológica Colombiana)*, 57, 1–30.
- Savage, J.M. (1967) A new tree-frog (Centrolenidae) from Costa Rica. *Copeia*, 1967, 325–331.
<http://dx.doi.org/10.2307/1442121>
- Savage, J.M. (2002) *The Amphibians and Reptiles of Costa Rica: A Herpetofauna between two Continents, between two Seas*. University of Chicago Press, Chicago, USA, 934 pp.
- Savage, J.M. & Heyer, W.R. (1967) Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, Central America. *Beiträge zur Neotropischen Fauna. Stuttgart*, 5, 111–131.
- Savage, J.M. & Starrett, P.H. (1967) A new fringe-limbed tree-frog (family Centrolenidae) from lower Central America. *Copeia*, 1967, 604–609.
<http://dx.doi.org/10.2307/1442239>

- Savage, J.M. & Heyer, W.R. (1997) Digital webbing formulae for anurans: a refinement. *Herpetological Review*, 28 (3), 131.
- Starrett, P.H. & Savage, J.M. (1973) The systematic status and distribution of Costa Rican glass-frogs, genus *Centrolenella* (Family Centrolenidae), with description of a new species. *Bulletin of the Southern California Academy of Sciences*, 72, 57–78.
- Taylor, E.H. (1949) Costa Rican frogs of the genera *Centrolene* and *Centrolenella*. *University of Kansas Science Bulletin*, 33, 257–270.
- Taylor, E.H. (1951) Two new genera and a new family of tropical American frogs. *Proceedings of the Biological Society of Washington*, 64, 33–40.
- Taylor, E.H. (1952) A review of the frogs and toads of Costa Rica. *University of Kansas Science Bulletin*, 35, 577–942.
- Taylor, E.H. (1958) Notes on Costa Rican Centrolenidae with descriptions of new forms. *University of Kansas Science Bulletin*, 39, 41–68.
- Twomey, E., Delia, J. & Castroviejo-Fisher, S. (2014) A review of Northern Peruvian glassfrogs (Centrolenidae), with the descriptions of four new remarkable species. *Zootaxa*, 3851, 1–87.
<http://dx.doi.org/10.11646/zootaxa.3851.1.1>
- Wen, A., Vasquez, N. & Castroviejo-Fisher, S. (2012) Description of the previously unknown advertisement calls of *Hyalinobatrachium fragile*, *H. pellucidum*, and *Vitreorana antisthenesi* (Amphibia: Centrolenidae). *Zootaxa*, 3480, 80–87.

APPENDIX I. Additional specimens examined.

- Hyalinobatrachium chirripoi*: COSTA RICA: Limón: KU 36865 (holotype), CRARC 1005, CRARC 1013, CRARC 1014.
- Hyalinobatrachium colymbiphyllum*: COSTA RICA: Alajuela: KU 23812 (holotype); Guanacaste: CRARC 0133; Limón: CRARC 1026; Puntarenas: CRARC 1000, CRARC 1001, CRARC 0132.
- Hyalinobatrachium fleischmanni*: COSTA RICA: Limón: CRARC 1006, CRARC 1023, CRARC 1036, CRARC 0184; Puntarenas: CRARC 1043.
- Hyalinobatrachium talamancae*: COSTA RICA: Cartago: KU 30887 (holotype); Limón: CRARC 1027, CRARC 1028, CRARC 1033, CRARC 0134, CRARC 0183.
- Hyalinobatrachium valerioi*: COSTA RICA: Limón: CRARC 1045, CRARC 0182, CRARC 0185; Puntarenas: CRARC 1002, CRARC 1003, CRARC 1029; San José: CRARC 1038, CRARC 1047.
- Hyalinobatrachium vireovittatum*: COSTA RICA: Puntarenas: CRARC 1008, CRARC 1009, CRARC 0131; San José: LACM 75141 (holotype).

APPENDIX II. DNA sequences and accession codes used for this study.

Genus	Species	Catalogue number	GenBank accession codes
<i>Cochranella</i>	<i>euknemos</i>	KRL 1054	FJ766601
<i>Hyalinobatrachium</i>	<i>chirripoi</i>	AJC 1841	KF604294
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	CH 6830	KF604296
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	CH 6844	KF604295
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	KLR 1557	FJ766710
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	USNM 572111	FJ766709
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	USNM 572121	FJ766713.1
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	USNM 572123	FJ766712
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	USNM 572125	FJ766711
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	USNM 572113	FJ766714
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	USNM 572116	FJ766715.1
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	CH 6773	KF604297
<i>Hyalinobatrachium</i>	<i>colymbiphyllum</i>	USNM 572112	FJ766708
<i>Hyalinobatrachium</i>	<i>dianae</i>	UCR 22033	KJ703103
<i>Hyalinobatrachium</i>	<i>fleischmanni</i>	JX564869i	JX564869
<i>Hyalinobatrachium</i>	<i>talamancae</i>	USNM 572134	FJ766718
<i>Hyalinobatrachium</i>	<i>valerioi</i>	CRARC 1045	KM925140
<i>Hyalinobatrachium</i>	<i>vireovittatum</i>	CH 6443	KF604298