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A new glassfrog (Centrolenidae: *Hyalinobatrachium*) from the Topo River Basin, Amazonian slopes of the Andes of Ecuador

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Abstract.—A new species of glassfrog (Centrolenidae) is described from the San Jacinto River, an affluent of the Topo River, on the Amazonian slopes of the Ecuadorian Andes. The new species, *Hyalinobatrachium adespinosai* sp. nov., can be differentiated from all other centrolenids by the combination of its coloration (transparent peritoneum and pericardium) and vocalization (call duration = 0.38–0.44 s, with 9–13 pulses per call; dominant frequency = 4,645–5,001 Hz). However, *H. adespinosai* sp. nov. is morphologically cryptic with *H. anachoretus, H. esmeralda,* and *H. pellucidum*, from which it differs by call traits (in *H. anachoretus:* call duration = 0.32–0.37 s, with 5 or 6 pulses per call, dominant frequency = 4,670–4,800 Hz; in *H. esmeralda:* call duration = 0.218–0.257 s, tonal call, dominant frequency = 4,739–5,580 Hz; in *H. pellucidum:* call duration = 0.112–0.140 s, tonal, dominant frequency = 5,000–5,710 Hz). Biogeographically, the new species is separated from *H. anachoretus* by a considerable distance and, also, the Marañon valley. Finally, following IUCN conservation criteria, the status of the new species is considered as Data Deficient.

Keywords. Amphibia, Anura, Ecuador, Pastaza basin, phylogeny, Tungurahua Province

Resumen.—Describimos una nueva especie de rana de cristal (Centrolenidae) del río San Jacinto, afluente del río Topo, en la vertiente amazónica de los Andes del Ecuador. La especie nueva, *Hyalinobatrachium adespinosai* sp. nov., se diferencia de todos los centrolénidos por la combinación de su coloración ventral (peritoneo y pericardio transparentes) y las características de su canto (duración del canto = 0.382–0.430 s, con 9–13 pulsos por canto; frecuencia dominante = 4,645–5,001 Hz). Sin embargo, es morfológicamente críptica con *H. anachoretus, H. esmeralda* y *H. pellucidum*, especies de las cuales difiere por su canto (en *H. anachoretus:* duración del canto = 0.32–0.37 s, con 5 or 6 pulsos por canto, frecuencia dominante = 4,670–4,800 Hz; en *H. esmeralda:* duración del canto = 0.218–0.257 s, tonal, frecuencia dominante = 4,739–5,580 Hz; en *H. pellucidum:* duración del canto = 0.112–0.140 s, tonal, frecuencia dominante = 5,000–5,710 Hz). Finalmente, siguiendo los criterios de la UICN, sugerimos que *Hyalinobatrachium adespinosai* sp. nov. sea ubicada en la categoría de Datos Insuficientes.

Palabras clave. Amphibia, Anura, Cuenca del Pastaza, Ecuador, filogenia, Tungurahua Province

Introduction

The glassfrog genus *Hyalinobatrachium* (sensu Ruiz-Carranza and Lynch 1991, as modified by Guayasamin et al. 2009) is one of the most charismatic anuran groups because of its peculiar morphological and behavioral traits. One of the most striking traits of this genus is its complete ventral transparency, produced by having a transparent ventral peritoneum (Ruiz-Carranza and Lynch 1991; Cisneros-Heredia and McDiarmid, 2007; Guayasamin et al. 2009). Males of all species in this genus also exhibit extended parental care towards

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fertilized egg clutches deposited on the leaves of trees (see Delia et al. 2017). Extended male parental care, a derived trait that has evolved at least twice in glassfrogs, is coupled with egg deposition on the underside of leaves in all *Hyalinobatrachium* and some *Centrolene* species (Ruiz-Carranza and Lynch 1991; Guayasamin et al. 2009; Delia et al. 2017; Salgado and Guayasamin 2018).

Although assigning species to *Hyalinobatrachium* is straightforward, distinguishing among members of this genus is more complicated because species of *Hyalinobatrachium* tend to be remarkably similar, both morphologically and ecologically. In recent years, species discovery in frogs has relied heavily on molecular and acoustic traits (Castroviejo-Fisher et al. 2009, 2011; Kubicki et al. 2015; Guayasamin et al. 2017). Calls, in particular, are especially useful for distinguishing among cryptic species since they function as efficient prezygotic mating recognition signals (Narins and Capranica 1976; Duellman and Trueb 1994; Zakon and Wilczynski 1988; Wilczynski and Ryan 1999; Wells 2007). As previously demonstrated in glassfrogs, the acoustic differences between species are often more pronounced than morphological differences (Escalona-Sulbarán et al. 2018).

We describe a new species of *Hyalinobatrachium* that is morphologically cryptic with *H. anachoretus* Twomey, Delia, and Castroviejo-Fisher 2014, *H. esmeralda* Ruiz-Carranza and Lynch 1998, and *H. pellucidum* (Lynch and Duellman 1973). The new species, which is known from a single locality in the Topo River basin, is differentiated from these two species by its call and genetics.

Materials and Methods

Ethics statement. Research was conducted under permits NoMAE-DNB-CM-2015-2017, 019-2018-IC-FAU-DNB/MAE, and 018-2017-IC-FAU-DNB/MAE, issued by the Ministerio del Ambiente del Ecuador. The study was carried out in accordance with the guidelines for use of live amphibians and reptiles in field research (Beaupre et al. 2004), compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR).

Taxonomy and species concept. Glassfrog generic and family names follow the taxonomy proposed by Guayasamin et al. (2009). Species are considered as segments of separately evolving metapopulation lineages, following the conceptual framework developed by Simpson (1951, 1961), Wiley (1978), and de Queiroz (2007). Assessing whether a given population is an independent lineage is a non-trivial task, especially when working with closely related taxa. In such cases, analyzing many different sets of characters provides tools for supporting species hypotheses (Dayrat 2005; de Queiroz 2007; Padial et al. 2009).

Morphological data. Lynch and Duellman (1973) and Cisneros-Heredia and McDiarmid (2007) are followed for the diagnosis and description of the new species. The webbing formula follows Savage and Heyer (1967), as

modified by Guayasamin et al. (2006). The taxonomy of centrolenid frogs follows the proposal by Guayasamin et al. (2009). Comparisons were made between various *Hyalinobatrachium* specimens (see Appendix 1) housed at the following collections: Instituto de Ciencia Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (ICN); University of Kansas, Museum of Natural History, Division of Herpetology, Lawrence, Kansas, USA (KU); Museo de Zoología, Universidad Tecnológica Indoamérica, Quito, Ecuador (MZUTI); National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM); and Museo de Zoología, Universidad San Francisco de Quito, Quito, Ecuador (ZSFQ). Morphological measurements were taken with a Mitutoyo® digital caliper to the nearest 0.1 mm, as described by Guayasamin and Bonaccorso (2004), except when noted, and are as follows: (1) snout–vent length (SVL); (2) tibia length; (3) foot length; (4) head length; (5) head width; (6) interorbital distance (IOD); (7) upper eyelid width; (8) internarial distance; (9) eye-to-snout distance; (10) eye diameter; (11) tympanum diameter; (12) radioulna length; (13) hand length; (14) Finger I length; (15) Finger II length = distance from outer margin of palmar tubercle to tip of Finger II; and (16) width of disc of Finger III. Sexual maturity was determined by the presence of vocal slits and calling activity in males.

Bioacoustics. Sound recordings were made with an Olympus LS-10 Linear PCM Field Recorder and a Sennheiser K6–ME 66 unidirectional microphone. The calls were recorded in WAV format with a sampling rate of 44.1 kHz/s with 16 bits/sample. Measurements and definitions of acoustic variables follow Köhler et al. (2017). Notes were divided into two classes—"pulsed" and "tonal"—based upon the distinct waveforms on the oscillogram (see Hutter and Guayasamin 2012). Pulsed (also termed peaked) notes are defined as those with one or more clear amplitude peaks and amplitude modulation (i.e., visible increases and decreases in amplitude on the oscillogram throughout the call). In contrast, tonal notes are defined as those with no clear amplitude peak (Dautel et al. 2011). In this study the call of *Hyalinobatrachium pellucidum* (Lynch and Duellman 1973) is also described from an individual (USNM 286708) recorded at the type locality of the species (Río Azuela, 0.1167°S, 77.617°W, 1,740 m, Napo province, Ecuador) by Roy McDiarmid; the recording is deposited at the Cornell University Macaulay Library (ML Catalogue No. 202401).

Evolutionary relationships. Mitochondrial sequences (16S) were generated for four individuals (ZSFQ 1647–48, 1650–51) of the new species of *Hyalinobatrachium*. Extraction, amplification, and sequencing protocols were as described in Guayasamin et al. (2008). The sequences obtained were compared with all those available for other species of glassfrogs (family Centrolenidae) and its sister taxon Allophrynidae (see Austin et al. 2002; Guayasamin et al. 2018). These sequences were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), and were generated mostly by Guayasamin et al. (2008), Castroviejo-Fisher et al. (2014), and Twomey et al.

(2014), but also included data from the newly described *H. yaku* Guayasamin et al. 2017 and *H. muiraquitan* de Oliveira and Hernández-Ruz 2017. Sequences were aligned using MAFFT v.7 (Multiple Alignment Program for Amino Acid or Nucleotide Sequences: http://mafft.cbrc.jp/alignment/software/), with the Q-INS-i strategy. MacClade 4.07 (Maddison and Maddison 2005) was used to visualize the alignment (no modifications were necessary). Maximum likelihood (ML) was run in the IQ-TREE 1.5.5 software (Nguyen et al. 2015). The best-fitting nucleotide substitution model was implemented using ModelFinder within IQ-TREE (Kalyaanamoorthy et al. 2017), which groups partitions with the same model and similar rates, and simultaneously searches the model and tree space. Node support was assessed via 1,000 ultra-fast bootstrap replicates, a method that shows less bias than other support estimates (Minh et al. 2013). Ultra-fast bootstrapping also leads to straightforward interpretation of the support values (e.g., support of $\geq$ 95 bootstrap should be interpreted as significant; Minh et al. 2013).

Results

Phylogenetic relationships. Based on the Bayesian Information Criterion, the best-fit model for this dataset was GTR+F+R5. Rate parameters were estimated as follows: A–C: 2.690, A–G: 9.067, A–T: 3.078, C–G: 0.346, C–T: 23.835, and G–T: 1.000. Base frequencies were: A = 0.346, C = 0.239, G = 0.181, and T = 0.234.

The phylogeny (Fig. 1) confirms the placement of the new species within the genus *Hyalinobatrachium*. The new species, described below, is inferred as part of a clade formed by four species that have very similar morphologies: the new species (described below) + *H. anachoretus + H. pellucidum + H. yaku.*

Species description

Hyalinobatrachium adespinosai new species

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Suggested English name: Adela's Glassfrog
Suggested Spanish name: Rana de Cristal de Adela

Holotype. ZSFQ 1648 (JMG 583, Fig. 2), adult male from riverine vegetation along the San Jacinto River (1.3447°S, 78.1814°W; 1,795 m asl), Tungurahua Province, Ecuador, collected by CRH, REG, and KC on 4 August 2017.

Paratypes. ZSFQ 1650–52, 1647, adult males with same data as holotype.

Generic placement. The new species is placed in the genus *Hyalinobatrachium* (Ruiz-Carranza and Lynch, 1991, as modified by Guayasamin et al. 2009) on the basis of morphological and molecular data. The main diagnostic phenotypic traits of *Hyalinobatrachium* are: (1) ventral parietal peritoneum completely transparent; (2) digestive tract and bulbous liver covered by iridophores; (3) humeral spines absent; (4) white bones in life; (5) males call from the underside of leaves; (6) females place the eggs on the underside of leaves; and (7) males provide extended parental care. All the aforementioned characteristics are shared by the new species. Additionally, analyses of the mitochondrial 16S gene place the new species as a close relative of other *Hyalinobatrachium* species (Fig. 1); thus, generic placement in *Hyalinobatrachium* is unambiguous.

Diagnosis. Within the genus *Hyalinobatrachium,* the new species is diagnosable mainly by having a transparent pericardium. However, the new species is morphologically cryptic with three closely related taxa (*H. anachoretus, H. pellucidum*, *H. esmeralda*). Based on comparisons with specimens examined (see Appendix 1), all these species display a similar size and color pattern (pale green dorsum with yellow dots and a transparent venter and pericardium; red heart visible ventrally). However, calls between species diverge noticeably; the major difference is the structure of the call, with two species (*H. adespinosai* **sp. nov.** and *H. anachoretus*) having pulsed calls and the others having tonal vocalizations (Fig. 3; Table 1). The call of *H. adespinosai* **sp. nov.** is further differentiated from that of *H. anachoretus* by being longer, having more pulses per note, and being produced at a higher rate (Table 1). Toe webbing (Toe IV) is less extensive in the new species ($2^{1/3}$ IV 2^+) than in *H. anachoretus* (1^+ IV 1^+; Twomey et al. 2014). Additionally, the new species and *H. anachoretus* are separated by considerable distance (airline distance = 473 km), including one of the most important biogeographic barriers in South America, the Marañon valley (see Duellman 1999; Winger and Bater 2015 and references therein). Uncorrected *p* genetic distances for the mitochondrial gene 16S between *H. adespinosai* **sp. nov.** and its closest relatives are summarized in Table 2.

Characterization. The following combination of characters is found in *Hyalinobatrachium adespinosai* **sp. nov.**: (1) dentigerous process of the vomer lacking teeth; (2) snout truncate in dorsal and lateral views; (3) tympanum barely visible, hidden under skin, with coloration similar to that of surrounding skin; (4) dorsal skin shagreen; (5) ventral skin areolate; cloacal ornamentation absent, paired round tubercles below vent absent; (6) parietal peritoneum transparent; pericardium with thin layer of iridophores (in life, a red heart is mostly visible ventrally); liver, viscera, and testes covered by iridophores; (7) liver bulbous; (8) humeral spines absent; (9) hand webbing formula: I (2–3) — (2–2$^+$) II (1–1$^+$) —3$^{1/3}$ III (2–2$^+$) — (2$^-$–2) IV; (10) foot webbing moderate; webbing formula: I 1— ($1^{2/3}$–2$^-$) II (1–1$^-$) — (2$^-$–$2^{1/3}$) III (1–1$^+$) —(2$^+$–$2^{1/3}$) IV 2$^+$— (1^+–$1^{1/3}$) V; (11) fingers and toes with thin lateral fringes; ulnar and tarsal folds present, but low and difficult to distinguish, with thin layer of iridophores that extends to ventrolateral edges of Finger IV and Toe V; (12) nuptial excrescence present as a small pad on Finger I (Type V), prepollex not enlarged; prepollical spine not projecting (spine not exposed); (13) when appressed, Finger I longer than II; (14) diameter of eye about two times wider than disc on
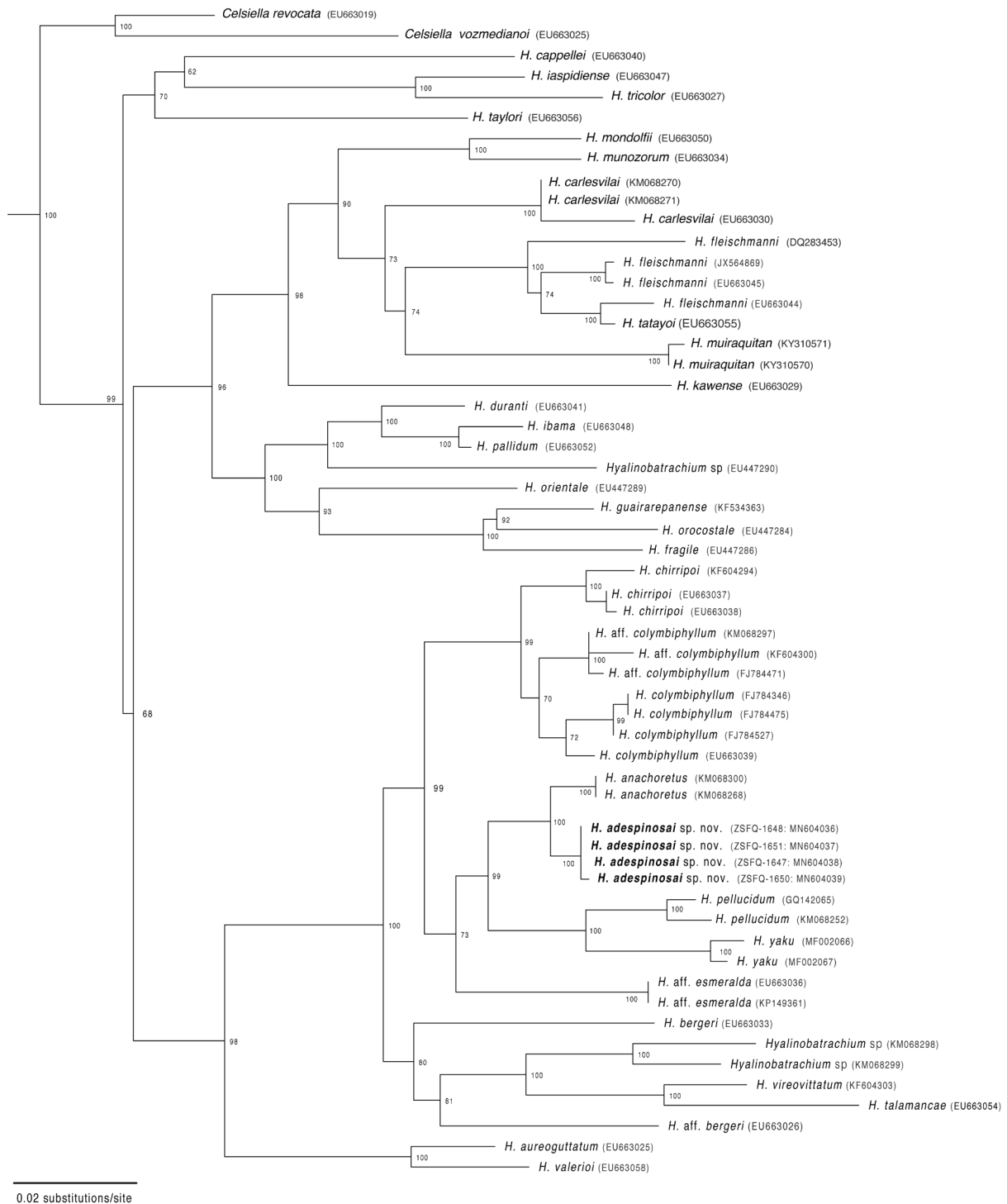
Fig. 1. Phylogenetic relationships of *Hyalinobatrachium* inferred from the 16S mitochondrial gene under ML criteria. All sequences were downloaded from GenBank, except for those of the new species. GenBank codes are listed next to each terminal. Associated locality data is available at GenBank, as well as in Guayasamin et al. (2008), Castroviejo-Fisher et al. (2014), and Twomey et al. (2014).

Finger III; (15) coloration in life: dorsal surfaces pale yellowish green with small pale yellow spots and minute gray to black melanophores; bones white; (16) coloration in preservative: dorsal surfaces pale cream with minute melanophores; (17) iris coloration in life: white with pale yellow hue and numerous minute lavender spots; (18) melanophores absent from most fingers and toes, but present on Finger IV and Toes IV and V; (19) males call from underside of leaves; advertisement call consisting of single note, distinctly pulsed (9–13 pulses per call), with duration of 0.382–0.430 s, and dominant frequency at 4,645–5,001 Hz; (20) males attend egg clutches located on the underside of leaves overhanging streams; clutch size of 22 embryos (*n* = 1); (21) SVL in adult males 20.5–22.2 mm (*n* = 3), females unknown; and (22) enameled tubercles absent from sides of head.

Fig. 3. Call of *Hyalinobatrachium adespinosai* **sp. nov.**, holotype, recorded in field conditions at the type locality. Air temperature: 18 °C.

Fig. 2. *Hyalinobatrachium adespinosai* **sp. nov.** in life, holotype.

Description of the holotype. ZSFQ 1648, adult male with SVL 22.2 mm. Head wider than long (head width 38% of SVL; head length 77% of head width). Snout truncate in dorsal and lateral views. Loreal region flat and nearly vertical, nostrils slightly protuberant, elliptical; internarial region concave anterodorsally; canthus rostralis well defined. Eyes small, directed anterolaterally, eyes about 45° relative to midline (where
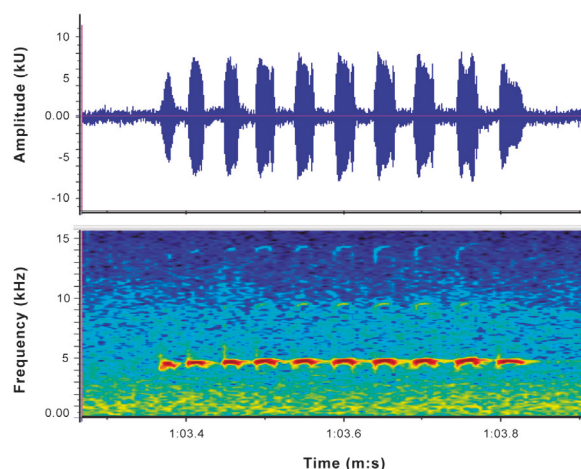
anterior-facing eyes would be 90° relative to midline). Tympanum annulus barely visible through the skin; tympanic membrane differentiated and pigmented as surrounding skin. Dentigerous processes on vomers absent; choanae large, oval, separated widely (distance about the same as between nostrils); tongue round, white in preservative, anterior 4/5 attached to mouth; vocal slits present, extending along floor of mouth lateral to tongue; enameled glands absent from lower part of upper jaw. Ulnar fold present, with a thin layer of iridophores, and continuing along the external edge of Finger IV; humeral spine absent. Relative lengths of fingers: I < II < IV < III; finger discs rounded, about the same size as discs on toes, disc on Finger III 54% of eye width; finger webbing reduced between Fingers I–III, moderate between Fingers III and IV, with formula I 3—2$^+$ II 1$^+$—3$^{1/3}$ III 2 — 2 IV. Prepollex concealed; subarticular tubercles round, faint; few small supernumerary tubercles present, palmar tubercle round and small, thenar tubercle ovoid; nuptial excrescences present as a small pad on external edge of Finger I (Type V). Hind limbs slender, tibia length 58% of SVL; tarsal fold present, with thin layer of iridophores; discs of toes round, inner metatarsal tubercle small; outer metatarsal tubercle round, but very difficult to distinguish. Webbing formula of feet: I 1— 2$^-$ II 1—2$^{1/3}$ III 1$^+$—2$^{1/3}$IV—2$^+$— 1V. In preservative, dorsal skin peppered with small dark lavender melanophores; dorsal skin shagreen; skin on venter areolate; cloacal opening at level of upper thighs, cloacal ornamentation present as a lightly enameled cloacal fold and small tubercles. Parietal peritoneum transparent; pericardium with a very thin layer of iridophores that, in life, exposes a red heart; urinary bladder lacking iridophores; liver, viscera, and testes fully covered by iridophores. Kidneys rounded, approximately bean-shaped; liver bulbous.

Coloration in life. Dorsal surfaces apple green to yellowish green with diffuse yellow spots and minute gray to black melanophores. Melanophores absent from fingers and toes, except Finger IV and Toes IV and V. Ventrally, parietal peritoneum and pericardium transparent, with a red heart always visible, even

Table 1. Comparison among calls of closely related species of *Hyalinobatrachium*. The calls of all species were recorded at the corresponding type locality, except for that of *H. esmeralda*. Call data were obtained from the following sources: *H. anachoretus* from Twomey et al. (2014), *H. esmeralda* from Acosta-Galvis (2017), and *H. yaku* from Guayasamin et al. (2017).

Species	Distribution	Sample size: # of individuals / # of calls	Call duration (s) range (mean and standard deviation)	# notes per call	Call structure	# pulses per call	Time between calls (s)	Frequency modulation	Dominant frequency at beginning of call (hz)	Dominant frequency at end of call (hz)	1st harmonic (hz)
H. adespinosai **sp. nov.**	Ecuador: Amazonian slopes of the Andes, 1,670–1,795 m	9/70	0.38–0.44 ($\bar{x}$ = 0.38 ± 0.017)	1	Pulsed	9–13 ($\bar{x}$ = 10.49 ± 1.281)	2.0–11.0 ($\bar{x}$ = 4.58 ± 2.317)	No	4,645–5,203 ($\bar{x}$ = 4,855 ± 152)	Same as at beginning	9,336–9,380
H. anachoretus	Peru: Amazonian slopes of the Andes, 2,001–2,050 m	1/4	0.32–0.37	1	Pulsed	5–6	13–16	No	4,670–4,800	Same as at beginning	No
H. esmeralda	Colombia: Amazonian slopes of the Andes, 1,026–1,700 m	1/14	0.218–0.257 ($\bar{x}$ = 0.239 ± 0.014)	1	Tonal	1 ($\bar{x}$ = 1 ± 0)	4.7–31.1 ($\bar{x}$ = 11.82 ± 7.01)	Yes, very subtle increase	4,739–5,581 (5,204 ± 298.2)	Slightly higher	No
H. pellucidum	Ecuador, Peru: Amazonian slopes of the Andes, 523–1,740 m	1/13	0.11–0.14 ($\bar{x}$ = 0.13 ± 0.009)	1	Tonal	1–3 ($\bar{x}$ = 2.15 ± 0.009)	2.3–3.3 ($\bar{x}$ = 2.7 ± 0.3)	Yes, very subtle increase	5,000–5,719 ($\bar{x}$ = 5,263 ± 207.1)	Slightly higher 5,531–5,719 ($\bar{x}$ = 5,595 ± 60.7)	11,213–11,721
H. yaku	Ecuador: Amazonian lowlands, 300–360 m	1/10	0.27–0.40 ($\bar{x}$ = 0.3 ± 0.03)	1	Tonal	1 ($\bar{x}$ = 1 ± 0)	5.3–8.9 ($\bar{x}$ = 7.1 ± 1.1)	No	5,219–5,330 ($\bar{x}$ = 5,284 ± 35.0)	Same as at beginning	No

when a very thin layer of iridophores is present on the pericardium of some individuals. Visceral peritoneum of gall bladder and urinary bladder transparent; hepatic and visceral peritonea white; ventral vein red. Iris pale yellowish white, with numerous minute lavender spots. Bones white.

Coloration in preservative. Dorsal surfaces cream, dotted with minute dark lavender melanophores; venter uniform cream; visceral peritoneum lacking iridophores; pericardium with a very thin layer of iridophores. Iris silvery white with minute lavender melanophores.

Measurements. Measurements of the type series are shown in Table 3.

Variation. Variation in hand webbing is as follows: I (2–3) — (2–2⁺) II (1–1⁺) —3¹ᐟ³ III (2–2⁺) — (2⁻–2) IV. Foot webbing variation is as follows: I 1— (1²ᐟ³–2⁻) II (1–1⁻) —(2⁻–2¹ᐟ³) III (1–1⁺) —(2⁺–2¹ᐟ³) IV 2⁺— (1⁺–1¹ᐟ³) V.

Vocalizations (Fig. 3). The description is based on recordings from nine individuals (Codes LBE-C: 048–057). The call of *Hyalinobatrachium adespinosai* **sp. nov.** has a striking resemblance to the chirp of a cricket, and was often confused for one in the field. Each call is composed of a single and high-pitched pulsed note, and has a duration of 0.38–0.44 s ($\bar{x}$ = 0.38 ± 0.017). Time between calls varied from 2.0–11.0 s ($\bar{x}$ = 4.58 ± 2.3). The fundamental frequency, the same as the dominant frequency, is at 4,645–5,203 Hz ($\bar{x}$ = 4,855 ± 152). There is no frequency modulation. The first harmonic is at 9,336–9,754 Hz and the second harmonic is at 14,159–14,444 Hz.

Ecology. All individuals of the new species were found on the underside of leaves of riverine vegetation along the San Jacinto River. The section of river was fast-flowing and had visible rapids. Although the population is locally abundant (as heard from numerous advertisement calls), individuals are very difficult to observe because they are usually found at the canopy level (4–16 m above ground level). The type series consists of males exclusively; they were calling in the months of July and August. One male (ZSFQ 1648) was apparently guarding an egg clutch containing 22 embryos; both the adult male and the egg clutch were on the same leaf most of the time, but the male also moved to nearby leaves (Fig. 4).

Distribution. *Hyalinobatrachium adespinosai* **sp. nov.** is only known from the type locality: San Jacinto River (1.3447°S, 78.1814°W; 1,795 m asl), Tungurahua Province, Ecuador (Fig. 5).

Evolutionary relationships. The phylogenetic analyses recover *Hyalinobatrachium adespinosai* **sp. nov.** haplotypes as sister to haplotypes sampled from *H. anachoretus* and nested within other members of a monophyletic clade comprised of all other sampled species of *Hyalinobatrachium* (Fig. 1). The most closely related species to *H. adespinosai* **sp. nov.** share several
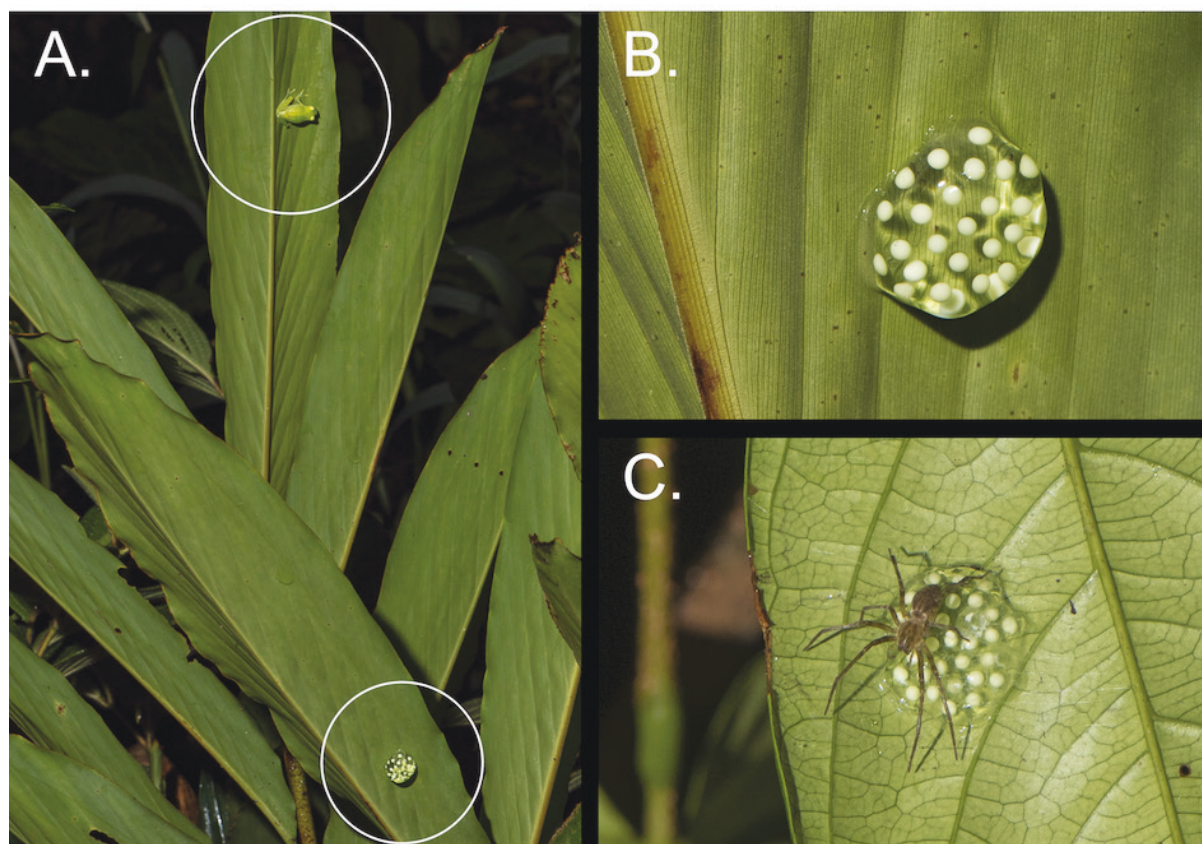
Fig. 4. Natural history and in-situ photographs of the new species. (A) Adult male of *Hyalinobatrachium adespinosai* near his egg clutch; other males were observed on the same leaf as the egg clutch. **(B)** Close-up of the egg clutch. **(C)** Spider predation on an unattended egg clutch.

morphological traits, including a red heart exposed ventrally (*H. adespinosai* + *H. anachoretus* + *H. pellucidum* + *H. yaku*).

Etymology. The specific epithet *adespinosai* honors Adela Espinosa, an Ecuadorian conservationist and board member of the Jocotoco Foundation (http://www.jocotoco.org). Adela's work has focused on the conservation of species and ecosystems. The new glassfrog described here is found only within the limits of a natural reserve owned by Adela and her husband, Antonio Páez. We are delighted to recognize Adela's devotion to nature with this marvelous species.

Conservation status. Available information is insufficient to fully assess the conservation status of *Hyalinobatrachium adespinosai* **sp. nov.** Therefore, following IUCN criteria, this species is considered as Data Deficient. The herpetological museums that house

specimens collected near the type locality (Topo basin) were consulted, but there were no additional specimens of the new species. Although this might suggest a conservation category other than Data Deficient, we actually prefer to maintain this status because the new species is very difficult to find (i.e., a canopy specialist). Therefore, in this case, absence in nearby localities where herpetological surveys have been carried out does not necessarily indicate a true absence of the species.

Discussion

Morphological stasis is expected in species under similar ecological conditions, whereas traits associated with social signaling tend to evolve more rapidly (Winger and Bater 2015; Arnegard et al. 2010; Safran et al. 2013; Escalona-Sulbarán et al. 2018). Species in the glassfrog genus *Hyalinobatrachium* exhibit a striking morphological homogeneity (see Ruiz-Carranza and

Table 2. Genetic distances (uncorrected *p* matrix for 16S, 813 base pairs) between *Hyalinobatrachium adespinosai* **sp. nov.** and closely related species.

	H. adespinosai	*H. anachoretus*	*H. esmeralda*	*H. pellucidum*	*H. yaku*
H. adespinosai	**0.000–0.001**				
H. anachoretus	0.010–0.011	**0.000**			
H. esmeralda	0.0254–0.0272	0.0272	**0.000**		
H. pellucidum	0.032–0.034	0.029–0.037	0.034–0.040	**0.007–0.009**	
H. yaku	0.036–0.037	0.033–0.041	0.038–0.040	0.025–0.030	**0.000–0.001**

Fig. 5. Distribution of *Hyalinobatrachium adespinosai* **sp. nov.** in Ecuador.

Lynch 1998; Guayasamin et al. 2009; Castroviejo-Fisher et al. 2011), perhaps because of the constraints associated not only with their similar ecology, but also with their derived reproductive strategy (prolonged parental care on the underside of leaves). The obvious consequence is that traditional morphological trait-based criteria provide an underestimation of the true biological diversity of the genus. In contrast, call traits in centrolenids have shown more variation that morphology (Escalona-Sulbarán et al. 2018). Acoustic signals can diverge because of the effects of multiple mechanisms, including drift (i.e., isolation-by-distance), natural selection (i.e., adaptation to local ecological conditions, reinforcement, character displacement), and/or sexual selection (i.e., sensory exploitation, divergent female choice; reviewed by Wilczynski and Ryan 1999; Wells 2007; Prum 2017; Köhler et al. 2017). However, this study represents another example of how vocalizations can be extremely useful for species discovery.

Given the lack of information for *Hyalinobatrachium adespinosai* **sp. nov.**, we consider the species as Data Deficient, following the IUCN criteria. The species is

locally abundant at the type locality and as currently known has a restricted distribution. However, given that the species is usually found at the canopy level, it is extremely difficult to locate individuals of this species, so we cannot infer its true distribution based solely on the lack of prior collection.

Establishing clear biogeographic patterns in groups where new species are often being described is challenging. However, *Hyalinobatrachium* species are generally found in the lowlands while *Centrolene* and *Nymphargus* species are predominantly Andean (Guayasamin et al. 2009; Hutter et al. 2017). In contrast to these general patterns, the clade formed by *H. adespinosai* **sp. nov.** + *H. anachoretus* + *H. pellucidum* + *H. yaku* + *H. esmeralda* is mostly found on the Amazonian slopes of the Andes (except *H. yaku*). Since tropical species tend to have narrow thermal niches (Shah et al. 2017, Polato et al. 2018), the linearity of the Andean mountain range might promote speciation by reducing contact and gene flow among parapatric populations (see Fig. 6), as suggested by Graves (1988). Similar patterns (i.e., closely related species along the same slope of
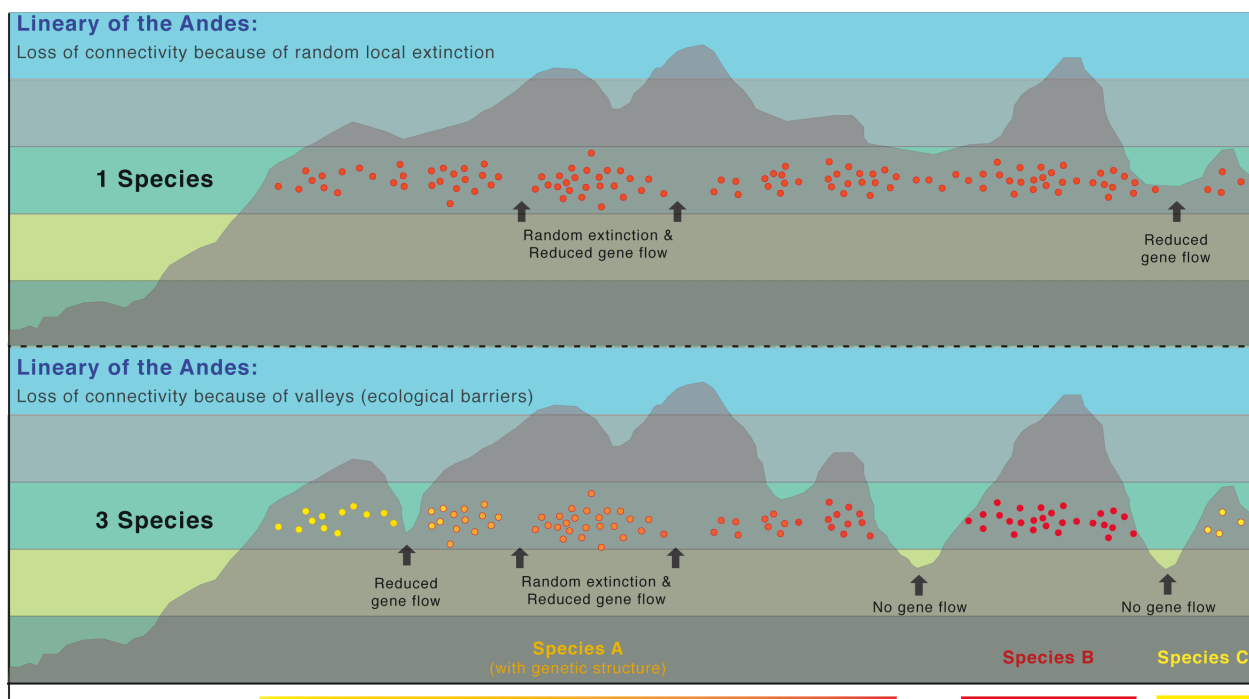
Fig. 6. Schematic graph illustrating how the linearity of the Andes facilitates the speciation process.

the Andes) have also been observed in other glassfrogs (e.g., *Nymphargus*; Guayasamin et al. 2019) and birds (Bonaccorso 2009; Benham et al. 2015; Cadena et al. 2019).

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Table 3. Meristic variation of *Hyalinobatrachium adespinosai sp. nov.* (in mm).

	ZSFQ 1647 (holotype)	ZSFQ 1651	ZSFQ 1647
Sex	Male	Male	Male
SVL	22.2	20.7	20.5
Femur	12.8	12.0	12.1
Tibia	12.8	11.5	11.9
Foot	11.0	10.5	10.5
Head length	6.5	6.4	6.2
Head width	8.4	7.7	7.9
IOD	2.8	2.5	2.6
Upper eyelid	1.6	1.4	1.6
Internarinal distance	1.8	1.7	1.7
Eye diameter	2.6	2.3	2.3
Eye-to-snout distance	3.2	2.8	3.0
Tympanum diameter	0.7	0.7	0.7
Radioulna	5.5	4.8	5.2
Hand length	6.7	6.4	6.4
Finger I	4.9	4.7	4.8
Finger II	4.5	4.4	4.5
Disc Finger III	1.4	1.2	1.2
Disc Toe IV	1.3	1.2	1.2

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Appendix 1. Examined Specimens

Hyalinobatrachium esmeralda: Colombia: *Boyacá Department:* Municipio de Pajarito, Inspección Policía Corinto, finca 'El Descanso', quebrada 'La Limonita', 1,600–1,650 m, ICN 9592–94, 9596, 9602–03 (type series of *H. esmeralda*).

Hyalinobatrachium pellucidum: Ecuador: *Morona Santiago Province:* Nueva Alianza, Finca Santa Catalina (78.1335°W, 2.100°S; 1,305 m), Límite del Parque Nacional Sangay, MEPN 14706. Quebrada del Río Napinaza (78.4070°W, 2.9266°S, 1,100 m), QCAZ 42000; km 6.6 on the Limón-Macas road (ca. 2.92816°S, 78.344°W; 1,013 m), QCAZ 29438; 6 km N of Limon, QCAZ 25950. *Sucumbíos Province:* Río Azuela (0.1167°S, 77.6167°W; 1,740 m), Quito-Lago Agrio road; KU 164691 (holotype), USNM 286708–10; Río Reventador, USNM 286711–12. *Zamora Chinchipe Province:* Cordillera del Cóndor, Miazi Alto (4.25044°S, 78.61356°W; 1,282 m), QCAZ 41560–61, 41648.

Hyalinobatrachium munozorum: Ecuador: *Sucumbíos Province:* Santa Cecilia (00°03'N, 76°58'W; 340 m), KU 118054 (holotype), 105251, 123225, 150620 (paratypes), 152488–89, 155493–96, 175504. *Orellana Province:* Tiputini Biodiversity Station, ZSFQ DFCH-USFQ D105. Colombia: *Meta Department:* Meta, ICN 5031–34, 39503. *Amazonas Department:* Leticia, ICN (field number JMR 4119).

Hyalinobatrachium yaku: Ecuador: *Pastaza Province:* stream affluent of the Kallana river (1.4696°S, 77.2784°W; 325 m), MZUTI 5001 (holotype), 5002 (paratype). *Orellana Province:* Timburi-Cocha Research Station (0.4800°S, 77.2829°W; 300 m) near San José de Payamino, QCAZ 55628, QCAZ 53352, 53354, 56664. *Napo Province:* Ahuano (1.0632°S, 77.5265°W; 360 m), ZSFQ 02322.

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