Addendum

A new frog of the *Pristimantis lacrimosus* group (Anura: Craugastoridae) from the montane forests of northwestern Ecuador

ALEJANDRO ARTEAGA[†], MARIO YÁNEZ-MUÑOZ[‡] & JUAN M GUAYASAMIN[§]

†Tropical Herping. Leonardo Tejada y Antonio Salguero. Quito, Ecuador. †División de Herpetología del Museo Ecuatoriano de Ciencias Naturales. Rumipamba 341 y Av. de los Shyris. Quito, Ecuador. §Centro de Investigación de la Biodiversidad y Cambio Climático, Universidad Tecnológica Indoamérica. Av. Machala y Sabanilla. Quito, Ecuador.

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Abstract. We describe a new *Pristimantis* from the valley of Mindo in the Pacific slopes of the Andes in northwestern Ecuador at elevations between 1243–1790 m. The species is assigned to the *Pristimantis lacrimosus* group, from whose members it differs by lacking a rostral papilla, having unique coloration features and smooth to weakly areolate ventral skin texture. Also, females of the new species are notably larger (SVL 40.0–40.8 mm) than females of the other members of the group. Calls of the new species are emitted constantly at about every two seconds. Each call is composed of a pulsed, frequency-modulated note, with a dominant frequency that peaks at 2698–2919 Hz. Comparisons of an 816 bp fragment of the 16s mitochondrial gene between the new species and the closest morphological species, *Pristimantis subsigillatus*, shows a genetic distance of 9.6–9.7%, providing independent evidence of the validity of the species.

Introduction. The diversity of *Pristimantis*, currently with 454 species¹, is far from being totally known. More than one hundred species of this genus have been described in the past 13 years and there is no reason to think that the description rate will slow down, especially with the advent of molecular techniques, which have facilitated the discovery of cryptic diversity²⁻⁵.

The observed species richness of *Pristimantis* is not well understood, but several factors might be involved, including high mutation rates, access to novel niches through its reproductive mode, phenotypic traits, the effect of mountain gradients and susceptibility to barriers that do not affect other amphibians^{6–10}. At least in the area of Mindo, the most likely scenario to explain the observed richness¹¹ of rainfrog species seems to have speciation along elevation gradients. Based on overall mor-

phological similarity, Mindoan rainfrogs such as *Pristimantis crenunguis*, *Pristimantis laticlavius* and *Pristimantis luteolateralis* seem to be upland vicariants of the Chocoan species *Pristimantis latioisus*, *Pristimantis latidiscus* and *Pristimantis walkeri*, respectively¹¹. This presumed pattern of speciation seems to explain the presence of a new Mindoan rainfrog species most closely related to *Pristimantis subsigillatus*. Below, we describe this new taxon and present notes on its ecology, distribution, phylogenetic relationships and vocalizations.

MATERIALS AND METHODS

Terminology and morphological data. Generic and family names follow the taxonomy proposed by Pyron & Wiens (2011). Specimens were euthanized with 20% benzocaine, fixed in 10% formalin and stored in 70% ethanol. The diagnosis and description generally follow Duellman & Lehr (2009). We examined comparative alcohol-preserved specimens from the herpetology collections at the MZUTI and MECN. See Appendix. When providing the standard deviation, we use the ± symbol. Morphological measurements were taken with digital calipers to the nearest 0.1 mm, as described by Lehr & Coloma (2008). These are as follows: (1) snout—vent length (SVL), (2) tibia length, (3) foot length, (4) head length, (5) head width, (6) eye diameter, (7) interorbital distance, (8) upper eyelid width, (9) internarial distance, (10) eye—nostril distance. Sexual maturity was determined by the presence of testis or vocal slits in males and by the presence of eggs or convoluted oviducts in females.

Vocalizations. To obtain vocalizations, we used an Olympus LS10 Linear PCM Recorder and Sennheiser M8 directional microphone. Calls were recorded in PCM format at a sampling rate of 44 Hz. Recordings were processed in RAVEN PRO 1.3 (Bioacoustics Research Program 2011) on MAC OS X. Call parameter definitions follow Hutter & Guayasamin (2012) and references therein. A call is defined as the sound produced in a single exhalation of air. Pulsed notes are defined as having one or more clear amplitude peaks¹². We analysed oscillograms, audio-spectrograms and power spectra for various temporal and spectral call components. The fast Fourier transformation size was set to 512 and frequency grid resolution to 86.1 Hz. Digital recordings are deposited at MZUTI.

Genetic data. In order to assess the molecular divergence between the putative new species and the closest morphological taxon, we obtained DNA data from four individuals of the new species (MZUTI 1381, 1382, 1755, 1756) and one individual of *Pristimantis subsigillatus* (MECN 10117). Specifically, we sequenced an 816 bp fragment of the 16s mitochondrial gene, using the primers 16SC and 16Sbr-H^{13,14} and following the protocols described in Guayasamin *et al.* (2008). Resulting sequences were aligned in MAFFT v7, using the Q-INS-I strategy, where secondary structure of RNA is considered. Genetic distances were calculated using the uncorrected distance matrix in PAUP¹⁵.



Figure 1. Adult male holotype of Pristimantis mindo, MZUTI 1382, SVL 26.2 mm.

RESULTS

Pristimantis mindo. New species. ARTEAGA, YÁNEZ-MUÑOZ & GUAYASAMIN

Mindo Rainfrog. Cutín de Mindo.

Holotype. MZUTI 1382, an adult male (Figures 1,2) obtained by Alejandro Arteaga and Paolo Escobar on June 2012, at Mindo (00.02470 S, 78.75909 W; 1794 m), Cantón San Miguel de los Bancos, Provincia Pichincha, Ecuador.

Paratopotypes. Five specimens (Figure 3) were collected by Alejandro Arteaga, Paolo Escobar and Lucas Bustamante between February and July 2012. From these, two (MZUTI 538,1383) are adult males, one (MZUTI 1765) is a juvenile female and two (MZUTI 1766, 1831) are adult females.

Paratypes. Two adult males (MECN 4341, 4342; Figure 3) were collected by Mario Yánez-Muñoz & Enrique Inga on June 2007 at Las Tolas, Pacto, Cantón Quito, Provincia Pichincha. One adult female (MECN 3534) was collected by Paúl Meza-Ramos on August 2005 at Junín, Cantón Cotacachi, Provincia Imbabura. Two other specimens were obtained by Fernando Rojas and Jaime García on March 2012 at Reserva Los Cedros, Cantón Cotacachi, Provincia Imbabura. From these, one (MZUTI 1755) is a subadult male, and one (MZUTI 1756) is an adult female.

Diagnosis. The new species is placed in the genus *Pristimatis*, as diagnosed by Hedges et al. 2008. It is included in the *Pristimantis lacrimosus* group because it has smooth dorsal skin texture, a rounded snout, moderately long limbs, Finger I shorter than Finger II, expanded digital disks, and noticeable tympanum. *Pristimantis mindo* is diagnosed by having the following features. (1) Skin texture of the dorsum smooth with no dorsolateral folds. Venter smooth to weakly areolate (Fig.

1). (2) Tympanic membrane and tympanic annulus evident, 40–42% the length of the eye in both males and females, with upper rim obscured by supratympanic fold. (3) Snout short, 14–17% of SVL, rounded in dorsal view and rounded in profile. (4) Upper eyelid bearing low, non-conical tubercles. In females, the upper eyelid width is 69–84% of the interorbital distance (87–119% of that distance in males). Cranial crests absent. (5) Dentigerous process of vomers well developed, oblique in outline, each bearing 3–6 teeth. (6) Males with a small subgular vocal sac and vocal slits, but no nuptial pads. (7) Finger I shorter than II. Discs on fingers broadly expanded, elliptical to slightly truncated, except for Finger I that is only barely expanded. (8) Fingers bearing narrow lateral fringes. Outer palmar tubercle cordate and distally bifid. Supernumerary tubercles low and indistinct. (9) There are 1–3 low ulnar tubercles. (10) Tarsal tubercles absent. The heel tubercles are round, low and almost undistinguishable from surrounding skin texture. (11) The toes bear narrow fringes but not webbing. Toe V is slightly longer than Toe III. The toe discs are broadly

Table 1. Character states in species currently placed in the *Pristimantis lacrimosus* group. Also included and written in bold are species similar to *Pristimantis mindo* that are not currently included in the group.

	Destrol			Heat and towns	
Species	Rostral Papilla	Eyelid tubercles	Ventral texture	Heel and tarsal tubercles	Snout shape
P. mindo	Absent	Present, low	Smooth	Present, low	Rounded
P. acuminatus	Present	Absent	Coarsely areolate	Absent	Acuminate
P. aureolineatus	Present	Absent	Areolate	Absent/indistinct	Acuminate
P. boulengeri	Present	One subconical	Areolate	Present, subconical	Acuminate
P. brevifrons	Present	One subconical	Areolate	Present, subconical	Subacuminate
P. bromeliaceus	Present	Present, low	Coarsely areolate	Present, low	Acuminate
P. dorsopictus	Present	Present, low	Areolate	Present, low	Subacuminate
P. eremitus	Present	Present, low	Areolate	Present, low	Subacuminate
P. galdi	Present	One conical	Areolate	Present, conical	Acuminate
P. lacrimosus	Present	Absent	Coarsely areolate	Absent	Broadly rounded
P. mendax	Present	One subconical	Coarsely areolate	Present, low	Acuminate
P. moro	Present	Absent	Coarsely areolate	Absent	Subacuminate
P. olivaceus	Present	Present, low	Areolate	Absent	Subacuminate
P. pardalinus	Present	Present, low	Coarsely areolate	Present, low	Acuminate
P. petersi	Present	One subconical	Coarsely areolate	Present, low	Rounded
P. prolixodiscus	Present	Present, low	Areolate	Present, low	Acuminate
P. pseudoacuminatus	Present	Absent/indistinct	Areolate	Absent	Acuminate
P. rhodostichus	Present	Present, low	Areolate	Present, low	Acuminate
P. royi	Absent	One, small	Coarsely areolate	Absent	Broadly rounded
P. schultei	Present	Present, low	Weakly areolate	Present, low	Acuminate
P. subsigillatus	Present	Absent	Coarsely areolate	Present, low	Acuminate
P. tantanti	Present	Absent	Areolate	Absent	Acuminate
P. tayrona	Present	Present, low	Areolate	Present, low	Acuminate
P. waoranii	Absent	Absent	Areolate	Absent	Subacuminate
P. zeuctotylus	Absent	Absent	Areolate	Absent	Subacuminate
P. zimmermannae	Present	Present, low	Coarsely areolate	Absent	Acuminate



Figure 2. Pristimantis mindo in life. MZUTI 1382, SVL 26.2 mm, adult male, holotype.

expanded and elliptical to slightly truncated. (12) The inner metatarsal tubercle is elliptical, about 4 times the size of the outer tubercle, which is rounded and low. The supernumerary plantar tubercles are round and weakly developed. (13) In ethanol (Figure 3), the dorsum of *Pristmantis mindo* is grayish-brown to sandy-brown in males (reddish-brown to olive in life; Figures 2,4,7) with dark, faint irregular markings and a pale middorsal spot. In females, the dorsum is slate gray (olive-drab to rich-dark-brown in life; Figures 4,7) with or without dark blotches and transverse bars, a dark canthal stripe, postocular stripe and faint supralabial bars. The lower flanks, groin and hidden surfaces of hind limbs are unmarked in males, but heavily barred or spotted in females and enclosing yellow to tawny flash-colors in living females. The background color of the throat, belly and ventrolateral surfaces is dingy white, with different levels of brown mottling. In life, the iris is golden with black reticulations and crossed by a coppery median streak. (14) SVL in females 40.0–40.8 mm. In males 24.9–27.4 mm.

Similar species. *Pristimantis mindo* differs from members of the *Pristimantis lacrimosus* group and other morphologically similar congeners (see Table 1) by lacking a rostral papilla and having smooth to weakly areolate ventral skin texture. Only two other members (*Pristimantis royi* and *Pristimantis waoranii*) of the group lack the rostral papilla and only one (*Pristimantis schultei*) has weakly areolate ventral skin texture. Moreover, the new species differs from the all other members except *Pristimantis petersi* by having a snout that is rounded in dorsal view. Finally, females of *Pristimantis mindo* are unique in the group based on their flash marks on the thighs and groin. Also, they are notably larger (SVL 40.0–40.8 mm) than females of the other members of the group. The most similar congener is *Pristimantis subsigillatus*. However, this other rainfrog bears a noticeable rostral papilla and has coarsely areolate ventral skin texture.

Description of the holotype. Adult male (MZUTI 1382; Figures 1,2). The head is slightly narrower than the body and slightly wider than long. The upper eyelid bears several feebly visible tubercles both in life and preserved. Head width is 36% of SVL. Head length is 34% of SVL. The snout is relatively short (snout-to-eye distance 15% of SVL), rounded in dorsal view (Figure 1) and rounded in profile. The

tongue is longer than wide, with the posterior half notched and not adherent to the floor of the mouth. Eye diameter slightly larger than eye-nostril distance. Nostrils not protuberant and directed anterolaterally. Canthus rostralis straight and loreal region weakly concave in profile. Upper eyelid width 92% of interorbital distance. Cranial crests are absent, but the tympanic annulus and tympanic membrane are distinct and round. The postrictal tubercles are barely visible. Choanae round and not concealed by palatal shelf of maxillary. The vomerine odontophores are oblique in outline and about the same size of choana. They are located posteromedial to the choanae and separated medially by distance less than width of odontophore. Each bears 5 teeth. Vocal slits and median, subgular vocal sac present. The skin on the dorsum is smooth (Figure 1). The skin on venter is also smooth and has no discoidal or thoracic folds (Figure 1). Cloacal sheath absent. Cloacal region bordered ventrally by small, closely packed warts. There are two low ulnar tubercles and the outer palmar tubercle is cordate and bifid. The subarticular tubercles are round in section and the supernumerary palmar tubercles are indistinct. Fingers bearing narrow lateral fringes. Finger I shorter than Finger II. The disc of Finger I is barely expanded. All other discs are expanded, twice the width of the proximal phalanx, and elliptical to slightly truncate. The ventral pads are well defined by circumferential grooves. Nuptial pads absent. Tibia length 47% of SVL and foot length 51% of SVL. There is an inner metatarsal tubercle about 3 times the size of the outer tubercle. The subarticular tubercles are round in section, but the plantar supernumerary tubercles are indistinct. Toes bearing narrow lateral fringes, but no webbing. Discs of Toe I barely expanded. All other toe discs expanded and elliptical to slightly truncated. The toes have ventral pads well defined by circumferential grooves. The relative length of the toes is: I < III < III < V < IV. Toe V slightly longer than Toe III.

Measurements of holotype (in mm). SVL 26.2; tibia length 13.3; foot length 12.0; head length 8.9; head width 9.4; eye diameter 3.9; interorbital distance 3.2; upper eyelid width 3.0; internarial distance 2.5; eye-nostril distance 2.9.

Table 2. Measurements (in mm) of adults of *Pristimantis mindo*. Range is followed by mean and standard deviation.

	Famalas (N = 2)	Malao (N = 4)
	Females (N = 3)	Males (N = 4)
SVL	40.0–40.8 (40.3 ± 0.5)	24.9–27.4 (26.1 ± 1.0)
Tibia length	20.9–21.2 (21.0 ± 0.2)	13.2–14.4 (13.6 ± 0.5)
Foot length	20.4–21.6 (21.0 ± 0.6)	12.0–14.3 (13.2 ± 0.9)
Head length	13.7–15.5 (14.6 ± 0.9)	8.9–10.5 (9.6 ± 0.7)
Head width	15.7–17.1 (16.4 ± 0.7)	9.4–10.8 (10.1 ± 0.5)
Interorbital distance	4.7-5.0 (4.8 ± 0.2)	2.5–3.2 (2.9 ± 0.3)
Upper eyelid width	3.4-3.9 (3.8 ± 0.3)	2.5–3.0 (2.8 ± 0.2)
Radioulna length	9.0-10.1 (9.5 ± 0.6)	5.6–6.8 (6.1 ± 0.5)
Eye-to-nostril distance	4.2-4.5 (4.3 ± 0.2)	2.5–4.5 (3.8 ± 1.1)
Snout-to-eye distance	5.9-6.7 (6.1 ± 0.5)	4.0-4.4 (4.2 ± 0.2)
Eye diameter	4.9-5.2 (5.1 ± 0.1)	3.7-4.3 (3.9 ± 0.2)
Tympanum diameter	2.0-2.4 (2.2 ± 0.2)	1.2–1.6 (1.4 ± 0.1)
Hand length	13.0-14.2 (13.4 ± 0.7)	7.6–8.5 (8.1 ± 0.4)
Finger I length	6.0-6.5 (6.3 ± 0.3)	3.8-4.3 (4.0 ± 0.2)

Coloration of holotype in preservative. Dorsal surfaces brownish-gray with a pale-gray middorsal spot and faint dark blotches on each half of the body (Figure 1). Toe pads tinged with black. A blackish canthal and supratympanic stripe is present. Background color of ventral surfaces dingy white with gentle, but localized brown mottling on the chest.

Coloration of holotype in life. Based on field notes by Alejandro Arteaga. Upper surfaces reddish-brown with faint dark blotches on the dorsum, a pale orangish middorsal spot and transverse bars on the hind limbs. Flanks cream with an olive tinge. Blackish canthal and postocular stripe present. Background color of ventral surfaces cream and finely mottled with brown pigment on the chest. Groin and hidden surfaces of hind-limbs immaculate cream. Iris golden with black reticulations and crossed by a coppery median streak.

Variation. Morphological variation is presented in Tables 2 and 3, and most of it corresponds to sexual dimorphism (Figure 3). Females are notably larger than males, with bluntier snouts, fewer supernumerary tubercles and with striking flash-colors on the groin and hidden surfaces of the hind limbs. Most males (MZUTI 1382, 1383, 1755 and MECN 4341, 4342) and most females (MZUTI 1755, 1765, 1766, 1831) have a pale middorsal spot, but this mark is feebly visible in MZUTI 538 and MECN 3534. The spot is accompanied by dark transverse bars in MZUTI 1766, and by dark chevrons in MECN 4342. Ventral pigmentation varies greatly, from heavily mottled in MZUTI 1756 to almost immaculate in MECN 4342 and MZUTI 1382. Finally, most of the type series has smooth ventral texture, but MECN 4341 and MECN 3534 have weakly areolate ventral texture.

Genetics. We obtained four sequences of 16S for *Pristimantis mindo* and one sequence for *Pristimantis subsigillatus*. The uncorrected distance between individuals of both species is 9.6–9.7%, whereas sequence variation within *Pristimantis mindo* is only 0.1–0.4%.

Table 3. Ranges of morphological proportions (in percentages) of adults of Pristimantis mindo.

	Females (N = 3)	Males (N = 4)
SVL	40.0-40.8 (40.3 ± 0.5)	24.9–27.4 (26.1 ± 1.0)
Tibia length	20.9–21.2 (21.0 ± 0.2)	13.2–14.4 (13.6 ± 0.5)
Foot length	20.4–21.6 (21.0 ± 0.6)	12.0-14.3 (13.2 ± 0.9)
Head length	13.7–15.5 (14.6 ± 0.9)	8.9–10.5 (9.6 ± 0.7)
Head width	15.7–17.1 (16.4 ± 0.7)	9.4–10.8 (10.1 ± 0.5)
Interorbital distance	4.7–5.0 (4.8 ± 0.2)	2.5–3.2 (2.9 ± 0.3)
Upper eyelid width	3.4-3.9 (3.8 ± 0.3)	2.5–3.0 (2.8 ± 0.2)
Radioulna length	9.0–10.1 (9.5 ± 0.6)	5.6–6.8 (6.1 ± 0.5)
Eye-to-nostril distance	4.2–4.5 (4.3 ± 0.2)	2.5-4.5 (3.8 ± 1.1)
Snout-to-eye distance	5.9-6.7 (6.1 ± 0.5)	4.0-4.4 (4.2 ± 0.2)
Eye diameter	4.9–5.2 (5.1 ± 0.1)	$3.7-4.3 (3.9 \pm 0.2)$
Tympanum diameter	2.0-2.4 (2.2 ± 0.2)	1.2–1.6 (1.4 ± 0.1)
Hand length	13.0–14.2 (13.4 ± 0.7)	7.6–8.5 (8.1 ± 0.4)
Finger I length	6.0-6.5 (6.3 ± 0.3)	3.8-4.3 (4.0 ± 0.2)



Figure 3. Color variation in the type series of *Pristimantis mindo*. From left to right, these are MECN 4342, 4341, MZUTI 1382, 1383, 1765, 1381, 1766. Males are shown in the first and third row.



Figure 4. Paratype male MZUTI 1382, shown amplecting female MZUTI 1766, and after amplexus.

Calls. In the type locality, the holotype male was recorded by Alejandro Arteaga at approximately 4:00 h. The vocalization is highly vigorous, persistent and consists of calls emitted every 1.842-3.136 s. Each call is composed by one frequency-modulated note with two to three pulses (mean = 2.4 ± 0.548 ; N = 5) (Figure 5). The dominant frequency peaks at 2698-2919 Hz (mean = 2848.4 ± 85.6 ; N = 5). The first harmonic is at 5466-5839 Hz (mean = 5716.8 ± 145.9 ; N = 5) and the second harmonic is at 5466-5839 Hz (mean = 5716.8 ± 145.9 ; N = 5). In the vicinity of Cunuco (00.00990 S, 78.81000 W; 1092 m), province of Pichincha, the call of a male *Pristimantis subsigillatus* was recorded by Alejandro Arteaga at approximately 21:42 h. This call differs from the call of *Pristimantis mindo* by having a faster rate (calls are produced every 0.150 s). Also, its calls are emitted in series, each containing an average of six calls. The dominant frequency of the call of *Pristimantis subsigillatus* peaks at 1961-2033 Hz.

Natural history. Uncommon. To our knowledge, twelve reported observations exist for *Pristimantis mindo* since it was first collected in 2002. Active individuals were found on January, February, March, June, July, August and October, but vocalizing males and amplectant pairs were found on January and June. This observation suggests that *Pristimantis mindo* is active year-round but reproduces seasonally. *Pristimantis mindo* is an arboreal, acrophilic, nocturnal and ombrophilic denizen of deep and well-preserved evergreen lower-montane forests, where it is found both near streams and far from significant watercourses. However, this rainfrog appears to be strongly dependent on high levels of substrate moisture. Therefore, it is most active after or during rains, when individuals perch and move about on wet vegetation 40–400 cm from ground and surely much higher. This acrophilic lifestyle may be the reason why *Pristimantis mindo* is frequently overlooked. However, individuals may descend to lower forest strata to reproduce and lay eggs. Indeed, two females where found close to the ground. Both were gravid and one (MZUTI 1766) contained 50 mature eggs. Most likely, these eggs undergo direct development. This is believed to be the case for the other nearly 450 congeneric rainfrogs. Amplexus is axillary. Under laboratory conditions, it may last for days if not disrupted. At the type locality, *Pristimantis mindo* is syntopic

with Pristimantis appendiculatus, Pristimantis calcarulatus, Pristimantis crenunguis, Pristimantis crucifer, Pristimantis illotus, Pristimantis luteolateralis, Pristimantis nyctophylax, Pristimantis parvillus, Pristimantis sobetes and Pristimantis verecundus. At Las Tolas, with Pristimantis achatinus, Pristimantis crenunguis, Pristimantis nyctophylax and Pristimantis parvillus. At Junín, with Pristimantis appendiculatus, Pristimantis calcarulatus, Pristimantis eugeniae, Pristimantis illotus, Pristimantis parvillus, Pristimantis romanorum and Pristimantis verecundus.

Distribution. 1243–1790 m. *Pristimantis mindo* is endemic to the Pacific slopes of the Andes in northwestern Ecuador (Figure 6). Here, the species has been reported in seven localities: at Sachatamia Lodge (00.02470 S, 78.75909 W; 1794 m), Hacienda San Vicente (00.04323 S, 78.75259 W; 1562 m), Séptimo Paraíso (00.02794 S, 78.76640 W; 1530 m), Las Tolas (00.04626 N, 78.78464 W, 1300 m), Nanegal (00.13111 N, 78.67632, 1243 m), Junín (00.2754 N, 78.6603 W, 1768), and Reserva Los Cedros (00.3184 N, 78.7837 W, 1790 m). The first three localities are separated from each other by an airline distance less than 2.2 km, and thus are grouped together under the name of Mindo. All records but the last two (Junín and Reserva Los Cedros, which fall within Imbabura) come from the province of Pichincha.

Etymology. The specific epithet is a noun in apposition and refers to the type locality, Mindo, an enchanting town and valley nestled in the cloudforests of northwestern Ecuador.

Conservation status. *Pristimantis mindo* is considered as Vulnerable following B2a IUCN criteria because it is known from just seven localities in a narrow strip of mountainous forest of only 6,158 km² across an elevation gradient of only 547 m. Our observations suggest that this species has low population densities and is closely associated to both rainfall patterns and well-preserved

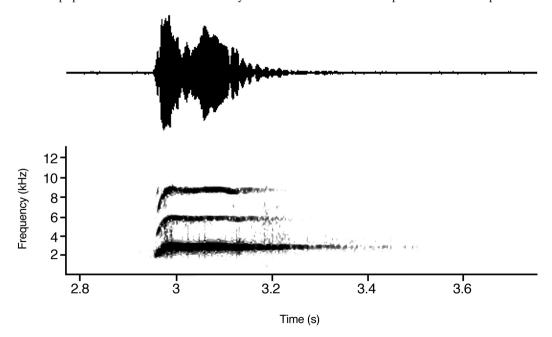


Figure 5. Oscillogram and sonagram of the advertisement call of Pristimantis mindo. MZUTI 1382, holotype.



Figure 6. Known distribution of Pristimantis mindo in Ecuador. Map by Belén Baus.

forested ecosystems. This makes *Pristimantis mindo* susceptible to habitat loss, climate change and prolonged periods of drought. The species is not included in a more threatened category because its populations, though small, are suspected to have remained stable for the last five years.

DISCUSSION

A recent but unpublished phylogenetic analysis 16 using the mitochondrial genes 12S and 16S of a sample of 178 species of *Pristimantis* place *Pristimantis mindo* most closely related to *Pristimantis*

subsigillatus¹⁶ (Figure 8). In the phylogeny, both rainfrogs cluster together with species traditionally included in the *lacrimosus* group¹⁶, a clade that has been shown to be largely monophyletic^{10,17,18}. However, the lineage includes at least five other morphologically similar species of rainfrogs typically included in other species groups (see Table 1). Conceivably, some species will be assigned to or removed from this group of rainfrogs once molecular data become available.

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References. ¹AmphibiaWeb 2013. ²Stuart *et al*. 2007. ³Fouquet *et al*. 2007. ⁴Elmer *et al*. 2007. ⁵Funk *et al*. 2011. ⁶Heatwole *et al*. 2011. ⁷González-Voyer *et al*. 2011. ⁸Wiens 2004. ⁹Duellman & Lehr 2009. ¹⁰Hedges *et al*. 2008. ¹¹Lynch & Duellman 1997. ¹²Dautel *et al*. 2011. ¹³Darst & Cannatella 2004. ¹⁴Palumbi *et al*. 1991. ¹⁵Swofford 2002. ¹⁶Rojas-Runjaic 2012. ¹⁷Pyron &



Figure 7. A large, gravid female of Pristimantis mindo.

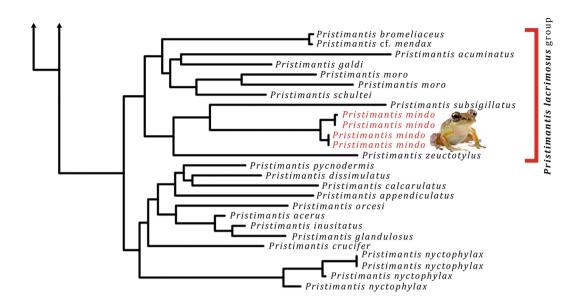


Figure 8. Extracted branch of a maximum likelihood phylogeny of 178 species of *Pristimantis*. The figure is part of an unpublished work by Fernando Rojas and collaborators. Reproduced with permission of the authors.

Wiens 2011. ¹⁸Pinto-Sánchez *et al*. 2012. Lehr & Coloma 2008. Hutter & Guayasamin 2012. Guayasamin *et al*. 2008.

Appendix. Specimens examined. Genbank accesion numbers are provided for *Pristimantis mindo* and *Pristimantis subsigillatus*.

Pristimantis acuminatus — Ecuador, Provincia Pastaza, Sacha Yacu (01.39553 S, 77.72952 W; 1081 m): MZUTI 236.

Pristimantis eremitus — Ecuador, Provincia Pichincha, vicinity of Bellavista Lodge (00.02077 S, 79.18734 W; 345 m): MZUTI 2277–79.

Pristimantis mindo — MZUTI 1756 (Genbank KF801581), MZUTI 1755 (Genbank KF801582), MZUTI 1381 (Genbank KF801583), MZUTI 1382 (Genbank KF801584).

Pristimantis subsigillatus — Ecuador, Provincia Pichincha, Hostería Selva Virgen (00.10547 N, 79.18007 W; 345 m): MZUTI 1999, 2653. Ecuador, Provincia Esmeraldas, Río Canandé Reserve (00.52615 N, 79.21282 W; 361 m): MZUTI 2228. Ecuador, Provincia Esmeraldas, Bilsa (00.34593 N, 79.71307; 532 m): MZUTI 2249. Ecuador, Provincia El Oro, Reserva Biológica Buenaventura (3.633334 S, 79.75001 W; 800 m): MECN 2566. Ecuador, Provincia Esmeraldas, Cresta San Francisco (0.69694 N, 80.01861 W; 117 m): MECN 2779. Ecuador, Provincia Manabí, Reserva Ayampe (1.654167 S, 80.818333 W; 60 m): MECN 10117 (Genbank KF801580).