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A new red-eyed treefrog of *Agalychnis* (Anura: Hylidae: Phyllomedusinae) from middle Magdalena River valley of Colombia with comments on its phylogenetic position

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Abstract

We describe a new species of the charismatic red-eyed treefrogs (genus *Agalychnis*) from middle Magdalena River valley of Colombia (05°50'8.04''N, 74°50'16.55''W, 380 m a.s.l.). The new species is readily distinguished from all species members of the group by having orange flanks with small white warts. Phylogenetic analysis of DNA sequences of 16S rRNA gene recovered the new species as a member of the *Agalychnis callidryas* group. The presence of a red hue in the iris and a golden reticulated palpebral membrane, putative synapomorphies of the clade, support this hypothesis. Our analysis suggests that *Agalychnis terranova* sp. nov is closely related to *A. callidryas* from Central America and is proposed as its sister species with an uncorrected genetic distance of 5.69% between these taxa. The phylogenetic position and the geographic distribution of the new taxon add new lights to the presence of a biogeographic disjunction between Middle America lowlands, the Pacific region and Magdalena River valley of Colombia.

Key words: Colombian inter-Andean valleys, molecular phylogenetics, morphology, species description, tadpole, taxonomy

Resumen

Describimos una nueva especie de las carismáticas ranas arbóreas de ojos rojos (género *Agalychnis*) del valle medio del Rio Magdalena, Colombia (05°50'8.04''N, 74°50'16.55''W, 380 m a.s.l.). La nueva especie es fácilmente distinguible de todas las especies del grupo por tener flancos naranjas con pequeñas y abundantes verrugas blancas. Análisis filogenéticos de secuencias de ADN del gen ribosomal 16S sitúan a la nueva especie como miembro del grupo *Agalychnis callidryas.* La presencia de iris rojo y membrana palpebral reticulada dorada, sinapomorfías sugeridas para este clado, apoyan ésta hipótesis. Nuestros análisis sugieren que *Agalychnis terranova* **sp. nov.** esta estrechamente relacionada con *Agalychnis callidryas* de América Central y la proponemos como su especie hermana con un 5.69% de distancia genética entre ellas. La posición filogenética y la distribución geográfica del nuevo taxón proporcionan nuevas luces sobre la existencia de una disyunción biogeográfica entre las tierras bajas de Centroamérica, la Región Pacífica y el valle del Río Magdalena de Colombia.

Palabras clave: Descripción de especie, filogenia molecular, morfología, renacuajo, taxonomía, valles interandinos colombianos

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Introduction

The charismatic genus *Agalychnis* Cope 1864, commonly known as red-eyed treefrogs, currently consists of 14 species (*sensu* Faivovich *et al.* 2010): *Agalychnis annae* (Duellman 1963), *A. aspera* (Peters 1873), *A. buckleyi* (Boulenger 1882), *A. callidryas* (Cope 1862), *A. dacnicolor* (Cope 1864), *A. danieli* (Ruiz-Carranza, Hernández-Camacho & Rueda-Almonacid 1988), *A. granulosa* (Cruz 1989), *A. hulli* (Duellman & Mendelson 1995), *A. lemur* (Boulenger 1882), *A. medinae* (Funkhouser1962), *A. moreletii* (Duméril 1853), *A. psilopygion* (Cannatella 1980), *A. saltator* Taylor 1955 and *A. spurrelli* Boulenger 1913. The genus is widely distributed from southern Mexico, throughout Central America, and the Pacific lowlands of Colombia and northwestern Ecuador. Fewer members of *Agalychnis* also occur east of the Andes, namely in the upper Amazon Basin and lower Andean slopes in Colombia, Venezuela, and northeastern Peru to the Atlantic forests of Bahia and Pernambuco, Brazil (Frost 2011). There are no records of *Agalychnis* from the lowland valleys of the Cauca or Magdalena rivers.

Recent fieldwork in the Magdalena River valley combined with historical collections highlighted an undescribed species within the genus *Agalychnis*. Using complementary sources of evidence, the goals of this study are 1) to describe a new species of *Agalychnis* using morphological analyses of adults and tadpoles, 2) to infer its phylogenetic position using mitochondrial DNA sequence evidence, 3) to estimate genetic divergence between the new species and its relatives and, 4) to discuss some biogeographical implications of this taxon for the Magdalena River valley region.

Materials and methods

Morphological analyses. Adults were captured by hand and tadpoles were collected with dip nets. Adults were sacrificed in 0.25% chloretone solution and fixed in 10% formaldehyde solution for five days before being transferred to 70% ethanol. Tadpoles were sacrificed, fixed and stored in 10% formaldehyde solution. Prior to fixation, tissue samples from some specimens including tadpoles were collected and stored in 96% ethanol. Webbing formula follows the notation of Savage and Heyer (1967) as modified by Myers and Duellman (1982). Sex and maturity was determined by examination of secondary sexual characters (i.e. nuptial excrescences and vocal slits) and observation of calling males in the field. Measurements (in millimeters) follow Duellman (1970) with some modifications and were taken with a digital caliper to the nearest 0.1 mm under a stereomicroscope. Acronyms for morphometric variables of adults were as follows: SVL (snout–vent length), HL (head length), HW (head width), ED (eye diameter), END (eye to nostril distance), NSD (nostril to tip of snout distance), IND (internarial distance), AMD (distance between the anterior margins of eyes), TD (tympanum diameter), FAL (forearm length), FAB (forearm breadth), HAL (hand length), THL (thigh length), TBL (tibia length), TAL (tarsal length), FL (foot length), TFD (third finger disk diameter) and FFD (fourth toe disk diameter).

Tadpoles were staged according to Gosner (1960), larval morphological terminology follows Altig and McDiarmid (1999) and meristic measurements follow Hoffmann (2004). Measurements of tadpoles (n = 37) correspond to values obtained from individuals at stages 25 to 39 excluding stages 32 and 36. Description of tadpole was based on specimen at stage 39 (MHUA-L 0198–1) and photo in life of specimen at stage 32 (MHUA-L 0199–1). To confirm the association of tadpoles to adults of the new species we: 1) sequenced a fragment of the mitochondrial ribosomal gene 16S rRNA (see below) from one adult (MHUA-A 7316) and one tadpole (MHUA-L 198–2), 2) reared several tadpoles to metamorphosis, one of which reached a sub-adult stage (MHUA-A 7320). Acronyms of tadpole measurements were: TL (total length), BL (body length), BW (body width), BH (body height), TMH (tail muscle height), TMW (tail muscle width), DFH (maximum dorsal fin height), VFH (maximum ventral fin height), OED (orbital eye diameter), IOD (interorbital distance), NW (nostril width), DIN (internarinal distance), DSN (distance snout nostril), DSE (distance snout eye), DSS (distance snout spiracle), DEN (distance eye nostril), DO (oral disc width).

Geographic locations were recorded with a global positioning system (GPS) device (Datum WGS84). Illustrations were made with a drawing tube attached to a Zeiss Stemi SV-11 stereomicroscope. Color and pattern descriptions were based on photographs taken in the field and on field notes. Information on other congeneric species was taken from preserved specimens and complemented with data from the literature (i.e. Taylor 1955; Duellman 1963; Duellman 1970; Cannatella 1980; Ruiz-Carranza *et al.* 1988; Cruz 1989; Duellman & Mendelson

1995). Institutional acronyms are: ICN (Instituto de Ciencias Naturales, Universidad Nacional de Colombia); MHUA-A and MHUA-L (Museo de Herpetología Universidad de Antioquia, Colombia, Amphibians and Larvae collections respectively) and QCAZ (Museo de Zoología, Pontificia Universidad Católica del Ecuador).

Amplification and sequencing of DNA. We inferred the phylogenetic position of the new species using a molecular approach in a statistical framework. We extracted total genomic DNA from ethanol preserved tissue using the DNeasy kit (Qiagen, Inc.). We amplified and sequenced a partial fragment of~1100 base pairs (bp) of the ribosomal gene 16S using the primers 16sAR–16sBR and 16sL2A–16sH10 (Palumbi *et al.*1991; Hedges 1994). Amplification protocol followed Faivovich *et al.* (2010). PCR products were purified using desalted and concentrated using either a GE GFX PCR purification kit or EXO⁄SAP (Fermentas) and cleaned products were sequenced in both directions using BigDye Terminator 3.1 (Applied Biosystems) and an ABI 3730XL at the Macrogen facilities in Korea (Macrogen, inc.). Chromatographs were aligned and contigs of the two strands were manually edited using Sequencer 4.1 (Gene Codes, Ann Arbor, MI, USA).

Molecular phylogenetic analyses. Genbank sequences (Appendix II) of the 12S, the intervening valine-tRNA and the 16S region were retrieved and an aligned matrix was generated using Clustal W (Larkin *et al.* 2007). Based on our previous assignment of the new species to the genus *Agalychnis*, we built a molecular character matrix including all available sequences of this genus in addition to representatives of the Phyllomedusinae clade (sensu Faivovich *et al.* 2010).

MrModeltest 2.3 (Nylander 2004) was used to find the best-fit model of molecular evolution and Bayesian phylogenetic inference was implemented in MrBayes 3.2.1 using Metropolis-Coupled Markov Chain Monte Carlo methods (Ronquist *et al.* 2011). As recommended by MrModeltest, we implemented the GTR+Γ+I model and initiated two independent runs from random starting trees that ran for 10 million generations. Chains were sample every 1000 generations and convergence and stationarity were verified by examining likelihood scores and parameter estimates using Tracer 1.4 (Rambaut & Drummond 2007). We approximated posterior distribution of nodes after a burnin period of two million generations based on a majority-rule consensus tree of remained trees.

Results

The inferred topology agreed with previous phylogenetic analyses of Phyllomedusines (Faivovich *et al.* 2010, Pyron & Wiens 2011). The new species is found nested within the *Agalychnis callidryas* group, with the nominal species as its sister taxon (Fig. 1). Uncorrected genetic distance between the new species and *A. callidryas* was 5.69%, similar to distances observed among other sister taxon pairs within the genus (Table 1). Lastly, we confirmed that the tadpole we sequenced belongs to this new species as no genetic difference was found between it and the adult.

Agalychnis terranova sp. nov

(Figs. 3–5)

Holotype. MHUA-A 7316, adult male, Colombia, Departamento de Antioquia, Municipio de Sonsón, Corregimiento La Danta, Vereda La Danta, 05°50’8.04’’N, 74°50’16.55’’W, 380 m.a.s.l., collected on April 15, 2011, by Felipe Duarte.

Paratypes. MHUA-A 7317–7319, three adult males and MHUA-A 7320 subadult, collected with the holotype. ICN 42966–42969, four adult males, Colombia, Departamento de Cundinamarca, Municipio de Yacopí, Vereda Guadualito, 05º37’52’’N, 74º8’23’’W, 820 m.a.s.l., collected on November 1, 1995, by Jose Vicente Rueda, Juan Manuel Renjifo and Freddy Castellanos. ICN 42970–42971, two adult males, Colombia, Departamento de Cundinamarca, Municipio de Yacopí, Vereda Cabo Verde, collected on October 29, 1995, by Jose Vicente Rueda, Juan Manuel Renjifo and Freddy Castellanos. MHUA-L 0198, 33 tadpoles, Colombia, Departamento de Antioquia, Municipio de Sonsón, Corregimiento La Danta, Vereda La Danta, 05°50’8.04’’N, 74°50’16.55’’W, 380 m.a.s.l., collected during April 15–18, 2011, by Felipe Duarte. MHUA-L 0199, two tadpoles, Colombia, Departamento de Antioquia, Municipio de Sonsón, Corregimiento La Danta, vereda La Danta, 05°50’8.04’’N, 74°50’16.55’’W, 380 m.a.s.l., collected on February 15, 2012, by Eliana Muñoz.

TABLE 1. Uncorrected genetic distances among *Agalychnis* species using 1100 base pairs of the 16S ribosomal mitochondrial gene region.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1 – *A. annae*	–										
2 – *A. callidryas*	0.0726	–									
3 – *A. spurrelli*	0.0635	0.0789	–								
4 – *A. moreletii*	0.0398	0.0660	0.0573	–							
5 – *A. saltator*	0.0717	0.0851	0.0735	0.0700	–						
6 – *A. terranova*	0.0766	0.0569	0.0901	0.0777	0.0903	–					
7 – *A. aspera*	0.1132	0.1199	0.1138	0.1095	0.1249	0.1305	–				
8 – *A. granulosa*	0.1103	0.1157	0.1083	0.1053	0.1207	0.1223	0.1065	–			
9 – *A. hulli*	0.1146	0.1167	0.1121	0.1116	0.1175	0.1194	0.1046	0.1158	–		
10 – *A. lemur*	0.1128	0.1186	0.1100	0.1098	0.1115	0.1261	0.1083	0.1174	0.1224	–	
11 – *A. dacnicolor*	0.0910	0.1031	0.0952	0.0906	0.0952	0.0884	0.0786	0.1222	0.1204	0.1159	–

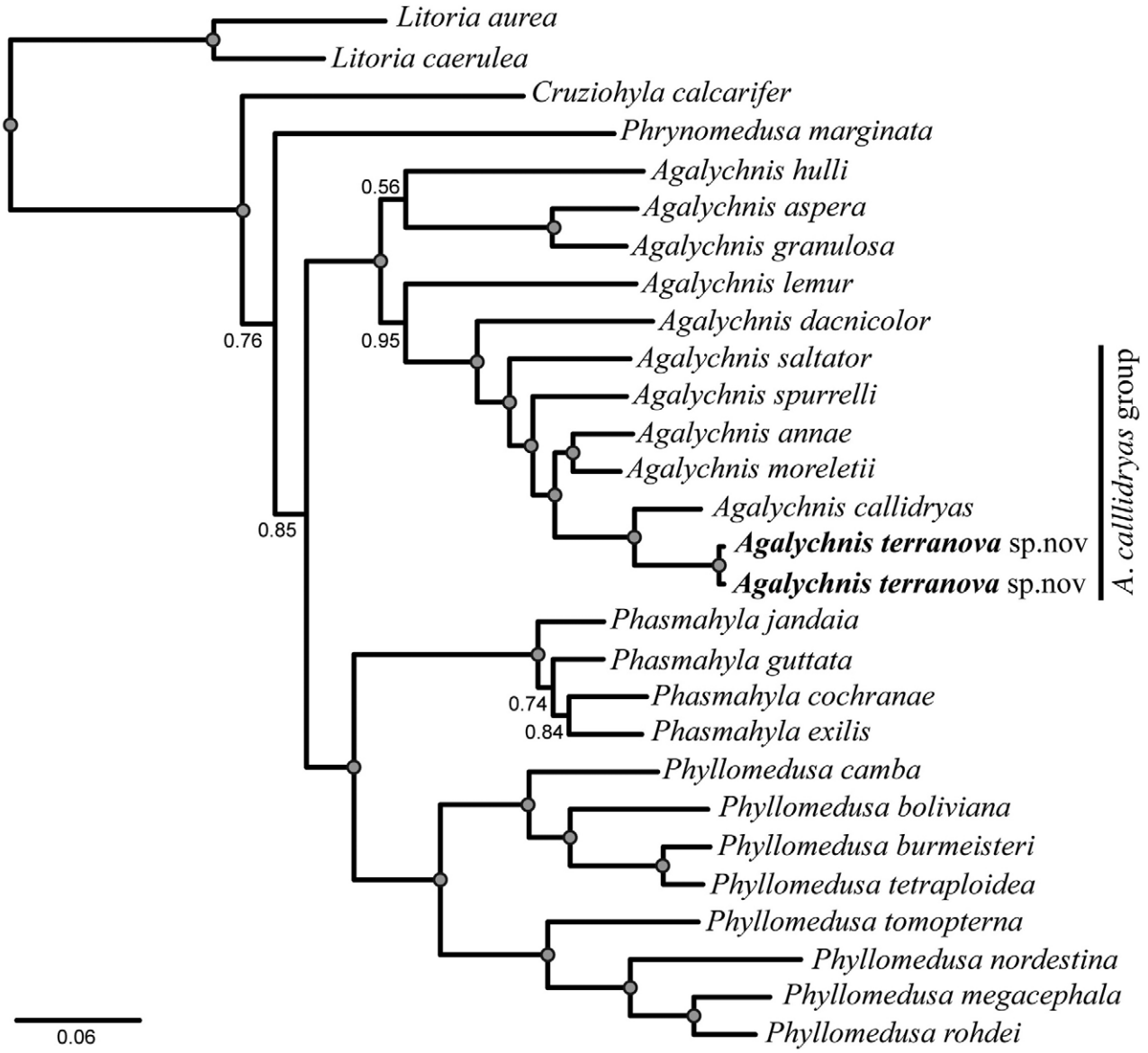

FIGURE 1. Bayesian consensus tree showing inferred evolutionary relationships within Phyllomedusinae and the phylogenetic position of *Agalychnis terranova* **sp. nov.** Bayesian support on nodes is shown as posterior probabilities and circles represent posterior probabilities of 1.0. We included as outgroups representatives of *Dendropsophus*, *Litoria* and *Hypsiboas.* We used *Dendropsophus ebraccatus* to root trees. *Dendropsophus* and *Hypsiboas*, not shown.

Diagnosis. We assigned the new species to the genus *Agalychnis* and the *A. callidryas* group on the basis of our phylogenetic results (Fig. 1). Our hypothesis is morphologically supported by the presence of the two putative synapomorphies in the new species: iris red and palpebral membrane reticulated with gold (Faivovich *et al.* 2010). The new species is characterized and defined by the following combination of characters (Figs. 2–3): (1) medium size for the genus (SVL 42.5 to 50.8 mm in adult males, 67.4 in one adult female); (2) slender body, head wider than body; (3) snout rounded in dorsal view and slightly sloping in lateral view; (4) loreal region slightly concave; (5) iris in life red wine with black reticulations; (6) pupil vertical, palpebral membrane with golden reticulations; (7) discs relatively wide, webbing formulae of fingers I ($2^{1/2}$–$2^{2/3}$) – $2^{1/2}$ II (2–2^{+}) – ($2^{2/3}$– 3) III (3–3^{-}) – ($2^{1/2}$–$2^{2/3}$) IV), (8) webbing formulae of toes I (2–2^{+}) – ($2^{1/3}$–$2^{1/2}$) II (2–2^{+}) – (3^{+}–$3^{1/3}$) III (2^{-}–2^{+}) – (3–3^{+}) IV ($3^{2/3}$–3^{+}) – (2^{-}–2) V; (9) dorsally green with or without white warts; (10) concealed surfaces of arm, forearm, thigh, tibia, tarsus, and digits light orange; (11) flanks light orange or orange with small white warts (12) ventral surfaces creamy in life (13) males with nuptial pad covering medial margin of prepollex with a distal expansion that partially covers the dorsal surface of the metacarpus and with dark brown colored epidermal projections; (14) dorsal skin slightly coarse; (15)

parotoid glands absent; (16) tympanum rounded with tympanic annulus distinct and upper edge covered by a supratympanic ridge; (17) inner ulnar and tarsal fold white present, both with smooth margin; (18) heel tubercles and calcars absent; (19) cloacal flap long, cloacal opening directed posteroventrally at level of ventral surfaces of thighs; cloacal sheath very short.

FIGURE 2. Dorsal and ventral view of *Agalychnis terranova* **sp. nov.** (MHUA-A 7316, holotype, adult male, SVL 47.9 mm)

FIGURE 3. *Agalychnis terranova* **sp. nov.** (ICN 42966, paratype, adult male in life, SVL 46.9 mm). Photo: P. Ruiz-Carranza.

Comparison with other species. *Agalychnis terranova* is readily distinguished from the other species of *A. callidryas* group by the orange flanks with small white warts. Additionally, *A. terranova* differs from *A. annae* by the presence of red iris, absence of light blue hue in the flanks (iris orange and light blue hue present in *A. annae*); from *A. callidryas* by absence of yellow vertical bars on the flanks on background blue or brown (present in *A. callidryas*); from *A. moreletii* and *A. spurrelli* by having less extensive webbing that does not reach beyond the distal subarticular tubercles between fingers III and IV and toes III and IV (webbing beyond distal subarticular tubercles in *A. moreletii* and *A. spurrelli*); from *A. saltator* in its larger size, without dark transverse stripes on the dorsum and, hidden areas dark blue or purple absent (presents in *A. saltator*). Furthermore, *A. terranova* is readily distinguished from *A. aspera*, *A. buckleyi*, *A. granulosa*, *A. hulli*, *A. lemur*, *A. medinae*, and *A. psilopygion* by their pale silvery bronze or cream iris (red iris in *A. terranova*), webbing on hands and feet absent (not extensive but present in *A. terranova*), and dorsal skin very thick and densely granular (slightly coarse in *A. terranova*). Furthermore none of these latter species has gold reticulated membrane palpebral (present in *A. terranova* and other *A. callidryas* group members); from *A. dacnicolor* by having golden iris with black thick reticulations (red iris in *A. terranova*) and membrane palpebral not reticulated (gold reticulated in *A. terranova*). *Agalychnis danieli* has iris golden, irregular black spots in lips, thighs, inner and outer margin of the arms and legs, and a calcar tubercle developed present (red iris present, blotches and calcar tubercle absent in *A. terranova*).

Description of the holotype. Body slender; head depressed, slightly wider than longer, head width 37% SVL; head length 32% SVL; snout rounded in dorsal view and sloping in lateral view from eyes to nostrils, then further inclined to tip of snout to the top of the head; lips flared; nostrils placed laterally, slightly protuberant and situated at about three-fourths the distance from the eyes to the tip of the snout; internarial area depressed; canthus rostralis broadly rounded, barely distinct; loreal region slightly concave; eye large, protuberant, directed anterolaterally, eye diameter approximately 34% of head length; internarial distance 20% of head width and 69% of eye diameter. Tympanum and tympanic annulus visible, round, laterally positioned, distinct dorsally, barely hidden by a thin dermal fold which extends in a curve from upper posterior corner of tympanum until the insertion of arm; tympanum diameter about one-half of eye length, nearly one-third of distance eye to nostril and separated from eye by distance equal to one-half of its diameter. Tongue lanceolated, free on the posterior half; choanae large, oval; dentigerous processes of vomers oblique and posteromeadially directed, between choanae, with processes separated by a relative distance of 40% of the diameter of choanae, each bearing six teeth. The vocal slits lie along the inner posterior margin of the jaw. The vocal sac is single, median, subgular, and not noticeably distensible.

Upper arm long and slender; forearms long and robust, with thin and smooth dermal ridges on ventrolateral edge of forearm from elbow to disc of fourth finger; fingers short, with circular and well-developed terminal discs, not opposable; disc diameter on the third finger equal to that of tympanum; finger lengths I < II < IV ≤ III (Fig. 5C); subarticular tubercle large size, ovoid, conical; supernumerary tubercles present, rounded, sub-conical, few and non-distinctive; inner metacarpal tubercle large, simple, elliptical, at the base of finger I and covering the metacarpal; outer metacarpal tubercle absent, but a tubercle present located almost in the center of the palm, round, simple, flat; nuptial pad covering medial margin of prepollex with a distal expansion that partially covers the dorsal surface of the metacarpus and with dark brown colored epidermal projections. Webbing formula of finger I $2^{1/2}$–$2^{1/2}$ II 2–$2^{1/2}$ III 3^{-}–$2^{1/2}$ IV

Legs long and slender; thigh and tibia of the same length; the smooth tarsal fold from heel to disc on the fifth toe; foot slender with toe lengths I < II < III < V < IV (Fig. 5D), with well-developed discs, circular, slightly smaller than those of fingers discs; toes not opposable; inner metatarsal tubercle medium sized, simple, flat, ovoid; outer metatarsal tubercle absent; subarticular tubercles medium sized, ovoid, and prominent; supernumerary tubercles are present on the proximal segments of all but the first toe; webbing formulae of toes I 2^{+}–$2^{1/3}$ II 2–$3^{1/3}$ III 2^{+}–3 IV 3^{+}–2 V.

Dorsal skin smooth with two medium rounded warts; granular flanks with small scattered warts; gular region smooth; belly and proximal area of thighs areolate; ventral surfaces of arms and limbs, smooth; cloacal region granulated. Cloacal flap long; cloacal opening directed posteroventrally at level of ventral surfaces of thighs. Paratoids and dorsolateral glands absent.

Color in life of holotype. Dorsal surfaces of body, forearms, legs, loreal, and tympanic regions green; orange-green color covering most of outer finger and outer toe, others fingers and toes and upper arms light orange; light orange dermal ridges on forearms and dermal flap on heel white; concealed surfaces of arm and thigh light orange; flanks light orange with white warts; hands, feet and ventral surfaces of limbs orange; throat and cloacal area are

white and the belly is creamy white; nuptial excrescence dark brown; vertical pupil black, iris red with black reticulations, and golden reticulations extending widely throughout palpebral membrane. Color in life of a paratype shown in Fig. 3.

Color in preservative of holotype. Dorsal surfaces of body, forearms, legs, loreal, and tympanic regions sky blue, dorsum with two white warts of different sizes; upper arms, fingers, and toes cream; iris light cupreous; flanks, inguinal region, internal and external sides of tibia, and internal side of tarsus cream; ventral surfaces cream; nuptial excrescence dark brown (Fig. 2).

Measurements of the holotype (in mm). SVL 47.9; HL 15.2; HW 17.3; ED 5.2; END 4.6; NSD 2.3; IND 3.0; AMD 12.2; TD 3.9; IOD 13.3; THL 27.1; TBL 26.1; TAL 15.5; FL 17.8; TFD 2.8; FFD 2.3

Variation. Measurements of the type series are summarized in Table 2. No sexual variation between the morphology of males and females, except for the sexually dimorphic structures (i.e. nuptial pads and vocal slits in males) and females are about 25% larger than males in SVL. Dorsal skin slightly coarse with scattered white warts without dark ring in some individuals: one wart (ICN 54248, MHUA-A 7318–7319), three warts (MHUA-A 7317) four warts (ICN 42967, 42970, 54249). Number of vomerine teeth range from 3 to 8, sometimes asymmetric (left/right): 4/3 (MHUA-A 7320), 4/4 (MHUA-A 7319), 4/5 (ICN 42970), 4/7 (MHUA-A 7318), 5/5 (ICN 42967, MHUA-A 7317), 6/7 (ICN 54248), 7/8 (ICN 54252). The white warts of flanks and ventral thighs are less numerous and more spaced in larger specimens, and some warts have minute melanophores on the margin. Nuptial excrescences less developed in ICN 54249 and undetectable in ICN 42970 and MHUA-A 7320. Variation in webbing formulae of fingers I ($2^{1/2}$–$2^{2/3}$) – $2^{1/2}$ II (2–2^{+}) – ($2^{2/3}$– 3) III (3–3) – ($2^{1/2}$) IV) and variation of webbing formulae of toes I (2–2^{+}) – ($2^{1/3}$–$2^{1/2}$) II (2–2^{+}) – (3^{+}–$3^{1/3}$) III (2^{-}–2^{+}) – (3–3^{+}) IV ($3^{2/3}$–3^{+}) – (2^{-}–2) V. In preservative, some individuals possess darker blotches, almost deep purple on different areas of dorsum and legs.

TABLE 2. Measurements (in mm) of adults *Agalychnis terranova* **sp. nov.** See text for abbreviations; min, minimum value; max, maximum value; mean, arithmetic mean; SD, standard deviation.

	Males (n=14)				Female (n=1)
Measurement	min	max	mean	SD	
SVL	42.5	50.8	46.9	2.63	67.4
HL	13.9	18.9	16.1	1.63	20.1
HW	13.4	17.6	15.1	1.52	22.8
ED	4.6	5.9	5.4	0.38	6.0
END	4.2	5.1	4.3	0.30	5.9
NSD	1.8	2.7	2.2	0.25	2.8
IND	3.3	4.1	3.8	0.22	4.6
AMD	9.4	11.7	10.4	0.65	13.1
TD	2.3	3.4	2.8	0.31	3.9
FAL	9.8	13.0	11.4	0.99	16.4
FAB	3.1	4.5	3.7	0.37	4.0
HAL	12.0	13.9	13.0	0.52	18.3
THL	22.6	27.1	24.9	1.36	37.6
TBL	22.8	26.1	24.6	0.82	36.4
TAL	13.1	15.8	14.6	0.71	21.4
FL	15.2	18.9	16.9	1.06	24.4
TFD	2.4	2.9	2.6	0.18	4.0
FFD	2.2	2.5	2.4	0.09	3.6

Tadpole description. Morphometric measurements are shown Table 3; external morphology in life (Fig. 5). Body ovoid in lateral view, elliptic in dorsal view. Body length slightly less than half total length, body two times longer than wide, 2.3 times longer than tall and 1.1 times wider than tall. Snout shape rounded to truncated in dorsal profile and rounded in lateral profile. Nostril dorsolateral, directed anteriorly, elliptical with slightly

flattened dorsal edge, margin in nostrils with rim; closer to tip of snout than eyes. Eyes dorsolateral and directed laterally; relatively large, eye diameter 40% of the maximum height of the body. Spiracle ventral sinistral to the midline, centripetal with wall absent and an opening 30% of the width of the oral disc. Neuromasts of the lateral line system not evident. Vent tube short, dextral, opening postero-laterally directed, connected along all its length with the ventral fin on the ventral edge. Caudal musculature robust anteriorly and gradually thinning towards the tip; dorsal fin emerging posteriorly to the body tail-junction, not extending onto body, more tall in the two thirds of the total length of the tail; ventral fin is slightly deeper than the dorsal fin and has about equal depth on the middle third length of tail, emerging anteriorly to vent tube and narrowing posteriorly. Hind limb length (stage 39) equals 32.4% of total length, many melanophores outer in femur and radio-ulna present; plantar tubercle is more conspicuous than subarticular tubercles and dorsum of phalanges are brownish; vestigial webs are in fingers III–IV, IV–V.

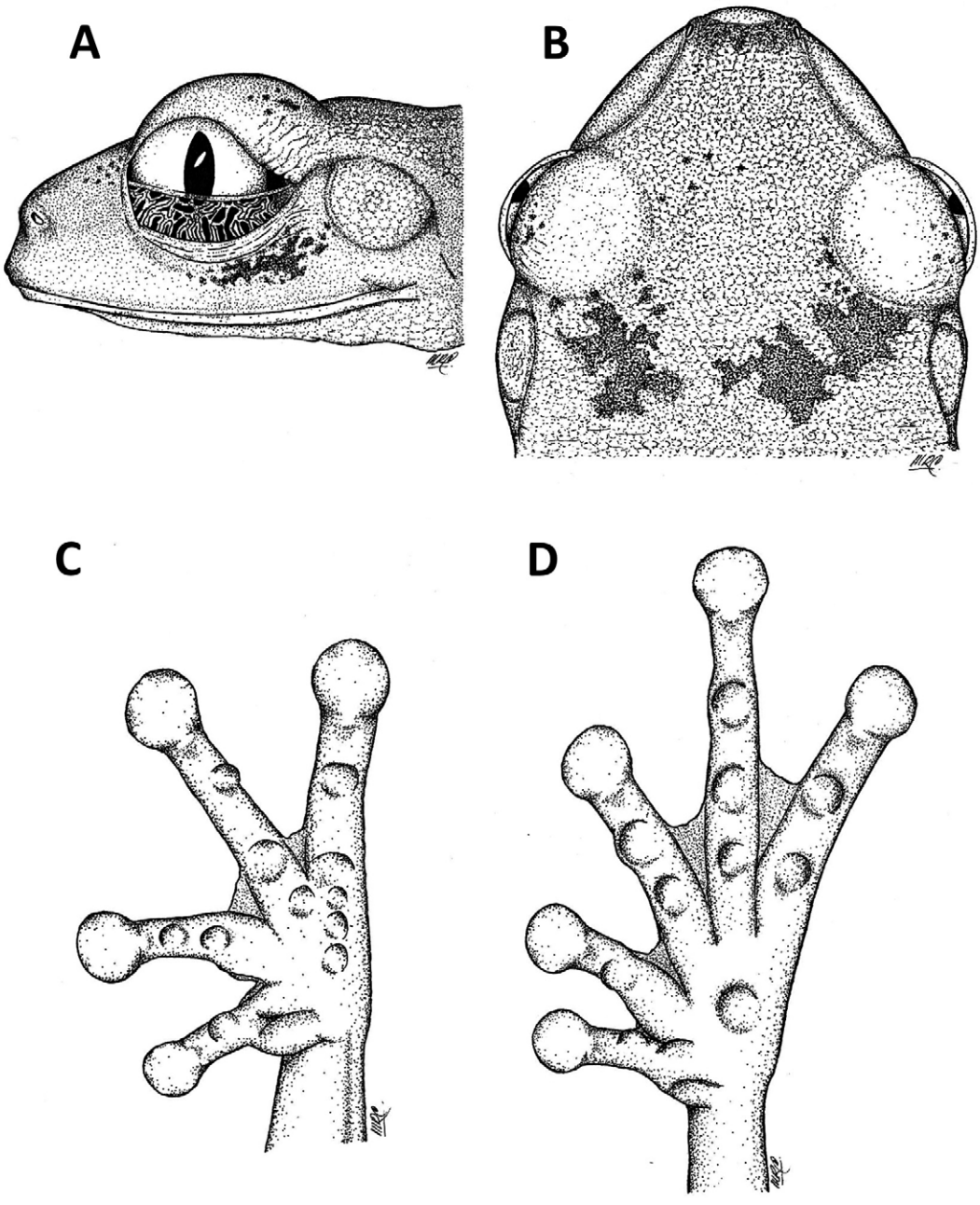

FIGURE 4. *Agalychnis terranova* **sp. nov.** (ICN 42970, paratype, adult male). (A) Head in lateral view; (B) head in dorsal view; (C) left hand in ventral view; (D) left foot in ventral view. Scale bar = 5mm. Drawings: M. Rivera-Correa

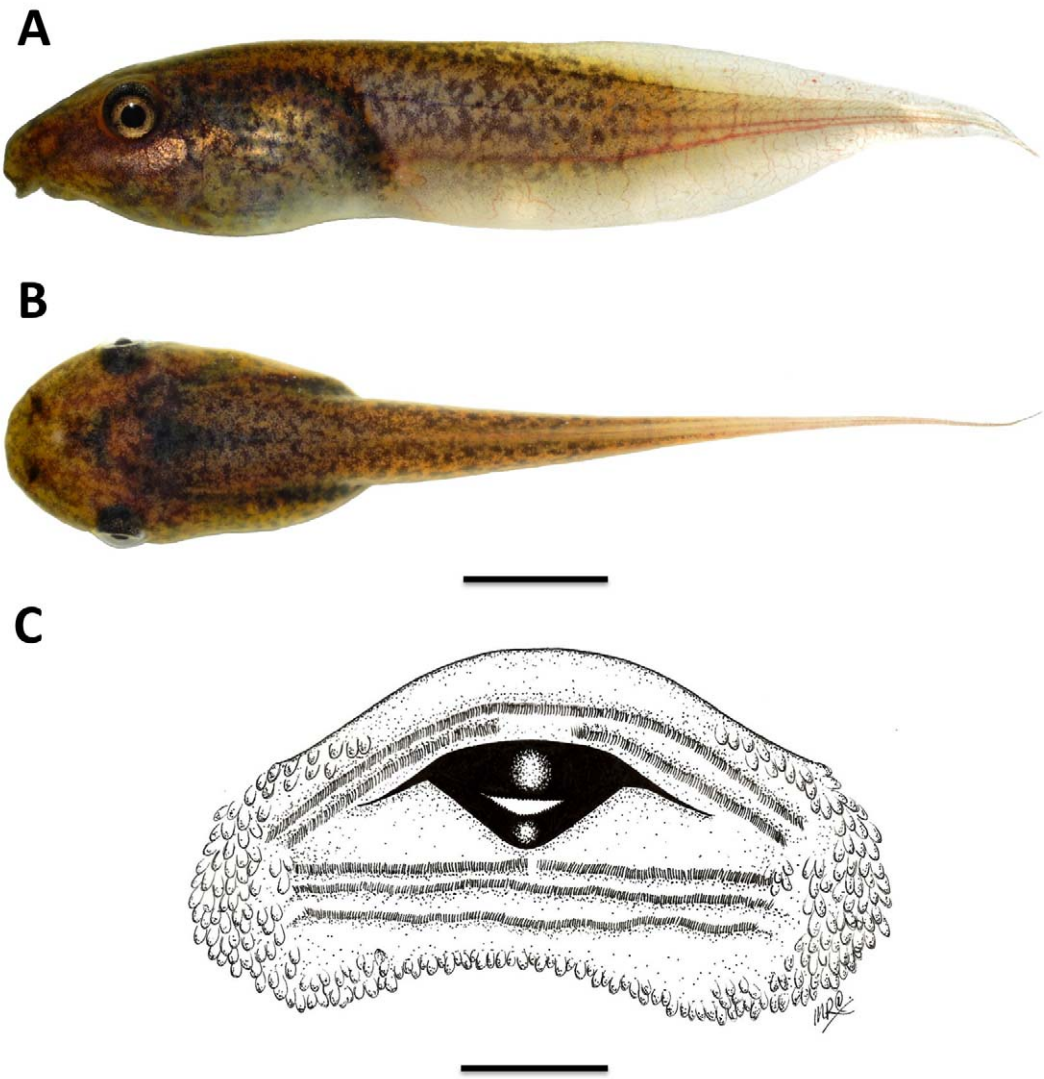

FIGURE 5. *Agalychnis terranova* **sp. nov.** (MHUA-L 0199–1, tadpole in life on stage 32. (A) Lateral view; (B) dorsal view, scale bar = 5 mm; (C) oral disc, scale bar = 1mm.

Oral disc medium, width 50% of maximum body width, anteroventral, directed anteroventrally, not emarginated; the median part of anterior labium is bare, width 50% of oral disc width; median part of posterior labium is bordered by single row of papillae; the rest of the mouth is bordered by two to four rows of papillae, laterally abundant submarginal papillae (Fig. 5A). Upper jaw moderately robust, keratinized, forming a broad arch with slender and large lateral process, expanded distally. The lower jaw is more slender and V-shaped. Both jaws have fine serrations, wide at the base; jaw sheath serrations closely spaced. Two rows of labial teeth on the anterior labium, three rows of labial teeth on the posterior labium; labial tooth row formula (LTRF) 2(2)/3(1), longitude of gap in A2 about 6% of total length of the row, gap in P1 narrowest, inconspicuous; rows of labial teeth on anterior labium equal in length and extend nearly to lateral papillae; second row of the labial teeth on posterior labium (P2) 6.5% shorter than first row (P1); third row of the labial teeth on posterior labium (P3) 4% shorter the second row (P2).

Color in life of all tadpoles. Background color of dorsum and flanks ocher-brown with blotches and olive brown and silver melanophores present. Oral disc with many spaced melanophores. Parietal peritoneum iridescent platinum-yellow. Caudal musculature light brown with blotches olive brown. Dorsal and ventral tail fins with transparent background highly vascularized with scattered red spots and scattered dark pigmentations that persist in preserved specimens with dendritic appearance. Inner ridges of ventral and dorsal fin above tail muscle with dense melanophores present in the proximal area of tail length. Iris gold.

TABLE 3. Measurements (in mm, n = 37) of tadpoles *Agalychnis terranova* sp. nov. See text for abbreviations; var., variables .

Var.	Stage 25 (n=4)	Stage 26 (n=5)	Stage 27 (n=5)	Stage 28 (n=5)	Stage 29 (n=4)	Stage 30 (n=1)	Stage 31 (n=4)	Stage 33 (n=2)	Stage 34 (n=2)	Stage 35 (n=1)	Stage 37 (n=2)	Stage 38 (n=1)	Stage 39 (n=1)
TL	27.5 – 32.2	32.9 – 41.9	37.4 – 43.3	35. 1 – 46.9	38.8 – 46.5	41.1	41.7 – 46.8	48.1 – 48.2	44.4 – 47.5	40.4	45.1 – 46.7	46.1	48.3
	12.7 ± 0.9	37.4 ± 3.2	41.1 ± 2.3	42.3 ± 4.4	44.5 ± 3.8		44.4 ± 2.7	48.1 ± 0.04	45.9 ± 2.0		45.9 ± 1.18		
BL	11.7– 13.9	7.6 – 17.1	15.8 – 18.2	18.8 – 19.9	11.7 – 18.4	17.1	19.9 – 21.9	16.6 – 19.6	19.3 – 22.2	19.0	18.8 – 20.1	21.3	21.4
	3.7 ± 0.6	14.4 ± 3.9	17.2 ± 1.0	16.7 ± 1.1	15.6 ± 2.8		19.6 ± 1.8	18.1 ± 1.1	19.7 ± 0.6		19.4 ± 0.71		
BW	2.5 – 8.1	7.7 – 8.8	8.3 – 9.7	6.6 – 11.5	3.7 – 8.9	4.8	4.7 – 10.9	10.3 – 10.4	10.6 – 11.1	10.1	10.6 – 10.9	0.3	10.5
	5.9 ± 2.3	8.1 ± 0.4	8.9 ± 0.7	8.9 ± 1.7	7.6 ± 6.8		9.2 ± 3.0	10.4 ± 0.04	10.8 ± 0.38		10.8 ± 0.09		
BH	5.0 – 6.6	6.6 – 7.7	7.0 – 8.8	7.2 – 10.4	7.2 ± 7.6	9.4	9.2 ± 10.9	8.5 – 9.3	8.1 – 9.6	9.1	10.0 – 11.4	10.5	9.3
	5.8 ± 0.8	7.3 – 0.6	8.1 ± 0.1	8.2 ± 1.4	7.6 ± 0.4		9.7 ± 0.8	8.9 ± 0.2	8.9 ± 0.6		10.7 ± 0.6		
TMH	3.2 – 4.6	4.4 – 5.8	5.6 – 6.1	4.5 – 6.6	5.2 – 6.5	6.3	5.9 – 6.2	6.6 – 6.8	6.2 – 6.3	6.4	6.4 – 6.9	7.0	6.8
	3.7 ± 0.6	5.2 ± 0.5	5.8 ± 0.2	5.7 ± 0.8	5.7 ± 0.6		6.2 ± 0.2	6.7 ± 0.1	6.2 ± 0.01		6.7 ± 0.3		
TMW	2.9 – 3.1	3.1 – 4.4	3.7 – 3.9	2.8 – 5.1	4.1 – 3.7	1.8	4.1 – 4.6	5.5 – 5.6	5.0 – 5.3	4.2	5.6 – 5.0	5.3	4.8
	2.5 ± 0.5	3.6 ± 0.6	3.8 ± 0.2	4.0 ± 0.1	4.0 ± 0.2		4.4 ± 0.2	5.6 ± 0.1	5.1 ± 0.2		4.8 ± 0.2		
DFH	1.1 – 1.6	1.1 – 1.9	1.1 – 2.1	1.5 – 2.6	1.9 – 2.5	1.6	2.0 – 2.5	1.8 – 2.2	2.3 – 2.9	2.5	2.1 – 2.8	2.6	2.8
	1.4 ± 0.2	1.5 ± 0.2	1.6 ± 0.4	2.0 ± 0.4	2.0 ± 0.4		2.3 ± 0.3	2.0 ± 0.2	2.6 ± 0.3		2.4 ± 0.4		
VFH	0.2 – 1.1	2.2 – 2.7	2.9 – 3.5	1.6 – 3.5	2.9 – 3.5	1.4	3.4 – 3.8	3.2 – 3.7	3.5 – 3.8	3.4	2.3 – 4.1	2.9	4.2
	2.1 ± 0.5	2.6 ± 0.2	3.1 ± 0.3	2.7 ± 0.8	3.2 ± 0.5		3.6 ± 0.2	3.4 ± 0.3	3.7 ± 0.1		3.2 ± 1.2		
OED	1.8 – 2.4	2.1 – 2.5	2.4 – 2.7	2.4 – 8.8	2.5 – 2.9	2.4	2.5 – 3.1	2.7 – 3.0	2.6 – 2.9	2.8	2.6 – 2.8	9.3	3.2
	2.0 ± 3.0	2.4 ± 0.1	2.5 ± 0.1	4.0 ± 2.7	2.7 ± 0.2		2.9 ± 0.3	2.8 ± 0.2	2.8 ± 0.1		2.7 ± 0.1		
IOD	6.1 – 7.3	7.2 – 9.1	7.7 – 9.6	8.3 – 10.6	8.2– 8.9	10.1	9.2 – 11.2	9.4 – 9.9	9.0 – 9.9	10.4	10.3 – 10.5	1.6	5.2
	6.7 ± 0.5	8.1 ± 0.8	8.8 ± 0.8	8.8 ± 1.3	8.7 ± 0.5		10.2 ± 0.8	9.6 ± 0. 21	9.5 ± 0.4		10.4 ± 0.1		
NW	0.2 – 0.4	0.3 – 0.4	0.2 – 0.5	0.4 – 0.5	0.3 – 0.5	0.4	0.3 – 0.5	0.4 – 0.6	0.3 – 0.6	0.3	0.4 – 0.6	0.4	4.6
	0.3 ± 0.1	1.5 ± 0.4	0.3 ± 0.1	0.5 ± 0.07	0.4 ± 0.08		0.4 ± 0.1	0.5 ± 0.01	0.5 ± 0.1		0.5 ± 0.1		
DIN	2.9 – 3.9	3.8 – 4.4	4.0 – 4.2	0.6 – 5.1	4.4 – 4.5	4.4	2.9 – 4.7	4.1 – 5.1	4.5 – 5.1	4.8	4.4 – 4.8	4.6	1.8
	3.4 ± 0.4	4.1± 0.2	4.1 ± 0.01	3.7 ± 1.8	4.5 ± 0.21		4.1 ± 0.9	4.6 ± 0.5	4.8 – 0.3		4.6 ± 0.2		
DSN	1.2 – 1.5	0.7 – 1.9	1.6 – 2.0	1.6 – 3.1	1.4 – 2.4	2.1	1.7 – 2.3	1.6 – 2.0	1.3 – 2.2	2.5	1.8 – 2.7	1.9	6.1
	1.5 ± 0.2	1.5 ± 0.4	1.9 ± 0.2	2.2 ± 0.5	1.9 ± 0.4		2.1 ± 0.3	1.8 ± 0.2	1.8 ± 0.4		2.2 ± 0.3		
DSE	3.1 – 4.7	3.7 – 5.2	3.4 – 5.6	3.8 – 5.7	1.4 – 5.8	5.9	5.8 – 6.9	5.7 – 5.8	5.5 – 6.1	6.3	3.6 – 6.0	6.1	20.6
	3.9 ± 0.6	4.8 ± 0.6	4.6 ± 1.0	4.7 ± 0.8	3.1 ± 1.9		6.2 ± 0.5	5.8 ± 0.04	5.8 ± 0.3		4.8 ± 1.2		
DSS	12.0 – 14.1	15.5 – 16.3	16.1 – 20.3	17.6 – 34.1	16.8 – 19.0	19.2	18.9 – 21.7	20.9 – 21.4	21.1 – 21.7	21.0	19.9 – 22.8	20.6	4.0
	13.0 ± 1.1	16.2 ± 0.7	17.8 ± 1.6	22.0 ± 6.8	1.8 ± 0.4		20.5 ± 1.3	21.1 ± 0.3	21.4 ± 0.2		21.3 ± 2.0		
DEN	2.3 – 2.8	2.7– 4.4	3.2 – 3.5	2.7 – 5.5	3.4 – 3.5	3.4	3.7 – 6.2	3.7 – 3.8	3.6 – 3.9	3.2	3.1 – 3.4	4.0	4.6
	2.6 ± 0.2	3.3 ± 0.6	3.4 ± 1.1	3.8 ± 1.1	3.4 ± 0.05		4.6 – 1.1	3.8 ± 0.07	3.7 ± 0.1		3.2 ± 0.2		
DO	3.7 – 3.3	4.1 – 5.2	4.4 – 5.0	3.7 – 4.9	4.7 – 5.0	5.0	4.8 – 5.6	4.2 – 4.8	5.0 – 5.3	5.1	4.7 – 5.8	4.6	4.1
	3.9 ± 0.4	4.5 ± 0.3	4.7 ± 0.2	4.6 ± 0.5	4.9 ± 0.24		5.2 – 0.3	4.5 ± 0.4	5.1 ± 0.1		5.2 ± 0.2		

Ontogenic changes. At stages 25 and 26 the body form is more depressed that at stage 27. The cement gland is not observed in the early stages. Melanophores at stages 25 to 28 are uniformly distributed, after stage 29 numerous blotches in the flanks and caudal musculature present. At stage 25 the papillae are widely spaced, others stages have numerous papillae with reduced space. Early stages (25 to 31) have diminutive and conspicuous spots above ventral tube. The tail-muscle junction is more protuberant after stage 31, and the wall of the ventral tube is largest after stage 33. In later stages (34 to 39) numerous melanophores appear closer to the tail-muscle junction. Finally, at stages 38 and 39 length of gap in anterior labium increases. The LTRF remains unchanged throughout development.

Etymology. The specific epithet *terranova* comes from the Latin words *terra* (Earth) and *novus* (new), meaning that which comes from a new land. Here we attribute the name, referring to the distribution of the new species in the Magdalena River valley of Colombia, previously not known to host any member of the genus *Agalychnis.*

Geographic distribution and natural history. *Agalychnis terranova* has been recorded at four localities in the Andean foothills between 380 and 900 meters along the Middle Magdalena River valley (Fig. 6). The region is characterized by a rugged relief covered by tropical humid forests (bh-T) *sensu* Holdridge (1987). Type locality is mostly represented by forest remnants having a plant community dominated by plant species such as *Cecropia insignis, Clusia palida, Cupania americana, Gleoespermum longifolium, Morisona oblongifolia, Inga umbellifera , Talisia oliviformis, Therocarpus officinali and Trichilia moschata*. The specimens from the type locality were observed active at night, calling from vegetation between 2 to 3 meters high, near artificial ponds. Tadpoles were collected in an artificial pool of 4 x 5 m length, forming schools or remaining motionless at an oblique angle to the sheet of water. The artificial pool where the tadpoles were observed was dominated by plants of the genus *Musa*. *Phyllomedusa venusta* tadpoles were also observed at the site. At the time of collection we did not observe photosensitive reaction in the tails of larvae and also did not register clutches or adults in amplexus. Other amphibians found in sympatry were *Craugastor raniformis, Dendrobates truncatus, Engystomops pustulosus, Hypsiboas boans, Leptodactylus bolivianus, Phyllomedusa venusta, Pristimantis taeniatus, Rhinella* gr. *margaritifera, Sachatamia punctulata, Scinax rostratus* and *Smilisca phaeota*. A KOH test revealed positive presence of *pterorhodin* in the skin of *Agalychnis terranova*.

Remarks. Recently Faivovich *et al.* (2010) assessed the phylogenetic relationships of the subfamily Phyllomedusinae based on a comprehensive molecular analysis, including 75% of the known species. In their taxonomic proposal, *Hylomantis* and *Pachymedusa* were synonymized with *Agalychnis.* In addition, Faivovich *et al.* (2010) proposed the *Agalychnis callidryas* species group, a well-supported clade including all of the species traditionally assigned to the genus *Agalychnis* (sensu Duellman 1970; Faivovich *et al*. 2005). Morphologically, the *A. callidryas* group is characterized by a gold reticulated palpebral membrane (Fig. 7) and a red hue of the iris, and both characters have been suggested as putative synapomorphies (red hue with a subsequent transformation into orange iris in *A. annae*). Furthermore, the study by Faivovich *et al*. (2010) suggested that the webbing on the hands and feet, which is more extensively developed than in other congeners outside of the group, could be an additional putative synapomorphy. These character states also are present in *A. terranova*, thus supporting the hypothesis of such character as a putative synapomorphy for the *Agalychnis callidryas* group, given that membership of *A. terranova* in the group is confirmed by independent (molecular) data.

In addition to the morphological evidence, the validity of *A. terranova* as a distinct species is also supported by genetic divergence given that the uncorrected genetic distance between this taxon and its sister species *A. callidryas* is higher than between other congeneric sister species, for example *A. annae* and *A. moreletii* (Table 1).

The phylogenetic position of *A. terranova* represents an opportunity to start building hypotheses about *Agalychnis* biogeography. Among the *A. callidryas* group, only *A. spurrelli* and *A. callidryas* reach the northwestern region of South America and are restricted mostly to the lowland forests of Choco. The records of two individuals of *A. callidryas* in an isolated population in the Botanical Garden of Cartagena, Department of Bolívar, northern Colombia (Ruiz-Carranza *et al.* 1997) could represent an introduced population established by accident via the ornamental plant trade. To date, *Agalychnis terranova* is the only species of this genus in the Magdalena River valley. The lowland rainforest in the Pacific coast of Colombia and Ecuador, the northern of the Cordillera Occidental and Central in Colombia and the humid portion of the Magdalena river valley has been considered a single biogeographic province (Hernandez-Camacho *et al.* 1992). However, recent studies suggest that a potential biogeographic break might occur between the Chocó and the Magdalena lowland forests where apparently no geographic barriers can be distinguished (Daza *et al.* 2009, Saldarriaga-Córdoba *et al.* 2009, Pinto-

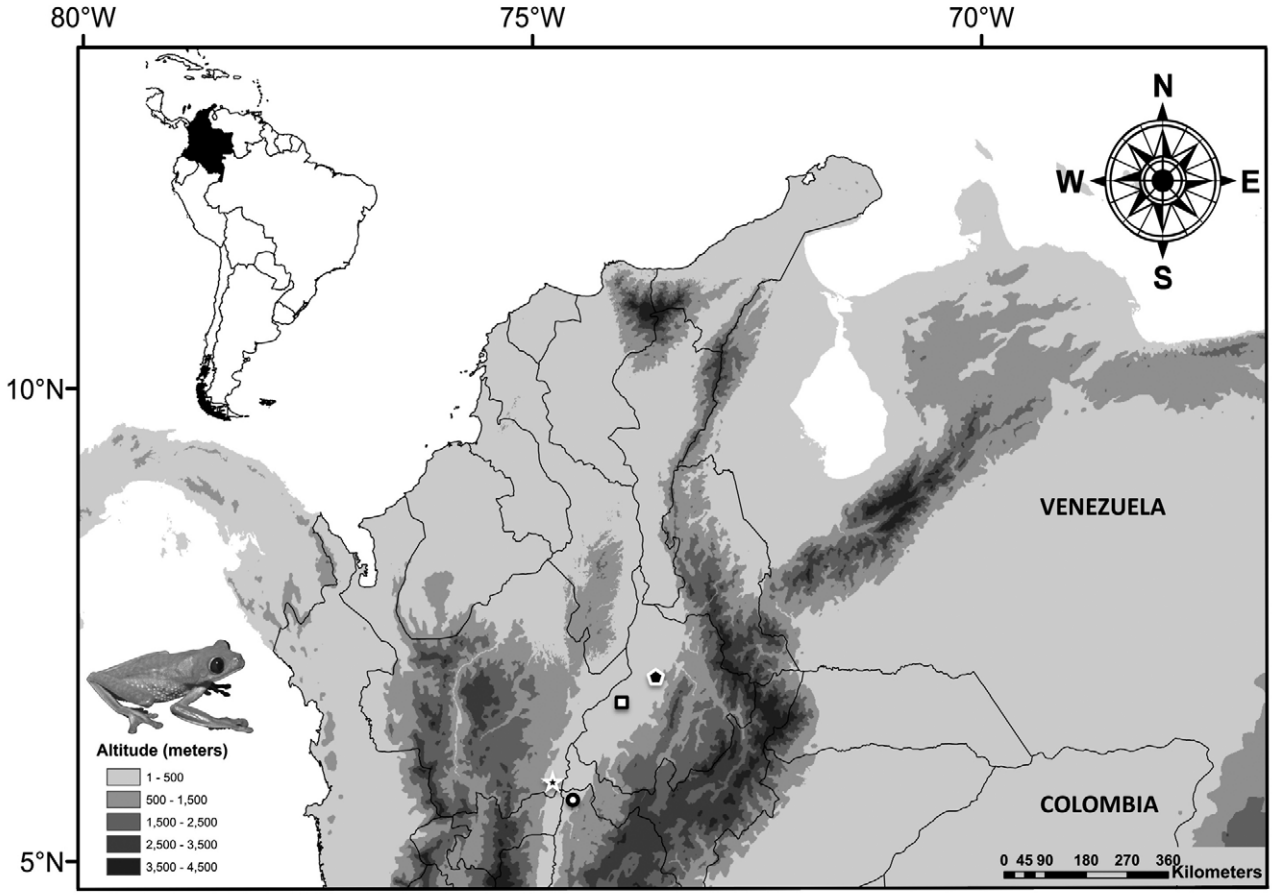

FIGURE 6. Geographic distribution of *Agalychnis terranova* **sp. nov.** Sonsón, Antioquia, type locality (star); Yacopí, Cundinamarca (circle); Puerto Parra, Santander (square); San Vicente del Chucurí (pentagon).

FIGURE 7. *Agalychnis terranova* **sp. nov.** at rest. Note the gold reticulated palpebral membrane characteristic of species of the *Agalychnis callidryas* group. Specimen not collected of Middle Magdalena River valley. Photo: Alejandro Campuzano.

Sánchez *et al.* 2011). This break will explain the presence of *A. terranova* in the Magdalena river valley and its sister taxa in the Choco region. Additional phylogeographic studies with a thorough sampling in northern Antioquia and the Chocó region will test this idea and contribute to understand the historical processes leading to the biotic divergence between Middle America lowlands and the Magdalena River valley regions.

Despite that the Magdalena River valley is relatively well-studied, *A. terranova* is known from only four localities and biological collections remain under-represented. This could be because the typical natural habitat of this species is the forest canopy, like some other species of the genus (Roberts 1994; Wells 2007). This would indicate that random encounters are associated with reproductive activity near the ground, because this species requires lotic water bodies for the development of the larval stages. Future studies on the reproductive biology will be needed to understand aspects of the natural history of this species.

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APPENDIX I. Additional specimens examined.

Agalychnis buckleyi: COLOMBIA: Santander, Charalá, Virolín, 1750 m. ICN 12402, 33171. Caquetá, Florencia, Vereda Tarqui, 1390 m. ICN 23958–23971
Agalychnis danieli: COLOMBIA: Antioquia, Frontino, Murri. ICN 16005
Agalychnis hulli: ECUADOR: Pastaza, Ecuador, Finca km 6 vía San Ramón-El Triunfo, pueblo más cercano Colonia Mariscal Sucre, Centro Ecológico Sancha Arajuno, QCAZ 37865, 37895, 40922–40924
Agalychnis spurrelli: COLOMBIA: Chocó, Quibdo, Tutunendo. ICN 52031–52034
Agalychnis psilopygion: ECUADOR: Esmeraldas, Durango, 7 km vía Durango -San Lorenzo, QCAZ 30215, 30217
Agalychnis terranova: COLOMBIA: Santander, Puerto Parra. ICN 54248–54254

APPENDIX II. GenBank accession numbers for sequences (12S and 16S rRNA) used for this study.

Species	Accession number	Species	Accession number
Agalychnis aspera	GQ366222	*Hypsiboas multifasciatus*	AY843648
Agalychnis annae	GQ366221	*Hypsiboas polytaenius*	AY843655
Agalychnis callidryas	DQ283423	*Litoria aurea*	AY843691
Agalychnis dacnicolor	AY843714	*Litoria caerulea*	AY843692
Agalychnis granulosa	GQ366224	*Phasmahyla cochranae*	AY843715
Agalychnis hulli	GQ366226	*Phasmahyla exilis*	GQ366231
Agalychnis lémur	AY843725	*Phasmahyla guttata*	GQ366232
Agalychnis moreletii	GQ366227	*Phasmahyla jandaia*	GQ366233
Agalychnis saltator	GQ366228	*Phrynomedusa marginata*	GQ366234
Agalychnis spurrelli	AY326043	*Phyllomedusa boliviana*	GQ366253
Agalychnis terranova	KC589394	*Phyllomedusa burmeisteri*	GQ366255
Agalychnis terranova	KC589395	*Phyllomedusa camba*	GQ366258
Cruziohyla calcarifer	GQ366229	*Phyllomedusa megacephala*	GQ366267
Dendropsophus ebraccatus	AY843624	*Phyllomedusa nordestina*	GQ366271
Dendropsophus microcephalus	AY843643	*Phyllomedusa rohdei*	GQ366236
Dendropsophus nanus	AY549346	*Phyllomedusa tetraploidea*	GQ366285
Hypsiboas boans	AY843610	*Phyllomedusa tomopterna*	GQ366286