A new species of marsupial frog (Anura; Gastrotheca) from the Cordillera de Colán in northeastern Peru

Pablo J. Venegas1,2, Luis A. García-Ayachi1,2, Lourdes Y. Echevarría2,3, Daniel J. Paluh4, Juan C. Chávez-Arribasplata1,2, Axel Marchelie2, Alessandro Catenazzi1,2,5

1 Instituto Peruano de Herpetología (IPH), Lima, Perú
2 División de Herpetología, Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Perú
3 Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Laboratório de Sistemática de Vertebrados, Porto Alegre, Brazil
4 Department of Natural History, Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA
5 Department of Biological Sciences, Florida International University, Miami, Florida, USA

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Corresponding author: Pablo J. Venegas (pvenegas@corbidi.org)

Abstract

We describe a new species of marsupial frog, genus Gastrotheca, using morphological characters and molecular data as lines of evidence. The new species was discovered in the páramo and the ecotone between páramo and humid montane forest of Cordillera de Colán, at elevations between 3136 and 3179 m a.s.l., in northeastern Peru. The new species is distinguished from all its congeners by the combination of the following characters: coarsely granular skin on dorsum, a green dorsal coloration without pattern, finger I shorter than finger II, turquoise iris, and a venter without blotches, flecks or dots. Furthermore, we include a detailed osteological description of the new Gastrotheca species based on Micro-CT scanning. Based on our phylogenetic analyses, the new species belongs to the Gastrotheca marsupiata species group, is sister to G. oresbios and closely related to G. psychrophila, G. spectabilis, G. stictopleura and one undescribed species. Additionally, we test for the presence of the fungal pathogen Batrachochytrium dendrobatidis (Bd). No Bd infection was detected for G. gemma sp. nov. specimens but Bd prevalence was detected among syntopic frogs.

Keywords

Amphibians, Andes, Batrachochytrium dendrobatidis, Gastrotheca abdita, Hemiphractidae, osteology, phylogeny

Introduction

With 75 described species, the genus Gastrotheca—commonly known as marsupial frogs because females brood eggs in closed dorsal pouches (Duellman 2015)—is the most speciose genus of the family Hemiphractidae (Frost 2020). Marsupial frogs are particularly diverse along the Andes, from Venezuela to central Argentina, with some species distributed above 4000 m a.s.l. (Duellman 2015). Yet, a number of species are also found in the upper Am-
azon Basin, Cordillera de la Costa and the Atlantic Coastal Forest, as well as in Central America (Blackburn and Duellman 2013; Castroviejo-Fisher et al. 2015; Duellman 2015). In addition to the variety of habitats that marsupial frogs occupy throughout its wide distribution, *Gastrotheca* includes species that undergo direct development, as well as species with an aquatic larval phase (Duellman 2015).

Among the thirty species of *Gastrotheca* that occur in Peru (Frost 2020), 40% have a distribution restricted to the northern Peruvian Andes (Duellman and Venegas 2005, 2016; Duellman 2015). In the Andes of northern Peru, marsupial frogs occur at elevations greater than 1500 m a.s.l. in the northern portion of Cordillera Occidental and Central and in smaller mountain ranges such as Cordillera de Colán, Cordillera del Cóndor, and Cordillera de Huancabamba (Duellman 2015; Duellman and Venegas 2016). However, the herpetofauna of this region is far from being well surveyed and new species of anurans are continuously discovered, especially in remote localities (e.g. Duellman and Venegas 2005; Venegas and Barrio 2005; Lehr and Catenazzi 2011; Rivera-Correa et al. 2016; Rodriguez and Catenazzi 2017).

The Cordillera de Colán is a mountain ridge of moderate elevation in the Andes (< 3700 m a.s.l.), isolated from the main chain of the Andes by the Chiriaco and Utcubamba rivers that flow northward into the upper Marañón River, the main tributary of the Amazon River (Duellman and Pramuk 1999). Few herpetologists have explored these mountains, and most of the herpetological knowledge comes from specimens collected by ornithologists of Louisiana State University (LSU) in 1978. Herpetologists from the University of Kansas described several species of frogs from these LSU specimens, such as *Gastrotheca abdita* Duellman, 1987, *Colostethus spinotogaster* Duellman, 2004, *Eleutherodactylus avicuprum* Duellman & Pramuk, 1999, *E. atrabrachus* Duellman & Pramuk, 1999, *E. serendipitus* Duellman & Pramuk, 1999, *E. cuneirostris* Duellman & Pramuk, 1999, *E. metabates* Duellman & Pramuk, 1999, and *Telmatobius colanensis* Wiens, 1993. However, as the herpetofauna from the Cordillera de Colán is still far from being well surveyed, the true dimension of its amphibian biodiversity remains unknown.

The amphibian disease chytridiomycosis has decimated frog communities in Andean montane forests and grasslands (Catanzazu et al. 2011, 2014). Marsupial frogs vary in their vulnerability to chytridiomycosis (Catanzazu et al. 2017), and populations of several species have experienced declines (Scheele et al. 2019). A comparison of skin defences in two sympatric *Gastrotheca* (*Bd*-tolerant *G. excubitor* and *Bd*-susceptible *G. nebulanastes*) supported the hypothesis that anti-*Bd* symbiotic bacteria, rather than skin peptides, may be associated with disease tolerance in marsupial frogs (Burkart et al. 2017). Direct development, associated with a predominantly terrestrial lifestyle, might also limit adult exposure to infective *Bd* zoospores, and eliminates the possibility of larval infection. Nevertheless, considering the catastrophic consequences of *Bd* epizootics in high-Andean frog communities, it is possible that *Bd* is a major threat to marsupial frogs in northern Peru.

The first author (PJV) conducted two herpetological surveys to the Cordillera de Colán in 2019, and discovered several putative new species of amphibians and reptiles inhabiting the montane forest and páramos of these remote mountains. Herein, we describe a new species of marsupial frog collected in a narrow páramo at the top of this poorly known mountain ridge. We integrate several lines of evidence, including a detailed osteological examination using computed tomography, external meristic and morphological traits, and molecular data to characterize and diagnose the new species. Finally, in recognition of the threat of chytridiomycosis for montane forest and páramo amphibians in the Andes, we quantified pathogen prevalence and intensity of infection in the new species and sympatric frogs.

**Materials and methods**

**Field surveys and skin swabbing for chytrid pathogen detection**

We surveyed frogs along existing trails and new open trails during five days, and captured frogs by hand. For each capture, we recorded coordinates and elevation with a GPS (Garmin, WGS84), and we swabbed the skin for detection of the fungal pathogen *Batrachochytrium dendrobatidis*. This procedure does not harm the frogs (Hyatt et al. 2007). Briefly, we gently stroked a sterile rayon-tipped swab (Medical Wire and Equipment MW113) across the skin a total of 30 times per frog: five strokes on each side of the abdominal midline, five strokes on the inner thighs of each hind leg, and five strokes on the foot webbing of each hind leg. We swabbed three specimens of the new species, and 59 specimens of eight species of sympatric frogs. We euthanized voucher specimens with 20% benzocaine gel, fixed them in 10% formalin for 24 hours, and stored them permanently in 70% ethanol. We deposited voucher specimens at the herpetological collection of the Centro de Ornitología y Biodiversidad (CORBIDI) in Lima, Peru.

**Morphology**

External morphological characters and format of description follow Trueb and Duellman (1978); Duellman and Hillis (1987); Duellman (2015). Coloration features included in the diagnosis refer to coloration in life. We determined sex and maturity of specimens by observing the presence of brood pouches, vocal sac, and vocal slits. We used digital callipers to measure the following meristic traits to the nearest 0.1 mm: snout-vent length (SVL); tibia length: straight-line distance measured between the upper edge of knee and the heel (TIBL); foot length: distance from the proximal edge of the inner metatarsal tubercle to the tip
of the fourth toe (FL); head length: straight-line distance from the posterior edge of the jaw articulation to the tip of the snout (HL); head width: the greatest width of head, measured in a straight line between the posterior edges of the jaw (HW), interorbital distance: straight-line distance between the inner edges of the upper eyelid (IOD); eyelid-width: the greatest width of the upper eyelid, measured from its medial most edge to its outer margin (EW); internarial distance: the distance between the openings of the nostrils (IND); eye diameter: the greatest length of the orbit (ED); eye-nostril distance: straight-line distance from the posterior edge of the opening of the nostril to the anterior corner of the orbit (EN); tympanum diameter: the greatest horizontal distance between the outer edges of the tympanic annulus (TD); thumb length: straight line distance between the proximal edge of the prepollical tubercle and the end of the Finger I (FFL); Finger III length: straight-line distance between the proximal edge of the palmar tubercle and the end of Finger III (TFL); and disc width: the width of the terminal disc on Finger III (TFD). For a better understanding of how the measurements were taken see figure 5.1 in Duellman (2015). Comparative lengths of Fingers I and II were determined when addressed against each other. Description of webbing follows Savage and Heyer (1967, 1997) with modifications of Myers and Duellman (1982). Specimens used for comparison and their associated locality data are listed in Appendix I.

We scanned the brooding female paratype of Gastrotheca gemma sp. nov. (CORBIDI 19396) on a SkyScan 1173 Micro-CT scanner at Instituto do Petróleo e dos Recursos Naturais (IPR). We set tube voltage and current at 60 kV and 75 µA, respectively, and voxel resolution was 25 µm. We visualized images using CT Vox 3.3.0 (Bruker MicroCT) and VG StudioMax (Volume Graphics, ver. 3.4.0). An image stack (TIFF) and 3D mesh files of the specimen were deposited in MorphoSource (doi:10.17602/M2/M160745). Cranial terminology follows Gómez and Turazzini (2016).

Phylogenetics

We performed phylogenetic analyses in order to infer the phylogenetic position of Gastrotheca gemma sp. nov. within the genus. We used muscle tissue of the holotype (CORBIDI 21238) to sequence a fragment of 16S rRNA for G. gemma sp. nov. We extracted DNA with a commercial extraction kit (IBI Scientific, Peosta, USA). For the polymerase chain reaction (PCR) we used the 16Sar (forward) primer (5'-3' sequence: CGCCTGTTTTATCAAAACAT) and the 16Sbr (reverse) primer (5'-3' sequence: CCGGTCTGAACCTCAGTACGT), and these thermocycling conditions: 1 cycle of 96 °C/3 min; 35 cycles of 95 °C/30 s, 55 °C/45 s, 72 °C/1.5 min; 1 cycle 72 °C/7 min (Palumbi et al. 2002). We ran the polymerase chain reaction (PCR) with a ProFlex thermal cycler (Applied Biosystems). We purified PCR products with Exosap-IT Express (Affymetrix, Santa Clara, CA), and shipped the purified products to MCLAB (South San Francisco, CA) for sequencing.

We downloaded sequences of the following genes from GenBank: 12S rRNA, 16S rRNA, NADH dehydrogenase subunit 1 (ND1), proopiomelanocortin A (POMC) and the recombination activating gene 1 (RAG1). We included terminals from all supraspecific clades currently considered for Gastrotheca (Echevarria et al. 2020). We used Hemiphractus proboscides to root the tree. Terminals and GenBank accession numbers of DNA sequences used are listed in Appendix II.

We aligned sequences of each gene in Aliview 1.17.1 (Larsson 2014), using Muscle (Edgar 2004) with default parameters. We concatenated alignments into a single matrix and exported them in Nexus format using SequenceMatrix 1.7.8 (Vaidya et al. 2011). We assessed the best-fit partition scheme and models of evolution in PartitionFinder2 (Lanfear et al. 2017), under the corrected Akaike information criterion (AICc), as implemented on the Cipres Science Gateway (Miller et al. 2010). We evaluated the following partition schemes in the partition analysis: (i) unpartitioned, (ii) two partitions (by mitochondrial or nuclear gene category), (iii) five partitions (by gene). We conducted Maximum likelihood analyses using GARLI v2.01 (Zwickl 2006) as implemented on the Cipres Science Gateway. We set the analysis at 200 independent tree searches and 1,000 bootstrap pseudoreplicates. We set the gencleshfortopterm (run termination threshold of generations without significant improvement in likelihood) at 100,000, and all other parameters as default. We used SunTrees 4.3.0 in DendroPy 4.3.0 (Sukumaran and Holder 2010) to summarize bootstrap pseudoreplicates. We used MEGA 7.0.14 (Kumar et al. 2016) to compute intraspecific uncorrected p-distances for the 16S rRNA (489 bp fragment).

To describe the results, we follow the taxonomy proposed by Echevarria et al. (2020).

Species concept

We considered multiple lines of evidence to support delimitation of the new species. For molecular analyses, we consider a species as the single lineage segment of ancestor-descendant populations or metapopulations evolving separately from other lineages (Simpson 1951; Wiley 1978; de Queiroz 1998; Wiley and Lieberman 2011). We used external morphological features to diagnose monophyletic groups inferred through phylogenetic analysis of DNA sequences.

Molecular assay for chytrid fungal infection

We used a real-time Polymerase Chain Reaction (PCR) assay on DNA material collected on skin swabs to quantify the level of Bd infection (Boyle et al. 2004). We extracted DNA from swabs using 40 ml of PrepMan Ultra,
Figure 1. Maximum likelihood optimal tree (log likelihood = –36764.630331) inferred from 4,919 bp of mitochondrial (12S rRNA, 16S rRNA, ND1) and nuclear (POMC and RAG1) gene sequences. The phylogenetic tree depicts the relationships of *Gastrotheca gemma* sp. nov. with 67 *Gastrotheca* terminals, including 59 described species. Bootstrap support values of 100% are represented by an asterisk. Species groups are abbreviated as s.g.
and analysed extracts with a QuantStudio 3 qPCR system (ThermoFisher Scientific) following the protocol of Boyle et al. (2004) and Hyatt et al. (2007). We analysed each extract once. We calculated the number of zoospore equivalents ZE (i.e., the genomic equivalent for Bd zoospores) by comparing the qPCR results to a serial dilution of standards (gBlock synthetic standards, IDT DNA, Iowa, USA) and considered any sample with ZE >1 to be infected, or Bd-positive. We deposited the dataset of Bd prevalence and infection load at the online database https://amphibiandisease.org.

Results

The molecular dataset includes 68 terminals, 59 of which represent described species of Gastrotheca, and 4,919 aligned base pairs. According to the PartitionFinder results, we considered the five loci independently and applied the GTR + I + G model to each. The maximum likelihood optimal tree has a log likelihood score of −36764.630331 (Fig. 1). Bootstrap values are overall high. Among the main clades, the clade including Gastrotheca ovifera and the species groups of G. marsupiata and G. microdiscus has a low support value. The clade including G. ovifera as sister to the G. microdiscus species group has also low support.

The Gastrotheca fissipes, G. longipes, G. marsupiata and G. microdiscus species groups were recovered as monophyletic. Only one terminal of the G. walkeri species group was included. Gastrotheca gemma sp. nov. was recovered within the Gastrotheca marsupiata species group, as sister to G. oresbios. Other closely related species to G. gemma are: G. psychrophila, G. spectabilis, G. stictopleura and one undescribed species. The matrix of uncorrected p-distances for a 16S rRNA fragment shows that G. oresbios and G. spectabilis had the lowest distances with respect to G. gemma (Table 1).

Gastrotheca gemma sp. nov.

http://zoobank.org/195F5DAF-F4FD-4392-9CB5-A40B-3BA2805F

Figs. 2–6 and 8

Holotype. PERU • 1 ♀, a brooding adult; Amazonas department, Ucubamba province, Cajamarca district, from the trail from Refugio Lechuca to El Hito; 5°36′58.7″S, 78°14′58.8″W; 3180 m a.s.l.; 25 Nov. 2019; P.J. Venegas, L.A. García-Ayachi, J.C. Chávez-Arribasplata, J.R. Ormeño, S. Bullard and A. Marchelie leg.; CORBIDI 21238.

Paratypes (3). PERU • 1 ♀, adult; Amazonas department, Ucubamba province, Cajamarca district, from El Hito; 5°36′45.3″S, 78°15′2.9″W; 3300 m a.s.l.; 09 Nov. 2017; A. García-Bravo leg.; CORBIDI 19396 • 2 ♀♂, adults; Amazonas department, Ucubamba province, Cajamarca district, from Bosque Quemado; 5°36′8.5″S, 78°14′54.9″W; 3140 m a.s.l.; 28 Nov. 2019; P.J. Venegas, L.A. García-Ayachi, J.C. Chávez-Arribasplata, J.R. Ormeño, S. Bullard and A. Marchelie leg.; CORBIDI 21246–47.

Table 1. Uncorrected p-distances of the mitochondrial 16S rRNA gene between Gastrotheca gemma sp. nov. and closely related Gastrotheca species.

|        | 1  | 2  | 3  | 4  | 5  |
|--------|----|----|----|----|----|
| 1. G. gemma CORBIDI 21238 |  |    | 2.3|    |    |
| 2. G. oresbios CORBIDI 11076 |    |    | 5.2 |    |    |
| 3. G. psychrophila KU 142634 |    |    | 4.5 | 4.8 | 4.3 |
| 4. G. spectabilis CORBIDI 11790 |    |    | 5.2 | 5.4 | 5.2 | 5.0 |
| 5. G. stictopleura MTD 45230 |    |    | 5.2 | 5.7 | 5.4 | 6.6 | 5.4 |
| 6. G. sp. CORBIDI 11776 |    |    | 5.2 | 5.7 | 5.4 | 6.6 | 5.4 |

Referred specimens (3). CORBIDI 21239–41, three froglets that emerged from the dorsal brooding pouch of the holotype on 26 November 2019.

Diagnosis. Assigned to the genus Gastrotheca by females possessing a closed brood pouch on the dorsum. A moderately large species (69.7 and 71.8 mm SVL in two females, 56.9 and 59.5 mm SVL in two males), with: (1) tibia length 57–59% SVL, longer than foot; (2) interorbital distance greater than width of upper eyelid (161–169%); (3) skin on dorsum coarsely granular in females and granular in males, not co-ossified with skull, lacking transverse ridges; (4) supraciliary processes absent; (5) heel lacking calcar or tubercle; (6) tympanic annulus wrinkled or tuberculate; (7) Finger I slightly shorter than Finger II, width of discs wider than digits; (8) finger webbing present basally, only between III and IV; (9) foot webbing between external toes extending to nearly antepenultimate subarticular tubercle on Toe IV, to penultimate subarticular tubercle on Toe V, (10) in life, dorsum green with numerous minute black flecks in females and green with scattered yellow dots in males, paravertebral marks absent; (11) head markings consisting of a chocolate or pale green labial stripe in females and males, respectively; (12) dorsolateral stripe absent; (13) flanks uniformly green in females and green with numerous dark green irregular flecks in males; groin yellowish green in both sexes; anterior surfaces of thighs green, posterior surfaces of thighs yellowish green with scattered irregular black flecks in females and dense black reticulations in males; ventrolateral region yellowish green; irises silvery with a light blue hue or turquoise with thin black reticulations with or without an orange ring; (14) gular region and chest green in females and yellowish green in males; venter yellowish green with or without a big dark grey patch in the middle in females and venter greenish cream in males; ventral surface of thighs yellowish green with a dark greyish brown patch on the centre in females and thighs pale brownish cream in males; palms, soles and ventral surface of tarsus dark grey or dark greyish brown; (15) brood pouch single, dorsal; (16) reproduction mode direct development.

Gastrotheca gemma closely resembles G. aguaruna, G. carinaceps, and G. dyprosita from Peru, and G. turnerorum from Ecuador. Gastrotheca gemma shares with the aforementioned species a granular dorsal skin and a green dorsum, however the skin texture in G. gemma is coarsely granular, whereas it is weakly or finely granular in G. aguaruna and G. carinaceps, and granular in...
Figure 2. Preserved holotype of *Gastrotheca gemma* sp. nov. (CORBIDI 21238; female, SVL = 69.7 mm) in dorsal (A) and ventral (B) views.
Furthermore, *G. aguaruna* differs by having finger I longer than II, and *G. carinaceps* finger I and II equal in size (finger I shorter than finger II in *G. gemma*). *Gastrotheca aguaruna* has a dorsolateral row of warts or tubercles, absent in *G. gemma*. *Gastrotheca turnerorum* also can be distinguished from *G. gemma* by having brown flanks and a dark brown venter with cream spots, while *G. gemma* has yellowish green flanks. The new species and *G. dysprosita* have a coarsely granular dorsum, however *G. dysprosita* differs from *G. gemma* (character in parentheses) by having the interorbital distance smaller than width of upper eyelid (interorbital distance greater than width of upper eyelid), finger I and finger II equal in size (finger I shorter than finger II), dorsum with middorsal and dorsolateral stripes (dorsum without pattern), and venter cream with small brown spots (spots or blotches absent).

*Gastrotheca gemma* can be confused with green individuals of *G. monticola* and *G. ossilaginis* that also occur in the Andes of northern Peru (Duellman and Venegas

**Figure 3.** Some morphological details of the preserved holotype of *Gastrotheca gemma* sp. nov.: dorsal (A) and lateral (B) views of the head, and views of palm (C) and sole (D). Scale bar 5 mm. Photographs by LAGA.
2005; Duellman et al. 2014). Nevertheless, *G. monticola* and *G. ossilaginis* have the dorsum finely shagreen (coarsely granular in *G. gemma*). Furthermore, *Gastrotheca monticola* differs from *G. gemma* by having the venter with scattered black spots (absent in the new species), and *G. ossilaginis* has the skin co-ossified with the skull (not co-ossified in *G. gemma*).

Phylogenetically, *G. gemma* is sister to *G. oresbios* and closely related to *G. psychrophila, G. spectabilis* and *G. stictopleura*. The skin on the dorsum of the adult female holotype of *G. oresbios*, the single known adult specimen of this species, was described as smooth with scattered tubercles (see Duellman and Venegas 2016). However, after the examination of the holotype of *G. oresbios*, we considered its skin texture shagreen with scattered tubercles, while in *G. gemma* is coarsely granular. Furthermore, *G. oresbios* has an acuminate snout in dorsal view and the dorsum tan with brown paravertebral marks (the new species possesses a rounded snout in dorsal view and green dorsum without marks). *Gastrotheca gemma* can be readily distinguished from *G. spectabilis, G. stictopleura* and *G. psychrophila* by differences in skin texture of dorsum: smooth in *G. spectabilis*, finely shagreen in *G. stictopleura* and granular in *G. psychrophila*; while in the new species is coarsely granular. *Gastrotheca spectabilis* has finger I equal in size to finger II whereas in the new species finger I is shorter than finger II. *Gastrotheca spectabilis* can be also distinguished from the new species by having a brown dorsum (dorsum green in *G. gemma*). *Gastrotheca stictopleura* is dorsally green, like *G. gemma*, but possesses a white dorsolateral stripe bordered below by brown (dorsolateral stripe absent in *G. gemma*). *Gastrotheca psychrophila* possess axilla, groins and hidden surfaces of hindlimbs blue or purple (yellowish green in *G. gemma*).

*Gastrotheca gemma* was found in syntopy with *G. abdita* in the páramo, however both can be easily distinguished by their skin texture, being smooth in *G. abdita*. Furthermore, the dorsum is usually brown with paravertebral stripes in *G. abdita*, but green in the new species. *Gastrotheca testudinea* also occurs in Cordillera de Colán but at lower elevations (from 1700 to 2200 m a.s.l.). *Gastrotheca testudinea* differs from the new species by having the dorsum smooth and finger I longer than finger II. Additionally, as in *G. abdita*, the dorsum of *G. testudinea* is usually brown.

Description of the holotype. An adult female (Fig. 2 and 3) in good state of preservation and with a piece of tissue removed from the left thigh for molecular analysis; SVL 69.7 mm; head wider than long; snout rounded in dorsal view, slightly inclined anteroventrally in profile; canthus rostralis round in section; loreal region slightly concave; lips rounded, broad; top of head granular; interorbital distance 173% of width of upper eyelid; intermaxillary area flat; nostrils not protuberant, directed anterolaterally, to the level of anterior margin of lower jaw; diameter of eye is less than its distance from nostril; tympanum round, separated from the eye by a distance larger than the diameter of tympanum; tympanic annulus barely evident; supratympanic fold ill-defined, extending from behind the tympanum near to the insertion of the forelimb. Denticulous vomerine processes narrowly separated mediially, one bearing five teeth and the other six teeth.

Arm robust; ulnar tubercles absent; hand and fingers moderately large (TFL 38% of SVL), fingers with basal web only between III and IV; discs large and rounded, width of disc of Finger III greater that diameter of tympanum; relative lengths of fingers I<II<IV<III; subarticular tubercles prominent, round in dorsal and profile views, none bifid; supranumerary tubercles, round; palmar tubercle ill-defined, bifid; prepollical tubercle large, elliptical. Hind limb robust; tibia length 59% of SVL; foot length 54% of SVL; calcare and tarsal tubercles absent; inner tarsal fold present; outer metatarsal tubercle absent; inner metatarsal tubercle elliptical, low; toes moderately long; relative length of toes I<II<III<IV; basal webbing between Toes I and II; webbing formula for other toes I–II–III–IV–IV; basilar webbing V between Toes I and II; webbing formula for other toes I–II–III–IV–IV–IV; subarticular tubercles moderately large, rounded; supernumerary tubercles, numerous, and rounded; outer edge of toe V bears a fringe extending along the edge of foot.

Skin on dorsum coarsely granular; skin on flanks covered by enlarged flattened warts; skin on throat and chest weakly granular, ventral surfaces of thighs and arms coarsely granular; skin on belly granular; ventral surface of shanks smooth; two vertical rows of enlarged brown tubercles (three on left and two on right) below the cloacal opening. Tongue broad, suboval, not notched posteriorly, fully attached to mouth floor. Pouch opening V-shaped with anterior border at level of posterior edge of sacrum.

Measurements of the holotype (in mm). SVL: 69.7; TIBL: 41.1, FL: 37.7, HL: 23.3, HW: 27.2, IOD: 9.7, EW: 5.6, IND: 3.9, ED: 5.8, EN: 7.2, TD: 3.9, FFL: 14.5, TFL: 26.8, TFD: 4.2.

Colour of holotype. In life: dorsal surfaces green with numerous black flecks on dorsum, chocolate brown labial stripe, groins and ventrolateral region yellowish brown;
forelimbs and fingers blotched with chocolate brown; hindlimbs with scattered black irregular flecks, numerous on shanks, larger on anterior surface of thighs, tarsus and feet; a chocolate brown stripe from the middle of thighs, running along knee, inner edge of shank and outer edge tarsus to toe V; toes chocolate brown. Ventral surface of throat, chest, forelimbs, belly and thighs yellowish green with a large greyish brown patch on the middle of the belly and a dark greyish brown patch on thighs; tibia and shanks green, ventral surface of tarsus, palms and soles dark greyish brown. Iris silvery with a light blue hue. In preservative (ethanol 70%): green coloration turns light blue and chocolate brown coloration turns pale brown on dorsal surface; ventrally, yellowish green coloration turns greyish light blue and dark greyish brown coloration turns light brown.

**Intraspecific variation.** Morphometric variation of two males and two females is summarized in Table 2. The single female paratype (CORBIDI 19396) is identical to the holotype varying only in lacking scattered black flecks on hindlimbs and the greyish patch in the middle of the belly, and by having turquoise irises. Females are larger than males (SVL in females 69.7–71.8 mm versus SVL in males 56.9–59.5 mm). However, *G. gemma* shows a strong sexual dimorphism in skin texture and coloration. Males have the skin on dorsum granular with scattered enlarged warts, while females have coarsely granular skin. In addition, have scattered large flattened warts on the dorsal surface of hindlimbs, whereas in females is smooth.

The dorsum in males (CORBIDI 21246–47; Fig. 4C–F) is green with scattered yellow dots, flanks green with numerous dark green irregular flecks, dorsal surface of hindlimbs greyish green with scattered yellow blotches,
posterior surface of thighs yellow with a dense black reticulation, gular region yellow, venter greenish cream, and ventral surface of thighs pale greyish brown. Iris coloration in both male specimens were turquoise with thin black reticulations and with or without an orange ring (Fig. 4).

The froglets (CORBIDI 21239–41) have the skin on dorsal and ventral surfaces smooth. In life, the dorsal coloration is green, sides of head brown, limbs pale brown, venter yellow and the iris is bronze.

Osteology. Osteological description of a brooding female paratype of *Gastrotheca gemma* (CORBIDI 19396) with a SVL of 71.85 mm.

Cranial osteology. The skull of *Gastrotheca gemma* is wider than long and measures 22.7 mm in length from the jaw joint to the tip of the snout and 27.0 mm in width at the level of the quadratojugal. The skull is hyperossified, with well-developed pit-and-ridge dermal sculpturing (i.e., exostosis) on the frontoparietals, squamosals, maxillae, and nasals (Fig. 5A). The exposed sphenethmoid has irregular vermiform ridging. The frontoparietals have a complete medial articulation with one another, and a moderately wide supraorbital and otic flange is present but does not form an articulation with the head of the squamosal (and therefore a temporal arcade is absent). The frontoparietal covers the anterior epiotic eminence, and the carotid canal is partially closed. The nasals are expanded, articulating with the pars facialis of the maxilla, and form a bony anterior orbital margin. The nasals overlap the anterior margin of the sphenethmoid and extend posteriorly but do not articulate with the anterior edges of the frontoparietal. The maxillary arcade is complete. The quadratojugal is broadly overlapped laterally by the maxilla. The large posterior process of the maxilla articulates with the zygomatic ramus of the squamosal via a broad diagonal articulation, forming the posterior margin of the orbit (Fig. 5C). The head of the squamosal has a moderately wide crest, and the otic plate of the squamosal covers 35% of the width of the crista parotica. The zygomatic ramus of the squamosal bifurcates distally, and the medial branch articulates with a high dorsal process of the anterior ramus of the pterygoid and the lateral branch articulates with the pars facialis of the maxilla. The anterior ramus of the pterygoid articulates with the lingual edge of the pars palatina of the maxilla. The premaxillae are broad and bear alary processes that are deflected posteriorly at a 25° angle, forming a high and slightly rounded snout in lateral profile. The premaxillae are wide and bear alary processes that are widely separated from one another and form a posterior margin to the choana (Fig. 5B). The prechoanal processes of the

Figure 5. Skull of *Gastrotheca gemma* sp. nov. (CORBIDI 19396) as visualized via Micro-CT scanning in dorsal (A), ventral (B), and right lateral (C) views. (D) Mandible in dorsal view. Scale bar = 5 mm.
vomers are long and articulate with the lingual surface of the pars facialis of the maxilla and the neopalatines, supporting the complete anterior margin of the choana. The postchoanal processes of the vomers are short and support half of the medial choanal margin. The dentigerous processes of the vomers are located at the level between the posterior portions of the choanae. The parasphenoid is synostosed to the overlying prootics and exoccipitals. The cultriform process narrows abruptly anterior to the optic fenestra and terminates posterior to the level of the neopalatines. The sphenethmoid is not synostosed with the prootic. There are 30 to 42 socketed, pedicellate teeth on each maxilla, 7 to 10 teeth on each premaxilla, and 5 teeth on each vomer. The dentary is edentate (Fig. 5D).

Postcranial osteology. Vertebral column. Eight presacral vertebrae, the low neural spines of the atlas and presacral II articulate while the remaining presacras are non-imbricate (Fig. 6A). Neural arches of presacras III–VIII lack neural spines. The atlas lacks transverse processes, presacras II–IV bear thicker and longer processes than presacras V–VIII. The transverse processes of presacral III are expanded distally, those of presacras II and IV are slightly expanded distally. The transverse process of presacras II and VIII are anteriorly directed, those of presacras III, VI and VII are perpendicular to the notochordal axis, and those of presacras IV and V are posteriorly directed. Lengths of the transverse process of presacras along with that of the sacral diaphyses (SD): SD > III > II > IV > V–VII > VIII. The anterior margin of the sacral diaphyses is perpendicular to the longitudinal axis of the vertebra column. The distal ends of sacral diaphyses are expanded, approximately twice the width of the base. Both anterior and posterior margins of the sacral diaphyses are convex. The urostyle is as long as the presacral portion of the vertebral column and has a bicondylar articulation with the sacrum. The shaft of the urostyle is dorsoventrally compressed in cross section. The width of the shaft of the urostyle is narrow anteriorly. The urostyle bears a dorsal crest along half of the bone shaft, the crest is higher anteriorly and gradually diminishes in height posteriorly.

Pectoral girdle. Arciform pectoral girdle (Fig. 6C). Clavicles curved and concave. Clavicles are uniform in width, only the lateral region is wider. Laterally each clavicle is
fused to the pars acromialis of the scapula. Medial tips of clavicles not in contact, reaching to the level of the anterolateral end of each clavicle. The clavicles do not reach the glenoid fossa. In ventral view, the sternal and glenoid ends of the coracoids have the same width and the midshaft has half the width of the lateral ends. The sternal end is flattened and the glenoid end is wide and slightly concave. The sternal ends of the coracoids are not in contact. The scapula is stout, longer than the coracoid. The pars glenoidalis is narrower than the pars acromialis. The suprascapular end is narrower than the zonal end. The coracoid and scapula form the margin of the glenoid fossa. Cleithrum and ossified portion of the suprascapula fused.

**Pelvic girdle.** In dorsal view, the ilial shafts have a V-shaped configuration. The ilial shaft has a low dorsal crest (Fig. 6D). The dorsal prominence is low but conspicuous, the associated dorsal protuberance is elongate, conspicuous and positioned above the dorsal margin of the acetabular fossa. The ventral anterior margin of the ventral acetabular expansion is straight and forms an angle of approximately 90° with the ilial shaft. The articulations of the ilium with the ischium and pubis are evident, but not the articulation of the pubis and the ilium.

**Forelimb and manus.** The humerus has a prominent ventral crest, extending along more than half the length of the bone, higher at the proximal end of the humerus and gradually diminishing in height distally. The distal head (eminentia capitata) is expanded and it is wider than the glenoid head (caput humeri). The radioulna is flattened and distinctly wide distally; the sulcus intermedius is indicated by a distinct groove on the distal half of the bone. The carpus is composed by radiale, ulnare, distal carpal 5–4–3, element Y fused to distal carpal 2, and elements of the prepollex. The phalangeal formula is 2–2–3–4–3 (Fig. 6B). The terminal phalanges have a rounded proximal base, from the base towards the tip become gradually narrower. The prepollex has two elements, including the base.

**Hind limb and pes.** The femur is slightly sigmoid, shorter than the tibiofibula. The sulcus intermedius of the tibiofibula is shallow. The tibiale and fibulare are separated more than the tibiofibula. The sulcus intermedius of the tibial head (caput humeri). The radioulna is flattened and the glenoid end is wide and slightly concave. The tibia and fibula form the margin of the glenoid fossa. Cleithrum and ossified portion of the suprascapula fused.

**Discussion**

The description of *Gastrotheca gemma* increases the number of *Gastrotheca* species reported from Peru to 31, among these 26 are endemic. *Gastrotheca gemma* is the second species of the genus described from Cordillera de Colán. The LSU ornithological expedition collected *G. testudinea*, also a direct-developer, more than 40 years ago. This poorly known species was considered the only *Gastrotheca* species inhabiting Cordillera de Colán. Duellman (2015) tentatively identified a juvenile *Gastrotheca* (CORBIDI 862) from Cordillera de Colán as *G. abdita*. We examined this juvenile specimen and observed characteristics in disagreement with the diagnosis of *G. abdita*, but in agreement with the diagnosis of *G. testudinea*. In support of this identification, the uncorrected p-distances for a 16S rRNA fragment between *G. sp. CORBIDI...
During the recent field expeditions to Cordillera de Colán we collected both *G. testudinea* and *G. abdita*, however *G. testudinea* was never found above 2200 m a.s.l.

The cranial morphology of *Gastrotheca* is morphologically complex, particularly in the Andean clade *Gastrotheca marsupiata* species group. *Gastrotheca galeata* possesses a highly derived, casqued skull that bears little
similarity to other species in the genus due to a unique sculpturing pattern and extensive synostosis (Trueb and Duellman 1978; Duellman 2015) and is more similar in skull shape to distantly related anurans known to use phragmatic behaviour (e.g., Smilisca fodiens, Peltophryne toads; Paluh et al. 2020). Species in the Gastrotheca marsupiata species group are highly variable and challenging to morphologically characterize (Duellman 2015). For example, variation exists across Andean Gastrotheca in the presence or absence of dermal sculpturing, supraorbital and otic flanges, a temporal arcade, and articulation between the zygomatic ramus of the squamosal and maxilla, prominently altering the overall architecture of the skull. The amount of homoplasy in these characters has not been investigated but is likely high. Gastrotheca gemma was recovered within a clade that includes

Figure 8. Skeleton and 34 embryos in pouch of Gastrotheca gemma sp. nov. (CORBIDI 19396) as visualized via Micro–CT scanning in dorsal (A) and right lateral (B) views. (A) Scale bar = 10 mm.
G. oresbios, G. psychrophila, G. spectabilis, and G. stictopleura. Of these closely related species, the skull anatomy has been described for only G. psychrophila (Duellman 2015). Gastrotheca gemma and G. psychrophila share several cranial characters, including the presence of wide supraorbital and otic flanges, a frontoparietal that covers the anterior epiotic eminence, a partially closed carotid canal, alary processes of the premaxillae that are deflected posteriorly, and the absence of a temporal arcade. Gastrotheca psychrophila differs from G. gemma in that the sphenethmoid is hyperossified with pit-and-ridge sculpturing and the nasals articulate with the anterior edges of the frontoparietal. The postcranial osteology of Gastrotheca has been less extensively studied, there are descriptions for G. carinaceps, G. galeata, G. lateonota and G. monticola (Duellman and Trueb 1978, 1988; Duellman et al. 2006). The presacral and sacral regions and ilium of G. gemma is very similar to those of the aforementioned members of the G. marsupiata species group. The clavicles are curved and robust as in G. lateonota and G. monticola. As stated by Duellman et al. (2006) the postcranial osteology, among these few species of the G. marsupiata species group, seems relatively conserved.

The herpetofauna of the isolated Cordillera de Colán deserves further investigation, in fact the expedition in which G. gemma was discovered is one of three expeditions targeting exclusively the unknown diversity of this group.

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Appendix 1

Examined specimens

Gastrotheca aguaruna — PERU: Amazonas: Luya: ACP Huiquilla, 2935 m a.s.l., CORBIDI 00038; Rodríguez de Mendoza: Vista Alegre: Hornillo, 3308 m a.s.l., CORBIDI 11747, 11752, 11763, 11767, 11770, 11773–74, 11788–89, 11792–94; Bongara: San Carlos: Corobamba, 3030–3050 m a.s.l., CORBIDI 21945, 21956, 21958. San Martín: Mariscal Cáceres: Camino Leimebamba-Los Chilchos, 3240 m a.s.l., CORBIDI 00592; San Martín: Tragadero-Las Piñas, 3235 m a.s.l., CORBIDI 10933.

Gastrotheca monticola — PERU: Amazonas: Bongara: Valera: Co-cachimba, 1620 m a.s.l., CORBIDI 492; Quebrada El Atajo, 2416 m a.s.l., CORBIDI 19931; Opelel, 2655 m a.s.l., CORBIDI 19946–47; Leimebamba: Centro Poblado dos de Mayo, 2370 m a.s.l., CORBIDI 15890–91; Luya: Tingo: Ruinas de Silic, 1804 m a.s.l., CORBIDI 18602, 18605; Ruinas imperio – Bajo Kuelap, 2869 m a.s.l., CORBIDI 18616. Piura: Huancabamba: Sondor: Agupampa, 2625 m a.s.l., CORBIDI 14865–66. Cajamarca: Jaén: Pomahuaca, 2772 m a.s.l., CORBIDI 11040.

Gastrotheca sp. — PERU: Amazonas: Rodríguez de Mendoza: Vista Alegre, 3308 m a.s.l., CORBIDI 11776, 11768, 11775, 11776–78.

Gastrotheca oresbios — PERU: Amazonas: Chachapo yas: Abra Barro Negro, 3290 m a.s.l., CORBIDI 11076 (Holotype); San Martín: Mariscal Cáceres: Quintecocha, 3119 m a.s.l., CORBIDI 11040.

Gastrotheca spectabilis — PERU: Amazonas: Rodríguez de Mendoza: Vista Alegre: Hornillo, 3308 m a.s.l., CORBIDI 11790 (Holotype), CORBIDI 11644, 11753, 11780–81, KU 342532.

Appendix 2

GenBank accession numbers for sequences used in this study.

| Species           | 12S   | 16S   | ND1   | POMC | RAG1 (1) |
|-------------------|-------|-------|-------|------|----------|
| Gastrotheca aguaruna | KU 212026 | —     | KF723438 | KF723462 | KF723484 | KF723505 |
| Gastrotheca albolineata | MNRJ 54401 | KR559919 | KF270407 + KR270425 | KC844949 | KR270365 | KR138423 |
| Gastrotheca antoniiochoai | MUSM 27944 | —     | KF723434 | KF723467 | KF723489 | KF723510 |
| Gastrotheca argenteovirens | KU 212056 | —     | KF723435 | KF723467 | KF723489 | KF723510 |
| Gastrotheca atympana | MHNSM 18692 | DQ679234 | DQ679384 | DQ679343 | DQ679312 | DQ679276 |
| Gastrotheca aureomaculata | KU 181194 | DQ679235 | DQ679385 | DQ679343 | DQ679312 | DQ679276 |
| Gastrotheca christiani | FML 2881 | DQ679236 | DQ679386 | DQ679343 | DQ679312 | DQ679276 |
| Gastrotheca chrysosticta | LM 58 | DQ679237 | DQ679387 | DQ679343 | DQ679312 | DQ679276 |
| Gastrotheca dissimilis | KU 181740 | DQ679238 | DQ679388 | DQ679343 | DQ679312 | DQ679276 |
| Gastrotheca dunni | ICN 10059 + MHUA A 4800 | DQ679239 | DQ679389 | DQ679343 | DQ679312 | DQ679276 |
| Gastrotheca ernestoi | MNRJ 57129 + MNRJ 64000 | KR559920 | KR270408 | KC844952 | KR270366 | KR138424 |
| Gastrotheca espeletia | KU203440 | KF723440 | KF723460 | KF723480 | KF723500 | KF723505 |
| Gastrotheca excubitor | MUSM 26280 | —     | —     | —    | —        | —        |
| Gastrotheca fissipes | ZUFRJ 7901 | —     | —     | —    | —        | —        |
| Gastrotheca fulvorufa | CMTM 07467 | —     | —     | —    | —        | —        |
| Gastrotheca galeata | KU 181700 | —     | —     | —    | —        | —        |
| Gastrotheca gracilis | DCC 006 | —     | —     | —    | —        | —        |
| Gastrotheca griswoldi | MHNSM 20588 | —     | —     | —    | —        | —        |
| Gastrotheca guentheri | KU 173112 | —     | —     | —    | —        | —        |
| Gastrotheca helenae | KU 181070 | —     | —     | —    | —        | —        |
| Gastrotheca pseustes 1 | QCAZ 45113 | —     | —     | —    | —        | —        |
| Gastrotheca pseustes 2 | QCAZ 42862 + TNHC 62492 | —     | —     | —    | —        | —        |
| Species                     | 12S          | 16S          | ND1          | POMC         | RAG1 (1)   |
|-----------------------------|--------------|--------------|--------------|--------------|------------|
| Gastrotheca marsupiata KU 214813 + KU 214814 | AY819356     | DQ679397     | AY819487     | AY819105    | DQ679289   |
| Gastrotheca megacephala JLG 90 + CFBH T377 | AY843592     | AY843592     | KC844953     | KC844976    | AY844381 + KC844996 |
| Gastrotheca microdiscus CFBH T 1250 + CFBH T 3068 | —            | —            | KC844932     | KC844958    | KC844979   |
| Gastrotheca monticola KU 212036 | AY819357     | DQ679398     | AY819488     | AY819106    | DQ679290   |
| Gastrotheca nebulanastes MUSM 27943 + MCZ 265218 | —            | —            | KC844959     | KC844992    | KC845001   |
| Gastrotheca nicefori KU 181071 | DQ679249     | DQ679399     | DQ679357     | DQ679325    | DQ679291   |
| Gastrotheca ochoai KU 173499 | DQ679250     | DQ679400     | DQ679358     | DQ679326    | DQ679292   |
| Gastrotheca orophylax KU 178568 | DQ679251     | DQ679401     | DQ679359     | DQ679327    | DQ679293   |
| Gastrotheca ovifera KU 185758 | DQ679252     | —            | DQ679360     | —            | —          |
| Gastrotheca pachachacae MUSM 28492 | —            | —            | KC844983     | —            | —          |
| Gastrotheca peruana KU 207815 | —            | —            | KC845002     | —            | —          |
| Gastrotheca phalarosa CORBIDI 11044 | —            | —            | —            | —            | —          |
| Gastrotheca phelloderma MUSM 33350 | —            | —            | —            | —            | —          |
| Gastrotheca plumbea KU 178499 | DQ679254     | DQ679403     | DQ679362     | DQ679328    | DQ679294   |
| Gastrotheca praesina MZUSP 147060 + MZUSP 17460 | JX262901     | KJ489476     | JX262922     | —            | KJ489602   |
| Gastrotheca psychrophila KU 142634 | DQ679255     | DQ679404     | DQ679363     | DQ679329    | DQ679295   |
| Gastrotheca rebeccae CORBIDI 08006 | KC844937     | KC844963     | KC844987     | —            | —          |
| Gastrotheca ruizi KU 200002 | DQ679257     | DQ679406     | DQ679365     | —            | DQ679297   |
| Gastrotheca yacuri QCAZ 21105 | —            | —            | KC844939     | KC844965    | KC844989   |
| Gastrotheca turnerorum QCAZ 47299 | —            | —            | KC844940     | KC844960    | KC844984   |
| Gastrotheca elicii QCAZ 21213 | —            | —            | KC844922     | KC844947    | —          |
| Gastrotheca spectabilis CORBIDI 11790 | —            | —            | KJ489464     | KJ489513    | KJ489592   |
| Gastrotheca stictopleura MTD 45230 | DQ679258     | DQ679407     | DQ679366     | DQ679330    | DQ679298   |
| Gastrotheca testudinea QCAZ 16444 | —            | —            | KC844940     | KC844966    | —          |
| Gastrotheca trachyceps KU 181189 | DQ679259     | DQ679408     | DQ679367     | DQ679331    | DQ679299   |
| Gastrotheca walkeri Vz 8996 | DQ679260     | DQ679409     | DQ679368     | DQ679332    | DQ679300   |
| Gastrotheca weinlandi KU 143105 | DQ679261     | DQ679410     | DQ679369     | DQ679333    | DQ679301   |
| Gastrotheca zeugocystis MHNMS 18675 | DQ679262     | DQ679411     | —            | DQ679334    | DQ679302   |
| Gastrotheca sp. 1 IDLR 4073 (MNCN/ADN 566) | —            | —            | KR270428     | —            | —          |
| Gastrotheca sp. 2 MNK 5286 + CBG 1020 | AY843590     | AY843590     | KC844955     | —            | AY844380   |
| Gastrotheca sp. 3 ZFMK 66954 | —            | —            | KR270429     | —            | —          |
| Gastrotheca sp. J CORBIDI 862 | —            | KJ489463     | KJ489511     | —            | KJ489590   |
| Gastrotheca sp. CORBIDI 11776 | —            | KJ489475     | KJ489525     | KJ489563    | —          |
| Gastrotheca sp. KU 173171 | DQ679241     | DQ679391     | DQ679350     | DQ679317    | DQ679283   |
| Gastrotheca gemma CORBIDI 21238 | —            | MW403923     | —            | —            | —          |

Hemiphractus proboscideus

| Species                     | 12S          | 16S          | ND1          | POMC         | RAG1 (1)   |
|-----------------------------|--------------|--------------|--------------|--------------|------------|
| Pablo J. Venegas et al. A new species of marsupial frog from the Cordillera de Colán in northeastern Peru | —            | —            | —            | —            | —          |