Two new syntopic species of glassfrogs (Amphibia, Centrolenidae, *Centrolene*) from the southwestern Andes of Ecuador

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Abstract

We describe two new species of glassfrogs of the genus *Centrolene* living in syntopy at La Enramada, province of Azuay, southwestern Ecuador. They were found in a small creek in montane evergreen forests at 2900 m elevation. The first new species is distinguished from all other members of the genus *Centrolene* by having the following combination of characters: dentigerous process of vomer absent; sloping snout in lateral view; thick, white labial stripe and a faint white line between the lip and anterior ¼ of body; humeral spine in adult males; parietal peritoneum covered by iridophores, visceral peritonea translucent (except pericardium); ulnar and tarsal ornamentation; dorsal skin shagreen with dispersed warts; uniform green dorsum with light yellowish green warts; and green bones. The new species is remarkable by being sister to a species from the opposite Andean versant, *C. condor*. The second new species is distinguished from all other *Centrolene* by having the following combination of characters: dentigerous process of vomer absent; round snout in lateral view; thin, yellowish labial stripe with a row of white tubercles between the lip and arm insertion, and a yellowish line between arm insertion and groin; uniform green dorsum; humeral spine in adult males; parietal peritoneum covered by iridophores, visceral peritonea translucent (except pericardium); dorsal skin shagreen with dispersed spicules; ulnar and tarsal ornamentation; and green bones. The second new species is the sister to *C. sabini* and an undescribed species of *Centrolene* from southeastern Ecuador. Based on nuclear and mitochondrial DNA sequences, we present a new phylogeny for *Centrolene* and comment on the phylogenetic relationships inside the genus.

Introduction

Glassfrogs of the genus *Centrolene* Jiménez de la Espada, 1872 are distributed across the Andes, from the Merida Massif in Venezuela to the Kosñipata Valley in southern Peru (Frost, 2021). While no synapomorphies are known for *Centrolene*, its monophyly is well-supported (Guayasamin et al., 2009, 2020; Catenazzi et al., 2012; Twomey, Delia & Castroviejo-Fisher, 2014). The following combination of morphological characters is helpful to diagnose *Centrolene*: presence of humeral spines in adult males of most species—except *Centrolene daidalea* (Ruiz-Carranza & Lynch, 1991a) and *C. savagei* (Ruiz-Carranza & Lynch, 1991a); liver lobed and covered by translucent hepatic peritoneum; pericloacal warts enamelled; bones green in life; background colouration of dorsum in preservative lavender (Ruiz-Carranza & Lynch, 1991b; Cisneros-Heredia & McDiarmid, 2007; Guayasamin et al., 2009; Catenazzi et al., 2012).

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Twelve species of *Centrolene* have been reported from Ecuador: *C. ballux* (Duellman & Burrowes, 1989); *C. buckleyi* (Boulenger, 1882); *C. charapita* Twomey, Delia, & Castroviejo-Fisher, 2014; *C. condor* (Cisneros-Heredia & Morales-Mite, 2008); *C. geckoidea* Jimenez de la Espada, 1872; *C. heloderma* (Duellman, 1981); *C. huilensis* (Ruiz-Carranza & Lynch, 1995), *C. lynchi* (Duellman, 1980), *C. medemi* (Cochran and Goin, 1970), *C. peristicta* (Lynch & Duellman, 1973), *C. pipilata* (Lynch & Duellman, 1973), and *Centrolene sanchezi* (Ruiz-Carranza & Lynch, 1991c) (Lynch & Duellman, 1973; Cisneros-Heredia & McDiarmid, 2005, 2006; Cisneros-Heredia & Yánez-Muñoz, 2007a; Cisneros-Heredia & Morales-Mite, 2008; Guayasamin et al., 2020). Six of them inhabit the north-western slopes of the Cordillera Occidental of the Andes of Ecuador: *C. ballux*, *C. buckleyi*, *C. geckoidea*, *C. heloderma*, *C. lynchi* and *C. peristicta*. Still, only *C. heloderma* has been reported from the southwestern slopes. Yánez-Muñoz et al. (2015) preliminarily informed of the presence of *C. heloderma* in the southwestern Andes of Ecuador based on three specimens collected at La Enramada, province of Azuay. However, molecular analyses show that they belong to two different and undescribed species of *Centrolene* found together at one of the last remnants of montane forests in the region. We are pleased to describe these two new species in this publication.

Materials & Methods

Ethics statement

Our study was authorised under framework contracts for access to genetic resources MAE-DNB-CM-2016-0045 and MAE-DNB-CM-2019-0120, issued by the Ministerio del Ambiente del

Ecuador. We followed the standard guidelines for using live amphibians and reptiles in field research by Beaupre et al. (2004).

Species concept

We consider species as separately evolving metapopulation lineages, recognisable from an operational point of view to the extent that isolation from their putative sister lineages can be inferred (De Queiroz, 2007).

Taxonomic sampling

Specimens from the following collections were examined: División de Herpetología, Museo Ecuatoriano de Ciencias Naturales, Instituto Nacional de Biodiversidad, Quito (DHMECN); University of Kansas Natural History Museum, Lawrence (KU); Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito (QCAZ); National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM).

Information on species for comparative diagnoses was obtained from the literature (Duellman & Schulte, 1993; Señaris & Ayarzaguena, 2005; Cisneros-Heredia & McDiarmid, 2007; Catenazzi et al., 2012; Twomey, Delia & Castroviejo-Fisher, 2014; Guayasamin et al., 2020) and the following examined specimens: *Centrolene ballux* (12 specimens): Ecuador: Carchi: KU 202798, 5 km. W La Gruel; Pichincha: QCAZ 40195–97, Las Gralarias; KU 164726–32, 14 km W of Chiriboga; KU 164733, Quebrada Zapadores. *Centrolene buckleyi* (44 specimens)*:* Ecuador: Bolívar: DHMECN 0866–67, Guanujo; Carchi: DHMECN 1246, Los Encinos; DHMECN 13375, 13376, 13828, 14180, Cabaña Las Orquídeas Morán; Cotopaxi: USNM 288428, Pilalo; Napo: USNM 311113–14, Santa Bárbara; Pichincha: USNM 288423, Quito; USNM 286626–27: 8.5 km (by road) NW of Nono; USNM 286628–29, Machachi; USNM 286630–31: 21.2 km (by road) ESE of Chiriboga; USNM 288424: 8 km to Chiriboga; Sucumbíos: DHMECN 868–893, near Santa Bárbara. *Centrolene condor* (6)*:* Ecuador: Zamora Chinchipe (6 specimens): QCAZ 37279, Destacamento Militar Cóndor Mirador; DHMECN11208–11210, Paquisha Alto, DHMECN 12049, Concesión Colibrí, DHMECN12053, Concesión La Zarza. *Centrolene heloderma* (11 specimens): Ecuador: Pichincha (9): USNM 211219–21, Quebrada Zapadores; USNM 211216–7: 13.1 km NW of Nono; USNM 211218: 8.6 km SE of Tandayapa; QCAZ 40200, 50722, Reserva Las Gralarias, QCAZ 44881, 14 km al W de Chiriboga; Carchi: DHMECN 14999-15000, Reserva Dracula, El Guapilal. Additional specimens examined during our studies in Centrolenidae are listed in Cisneros-Heredia & McDiarmid (2007) and Guayasamin et al. (2020).

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Fieldwork

Fieldwork was conducted at La Enramada (3.161074 ºS; 79.600045 ºW, 2900 m), province of Azuay, Ecuador, during expeditions of the Instituto Nacional de Biodiversidad INABIO on 21–31 March 2015, 13–17 April 2019 and 06–11 December 2022. We used visual encounter surveys for herpetological searches (Crump & Scott, Jr., 1994). Only the first expedition in March 2015 resulted in the collection of specimens of the new species described herein. Individuals were photographed alive and euthanised with benzocaine, a muscle tissue sample was extracted and preserved in 95% ethanol, and whole specimens were fixed in 10% formalin and preserved in 75% ethanol.

Morphology and colouration

Diagnosis, terminology, and adult characters and measurements follow the format and definitions proposed by Cisneros-Heredia & McDiarmid (2007). All characteristics reported in the description of the type series are from adult specimens. Sex and maturity were determined by directly examining gonads through dissections and noting the presence of secondary sexual characters (i.e., vocal slits and nuptial pads). All morphometric data were measured with a digital calliper (0.05 mm accuracy, rounded to the nearest 0.1 mm) under a stereomicroscope, reported as a range (mean ± standard deviation), and included: snout-vent length (SVL), head length (HL), head width (HW), interorbital distance (IOD), eye diameter (ED), internarial distance (IND), eye-nostril distance (EN), tympanum diameter (TD), tibia length (TL), foot length (FL), hand length (HAL), Finger III disk width (Fin3DW). Colour patterns are described based on photographs of life specimens taken in the field. The adjective "enamelled" describes the shiny white colouration produced by an accumulation of iridophores (Lynch & Duellman, 1973; Cisneros-Heredia & McDiarmid, 2007).

Phylogenetic analyses and genetic distances

To assess the evolutionary affinities of the new species, we sequenced two mitochondrial genes (12S rRNA and NADH dehydrogenase subunit 1, ND1) and two nuclear genes (RAG1 and C-MYC 2). DNA was extracted from muscle or liver tissue preserved in 95% ethanol or tissue storage buffer using standard phenol-chloroform extraction protocols (Sambrook, Fritsch & Maniatis, 1989). PCR amplification was performed under standard protocols and sequenced by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea). We also added a short new sequence of *C. lynchi* QCAZ 40192 (3' end of 16S, tRNA-Leu, and 5' beginning of ND1) because in a preliminary phylogeny, *C. lynchi* GenBank sequences QCAZ 40192 and QCAZ 40191, from the same population, unexpectedly, came out separate. Upon further inspection, we realised they lacked overlapping sequences, and the new sequence overlapped with a fragment of QCAZ 40191.

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Our phylogeny is based on sequences of *Centrolene* from GenBank (published by Guayasamin et al., 2008, 2020; Castroviejo-Fisher et al., 2014; Twomey, Delia & Castroviejo-Fisher, 2014) and new sequences of the new species. We analysed the mitochondrial genes 12S rRNA, 16S rRNA, ND1 and the nuclear genes BDNF, C-MYC 2, CXCR4, POMC, RAG1, SLC8A1, SLC8A3 for a total of 10 loci and up to 6355 bp. We also included Genbank sequences of *Allophryne*, *Celsiella*, *Chimerella*, *Cochranella*, *Espadarana*, *Hyalinobatrachium*, *Ikakogi*, *Nymphargus*, *Rulyrana*, *Sachatamia*, *Teratohyla*, and *Vitreorana*. The phylogeny was rooted with *Allophryne ruthveni* (specimen MAD1857; outgroup choice based on Twomey, Delia & Castroviejo-Fisher, 2014). The matrix had 61 terminals. GenBank accession numbers for newly generated sequences are in Table 1.

Raw sequences were assembled with Geneious 9.1.8 software (Biomatters Ltd.). Sequences were aligned using MAFFT 7.017 and the L-INS-i algorithm (Katoh & Standley 2013). The alignment was visually inspected in Mesquite (version 3.61; Maddison & Maddison 2019), and alignment errors were adjusted manually. We partitioned the matrix to allow separate evolution models for each gene and codon position (except for 12S and 16S non-coding) for a total of 26 partitions. We used the command *-m MPF* (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017) in the software IQ-TREE multicore version 2.2.0 (Minh et al. 2020). The phylogeny was estimated under maximum likelihood using IQ-TREE 2.2.0 under default settings. To assess branch support, we made 200 non-parametric bootstrap searches (-b 200 command) and 1000 replicates for the SH-like approximate likelihood ratio test (-alrt 1000 command; (Guindon et al. 2010). We considered that branches with bootstrap values > 70 and SH-aLRT values > 80 had strong support. Pairwise uncorrected p-genetic distances were calculated with the software MEGA 11.0.13 (Tamura, Stecher & Kumar, 2021). The standard error of the genetic distance was estimated with the bootstrap method. For accuracy, we only compared overlapping fragments longer than 400 bp.

Nomenclatural acts

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN). Hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved, and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid: zoobank.org:pub:A2A88B00-DA2C-443E-BC8B-9922980F8789. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

Results

Phylogenetic relationships

Our phylogeny (Fig. 1) is generally consistent with previous phylogenies of Centrolenidae (e.g., Twomey, Delia & Castroviejo-Fisher, 2014; Guayasamin et al., 2020). Unlike Guayasamin et al. (2020), we found a clade that excludes *C. charapita* and *C. geckoidea* and unites two sister subclades: (*C. savagei* + (*C. daidalea* + *C.* sp. Ca01)) + (*C. antioquiense* + *C. peristicta*)) and a clade containing all remaining species of *Centrolene*. The two new species described herein belong to the later subclade.

The first new species is strongly supported as sister to *C. condor*, a species only known from the Cordillera del Cóndor, southeastern Ecuador. The uncorrected-p genetic distance between them is 1.04% (SE = 0.338%) for the gene 12S. In *Centrolene*, at least two pairs of sister species are separated by distances lower than 1%: *C. altitudinale* vs *C. notosticta* (0.8%) and *C. peristicta* vs *C. antioquiense* (0.6–0.7%). Therefore, the 12S genetic distance between the first species and *C. condor* falls within the observed range of interspecific distances for the genus. The genetic distance between *C. condor* and the first new species for ND1 ranges from 6.1% (SE = 0.786%) to 6.5% (SE = 0.818%). The second new species is sister to a clade composed of *C. sabini* (from southeastern Peru) and an undescribed species of *Centrolene* from southeastern Ecuador (MRy 547, referred to as [Ca1] by Amador et al. 2018). The uncorrected p-genetic distance between the second new species and *C. sabini* is 2.9% (SE = 0.549%), while the distance with *Centrolene* sp. (MRy 547) is 3.7% (SE = 0.709%).

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Species descriptions

Centrolene camposi sp. nov.

LSID urn:lsid:zoobank.org:act:868316B5-0ED5-4A21-AE3A-0488D98E418B
(Figs. 2–6)

Centrolene heloderma Yánez-Muñoz et al. (2015)
Centrolene sp. 2. Bejarano-Muñoz et al. (2019)

Comentado [DAO5]: Does not appear in references

Proposed Spanish common name. Rana de Cristal de Campos
Proposed English common name: Campos' Glassfrog

Holotype. DHMECN 11407 (field number 3566), adult male (Fig. 2–3) from La Enramada (3.1628ºS; 79.5886ºW, 2950 m), provincia de Azuay, República del Ecuador, collected by J. Sánchez-Nivicela on 31 March 2015.

Comentado [DAO6]: Azuay province, Ecuador

Paratype. DHMECN 11408, adult male, same data as holotype.

Diagnosis. *Centrolene camposi* sp. nov. is distinguished from all other Centrolenidae by the following combination of characters: (1) dentigerous process of vomer absent; (2) snout rounded to subacuminate in dorsal view, sloping in lateral view; (3) tympanic annulus barely visible, lower ¾ visible, tympanic membrane coloured as dorsal skin, supratympanic fold present and low; (4) dorsal skin shagreen with dispersed low and rounded warts, and microspicules and spicules present (at least in males); (5) ventral skin granular, subcloacal area enamelled, strongly

Comentado [DAO7]: I suggest not using after description granular with two large subcloacal warts and with enamelled cloacal sheath; (6) parietal peritoneum white, iridophores covering 2/3 the parietal peritoneum; pericardium covered by iridophores, all other visceral peritonea clear (condition V1); (7) liver lobed (five lobes) and hepatic peritoneum clear (lacking iridophore layer, condition H0); (8) adult males with projecting humeral spine; (9) basal webbing between fingers I and II, moderate webbing between fingers II and IV, II (2^{-}–2)–3^{+} III 2½–2^{+} IV; (10) toe webbing I (1^{-}–1½)–(2–2^{+}) II (0^{+}–1^{-})–(2½–2^{+}) III (1^{+}–1½)–2½ IV 2½–1½ V; (11) low, enamelled metacarpal fold continuing with elevated, thick, enamelled ulnar fold; elevated, low, enamelled metatarsal and tarsal fold; low tarsal fringe on inner tarsus; (12) nuptial excrescences type I; concealed prepollex; (13) Finger I shorter than Finger II; (14) diameter of eye larger than width of disc on Finger III; (15) colour in life, bright green dorsum, thick yellowish-white labial stripe continuing into a faint yellowish line between lip and anterior ¼ of body, yellowish green flanks, hidden surfaces of limbs and digits, enamelled metacarpal, ulnar, metatarsal and tarsal folds, bones green; (16) colour in preservative, lavender dorsum with translucent spicules, enamelled labial stripe continuing into a faint enamelled line between lip and anterior ¼ of body, faint enamelled metacarpal, ulnar, metatarsal and tarsal folds; (17) iris coloration in life, white background, fleshed coloured towards the centre, fine brown reticulations; (18) melanophores present on dorsal surfaces of hands and feet and at the base of Finger IV, Toe IV, and Toe V; (19) males call from upper side of leaves; advertisement call unknown; (20) fighting behaviour unknown; (21) egg masses and parental care unknown; (22) tadpoles undescribed; (23) snout-vent length in adult males 29.1–31.2 mm (n=2), females unknown.

Comparisons. *Centrolene camposi* sp. nov. differs from all other glassfrogs, except *C. altitudinale*, *C. buckleyi, C. heloderma, C. hesperia*, *C. lemniscata*, and *C. venezuelense* by having a combination of the following characters: absence of dentigerous process of vomer, sloping snout in lateral view, light labial stripe, humeral spine in adult males, parietal peritoneum covered by iridophores, visceral peritonea translucent (except pericardium), ulnar and tarsal ornamentation, green bones. *Centrolene altitudinale* differs from *C. camposi* sp. nov. by having (characters of *C. camposi* sp. nov. in parentheses) truncate snout in dorsal view (rounded to subacuminate), tympanic annulus ½ visible (tympanic annulus barely visible), green dorsum with white dorsal spots in life (uniform green dorsum with light green warts); row of small, non-connected, enamelled tubercles on outer borders of hand, ulna, and tarsus (enamelled folds). *Centrolene buckleyi* and *C. venezuelense* differ by having ulnar and tarsal folds low or absent (elevated and thick ulnar and tarsal folds). *Centrolene heloderma* differs by having pustular dorsal skin (shagreen with dispersed warts), tympanic annulus completely visible (tympanic annulus barely visible), grey lavender dorsum in preservative (lavender), outer tarsal fold with low white tubercles (enamelled fold without tubercles), and humeral spine distinctly projected from arm (humeral spine curved towards arm). *Centrolene hesperia* differs by having weakly truncate snout in dorsal view (rounded to subacuminate), less hand webbing, II 2^{+}–3½ III 3^{-}–2½ IV (II (2^{-}–2)–3^{+} III 2½–2^{+} IV). *Centrolene lemniscata* differs by having round snout in dorsal and lateral views (sloping in lateral view), arms and legs lacking dermal folds (present), and a white lateral stripe extending from arm insertion to groin. The second new species of *Centrolene* described in this work differs from *C. camposi* sp. nov. (characters of the later in parenthesis) by its round snout in lateral view (sloping), thin yellowish labial stripe (thick, white labial stripe), row of white tubercles between lip and arm insertion (white tubercles absent), yellowish line between arm insertion and groin (faint white line between lip and anterior ¼ of body), warts and spicules on dorsum with same colour as surrounding dorsal surfaces (warts and spicules on dorsum lighter than surrounding dorsal surfaces). *Centrolene condor*, sister species of *C. camposi* sp. nov., differs by having a green dorsum with abundant yellowish–white flecks and abundant dark flecks (bright uniformly green dorsum); iris cream–yellow with fine dark reticulation (white background, fleshed coloured towards the centre, fine brown reticulations); and vomerine teeth present (absent). Molecular analyses clearly differentiate *C. camposi* sp. nov. from morphologically similar species found in the Andes.

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Description of the holotype. Adult male, moderate-sized, SVL = 29.1 mm (Figs. 2–5). Head distinct, wider than long, and wider than body; HW/HL = 1.10, HW/SVL = 0.38, HL/SVL = 0.35. Snout short, EN/HL = 0.24; nostrils slightly elevated, producing a shallow depression in the internarial area, loreal region concave; canthus rostralis rounded; lips flared. Small-size eyes, ED/HL = 0.31, directed anterolaterally at about 50º from midline, interorbital area wider than eye diameter, IOD/ED = 1.71, EN/ED = 0.77, EN/IOD = 0.58. Tympanic annulus oriented dorsolaterally, weak supratympanic fold above upper portion of tympanum and extending down to shoulder. Dentigerous processes of vomers absent; choanae rounded, large; tongue rounded, indented posteriorly; vocal slits present, extending from anterior base of tongue to angles of jaws.

Skin of dorsal surfaces of head, body and limbs shagreen with dispersed low warts, some warts are non-clustered translucent spicules, and the skin is covered by non-clustered microspicules, infratympanic area with spicules. Skin of ventral surfaces of body granular, on throat, chest, and limbs fairly granular. Cloacal opening directed posteriorly at upper level of thighs, distinct enamelled cloacal sheath; subcloacal area enamelled and granular, with a pair of large, round, flat subcloacal warts on ventral surfaces of thighs below vent.

Upper arm thin, forearm moderately robust. Humeral spine present and externally visible, but not piercing the skin. Relative lengths of fingers III > IV > II > I; finger discs wider than the adjacent phalanx, nearly truncate; disc on third finger about the same size than those on toes, ED/Fin3DW = 1.48; subarticular tubercles rounded and elevated, supernumerary tubercles present; palmar tubercle large, rounded, elevated; thenar tubercle elliptic. Concealed prepollex, unpigmented nuptial excrescences present, Type I on dorsolateral side of thumbs.

Hind limbs slender; TL/SVL = 0.54, FL/SVL = 0.49. Inner metatarsal tubercle large and elliptical; outer metatarsal tubercle indistinct. Subarticular tubercles rounded and low, supernumerary tubercles small, rather indistinct. Toe discs bluntly truncate, no papillae on tip of disc of toes.

Colouration of holotype in life. (Fig. 2–3) Bright green dorsal colouration, with some warts slightly lighter green; thick, yellowish-white labial stripe continuing into a faint yellowish line between lip and anterior ¼ of body; yellowish-green flanks and hidden surfaces of limbs; enamelled metacarpal, ulnar, metatarsal, and tarsal folds; yellowish white venter. Iris with grey background, fleshed coloured towards the centre, fine brown reticulations. Discs orange to red in Fingers II, III and IV on the left hand, Fingers II and IV on the right hand, and Toe V on both feet. Yellowish green webbing between fingers and toes. Bones green.

Colouration of holotype in ethanol. (Figs. 4–5) Lavender dorsum with translucent spicules; enamelled labial stripe continuing into a faint enamelled line between lip and anterior ¼ of body; flanks lighter lavender than dorsal surfaces; faint enamelled metacarpal, ulnar, metatarsal, and tarsal folds; venter cream. Melanophores present on dorsal surfaces of hands and feet and at the base of Finger IV, Toe IV, and Toe V. Parietal peritoneum white, iridophores covering 2/3 the parietal peritoneum; pericardium covered by iridophores, all other visceral peritonea clear.

Measurements in mm: Measurements of the holotype are followed by those of the paratype in parentheses: SVL = 29.1 (31.2), HL = 10.1 (11.1), HW = 11.1 (11.5), IOD = 5.3 (5.7), ED = 3.1 (3.4), IND = 2.7 (2.9), EN = 2.4 (2.4), TD = 1.0 (1.2), TL = 15.8 (17.0), FL = 14.3 (14.8), HAL = 11.1 (11.6), Fin3DW = 2.1 (2.2).

Variation. Morphologically the paratype is very similar to the holotype, except for its snout subacuminate in dorsal view. Morphometric variation is reported in the previous section. The paratype shows dorsal warts lighter green than the holotype, almost looking like bright yellowish green dots, and has some dorsal dark flecks. Enamelled line on anterior ¼ of body thinner than in the holotype.

Etymology. The specific name of this new taxon is a patronymic in honour of Felipe Campos-Yánez, a distinguished Ecuadorian zoologist, free thinker, and passionate conservationist. His biological collections are deposited in the main museums of the country, and he has left a legacy of contributions to biodiversity conservation throughout his 30 years of professional career.

Distribution and Natural History. *Centrolene camposi* sp. nov. is known only from its type locality in the province of El Oro, on the southwestern slopes of the Andes (on Cordillera Occidental) of Ecuador (Fig. 7). Inhabits montane evergreen forests at 2900 m elevation. This ecosystem is characterized by an architecture of vegetation, with trees greater than 15 m in height and densely loaded with epiphytes, such as bromeliads, mosses, and orchids. Both individuals of *C. camposi* sp. nov. were found together with *C. ericsmithi* sp. nov., in a steep creek in the area. *Centrolene camposi* sp. nov. is also sympatric with *Pristimantis allpapuyu* Yánez-Muñoz, Sánchez-Nivicela & Reyes-Puig, 2016, four undescribed species of *Pristimantis* and one *Gastrotheca*.

Centrolene ericsmithi sp. nov.

LSID urn:lsid:zoobank.org:act:BA14CDBB-9BEB-4245-889F-ADB975775E74
(Figs. 2–6)

Centrolene heloderma Yánez-Muñoz et al. (2015)
Centrolene sp. 1. Bejarano-Muñoz et al. (2019)

Comentado [DAO9]: Does not appear in references

Proposed Spanish common name: Rana de Cristal de Smith
Proposed English common name: Smith's Glassfrog

Holotype. DHMECN 11406 (field number 3546), adult male (Fig. 2–3) from La Enramada (3.1628°S; 79.5886ºW, 2950 m), provincia de Azuay, República del Ecuador, collected by J. Sánchez-Nivicela on 31 March 2015.

Comentado [DAO10]: Azuay province, Ecuador

Diagnosis. *Centrolene ericsmithi* sp. nov. is distinguished from all other Centrolenidae by the following combination of characters: (1) dentigerous process of vomer absent; (2) snout round in dorsal and lateral views; (3) tympanic annulus barely visible, lower ¾ visible, tympanic membrane coloured as dorsal skin, supratympanic fold present and low; (4) dorsal skin shagreen with dispersed spicules, and covered by microspicules; (5) ventral skin granular, subcloacal area enamelled, strongly granular with two slightly larger subcloacal warts and enamelled cloacal sheath; (6) parietal peritoneum white, iridophores covering ½ parietal peritoneum; pericardium covered by iridophores, all other visceral peritonea clear (condition V1); (7) liver lobed and hepatic peritoneum clear (lacking iridophore layer, condition H0); (8) adult males with projecting humeral spine; (9) basal webbing between fingers I and III, moderate webbing between fingers III and IV, III 2⅔ –2½ IV; (10) toe webbing I 2^{-}–2^{+} II 1^{-}–2⅓ III 2^{-}–2½ IV 2½–2^{-} V; (11) enamelled metacarpal area without fold continuing with low, slightly elevated, enamelled ulnar fold; low, enamelled metatarsal and tarsal fold; low tarsal fringe on inner tarsus; (12) nuptial excrescences type I; concealed prepollex; (13) Finger I shorter than Finger II; (14) diameter of eye larger than width of disc on Finger III; (15) colour in life, bright green dorsum, thin yellowish labial stripe continuing with a row of white tubercles towards arm insertion, yellowish line between arm insertion and groin, enamelled metacarpal area, enamelled ulnar, metatarsal and tarsal fold, bones green; (16) colour in preservative, lavender dorsum with translucent spicules, enamelled labial stripe, enamelled line between arm insertion and groin;

Comentado [DAO11]: I suggest not using sp. nov. after description

(17) iris coloration in life, flesh colour background, fine brown reticulations; (18) few melanophores present on dorsal surfaces of hands and feet and at the base of Finger IV, Toe IV, and Toe V; (19) males call from upper side of leaves; advertisement call unknown; (20) fighting behaviour unknown; (21) egg masses and parental care unknown; (22) tadpoles undescribed; (23) snout-vent length (SVL) in adult male 27.3 mm (n=1), females unknown.

Comparisons. *Centrolene ericsmithi* sp. nov. differs from all other glassfrogs, except from *C. altitudinale*, *C. buckleyi, C. heloderma, C. hesperia*, *C. lemniscata*, *C. sabini*, and *C. venezuelense* by having a combination of the following characters: absence of dentigerous process of vomer, light labial stripe, uniform green dorsum, humeral spine in adult males, parietal peritoneum covered by iridophores, visceral peritonea translucent (except pericardium), ulnar and tarsal ornamentation, green bones. *Centrolene altitudinale* differs from *C. ericsmithi* sp. nov. by having (characters of *C. ericsmithi* sp. nov. in parentheses) truncate snout in dorsal view (rounded), tympanic annulus ½ visible (tympanic annulus barely visible), dorsum shagreen with small spicules (shagreen with large spicules), green dorsum with white dorsal spots in life (uniform green dorsum); row of small, non-connected, enamelled tubercles on outer borders of ulna and tarsus (enamelled folds). *Centrolene buckleyi* and *C. venezuelense* differ by having sloping snout (round), supratympanic fold moderately heavy (low), outer tarsal fold absent (present); iris with a horizontal brown stripe (brown stripe absent). *Centrolene camposi* sp. nov. differs by having sloping snout in lateral view (round), thick, white labial stripe (thin yellowish labial stripe), absence of row of white tubercles between lip and arm insertion (present), faint white line between lip and anterior ¼ of body (yellowish line between arm insertion and groin), warts and spicules on dorsum lighter than surrounding dorsal surfaces (warts and spicules on dorsum with same colour as surrounding dorsal surfaces). *Centrolene heloderma* differs by having pustular dorsal skin (shagreen with dispersed spicules), tympanic annulus completely visible (tympanic annulus barely evident), grey lavender dorsum in preservative (lavender); outer tarsal fold with low white tubercles (enamelled fold without tubercles), and humeral spine distinctly projected from arm (humeral spine curved towards arm). *Centrolene hesperia* differs by having weakly truncate snout in dorsal view (rounded) and white labial stripe continuous with stripe along the flanks to the groin (labial stripe separate from body line by a row of tubercles). *Centrolene lemniscata* differs by arms and legs lacking dermal folds (present) and white labial stripe continues along the body to the groin (labial stripe separate from body line by a row of tubercles). *Centrolene sabini* differs by having sloping snout in lateral view (round), dorsum green with yellowish-green spots and patches (uniformly green), white labial stripe continuous with stripe along the flanks (labial stripe separate from body line by a row of tubercles), and strongly protruding nostrils (not strongly protruding). *Centrolene lynchi* differs by having snout truncate to sloping in lateral view (round), dorsal skin shagreen in males and females, males with low, white warts, and spicules and spiculated warts on sides of head (dorsal skin shagreen with dispersed spicules); dorsum dull green with minute yellowish–white warts and small diffuse black spots (green dorsum), tarsal fold absent (present), nuptial pad Type II (Type I), and humeral spine distinctly projected from arm (humeral spine curved towards arm). Molecular analyses clearly differentiate *C. ericsmithi* sp. nov. from morphologically similar species found in the Andes.

Description of the holotype. Adult male, moderate-sized, SVL = 27.3 mm (Fig. 2–5). Head slightly distinct, wider than long, and wider than body; HW/HL = 1.06, HW/SVL = 0.33, HL/SVL = 0.31. Snout short, EN/HL = 0.21; nostrils slightly elevated, producing a shallow depression in the internarial area, loreal region concave; canthus rostralis rounded; lips not flared. Small-size eyes, ED/HL = 0.33, directed anterolaterally at about 50º from midline, interorbital area wider than eye diameter, IOD/ED = 1.43, EN/ED = 0.64, EN/IOD = 0.70. Tympanic annulus oriented dorsolaterally, weak supratympanic fold above upper portion of tympanum and extending down to shoulder. Dentigerous processes of vomers absent; choanae squarish, large; tongue rounded, indented posteriorly; vocal slits present, extending from anterior base of tongue to angles of jaws.

Skin of dorsal surfaces of head, body and limbs shagreen, covered by non-clustered translucent spicules, spicules more concentrated on body surfaces, infratympanic area with few, slightly enlarged spicules. Dorsal surfaces with non-clustered microspicules. Skin of ventral surfaces of body granular, on throat, chest, and limbs fairly shagreen. Cloacal opening directed posteriorly at upper level of thighs, distinct enamelled cloacal sheath; subcloacal area enamelled and granular, with a pair of slightly large, round, flat subcloacal warts on ventral surfaces of thighs below vent.

Upper arm thin, forearm slightly robust. Humeral spine present and externally visible, but not piercing skin. Relative lengths of fingers III > IV > II > I; finger discs wider than the adjacent phalanx, truncate; disc on third finger about the same size than those on toes, ED/Fing3DW = 1.87; subarticular tubercles rounded and elevated, supernumerary tubercles present, small, and flat; palmar tubercle large, rounded, elevated; thenar tubercle elliptic. Concealed prepollex, unpigmented nuptial excrescences present, Type I on dorsolateral side of thumbs.

Hind limbs slender; TL/SVL = 0.55, FL/SVL = 0.47. Inner metatarsal tubercle large and elliptical; outer metatarsal tubercle indistinct. Subarticular tubercles rounded and low, supernumerary tubercles small and flat, rather indistinct. Toe discs bluntly truncate, no papillae on tip of disc of toes.

Colouration of holotype in life. (Fig. 2–3) Bright, uniform green dorsum, thin yellowish labial stripe continuing with a row of white tubercles towards arm insertion, yellowish line between arm insertion and groin, enamelled metacarpal area, enamelled ulnar, metatarsal, and tarsal fold, yellowish white venter. Iris flesh colour background, fine brown reticulations. Fingers, toes, and membrane yellowish green. Bones green

Colouration of holotype in ethanol. (Figs. 4–5) Lavender dorsum with translucent spicules, enamelled labial stripe, enamelled line between arm insertion to groin. Faint enamelled metacarpal area, faint enamelled ulnar, metatarsal, and tarsal fold. Few melanophores present on dorsal surfaces of hands and feet and at the base of Finger IV, Toe IV, and Toe V. Parietal peritoneum white, iridophores covering ½ parietal peritoneum; pericardium covered by iridophores, all other visceral peritonea clear.

Measurements of the holotype: SVL = 27.3, HL = 8.5, HW = 9.0, IOD = 4.0, ED = 2.8, IND = 2.5, EN = 1.8, TD = 0.9, TL = 15.1, FL = 12.8, HAL = 9.4, Fin3DW = 1.5.

Etymology. The specific name of this new taxon is a patronymic in honour of Eric Nelson Smith, U.S. herpetologist and curator of the amphibian and reptile collections at The University of Texas at Arlington. A collector prodigy that has been describing 50 new species from the most remote corners of the planet for almost three decades. This is a small recognition of his extensive career.

Comentado [DAO12]: This sentence is confusing

Distribution and Natural History. *Centrolene ericsmithi* sp. nov. is currently known only from its type locality in the province of El Oro, on the southwestern slopes of the Andes (on Cordillera Occidental) of Ecuador (Fig. 7). *Centrolene ericsmithi* sp. nov. and *C. camposi* sp. nov. are syntopic. Both new species vocalized during the samplings carried out in March 2015. Other relevant information is described in the section corresponding to *C. camposi*.

Discussion

Due to their sympatry, with overlapping microhabitat occupancy, Yánez-Muñoz et al. (2015) initially assumed that the three specimens of *Centrolene* from La Enramada, province of Azuay, belonged to the same species and were phylogenetically close to *C. heloderma*. However, the phylogenetic reconstruction showed that they were two different, syntopic, not closely related lineages, *C. camposi* sp. nov. and *C. ericsmithi* sp. nov.

The two new *Centrolene* described herein inhabit the montane evergreen forests on the south-western Andes of Ecuador. *Centrolene camposi* shows an unusual biogeographic pattern because it is sister to a species from the opposite versant of the Andes (Fig. 7), and both species are separated by relatively low genetic distances (1.08% for gene 12S), suggesting a recent divergence. The Andes are a formidable dispersal barrier for amphibians which results in almost entirely different amphibian communities on opposite versants of the Andes of Ecuador, despite having ecologically similar forests. In Centrolenidae, only two other sister species occur on opposite versants of the Andes, *T. amelie* (Cisneros-Heredia & Meza-Ramos, 2007) + *Teratohyla pulverata* (Peters, 1873) and *Cochranella granulosa* (Taylor, 1949) + *C. resplendens* (Lynch & Duellman, 1973) (Guayasamin et al., 2020). *Teratohyla amelie* and *T. pulverata* diverged 15 My ago, suggesting vicariant speciation as a result of the Andean uplift. *Cochranella granulosa* + *C.*

resplendens are also old lineages, diverging over 7 My ago (Guayasamin et al. 2020). In contrast, *C. camposi* + *C. condor* likely diverged much more recently because their genetic distances are at the lower end for species pairs within Centrolenidae. This unusual biogeographic pattern suggests a unique combination of topography and environmental history in the Andes of southern Ecuador. The pattern of southwestern Andean clades in Ecuador and Peru having a closer relationship with eastern Andean clades should be much more common in species with high dispersal ability like spiders (e.g., *Gasteracantha cancriformis* [Linnaeus, 1758], Salgado-Roa et al., 2022) and birds (e.g., *Pachyramphus* spp., Musher & Cracraft, 2018).

Centrolene condor is endemic to the Cordillera del Condor, a sub-Andean mountain range running parallel to the southeastern Andes of Ecuador, about 140 km W from the type locality of *C. camposi* (Fig. 7) (Cisneros-Heredia & Morales-Mite, 2008; Almendáriz & Batallas, 2012; Guayasamin et al., 2020). *Centrolene sabini* is only known from the Kosñipata valley, on the southeastern Andes of Peru, more than 1400 km south of the type locality of *C. ericsmithi*. (Catenazzi et al., 2012; Catenazzi, 2017). The undescribed *Centrolene* sp. [Ca1] was collected at the province of Zamora-Chinchipe, on the southeastern Andes of Ecuador, about 120 km W from the type locality of *C. ericsmithi* (Amador et al., 2018). The undescribed *Centrole*ne sp. [Ca1] is more closely related to the geographically distant *C. sabini* than to the geographically close *C. ericsmithi* sp. nov., a relationship that counters the usual isolation by distance pattern of genetic differentiation among populations of a single species. Lack of consistency with isolation by distance suggests the existence of reproductive barriers between the three populations (i.e., the populations belonging to three species).

Conclusions

We provide congruent lines of evidence supporting the diagnosis and description of two new species of *Centrolene* from the southwestern high Andes of Ecuador. The diversification and adaptation of a high diversity of vertebrates in southwestern Ecuador, specifically in the province of Azuay and El Oro, is apparently due to the topographic complexity of the area, with the presence of the Jubones River basin and the Gulf of Guayaquil, the Andes and its proximity to the Pacific coast, and the biogeographic influence of different climatic zones (INABIO, 2015; Arteaga et al., 2016, 2017, 2018; Yánez-Muñoz, Sánchez-Nivicela & Reyes-Puig, 2016; Torres-Carvajal et al., 2020; Brito et al., 2022). Evidence accumulated in several clades of small vertebrates suggests that the River Jubones basin might be an important isolation barrier between lineages north and south of the Andes of Ecuador, including frogs of the genera *Pristimantis*, *Elachistochleis* and *Hyloscirtus*, lizards *Anadia* and *Enyalioides*, and snakes *Atractus*, *Dipsas* and *Leptodeira* (Torres-Carvajal, 2007; Cisneros-Heredia & Yánez-Muñoz, 2007b; Passos et al., 2012; INABIO, 2015; Arteaga et al., 2016, 2018; Yánez-Muñoz, Sánchez-Nivicela & Reyes-Puig, 2016; Betancourt et al., 2018; Sánchez-Nivicela et al., 2019, 2020; Guayasamin et al., 2020; Torres-Carvajal et al., 2020).

Comentado [DAO13]: In results or discussion, nothing is mentioned about this topic

The Andes of southern Ecuador show high geologic, geographic and environmental heterogeneity (Gentry, 1982; Duque-Caro, 1990; Veblen, Young & Orme, 2015; Morrone, 2017). The combination of these factors has fostered the evolution of a complex and rich biological diversity, with several local hotspots concentrating high levels of endemism (Chapman, 1917, 1926; Gentry, 1982; Cracraft, 1985; Duellman, 1988; Dodson & Gentry, 1991; Morrone, 2014, 2015, 2017). Unfortunately, its biodiversity remains poorly studied and heavily threatened by unceasing habitat loss, degradation, and fragmentation due to legal and illegal logging, expansion of the agricultural frontier, and mining activities (MAE, 2012, 2015; MAE et al., 2013; Sierra, 2013). The remnants of native montane forests in the province of El Oro are restricted and scarce. Even though we have carried out extensive surveying of amphibians in the region, no more individuals or localities of the new species have been reported, so we consider that both species should be assigned to the IUCN Red List category Data Deficient at the national and global levels (Ortega-Andrade et al., 2021). The discovery of these new species of anurans in small Andean remnants evidences the need to carry out urgent conservation actions, to avoid the collapse of these ecosystems in southwestern Ecuador (INABIO, 2015; Ortega-Andrade et al., 2021).

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