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ON THE IDENTITY OF HOG-NOSED PIT-VIPERS FROM WESTERN PANAMA: A REVIEW OF SPECIMENS OF *PORTHIDIUM LANSBERGII* (SCHLEGEL, 1841) IN LOWER CENTRAL AMERICA.

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ABSTRACT

Porthidium lansbergii is a relatively abundant pit viper in semiarid environments from eastern Panama, through the Caribbean plains and Magdalena Valley in Colombia to northeastern Venezuela. Like other members of this genus, *P. lansbergii* exhibits great variation in scutellation and other morphological characters, a situation that has complicated the taxonomy and identification of populations assigned to the species. In Lower Central America, *P. lansbergii* is known to inhabit seasonally-dry environments of the Pacific slopes of western Panama. Here, we aimed to clarify the taxonomic status of some enigmatic specimens collected in western Panama, with morphological

ambiguity, resembling *P. lansbergii*. We used the mitochondrial marker cytochrome oxidase b to perform a Bayesian phylogenetic analysis, to evaluate the affinities ofthese specimens with *P. lansbergii* individuals from central and eastern Panama, and Venezuela. We also analyzed meristic morphological characters to discriminate among populations of *P. lansbergii* in Panama, including other species of the *Porthidium* genus. Our analyses indicate that the enigmatic individuals do not differ from others individuals identified as *P. lansbergii*, thus forming a monophyletic group. We conclude that *P. lansbergii* has a more extended range than currently recognized including the western Pacific in Panama.

KEY WORDS

Porthidium lansbergii, Viperidae, morphometric characters, mitochondrial DNA, distribution, taxonomy.

SOBRE LA IDENTIDAD DE LAS VÍBORAS DE NARIZ DE CERDO DEL OESTE DE PANAMÁ: UNA REVISIÓN DE ESPECÍMENES DE *PORTHIDIUM LANSBERGII* (SCHLEGEL, 1841) EN BAJA CENTROAMERICA

RESUMEN

Porthidium lansbergii es una víbora relativamente abundante en ambientes semiáridos. Se distribuye desde el oeste panameño, hacia las llanuras del Caribe y el Valle de Magdalena en Colombia, hasta el noreste de Venezuela. Similar a otras especies del género, *P. lansbergii*, exhibe gran variación en su lepidosis y otros caracteres morfológicos. Esta situación taxonómica crea escenarios complejos para la identificación de ejemplares de esta especie. En Centro América, *P. lansbergii* es conocida por habitar ambientes estacionales, mayormente secos, en el este y sur de Panamá. Sin embargo, este trabajo procura aclarar el estado taxonómico de algunos especímenes enigmáticos colectados en el oeste de Panamá, con ambigüedad morfológica y que se asemejan a *P. lansbergii*. Utilizamos el marcador mitocondrial citocromo oxidasa b para evaluar las afinidades de estos especímenes con individuos de *P. lansbergii* de centro y este de Panamá, así como de Venezuela, mediante análisis filogenético Bayesiano. Realizamos un análisis de caracteres morfométricos para discriminar entre poblaciones de *P. lansbergii* en Panamá, incluyendo otras especies del género. Nuestros resultados sugieren que los individuos enigmáticos no difieren de *P. lansbergii*, formando un grupo monofilético. Concluimos que la distribución de *P. lansbergii* incluye tierras del oeste pacifico panameño y es más amplia de lo que se reconoce actualmente.

PALABRAS CLAVES
Porthidium lansbergii, Viperidae, caracteres morfométricos, ADN mitocondrial, distribución, taxonomía

INTRODUCTION

The genus *Porthidium* was described by Edward D. Cope (1871) to assign the nominal species *P. nasutum* and *P. lansbergii*, recognizing several anatomical features that separate them from other species then included in *Bothrops* (Bocourt, 1868) and *Trigonocephalus* (Schlegel 1841), respectively. As currently accepted, *Porthidium* includes nine small to medium sized pit-viper species that exhibit cryptic coloration and are often referred to as the *hog-nosed pit-vipers* due to their characteristic high rostral scale that tends to elevate –slightly or greatly– the tip of the snout (Campbell and Lamar, 2004; Köhler, 2003). Snakes in this genus are distributed at low to mid elevations in mesic and dry regions throughout most of Mesoamerica south to northwestern South America (Campbell and Lamar, 2004), comprising a monophyletic clade that has close affinities with the genera *Cerrophidion* and *Metlapilcoatlus* (Castoe *et al*., 2005; Campbell *et al*., 2019).

Most species within *Porthidium* are relatively abundant within their distributions, but the extent of variation in morphological and color pattern traits –and the great overlap of meristic characters among nominal species– have complicated proper identification throughout their range. Furthermore, early researchers created taxonomic confusion by including closely related *Porthidium* species into their description of nominal species (see Campbell and Lamar, 2004 for a review). Thus, it is not surprising that comprehensive morphological and/or molecular analyses of wide ranged species have revealed the existence of distinct populations that represent cryptic species, following their own evolutionary trajectory. For instance, *P. porrasi*, a hog-nosed pit-viper that inhabits lowland mesic forest in the southern Pacific of Costa Rica (Lamar and Sasa, 2003), was previously recognized as a distinct population of *P. nasutum* (Porras *et al*., 1981; Savage, 2002).

In the case of *P. lansbergii* (type-locality: Turbaco, Colombia), the

problem has been even more complicated. For this species, several subspecies have been previously proposed, and it was suspected that *P. lansbergii* may be polytypic (Campbell and Lamar 1989, 2004). The distributional range of *P. lansbergii* includes the Caribbean plains and the Magdalena Valley in northern Colombia eastward to northern and central Venezuela, including the Maracaibo basin, ranging in elevation from sea level to at least 1270 m (Campbell and Lamar, 2004). The species also occurs in coastal plains of the Pacific from eastern (Darien region) to central Panama (Fig 1.). Along its range, *P. lansbergii* occurs mainly in seasonally dry environments, although it also inhabits humid forests of eastern Panama and Colombia (Campbell and Lamar, 2004).

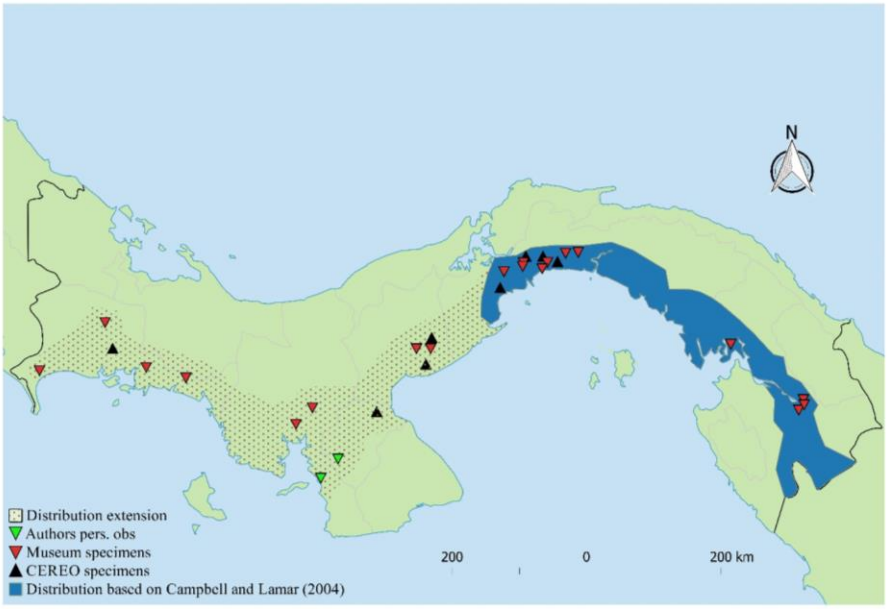

Fig. 1 Distribution of *P. lansbergii* in Panama. Map of Panama showing the distribution based on Campbell and Lamar (2004) in blue; specimens from the CEREO collection in black triangles, from museums in red triangles, and observed by authors in green triangles. Point patterns represents the extended distribution for *P. lansbergii* presented herein

Although *P. lansbergii* has been extensively collected in central and eastern Panama, its status in western Panama remains unclear. A few

museum records account for collection localities scattered throughout the Pacific coast on the occidental provinces of Chiriqui, Veraguas, and Cocle. For instance, H. Clark recorded an individual in 1936 from Puerto Armuelles, in Chiriqui Province (MCZ:Herp:R-42731, Fig. 2), which represents a range extension over 350 km from known localities in central Panama. Moreover, several enigmatic individuals, with ambiguous morphology, were collected at some localities in the western Pacific of Panama (Fig. 3). These specimens were housed at the snake collection in the 'Centro para Investigaciones y Respuestas en Ofidiología (CEREO), Facultad de Ciencias Naturales, Exactas y Tecnologia' at the University of Panama. Based only in the folidosis, these specimens were, tentatively, identified as *P*. cf. *lansbergii* by Martinez, V. However, the atypical coloration patterns observed (V. Martinez, pers. obs.) in these individuals made their taxonomy confusing (Fig. 3). Therefore, we aimed to clarify these enigmatic individuals' identification. We compared morphometric characters, sequenced mitochondrial DNA fragment *cytochrome oxidase b* and conducted an intensive literature search. We formally assigned them to *P. lansbergii*, thus providing strong evidence to support that its distributional range should be extended to the western Pacific slope of Panama.

Fig. 2 Specimen collected by H. Clark in Puerto Armuelles, Chiriqui (western Panama), with museum record MCZ:Herp:R-42731

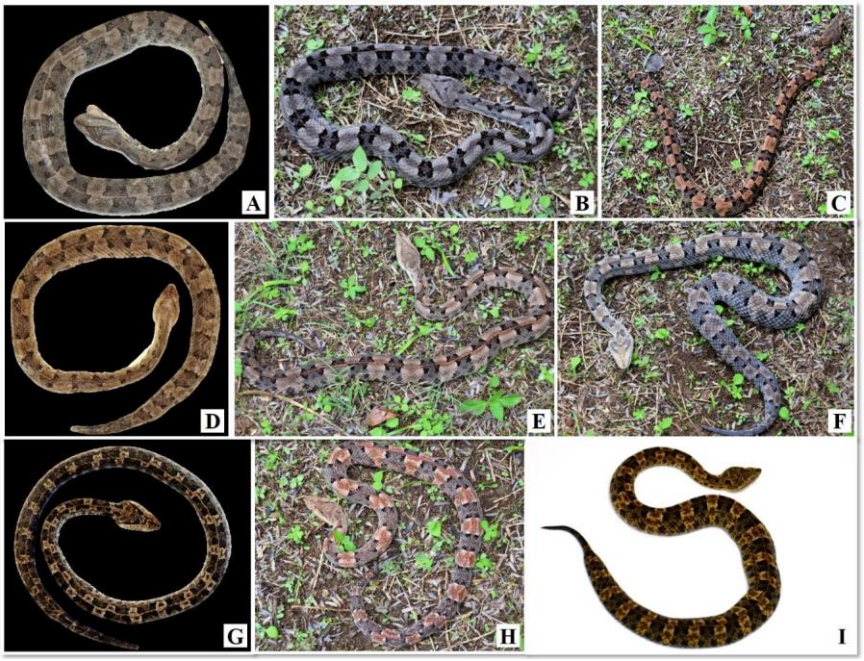

Fig. 3 *P. lansbergii* specimens collected from central Panama (figs. 3B, C, D, G, H) and western Panama (figs. 3A, E, F, I)

METHODS

We assessed the morphological traits that discriminate between *Porthidium lansbergii* from Panama and related species within the genus. We performed morphological analysis on 9 enigmatic *P.* cf *lansbergii* individuals held at the CEREO and compared them to 114 *Porthidium* specimens kept in the following institutions: California Academy of Science (CAS), Field Museum of Natural History, Chicago, IL (FMNH), Florida Museum of Natural History (FLMNH), University of Kansas Natural History Museum and Biodiversity Center (KU), Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), Museo de Zoologia, Universidad de Costa Rica (MZUCR), and the National Museum of Natural History (NMNH).

In total, we included 4 *P. lansbergii* specimens from eastern Panama, 15

from central Panama, and 9 *P*. cf *lansbergii* from western Panama (see Table 1 for specimens' localities); 39 *P*. *ophryomegas*, 3 *P*. *volcanicum*, 3 *P*. *porrasi*, and 50 *P*. *nasutum* (see Table 2 for meristic data). For each specimen, we identified sex and counted the number of body blotches, as well as the number of the following scales: dorsal (at head, mid-body, and anal region), ventral, caudal, supralabial, infralabial, canthal and internasal. We also measured total body length and tail length (to the nearest 0.1cm). Sexual dimorphism in meristic characters was evaluated using parametric mean comparisons. We applied Principal Component Analysis (PCA) to the correlation matrix to determine the existence of patterns in scale meristic characters among populations of all *Porthidium* species included, as well as among specimens of *P.* cf *lansbergii* and *P. lansbergii* to distinguish subpopulations. We implemented all statistical analyses in JMP Pro 13® (SAS Institute Inc, 1989-2019).

We evaluated phylogenetic relationships between P. cf. lansbergii and other members of the group. We collected blood samples from 7 *P. cf. lansbergii* individuals located at the CEREO, and extracted DNA using the DNeasy® Blood & Tissue kits following the manufacturer's protocol (QIAGEN, 2006). We used the primers Gludg (5'-TGACTTGAA**R**AACCA **Y**CGTTG-3') and Atrcb3 (5'-TGAGAAGTTTTC **Y**GGGTC**R**T T-3') (Palumbi, 1996). We adjusted the protocol described by Chippindale et al (2000) and amplified the subunit *cyt-b* in a Perkin-Elmer 2400 thermocycler under the following conditions: 1 minute at 95°C and 15 seconds at 94°C, one minute at 54°C, and one minute at 72°C with a final extension of 7 minutes at 72°C. We purified the PCR products using MinElute reaction cleanup kit (QIAGEN) and then sequenced using BigDye® technology (PE Applied Biosystems) in an ABI PRISM® 310 sequencer. All *Porthidium lansbergii* sequences were deposited in GenBank with the following accession number: MN597002 (CEREO109), MN597003 (CEREO115), MN597004 (CEREO116), MN597005 (CEREO118), MN597006 (CEREO120), MN597007 (CEREO213), MN597008 (CEREO235). The phylogenetic analysis was performed under Metropolis-Hastings coupled Markov Chain Monte Carlo Bayesian methods (BMCMC) using the program MEGAS 5.10 and results were

visualized with TreeView. In addition to our Panamanian samples (sequences for *P. lansbergii* specimens are available per request to the corresponding author), we included the following *Porthidium* sequences as ingroups or near outgroups (GenBank® accession numbers): *P. lansbergii* (DQ061206, central Panama) and (AY223582, Venezuela); *P. arcosae* (AF292575); *P. dunni* (DQ061218); *P. nasutum* (AY223579); *P. ophryomegas* (DQ061241, plus two individuals from Costa Rica housed at Instituto Clodomiro Picado); *P. porrasi* (DQ061211); and *P. yucatanicum* (DQ061215). In addition, sequences from *Metlapilcoatlus nummifer* (=*mexicanus*; AY223584), *Atropoides. picadoi* (DQ061197), *Cerrophidion godmani* (AY223578), *C. petlalcalensis* (DQ061202.1), and *C. tzotzilorum* (DQ061204) were assigned as outgroups.

Table 1. Information of collection for P. lansbergii specimens from Panamá

Voucher	Region	Locality	Year	Collector
CEREO-120	Central	Las Pavas, Arraijan, Panama Oeste province	NA	NA
CEREO-213	Central	Tocumen, Panama province	NA	NA
CAS-21061	Central	Canal Zone, vic. Fort Clayton, Panama province	1958	"H.M. Smith"
CAS-21062	Central	Canal Zone, vic. Fort Clayton, Panama province	1958	"H.M. Smith"
MVZ-78766	Central	Chepo (9.16667, -79.1), Panama province	1964	N. Gale
FLMNH-39818	Central	NA	1968	Sam R. Telford
FLMNH-74277	Central	Canal Zone, Panama province	1967	D. Bruce Means
FMNH-Chi-68051	Central	NA	NA	NA
CEREO-112	Central	San Juan de Pequeni, Pacora, Panama province	NA	NA
CEREO-116	Central	NA	NA	NA
CEREO-118	Central	El Palmar, Panama province	NA	NA
CAS-98362	Central	Tocumen Airport (8.18333, -77.68333), Panama province	1964	"T.J. Papenfuss"
FLMNH-39819	Central	NA	1968	Sam R. Telford
KU-112588	Central	Juan Diaz, Panama province	1961	NA
KU-112576	Central	28 km ENE Panama City, Rio Pacora, Panama province	1965	NA
KU-112579	Eastern	Rio Tuira at Rio Mono, Darien province	1965	NA
NMNH-140676	Eastern	El Real de Santa Maria, Darien province	1958	J. Hardy
MVZ-83442	Eastern	Yaviza, Darien province	1967	C. M. Cavalier
KU-112578	Eastern	3 km E El Real, Rio Tuira, Darien province	1965	NA
CEREO-109	Western	Santiago, Veraguas province	NA	NA
CEREO-115	Western	Puerto Frio, Penonome, Cocle province	NA	NA
CEREO-235	Western	Valle de Anton, Cocle province	NA	NA
CEREO-329	Western	Las Lomas, Boquete, Chiriqui province	NA	NA
NMNH-129876	Western	Mojarras, Veraguas province	1951	M. Stirling
NMNH-297735	Western	Cerro Colorado, Escopeta Camp, ca. 23 km NE of San Felix, Chiriqui province	1969-1980.	A. Forero
FLMNH-127697	Western	NA	1970	Nicholas Nell
KU-112580	Western	5 km NW San Lorenzo, Chiriqui province	1966	NA

Table 2. Data for meristic characters for the five Porthidium species included in this study. Species 1= *P. nasutum*, 2= *P. ophryomegas*, 3= *P. lansbergii*, 4= *P. volcanicum*, 5= *P. porrasi*; (1) ventral scales, (2) caudal scales, (3) head dorsal scales, (4), mid-dorsal scale (5) anal dorsal scale row, (6) supralabial scales, (7) infralabial scales, (8) canthal scale, (9) internasal scale, (10) gender (0= female, 1= male), (11) body blotches, (12) total body length (cm), and (13) tail length

Museum Catalog	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
MZUCR-109	1	143	28	25	23	19	10	12	1	6	1	30	51.5	4.9
MZUCR-110	1	139	27	25	23	19	10	12	1	5	1	21	58.1	6
MZUCR-162	1	138	28	23	23	19	10	12	1	5	1	22	44.2	4.5
MZUCR-1870	1	130	27	23	23	19	9	11	1	6	1	18	26	3.6
MZUCR-2721	1	136	31	23	23	19	8	10	1	6	1	28	34.9	5.5
MZUCR-2739	1	139	34	23	23	23	9	11	1	4	1	25	31.4	5
MZUCR-2941	1	144	32	25	23	19	10	12	1	6	1	25	56.5	6.5
MZUCR-4647	1	139	28	23	23	19	9	11	1	5	1	24	21.6	1.8
MZUCR-5743	1	145	27	25	23	19	10	12	1	4	1	24	41.7	4
MZUCR-5998	1	144	30	27	25	21	10	12	1	5	1	18	25.5	3.2
MZUCR-6767	1	137	28	25	23	19	10	12	1	5	1	21	34.1	4.5
MZUCR-7238	1	139	27	23	23	19	10	12	1	5	1	22	48	5.6
MZUCR-10230	1	138	27	25	23	21	9	11	1	5	1	26	30.5	4.2
MZUCR-10304	1	143	31	23	23	21	9	11	1	5	1	34	32.5	4.8
MZUCR-10305	1	127	27	25	25	21	10	12	1	5	1	27	64.6	6.6
MZUCR-10362	1	131	27	25	25	21	10	12	1	5	1	19	25.1	3
MZUCR-10413	1	132	26	25	25	19	9	11	1	5	1	22	34.4	4.1
MZUCR-11150	1	135	28	23	23	19	10	12	1	5	1	27	49.8	5.7
MZUCR-12612	1	144	27	25	23	19	10	12	1	5	1	25	53	5.9
MZUCR-12809	1	141	23	27	25	21	11	13	1	5	1	20	58.4	5.5
MZUCR-13800	1	143	27	25	23	19	10	12	1	5	1	25	48.3	4.6
MZUCR-13887	1	141	27	25	23	19	9	11	1	5	1	31	61.5	6.1
MZUCR-14058	1	141	26	25	23	19	9	11	1	6	1	31	38	4.3
MZUCR-14131	1	148	30	25	23	21	10	12	1	6	1	22	60.8	6.5
MZUCR-15328	1	134	27	23	23	19	9	11	1	4	1	21	46.5	6
MZUCR-15329	1	142	29	23	23	19	10	12	1	6	1	18	58.6	6.6
MZUCR-15330	1	139	26	25	23	19	10	12	1	5	1	21	58.5	5.6
MZUCR-15331	1	142	28	25	23	19	10	12	1	6	1	23	62	6.1
MZUCR-15332	1	140	29	23	23	19	10	12	1	5	1	23	52.3	5.2
MZUCR-16540	1	143	27	23	23	19	10	12	1	5	1	25	61.5	7
MZUCR-16803	1	142	33	23	23	19	10	12	1	6	1	29	35	4.3
MZUCR-17025	1	139	27	25	23	19	11	13	1	5	1	24	46.2	5.4
MZUCR-19212	1	144	27	23	23	19	10	12	1	6	1	20	53.3	5.3
MZUCR-19821	1	141	28	25	23	19	10	12	1	6	1	20	71.3	8.1
MZUCR-20780	1	136	25	23	23	19	9	11	1	6	1	22	22.3	2.9
MZUCR-41	1	141	28	25	23	21	9	12	1	5	2	32	44.4	4.8
MZUCR-308	1	137	33	25	23	19	9	11	1	5	2	26	33.4	5

Continue Table 2.

Museum Catalog	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
MZUCR-1431	1	135	31	25	23	19	9	11	1	5	2	19	33.6	5.5
MZUCR-2722	1	136	29	25	25	21	10	12	1	5	2	19	35.1	4.5
MZUCR-2738	1	137	29	23	23	19	9	11	1	5	2	25	33.8	5.1
MZUCR-2940	1	140	29	25	23	19	9	11	1	5	2	23	33.4	3.5
MZUCR-2990	1	138	34	23	23	19	9	11	1	6	2	27	39.5	6
MZUCR-7584	1	137	31	23	23	19	10	12	1	5	2	23	19	2.2
MZUCR-8552	1	138	27	25	23	19	9	11	1	5	2	28	27	3.5
MZUCR-10965	1	138	31	25	25	21	10	12	1	3	2	23	51	5.5
MZUCR-12743	1	135	32	25	23	21	10	10	1	6	2	22	37.2	5.8
MZUCR-15326	1	139	32	25	23	19	9	11	1	5	2	22	40.1	5.8
MZUCR-15327	1	136	33	25	23	19	9	11	1	5	2	21	44.5	6.5
MZUCR-17122	1	134	28	23	23	19	10	10	1	5	2	24	28.1	4.4
MZUCR-17370	1	142	30	23	23	19	9	11	1	5	2	21	39.8	5.9
MZUCR-3411	2	168	35	25	25	19	9	11	2	6	1	28	33.8	4.6
MZUCR-7188	2	168	39	27	27	21	11	13	2	6	1	28	62.6	8.1
MZUCR-7761	2	163	34	25	25	19	9	11	2	7	1	28	49.8	5.7
MZUCR-12357	2	160	40	25	25	19	10	12	2	5	1	35	36.7	5.5
MZUCR-13920	2	168	33	25	25	19	11	13	2	7	1	30	17.5	2.5
MZUCR-15286	2	176	37	21	27	19	10	12	2	6	1	28	22	4
MZUCR-15289	2	174	33	25	25	19	10	12	2	5	1	28	66.1	8
MZUCR-15290	2	172	34	27	27	19	10	12	2	6	1	28	78.8	9.2
MZUCR-15291	2	173	27	25	25	19	11	11	2	6	1	30	67.4	8
MZUCR-15292	2	165	35	25	25	19	10	11	2	6	1	29	73.3	9.1
MZUCR-15293	2	175	34	27	27	19	11	12	2	7	1	28	76.1	9.1
MZUCR-15295	2	167	41	25	25	19	10	12	2	6	1	28	22	3.5
MZUCR-15297	2	160	29	23	25	19	9	11	2	5	1	29	22.4	3.2
MZUCR-15299	2	181	36	25	27	19	11	13	2	6	1	30	22.2	2.4
MZUCR-15303	2	176	35	25	25	19	10	10	2	6	1	30	87.2	9.6
MZUCR-18016	2	154	38	25	25	19	9	11	2	5	1	38	42	6
MZUCR-18018	2	165	38	25	25	19	10	12	2	6	1	34	75.2	8.9
MZUCR-19060	2	164	35	23	25	19	9	11	2	7	1	34	36.1	5.3
MZUCR-180	2	160	26	25	25	19	10	12	2	5	2	34	22.5	3.1
MZUCR-1034	2	160	38	27	25	21	10	12	2	6	2	36	33.5	5
MZUCR-6998	2	163	39	25	23	19	10	12	2	6	2	48	47.2	6.7
MZUCR-6999	2	167	42	25	25	19	11	11	2	5	2	42	48.2	7
MZUCR-7762	2	166	33	25	25	19	9	11	2	5	2	33	18.4	3.5
MZUCR-8065	2	166	39	25	25	19	11	13	2	6	2	43	52.7	8.8
MZUCR-8592	2	164	41	25	25	19	10	12	2	6	2	34	59.2	9
MZUCR-13838	2	162	38	25	25	19	10	12	2	6	2	38	33.6	5.2
MZUCR-13919	2	161	35	25	25	19	10	10	2	5	2	33	17.7	3.5
MZUCR-14108	2	163	39	25	25	19	9	11	2	5	2	32	58	8.5
MZUCR-14609	2	164	40	25	25	19	10	12	2	5	2	48	59	9
MZUCR-15287	2	162	39	23	25	19	9	11	2	5	2	36	64.6	9

Continue Table 2.

Museum Catalog	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
MZUCR-15288	2	170	39	25	25	19	10	12	2	5	2	39	65.2	9.1
MZUCR-15294	2	166	39	25	23	19	10	12	2	6	2	39	68	9.5
MZUCR-15296	2	168	44	25	25	19	10	12	2	6	2	31	23	3.2
MZUCR-15298	2	153	35	25	25	19	10	12	2	7	1	24	26.3	3.5
MZUCR-15300	2	145	28	23	25	19	10	10	2	4	2	32	21.6	3.8
MZUCR-15301	2	167	42	25	25	19	10	12	2	6	2	35	75.2	10.1
MZUCR-15302	2	166	37	21	23	19	9	11	2	6	2	27	62.5	8.6
MZUCR-15476	2	166	37	25	25	19	10	10	2	7	2	33	70.9	7.6
MZUCR-20838	2	161	35	25	25	19	9	11	2	5	2	33	45.5	6.6
CEREO-109	3	153	26	25	25	19	9	11	1	5	1	18	54	5
CEREO-115	3	162	32	25	25	19	11	13	1	5	1	19	73	7
CEREO-120	3	155	28	25	25	19	9	11	1	5	1	19	62	5
CEREO-213	3	156	23	23	23	19	10	12	1	5	1	23	54	4.2
CEREO-235	3	164	32	23	23	19	9	11	1	5	1	21	52	5.5
CEREO-329	3	154	29	23	25	19	9	11	1	5	1	26	62	5.3
NMNH-129876	3	165	33	25	25	19	9	11	1	5	1	17	50.9	4.8
NMNH-297735	3	161	32	25	27	21	10	14	1	6	1	20	21.5	2.2
CAS-21061	3	154	29	27	27	21	10	12	1	6	1	20	55.8	5.4
CAS-21062	3	155	31	27	25	19	10	12	1	5	1	16	35.8	3.2
MVZ-78766	3	153	33	25	25	19	9	11	1	5	1	18	39.2	4.8
FLMNH-39818	3	156	29	27	27	21	9	12	1	5	1	18	60.3	5.1
FLMNH-127697	3	159	31	27	25	19	9	12	1	5	1	22	55.6	4.7
FLMNH-74277	3	154	29	25	25	19	10	12	1	3	1	17	24.9	2.1
KU-112579	3	159	30	25	25	21	11	14	1	7	1	18	23.2	1.6
KU-112580	3	162	33	25	25	19	9	11	1	5	1	24	46.3	4.7
FMNH-Chi-68051	3	152	32	25	25	19	10	12	1	6	1	17	33.4	3.3
CEREO-112	3	147	26	23	23	19	9	11	1	5	2	27	45.7	4.5
CEREO-116	3	154	29	25	27	19	10	12	1	5	2	21	51	5.5
CEREO-118	3	158	31	25	25	21	10	12	1	5	2	18	57	5.5
NMNH-140676	3	154	34	25	25	19	9	12	1	5	2	18	45.9	3.6
CAS-98362	3	153	34	25	25	19	9	10	1	6	2	19	25.1	2.5
MVZ-83442	3	157	34	23	25	19	9	11	1	5	2	15	31.4	3.3
FLMNH-39819	3	155	34	23	23	19	9	11	1	5	2	18	42.3	4.5
KU-112588	3	149	31	25	25	19	9	11	1	5	2	17	41.6	4.1
KU-112578	3	158	34	27	25	17	9	12	1	6	2	18	48.9	4.2
KU-112576	3	153	34	25	25	19	9	10	1	4	2	19	38.4	3.9
MZUCR-11643	4	159	31	27	25	19	10	12	1	8	1	27	44.2	4.2
MZUCR-11644	4	161	19	27	23	19	9	11	1	6	1	24	45.4	3.4
MZUCR-11642	4	157	35	27	25	21	9	11	1	7	2	28	25.5	2.6
MZUCR-3310	5	144	27	25	25	21	10	12	1	5	1	19	29	3.6
MZUCR-3359	5	142	30	23	23	19	9	11	1	5	2	22	37.3	3.9
MZUCR-11479	5	140	31	25	23	21	10	12	1	7	2	21	20.1	2.1

RESULTS

Porthidium lansbergii and *Porthidium* cf *lansbergii* from Panama show great variation in meristic characters (Table 2). Briefly, ventral scales ranged, including both sexes, from 147 to 165 and caudal scales from 23 to 34. The mean (±S.E.) number of ventral scales is higher in females (157.3 ± 1.0) than males (153.8 ± 1.1), and the differences are statistically significant (*Student t* = 2.3, *df* = 25, *p* = 0.03). Conversely, males tend to have a slightly higher mean number of subcaudals (32.1 ± 0.8) than females (30.1 ± 0.6), but the differences were marginally significant (*Student t* = 1.8, *df* = 25, *p* = 0.08). Males are slightly smaller (mean: 42.7 ± 2.9 cm) than females (mean: 47.2 ± 3.6 cm), with no significant differences in their total length ($F_{1,22}$ = 0.99, *p* = 0.33). Overall, body size did not differ among *P. lansbergii* individuals ($F_{2,22}$ = 2.30, *p* = 0.12).

Dorsal patterns of *P. lansbergii* consist mostly of pairs of rectangular blotches that are mostly arranged alternately, at each side of the vertebral line. However, in a few individuals, blotches are placed opposite to each other. Coloration pattern and body hue vary slightly among individuals (Fig. 3), but not clear differences in coloration were observed among individuals from eastern, central, or western Panama. The number of body blotches ranged from 15 to 34 (Table 2), but no differences were observed in this variable between sexes ($F_{1,22}$ = 0.09, *p* = 0.76), or among regions ($F_{2,22}$ = 0.96, *p* = 0.39). Intersupraoculars, canthals, and the number of dorsal scale rows showed the lowest variation in our *P. lansbergii* sample.

A PCA performed over meristic characters allowed partial discrimination among some hog-nosed pit-viper species (Fig. 4A). The first component accounts for 77.9% of the variance and mainly differentiated three groups: *P. ophryomegas*, *P. nasutum/P. porrasi*, and *P. lansbergii/P. volcanicum*. This component is correlated with ventral scale count (r = 0.92), and less strongly with number of subcaudals (r = 0.24), as well as the number of blotches (r = 0.28). The second component is accounts for 15.9% of the variation and is mainly explained by the number of blotches (r = 0.91), separating *P. lansbergii* from other species. No discrimination between *P nasutum* and *P. porrasi*

was possible from this data set; and *P. volcanicum* shares characteristics with *P. ophrymegas* and *P. lansbergii* (Fig. 4A). Our PCA results for *P. lansbergii* from Panama (Fig. 4B) accounted for 77% of the total variance with the first two components, suggesting a relatively high morphological overlapping among specimens from the three regions studied.

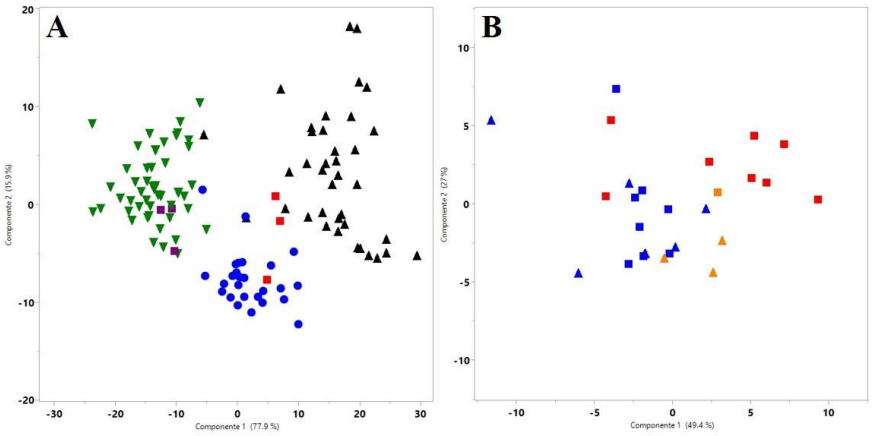

Fig. 4 Principal Component Analysis with Manhattan distance for all species in this study (A) (dark blue= *P. lansbergii*; green= *P. nasutum*; purple= *P. porrasi*; black= *P. ophryomegas*; and red = *P. volcanicum*) and (B) only for P. lansbergii specimens from Panama (Females=triangle, Males=squares; red=western, blue=central, orange=eastern Panama)

Our phylogenetic analyses recovered a partial phylogeny of *Porthidium* that is consistent with previous reconstructions of hog-nosed pit-viper phylogenies (Fig. 5). Briefly, an early divergence produces two main clusters, one including *P. dunni* and *P. ophryomegas*, and the other includes *P. yucatanicum* as the sister taxon of a clade including *P. nasutum*/*P. porrasi* and *P. lansbergii*. Further, *P. lansbergii* comprises a monophyletic group that includes both western, central and eastern Panama specimens, as well as the individual from Venezuela. Therefore, the enigmatic western Panama specimens are identified as members of this species.

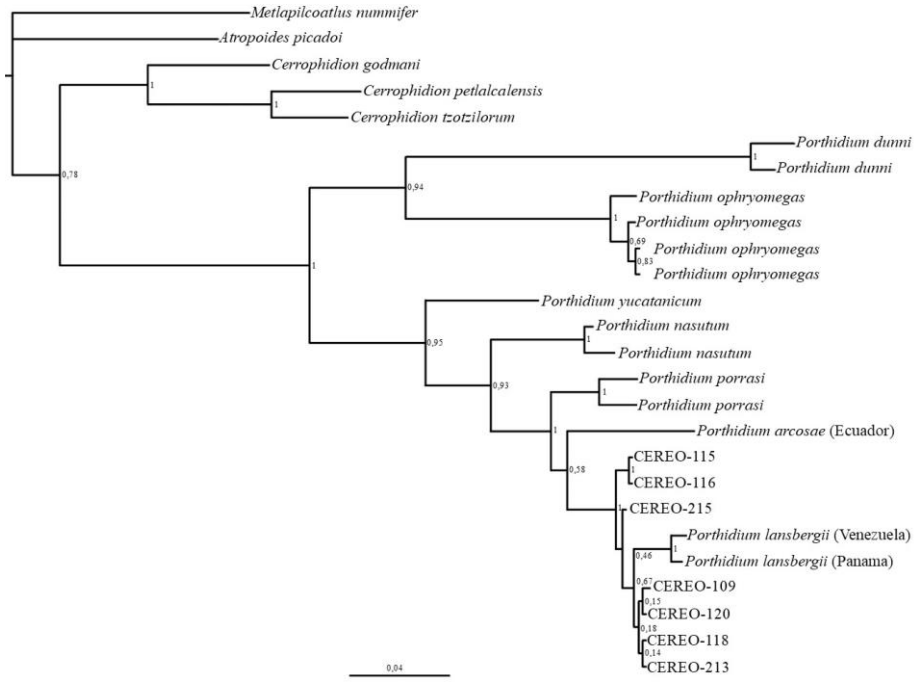

Fig. 5 Phylogram Phylogenetic tree constructed based on Bayesian inference

DISCUSSION AND CONCLUSIONS

Campbell and Lamar (1989, 2004), and Kohler (2003) provided several records of *P. lansbergii* in Panama as part of their claim that it was restricted to the central and eastern half of the country. Ray and Knight (2013) considered the distribution of *P. lansbergii* throughout the Pacific slope of Panama, but localities or records were not given. Our extensive literature search recovered records, omitted in previous reviews, of this species from the western Pacific slope of Panama. For instance, Dunn (1949) mentioned specimens described as *Bothrops* (=*Porthidium*) *lansbergii* collected in localities within the Cocle-Herrera region. Recently, the species was reported by Martínez-Cortés et al (2005) in 'El Montuoso' Forest Reserve in the Herrera province and by Monteza-Moreno, C. M. (pers. obs., 2017) in the locality of Mariato, Veraguas Province. Furthermore, museum records and new collections of

individuals show that this species inhabit in the provinces of Chiriqui and Veraguas. The distribution of the nominal *P. lansbergii* in Lower Central America extends to the western Pacific of Panama (Fig. 1). Thus, since our records lie near the border of Costa Rica, the species could potentially occur there.

Despite putative differences in coloration patterns, we cannot distinguish enigmatic individuals from those identified as *P. lansbergii* from central and eastern Panama based in scutellation. In addition, our phylogenetic analysis clearly supports the monophyly of *P. lansbergii* from Panama, and does not provide any evidence for the existence of multiple lineages within the nominal species in this country. However, our dataset does not include specimens from the entire distribution range of *P. lansbergii* and the fact that the species shifts its distribution from the Pacific slopes in Panama to the Atlantic in Colombia up to Venezuela is interesting and warrants further scrutiny.

Two other members of *Porthidium* are also know to occur in Panama: *P*. *nasutum,* and *P*. *volcanicum.* The first one inhabits the humid environments along the Caribbean lowlands and piedmonts, crossing to the Pacific side in Darien; therefore, it is allopatric to *P. lansbergii* populations. *Porthidium*. *volcanicum,* a species ostensibly endemic to a small area in Costa Rica, was reported in Boquete, Chiriquí, in western Panama (Dwyer and Van den Burgh, 2012). It is likely that this species can be sympatric with *P. lansbergii*; however, further studies are required to depict distribution ranges for both species.

Our data showed that ventral scale counts and number of blotches are sufficient to distinguish among hog-nosed pit-vipers' species. Differences in ventral scales, and subtle color variation, were used to distinguish three subspecies of *Porthidium lansbergii* (Roze, 1959; Peters, 1968; Sandner-Montilla, 1989; Fuentes and Rodriguez 1997). However, our data suggest a great variation in meristic characters, including a higher ventral scale range than considered in these studies. Additional studies are highly recommended for a comprehensive understanding of the variability in morphological traits across the species geographic distribution. Moreover, since the species occurs in

seasonally-dry and humid environments along its distribution, it is possible that variation in scutellation characters results from environmentally driven effects, as it has been observed in other wide ranged snakes. For instance, Saldarriaga-Córdoba et al. (2009) found that geographic variation of dorsal blotches, ventral scales and other meristic characters in *Bothrops asper* correlates to external factors, such as latitude, the number of dry months, and precipitation. *P. lansbergii*, as well as *P. nasutum* which also shifts its distribution from the Atlantic into the Pacific in eastern Panama down to Ecuador, provides an ideal opportunity to understand morphometric responses to environmental variables.

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