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A new species of frog (Strabomantidae: Pristimantis) from Peru with comments on its ectoparasites (Acari: Trombiculidae)

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by

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ABSTRACT

In South America, frogs of the genus *Pristimantis* are diverse and can be found from lowland forests to elevations of about 4000 m in the Andes. The 444 known species of *Pristimantis* (AmphibiaWeb 2012) belong to 16 species groups (Frost 2011, Hedges *et al.* 2008). One of these groups is the *Pristimantis orestes* Group, the 14 members of which inhabit the páramo, puna, and upper montane forests in southern Ecuador (3 species) and Peru (11 species; Duellman and Lehr 2009). Species of the *Pristimantis orestes* Group are characterized by having snout-vent lengths ranging from 18.0 to 29.4 mm, short robust bodies, relatively short snouts, narrow digital discs, and areolate ventral skin. Some species have variously colored pale spots in the groin. Herein, I describe a new, diminutive species of *Pristimantis* from the Andes of northern Peru that I assign to the *Pristimantis orestes* Group. The new species, denoted *Prsitimantis* sp. 1, has a snout-vent length of 17.35–29.08 mm (n = 47) in adult females, and 14.39–22.97 mm (n = 40) in adult males, and it differs from all other members of the *Pristimantis orestes* Group in having prominent scapular tubercles. Ectoparasitic mites (Trombiculidae) of the new species were studied to determine any relation between the degree of infestations and body regions, size, sex, and age. No relationships were found among sexes or ages of frogs. Larger females were 3.85 times more likely to be infested than small females, but no difference was seen between different sized males. The throat had significantly more mites than other body regions and the legs had significantly fewer mites than other regions. Mites were examined using scanning electron microscopy and their morphology was compared to drawings of a previously described mite. The mite on *Pristimantis* sp. 1 was not *Hannimania* sp., the genus commonly reported to infest frogs.

INTRODUCTION

In Peru, there are 492 known anuran species, 166 of which belong to the family Strabomantidae (AmphibiaWeb 2012). Biodiversity in Peru results from the complex physiography of the country. Three physiographic regions characterize Peru—the narrow Pacific coast, the Andes Mountains, and the Amazon Basin. Varying ecoregions and climates within

these physiographic divisions have created observable distribution barriers, which are responsible for the high rates of endemism and diversity in Peru (Tuomisto *et al.* 1995). In many of these regions, adequate scientific exploration is lacking, especially in the Andes, which are difficult to access. Consequently, exploration of these areas can result in the discovery of new species, often endemic to the locations in which they are identified. One of these unexplored areas is the Río Abiseo National Park (Figure 1), which is situated in the Cordillera Oriental of the Andes Mountains in northern Peru and encompasses the Abiseo river basin and the surrounding environments, including montane and puna regions, ranging in elevation from 500 to 4200 m (McGinley 2008). Montane forests extend to elevations of 3000–3400 m and are characterized by copious rainfall (1000–3000 mm annually), elfin trees, mosses, lichens, and ferns. Puna ecoregions occur at altitudes above upper montane regions. Bunch grasses and scattered trees dominate in northeastern Peruvian puna zones (Duellman and Lehr 2009). A. Catenazzi and R. von May collected a large series of *Pristimantis* in the Río Abiseo National Park in 1999. This material was loaned to Dr. Edgar Lehr who recognized two new species in the material (*Pristimantis* sp. 1 and *Pristimantis* sp. 2); Cindy Gregory and Alan Brus separated these species.

The common lifecycle of frogs includes an aquatic, free-living larval stage, the tadpole, which hatches from aquatic eggs; however, members of the family Strabomantidae are terrestrial breeders. Females of this group lay eggs in moist terrestrial environments (e.g. under moss or in the leaf litter). From these eggs, hatch minute, juvenile frogs, which do not pass through a free-living larval stage, a process known as direct development. The family Strabomantidae contains 18 genera (AmphibiaWeb 2012), including *Pristimantis*. Frogs of the genus *Pristimantis* were formerly classified within the genus *Eleutherodactylus*. In South America, this genus of frog is common to the lowland forests to elevations of about 4000 m in the Andes (Duellman and Lehr 2009). The 440 currently known species of *Pristimantis* belong to 16 species groups (Frost 2011, Hedges *et al.* 2008). One of the groups is the *Pristimantis orestes* Group which includes 14 members (Duellman and Lehr 2009) that inhabit páramo, puna, and upper montane forests in southern Ecuador (3 species) and Peru (11 species).

The *Pristimantis orestes* Group is an informal taxonomic grouping of frogs. The grouping is based on morphological similarities of the representatives, and while a close phylogenetic relationship is assumed, the members do not constitute a subgenus. The

Prisimantis orestes Group is unlikely to be a monophyletic grouping due to differing sequence data from mitochondrial and nuclear DNA (Hedges *et al.* 2008). Species of the *Pristimantis orestes* Group are characterized by having snout-vent lengths ranging from 18.0 to 29.4 mm, short robust bodies, relatively short snouts, narrowly expanded digital discs, and areolate ventral skin. Some species have variously colored pale spots on the groin (Appendix 1).

Anuran species are infrequently reported with serious infestations. In North and South America and Africa, flat worms of the family Trematoda have been reported in larval and adult stages of frogs (Johnson *et al.* 2001a; Johnson *et al.* 2001b; Johnson *et al.* 1999); however, in North and South America, mites of the family Trombiculidae are most commonly reported on frogs (Duszynski *et al.* 1973; Hatano *et al.* 2007; Jung *et al.* 2001; Malone & Paredes-León 2005; Rey 1992; Wohltmann *et al.* 2006). These reports all focus on ectoparasitic mites in the genus *Hannemania.* Trombiculid mites, commonly known as chiggers, are parasites of vertebrates and are classified by a parasitic larval stage (Hatano *et al.* 2007; Malone and Paredes-León 2005; Rey 1992; Ruppert *et al.* 2004; Robert *et al.* 2000; Wohltmann *et al.* 2006). Larval mites remain in epidermal capsules for a few weeks feeding on the frog cellular material. The capsules form after the mite attaches the skin when mite salivary secretions stimulate the proliferation of the epidermal and connective tissues. These tissues eventually envelop the larval mite (Ruppert *et al.* 2004; Robert *et al.* 2000). Prior to metamorphosis, the capsules rupture and the mites emerge (Hatano *et al.* 2007; Ruppert *et al.* 2004; Robert *et al.* 2000). Adult stages of these mites are predaceous and free living in the soil (Ruppert *et al.* 2004; Robert *et al.* 2000). In the wild, the mechanism of host identification employed by the mite is unknown (Hatano *et al.* 2007).

The primary goal of this study is to describe a new, diminutive species, a member of the *Pristimantis orestes* Group, from the Río Abiseo National Park. Second, to provide data on trombiculid mites parasitizing the skin of these frogs. I characterized the infestations and the morphology of the extracted mites. The number of mite capsules were counted and recorded based on the sex, body size, and body region of the frog. Incidences of mite capsules per mm^2 were used in order to compensate for the differences in surface area among the various body regions; the present study is the first to incorporate these differences. I provide statistical results of the above relationships and discuss the degree of parasitism in the frogs and the variation in parasitism as related to gender, body size, and body region of the new species.

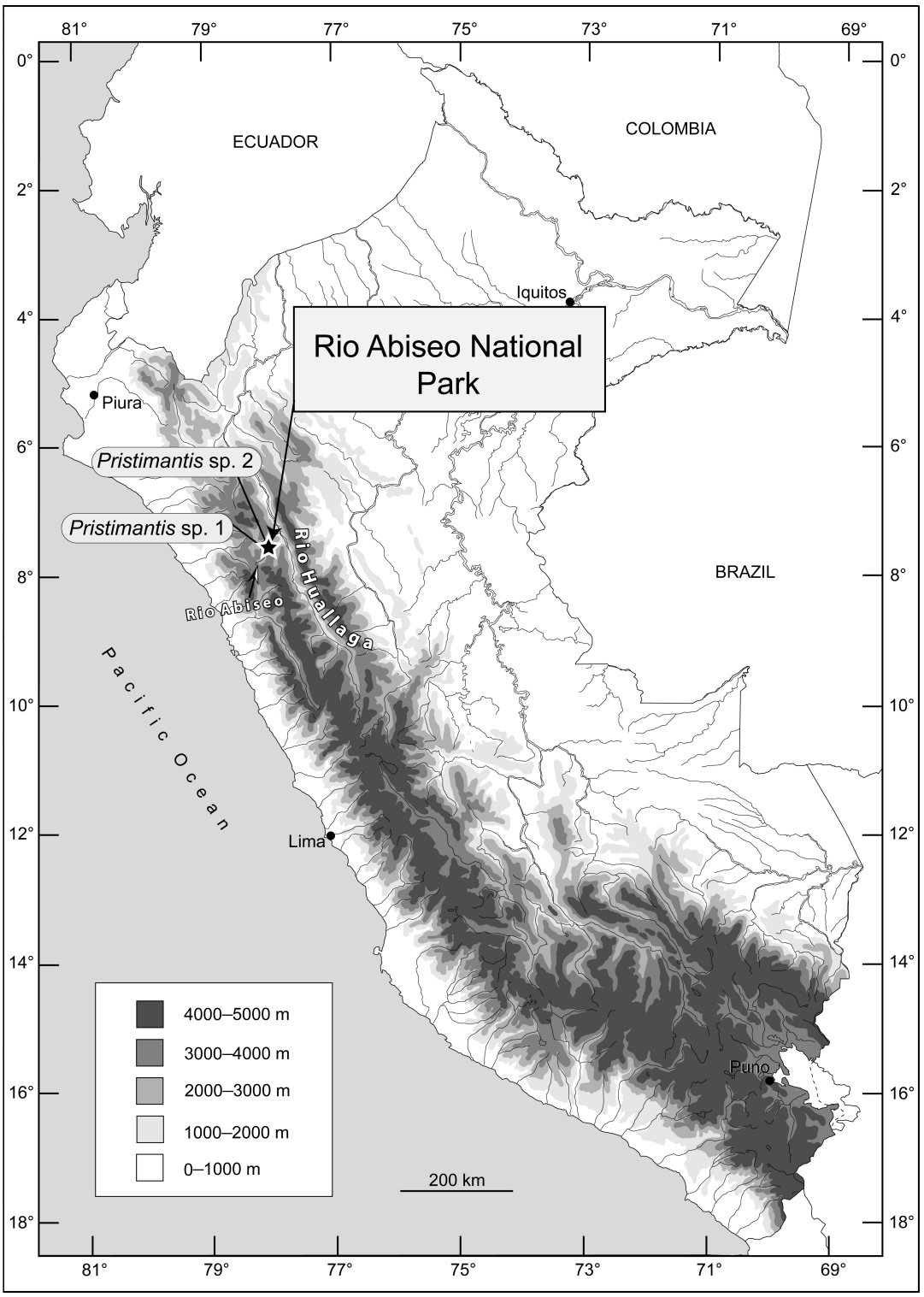

Figure 1. A map of Peru showing the location of the Río Abiseo National Park (star) where two new species of *Pristimantis* were collected. The species described herein is indicated as *Pristimantis* sp. 1. Map created by E. Lehr.

MATERIALS AND METHODS

Collection Site

The Río Abiseo National Park (Figure 1), situated in the Cordillera Oriental of the Andes Mountains in northern Peru, was founded in 1983 in an effort to protect the characteristic flora and fauna of the Andes. The park is a major site of Andean conservation research because of the high degree of endemism in the region and has been closed to the public since 1986 (Lehr and Catenazzi 2011). Peruvian herpetologists A. Catenazzi and R. von May collected a large series of strabomantid frogs (n = 101) of the genus *Pristimantis* from the puna and montane ecoregions in the Río Abiseo National Park in 1999. Their material, containing two new species (*Pristimantis* sp. 1 and *Pristimantis* sp. 2), was stored in 70% ethanol and deposited in the herpetological collection of the Museo de Historia Natural Universidad Nacional Mayor de San Marcos (Lima, Peru, MHNSM and MHNJP). The material was loaned to Dr. Edgar Lehr and the senior author used one of the species in the present study.

Characters and Measurements of Pristimantis sp. 1

Format of the description follows Lynch and Duellman (1997), and diagnostic characters are taken from Duellman and Lehr (2009). Adult specimens were sexed externally by the presence or absence of vocal slits and internally by the condition of the gonads. Juvenile (SVL 10.29–18.55 mm) frogs lack sexual markers and were not sexed (n = 47 females, n = 40 males, and n = 14 juveniles). Measurements of the collection, or series, of frogs were taken with a digital caliper (Appendix A) using a stereomicroscope to the nearest 0.01 mm, and are as follows: snout–vent length (SVL); tibia length (TL); foot length (FL), from the proximal margin of inner metatarsal tubercle to tip of Toe IV; head length (HL), obliquely from angle of jaw to tip of snout; head width (HW), at level of the angle of jaw; eye diameter (ED); interorbital distance (IOD); upper eyelid width (EW); internarial distance (IND); eye–nostril distance (E–N), the straight line distance between anterior corner of orbit and posterior margin of external nares and tympanic membrane diameter (TY). Fingers are numbered preaxially to postaxially from I–IV. Toes are numbered preaxially to postaxially from I–V. Comparative lengths of Toes III and V were determined when both were adpressed against Toe IV, and lengths of Fingers I and II were compared when pressed against each other. Holotype is a single, well preserved frog selected as the frog that best exhibits the characteristics of the new species. Drawings were complete using a stereomicroscope with a camera lucida attachment. Description of the coloration in life is

based on photos taken by A. Catenazzi. *Pristimantis* sp. 1 was compared to members of the *Pristimantis orestes* Group; including *P. corrugatus*, which I assign as most similar to the new species based on morphological characters. Variation of the series was described based on major trends within the collection.

Analysis of the Mite Infestation

The infestations of *Pristimantis* sp. 1 with ectoparasites (Trombiculid mites) were recorded based on domed distortions of epithelium observed using a stereomicroscope. Data on the infestation were collected using both closed and ruptured capsules (Figure 2).

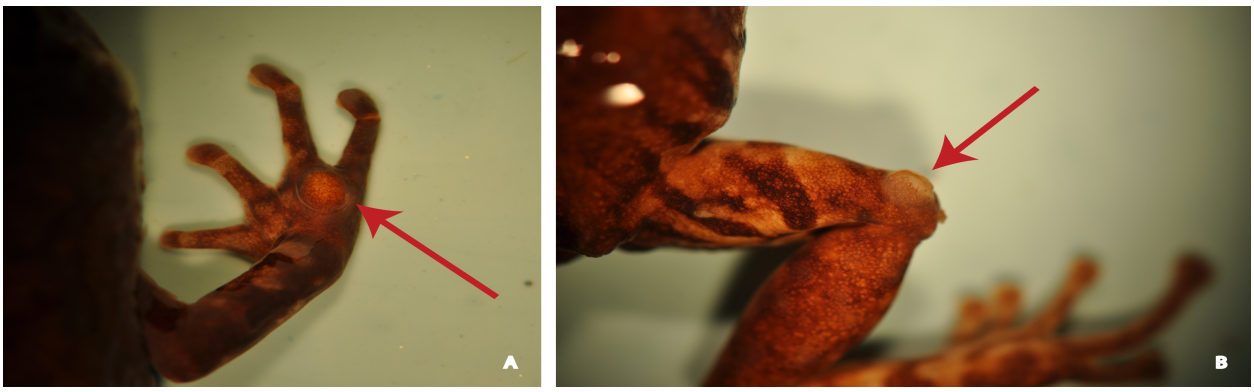

Figure 2. Photographs of *Pristimantis* sp. 1 showing **A.** a closed mite capsule on the dorsal surface of the right hand (arrow; MHNJP 3776) and **B.** a ruptured mite capsule on the right knee (arrow; MHNSM 8754).

Examination of the Mites: A Jeol JSM-5800LV Scanning Microscope was used to capture scanning electron micrographs of two mites, one mite discovered in ethanol solution and another removed from a female frog. These images were produced in order to determine the genus of the mite. The female frog (MHNSM 8765) with the most mite capsules (n = 11) was selected to have a single capsule opened. A single mite was present in the capsule. Micrographs were compared with images by Wohltmann *et al.* (2005), who examined and described *Hannemania yungicola,* isolated from *Eleutherodactylus platydactylus* from a montane forest in Bolivia. The mites were preserved in 100% ethanol prior to preparation. Mites were dried in a CPD2 Pelco Critical Point Dryer following the manufacturer's instructions. Specimens were then mounted on an SEM pin primed with two-sided carbon tape using an adhesive probe and a stereoscope. A Gold-Palladium mixture was used to increase conductance of specimens; two layers were added using a Pelco SC-7 Auto Sputter Coater following the manufacturer's instructions. Use of the scanning electron microscope followed the manufacture's instructions. To facilitate comparisons,

regions of mites were photographed to match the drawings of *Hannemania yungicola* in Wohltmann *et al.* (2005), and are as follows: ventral view of the whole body, the gnathosoma (the region containing the mouth, cheliceral digtus, and palp claw), and the legs.

Statistical Examination of the Infestations: The number of mite capsules on each frog was assessed with respect to sex, size within each gender (sexes were divided into large and small groups by the median SVL), and age. Pearson's X^2 test was utilized to determine if the associations were significant. Juveniles were not studied in tests categorized by sex. Differences in the mean number of mite capsules on different body regions were also determined. Body regions are defined as throat, dorsal surface of the head, chest and belly, dorsum, arms, legs, and flanks (between dorsolateral folds and venter). The body regions study followed the procedure of Malone and Paredes-León (2005). For each infested individual, the number of mite capsules per mm^2 of each body region was calculated. The surface area of each body region was determined using measurements of the body parts in the regions and the surface area formula matching the approximate geometric shape the each portion. Measurement data were collected as described above. Data were tested for normality with the Kolmogorov-Smirnov and Shapiro-Wilk tests, and variance homogeneity was tested using a Levene statistic. If necessary, data were normalized using a natural logarithmic transformation. The infestation means at these sites were analyzed using a one-way ANOVA with Hochberg's GT2 post-hoc tests. For significant analyses, effect sizes were calculated using equation 1 (Fields 2009):

Equation 1. $$\omega = \sqrt{\frac{SS_M - (df_M)MS_R}{SS_T + MS_R}}$$

In the above equation, SS_M is the model sum of squares, df_M is the degrees of freedom, MS_R is the mean squares of the model, and SS_T is the total sum of squares. All statistical tests were performed using SPSS 19.

RESULTS

New species of Pristimantis

Holotype: MHNSM 8796, adult male, from Río Abiseo National Park, Region San Martin, Peru (Figure 3).

Paratypes: 47 adult females, 40 adult males, and 14 juveniles: collected at type locality along with the holotype by A. CATENAZZI and R. VON MAY in 1999. A drawing of a paratype can be found in Figure 4.

A. B

Figure 3. Photographs of a preserved specimen of *Pristimantis* sp. 1 (holotype, SVL 19.50 mm) in **A.** dorsal and **B.** ventral views. Photographs by A. Brus.

Diagnosis: A member of the *Pristimantis orestes* Group having: (1) skin on dorsum shagreen with scattered tubercles; skin on venter coarsely areolate; discoidal fold and dorsolateral folds absent; (2) tympanic membrane present; tympanic annulus distinct, round, about half the diameter of eye; (3) snout short, rounded in dorsal view and profile, having small rostral papillae; (4) upper eyelid bearing a variable number of tubercles, one prominent in females, minute in males; upper eyelid width narrower than IOD; cranial crests absent; (5) dentigerous processes of vomers absent; (6) males having vocal slits and vocal sac, nuptial pads absent; (7) Finger I shorter than Finger II; discs on fingers narrow, barely wider than digit; (8) fingers with narrow lateral fringes; (9) ulnar tubercles low; (10) heel with small tubercle; inner tarsal fold weak.

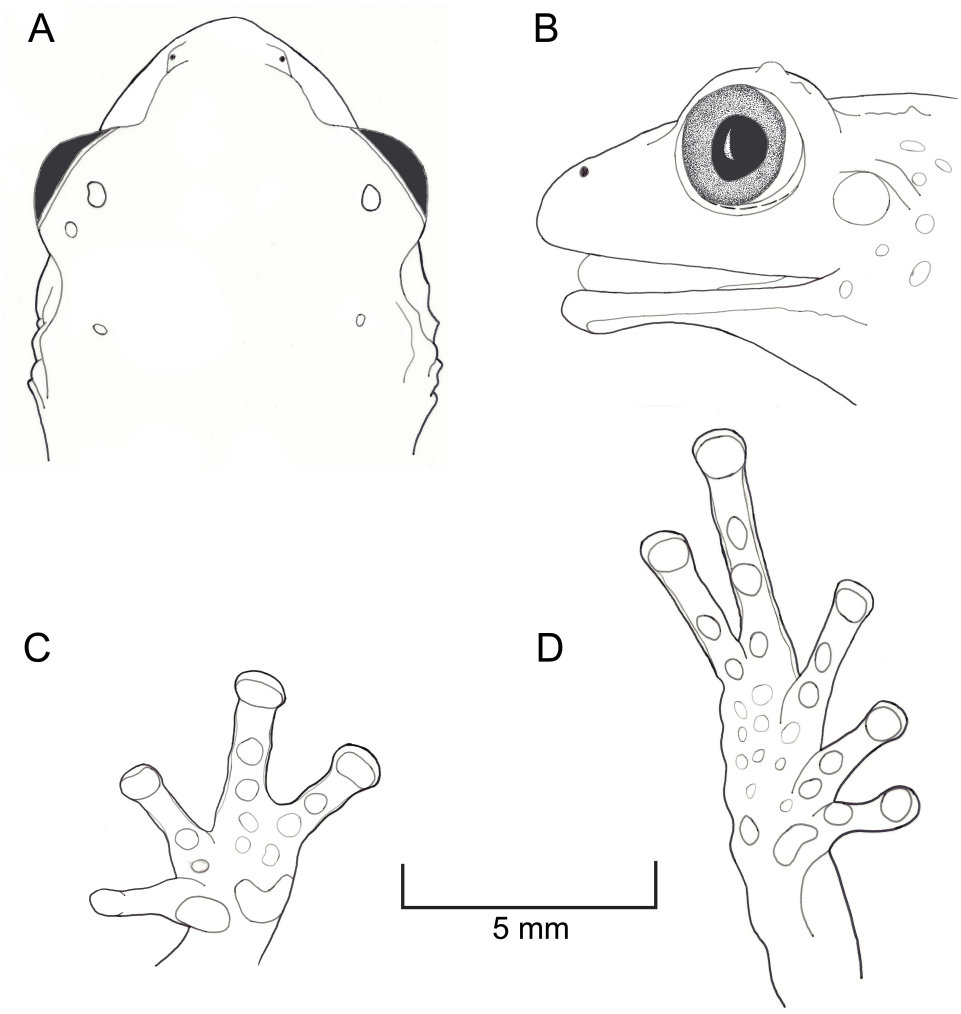

Figure 4. Drawings of *Pristimantis* sp. 1 (MHNSM 8776). **A.** Head in dorsal view. **B.** Head in lateral view **C.** Left hand in ventral view **D.** Right foot in ventral view. Drawings by A. Brus.

(11) Inner metatarsal tubercle ovoid, larger than subconical outer metatarsal tubercle, elevated, subconical in lateral view; many low, supernumerary plantar tubercles; (12) toes with narrow lateral fringes; webbing absent; Toe V slightly longer than Toe III; toe discs similar to those on fingers, narrow; (13) in ethanol, dorsum reddish brown to tan becoming tan laterally, with brown occipital scapular markings, venter tan with scattered brown blotches, groin pale tan with brown reticulations; (14) SVL 22.97–14.39 mm in males (n = 40), 29.08–17.35 mm in females (n = 47). The maximums, minimums, and means of the males and females measurements are given in Table 1.

Pristimantis sp. 1 is readily distinguished from other members of the *Pristimantis orestes* Group by the presence of prominent scapular tubercles. *Pristimantis* sp. 1 differs from five other species—*Pristimantis chimu, Pristimantis cordovae, Pristimantis pataikos, Pristimantis pinguis, Pristimantis seorsus*—in the absence of a discoidal fold. The new species lacks dorsolateral folds, which are present in five other species: *P. chimu, cordovae, seorsus, simonsii,* and *ventriguttatus*. Vocal slits are present in the new species, *P. atrabracus, cordovae, pataikos, pinguis,* and *ventriguttatus* but absent in all others. *Pristimantis chimu, P. melanogaster,* and *P. pataikos* lack a vocal sac, which is present in *Pristimantis* sp. 1 and *Pristimantis cordovae* but were not reported the other species of the group. *Pristimantis cordovae, P. corrugatus,* and *P. simonsii* are unique in the *Pristimantis orestes* Group in the presence of nuptial pads. A rostral tubercle is weakly present in *Pristimantis* sp. 1 and *P. cordovae* but absent or not reported in all other group members.

The new species is distinct from *P. cordovae, corrugatus, melanogaster, pinguis, simonsii,* and *strictoboubonus* because of the presence of heel tubercles. *Pristimantis atrabracus, P. melanogaster,* and *P. strictoboubonus* lack ulnar tubercles, which are present in *Pristimantis* sp. 1. Upper eyelid tubercles occur in *P. chimu, cordovae, corrugatus,* and *ventriguttatus,* as well as in the new species, but not in other species of the *Pristimantis orestes* Group. *Pristimantis simonsii* lacks a tympanic annulus, present in all other members of the group. *Pristimantis melanogaster, P. pataikos, seorsus,* and *simonsii* do not have a tympanic membrane, but the new species as well as all others do. *Pristimantis* sp. 1 lacks dentigerous processes of vomers, which is different from *P. chimu, cordovae, pinguis, seorsus,* and *ventriguttatus.* All members of the *Pristimantis orestes* Group, including the new species, have digits with narrow expansion except for *Pristimantis corrugatus* and *Pristimantis ventriguttatus*. Only *P. cordovae, seorsus, simonsii,* and *ventriguttatus* posses toe webbing. Appendix B shows a comparison of selected characters of the 11 known Peruvian group members and the new species.

Description of the Holotype: Head width equal to the body, wider than long; head width 43.0% of SVL; head length 39.0% of SVL; snout short, rounded in dorsal view, rounded in lateral view with small rostral papilla at tip of snout, eye-nostril distance less than eye diameter, nostrils protuberant, directed laterally; canthus rostralis slightly concave in dorsal view, rounded in profile; loreal region slightly concave; lips rounded; upper eyelid with minute tubercles; width of upper eyelid narrower less than IOD (upper eyelid width 62.0% of IOD); supratympanic fold

absent, tympanic membrane and tympanic annulus present, about half the size of ED (49.0%), separated from eye by more than half its diameter; postrictal tubercles prominent. Choanae large, round, not concealed by palatal shelt of maxilla; dentigerous processes of the vomers absent; tongue over two times as long as wide (tongue width is 63.9% of the length), oval, posterior half free.

Skin on dorsum is shagreen with low scattered tubercules more dense on posterior, scapular tubercle ovoid in dorsal view, subconical in lateral view, dorsolateral fold absent; skin on flanks areolate; skin on throat, chest, and belly areolate; discoidal fold absent, thoracic fold weak; cloacal sheath short, large tubercles absent from the cloacal region. Outer surface of the ulnar with minute tubercles; palmar tubercles low, outer palmer tubercle bifid, ovoid, approximately equal to elongate inner tubercle; one subconical supernumerary tubercle at the base of each finger, about a quarter of the size of the subarticular tubercles; subarticular tubercles are well defined, round in dorsal view, subconical in lateral view; fingers with narrow lateral fringes broadest at base; Finger I shorter than Finger II; discs on digits of outer fingers expanded, truncate.

Hind limbs moderate in length, tibia length 47% of SVL; foot length 46% of SVL; upper surface of the hind limbs shagreen; posterior and ventral surfaces weakly areolate; heel with low tubercle; tarsal fold weak; metatarsal tubercles subconical, inner metatarsal tubercle ovoid, about three times the size as the round outer metatarsal tubercle; plantar supernumerary tubercles low, diffuse, about one half to one forth the size of subarticular tubercles; subarticular tubercles well defined, less prominent than those on fingers, ovoid in dorsal view, slightly conical in profile; toes with narrow lateral fringes, basal webbing absent; discs on digits of toes narrowly expanded, slightly wider than digit proximal to the disc, discs on toes larger than those on fingers; relative lengths of toes: $1 < 2 < 3 < 5 < 4$; Toes I and V on left foot are abnormally bulbous and short, discs absent from the toes, appears to have healed after injury. Images of the holotype are found in Figure 3.

Measurements (in mm) of holotype: SVL 19.50; tibia length 9.16; foot length 9.02; head length 7.64; head width 8.42; eye diameter 2.83; tympanum diameter 1.38; interorbital distance 3.02; upper eyelid width 1.87; internarial distance 3.30; eye–nostril distance 2.35.

Coloration of holotype in preservative: Dorsum grayish brown, occiptal-scapular region H-shaped, light brown; outer surface of forelimbs grayish brown with darker brown blotches,

inner surface tan, freckled with brown spots; hind limbs reddish brown without bars, heels grayish brown, darker than hind limbs; Fingers I and II tan with minute brown spots, Fingers III and IV grayish brown, Toes I-IV tan with scattered brown freckles, Toe V grayish brown; head laterally grayish tan without canthal stripe, supratympanic stripe brown and faint labial stripes; upper half of tympanum brown, contiguous with supratympanic stripe, lower half tan with blown specks; flanks grayish tan with aberrant brown stripes; axilla tan with scattered brown flecks; groin tan with brown specks, grayish brown at leg joint; anterior surface of thigh tan with light brown blotches; posterior surfaces of thigh reddish brown; concealed surfaces of tibia grayish brown dorsally becoming tan with brown freckles ventrally; throat, chest and belly grayish tan with brown speckles concentrated above thoracic fold; ventral surface of the thighs reddish brown spots posteriorly, become more diffuse anteriorly; hands and feet tan; iris dark grayish black.

Table 1. Measurements (in mm) and proportions of type series of *Pristimantis* sp. 1*;* minimum and maximum values followed by means and one standard deviation in parentheses.

Characters	Females (*n* = 47)	Males (*n* = 40)
SVL	29.08–17.35 (23.39 ± 3.43)	22.97–14.39 (18.08 ± 2.15)
TL	13.11–8.07 (10.86 ± 1.25)	10.37–6.75 (8.77 ± 0.72)
FL	12.98–7.80 (10.79 ± 1.48)	10.49–6.12 (8.37 ± 0.92)
HL	10.50–6.28 (8.60 ± 1.05)	8.77–5.49 (7.03 ± 0.83)
HW	11.59–6.43 (8.84 ± 1.26)	8.42–5.46 (6.96 ± 0.88)
ED	3.55–2.22 (3.05 ± 0.31)	3.49–1.80 (2.59 ± 0.40)
TY	1.82–0.90 (1.32 ± 0.21)	1.85–0.66 (1.10 ± 0.26)
IOD	4.30–2.07 (3.25 ± 0.53)	3.49–1.81 (2.70 ± 0.40)
EW	2.76–1.34 (1.96 ± 0.29)	2.96–1.21 (1.77 ± 0.35)
IND	2.80–1.70 (2.15 ± 0.27)	3.30–1.43 (1.99 ± 0.43)
E–N	2.90–1.47 (2.21 ± 0.35)	2.34–1.10 (1.71 ± 0.24)
TL/SVL	0.57–0.40	0.59–0.42
FL/SVL	0.52–0.38	0.53–0.40

Table 1 (continued). Measurements (in mm) and proportions of type series of *Pristimantis* sp. 1*;* minimum and maximum values followed by means and one standard deviation in parentheses.

Characters	Females (*n* = 47)	Males (*n* = 40)
HL/SVL	0.42–0.32	0.45–0.35
HW/SVL	0.43–0.34	0.44–0.35
HW/HL	1.19–0.89	1.10–0.83
E–N/ED	1.06–0.46	0.92–0.52

Variation: The morphology of the paratypes is similar to the holotype. Male specimens are smaller than females and have vocal slits and vocal sacs, but lack nuptial pads. Several specimens (males: MHNSM 8722, 8727, 8729, 8757, 8790 and MHNJP 3789; females: MHNSM 8733, 8767, 8775, and MHNJP 3768) have prominent dorsal stripes, dorsal coloration ranging from cream and light brown to reddish brown and brown. Some of the series (males: MHNSM 8744, 8761, 8776, 8911, 8925 and MHNJP 3771; females: MHNSM 8724, 8738, 8782 and MHNJP 3779) have a middorsal hairline stripe. A single female (MHNJP 3779) has prominent dorsolateral strips. One male (MHNSM 8746) has reddish brown dorsum with irregularly shaped tan blotches. Eight females (MHNSM 8718, 8726, 8751, 8752, 8758, and MHNJP 3774, 3776) and one male (MHNSM 8761) have uniformly black dorsums and cream to reddish brown coloring on the media edge of the hands and dorsal surface of thighs. One male (MHNSM 8760) has a brownish red dorsum with cream speckles. Compared to the holotype, numerous specimens (males: MHNSM 8736, 8742, 8746, 8763, 8768, 8773, 8778, 8797, 8911, 8919, 8921, 8925, 8935, 8947, 8948 and MHNJP 3786; females: 8748, 8755, 8756, 8785, 8789, 8791, 8795, 8800, 8931, 8938, 8949 and MHNJP 3772, 3773, 3775) have a more defined but incomplete X-shaped occipital-scapular ridge. Four specimens (males: MHNSM 8776, 8797; females: MHNSM 8764, 8791) have a W-shaped occipital-scapular ridge. Some specimens (males: MHNSM 8721, 8757, 8762, 8790, 8794, 8921, MHNJP 3789; females: MHNSM 8725, 8735, 8800 and MHNJP 3775, 3779) possess an interorbital blotch. Five specimens (males: MHNSM 8778 and MHNJP 3771; females: MHNSM 8724, 8750, 8759) have an interorbital bar extending over the upper eyelid. One female (MHNSM 8759) has a cream dorsum with a single, posterior, brownish-black bifid blotch. One female specimen (MHNSM 8781) has highly pronounced tubercles on the limbs and dorsum, and scapular tubercles set upon vertical, sinusoidal ridges. One male (MHNSM 8794) has pale tan groin and axilla, lacking all patterns

present in others. Several specimens (males: MHNSM 8722, 8736, 8740, 8742, 8744, 8757, 8762, 8776, 8790, 8797, 8911, 8923, 8935, 8948; females: MHNSM 8724, 8725, 8733, 8738, 8755, 8756, 8765, 8775, 8795, 8800, 8931, 8938, 8949 and MHNJP 3782, 3785, 3786) have venters with little to no patterning; however, reddish brown spots on ventral surface of thighs are still present. Some females (MHNSM 8718, 8735, 8748, 8750, 8751, 8754, 8759, 8764 and MHNJP 3774) have scattered brown blotches on the ventral surface. One male (MHNJP 3771) has three, thin white lines on the venter, one continuous from right Finger 1 to left Finger 1, another line extending midventrally from the tip of the snout to cloaca, and a final line between the left and right inner metatarsal tubercles. Three males (MHNSM 8773, 8779, 8794) have dark tanish red ventral sides. Ventral coloration of series ranges from cream to orangish red. Two males (MHNSM 8721, 8921) are finely areolate, and six males and seven females (males: MHNSM 8727, 8729, 8740, 8749, 8763; females: 8730, 8733, 8738, 8752, 8758, 8767, 8789 and MHNJP 3776) are coarsely areolate.

A photograph of a living specimen (taken by A. Catenazzi) shows a grayish brown dorsum becoming tanish gray on lateral sides. In this specimen a brown, triangular interorbital blotch is present, peak oriented posteriorly. Additionally, a brown, X occipital-scapular ridge is noticeable. Labial region is cream colored with dark brown labial stripes and supratympanic stripe, some brown canthal coloration evident. Lateral sides are two toned, the dorsal side being tanish gray and the ventral side being pale yellow; grayish brown bars extend dorsocranially to ventrocaudally. Dorsal surface of the tibia and lateral surface of the forearm have faint grayish brown bars. Groin, concealed surface of the thigh, and anterior dorsal region of the tarsal are deep red in color. Axilla and venter are creamy yellow. The iris is darker ventrally. An additional photograph (taken by A. Catenazzi) of a different specimen shows a moss green dorsal coloration.

Distribution: The new species is only known from puna and montane forests of the Río Abiseo National Park (Figure 1). The exact elevations and coordinates at which the series was collected were not recorded.

Analysis of the Mite Infestation

Examination of the Mites: The majority of domed distortions were complete, and no entrances were observed in any of the capsules examined (Figure 5A). These observations suggest that the mites did not burrow into the skin, which is consistent with the mites of the family Trombiculidae. Numerous capsules were ruptured and no mites were present. The inner boundary of the capsule was smooth and had a pale tan coloration. The extracted mite was positioned with its dorsal surface to the outer half of the capsule. Both the free mite and the extracted mite were engorged. The presence of six legs confirmed the larval stage of the mite and the fact that the larval stage was parasitic confirmed the classification in the family Trombiculidae. Adult stages of these mites possess four pairs of legs and are free living in the soil (Ruppert *et al.* 2004; Robert *et al.* 2000; Wohltmann *et al.* 2005; Hatano *et al.* 2007). The scanning electron micrographs of the mite that were compared to Wohltmann *et al.* (2005) are presented in Figure 6. The examination of the mites did not confirm the genus *Hannemania*. According to Wohltmann *et al.* (2005), the cheliceral digitus of mites of the genus *Hannemania* have a characteristic bottle-opener-shaped notch on the non-serrated, internal edge. This character was absent from both mites examined. This new species of mite may belong to a new genus; however, it is possible that these findings represent a novel condition of the genus *Hannemania*.

Statistical Examination of the Infestations: The red coloration of the mite capsules on the epidermis of a live and fresh-killed frogs made the infestation easy to detect (Figure 5A). Three frogs (MHNSM 8752, 8796, and MHNJP 3783) had damage to their hands or feet (Figure 5B). Damage was considered to be any displacement or deformation of the hands and feet as a result of a previous mite. A breakdown of the mite capsules found in the series is given in Table 3. Incidences of mites recorded in the series are presented in Table 2.

Table 2. Frogs with at least one capsule were considered infested (*n* = 101)

Sex	Number of Infested Individuals (Infested / Total Frogs)	% Infested
Female	14 / 47	29.79
Males	13 / 40	32.50
Juveniles	4 / 14	28.57
Total	31 / 101	30.69

No significant association between the gender of the frog and the number of infested individuals was found ($X^2_1 = 0.074$, $p = 0.79$). The percent of infested small males (SVL < 17.79 mm; 30.0 %) did not differ significantly from the percent of infested large males (SVL > 17.79 mm; 35.0 %; $X^2_1 = 0.114$, $p = 0.736$). However, large females (SVL > 23.25 mm) showed a significantly higher level of infestation than smaller females (SVL < 23.25 mm; $X^2_1 = 4.037$, $p = 0.045$). Based on the odds ratio (Fields 2009), the likelihood of infestation was 3.85 times higher in larger females. The adult and juveniles showed no significant differences in the percent of infested individuals ($X^2_1 = 0.034$, $p = 0.853$).

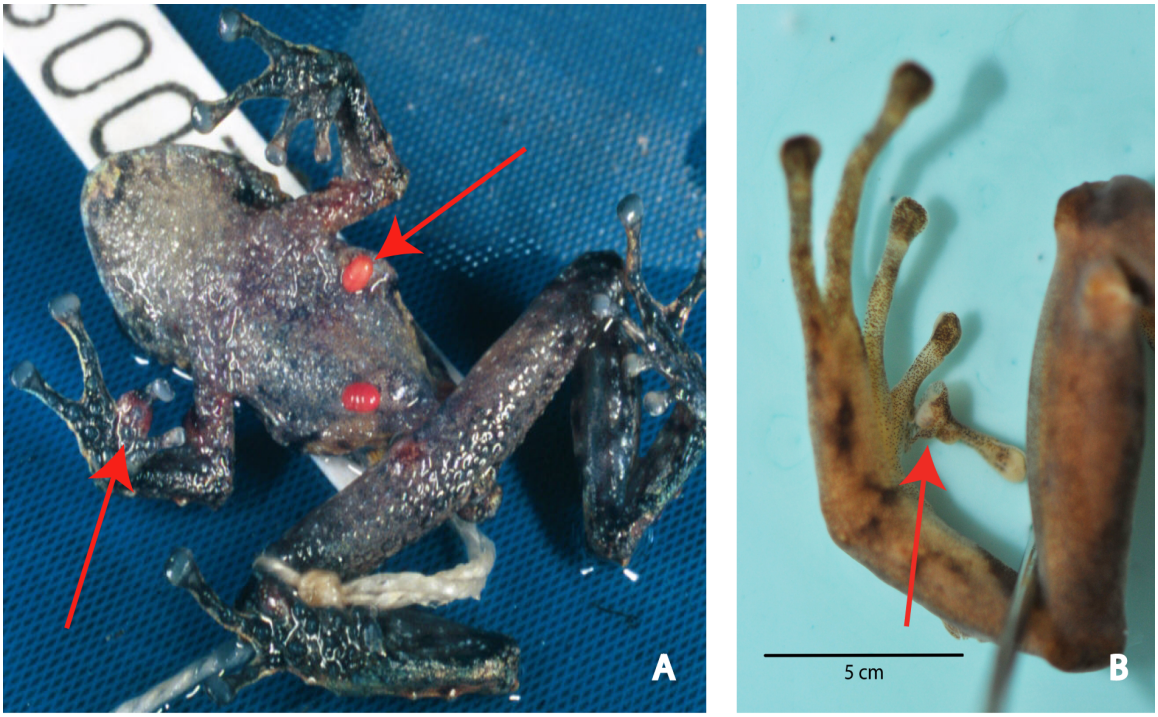

Figure 5. A. Photograph of an unknown specimen of *Pristimantis* sp. 1 showing several mite capsules (Arrows). After preservation red coloring is lost. Note the capsule on Finger II of the right hand. Photograph by A. Catenazzi. **B.** Photograph of a preserved specimen (MHNJP 3783) showing toe I of the left foot being displaced due to a mite at its base. Photograph by A. Brus.

Table 3. Mite capsules recorded in the sexes and juvenile frogs ($n = 83$).

Sex*	Number of capsules	% Of total capsule present
Female ($n = 14$)	47	56.63
Male ($n = 13$)	24	28.92
Juvenile ($n = 4$)	12	14.46
Total ($n = 31$)	83	100.00

*n *values report the number of infested frogs in each category.*

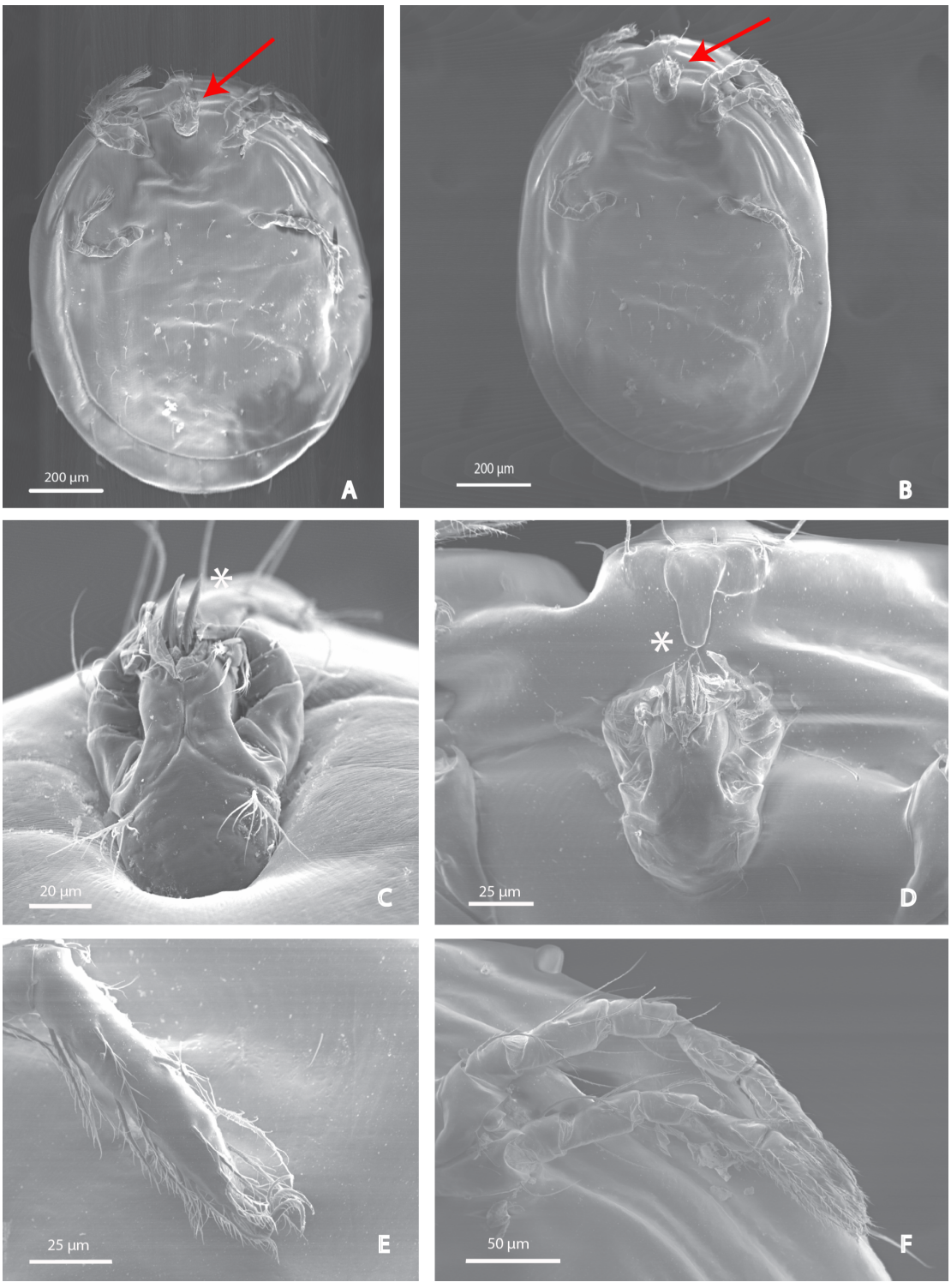

Figure 6. Scanning electron micrographs of mites. Images of the left of the figure are from the collected specimen and those on the right are of the mite found in ethanol. **A–B.** Ventral surface of the mite is visible with legs and gnathosome present (arrows). **C–D.** Micrographs show a detailed view of the gnathosome, the feeding structure of the larva; note the smooth inner surface of the cheliceral digiti (asterisk). **E.** The distal portion of the front-most right leg. **F.** Two front, left legs. Photographs by A. Brus.

Because no differences were found in the frequency of infestations in males and females, the entire adult infested population was utilized in further analyses. Due to insufficient sample size ($n = 2$), the mite capsules on the dorsal surface of the head were omitted. Means and standard deviations of the surface area of the body regions are presented in Table 4, while the number of mite capsules found in each body region and the mean number of mite capsules per mm^2 of each region are in Table 5. A highly significant difference existed in the mean number of capsules among body regions ($F_{6, 56} = 10.72$, $p < 0.001$, $\omega = 0.71$). The throat showed a significantly higher rate of infestation compared to all other body regions examined, except for the chest–belly (Table 6). Conversely, the legs had significantly fewer capsules than all body regions used in the study, except for the arms. There were no other significant differences in the mean number of capsules among body regions. All capsules recorded on juveniles were present on the arms ($n = 3$) and legs ($n = 9$).

Table 4. Mean surface areas (mm^2) with standard deviations reported for each body region. Mean and standard deviations were calculated using the entire infested adult population ($n = 27$).

Body Region	Mean ± Standard Deviation	Maximum	Minimum
Dorsum	113.26 ± 46.04	204.68	51.99
Arms	163.90 ± 65.38	299.66	79.81
Legs	265.63 ± 96.10	436.69	140.31
Flanks	133.73 ± 53.51	228.39	60.98
Throat	35.36 ± 12.40	60.88	21.35
Chest–Belly	103.22 ± 42.65	172.98	46.70
Head [Dorsal]	102.55 ± 39.37	181.17	52.67

Table 5. Number of mite capsules and means ± standard deviations of the incidences of mite capsules per mm^2 for the body regions. Mean and standard deviations were calculated using the entire infested adult population ($n = 27$).

Body Region*		Total Number of Capsules (% of total)	Mean ± Standard Deviation of Incidences of Mite Capsules per mm^2	Maximum	Minimum
Dorsum	($n = 14$)	16 (22.5)	0.011 ± 0.004	0.018	0.005
Arms	($n = 12$)	15 (21.1)	0.008 ± 0.004	0.016	0.003

*n *values are given for the number of frogs with capsules in each location.*

Table 5 (continued). Number of mite capsules and means ± standard deviations of the incidences of mite capsules per mm2 for the body regions. Mean and standard deviations were calculated using the entire infested adult population (n = 27).

Body Region*		Total Number of Capsules (% of total)	Mean ± Standard Deviation of Incidences of Mite Capsules per mm^2	Maximum	Minimum
Legs	(*n* = 11)	15 (21.1)	0.005 ± 0.002	0.010	0.002
Flanks	(*n* = 9)	12 (16.9)	0.010 ± 0.005	0.017	0.004
Throat	(*n* = 5)	6 (8.5)	0.03 ± 0.012	0.045	0.017
Chest–Belly	(*n* = 4)	5 (7.0)	0.011 ± 0.004	0.015	0.007
Head [Dorsal]	(*n* = 2)	2 (2.8)	0.011 ± 0.006	0.015	0.007

**n* *values are given for the number of frogs with capsules in each location.*

Table 6. Results of Hochberg's GT2 post-hoc analyses comparing mean number of capsules in body regions. Significant values are indicated with an asterisk.

Comparison	*P* value	Comparison	*P* value
Throat v. Chest–Belly	0.052	Chest–Belly v. Flanks	1.00
Throat v. Dorsum	0.001*	Dorsum v. Arms	0.24
Throat v. Arms	< 0.001*	Dorsum v. Legs	0.001*
Throat v. Legs	< 0.001*	Dorsum v. Flanks	1.00
Throat v. Flanks	0.001*	Arms v. Legs	0.45
Chest–Belly v. Dorsum	1.00	Arms v. Flanks	0.88
Chest–Belly v. Arms	0.53	Legs v. Flanks	0.19
Chest–Belly v. Legs	0.017*		

DISCUSSION

New species of Pristimantis

Because of remote habitats, a complex physiography, and protection of the ecosystems, new anuran species are often identified in Peru. The new species of *Pristimantis* is a member of the *Pristimantis orestes* Group and is unique from all other members in the presence of prominent scapular tubercles. The identification of new species is an important part of conservation as protection can only be provided for those ecosystems and species. The new species will be named in the publication of the results.

Analysis of the Mite Infestations

Examination of the mites: The morphological description of the outside and inside of the capsules was similar to other studies (Wohltmann *et al*. 2006; Duszynski *et al*. 1973; Malone and Paredes-León 2005). Previous reports of mite infestations on anuran species have classified the mites as *Hannemania* sp. (Wohltmann *et al.* 2006; Hatano *et al.* 2007; Jung *et al.* 2001; Malone and Paredes-León 2005). The images of the mites taken in this study do not confirm the generic affiliation with *Hannemania*. The bottle-opener shape on the inner surface of the cheliceral digiti is considered a synapomorphic character of the genus *Hannemania* (Wohltmann *et al.* 2006) but was not recorded in the mite infesting specimens of *Pristmantis* sp. 1. The inner surfaces of the cheliceral digiti have a distinctly smooth appearance (Figure 6). The mites had only three pairs of legs as opposed to four pairs found in adult stages; this condition, a parasitic larval stage, confirmed the mites as trombiculids. Given that no reports reviewed discussed genera other than *Hannemania*, the mites of the new species could constitute a new genus or an unknown characteristic of the genus *Hannemania*. An acarologist is needed to confirm these findings and compare the mite found in this study to other species.

Statistical Examination of the Infestation: The series of frogs examined was unusually large (n = 101) and provided an opportunity for a detailed analysis of the infestations. Previous studies had smaller sample sizes, the largest being Hatano *et al.* (2007) in which 49 frogs were examined. Over a quarter of the frogs in the series were infested with at least one mite capsule, and, within the adult population, at least two mite capsules were found in each of the body regions. The percentage of frogs infested suggests that *Pristimantis* sp. 1 is a major host in the life cycle of the mite; however, Hatano *et al.* reported rates of infestation as high as 91.7 %, so it is likely that the new species is not the sole host of the mite.

No differences were found in the number of mite capsules between sexes. These findings are consistent with previous studies (Hatano *et al.* 2007; Malone and Paredes-León 2005) and suggest that males and females do not differ in their microhabitat. Jung *et al.* (2001) suggest that differences in infestations between males and females of *Rana berlandieri* reflect the sexes proximity to water supplies. They reported that males in the study were more heavily infested compared to females and were more associated with the Rio Grande. Further study is needed to determine the microhabitat of the new species. Those members of the series with infestations are

likely to have a unique microhabitat or were present in an area that overlapped with the geographic distribution of the mites. The month in which the collection occurred is also unknown and could help explain the differences in the number of mites among the frogs in the series. Geographic coordinates were not available for the frogs, so it was not possible to determine if the infested individuals were isolated to a subarea of the collection site.

Malone and Paredes-León (2005) reported a strong relationship between the size of the frog and the number of capsules present, which contradicts the findings of Hatano *et al.* (2007). The present study found a relationship between size and degree of infestation in females but not in males. However, the small sample size of infested males and females may have distorted these results. No differences were seen between the number of infested adults and juveniles. This differs from Jung *et al.* (2001), but *R. berlandieri,* the frog used in their study, has indirect development and this could affect the number of mites present in juveniles.

The majority of the mite capsules were present on the dorsum (22.5 %) and limbs (42.2 %), which is consistent with previous studies examining body regions (Hatano *et al.* 2007; Malone and Paredes-León 2005). Normalizing the number of mite capsules using the surface area of the body regions is a new approach that has not been preformed previously. A strong inverse relationship was seen between the regional surface area and the number of mite capsules per mm^2. Despite having the lowest surface area, the throat showed significantly more mite capsules per mm^2 than all other regions except for the chest–belly. The legs had the opposite condition: a larger surface area but a lower concentration of mite capsules than all other regions except for the arms from which the legs did not differ significantly. These results have not been documented in previous reports, which did not account for the surface area of the different regions. Two factors may explain the heterogeneous distribution of the capsules: 1) The method of attachment utilized by the mite or the regions exposed to the mite (Malone and Paredes-León 2005). The ability of the frog to clean the dorsal surface of the head could explain the low occurrence of capsules in this region. The low number of visible capsules on the hands and feet combined with the large surface area of these regions could have artificially lowered the number of mite capsules per mm^2 of the arms and legs. Future studies could be made more accurate by isolating the hands and feet as distinct body regions. 2) Physiochemical differences among the regions, such as the presence of different compounds or concentrations of compounds secreted

onto the skin, could differentially affect the survival of the mites and thereby alter their distribution (Rey 1992).

Studies on infestations in frogs have found malformed appendages in North America and Brazil (Johnson *et al.* 2001a; Johnson *et al.* 2001b; Johnson *et al.* 1999). These reports, however, differed from the present study in several ways: 1) The infesting agent was a trematode in all three studies and environmental damages, including ultraviolet and pesticide exposure, were discussed as possible causes; 2) the host frogs showed indirect development and tadpoles were most commonly afflicted by the trematode; 3) polydactyly was the most commonly reported malformation; however, missing appendages were also discovered. Damages reported above are restricted to hands (n = 1) and feet (n = 3) and consisted only of missing or malformed digits. Johnson *et al.* (2001b) reported a larger presence of malformations in the hind limbs, which is consistent with our findings. However, Johnson *et al.* (2001b) also reported a lower level of malformation in adults, which is contrary to the findings in the new species. The juveniles of the series had a similar level of infestation (n = 4; 28.57 %) as the whole series (30.69 %), and while all the mite capsules were located on the arms and legs, the juveniles showed no damages or malformations. Damages may occur with rupture, and capsules on juveniles were rarely found to be open. While predation as a cause of injury cannot be discounted, the placement of the juvenile infestation, along with a mite causing the displacement of a toe (MHNJP 3783; Figure 5B), supports our hypothesis that mites on the hands or feet can damage the delicate digits. Further controlled studies are required to confirm this hypothesis. The multitude of mites present on some frogs (n = 1–11 capsules on a single frog) suggests that the majority of mite capsules are not detrimental to the frog; this is consistent with Hatano *et al.* (2007) who reported no changes in body conditions as a result of infestation. In spite of the damages observed, the association of *Pristimantis* sp. 1 and the mites is likely the result of coevolution balancing the impairment of the infested frogs and the benefits received by the mites (Cheng 1964; Ávila-Pires 1989).

Peru has a high degree of biodiversity, resulting from the complex geographic regions characteristic of the country. Tuomisto *et al.* (1995) reports that divisions in these physiographic regions creates observable distribution barriers, and produces high rates of endemism and diversity. The exploration Río Abiseo National Park resulted in the discovery of several new species, including the new *Pristimantis* species presented herein. Isolation allows biodiversity to

thrive, and continued protection and study are needed to maintain the endemic species and conservation effort in Peru. Human agricultural development has caused increased habitat destruction over the past century, impacting the distribution of many highland anurans. Global warming has a disproportionately large effect on high elevations due to a more intense exposed to solar radiation (Duellman and Lehr 2009). Studies such as this enhance understanding of the ecological balance in the Peruvian forests, which will assist in the protection of the flora and fauna in these regions. Continued efforts are required to classify new species so that they can be protected and the human impact on these remote environments can be better understood.

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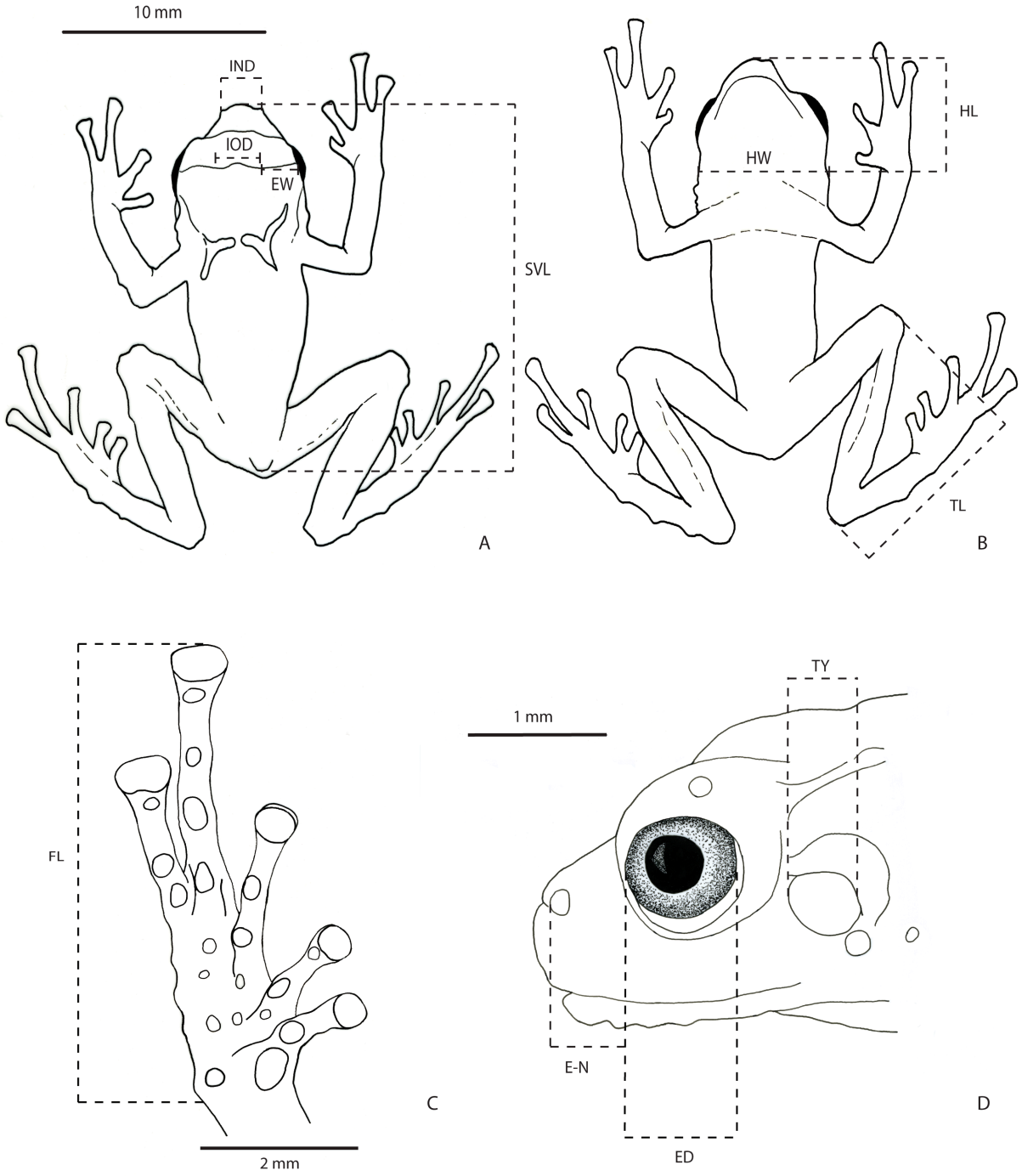
Appendix A. The figure is a series of drawing on which are indicated the measurements taken of *Pristimantis* sp. 1. **A.** Dorsal view of body (SVL = snout-vent length, IND = internarial distance, IOD = interorbital distance, EW = width of upper eyelid), **B.** Ventral view of body (TL = tibia length, HL = head length, HW = head width). **C.** Ventral surface of the foot (FL = foot length). **D.** Lateral view of the head (E-N = eye-nostril distance, ED = eye diameter, TY = tympanum diameter). Diagram drawn after Duellman and Lehr (2009).



Appendix B. *Pristimantis* sp. 1 compared to the other eleven Peruvian *Pristimantis* species. ND = Not Described

Character	*Pristimantis* sp. 1	*P. atrabracus*	*P. chimu*	*P. cordovae*	*P. corrugatus*	*P. melanogaster*	*P. pataikos*
Dicoidal fold	–	–	+	+	–	–	+
Dorsolateral fold	–	–	+	+	–	–	-
Vocal Slits	+	+	–	+	–	–	+
Vocal Sac	+	ND	–	+	ND	–	-
Nuptial Pads	–	–	–	+	+	–	-
Rostral Tubercle	+	ND	–	+	ND	ND	-
Heel Tubercles	+	–	–	+	+	+	-
Ulnar Tubercle	+	–	+	+	+	–	+
Scapular Tubercle	+	–	ND	–	ND	–	-
Upper Eyelid Tubercles	+	–	+	+	+	–	-
Tympanic annulus	+	+	+	+	+	+	+
Tympanic membrane	+	+	+	+	+	–	-
Dentary Processes of Vomers	–	–	+	+	–	–	-
Digit Discs	Narrow	Narrow	Narrow	Narrow	Expanded	Narrow	Narrow
Toe Webbing	–	–	–	+	–	–	-

Appendix B (continued). *Pristimantis* sp. 1 compared to the other eleven Peruvian *Pristimantis* species. ND = Not Described

Character	*Pristimantis* sp. 1	*P. pinguis*	*P. seorsus*	*P. simonsii*	*P. strictoboubonus*	*P. ventriguttatus*
Dicoidal fold	–	+	+	–	–	+
Dorsolateral fold	–	–	+	+	–	+
Vocal Slits	+	+	–	–	–	+
Vocal Sac	+	ND	ND	ND	ND	ND
Nuptial Pads	–	–	–	+	–	–
Rostral Tubercle	+	–	ND	ND	ND	ND
Heel Tubercles	+	–	+	–	–	+
Ulnar Tubercle	+	+	+	+	–	+
Scapular Tubercle	+	–	ND	ND	ND	ND
Upper Eyelid Tubercles	+	–	–	–	–	+
Tympanic annulus	+	+	+	–	+	+
Tympanic membrane	+	+	–	–	+	+
Dentary Processes of Vomer	–	+	+	–	–	+
Digit Discs	Narrow	Narrow	Narrow	Narrow	Narrow	Expanded
Toe Webbing	–	–	+	+	–	+